Chapter Four:

Investigation of ALT in tumours using the APB assay

Investigation of ALT in tumours using the APB assay

4.1   Introduction

This chapter demonstrates the utility of the APB assay for ALT by using it to survey for the presence of ALT in a variety of tumour types. By analysing the clinical data, the significance of the ALT mechanism for patient outcome and tumour behavior was examined. The APB assay was also used to test for ALT in tumours from patients with known mutations in tumour suppressors to further investigate genes that may be involved in the ALT mechanism or its regulation.

4.2   Prevalence of ALT in tumours

We used the APB assay to test for ALT in paraffin sections of five major tumour types, namely osteosarcoma, soft tissue sarcoma (STS), astrocytoma, non small cell lung carcinoma (NSCLC) and papillary carcinoma of the thyroid (Table 4.1; examples in Fig. 4.1 - 4.3 and Fig. 3.11), as we considered these more likely to have a substantial proportion of ALT[+] tumours[4,270] (also see Section 1.11). The assay detected ALT in 27/58 (47%) osteosarcomas, 35/101 (35%) STS (including the 26 STS in Section 3.7), 17/50 (34%) astrocytomas, 22/291 (8%) NSCLC and none of the 17 papillary carcinomas. We also tested the prevalence of ALT in paraffin sections of colorectal carcinomas which are reported to have a high prevalence of telomerase activity (Table 1.3) and found none of the 31 to be ALT[+] (Table 4.1; Fig. 4.3).
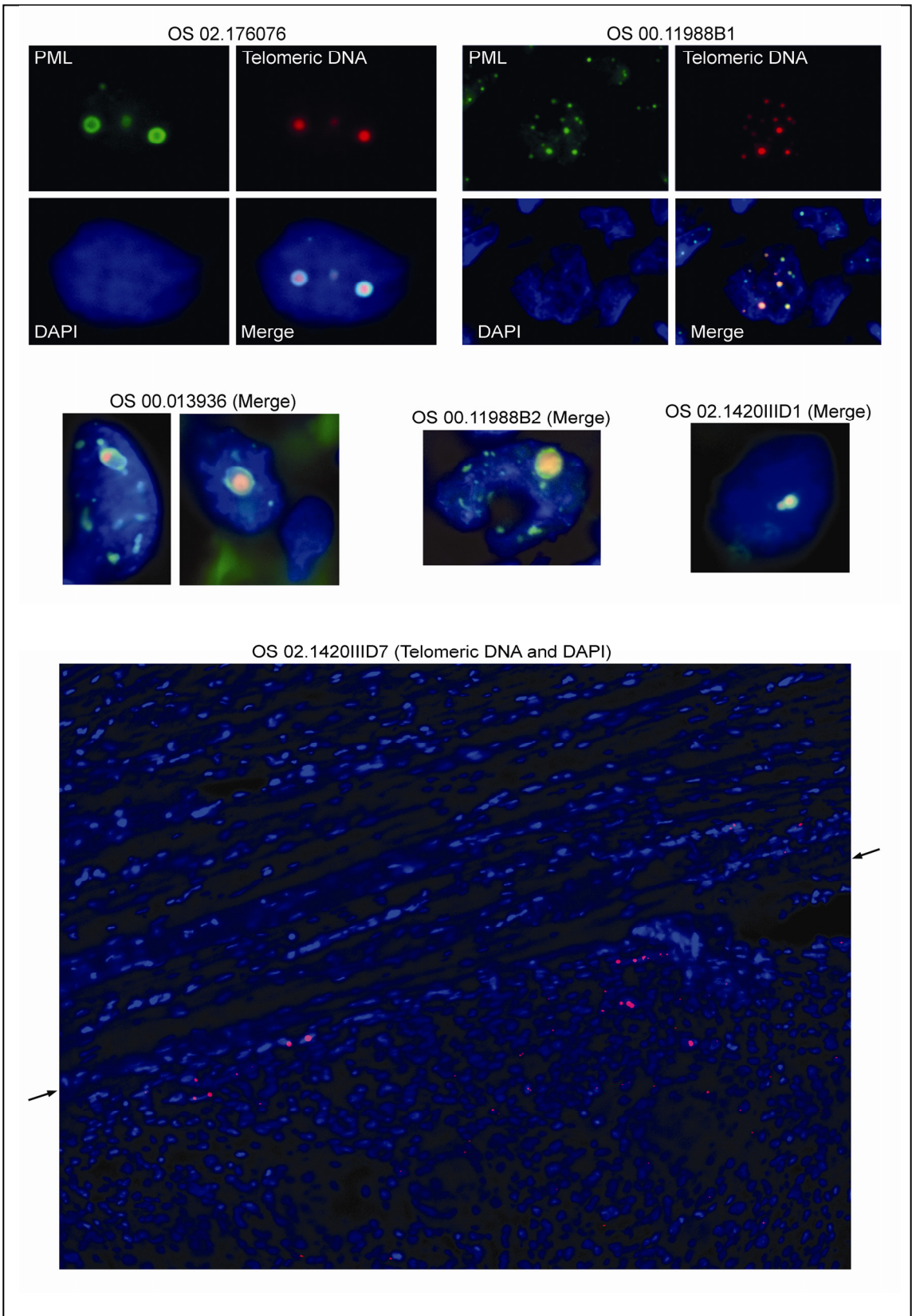
STS include tumours derived from or resembling a diverse group of tissue types and the prevalence of ALT varied greatly between the subtypes (Table 4.1). Malignant

Table 4.1: Prevalence of ALT in tumours

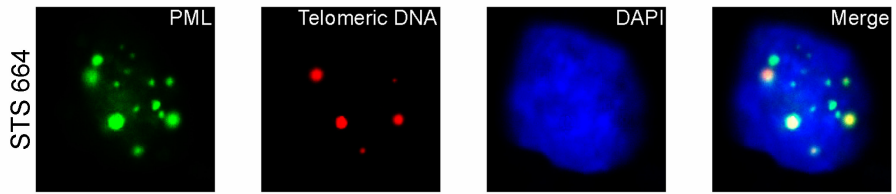| Tumour Type	ALT[+]/Total	% ALT[+]
Osteosarcoma	27/58	47%
Total STS	35/101	35%
Malignant Fibrous Histiocytoma	17/22	77%
Leiomyosarcoma	8/13	62%
Liposarcoma	3/9	33%
Synovial Sarcoma	1/11	9%
Rhabdomyosarcoma	2/35	6%
Fibrosarcoma	2/2	N/A
Alveolar Soft Part Sarcoma	1/4	N/A
Chondrosarcoma	1/3	N/A
Epithelioid Sarcoma	0/2	N/A
Telomerase[-] set of STS	10/16	52% [a]
Malignant Fibrous Histiocytoma	8/10	73% [a]
Leiomyosarcoma	1/3	N/A
Liposarcoma	1/3	N/A
Astrocytoma	17/50	34%
Non Small Cell Lung Carcinoma	22/291	8%
Colorectal Carcinoma[b]	0/31	0%
Papillary Carcinoma of the Thyroid	0/17	0%

[a]This separate set of STS were from a group of 24 STS of which 83% were telomerase[-] and included 12 Malignant Fibrous Histiocytoma (MFH), of which 11 (92%) were telomerase[-][297]. Thus 52%=10/16 x 83% and 73%= 8/10 x 92%. [b]12 of the 22 colorectal carcinomas were known to have MSI, 10 were MSS and 9 unknown. N/A, not applicable.

Figure 4.1: The APB assay in osteosarcomas. Combined PML immunofluorescence and telomere FISH in paraffin sections of ALT[+] osteosarcomas. Indirect immunofluorescence (FITC label) was used for the *PML* protein, telomere FISH was performed using a Cy3-conjugated telomeric peptide nucleic acid probe and slides were photographed using a 100x objective. The bright foci of *telomeric DNA* that colocalise with *PML* represent APBs. In APB[+] sections the telomeres are not bright enough relative to the *telomeric DNA* in APBs to be visible with the exposure time used. Similar to those in cell lines, tumour APBs can have *PML* protein detected in the outer rim and *telomeric DNA* inside (*OS 02.176076*) or *PML* staining throughout the foci (*OS 00.11988B1*). APBs in tumours can sometimes be extremely large (*OS 00.013936* and *OS 00.11988B2*) and can also appear to be dividing or fusing (*OS 02.1420IIID1*). The image of *OS 02.1420IIID7* was taken with low magnification (40x objective) to illustrate what appears to be a distinct border (*arrows*) between normal tissue and a tumour mass with APBs; bright telomeric foci (red) in the nuclei (blue). In one region, a few cells on the normal tissue side of the border also have APBs.
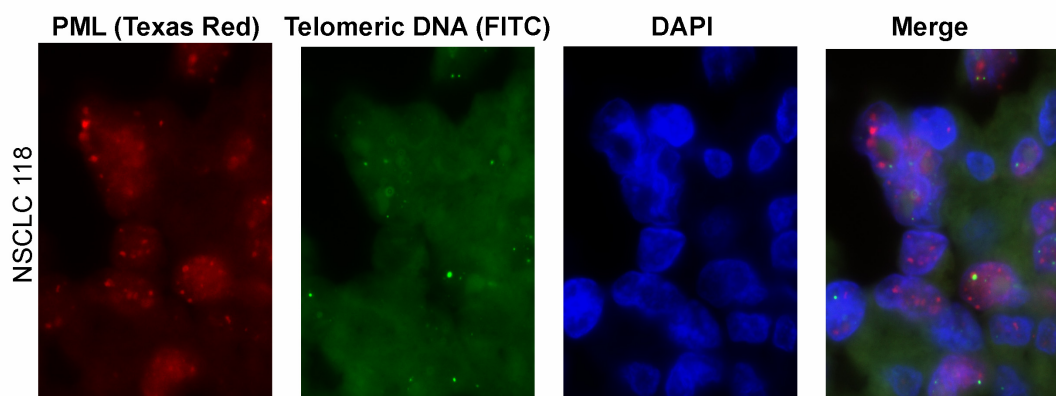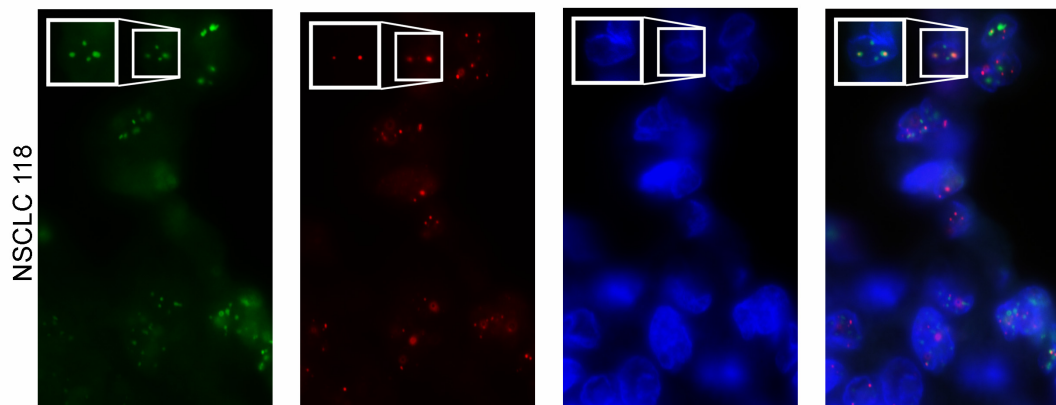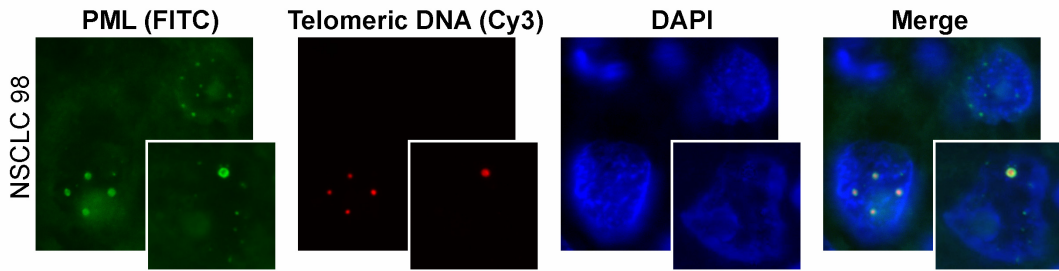
OS 02.176076

PML | Telomeric DNA

DAPI | Merge

OS 00.11988B1

PML | Telomeric DNA

DAPI | Merge

OS 00.013936 (Merge)

OS 00.11988B2 (Merge)

OS 02.1420IIID1 (Merge)

OS 02.1420IIID7 (Telomeric DNA and DAPI)

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Figure 4.2: The APB assay in *A: STS* and *B: NSCLC*. Combined PML immunofluorescence and telomere FISH in paraffin sections. Unless specified otherwise, indirect immunofluorescence (FITC label) was used for the *PML* protein, telomere FISH was performed using a Cy3 conjugated telomeric peptide nucleic acid probe and slides were photographed using a 100x objective. *A:* *STS 664* is an example of an APB[+] STS. *B:* *NSCLC 98* and *102* were APB[+] however, *NSCLC 102* had less than <0.5% of its nuclei APB[+] and was classified as a low frequency APB[+] tumour. The inset in *NSCLC 98* shows a nucleus from another region of the slide. The inset in *NSCLC 118* shows a nucleus in a more appropriate focal plane than used for the other nuclei in this image (and slightly magnified post-imaging) – the APBs in 8 μm sections are in different focal planes. *NSCLC 118* was classified as APB[-] despite having colocalisations between telomeric DNA and PML protein (see Section 4.3). Cy3 generally gives better distinction between telomeric foci and artifact, however, as shown in the lower panel (*NSCLC 118*), when appropriate, telomere FISH was performed using a FITC-conjugated telomeric peptide nucleic acid probe to ensure exact colocalisations were not false positives due to bright Cy3 signal being detected through the green filter.

A: Soft Tissue Sarcoma

STS 664 | PML | Telomeric DNA | DAPI | Merge

B: Non Small Cell Lung Carcinoma

NSCLC 98 | PML (FITC) | Telomeric DNA (Cy3) | DAPI | Merge

NSCLC 102

NSCLC 118

NSCLC 118 | PML (Texas Red) | Telomeric DNA (FITC) | DAPI | Merge

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Figure 4.3: The APB assay in *A: Papillary Carcinoma of the Thyroid* and *B: Colorectal Carcinoma*. Combined PML immunofluorescence and telomere FISH in paraffin sections. Indirect immunofluorescence (FITC label) was used for the *PML* protein, telomere FISH was performed using a Cy3 conjugated telomeric peptide nucleic acid probe and slides were photographed using a 100x objective. *A:* *PC 97.4778* is an example of a APB[-] papillary carcinoma of the thyroid. *B:* examples of APB[-], minisatellite unstable colorectal carcinomas that were scored as having most telomeres undetectable (*CRC 1146*) or staining at medium intensity (*CRC 863*).

A: Papillary Carcinoma of the Thyroid

PC 97.4778

PML | Telomeric DNA
DAPI | Merge

B: Colorectal Carcinoma

CRC 1146

PML | Telomeric DNA
DAPI | Merge

CRC 863

Telomeric DNA
Telomeric DNA and DAPI
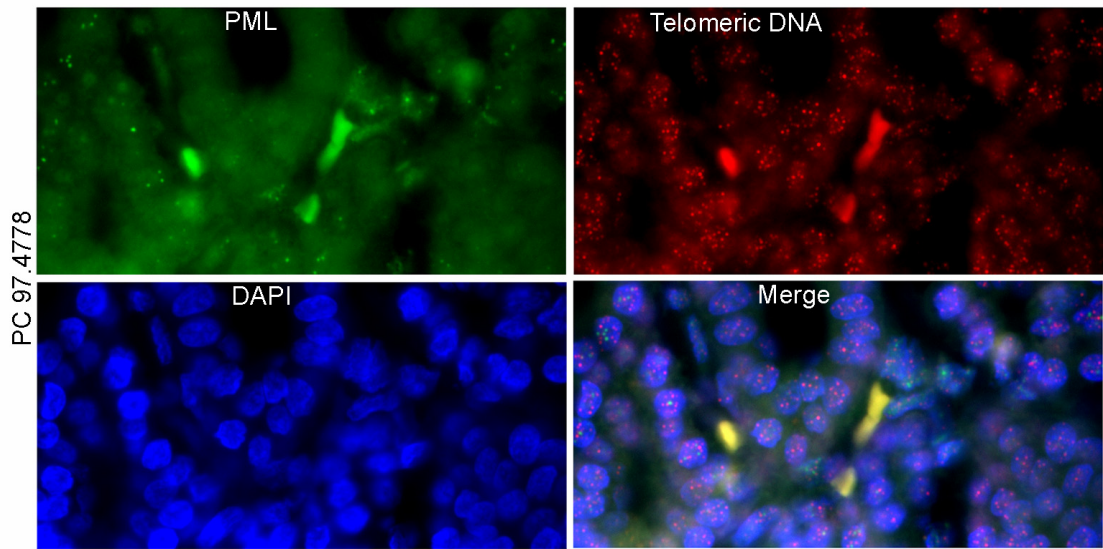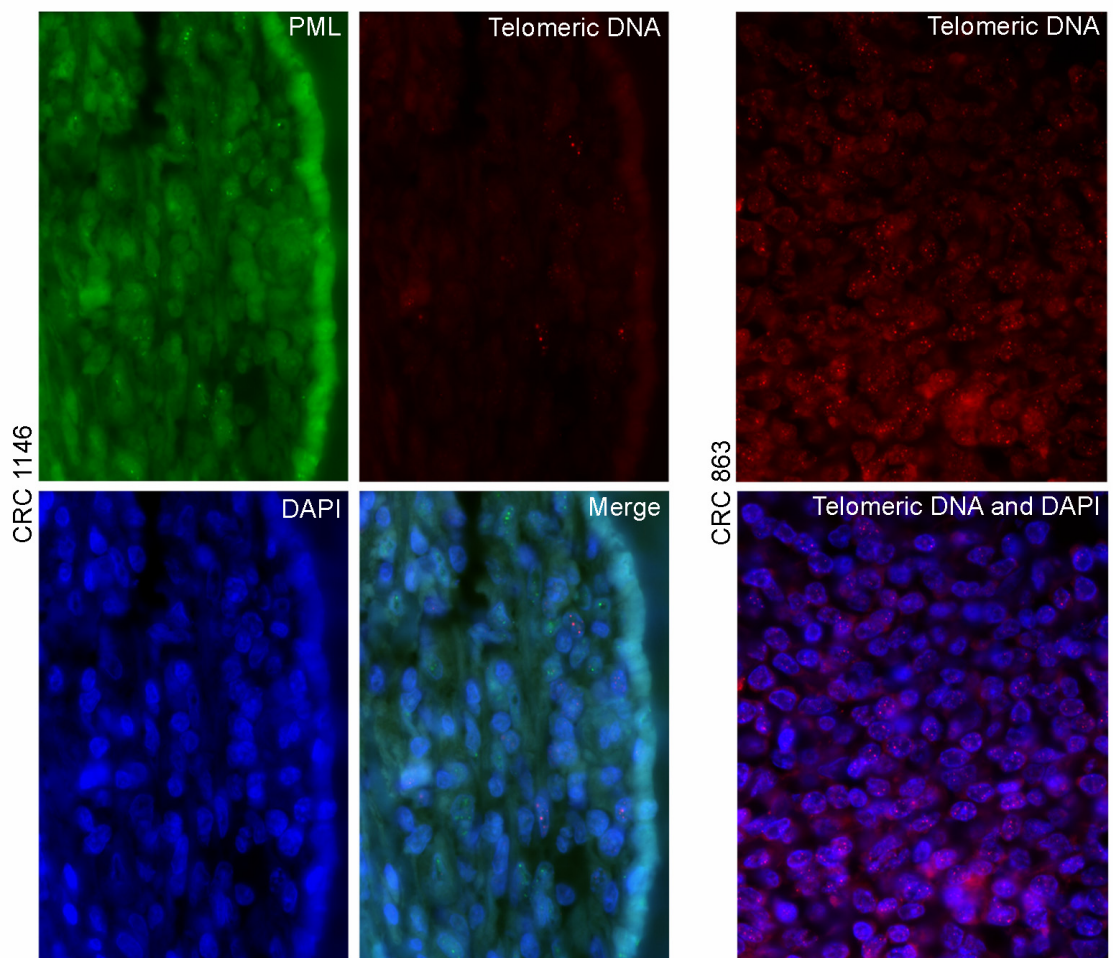
Fibrous Histiocytomas (MFH), the most common STS subtype in adults[353,354], had the highest prevalence of ALT, with 77% being ALT[+]. ALT was also common in leiomyosarcomas (62%) and liposarcomas (33%). Rhabdomyosarcomas (6%) and synovial sarcomas (9%) had a significantly lower prevalence of ALT compared to MFH (Fisher's exact test; $p < 0.0001$ for each). Results from a separate set of 16 STS selected on the basis of being telomerase[-] (Table 4.1) supported the high prevalence of ALT in MFH. Both of the ALT[+] rhabdomyosarcomas were of the embryonal subtype (the rhabdomyosarcomas consisted of 15 embryonal, 4 alveolar and 16 unspecified subtypes). Of the liposarcoma subtypes, 0/1 myxoid, 1/2 pleomorphic, 1/2 dedifferentiated and 1/4 unspecified were ALT[+].

4.3 Tumours with low frequency APBs and other diagnostic dilemmas

In 5-10% of ALT[+] sarcomas and astrocytomas (3/45 ALT[+] STS, 2/27 ALT[+] osteosarcomas and 1/17 astrocytomas) and 50% (11/22) of ALT[+] NSCLC, the frequency of APB[+] nuclei was <0.5%, which is more than 10-fold less than the frequency of APB[+] nuclei reported for ALT[+] cell lines[83]. In this study most ALT[+] tumours had 10-25% of their nuclei APB[+]. Examples of tumours with a low frequency of APB[+] nuclei include NSCLC 102 (Fig 4.2B) and STS 2 and 12 in Fig 3.7A and Fig 3.9. The TRF analysis of STS 12 may be consistent with it having a relatively small (<5%) subpopulation of ALT^{+} cells (Fig 3.7B and E). Whether tumours with < 0.5% of nuclei APB[+] should be classified as ALT[+] may ultimately depend on which conclusions are being drawn from the data.

Another finding that warranted review of the APB assay criteria arose in a NSCLC, NSCLC 118 (Fig 4.2B). NSCLC 118 was classified as APB[-] despite having colocalisations between telomeric DNA and PML protein. The colocalisations did not fit the APB criteria because the telomeric foci were not bright enough and/or were not completely within the PML bodies. Even if the intensity requirement was relaxed, less than 10 telomeric foci in the entire slide (~10 000 nuclei) were found to be completely inside the PML bodies. With a low proportion of exact colocalisations and relatively small foci, there is a significant chance that the exact colocalisations observed are false positives due to separation in the vertical axis but colocalisation in the horizontal plane. Since bright telomeric foci adjacent to PML bodies has been reported for the cell line, AG11395[95], the telomeric foci in NSCLC 118 were compared to telomeric foci in AG11395. The telomeric foci in NSCLC 118 appeared very different (much more distinct) than the telomeric foci in AG11395. NSCLC 118 more resembled the LOX-IMVI melanoma cell line (Section 3.3). This type of difficulty did not arise in any other tumour.

4.4 ALT and tumour aggressiveness

Patient data were analysed to determine if ALT is associated with tumour aggressiveness (Table 4.2). For both osteosarcomas and adult STS, approximately 50% of the high grade tumours were ALT[+]. Thus, in this sample of sarcomas the prevalence of ALT was not reduced in the high grade tumours compared to the prevalence of ALT overall. In astrocytomas ALT was more prevalent in the lower grades, 88% compared with 24% in GBM (Fisher's exact test; $p = 0.001$). Consistent with this observation, work performed by Hakin-Smith *et al.*[270] showed that 0/7 of

these grade II-III astrocytomas were telomerase[+] compared with 13/34 (38%) of the GBM, which is in agreement with other reports that show the prevalence of telomerase[+] in astrocytoma correlates with grade[355,356].

Although 33% of the metastases in adult STS were ALT[+], this was significantly less than the 67% of primary tumours that were ALT[+] (p = 0.025). There was no significant difference between the prevalence of ALT in the recurrences (50%) and either the primaries or the metastases (p = 0.36 and p = 0.37, respectively). Whenever paired samples were available, metastases and recurrences always had the same ALT status as the primary tumour; for all tumour types this totalled 5 metastases (2/5 ALT[+]) and 2 recurrences (1/2 ALT[+]).

Table 4.2: Association of ALT with tumour grade and metastasis

Tumour Type	Total Tumours	Grade Low[a]	High	Tumour Stage Sampled Primary	Recurrence	Metastasis
Osteosarcoma	27/58 (47%)	1/7	20/39 (51%)	27/57 (47%)	1/1	2/3
Adult STS	34/68 (50%)	3/5	8/16 (50%)	16/24 (67%)	10/20 (50%)	9/27 (33%)
Astrocytoma	17/50 (34%)	7/8[b] (88%)	10/42 (24%)	17/50 (34%)	1/1	N/A

Data are presented as number of tumours ALT[+]/total tumours in partition (percentage ALT[+]). Grading was not available for all tumours. [a]Low or intermediate grade. [b]1/2 grade II and 6/6 grade III astrocytomas were ALT[+]. Metastasis is not applicable (N/A) for astrocytomas.
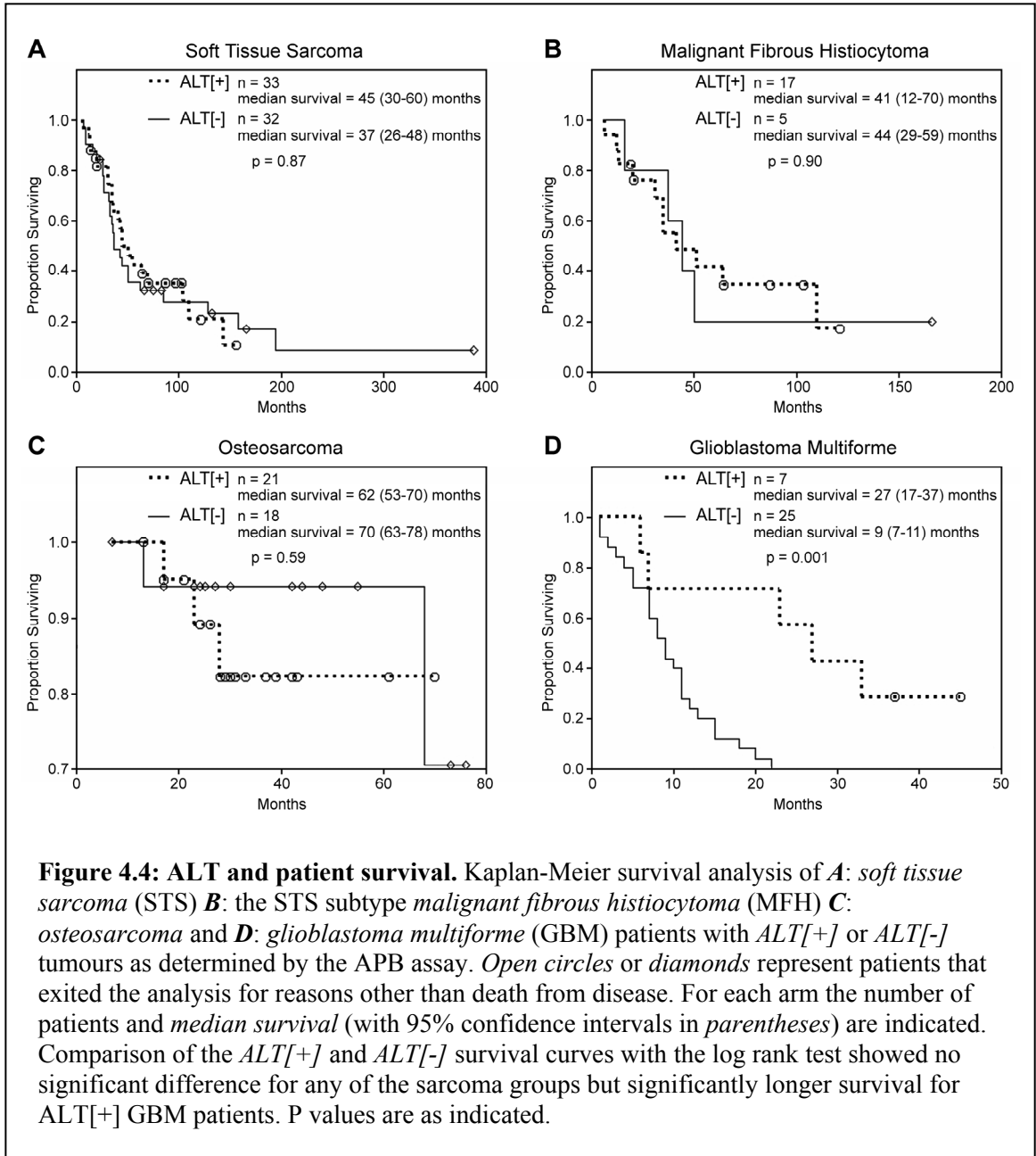
4.5   ALT and outcome of STS

The relationship between patient survival and ALT had not previously been investigated for STS. Patient survival data were available for all adult STS patients except the three patients with chondrosarcoma. The median survival for the entire group of 65 patients was 44 months, with no significant variation between the component STS subtypes. Kaplan-Meier analysis (Fig 4.4*A* and 4.4*B*) showed that there was no significant difference in survival between the ALT[+] and ALT[-] patients in STS or the MFH subgroup (log rank p = 0.87 and 0.90, respectively; Hazard Ratio = 1.0 (95% CI = 0.5 to 1.7) and 0.9 (95% CI = 0.3 to 3.0), respectively). If the two-fold longer median survival reported for ALT[+] compared with ALT[-] GBM patients[270] had been present in STS, our sample size was sufficient to detect this (based on the 95% confidence intervals described in Fig 4.4*A*).

4.6   ALT and outcome of osteosarcoma

Kaplan-Meier analysis found no significant survival difference between the ALT[+] and ALT[-] osteosarcoma patients (Fig 4.4*C*; log rank p = 0.59, Hazard Ratio = 1.6 (95% CI = 0.3 to 9.3)). Although median follow up time (28 months) was short, these results are consistent with recently published data[271] indicating that survival of patients with ALT[+] osteosarcomas is the same or less than in the ALT[-] group.

We investigated if response to chemotherapy correlated with ALT status in osteosarcoma as there is currently no adequate predictor of osteosarcoma response that could facilitate tailoring of preoperative chemotherapy to increase survival[357]. However, there was no significant difference between the proportion of ALT[+]

(6/17; 35%) and ALT[-] (8/24; 33%) osteosarcomas that responded (>90% necrosis) to chemotherapy (p = 1.00; mean difference of response = 2%, 95% CI = -33% to +29%).

Figure 4.4: ALT and patient survival. Kaplan-Meier survival analysis of *A*: *soft tissue sarcoma* (STS) *B*: the STS subtype *malignant fibrous histiocytoma* (MFH) *C*: *osteosarcoma* and *D*: *glioblastoma multiforme* (GBM) patients with *ALT[+]* or *ALT[-]* tumours as determined by the APB assay. *Open circles* or *diamonds* represent patients that exited the analysis for reasons other than death from disease. For each arm the number of patients and *median survival* (with 95% confidence intervals in *parentheses*) are indicated. Comparison of the *ALT[+]* and *ALT[-]* survival curves with the log rank test showed no significant difference for any of the sarcoma groups but significantly longer survival for ALT[+] GBM patients. P values are as indicated.

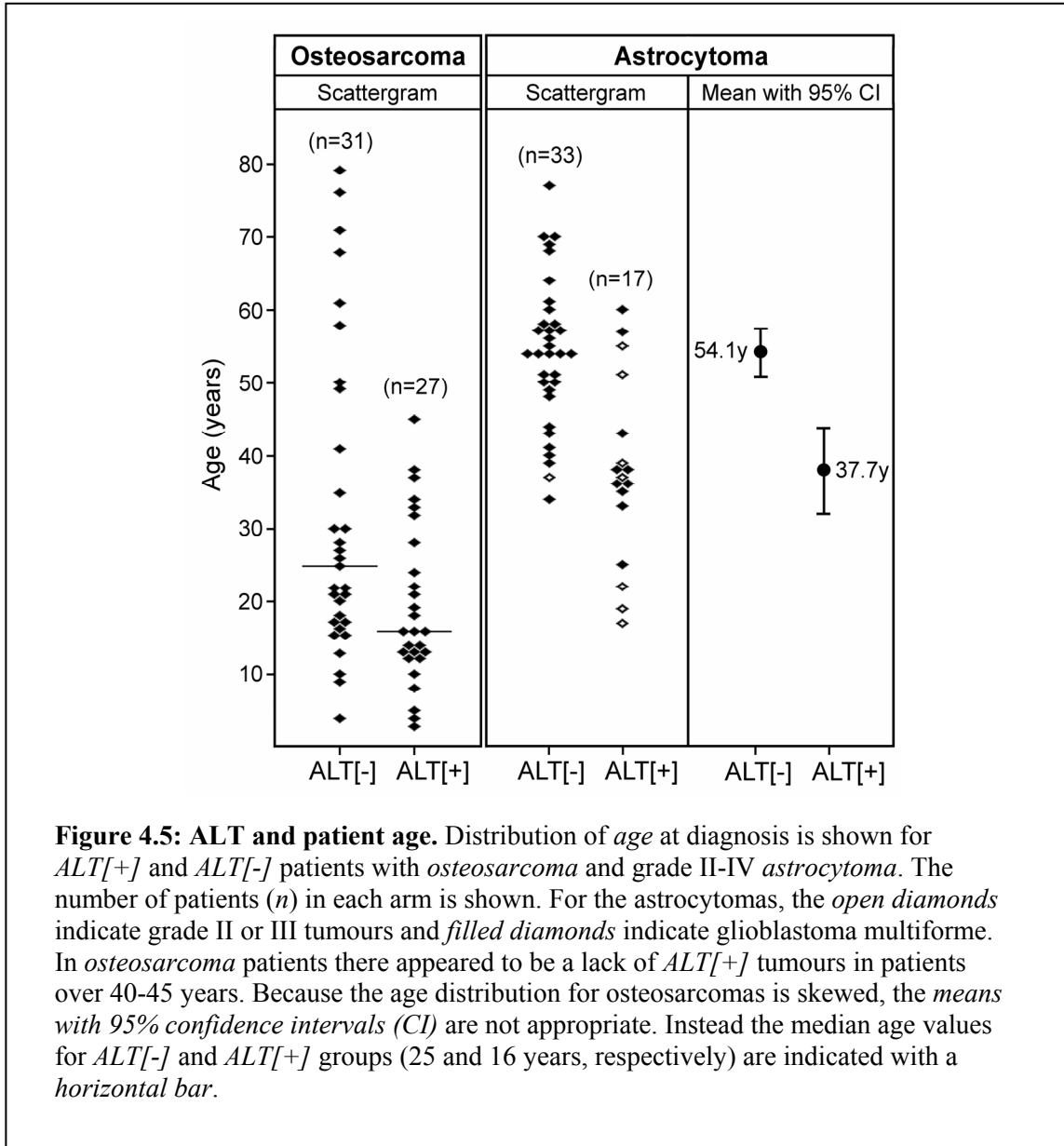## 4.7   ALT and outcome of glioblastoma multiforme

Because of the concordance between the assays for ALT, we expected the APB assay to be a prognostic indicator for patients with GBM as has been reported for ALT detected by TRF analysis[270]. Kaplan-Meier analysis (Fig 3*D*) showed that the patients with APB[+] GBM had better survival than the APB[-] group (log rank p = 0.001). The APB[+] GBM patients had a 3-fold longer median survival (Fig 3*D*; Hazard Ratio = 0.3 (95% CI = 0.1 to 0.6)).

4.8   ALT and patient age

As illustrated in Fig. 4.5, there was a significantly lower age at diagnosis for the ALT[+] osteosarcoma patients compared with the ALT[-] osteosarcoma patients (Mann-Whitney Test, p = 0.01). This appeared to be due to a marked lack of ALT[+] osteosarcomas diagnosed in patients older than 40 years (1/10 or 10% ALT[+]) compared with prevalence of ALT[+] osteosarcomas diagnosed in patients younger than 40 years (26/48 or 54% ALT[+]; p = 0.01). However Ulaner *et al.*[271] did not observe a significant difference in the mean age at diagnosis of ALT[+] osteosarcomas, so this association needs to be tested in a larger tumour set.

ALT has been associated with a younger patient group in GBM[270]. This study also found that patients with ALT[+] astrocytomas had a significantly lower mean age at diagnosis than that of patients with ALT[-] astrocytomas (Fig 4.5; mean difference of age at diagnosis = 16.4 years, 95% CI = 9.0 to 23.7 years). If grade II and III astrocytomas (which can progress to GBM) are excluded from analysis, there was still a significantly lower mean age of the remaining ALT[+] GBM (mean age of 40.1 years, 95% CI = 33.4 to 46.8 years for the ALT[+] GBM compared to 54.6 years,

95% CI = 51.2 to 58.0 years for ALT[-] GBM; mean difference = 14.5 years, 95% CI = 6.3 to 22.7 years).

Figure 4.5: ALT and patient age. Distribution of *age* at diagnosis is shown for *ALT[+]* and *ALT[-]* patients with *osteosarcoma* and grade II-IV *astrocytoma*. The number of patients (*n*) in each arm is shown. For the astrocytomas, the *open diamonds* indicate grade II or III tumours and *filled diamonds* indicate glioblastoma multiforme. In *osteosarcoma* patients there appeared to be a lack of *ALT[+]* tumours in patients over 40-45 years. Because the age distribution for osteosarcomas is skewed, the *means with 95% confidence intervals (CI)* are not appropriate. Instead the median age values for *ALT[-]* and *ALT[+]* groups (25 and 16 years, respectively) are indicated with a *horizontal bar*.

Due to significant differences in both the mean ages and prevalence of ALT in the different STS subtypes they could not be combined and the sample sizes did not allow significant results when analysed separately. No significant gender difference was found for the prevalence of ALT in osteosarcoma, STS or astrocytoma (Table 4.3).

Table 4.3: Gender and the prevalence of ALT

| Tumour	Gender	N	ALT[+]	P-value[a]	Difference (f - m)	95% CI of the difference
Osteosarcoma	f	34	56%	0.11	23%	-4% to +49%
	m	24	33%			
STS[b]	f	26	42%	0.32	-14%	-40% to +11%
	m	39	56%			
Astrocytoma	f	20	40%	0.55	10%	-18% to +38%
	m	30	30%			

[a]Fisher's exact test. [b]The 65 adult STS used for survival analysis.

4.9   Prevalence of ALT and tumour predisposition syndromes

4.9.1   Prevalence of ALT in Li Fraumeni syndrome

It is possible that Li Fraumeni syndrome (LFS) patients are predisposed to ALT due to loss of putative p53 suppression of ALT (Section 1.9). Therefore, APBs were assayed in paraffin sections of fourteen cancers from LFS patients including five sarcomas (one sclerosing spindle cell sarcoma, one leiomyosarcoma and three ovarian sarcomas) three breast carcinomas, three lung adenocarcinomas, two endometrial carcinomas and one squamous cell carcinoma. Only one sarcoma, the sclerosing spindle cell sarcoma, was ALT[+] (Fig 4.6A). However, 13/14 of these tumours (including the ALT[+] sarcoma) were from LFS patients with G:C to A:T germline mutations in p53 codon 273. The remaining tumour, an ALT[-] breast carcinoma, was from a LFS patient with a G:C to A:T germline mutation in p53 codon 342 (R Eeles, personal communication)

Figure 4.6: ALT in cancer predisposition syndromes. *A:* The APB assay for ALT using PML immunofluorescence with (indirect) FITC label and telomere FISH with a Cy3 conjugated telomeric peptide nucleic acid probe in paraffin sections of cancer predisposition syndromes, photographed using a 100x objective. The bright foci of *telomeric DNA* that colocalise with *PML* represent APBs. APB[+] and hence ALT[+] examples of Li Fraumeni syndrome, *LFS 95.1033*, Werner syndrome, *WS 6102* and Rothmund-Thompson syndrome, *RTS 117* are shown. *B:* The mismatch repair protein, *MLH1*, colocalises with APBs (bright *TRF1* foci) in the ALT[+] cell line, *JFCF-6/T.1J/1-3C*. Bright *MLH1* foci were not seen in the ALT[-] cell line, *JFCF-6/T.1J/6G*. *MLH1* and *TRF1* were detected by indirect immunofluorescence with FITC and Texas Red labels, respectively.
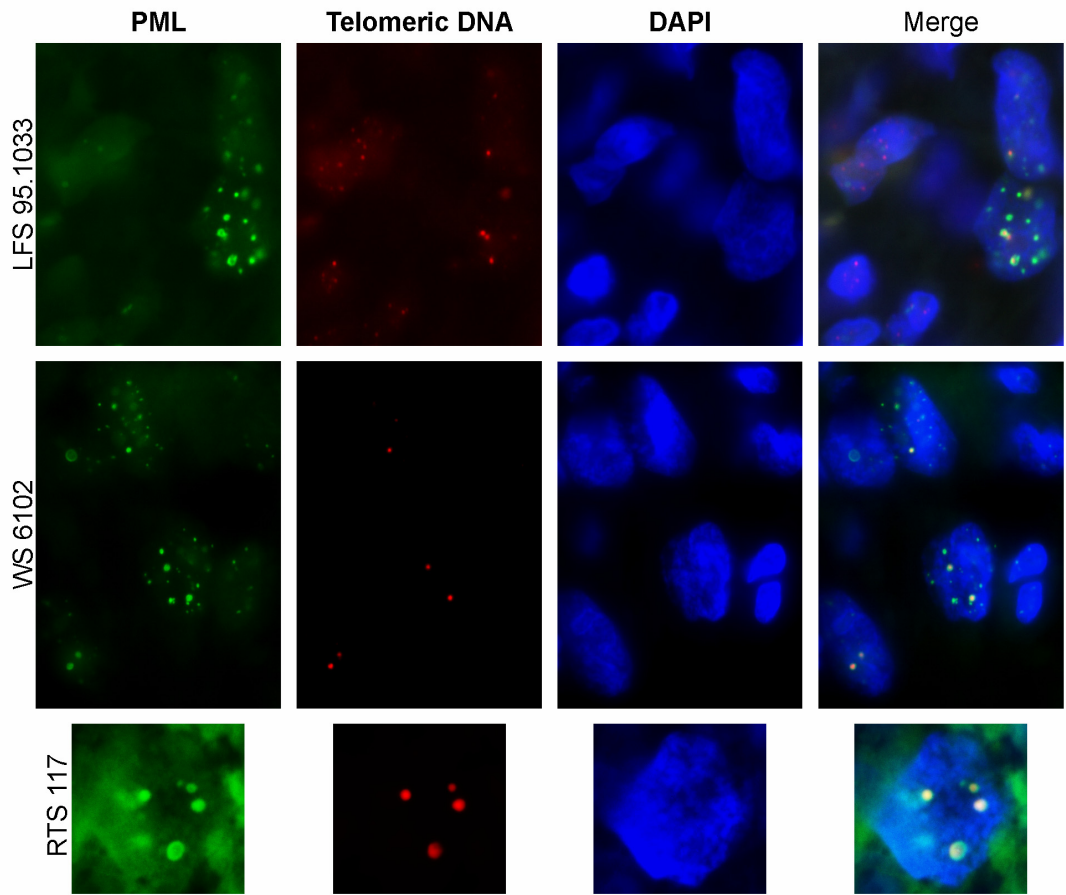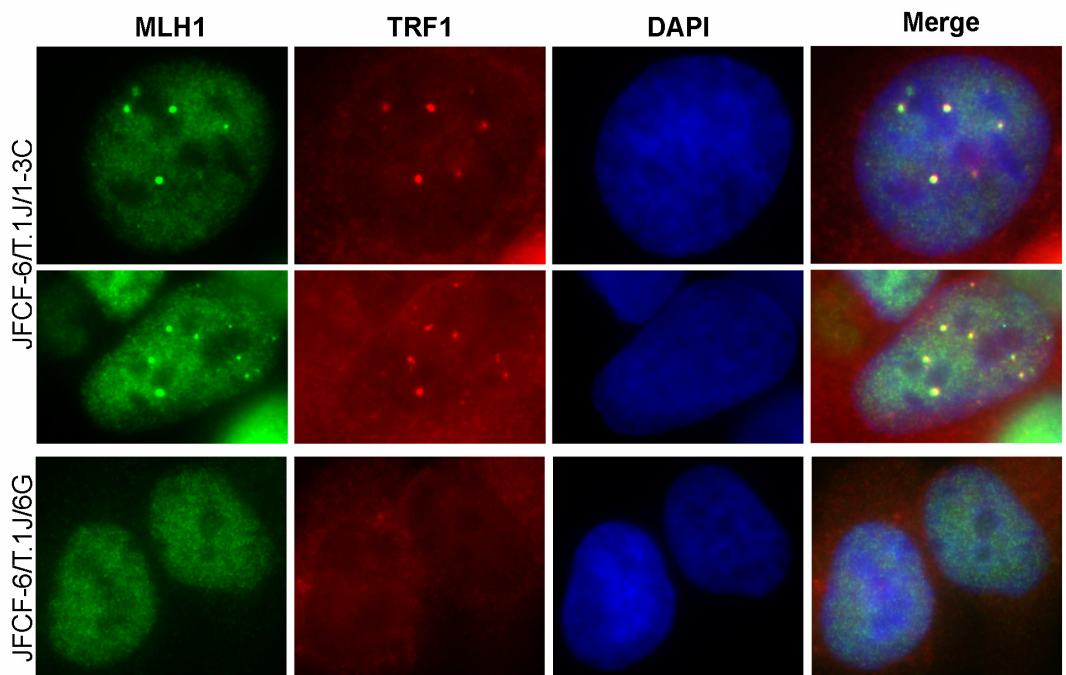
4.9.2   *Prevalence of ALT in Werner and Rothmund-Thompson syndromes*

To determine if RecQ helicases are required for ALT, as in the *S. cerevisiae* analogue of ALT[134,166,358], ALT was assayed in tumours from patients with Werner syndrome (WS) and Rothmund Thompson syndrome (RTS) who had known mutations in the RecQ helicase genes, WRN and RecQL4 respectively.

For WS, APBs were assayed in paraffin sections of a fibrosarcoma from a patient with homozygous G to C mutation in the splice donor site immediately before nucleotide 3370 of the WRN cDNA[359] (M Goto, personal communication) and an adrenal adenocarcinoma and a ureteric transitional cell carcinoma, both from the same patient with a homozygous C to T mutation at nucleotide 1336 of the WRN cDNA[360,361]. Both these mutations result in truncations before the nuclear localisation signal and are considered to be functionally null with identical phenotypes[362,363]. However the possibility of a difference between the two mutations has been suggested by one study[364] that found the latter mutation (that retains the helicase domain) to be associated with follicular thyroid carcinomas and the former muation (that causes a truncation before the helicase domain) to be associated with papillary thyroid carcinomas. The fibrosarcoma tested ALT[+] (Fig 4.6A) and the remaining two tumours were ALT[-].

Paraffin sections of two osteosarcomas from different RTS patients with known mutations in REQL4 (L Wang personal communication) were both ALT[+] (Fig 4.6A).

4.9.3 Prevalence of ALT in carcinomas with microsatellite instability

To determine if MMR may be involved in suppressing ALT, an increased prevalence of ALT was looked for in colorectal cancers with microsatellite instability (MSI), which is a marker for defective MMR[266]. APBs were assayed in paraffin sections from 22 colorectal tumours with known microsatellite status; twelve had MSI and ten were microsatellite stable (MSS; Ward, R. personal communication). None were APB[+] (Table 4.1, Fig 4.3B), which is supported by recent results[365]. Carcinomas with MSI appeared to have longer telomeres (by telomere FISH; Table 4.4), however larger sample sizes are needed to obtain statistical significance. Figure 4.3B shows an example of two MSI carcinomas with average telomeres scored by telomere FISH as not visible and medium length, respectively.

Table 4.4: Telomere length and MSI in colorectal carcinomas

Telomere length[a]	MSI tumours	MSS tumours	Total
not visible	6	8	14
small	3	2	5
small medium	2	0	2
medium	1	0	1
Total	12	10	

[a]Telomere length determined by telomere FISH. MSI, microsatellite instability. MSS, microsatellite stable.

Although there is evidence that MMR deficiency may facilitate ALT in yeast[265], there is also evidence that MMR deficiency is associated with deficiency of the MRN complex[366,367] which is thought to be necessary for ALT[2]. Thus ALT may be blocked

in most MMR deficient cancers. Consistent with ALT requiring intact components of MMR, we found MLH1, the protein most commonly deficient in MSI carcinomas[266], to be present in APBs (Fig 4.6B).

4.10 Summary of results (see chapter 6 for discussion)

In this chapter, the APB assay was used to show that ALT is a significant concern for oncology. ALT was utilised in approximately one quarter of GBM, one third of STS including three quarters of MFH, half of osteosarcomas and one tenth of NSCLC. Furthermore, the patients with these ALT[+] tumours had poor survival; median survivals were 2 years for ALT[+] GBM, 4 years for ALT[+] STS including 3.5 years for ALT[+] MFH and 5 years for ALT[+] osteosarcoma. ALT[+] STS and osteosarcomas were also just as aggressive as their ALT[-] counterparts in terms of grade and patient outcome. ALT status was not found to be associated with response to chemotherapy in osteosarcomas or survival in STS. ALT was however, less prevalent in metastatic STS.

The APB assay was a prognostic indicator for GBM and was correlated with three-fold increased median survival in GBM (although this survival was still poor). ALT was more common in lower grade astrocytomas (88% ALT[+]) than GBM (24% ALT[+]) and ALT[+] GBM had an identical median age at diagnosis to that reported for secondary GBM[368]. In osteosarcoma, ALT was also associated with a younger age at diagnosis.

ALT varied among the different STS subtypes. It was common in MFH (77%), leiomyosarcoma (62%) and liposarcoma (33%) but rare in rhabdomyosarcoma (6%)

and synovial sarcoma (9%). ALT was not found in thyroid papillary carcinoma (0/17) or colorectal carcinoma (0/31).

Diagnostic difficulties were identified for the APB assay in tumours. Occurrences of cell populations with a low frequency (<0.5%) of APB[+] nuclei was seen in 2-4% of sarcomas, astrocytomas and NSCLC. Although the variant APBs reported for the in vitro transformed cell line, AG11395[95], were not seen in any of the 583 tumours, one NSCLC did have telomeres appearing to colocalise with PML nuclear bodies without any APBs detected, similar to observations in the melanoma cell line, LOX-IMVI (Section 3.3).

Using the APB assay to test for the presence of ALT in tumours from patients with known mutations in either WRN or RECQL4 it was demonstrated that neither of these RecQ helicases was essential for ALT. There was no apparent increase in the frequency of ALT in tumours from patients with germline G:C to A:T mutations in p53 codon 273 or in colorectal carcinomas that had MSI and thus MMR deficiency. The MMR protein, MLH1 was demonstrated to be present in the APBs of an ALT[+] cell line.

Chapter Five:

Gene expression associated with ALT

Gene expression associated with ALT

5.1 Introduction

The research described in this chapter investigated gene expression changes associated with the ALT mechanism. It seemed possible that expression changes directly or indirectly associated with ALT activity may make ALT[+] tumours more susceptible to existing treatments or be potential targets for new therapies. Support for suspecting a different pattern of gene expression associated with the ALT mechanism arose from the investigations of ALT and patient outcome. The reason the presence of the ALT mechanism was associated with a better prognosis in GBM but not in sarcomas could possibly be due to tissue specific effects of changes in gene expression associated with an active ALT mechanism. In this chapter, gene expression levels were compared between ALT^{+} cells lines and telomerase[+] cell lines using RNA microarray analysis. The results from this analysis motivated the further investigation of three proteins: DRG2, AGT and SATB1.

5.1.1 DRG2

DRG2 is one of two known members of the *D*evelopmentally *R*egulated *G*TP binding protein (DRG) subfamily of GTPases[369,370]. DRG1 and DRG2 are both thought to have similar functions and share 62% sequence identity[371]. DRG1 has been better characterised and was originally identified as being highly expressed in the developing mouse brain[372,373]. DRG1 is a putative tumour and cell growth suppressor with low levels of DRG1 associated with more advanced cancers and worse prognosis. However, high levels of DRG1 appear to improve cancer cell resistance to some chemotherapy agents and DRG1 expression is enhanced by DNA damage in a p53 dependent manner[374-376].

In humans, DRG2 may not be regulated developmentally with similar mRNA levels reported for foetal and adult organs[377]. In adult humans DRG2 mRNA is highly expressed in heart, skeletal muscle and kidney, and to a lesser extent in liver, brain and placenta[369,371]. In *Xenopus laevis,* DRG2 is highly expressed in developing tissues and gonads[378] and similarly in *Arabidopsis thaliana* DRG proteins are highly expressed in growing tissue and reproductive organs[379].

The possibility that repression of DRG2, like DRG1, may promote carcinogenesis is supported by the reduced expression of DRG2 found in some sarcoma cell lines relative to the moderate levels found in a normal fibroblast strain[371]. DRG2 RNA levels have been reported to be low in the cell line WI38-VA13/2RA[369] which is ALT[+] and moderate in the osteosarcoma cell line MG-63[371] which is telomerase[+]. DRG2 is located in a region deleted in Smith-Magenis Syndrome[377,380] that has also been associated with neuroblastoma[381].

The fact that DRG proteins are highly conserved in eukaryotes and Achaea indicates that they may have an important function[369,371]. DRG2 appears to be involved in regulating cellular proliferation. Overexpression of DRG2 leads to decreased cellular proliferation, accumulation of cells in G2/M phase, decreased cellular adhesion and decreased apoptosis after nocodazole exposure[382,383]. Furthermore, DRG2 is increased in fish cells infected with rhabdovirus[384]. Although DRG2 is found in the cytoplasm[369], it is a basic protein and may have RNA binding activities[378]. DRG2 protein levels may be regulated by ubiquitylation dependent proteolysis and DFRP2 (hGIR2) may stabilise DRG2 by inhibiting ubiquitylation[385].

5.1.2 AGT

The O^6-methylguanine DNA methyltransferase (MGMT) gene encodes the DNA repair protein O^6-alkylguanine DNA transferase (AGT). MGMT expression is an important determinant of cancer-causing mutations and resistance of cancer cells to chemotherapy agents. AGT acts alone to detect and remove alkylating lesions at the O^6 position of guanine, including its preferred substrate, O^6-methylguanine (O^6-MG)[386,387]. Although nucleotide excision repair (NER) is capable of removing O^6-alkylguanine (O^6-AG lesions), AGT appears to be the critical repairer, especially for O^6-MG[386-389]. Repair of all O^6-AG lesions is essential for avoiding both mutation and cell death[386].

From prokaryotes to humans, AGT has a conserved function and sequence, especially at its active site[386]. AGT is a small protein of 207 amino acids and 22 kDa[387]. Each AGT enzyme scans DNA for one O^6-AG adduct, which it removes in a suicide reaction that corrects the lesion. The AGT enzyme is inactivated by the covalent transfer of the alkyl group to its active site which causes a conformational change causing both its release from DNA and ubiquitylation which marks it for degradation[386,390]. This makes AGT a simple target for inhibition, as any substrate is an irreversible inhibitor.

Although O^6-MG comprises less than 10% of nucleotide adducts due to methylating agents, it is one of the main carcinogenic lesions. Methylating agents can be endogenous such as S-adenosylmethionine, environmental such as N-nitroso compounds in food and cigarette smoke or therapeutic such as temozolomide and dacarbazine which are used as chemotherapy agents for a variety of tumours including gliomas and STS, respectively. If O^6-MG is not repaired before DNA replication, 90% of the time O^6-MG is paired with thiamine which can cause G:C to

A:T transition mutations. MMR will recognise O^6-MG:T, degrade the newly synthesised strand surrounding the T and then resynthesise it but as O^6-MG:T 90% of the time. This results in several rounds of repair and single strand gap. If replication occurs while there is a single strand gap, a double strand break will occur in one daughter chromatid that may be repaired by HR or NHEJ (possibly leading to SCE or cytogenetic changes) or cell death may occur - usually apoptosis initiated by p53[386,387,391]. Thus MMR is required for the creation of the single strand gap necessary for the cytotoxic effect of methylating agents. Drug resistance has been seen to emerge in AGT deficient tumours due to mutation or promoter methylation which inactivates MMR genes[386,392,393]. Knockout of both AGT and MMR protein MLH1 in mice leads to mutagenesis but not cell death[394] and the level of MLH1 was found to be important for the control of mutation and apoptosis.

AGT also repairs branched and chlorinated alkyl groups such as O^6-chloroethylguanine formed by chemotherapeutic agents such as bis-(2-chloroethyl)-nitrosourea (BCNU) that has also been used to treat gliomas. If O^6-chloroethylguanine lesions are not removed within 8-12 h they form G-C interstrand crosslinks, which are poorly repaired and extremely cytotoxic[386,391].

MGMT is ubiquitously expressed in normal human tissue but levels vary, presumably reflecting genetic background and exposure to alkylating agents. AGT activity can be heterogeneous in tumours but average activity is usually many fold higher than the normal tissue counterpart[386,388,395]. Many tumour cell lines and a small subset of tumours lack AGT expression due nearly always to gene silencing. Methylation of specific CpG regions in the MGMT promoter is associated with a condensed chromatin structure and turns off expression of the gene. This is sometimes associated with similar changes in other cancer associated genes such as CDKN1A (p21), MLH1

and CDKN2A (p16)[386,396]. Exclusion of a putative MGMT enhancer binding protein from the nucleus may also be involved in silencing of MGMT[397].

AGT function can be disrupted by loss of nuclear transport due to mutation in its nuclear recognition sequence or a sequence for binding an accessory protein thought to facilitate its transfer to the nucleus. Phosphorylation of AGT increases its nuclear localisation and decreases its ubiquitylation and degradation. MGMT can be induced by glucocorticoids, cAMP and PKC activators. Modest p53-dependent induction of MGMT occurs after DNA damage such as ionising radiation and other non alkylating damaging agents[386,387].

AGT may have another role as it is expressed in normal and cancer cells at levels higher than necessary to repair endogenous or therapeutic damage respectively[386,388], and is known to make mycobacteria more virulent[398]. Human AGT does appear to have some role in inhibiting cell cycle progression in the presence of DNA damage. The most critical times for DNA repair are during transcription and replication. Not only are these the processes that realise the mutations into mutated mRNA and fix them into daughter chromosomes, but at sites of transcription and replication the DNA is open and accessible to mutagenic agents. O^6-MG is of particular concern as it does not appear to halt the respective polymerases[386,399]. AGT has been found to accumulate at these sites, possibly by binding other proteins[399,400]. There is no evidence that non-alkylated AGT influences transcription or replication, but it does bind CREB-binding protein (CBP)[401] and DNA replication licensing factors[400]. Alkylated AGT formed by the suicidal removal of O^6-AG adducts initiates a conformational changes that stops it binding CBP and allows AGT to bind the oestrogen receptor, inhibiting the latter from transcriptional activation that mediates cellular proliferation - including progression to S phase of the cell cycle[401]. Alkylated

AGT may also be linked to cell signalling events leading to a G2/M arrest in response to DNA damage[402]. Evidence that increased levels of AGT help survival of human cancer cells treated with cyclophosphamide that is not known to cause an AGT repairable lesion[387] and that AGT binds DNA repair proteins (such as MSH2 and DNA dependent protein kinase) and cell cycle and signalling proteins[400] suggest that AGT may be part of a more general cell cycle arrest pathway. Interestingly, AGT has also been found to bind two proteins, DNA-PK and heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1[400] that are known to reside at telomeric DNA[139,403].

Loss of MGMT expression may be an early event in carcinogenesis by allowing mutagenesis of endogenous O^6-MG adducts in oncogenes and tumour suppressors. Evidence suggests that both MGMT promoter methylation and p53 mutation are early events in the progression of astrocytoma to secondary GBM in about two thirds of these tumours[404,405]. The possibility that the lack of AGT may be the cause of the p53 mutations in these astrocytomas was supported by a correlation between MGMT promoter methylation and G:C to A:T transition mutations in p53[404]. Loss of MGMT expression is also associated with p53 G:C to A:T mutations in NSCLC and mutations of K-ras in gastrointestinal tract cancer[406-408]. Thus, one effect of MGMT loss on prognosis could be to worsen it due to increased oncogenic mutations.

Contrary to this, the overall effect of MGMT expression loss in GBM and anaplastic astrocytoma was improved survival, especially when patients receive temozolomide chemotherapy[409,410]. In GBM loss of MGMT expression as determined by MGMT promoter methylation, was associated with an increase in median survival of 50% overall and 70% in the treatment group who received adjuvant temozolomide chemotherapy. However, AGT status is not always indicative of response to

temozolomide[387,410]. Other determinants of drug sensitivity are likely to include MMR status[386,392-394], p53 status[395] and the ability of MGMT expression to re-emerge[386]. Other putative changes common to cells with MGMT promoter methylation such as concomitant methylation of other promoters may also make these tumours more sensitive to chemotherapy.

5.1.3 SATB1

SATB1 is a chromatin organiser that is necessary for cell-type specific, coordinated gene regulation. SATB1 forms a cage-like structure in the nucleus[411] and binds specific sites on the DNA, organising it into loop domains. It also acts as a landing platform for chromatin remodelling factors to act in the neighbourhood of the tethered DNA (within about 50 kb) to establish histone modifications and inhibit or enhance gene expression in a tissue specific manner[411,412].

SATB1 is found at high levels only in the thymus, where it is essential for the maturation of T-cells and its loss at this stage affects the expression of over 2% of total genes[413]. It has also been found to have a role in the activation of mature T-cells[413] and differentiation in both myeloid and erythroid cells[414,415,415]. In erythroid progenitor cells SATB1 binds and affects gene expression in conjunction with CREB-binding protein (CBP) but not p300[415]. However in myeloid progenitor cells the reverse occurs and SATB1 binds and affects gene expression in conjunction with p300 but not CBP[416].

SATB1 binding appears to coincide with Base Unpairing Regions (BURs) which are 20 -30 bp regions which can readily unpair to form a single stranded conformation, depending on local supercoiling[417-419]. BURs are often found in Matrix Attachment Regions (MARs). MARs (about 1 kb long) allow tethering of the chromatin either

side of a loop (of 70kb on average) that is an independent torsional, transcriptional and replication domain[418,420]. The BURs' ability to base unpair appears to be important for binding to the nuclear matrix[421], and the regions immediately adjacent to matrix binding may be hotspots for recombination[420,422,423]. BURs can contain short (several nucleotides long) subregions termed core unwinding elements that stay unpaired even under conditions that favour the double stranded conformation[424]. SATB1 makes direct contact with the core unwinding elements[419,421], but there does not appear to be an exact consensus sequence for its binding, which also depends on the surrounding BUR sequence[419]. BURs tends to be AT rich, have the Gs and Cs segregated on different strands and be well mixed in terms of not having runs of purines or pyrimidines[419]. However one reported SATB1 site in the MAR of the ε-globin promoter only contained 12 A/Ts out of 22 bases[415]. Telomeric DNA is also a MAR despite not being AT rich[425]. SATB1 binds BURs in the double stranded conformation and stabilises them against unpairing[419,426]. This may inhibit BUR binding to the nuclear matrix and other proteins that bind the unpaired single strands of BUR[426].

5.2   RNA microarray

5.2.1   Cell lines and RNA isolation

For RNA microarray analysis, 12 pairs of ALT[+] and telomerase[+] cell lines were chosen that best provided an unbiased sample of ALT[+] cell lines and could be closely matched by cell or tissue type and method of transformation with telomerase[+] cell lines (Table 5.1). RNA was harvested immediately from 50-70% confluent monolayers. RNA purity was confirmed for all samples; the A_{260}/A_{280} ratio was between 1.95 and 2.05 (pH 7.5) and no degradation of the RNA was seen on analysis by agarose gel electrophoresis.

Table 5.1: Cell lines used for RNA microarray analysis

Cell/Tissue Type	Transformation	ALT[+]	Telomerase[+]
Fibroblast, jejunal[a]	SV40	JFCF-6/T.1J/1.3C [m]	JFCF-6/T.1J/6G [m]
Fibroblast, jejunal[a]	SV40	JFCF-6/T.1R [m]	JFCF-6/T.1P [m]
Fibroblast, lung	SV40	MRC5-V2 [m]	MRC5-V1 [m]
Fibroblast, lung	SV40	WI38-VA13/2RA [f]	BFT-3K [xo]
Fibroblast, skin	SV40	GM847 [m]	GM639 [m]
Fibroblast, AT	SV40	AT1BR44neo [m]	AT22IJE-T [f]
Mesothelial	SV40	MeT-4A [f]	MeT-5A/6TGR-B [m]
Osteosarcoma	tumour	Saos-2 [f]	TE-85 [f]
Osteosarcoma	tumour	U-2 OS [f]	MG-63 [m]
Adenocarcinoma, lung	tumour	SK-LU-1 [f]	A2182 [m]
Fibroblast, liver	chemical	SUSM-1 [m]	
Fibroblast, breast, LFS	Spontaneous	IIICF/c [f]	
Fibrosarcoma	tumour		HT1080 [m]
Fibroblast, breast	SV40, hTERT		F80-TERT/K1-1 [f]

[a]JFCF-6/T.1J/1-3C, 1J/6G, 1P and 1R all were derived from the JFCF-6 primary cell strain. JFCF-6/T.1J/1-3C and 1J/6G were subclones from the same transformation event. [f] or [m] indicates the cell line was derived from a female or male patient, respectively. [xo]BFT-3K was derived from a male primary cell donor, but the cell line is aneuploid X0. AT and LFS denotes the cells were from a patient with Ataxia Telangiectasia and Li Fraumeni syndrome, respectively.

5.2.2 *Microarray analysis and identification of differentially expressed genes*

The level of mRNA from 27868 genes (33096 probes or gene elements) was determined for each cell line separately on a Human Genome Survey Microarray (Applied Biosystems), using the commercial microarray service provided by Human Genetic Signatures (Macquarie Park NSW, Australia) with no amplification of the cDNA; see section 2.6.2. Genome wide median expression was used to normalise each gene for comparison with other arrays. In total, 19469 gene elements had a signal to noise ratio of greater than or equal to 3 in at least 6/12 ALT or 6/12 telomerase cell lines, and only these genes were included in further analysis.

The gene expression among the different cell lines was not normally distributed and contained outliers and noise, all of which has been reported to be standard for microarray data[427]. The nonparametric Mann-Whitney test was considered the most

appropriate method to identify the differentially expressed genes due to the above characteristics of the data. Appendix A shows the expression levels of the 240 genes (250 gene elements) that were differentially expressed between the ALT[+] and telomerase[+] groups (Mann-Whitney test, p < 0.005) and fourteen of these genes that are further analysed in this chapter are summarised in Fig 5.1. Some of the differentially expressed genes with known functions that may be relevant to telomere maintenance are shown in Table 5.2. Hierarchical clustering of the cell lines based on these 250 gene elements yielded a clear separation of ALT[+] cell lines from telomerase[+] cell lines (Fig 5.2).

There are not currently any published studies comparing the effect on gene expression of the ALT and telomerase telomere maintenance mechanisms. However, Nautiyal *et al.*[428] have investigated in *S. cerevisiae* the gene expression changes in response to deletion of the telomerase RNA component. Nautiyal *et al.* described a set of twelve genes that were upregulated specifically in response to the telomere shortening associated with telomerase deletion (and not generally in other stress conditions) and that remained upregulated in telomerase negative survivors. However, none of these genes corresponded to the genes found in this chapter to be differentially expressed between the ALT[+] and telomerase[+] groups. One gene, RPL37A, had a human homologue that had weak differential expression between the ALT[+] and
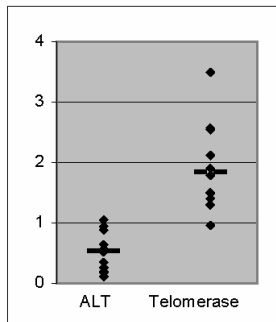
DRG

developmentally regulated GTP binding protein 2

Rank: 1

P-value: 1.0×10^{-7}

Fold change: 0.3
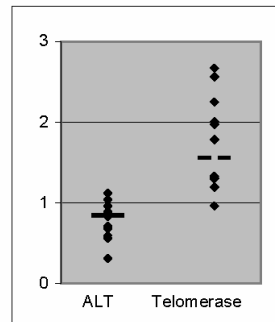
SFXN4

sideroflexin 4

Rank: 2

P-value: 1.0×10^{-6}

Fold change: 0.5
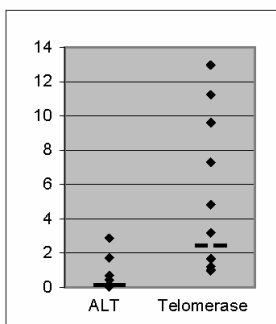
MGMT

O-6-methylguanine-DNA methyltransferase

Rank: 10

P-value: 6.7×10^{-5}

Fold change: 0.1
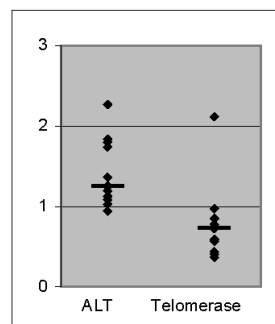
IRAK1BP1

interleukin-1 receptor-associated kinase 1 binding protein 1

Rank: 12

P-value: 1.0×10^{-4}

Fold change: 1.7

EGF
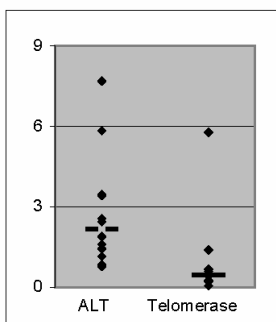
epidermal growth factor

Rank: 13

P-value: 1.0×10^{-4}

Fold change: 4.8
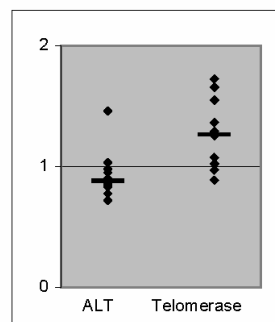
HNRPK

heterogeneous nuclear ribonucleoprotein K

Rank: 43

P-value: 6.0×10^{-4}

Fold change: 0.7
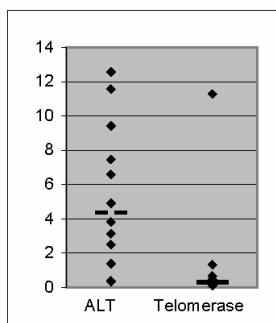
SATB1

special AT-rich sequence binding protein 1

Rank: 50

P-value: 5.9×10^{-4}

Fold change: 14.2
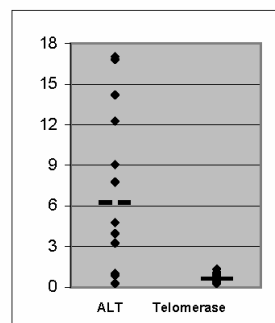
NSBP1

nucleosomal binding protein 1

Rank: 51

P-value: 6.8×10^{-4}

Fold change: 9.9

5-143

CCNG2

cyclin G2

Rank: 73

P-value: 1.3×10^{-3}

Fold change: 2.3

LEF1

lymphoid enhancer-binding factor 1

Rank: 108

P-value: 1.9×10^{-3}

Fold change: 5.3

TACC2

transforming, acidic coiled-coil containing protein 2

Rank: 112

P-value: 1.9×10^{-3}

Fold change: 0.5

NSE1

Rank: 130

P-value: 2.1×10^{-3}

Fold change: 6.7

CEBPA

CCAAT/enhancer binding protein (C/EBP), alpha

Rank: 203

P-value: 4.0×10^{-3}

Fold change: 0.6

SIGIRR

single Ig IL-1R-related molecule

Rank: 206

P-value: 4.0×10^{-3}

Fold change: 0.3

Figure 5.1: Differential gene expression between ALT[+] and telomerase[+] cell lines. Gene expression levels for 12 ALT[+] and 12 telomerase[+] cell lines were determined by RNA microarray analysis and 19469 gene elements had levels three-fold higher than background noise in at least half of the ALT[+] or at least half of the telomerase[+] cell lines. Of these gene elements, 240 genes found to be differentially expressed (Mann-Whitney test, $p \leq 0.005$) between the ALT[+] and telomerase[+] cell lines (Appendix A). Of these genes, the fourteen further analysed in Chapter 5 are summarised here with the significance of their differential expression (Mann-Whitney test *p-value*) and their *rank* according to this test. The *fold change* was determined by dividing the median gene expression level in the ALT[+] cell lines by the median gene expression level in the telomerase[+] cell lines.

Table 5.2: Differentially expressed genes with known roles that are potentially relevant to telomere maintenance.

Gene	Change[a]	Rank	Relevant Functions	Comments	References
DRG2	0.3	1	Cellular proliferation, RNA binding	Cytoband 17p11.2 contains DRG2, FLJ36674 and FAM18B that are all down-regulated in ALT.	See section 5.1.1
MGMT	0.1	10	DNA repair, chemotherapy resistance	Located near TACC2 and SFXN4 on 10q23-26 which contains 13 genes that are all expressed at significantly lower levels in ALT.	See section 5.1.2
EGF	4.8	13	Cellular proliferation, may negatively regulate MGMT and MLH1	Chromosome 4 contains high proportion of genes with higher expression in ALT. Other evidence of up-regulation of MAPK pathway in ALT includes higher levels of IRAK1BP1, LEF1, TGFB2 and lower levels of SIGIRR .	[430-432]
HNRPK	0.7	43	Binds telomeric C-strand DNA. Transcriptional coactivator of p53 in DNA damage response.	Single stranded C-strand telomeric DNA is increased in ALT. Also binds the CT-rich element in the c-myc promoter	[433-435]
SATB1	14.2	50	Establishment/maintenance of chromatin architecture, Transcriptional regulator	Only 4/16 differentially expressed genes on chromosome 3 are elevated in ALT.	See section 5.1.3
NSBP1	9.9	51	May be a transcriptional regulator	On X chromosome; fold median expression change in NSBP1 between ALT and telomerase for female cell lines, 29; male cell lines 3.7 (all cell lines 9.9).	[436]
CCNG2	2.3	73	Cell cycle checkpoint	Chromosome 4 contains high proportion of genes with higher expression in ALT. CCNG2 levels are increased by DNA damage dependent on p53. Expression may be positively regulated by hTR.	[429,437]
TACC2	0.5	112	Gene regulation, chromatin remodelling	Located near MGMT and SFXN4. 10q23-26 contains 13 differentially expressed genes all lower in ALT.	[438,439]
NSE1	6.7	130	DNA HR and repair in yeast (non SMC)	Lower significance but higher in ALT in all ALT-telomerase pairs from the same parental cell strain.	[440]
CEBPA	0.6	203	Transcriptional regulation, cell proliferation and differentiation	May act with pRb proteins to impose G1 arrest in response to DNA damage.	[441,442]

[a]Fold change in median expression levels in ALT[+] compared to telomerase[+] cell lines
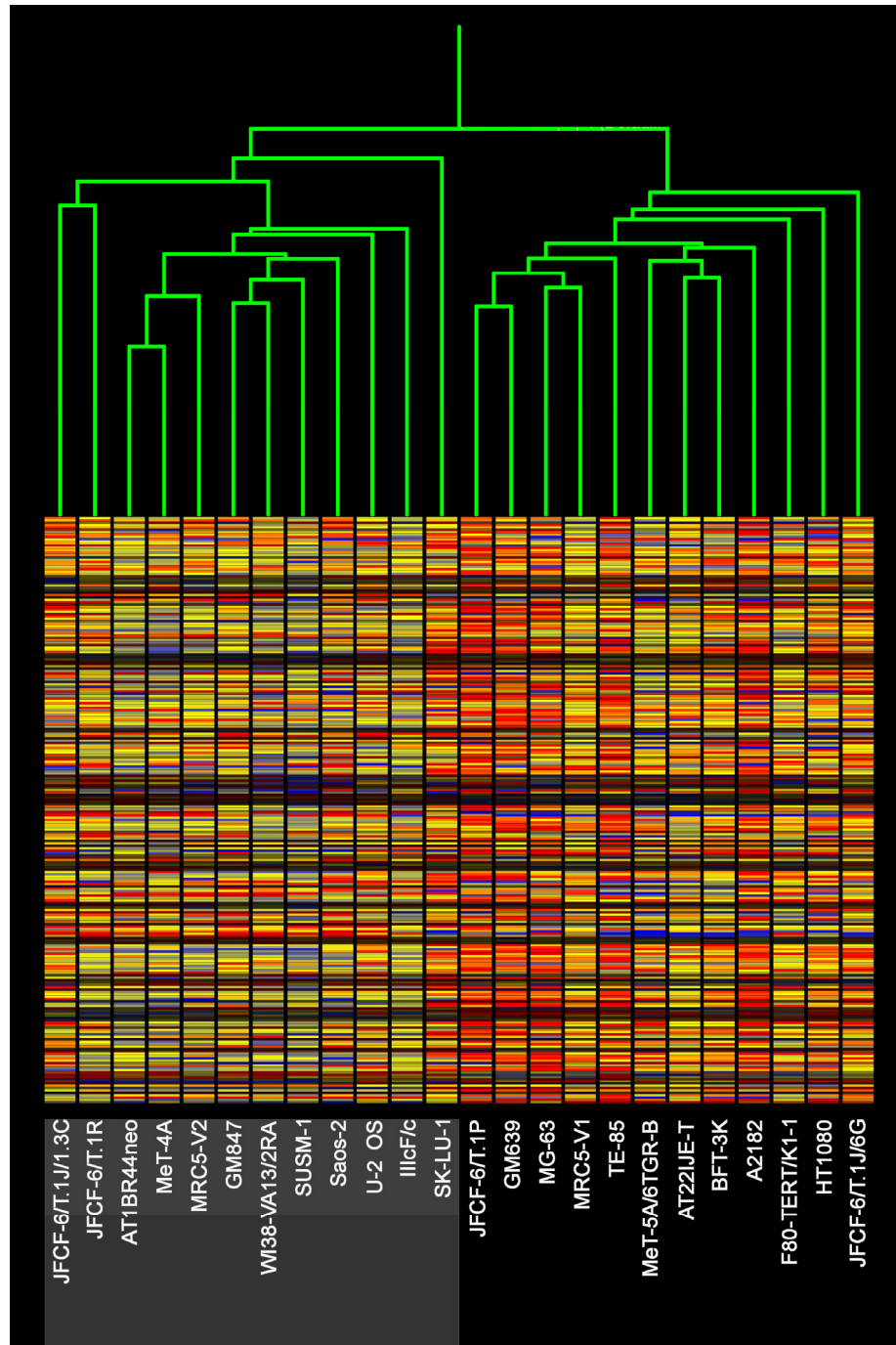
Figure 5.2: Hierarchical clustering of the differential expressed genes. Hierarchical clustering of the cell lines based on their expression levels of the 250 differentially expressed gene elements (Mann-Whitney test, p < 0.005). For each cell line, gene expression relative to the median level for all cell lines is illustrated by a red bar for increased expression, a blue bar for decreased expression and a yellow bar indicates expression not different from the median level. The individual gene elements are not indicated but are described in detail in Appendix A. This figure illustrates that the set of 250 differentially expressed gene elements successfully separates all the ALT[+] cell lines (*grey background*) from the telomerase[+] cell lines.

telomerase[+] groups (Mann-Whitney test, p = 0.055). However, RPL37A was > 3-fold upregulated in the *S. cerevisiae* telomerase negative survivors[428] and 1.4-fold downregulated in the ALT[+] group. Li *et al.*[429] have described a set of genes, including Cyclin G2, that are downregulated in immediate response to depletion of the telomerase RNA component in human cell lines. Although Cyclin G2 was differentially expressed between the ALT[+] and telomerase[+] groups (CCNG2, Appendix A; Mann-Whitney test, p = 0.001) Cyclin G2 was upregulated in ALT.

One problem with analysing a large pool of genes is that the number of false positives for differential expression will be high. That is, a significant number of the differentially expressed genes will have a higher level of expression in one group solely due to chance (and would show equal expression if we had sampled an infinite number of cell lines). Two simple ways of managing this problem are the conservative Bonferroni method of adjusting the p-value to take into account the chance of false positives and the less stringent Benjamini and Hochberg method of estimating the false positive rate[443]. Table 5.3 shows the Bonferroni corrected p-values and the Benjamini and Hochberg estimate of the false positive rate for the 250 differentially expressed gene elements (Mann-Whitney test, p < 0.005). Both methods estimate that only two genes, DRG2 and SFXN4 are significantly likely to be both differentially expressed and not false positives (p < 0.05). The Benjamini and Hochberg method also provides an estimate of the proportion of false positives for each p-value cut-off (Table 5.3). For example, 39% or 98 of the 250 differentially expressed gene elements (Mann-Whitney test, p < 0.005) are expected to be false positives. The Bonferroni corrected p-values and the Benjamini and Hochberg estimate of the false positive rate are plotted for the 1580 most significantly differentially expressed gene elements (Mann-Whitney test p < 0.05; Fig 5.3).

Table 5.3: Adjusting the significance of differentially expressed genes to include the estimated false positive rate.

Gene Rank	P-value	Adjusted P-value[a]	False +ve rate[b]	Gene Rank	P-value	Adjusted P-value[a]	False +ve rate[b]
1	1.05E-07	0.0020	0.2%	87-99	1.42E-03	27.6	27.9%
2	1.00E-06	0.019	1.0%	100-102	1.61E-03	31.3	30.7%
3-4	1.75E-05	0.3	8.5%	103	1.84E-03	35.8	34.8%
5	3.51E-05	0.7	13.7%	104-110	1.85E-03	36.0	32.7%
6-8	4.40E-05	0.9	10.7%	111-127	1.86E-03	36.2	28.5%
9	6.07E-05	1.2	13.1%	128	1.99E-03	38.7	30.3%
10	6.69E-05	1.3	13.0%	129	2.06E-03	40.1	31.1%
11	8.22E-05	1.6	14.5%	130	2.10E-03	40.9	31.4%
12-13	1.00E-04	1.9	15.0%	131-135	2.11E-03	41.1	30.4%
14-16	1.01E-04	2.0	12.3%	136	2.38E-03	46.3	34.1%
17-18	1.48E-04	2.9	16.0%	137	2.40E-03	46.7	34.1%
19-22	2.14E-04	4.2	18.9%	138-155	2.41E-03	46.9	30.3%
23-24	2.98E-04	5.8	24.2%	156	2.67E-03	52.0	33.3%
25-30	3.04E-04	5.9	19.7%	157	2.73E-03	53.2	33.9%
31-35	4.24E-04	8.3	23.6%	158	2.73E-03	53.2	33.6%
36	4.93E-04	9.6	26.7%	159	3.06E-03	59.6	37.5%

37	4.95E-04	9.6	26.0%	160	3.06E-03	59.6	37.2%
38-39	4.97E-04	9.7	24.8%	161-185	3.10E-03	60.4	32.6%
40	5.76E-04	11.2	28.0%	186	3.49E-03	67.9	36.5%
41	5.82E-04	11.3	27.6%	187	3.50E-03	68.1	36.4%
42-50	5.85E-04	11.4	22.8%	188	3.90E-03	75.9	40.4%
51	6.80E-04	13.2	26.0%	189	3.92E-03	76.3	40.4%
52	7.84E-04	15.3	29.4%	190-192	3.93E-03	76.5	39.9%
53-56	7.92E-04	15.4	27.5%	193	3.94E-03	76.7	39.7%
57-68	7.95E-04	15.5	22.8%	194-215	3.95E-03	76.9	35.8%
69	9.04E-04	17.6	25.5%	216	4.36E-03	84.9	39.3%
70	1.05E-03	20.4	29.2%	217	4.43E-03	86.2	39.7%
71-72	1.06E-03	20.6	28.7%	218-222	4.44E-03	86.4	38.9%
73-84	1.07E-03	20.8	24.8%	223-225	4.98E-03	97.0	43.1%
85	1.15E-03	22.4	26.3%	226-250	4.99E-03	97.2	38.9%
86	1.22E-03	23.8	27.6%				

[a]P-value was adjusted to take into account the chance of false positives by the Bonferroni method. [b]False positive rate was estimated by the Benjamini and Hochberg method.

Figure 5.3: Effect of the false positive rate on the significance of differential expression. *A*: P-values were adjusted by the Bonferroni method to take into account the chance of false positives and plotted for the 1580 most significantly differentially expressed gene elements ranked in order of their significance. *B*: The false positive rate, as estimated by the Benjamini and Hochberg method is graphed for the 1580 most significantly differentially expressed gene elements.

5.2.3   Characteristics of differentially expressed genes

Of the 240 genes that were differentially expressed (Mann Whitney test $p < 0.005$), only 93 or 39% (95% CI; 33% to 45%) had higher expression in ALT[+] cell lines. If we estimate (Benjamini and Hochberg method) that 94 of the 240 differentially expressed genes are false positives - with half of the false positives expressed at a lower level in ALT cells, then 46 (32%) of 146 truly differentially expressed genes have higher levels of expression in ALT[+] cell lines. The distribution of the differentially expressed genes among the chromosomes is shown in Table 5.4. Chromosomes 1, 4, 14 and X contain a significantly high proportion of genes upregulated in ALT cell lines. Chromosomes 10 and 15 contain a significantly high proportion of genes down-regulated in ALT cell lines. The high proportion of the differentially expressed genes on chromosome X that are up-regulated in ALT, could be in part due to the higher proportion of ALT cell lines derived from female donors; six ALT compared with three telomerase cell lines.

Table 5.4: Distribution of the differentially expressed genes among the chromosomes.

Chromosome	1	2	3	4	5	6	7	8	9	10	11	12	13
No. genes changed	17	17	16	13	5	6	13	10	8	14	15	2	6
No. genes up[a]	13	5	4	11	3	3	2	2	3	1	6	0	2
% genes up[a]	76.5%	29.4%	25.0%	84.6%	60.0%	50.0%	15.4%	20.0%	37.5.0%	7.1%	40.0%	0.0%	33.3%
lower 95% CI	54.0%	5.3%	1.2%	62.0%	-8.0%	-7.5%	-7.0%	-10.2%	-5.8%	-0.8%	11.9%	-	-20.9%
upper 95% CI	99.0%	53.6%	48.8%	107.0%	128.0%	107.5%	38.0%	50.2%	80.8%	22.6%	68.1%	-	87.5%

Chromosome	14	15	16	17	18	19	20	21	22	X	N/A	total
No. genes changed	7	13	4	7	2	8	3	3	4	18	29	**240**
No. genes up[a]	6	1	1	2	0	3	1	1	1	14	8	**93**
% genes up[a]	85.7%	7.7%	25.0%	28.6%	0.0%	37.5%	33.3%	33.3%	25.0%	77.8%	27.6%	**38.8%**
lower 95% CI	50.8%	-9.1%	-54.6%	-17.0%	-	-5.8%	-110.0%	-110.0%	-54.6%	56.5%	10.3%	**32.5%**
upper 95% CI	120.7%	24.5%	104.6%	74.0%	-	80.8%	180.0%	180.0%	104.6%	99.1%	44.9%	**45.0%**

[a]in ALT[+] compared to telomerase[+]. N/A indicates that the gene location was not available on the public domain.

5.2.4   Co-regulation of differentially expressed genes

Analysis was carried out to elucidate differentially expressed genes that may be co-regulated or genes that may be responsible for regulating ALT specific expression. For practical reasons analysis was carried out on a small number of genes. Also to allow immediate interpretation of results, the genes chosen for analysis were the differentially regulated genes with known roles that are potentially relevant to telomere maintenance (Table 5.2). SFXN4 and differentially expressed genes in the MAPK pathway, IRAK1BP1, LEF1 and SIGIRR were also included in the analysis. Spearman's correlation coefficient was used to determine pair-wise correlation between expression levels (Table 5.5). In Table 5.5 all gene pairs with a Spearman's correlation coefficient > 0.4 (or < -0.4) are correlated (or negatively correlated) with $p < 0.05$. This means that there is a less than 5% chance that they are not correlated (or not negatively correlated). The problem with this is that this set of differentially expressed genes is a biased set from an unbiased set of the 19469 gene elements analysed in section 5.2.2. This provides 1.9×10^8 gene (probe) pairs and thus a large number of false positive pair-wise correlations over the 24 cell lines that may be overrepresented in the biased set of differentially expressed genes analysed in Table 5.5. To reduce the chance of false positives a stringent cut-off for the Spearman's correlation coefficient of > 0.71 (or < -0.71) was used, which corresponds to $p < 5 \times 10^{-5}$.

Two groups of genes appeared to have correlated expression levels that fitted these criteria (Fig 5.4 and Fig 5.5A). One group centred on SIGIRR. Both NSBP1 and LEF1 expression levels were strongly negatively correlated with expression of SIGIRR (Spearman's correlation coefficient = -0.77 and -0.76, respectively; Table 5.5, Fig 5.5A). There was a weaker correlation between SATB1 and NSBP1 but the

Table 5.5: Correlation of gene expression levels determined by Spearman's correlation coefficients

	DRG2	SFXN4	MGMT	EGF	HNRPK	SATB1	NSBP1	CCNG2	TACC2	NSE1	CEBPA	IRAK1BP1	LEF1	SIGIRR
DRG2	1	0.69	0.63	-0.56	0.60	-0.53	-0.48	-0.59	0.54	-0.61	0.44	-0.60	-0.58	0.48
SFXN4	0.69	1	0.73	-0.45	0.64	-0.49	-0.56	-0.42	0.77	-0.41	0.76	-0.49	-0.40	0.63
MGMT	0.63	0.73	1	-0.60	0.52	-0.43	-0.60	-0.38	0.59	-0.45	0.67	-0.53	-0.49	0.63
EGF	-0.56	-0.45	-0.60	1	-0.35	0.41	0.57	0.44	-0.27	0.43	-0.22	0.61	0.53	-0.40
HNRPK	0.60	0.64	0.52	-0.35	1	-0.21	-0.25	-0.32	0.73	-0.54	0.73	-0.36	-0.28	0.37
SATB1	-0.53	-0.49	-0.43	0.41	-0.21	1	0.71	0.39	-0.31	0.56	-0.27	0.50	0.66	-0.59
NSBP1	-0.48	-0.56	-0.60	0.57	-0.25	0.71	1	0.31	-0.51	0.26	-0.38	0.43	0.68	-0.76
CCNG2	-0.59	-0.42	-0.38	0.44	-0.32	0.39	0.31	1	-0.11	0.46	-0.16	0.43	0.33	-0.18
TACC2	0.54	0.77	0.59	-0.27	0.73	-0.31	-0.51	-0.11	1	-0.32	0.82	-0.31	-0.40	0.65
NSE1	-0.61	-0.41	-0.45	0.43	-0.54	0.56	0.26	0.46	-0.32	1	-0.35	0.36	0.44	-0.22
CEBPA	0.44	0.76	0.67	-0.22	0.73	-0.27	-0.38	-0.16	0.82	-0.35	1	-0.27	-0.37	0.61
IRAK1BP1	-0.60	-0.49	-0.53	0.61	-0.36	0.50	0.43	0.43	-0.31	0.36	-0.27	1	0.32	-0.33
LEF1	-0.58	-0.40	-0.49	0.53	-0.28	0.66	0.68	0.33	-0.40	0.44	-0.37	0.32	1	-0.77
SIGRR	0.48	0.63	0.63	-0.40	0.37	-0.59	-0.76	-0.18	0.65	-0.22	0.61	-0.33	-0.77	1

A Spearman's correlation coefficient of > 0.40 (< -0.40) indicates significant correlation (negative correlation) with $p < 0.05$.
A Spearman's correlation coefficient of > 0.71 (< -0.71) indicates significant correlation (negative correlation) with $p < 5 \times 10^{-5}$
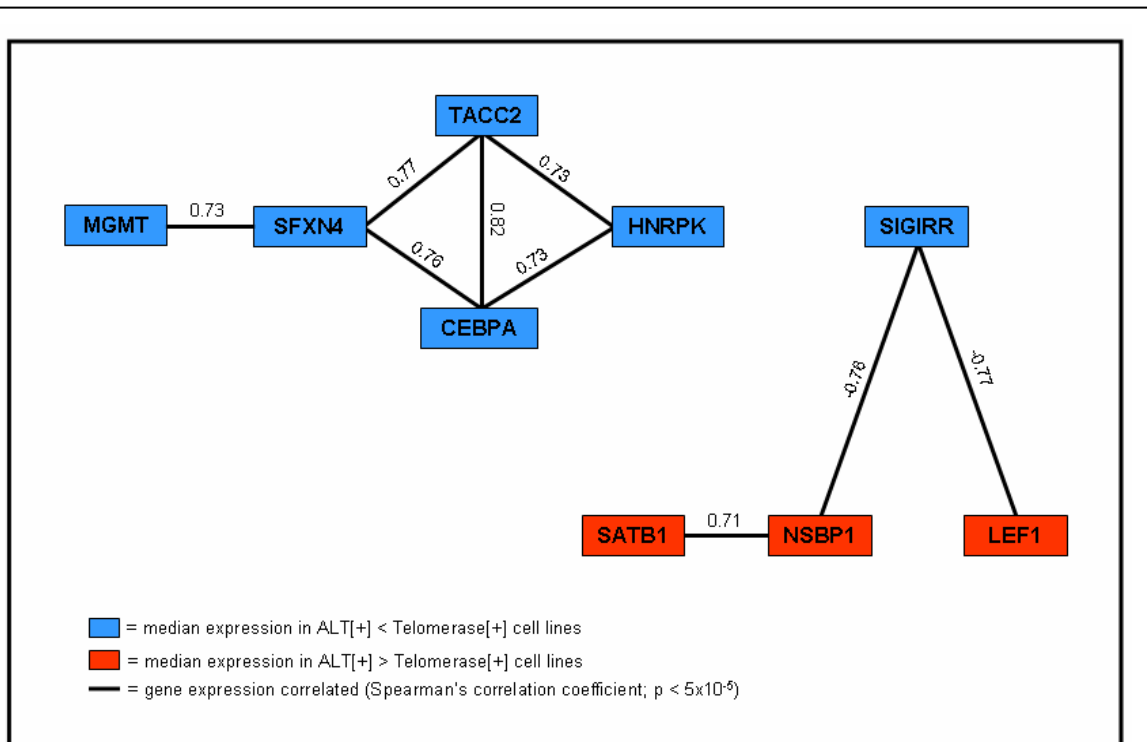
Figure 5.4: Co-regulated groups of differentially expressed genes. A subset of differentially expressed genes were analysed for correlated gene expression. The *lines* between the gene pairs represent highly significant correlations (Spearman's correlation coefficient > 0.71 or < -0.71 corresponding to a p-value cut-off of $p < 5 \times 10^{-5}$). The *numbers* beside the *lines* indicate the Spearman's correlation coefficient for the gene pair. Gene pairs down-regulated in ALT (Mann-Whitney test, $p < 0.005$) have a *blue* background, where as a *red* background denotes genes up-regulated in ALT (Mann-Whitney test, $p < 0.005$).

A

B
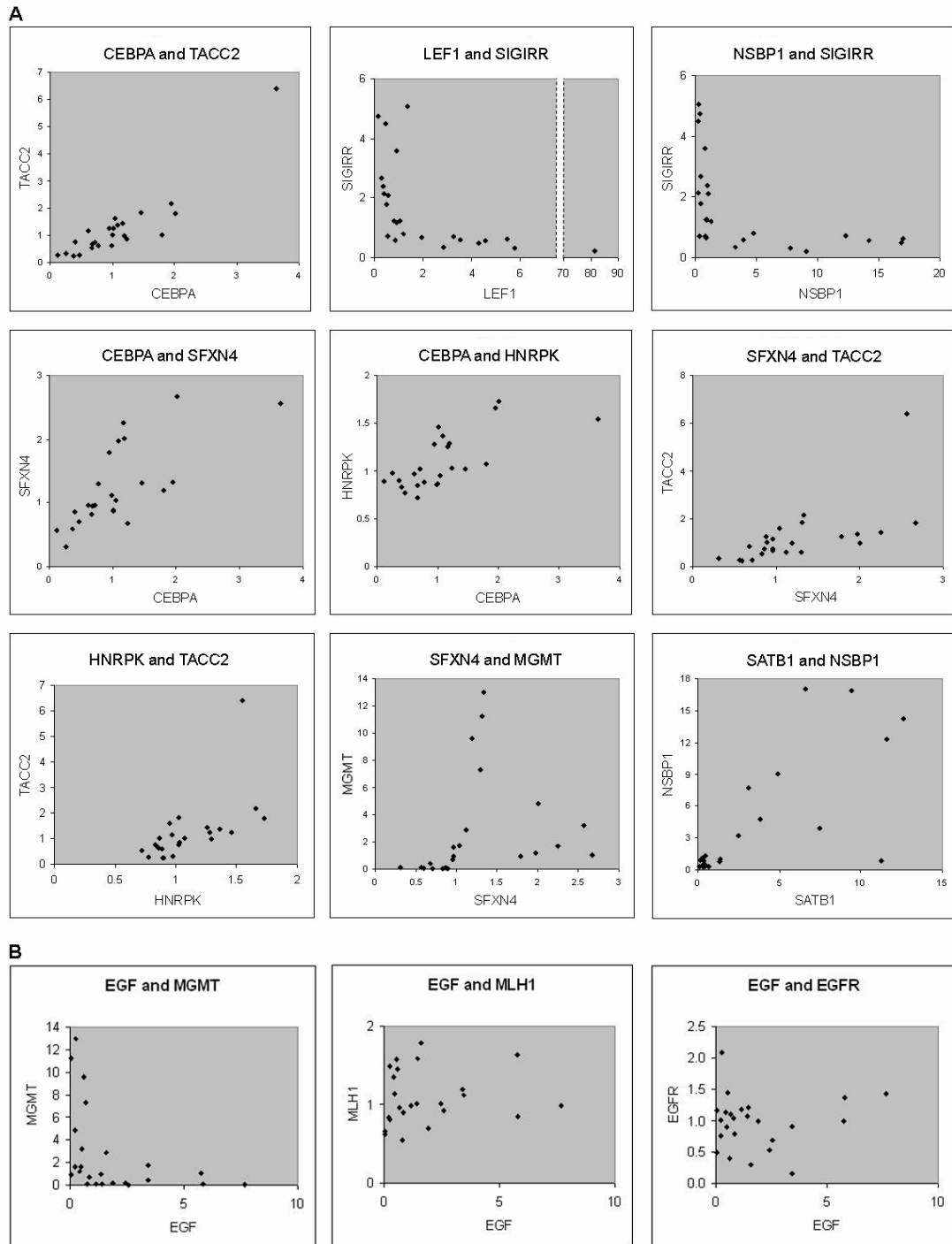
Figure 5.5: Pair-wise gene expression correlations. The pair-wise correlations between the expression levels of selected genes are illustrated. Each one of the 24 cell lines are represented by a point on the graph. *A:* Shows the correlations for co-regulated gene groups shown in Fig 5.4. There was a highly significant correlation (Spearman's correlation coefficient, $p < 5 \times 10^{-5}$; Table 5.5) between the expression levels of all gene pairs. *B:* The correlation between *EGF* and *MGMT*, *MLH1* and EGFR. There was a significant negative correlation between *EGF* and *MGMT*, Spearman's correlation coefficient = -0.6 ± 0.4, $p < 0.05$. However, this was below the more stringent p-value cut-off used to select the gene pairs in part *A* of this figure. No significant correlation was found between *EGF* and either *MLH1* or *EGFR*; Spearman's correlation coefficients = 0.26 ± 0.4, and -0.07 ± 0.4, respectively ($p < 0.05$).

Spearman's correlation coefficient = 0.71 was above the $p < 5 \times 10^{-5}$ cut-off (Table 5.5, Fig 5.5A). SATB1, NSBP1 and LEF1 were up-regulated in ALT (Mann-Whitney test $p = 5.9 \times 10^{-4}$, $p = 6.8 \times 10^{-4}$ and $p = 1.9 \times 10^{-3}$, respectively, Fig 5.1) and SIGIRR was down-regulated in ALT (Mann-Whitney test $p = 4.0 \times 10^{-3}$, Fig 5.1).

The second group of co-regulated genes centred on CEBPA and TACC2 (Fig 5.4 and Fig 5.5A). The expression levels for this pair of genes were strongly correlated (Spearman's correlation coefficient = 0.82; Table 5.5, Fig 5.5A). Both CEBPA and TACC2 had expression levels that correlated with both SFXN4 and HNRPK expression, but only SFXN4 expression correlated with MGMT expression levels (Spearman's correlation coefficients were between 0.73 to 0.77, all above the $p < 5 \times 10^{-5}$ cut-off; Table 5.5, Fig 5.5A). This group of genes were all down-regulated in ALT (Mann-Whitney test $p = 1.0 \times 10^{-6}$, 6.7×10^{-5}, 5.9×10^{-4}, 1.9×10^{-3} and 4.0×10^{-3} for SFXN4, MGMT, HNRPK, TACC2 and CEBPA, respectively; Fig 5.1).

Since Epidermal Growth Factor (EGF) has been reported to down-regulate MGMT and MLH1 expression[432], these relationships were looked at separately. EGF was up-regulated in ALT (Mann-Whitney test $p = 1 \times 10^{-4}$, Figure 5.1), MGMT expression was down-regulated in ALT (Mann-Whitney test $p = 1 \times 10^{-4}$, Fig 5.1) and there was no difference in expression levels of MLH1 between the ALT[+] and telomerase[+] cell lines (Mann-Whitney test $p = 0.72$, Fig 5.6A). The telomerase[+] cell lines that had a marked elevation in MGMT levels had below median levels of EGF expression (Fig 5.5B). There was a significant negative correlation between the expression of EGF and MGMT (Spearman's correlation coefficient = -0.60 ± 0.40, $p < 0.05$; Fig 5.5B, Table 5.5), but not as significant as the correlations shown in Figure 5.4 with $p <$

5x10^{-5}. No correlation was seen between EGF and MLH1 (Spearman's correlation coefficient = 0.26 ± 0.40; Fig 5.5B). The relationship between EGF and EGF receptor (EGFR) expression was also investigated. ALT appears to be more common in secondary GBM than primary GBM (Section 6.3). EGFR has been reported to be over-expressed in primary GBM[444]. No correlation was observed between the expression levels of EGF and EGFR in the 24 cell lines (Spearman's correlation coefficient = -0.07 ± 0.40; Fig 5.5B) and EGFR expression was not significantly associated with ALT[+] or telomerase[+] cell lines (Mann-Whitney test p = 0.50, Fig 5.6B).

A

MLH1: mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)

P-value:	0.71							Fold change:	0.9		

Chromosome: 3p21.3

Genbank: NM_000249.2   Gene ID: hCG17142.2

Biological process: mismatch repair; negative regulation of cell cycle

Molecular function: ATP binding

Cellular component: nucleus

Expression levels in ALT cell lines   median: **1.0**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	0.93	0.55	0.85	1.59	0.98	0.69	1.13	1.79	1.01	0.99	0.90

Expression levels in Telomerase cell lines   median: **1.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	A2182	F80	HT1080	MG63	TE85
1.45	1.49	0.97	1.01	1.35	0.62	0.66	1.58	0.83	1.14	0.81	1.64

B

EGFR: epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)

P-value:	0.50							Fold change:	0.9		

Chromosome: 7p12   Genbank: NM_005228.3   Gene ID: hCG1811404.2

Biological process: cellular proliferation;EGFR signalling pathway;protein phosphorylation;electron transprt

Molecular function: iron ion and ATP binding;EGFR, transferase and electron transporter activity

Cellular component: integral to plasma membrane;endosome;cytoskeleton

Expression levels in ALT cell lines   median: **0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.90	0.69	1.04	1.37	1.22	1.18	0.99	0.15	0.29	0.53	1.43	0.79

Expression levels in Telomerase cell lines   median: **1.0**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	A2182	F80	HT1080	MG63	TE85
0.41	2.07	1.09	1.07	1.13	1.17	0.49	1.45	1.00	0.90	0.76	1.00

Figure 5.6: Expression levels of MLH1 and EGFR in ALT[+] and telomerase[+] cell lines. *A*: MLH1 and ***B*:** EGFR expression levels for 12 ALT[+] and 12 telomerase[+] cell lines determined by RNA microarray analysis. MLH1 and EGFR were not differentially expressed between the ALT[+] and telomerase[+] cell lines (Mann-Whitney test; *p-value* = 0.71 and 0.50, respectively) and the *median* expression of MLH1 and EGFR in ALT[+] cell lines were both 0.9 fold the *median* of the telomerase[+] cell lines. Descriptions of MLH1 and EGFR are shown[1].

5.3   Western blot analysis and confirmation of RNA microarray results

Three genes, DRG2, MGMT and SATB1, were prioritised for further investigation. Of the genes for which investigations could be facilitated by existing antibodies and expression plasmids, these genes were thought to be most relevant to the ALT mechanism or the therapy of ALT[+] cancer. This was based on the strength of their differential expression and their known functions.

Microarray results were validated and extended by testing the protein levels of DRG2, AGT and SATB1 in 37 cell lines (Table 5.6) including the 22 of the 24 cell lines used for the microarray analysis. Protein levels were determined by western blot analysis with 19 cell lines per gel to minimise problems of comparing protein levels between gels. Gels were probed for DRG2, AGT and actin before stripping and probing for SATB1. Gel conditions were optimised for the 22 and 41 kDa size of AGT[387] and DRG2[369] and not the 86 to 104 kDa size of SATB1[419,445]. As confirmed by Ponceau S staining (Fig 5.7A) the gels had equal loading.

The protein levels for DRG2, AGT, SATB1 and actin are shown in Figure 5.7B and have been quantitated (Appendix B) and plotted in Figure 5.8. The DRG2 antibody bound a band at the correct position (41 kDa[369]), labelled as DRG2 in Figure 5.7B, but also bound to a lower band at approximately 35 kDa (DRG2lower; Fig 5.7B). Because the intensity of DRG2 relative to DRG2lower seemed to be more strongly related to ALT status than DRG2 intensity alone, DRG2lower was also quantitated and the ratio between DRG2 and DRG2lower was analysed. The resolution of the SATB1 band in the gel 2 (Fig 5.7B) was considered unsatisfactory and the intensity results were omitted from further analysis. MeT-5A results were not included in the telomerase[+] cell line group as this cell line has a low frequency of APBs suggesting it may have some ALT activity (Section 3.2).

Table 5.6: Cell lines used for Western blot analysis

Cell/Tissue Type	Transformation	Primary strain	ALT[+]	Telomerase[+]
Fibroblast, jejunal*	SV40	JFCF-6 [m]	JFCF-6/T.1R [m]	JFCF-6/T.1P [m]
Fibroblast, lung	SV40	MRC-5 [m]	MRC5-V2 [m]	MRC5-V1 [m]
Fibroblast, lung	SV40	WI-38 [f]	WI38-VA13/2RA [f]	
Fibroblast, bronchial	SV40	BF10* [m]		BFT-3K* [xo]
Epithelial, bronchial	SV40	ND	BET-3M* [m]	BET-3K* [m]
Fibroblast, breast, LFS	spontaneous	IIICF [f]	IIICF/c [f]	
Fibrosarcoma	tumour	ND		HT1080 [m]
Fibroblast, skin	SV40	HFF5 [m]		
Fibroblast, skin		ND	GM847 [m]	
Fibroblast, skin		ND		GM639 [m]

Cell/Tissue Type	Transformation	ALT[+]	Telomerase[+]
Fibroblast, AT	SV40	AT1BR44neo [m]	AT22IJE-T [f]
Mesothelial	SV40	MeT-4A [f]	MeT-5A/6TGR-B [m]
Mesothelial	SV40		**MeT-5A [m]
Osteosarcoma	tumour	Saos-2 [f]	TE-85 [f]
Osteosarcoma	tumour	G292 [f]	SJSA-1 [m]
Osteosarcoma	tumour	U-2 OS [f]	MG-63 [m]
Adenocarcinoma, lung	tumour	SK-LU-1 [f]	A2182 [m]
Fibroblast	SV40	GM637 [f]	
Adenocarcinoma, lung	tumour		A549 [m]
Fibroblast, embryonal	γ - irradiation	KMST6 [f]	
Adenocarcinoma, cervix	tumour		HeLa [f]
Fibroblast, liver	chemical	SUSM-1 [m]	
Fibroblast, breast	SV40		F80-TERT/K1-1 [f]

*BF10, BFT-3K, BET-3M and BET-3K were derived from the same donor. [f] or [m] indicates the cell line was derived from a female or male patient, respectively. [xo]BFT-3K is aneuploid X0. **MeT-5A had a low frequency of APBs (Table 3.1). ND indicates the primary cell strain was not investigated.

Figure 5.7: Western blot analysis of DRG2, AGT and SATB1 protein levels. *Gel 1* contained lysates from six *ALT[+] cell lines* with matched *mortal cell strains* (early to mid life span) and *telomerase[+] cell lines* as well as an additional telomerase[+] cell line, A2182, which was included on both gels. Gel 2 contained lysates from nine *ALT[+] cell lines* with matched *telomerase[+] cell lines* and the *telomerase[+] cell line* MeT-5A that may have some ALT activity (Section 3.2). *A*: Ponceau S staining confirmed equal loading. *B*: Immunoblots for *DRG2*, *AGT*, *SATB1* and *actin*. As well as the expected 41 kDa band (doublet), the DRG2 antibody also recognised a second band, *DRG2-lower*, at about 35 kDa.

Figure 5.8: Association of TMM with DRG2, AGT and SATB1 protein levels. *Protein levels* for *A*: *DRG2*, *B*: *AGT* and *C*: *SATB1* are plotted for the *mortal* cell strains and the *ALT*[+] and *telomerase*[+] cell lines. The *horizontal bars* indicate the median levels. For SATB1, only cell strains/lines from gel 1 were analysed (unmatched A2182 omitted). The values in boxes linking two groups indicate the p-value from the Mann-Whitney test that evaluates the significance of the difference in the protein levels between those two groups.

The expression level for DRG2 was lower in ALT[+] cell lines (median = 0.38; Fig 5.8A, Appendix B) compared to the telomerase[+] cell lines (median = 0.64) and mortal cell strains (median = 0.55), however, only the former was significant (Mann-Whitney test, ALT[+] compared to telomerase[+]; p = 0.045, ALT[+] compared to mortal; p = 0.42). Analysing the level of DRG2 relative to the lower band, DRG2lower, improved the discrimination between the ALT[+] cell lines and the ALT[-] cell lines (Mann-Whitney test, ALT[+] compared to telomerase[+] p = 0.001, ALT[+] compared to mortal p = 0.38) and also in contrast to the DRG2 levels the DRG2:DRG2lower levels were significantly different between the telomerase[+] and mortal cell lines (Mann-Whitney test p = 0.045).

The expression level for AGT in ALT[+] cell lines (median = 0.08) was lower than in telomerase[+] cell lines (median = 0.70) and mortal cell strains (median = 1.19), however only the former was significant (Mann-Whitney test, p = 0.01 and 0.06, respectively; Fig 5.8B).

The expression of SATB1 protein in ALT[+] cell lines (median = 0.68) and telomerase[+] cell lines (median = 0.40; unmatched A2182 omitted) was significantly higher than in the mortal cell strains (median = 0.20; Fig 5.8C, Mann Whitney test p=0.01 and 0.02 respectively). Although 5/6 of the ALT[+] cell lines had a higher level of SATB1 than 5/6 of the telomerase[+] cell lines, one ALT[+] and one telomerase[+] outlier meant that the difference between the ALT[+] and telomerase[+] cell lines was not significant (Fig 5.8C, Mann Whitney test p=0.31). Only a small sample size was available for SATB1; a larger sample size may reveal a significant difference.

As the contexts in which these proteins are expressed may be different for the different cell lines, the change in the protein levels was analysed between the parental (mortal) cell strains and the ALT[+] and telomerase[+] cell lines derived from them (Fig 5.9). This analysis is most clear cut for SATB1 levels. SATB1 increased as the mortal cell strains activated a TMM but to higher levels for ALT activation. There was also a clear pattern for AGT. As the mortal cell lines activated ALT, the levels of AGT decreased, while AGT levels increased during activation of telomerase. It is interesting that the only exception to this pattern, BFT-3K (a telomerase[+] cell line which had a lower level of AGT than its parental strain), had an abnormally high level of EGF expression for a telomerase[+] cell line (Appendix A). While all ALT[+] cell lines had a lower level of DRG2 than their corresponding telomerase[+] cell line derived from the same parental cell strain, the change during activation of the TMM from the mortal cell strains was less consistent. Nevertheless, DRG2 levels were reduced during activation of ALT in 3/4 of the mortal:ALT[+] pairs (Fig 5.9).

The RNA microarray results were also tested for a gene, FEN1 which showed no differential RNA expression (Mann-Whitney test p = 0.51; Fig 5.10). Western blot analysis of FEN1 in seven ALT[+] and seven telomerase[+] matched cell lines (Fig 5.11) showed no difference in protein levels between ALT[+] and telomerase[+] cell lines.

Although the microarray results indicating a down-regulation of DRG2 and MGMT, and an up-regulation of SATB1 in ALT[+] cell lines were confirmed by the Western analysis, it is also important for future work to assess the general accuracy of the microarray results. Although mRNA levels are only one factor influencing protein levels a significant correlation between the two would imply that the microarray
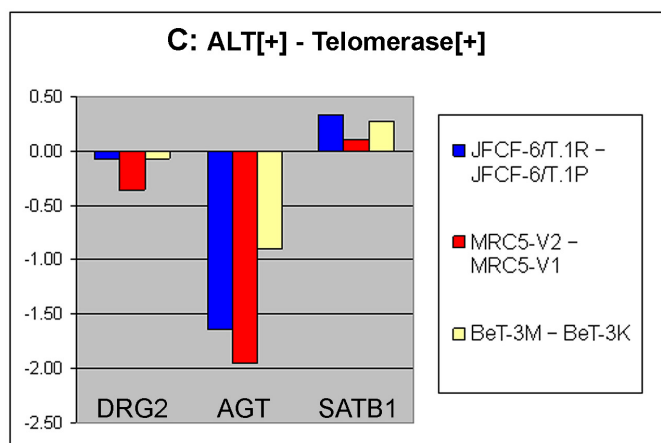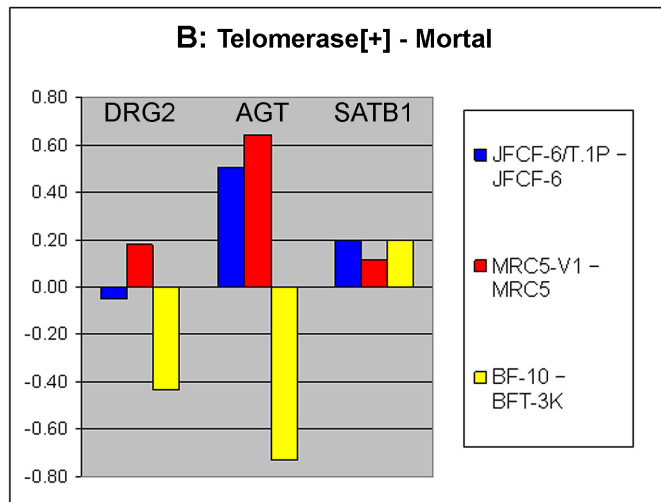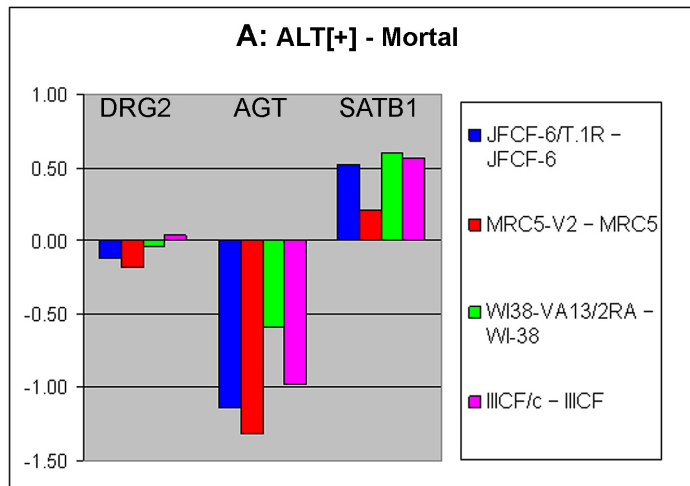
Figure 5.9: Changes in DRG2, AGT and SATB1 with TMM activation. Difference in *DRG2*, *AGT* and *SATB1* levels between *A*: *ALT[+]* cell lines and the parental (*mortal*) strains they were derived from, *B*: *telomerase[+]* cell lines and the parental (*mortal*) strains they were derived from and *C*: *ALT[+]* cell lines and *telomerase[+]* cell lines derived from the same parental strains.

Figure 5.10: Expression levels of FEN1 in ALT[+] and telomerase[+] cell lines. FEN1 expression levels for 12 ALT[+] and 12 telomerase[+] cell lines was determined by RNA microarray analysis. FEN1 was not differentially expressed between the ALT[+] and telomerase[+] cell lines (Mann-Whitney test; *p-value* = 0.48) and the *median* expression of FEN1 in ALT[+]and telomerase[+] cell lines was equal. A description of FEN1 is shown[1].
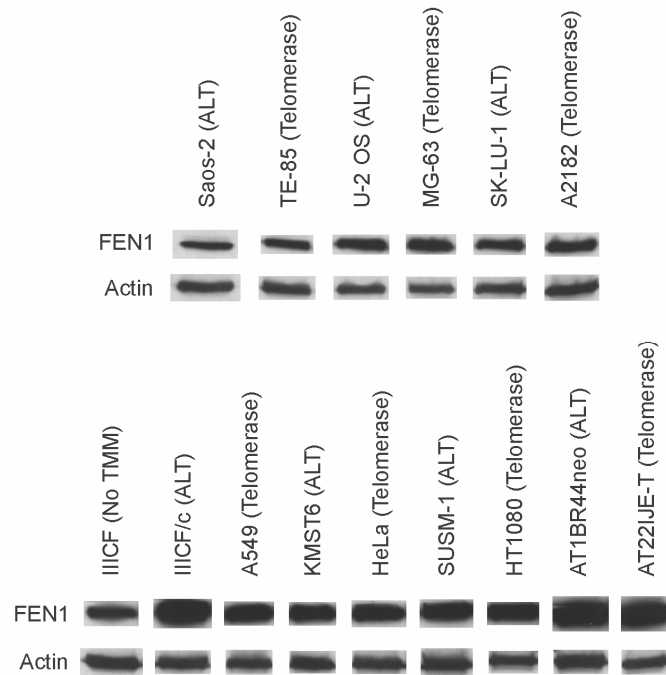
Figure 5.11: Western analysis of FEN1 protein levels in ALT[+] and telomerase[+] cell lines. Protein lysates from seven *ALT*[+] and seven *telomerase*[+] cell lines and one mortal cell strain (*no TMM*) were run on two gels (upper and lower panel each represent one gel). The immunoblots for *FEN1* and *actin* are shown.

results were generally accurate. The general accuracy of the RNA microarray results were tested by comparing the RNA levels for DRG2, MGMT and SATB1 to the

protein levels for each cell line (Fig 5.12). All three genes showed a significant correlation between the RNA levels from the microarray and the protein levels from the Western analysis. However the correlation was strongest for MGMT/AGT (Pearson's coefficient = 0.91 ± 0.23) which showed the largest range in expression levels. DRG2, which had the lowest range of expression and SATB1, for which the gel conditions were not optimal, had weak correlations between the RNA and protein levels (Pearson's coefficient = 0.36 ± 0.23 and 0.62 ± 0.36, respectively).

5.4   Loss of MGMT could mutate telomeric DNA

Loss of AGT activity has been associated with G:C to A:T transition mutations that could give rise to the mutated telomeric repeat 5′-TTAAGG-3′. This would introduce a restriction site for MseI, which recognises TTAA, into the telomere. To investigate the presence of MseI sites in ALT telomeres, TRF analysis was performed on a panel of six ALT[+] and four telomerase[+] cell lines using a variety of different restriction enzymes to create the terminal restriction fragments – including MseI or the standard TRF endonucleases HinfI or RsaI (Fig 5.13). These results indicated that the (mutated) TTAAGG repeats may exist in the ALT cell line GM847 that lacks MGMT expression (Fig 5.13 and Fig 5.7). The median TRF length of GM847 genomic DNA cut with MseI was 2.7kb compared with 10kb for the same genomic DNA cut with the usual enzymes, HinfI or RsaI. MseI did not appear to generate shorter TRFs than HinfI or RsaI in KMST6 or IIICF/c which express MGMT (Fig 5.13 and Fig 5.7). Other ALT cell lines JFCF-6/T.1J/1-4D, WI38-VA13/2RA and SUSM-1 and telomerase cell lines JFCF-6/T.1J/6B, HeLa, HT1080 and 293 showed shorter TRFs with MseI

A: DRG2

Pearson's:
0.36 ± 0.23

B: MGMT/AGT

Pearson's:
0.91 ± 0.23

C: SATB1

Pearson's:
0.62 ± 0.36
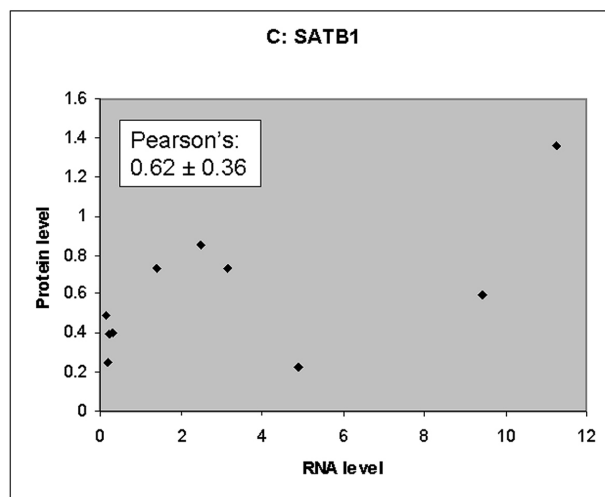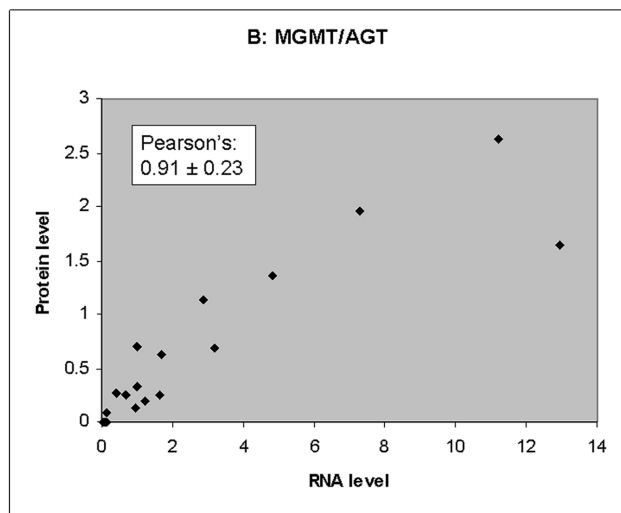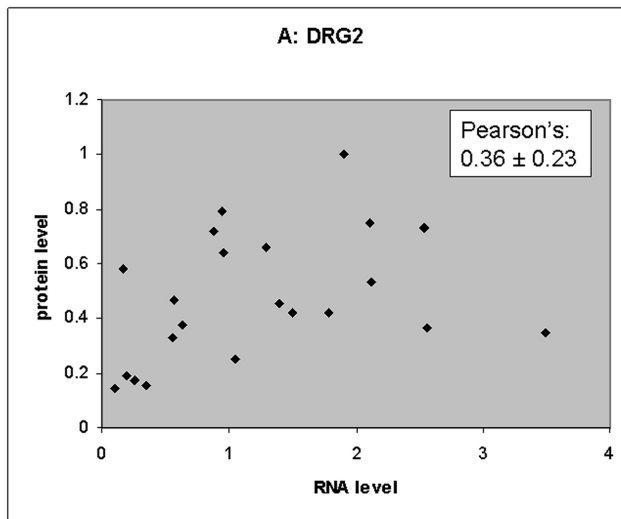
Figure 5.12: Correlation between microarray RNA levels and Western analysis protein levels. A subset of 22 cell lines were analysed by both RNA microarray and Western analysis. For *A: DRG2*, *B: MGMT/AGT* and *C: SATB1* the RNA levels from the microarray and protein levels from Western analysis are plotted for each of these cell lines. The significance of the correlations between the microarray and Western results was evaluated using *Pearson's* correlation coefficient.

expressed (p<0.005) between ALT[+] and telomerase[+] cell lines. Only DRG2 and

Figure 5.13: Presence of 5′-TTAA-3′ sites in GM847 telomeres . Genomic DNA from ALT[+] cell lines *GM847*, *KMST6*, *JFCF-6/T.1J/14-D*, *IIICF/c*, *WI38-VA13/2RA* and *SUSM-1* and telomerase[+] cell lines *JFCF-6/T.1J/6B*, *HeLa*, *HT1080* and *293* were digested with one of nine restriction enzymes and analysed by TRF analysis. The nine *restriction enzymes* are shown with the corresponding TRF *lanes* and the DNA *sequence* recognised by the enzyme. The TRF length distribution was measured by Telometric[341] and the *median* lengths shown below their respective lanes.

Lane	Restriction Enzyme	Sequence
1	Hinf I	GANTC
2	Rsa I	GTAC
3	Apa I	GGGCCC
4	Hpa I	GTTAAC
5	Stu I	AGGCCT
6	Alu I	AGCT
7	Mse I	TTAA
8	BstE II	GGTNACC
9	Hae III	GGCC
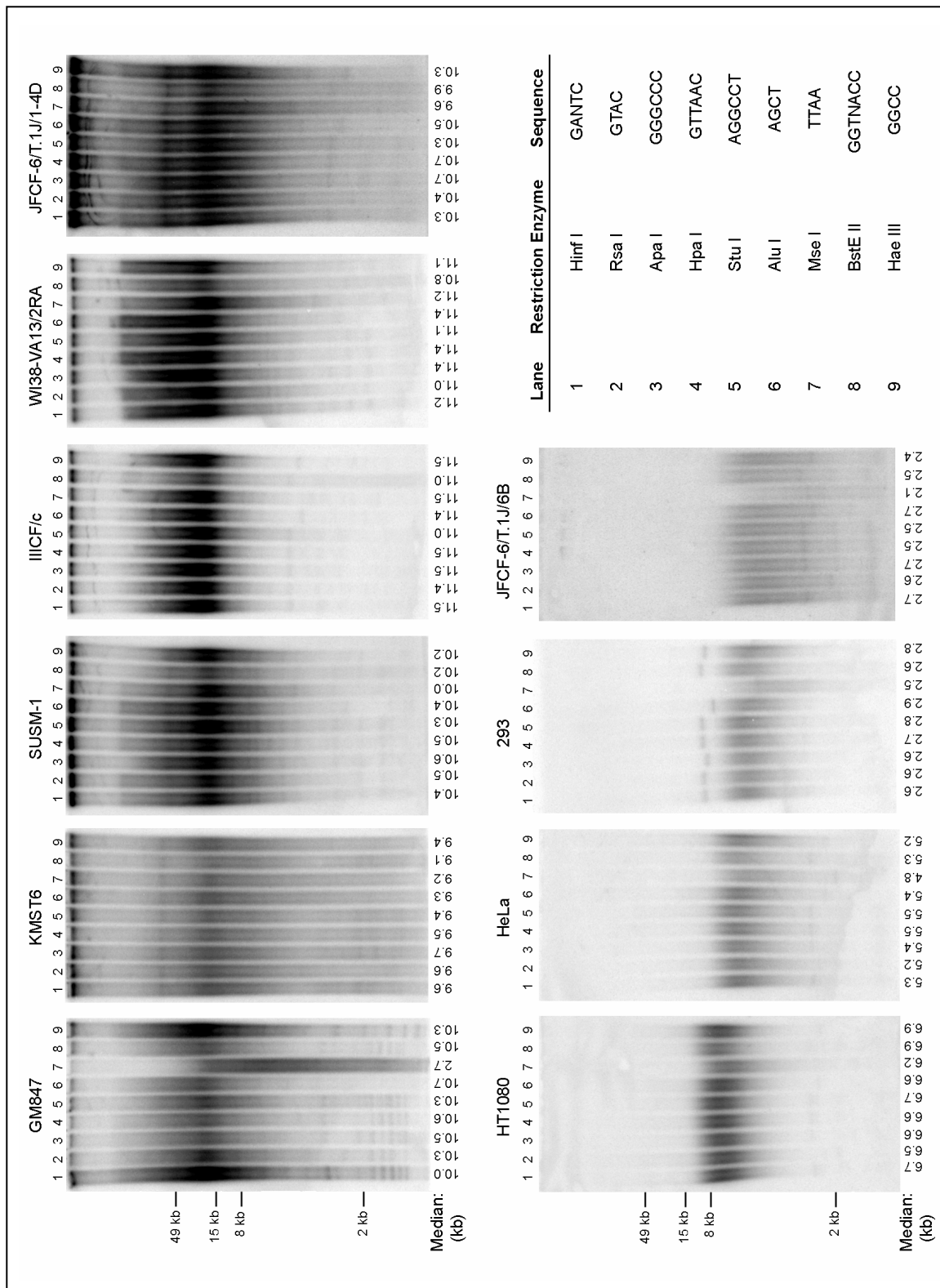
digestion of amount and pattern consistent with a restriction site in the distal subtelomeric region or possibly in the proximal telomere which is known to harbour variant repeats. This is not unexpected for a frequent restriction site and thus these cell lines show no evidence of mutated telomeric repeats. Further work is needed to determine if the dramatic shortening in GM847 suggestive of a TTAA restriction site in the telomere is due to TTAAGG repeats and if these repeats occur in other ALT and telomerase cell lines but were below the detection threshold of TRF analysis.

5.5   Summary of results (see chapter 6 for discussion)

By microarray analysis 240 genes were identified that were significantly differentially SFNX4 were significantly differentially expressed after adjusting for the estimated false positive rate. The genes, CEBPA, TACC2, SFXN4, HNRPK and MGMT that were down-regulated in ALT ($p < 0.005$), formed a group of co-regulated genes ($p < 5\times10^{-5}$) with the strongest correlation of expression between CEBPA and TACC2. Another group of co-regulated genes involved SIGIRR, LEF1, NSBP1 and SATB1 ($p < 5\times10^{-5}$) with strong negative correlation between SIGIRR and both NSBP1 and LEF1. The microarray data were consistent with MGMT expression being suppressed by EGF ($p < 0.05$). Two thirds of differentially expressed genes were down-regulated in ALT. Chromosomes 10 and 15 had a bias towards genes with lower expression in ALT while chromosomes 1, 4, 14 and X had a bias towards genes with higher expression levels in ALT.

Considering the known functions of the 240 differentially expressed genes, three genes, DRG2, MGMT and SATB1 were identified as most likely to be relevant to ALT[+] tumours and their differential expression was confirmed by Western analysis, although for SATB1 larger sample sizes are needed to confirm statistical significance. Investigating the levels of these proteins in the parental cell strains from which some

of the ALT[+] and telomerase[+] cell lines were derived, indicated that DRG2 and AGT (MGMT) protein levels were down-regulated after activation of ALT and up-regulated after activation of telomerase. SATB1 protein levels appeared to be up-regulated after immortalisation but to a higher degree with activation of ALT compared to telomerase. GM847 which did not express AGT had TTAA sequence in its telomeres that possibly could have resulted from mutations due to lack of AGT expression.

Chapter Six:

Discussion

Discussion

6.1   Development of the APB assay for ALT in tumours

This study has developed a robust assay for detecting ALT in archived tumour specimens that does not require extraction of high molecular weight genomic DNA for Southern analysis of TRF length. TRF Southern analysis has previously been used as the definitive test for ALT and detects the long and heterogeneous telomere length distribution that is a hallmark of ALT activity. For a homogeneous population of cells in culture, the presence of ALT is determined by demonstrating telomere maintenance (over many population doublings) in the absence of telomerase activity and also the presence of the characteristic ALT telomere length pattern. However, it cannot be assumed that every tumour contains cells that are immortal and therefore have a TMM[269], so a telomerase[-] tumour may not necessarily be ALT[+]. Conversely, it has been demonstrated that some tumours utilise both TMMs[73,270,271], so a telomerase[+] tumour cannot be assumed to be ALT[-]. Consequently, TRF Southern analysis alone (i.e., regardless of telomerase activity) has been used as the test for ALT in tumours.

The APB assay provides another method of testing for ALT in tumours. Although this study and other work has demonstrated that the presence of APBs is a hallmark of ALT, detection of APBs in tumours had not previously been compared to TRF Southern analysis and validated as an assay for ALT. Section 3.4 showed an excellent correlation between the results of these two assays. Both in a set of frozen STS specimens and also in a set of paraffin sections of grade II – IV astrocytomas there was an exact correspondence between the APB assay and the TRF Southern assay for

every tumour sample where the TRF Southern analysis yielded a result (total of 20 ALT[+] and 40 ALT[-] tumours).

The APB assay may be more appropriate than TRF analysis for detecting ALT in tumours, as the APB assay analyses individual cells in contrast to Southern analysis which uses genomic DNA extracted from a mixture of cells in the tumour. This may be the reason that the presence of ALT could be determined by APB status in six STS where the TRF pattern was equivocal (Fig 3.7). In addition to frozen and paraffin sections, the APB assay can be applied readily to fine needle aspiration biopsy samples.

For both the APB and TRF assays for ALT in tumours there will need to be a standard set of criteria for scoring ALT status. For the APB assay a stringent set of criteria was used to avoid false positive results. In particular, the only APBs that were counted were ones with an order of magnitude more telomeric signal than the telomeres on the same slide. Also to avoid false positives, samples with APBs an order of magnitude less frequent than usual for ALT[+] cell lines[83] were not scored as ALT[+] unless the APBs had a morphology that made them very unlikely to be an artefact. Since a very low frequency of APBs could reflect a low amount of ALT activity, whether tumours with a low frequency of APBs should be classified as ALT[+] may depend on the study design.

For the TRF assay a similar approach to that of Hakin-Smith et al.[270] was employed. The median telomere length of all tumours was plotted and a high, intermediate and low cluster was noted with only tumours in the high cluster being classified as ALT[+]. Since ALT[+] TRF length distributions are characteristically heterogeneous[4], it may be prudent to also include measures of variance and range in determining ALT

status. However, combining the three characteristics of median, variance and range to determine ALT status gave identical results to using the median alone. Thus the simplicity of just using the median may be preferable.

Evidence of intratumoral spatial heterogeneity for ALT was found in 2/10 (20%) osteosarcomas, which is similar to reported frequencies of intratumoral spatial heterogeneity for telomerase activity of 7-38%[271,323,325]. The lack of intratumoral spatial heterogeneity found for ALT in 15 osteosarcomas[271] or 13 GBM (this study) may be due to the sample sizes involved. Alternatively, the lack of heterogeneity for ALT in GBM may be due to ALT being an early event in the progression of lower grade astrocytomas (88% were ALT[+]) into secondary GBM. Loss of heterogeneity due to clonal expansion during progression to secondary GBM has been reported for p53 mutation[446,447]. The occurrence of intratumoral spatial heterogeneity for TMM may mean that several sites need to be tested for each tumour to reliably assess its TMM status. Where studies have not addressed this issue, the reported prevalence values for ALT in tumours may be underestimates.

6.2   Potential limitations of APBs as a test for ALT

This study and recent publications have uncovered some reasons to reassess whether APBs only occur in the presence of ALT activity and if ALT activity always gives rise to APBs. Although no telomerase[+] cell lines have > 0.5% of their nuclei APB[+], the fact that 3/31 (10%) telomerase[+] cell lines (Table 3.1) as well as 2-4% of sarcomas, astrocytomas and NSCLC have a low frequency of APBs, raises the concern that APBs could be occurring in tumours without ALT activity. However, known phenomena could provide an alternative explanation for a significantly lower frequency of APBs in these situations. *In vivo* intratumoral heterogeneity for ALT due to a mix of immortal clones or an emerging ALT[+] population would obviously be

associated with a low frequency of APB[+] nuclei. This is supported by the finding in collaboration with the Dr N Royle laboratory, that the subset of ALT[+] STS that lacked MS32 instability had the lowest frequency of APB[+] nuclei (Table 3.2; MS32 instability is a characteristic of ALT[+] cell lines[121]). Since 3/5 of these STS had MS32 mutation frequencies within approximately one tenth of the threshold level for MS32 instability[121], it is possible that the lower frequencies in some ALT[+] STS was due to intratumoral heterogeneity, especially if the heterogeneity was due to an emerging ALT[+] population that may have had insufficient PDs to generate high levels of MS32 instability in the ALT[+] population. Also, similarly low frequencies of APBs have been observed in a minority of clones of an ALT[+] cell line forced to express telomerase[227], suggesting that coexpression of telomerase activity with ALT in the same cells may sometimes partially mask ALT.

It remains to be determined if the three telomerase[+] cell lines with a low frequency of APBs have some ALT activity. All three of these cell lines could be considered likely to activate ALT. JFCF-6/hTERT/1 is derived from the same parental cell strain that has given rise to several ALT[+] cell lines. The other two APB[+]/telomerase[+] cell lines, SKOV-3 and MeT-5A are an ovarian adenocarcinoma and a SV40 transformed mesothelial cell line, respectively, and both ovarian epithelial cells and mesothelial cells are known to be transformable into ALT[+] cell lines. Further testing of these cell lines for other characteristics of ALT is required; however, at this stage there is no reason to believe that APBs can occur without ALT activity, especially considering the stringent criteria used to determine APB-positivity in the APB assay. Furthermore, none of the ten mortal cell strains or two telomerase[+] cell lines with long telomeres had any APBs.

Although this study and others[448] have not found evidence of ALT in tumours (by TRF analysis) without APBs, rarely in artificial situations it appears that the ALT mechanism can be varied such that APBs and the long heterogeneous TRF length hallmark of ALT are varied or lost. One of the two APB[-]/ALT[+] cell lines (see Section 3.2), AG11395, had SV40 sequence integrated into its telomeres[95,449], which is an unusual situation; SV40 sequence integrated into telomeres has not been reported in human cancers. Furthermore, AG11395 had a variant of APBs: bright telomeric foci colocalising with the recombination proteins usually found in APBs and adjacent to PML nuclear bodies instead of inside them. This situation was not seen in any of the 583 tumours tested with the APB assay. Thus this phenomenon is unlikely to occur often enough in human cancer to significantly affect the sensitivity of the APB assay for ALT. The second cell line that had APB[-] telomere maintenance without telomerase activity, C16-c3, was a clone from the WI38-VA13/2RA ALT[+] cell line that had been transformed with hTR and a catalytically inactive hTERT mutant[110]. Although other clones from the same transformation retained their ALT characteristics, this clone, despite continued lack of telomerase activity, had lost all its ALT characteristics except a high rate of postreplicative telomeric exchanges. It had short homogeneous telomeres by TRF and telomere FISH, no ECTR (linear or circular) and no APBs. Intertelomeric recombination and fluctuations in the ratio of p:q arm telomere lengths were not checked. This may represent an altered or different ALT mechanism and although it also occurred in a SV40 transformed cell line it may represent a possible cancer TMM. In both these cases the standard assay for ALT in tumours, TRF analysis, would have also given a negative result. Thus it is possible that a subset of cancers that test negative for telomerase and ALT do have a TMM.

It seems possible that APBs are associated with the production and/or amplification of ECTR. Hence an ALT mechanism that manages to suppress the formation and/or amplification of ECTR but maintain intertelomeric recombination-mediated replication could occur without APBs, possibly despite a very similar set of proteins being involved. Although it is not known exactly how APBs relate to the ALT mechanism, Section 3.2.2 showed that in an ALT[+] cell line in which the APBs were suppressed, ALT activity was also inhibited (this has been confirmed by loss of other hallmarks of ALT in Jiang *et al.*[2]). This combined with evidence that APBs contain the Mre11-RAD50-Nbs1 complex[132,133] and RAD51D[138], both of which are implicated in the ALT mechanism[2,138] and that ATM/ATR dependent DNA synthesis has been found to occur in APBs[131,132,140] support a functional link between APBs and ALT. Thus there is a strong basis to use APBs as a marker for ALT.

6.3 Using the APB assay to show the significance of ALT in tumours

Before the commencement of this study, ALT was generally assumed to be limited to rare sarcomas[450]. Chapter 4 demonstrated the utility of the APB assay by using it to investigate ALT in sarcomas, astrocytomas and various carcinomas including NSCLC, papillary carcinomas of the thyroid and colorectal carcinomas. The prevalence of ALT in STS had not previously been investigated in detail. Guilleret *et al.*[345] reported that 4/22 telomerase[-] STS were ALT[+] and a recent study[448] found 6/18 STS to be ALT[+]. In the current study the prevalence of ALT in STS was found to be 35/101 (35%), and ALT was found to be common among some types of STS: 77% of MFH, 62% of leiomyosarcomas and 33% of liposarcomas were ALT[+]. The prevalence of ALT in MFH was supported by the independent set of telomerase[-] STS in which 8/10 MFH were ALT[+]. ALT was significantly less common in other types of STS: only 9% of synovial sarcomas and 6% of rhabdomyosarcomas were ALT[+]. The APB assay also showed ALT to be common in osteosarcomas and grade

II-IV astrocytomas (47% and 34%, respectively), which is consistent with recent reports using TRF analysis to test for ALT[270,271].

This study is the first substantial study of ALT in carcinomas and the finding that 8% (22/291) of NSCLC were ALT[+] is the first evidence that ALT is a significant TMM for carcinomas. The higher prevalence of ALT in NSCLC compared to the prevalence of ALT in colorectal carcinomas of 0% (0/31), complements the reported prevalence of telomerase-negativity in these carcinomas of 27% and 11%, respectively (Table 1.3). In contrast, although over half of all papillary carcinomas of the thyroid are telomerase[-] (Table 1.3), none of the 17 papillary carcinomas were ALT[+]. This may reflect less need for a TMM in a cancer with a low cell turnover[451,452]. It will be of interest to determine the prevalence of ALT in other thyroid cancers, such as follicular and anaplastic carcinoma, that have a higher cell turnover but still have a low prevalence of telomerase activity (70% and 62% telomerase[-], respectively; Table 1.3)

Thyroid follicular cancer is an example of a cancer for which development of a marker capable of differentiating benign from malignant neoplasia would significantly help management. A common problem in management is distinguishing between follicular adenoma and follicular cancer with many patients undergoing unnecessary surgery and some patients undergoing inadequate surgery. There are a number of studies attempting to use the presence of telomerase activity to aid in this diagnosis[306,313,315,316], as well as to distinguish more aggressive thyroid tumours in general[308,310-312]. It is possible this correlation would be improved if ALT were also taken into account.

The reason for the differing proportions of ALT[+] tumours in various tumour types is unknown. It is possible that the prevalence of ALT may depend on the selection pressure for an alternative TMM and the presence of ALT repressors. In cancers originating from cell types where telomerase is repressed, there may be more selection pressure for ALT activation than in cancers originating from cell types where telomerase can be easily and quickly up-regulated. The higher frequency of ALT in cancers and cell lines of mesenchymal origin may be due to a tighter repression of telomerase in normal mesenchymal cells than in epithelial cells[4,453,454]. Similarly, the cells that give rise to astrocytomas may have tight repression of telomerase given that telomerase activity is undetectable in normal brain tissue[355,455-458] and normal human astrocyte cells[459,460]. However, different sarcoma subtypes have widely varying proportions of ALT[+] tumours. Somatic cell hybridisation studies have shown that ALT repressors exist[230] and it seems possible that some tissues have tighter repression of ALT than others.

Sarcoma subtypes can be classified into two groups: one group with near-diploid karyotypes with few chromosome rearrangements that often include a specific translocation, and another group with complex karyotypes which are characteristic of severe genetic and chromosomal instability[461]. A recent report suggests an association between ALT and complex karyotype STS[448]. My data are partly consistent with this, as I found ALT to be common in osteosarcomas, MFH, leiomyosarcomas and non-myxoid liposarcomas which typically have complex karyotypes, and rare in synovial sarcomas which typically have simple karyotypes[461]. However, embryonal rhabdomyosarcomas typically have complex karyotypes, but I found only 2/15 (13%) to be ALT[+]. It has been proposed that the ALT mechanism causes chromosome instability because of chromosome end-to-end fusion events resulting from the subset of telomeres within ALT^{+} cells that are very short[96]. If such a causal association

exists between ALT and complex karyotypes, then most or all ALT[+] tumours would have complex karyotypes, but because ALT activity is not the only source of chromosomal instability some types of tumours with complex karyotypes will be found to be ALT[-]. Thus my data are consistent with ALT being one cause of complex karyotypes.

The APB assay was also useful for investigating associations between ALT and clinical parameters. This study supported the view that the ALT TMM can support fully malignant sarcomas[462]. It was recently reported that ALT[+] osteosarcomas were just as clinically aggressive as telomerase[+] osteosarcomas in terms of clinical outcome, and were also able to metastasise[271]. My results for osteosarcomas were consistent with this, and I showed for the first time a similar pattern in STS. I found no difference between the survival rates of patients with ALT[+] STS and the ALT[-] group, and there was no reduction in the proportion of ALT[+] STS in the high grade group or in the group with local recurrence.

While a significant proportion of STS metastases were ALT[+] (9/27), the prevalence of ALT was lower in the metastases than in the primary tumours. A similar pattern was described in ALT[+], mTerc-/- mouse cell lines that were able to form extremely aggressive fast growing tumours in SCID mice, but had poor metastatic activity without the reconstitution of mTerc[74]. There is growing evidence that telomerase has cancer promoting properties independent of its role in telomere maintenance[74,463,464], so it is possible that cancers utilising ALT may take longer than telomerase[+] cancers to acquire properties such as the ability to metastasise. However, the data for human tumours presented here and elsewhere[271] indicate that ALT[+] tumours may nevertheless be highly malignant in contradiction to suggestions based on *in vitro* data, that ALT may not be able to support fully malignant cancers[465]. The apparent

paradox of ALT[+] STS being less likely or slower to metastasise but having an equally poor prognosis as ALT[-] STS, could be explained if other factors, such as ability to invade and resistance to chemotherapy are more important for patient survival.

The survival analyses underscore the relevance of investigating ALT in cancer. ALT was common in GBM, STS (including MFH) and osteosarcoma and in all these cancers the ALT[+] tumour subsets tested were found to have poor prognosis (Table 6.1). Thus ALT is an important cancer therapy target. Despite ALT[+] GBM only having a median survival of 2.3 years, this was three fold higher than the ALT[-] subgroup. This is consistent with a previous study that used the TRF assay to show ALT was a better independent predictor of survival in GBM than the currently used prognosticator, patient age[270]. Results here confirm that the APB assay can be used as a prognostic indicator in GBM.

Table 6.1: Prognosis of ALT[+] tumours

Cancer type	Percentage ALT[+]	Median survival in ALT[+] group (years)	Median survival in ALT[-] group (years)
GBM	24	2.3	0.8
STS	35	3.8	3.1
MFH	77	3.4	3.7
Osteosarcoma	47	5.2	5.8

This study has shown for the first time that the prevalence of ALT is significantly higher in grade II-III astrocytomas compared to GBM (grade IV astrocytomas). Grade II-III astrocytomas slowly progress to GBM over 5-10 years (these GBM are referred to as "secondary" in contrast to "primary" GBM which arise *de novo* in the absence of a pre-existing low grade lesion[444]). This suggests an association between ALT and secondary GBM, which is strengthened by the observation of Hakin-Smith

et al.[270] that ALT is associated with younger GBM patients. In my data set the mean age of ALT[+] GBM was 40 years compared with 55 years for ALT[-] GBM, possibly reflecting the mean ages of secondary and primary GBM patients of 39 and 55 years, respectively[368]. This is of interest because secondary GBM have genetic alterations (such as p53 and Rb mutations)[444] that are distinct from those of primary GBM and which conceivably may facilitate activation of the ALT mechanism. An association between ALT and p53 mutation has recently been confirmed in GBM tumours[466], however it still remains to be determined if p53 mutation facilitates the ALT mechanism.

This study also found that ALT is more common in younger patients with osteosarcoma which, unlike astrocytoma, appeared to be due to a marked lack of ALT in patients over 40 years of age. Ulaner *et al.*[271] did not observe a significant difference in the mean age at diagnosis of ALT[+] osteosarcomas, so this association needs to be tested in a larger tumour set.

6.4   The prevalence of ALT in tumour predisposition syndromes

The association between ALT and tumour predisposition syndromes was investigated in this study in an effort to elucidate proteins involved in the ALT mechanism or its regulation. This study found that MLH1, the protein most commonly deficient in MSI carcinomas[266] was present in APBs. MLH1 is known to interact with MRE11[367], a component of the MRE11-RAD50-NBS1 complex that is essential for ALT[2]. Given the potential that MLH1 may be functionally involved in the ALT mechanism, combined with reports of at least 92% of MSI colorectal cancers (and at least 43% of non-colorectal MSI cancers) having mutations in a MRE11-RAD50-NBS1 complex member[366], it is not surprising that this study and others[365] found no evidence of ALT in MSI cancers. However, as ALT has never been detected in these MSI cancer types

(colorectal cancer, endometrial cancer and transitional cell carcinoma) it will be necessary to determine the change in the prevalence of ALT with the presence of MSI in cancer types that commonly use ALT. MSI has been reported in MFH, leiomyosarcomas and osteosarcomas[467-471]. Involvement of MLH1 in ALT could also be investigated by knockdown experiments in ALT^{+} cells.

The finding that ALT occurs in tumours from patients with known mutations in WRN and RecQL4 indicate that these RecQ helicases are not required for ALT. This is consistent with recent evidence in mouse cells that WRN is not required for ALT and deficiency of WRN may even facilitate ALT in mouse cells lacking telomerase activity[472]. Given that there are three other human RecQ helicases that could be used in the ALT mechanism it is still possible that ALT is mechanistically similar to the *S. cerevisiae* analogue of ALT (that depends on the yeast RecQ helicase, *sgs1*[134,166]). It has been suggested that the RecQ helicase, BLM is involved in the ALT mechanism[136], and BLM deficiency is the cause of a cancer predisposition syndrome, Bloom syndrome[243]. However, if the prevalence of ALT is indeed reduced in patients with Bloom syndrome, it may be difficult to prove as the tumour spectrum of Bloom syndrome does not contain a high proportion of cancer types for which ALT is known to be common (Section 1.9).

Although only 1/14 LFS tumours were ALT[+], it is difficult to draw any conclusions about the influence p53 mutation has on the prevalence of ALT because very few of the tumours were of types in which ALT is known to be common. However, there was not as high a prevalence of ALT as was expected given that 2/2 previously tested LFS tumours and 70% of 37 LFS cell lines are ALT[+][68,73,90,92,260,261] (L Huschtscha and R Reddel, unpublished results). One possible reason could be that different types of p53 mutations have a different effect on ALT. 13/14 of the LFS tumours in this

study (including the ALT[+] tumour) had a G:C to A:T germline mutation in the 273 codon. The previously reported ALT[+] LFS tumours and the LFS cell lines with a high prevalence of ALT were either from one patient with a germline mutation in p53 codon 248, or from a family with a p53 truncating mutation (intron 4 inclusion). It may be interesting to test for a higher prevalence of ALT in sarcomas from LFS families with germline mutations in p53 codon 248, compared to those with codon 273 mutations. Although p53 with the G:C to A:T mutation in codon 273 has been shown to be compatible with ALT in cell lines[258], other p53 mutations such as mutation in codon 248 may predispose to ALT. However, recent work has failed to find any association between ALT and specific p53 mutations in GBM[466].

6.5   Single stranded C-rich DNA in ALT telomeres

It is interesting that ALT[+] cell lines had detectable single stranded C-rich and G-rich telomeric DNA, whereas telomerase and mortal cell lines only had single stranded G-rich telomeric DNA. Large amounts of single stranded C-rich DNA could be seen colocalising with similar amounts of single stranded G-rich DNA inside APBs. It is possible that the single stranded C-rich DNA could promote recombination mediated DNA synthesis or could simply be a by-product of the telomere metabolism in ALT^{+} cells[473].

The single stranded DNA nuclease, MBN, destroys the ALT telomeres electrophoresing above the 20-25kb marker proportionally to its effect on the telomeric C-rich single stranded DNA. Since creation of C-rich telomeric single stranded DNA in HT1080 decreased the mobility of the telomeres so that they ran run above the 20-25 kb marker, ALT^{+} cells may have fewer telomeres larger than 20 kb than previously thought.

A potential use of this finding may be to make the APB assay quicker and more appropriate for pathology labs. If an antibody could be raised against C-rich telomeric single stranded DNA it would allow single step immunodetection of APBs. It would also be interesting to determine if C-rich telomeric single stranded DNA is detectable in the blood of patients with ALT[+] tumours. Further characterisation of this C-rich telomeric single stranded DNA and possible binding proteins is warranted.

6.6   ALT associated gene expression

The reason that ALT predicts better survival in GBM but same or worse survival in sarcomas[270,271,474] (Section 4.5 – 4.7) may be due to putative gene expression patterns that favour ALT activation. Different cancer types have characteristic gene expression patterns arising from their tissue origin and characteristic genetic modifications during carcinogenesis. It is possible that these different expression patterns could independently influence ALT activation and survival. Thus the gene expression pattern in GBM that results in activation of ALT may independently favour increased survival while the gene expression pattern in sarcomas that activates ALT may independently decrease or have no effect on survival. A potential example of this is the ALT associated loss of MGMT expression found in this study. Depending on genetic background, loss of MGMT expression could worsen prognosis due to increased oncogenic mutations or improve prognosis due to increased sensitivity to chemotherapy (Section 5.1.2). The association between ALT-positivity and secondary GBMs, which have been shown to have a distinct set of genetic alterations, further suggests that there exist activators or repressors of ALT and/or an ALT associated gene expression pattern. Identifying genes involved in ALT may allow the design of strategies to target ALT^{+} cancer cells. Identifying ALT associated gene expression changes may also identify which treatment strategies may be more effective in the ALT[+] subpopulation within specific tumour types.

This study identified genes that were differentially expressed between ALT[+] and telomerase[+] cell lines using RNA microarray analysis. The expression levels of two genes, DRG2 and SFXN4, were found to be significantly lower in the ALT[+] cell lines after taking into account the expected rate of false positives from the 19469 gene elements analysed. The 240 most significantly differently expressed genes were also considered, although 94 (39%) of these are expected to be false positives. Generally ALT[+] cell lines appeared to have a more repressive transcriptional environment than the telomerase[+] cell lines with two thirds of the differentially expressed genes being down-regulated in the ALT[+] cell lines. Two chromosomes in particular, 10 and 15 had a high proportion of genes with decreased expression in the ALT[+] cell lines. Chromosome 7, which has been suggested to harbour ALT inhibitors[87,233], appeared transcriptionally less active in ALT[+] cell lines, but the proportion of genes with lower levels of expression in ALT was not significantly different to the overall proportions.

The quality of the microarray results was assessed by comparing the microarray transcription levels with Western of analysis protein levels for three differentially expressed genes, DRG2, MGMT and SATB1. Despite the potential presence of factors other than mRNA levels affecting protein levels, there was a significant correlation between mRNA and protein levels on a cell line by cell line basis for all three genes. The strongest correlation was observed for MGMT which had the largest range of both RNA and protein levels.

One potential use of the microarray data is the identification of genes that will allow treatment of ALT[+] cancers either by inhibiting the ALT mechanism directly or by targeting common weaknesses in ALT[+] cancers. In order to increase the chance of

avoiding false positives and for the sake of practicality this study focused on known genes that have functions that are potentially relevant to telomere maintenance (Table 5.2). Three genes were chosen for initial further study, DRG2, MGMT and SATB1. The lower expression of DRG2 and MGMT in ALT was confirmed by comparing protein levels in 15 ALT[+] cell lines to 15 matched telomerase[+] cell lines. By also comparing the protein levels between the ALT[+] cell lines and the mortal cell lines they were derived from, it seemed that DRG2 and MGMT were repressed in conjunction with the activation of ALT. SATB1 levels were significantly increased in conjunction with activation of a telomere maintenance mechanism. The microarray results showing higher levels of SATB1 in ALT[+] than telomerase[+] cell lines were supported by the Western analysis of matched and related cell lines, but larger sample sizes than used in this study are necessary to confirm this with statistical significance (for SATB1 only six ALT[+] cell lines were compared to six telomerase[+] cell lines).

The potential role of DRG2 in cell proliferation and cell cycle progression (Section 5.1.1) is consistent with DRG2 being involved in telomere maintenance, however the main reason for interest in DRG2 was its marked differential expression. DRG2 was the most significantly differentially transcribed gene identified and even after allowing for false positives (Bonferroni method) there was only a 0.2% chance of no difference in the level of transcription between ALT[+] and telomerase[+] cell lines. DRG2 RNA levels have previously been reported to be low in the WI38-VA13/2RA cell line[369] which is ALT[+] and moderate in the osteosarcoma cell line MG-63[371] which is telomerase[+], in keeping with our results.

The association between ALT and MGMT in relation to GBM treatment is intriguing. Lower levels of AGT in ALT[+] cell lines were confirmed by western analysis with 7/15 ALT[+] cell lines having complete loss of AGT (none of the 16 telomerase[+] or

mortal cell lines had undetectable AGT levels). Both ALT-positivity and AGT loss (Sections 5.1.2 and 6.3) have been associated as early events in the progression to secondary GBM and both have been identified with better survival in GBM. This could be further investigated by correlating ALT status and MGMT status in GBM directly (preferably secondary GBM as this is probably a different disease entity to primary) and comparing them as predictors of survival and temozolomide response.

ALT and the APB assay may be a determinant that is complementary to, or better than AGT loss, for treatment stratification. Clearly AGT loss is not the only determinant for response to temozolomide (Section 5.1.2). If AGT loss is required for ALT, then in ALT[+] GBM there may be selection pressure against the re-expression of AGT. Alternatively other genetic characteristics of ALT^{+} cells such as a putative need for MLH1 (see Section 6.4) may increase alkylating agent sensitivity. The increased median survival found for ALT[+] GBM here was 3-fold, compared with only 1.5-fold for AGT[-] GBM[409]. Even if the association between ALT and increased survival in GBM is dependent on AGT status, it may help identify responsive tumours for appropriate chemotherapy regimens. Currently MGMT status is determined by PCR for promoter methylation or by MGMT immunostaining, both of which may have inaccuracies arising from contamination with normal cells and lymphocytes. Furthermore, PCR analysis of promoter methylation would miss other causes of AGT activity loss, and immunostaining for MGMT may not pick up low levels of MGMT expression that could be induced during chemotherapy. The APB assay could complement these techniques. ALT status may also indicate subgroups of other cancers for which methylating agents may useful. For example, it has been shown that temozolomide has some activity against previously treated STS[475] and any identification of responsive subgroups would be of benefit.

It is theoretically possible that AGT loss may be involved in the ALT mechanism. One possibility is that if telomeric chromatin is altered by O^6-G methylating agents, then AGT loss may allow O^6-MG lesions to persist. If MMR remains intact then it could remove segments of the C-strand, causing single strand gaps and double strand breaks in the telomeric DNA. The latter could lead to ECTR, as well as increased HR that could elongate the telomere as previously proposed[3] and enhance post replicative telomere exchanges as has been observed in ALT[117-119]. The single strand gaps as illustrated in Fig 6.1 could also promote HR between telomeres allowing elongation or HR from the proximal telomeric repeats leading to telomeric deletions and telomeric circular DNA as have been seen in ALT[81,108,109](Fig 6.1). Whether this could occur frequently enough is difficult to assess as the frequency of endogenous O^6-MG has not been determined. It would also not explain the specificity for increased recombination at the telomere, although only repetitive DNA may be able to undergo HR before S-phase and telomeres have been reported to be predisposed to alkylating damage[202]. Another possibility is that AGT normally represses ALT as part of its putative role in regulating DNA repair and replication (Section 5.1.2).

Figure 6.1: Loss of AGT could give rise to ALT characteristics. Persistence of an O^6-*MG* lesion in the telomere with the presence of functional MMR would be expected to give rise to repeated cycles of degradation of the opposite C-strand and resynthesis (Section 5.1.2). In this figure the G-rich telomeric strand is *black* and its *3′* terminus is indicated, the O^6-*MG* lesion is indicated by a *square*, the C-rich telomeric strand is *grey* and a mismatched T (opposite the O^6-*MG* lesion) is represented by a *triangle*. The repeated presence of single strand gaps (opposite O^6-*MG*) could give rise to the various hallmarks of ALT. *A:* Replication through a single strand gap could give rise to a telomeric double strand break (*DSB*) and thus a shortened telomere and linear ECTR. *B:* Elongation could occur by HR. For example, this figure illustrates a single strand gap in a short telomere initiating repair by HR, with strand invasion from a longer telomere (by creation of a 3′ end inside the C-strand of the longer telomere). If a Holliday junction is then formed and resolved with crossover, two long telomeres would result without any DNA synthesis but one with a long 3′ overhang and the other with a long 5′ overhang. Fill in repair could result in two long telomeres with a normal and a blunt end. *C:* Telomeric shortening with circular ECTR could occur if the single strand gap initiates HR repair from its own strand (on its centromeric side). As for *B*, Holliday junction formation and resolution with crossover would give rise to a short telomere and a circular ECTR.

The fact that not all ALT lines were devoid of MGMT could have a number of explanations. Firstly, MGMT silencing may not be directly related to ALT but part of a wider gene silencing required for ALT and is consistent with the general down-regulation of genes found in this study. Even so, the mechanistics of the gene silencing may still be of interest in understanding the epigenetics of ALT activation.

Secondly, ALT may have a number of mechanisms, with only one requiring loss of AGT. Thirdly, the function of AGT that suppresses ALT may not be related to its repair function and could be lost via mutation or loss of another protein. Fourthly it is possible that the AGT which is present in some ALT[+] cell lines is inactive due to mutation and/or mislocalisation. Immunostaining could test if AGT in these cell lines is not localised properly to the nucleus.

Although SATB1 is a transcriptional regulator, its expression was only found to be correlated with that of NSBP1 ($p < 5x10^{-5}$). The actual levels of SATB1 mRNA may not be as important as its expression above a threshold level. Alternatively, SATB1 could also have a more direct effect on telomeric DNA. A SATB1 binding site has been found at a telomere flank in mice[411] and telomeric DNA is a matrix attachment region (MAR)[425]. The telomeric T-loop is a candidate for a chromosomal torsional loop domain. The D-loop region of the T-loop is often near the start of the telomeric repeats[32] and its single stranded nature, together with that of the terminal overhang, could make good candidates for nuclear matrix tethering. Since SATB1 coordinates chromatin modification, if it bound the MAR of the telomere loop, the effect of aberrantly increased SATB1 expression in ALT^{+} cells could be to make the telomeres more open. In the absence of MGMT, it may be possible for a few G:C to A:T transition mutations in the telomere to change the telomeric sequence into a site more conducive to SATB1 binding and being recognised as a BUR in general. The potential of telomeric DNA to form G-quartets and I-motifs on the G- and C-strand, respectively[476], could stabilise any unpairing. A BUR at a telomere flank[411] could also become involved through recombination and/or telomere erosion. BURs are known to be conducive to recombination[420,422,423] and unscheduled DNA synthesis[477]. The association between secondary GBM and both p53 mutation[404,405] and ALT (this study) may be relevant since certain mutant p53s and not wild type p53 bind MAR[478].

EGF should not be overlooked as a possible upstream inhibitor of MGMT[432]. EGF showed negative correlation with MGMT expression ($p < 0.05$). Also, BFT-3K, the only telomerase[+] cell line that had a lower level of AGT than its parental cell strain (Fig 5.12) had an abnormally high level of EGF for a telomerase[+] cell line (Fig 5.1). If EGF is a repressor of MGMT, then caution would need to be taken with recent trials of combining EGFR inhibitors with temozolomide to treat GBM[479-481]. In ALT[+] GBM, the EGFR inhibitors could relax MGMT suppression decreasing sensitivity to temozolomide.

Other MAPK pathway members, LEF1 and SIGIRR appeared to be part of a co-regulated group including NSBP1 and SATB1 ($p < 5 \times 10^{-5}$). Another co-regulated group was centred around a strong pair-wise correlation between gene expressions of CEBPA and TACC2. CEBPA may need to be down-regulated in ALT to avoid DNA damage-induced growth arrest for which CEBPA is critical in at least some contexts[442]. Interestingly, chromatin remodelling in which TACC2 is involved, may be required for the ability of CEBPA to impose a G1 arrest[438,439,442]. SFNX4 and MGMT expression may be part of this co-regulated group due to their proximity to TACC2 on chromosome 10q26.

6.7   Future directions

The primary aim of this study was to establish and validate a method for detecting for ALT in routinely archived paraffin-embedded tumours to facilitate the investigation of ALT in cancer. It is expected that the APB assay can be used to further our knowledge of the prevalence of ALT in cancer and its association with patient prognosis and tumour behaviour such as metastasis. It would be of interest to investigate further the association of ALT with survival in the various STS subtypes,

osteosarcoma and NSCLC. Follicular cancer of the thyroid is another tumour type in which investigation of ALT may prove beneficial given the need for differentiating between benign and potentially malignant thyroid follicular neoplasias and the low prevalence of telomerase in these tumours. There is a wide variety of cancers for which the existence of ALT activity has not yet been determined.

The association between ALT and secondary GBM is of potential interest for understanding both ALT and GBM. It is necessary to directly prove an association between ALT and secondary GBM, and whether ALT is an early step in the development of secondary GBM from lower grade astrocytomas. In other tumour types ALT may also associate with distinct subtypes in terms of genetic alterations. In this context it may be worthwhile to confirm an association between ALT and patient age in osteosarcoma.

There is currently no clinically practical inhibitor of ALT and as argued here this would be of potential benefit. Further investigation of a functional link between MGMT, DRG2 and SATB1 and ALT by overexpression or knockdown studies may contribute to this. Testing ALT[+] cell lines for sensitivity to alkylating agents, especially temozolomide, would determine the utility of the observed association between AGT loss and ALT. If lack of AGT does cause ALT^{+} cells to be sensitive to alkylating agents then given the current interest in EGFR inhibitors as chemotherapy agents, it will be important to understand the mechanism of the apparent EGF inhibition of MGMT expression and if EGFR inhibitors reduce the sensitivity of ALT[+] cancers to alkylating agents.

Further investigation of the association between the tumour predisposition syndromes and ALT may lead to a better understanding of the ALT mechanism and its

regulation. One example would be to investigate the association between ALT and the different germline p53 mutations (i.e. codon 273 and 248 mutations). There is also good reason to determine if BLM and the MMR system (in particular MLH1) are necessary for ALT, or if deficiency of MMR promotes ALT. For all these investigations, the difficulty will be identifying sufficient numbers of samples of tumours that have a high prevalence of ALT, e.g., MFH, leiomyosarcomas and osteosarcomas, in these relatively rare cancer predisposition syndromes.

Since telomerase inhibitors may be used alone as cancer therapeutics, it may be important to determine if the three telomerase[+] cell lines found in this study to have a low frequency of APB[+] nuclei, have a homogeneous low level of ALT activity or a discrete APB[+] subpopulation. One of these cell lines, SKOV-3, has been found to be resistant to a telomerase inhibitor[482].

Finally, further characterisation of the existence of the single stranded C-rich telomeric repeats in APBs may provide for the development of a more rapid APB assay that may be more appropriate for routine use in a clinical pathology laboratory. Development of an antibody against single stranded C-rich telomeric repeats may allow such an assay.

6.8   Conclusion

This study has developed APB analysis as a robust assay for detecting ALT in archived tumour specimens. Whenever ALT was clearly present or absent according to TRF Southern analysis the APB assay gave a concordant result and the APB assay appeared to be superior to TRF Southern analysis when there was intratumoral

heterogeneity. This study found the APB assay very useful for testing archives of paraffin-embedded specimens as well as assessing prognosis for GBM.

ALT is an important target for anticancer treatments. This study has shown ALT to be common in a range of tumour types and unfortunately all ALT[+] tumour subsets tested were found to have poor prognosis (Table 6.1). Although inhibitors for telomerase are entering clinical trials[483], there is no known inhibitor of ALT that could be used clinically. Yet *in vitro*, inhibition of ALT can lead to rapid senescence and cell death[87,230]. Therefore it is important to identify proteins that may facilitate the design of a clinically practical method of inhibiting ALT in ALT[+] cancers and thus killing cancer cells with minimal side effects. This study has started to address this by identifying genes expressed differently between ALT[+] and telomerase[+] cell lines.

Appendix

Appendix

A. Differential gene expression between ALT[+] and telomerase[+] cell lines.

Gene expression levels for 12 ALT[+] and 12 telomerase[+] cell lines were determined by RNA microarray analysis and 19469 gene elements had levels three-fold higher than background noise in at least half of the ALT[+] or at least half of the telomerase[+] cell lines. Of these gene elements, the 250 genes found to be differentially expressed (Mann-Whitney test, p ≤ 0.005) between the ALT[+] and telomerase[+] cell lines are summarised here with a brief description[1], the *fold change* of the *median* gene expression level in the ALT[+] cell lines compared to the *median* gene expression level in the telomerase[+] cell lines and the significance of the differential expression (Mann-Whitney test *p-value*). *N/A* indicates this gene has not yet been named. Descriptions are left blank were gene ontology descriptions were not available[1]. Some cell lines have been abbreviated: *1.3C* denotes JFCF-6/T.1J/1-3C, *1R* denotes JFCF-6/T.1R, *V2* denotes MRC5-V2, *2RA* denotes WI38-VA13/2RA, *AT1* denotes AT1BR44neo, *6G* denotes JFCF-6/T.1J/6G, *1P* denotes JFCF-6/T.1P, *V1* denotes MRC5-V1, *AT22* denotes AT22IJE-T, *6TGR* denotes MeT-5A/6TGR-B and *F80* denotes F80-TERT/K1-1.

Appendix A: Expression levels and descriptions of differentially expressed genes

Index of genes

Gene name[a]	Rank	Gene name[a]	Rank	Gene name[a]	Rank	Gene name[a]	Rank	Gene name[a]	Rank
ABCB4	153	FLJ14803	77	hCG2041462	191	LOC149134	213	SATB1	50
ABHD2	250	FLJ20032	54	hCG2042185	17	LOC284014	221	SCAMP2	177
ACVR2	91	FLJ21159	158	hCG2042587	212	LOC284475	182	SCARB2	118
ADP-GK	64	FLJ23033	119	hCG22106.2	184	LOC338645	126	SERP1	95
ADP-GK	125	FLJ30046	129	hCG23704.1	30	LOC339803	141	SFXN4	2
ALDH6A1	157	FLJ32685	156	hCG24202.3	114	LOC375449	227	SH3MD2	146
AMOTL2	228	FLJ36674	48	hCG26523.2	185	LOC51234	181	SHOC2	233
ANXA2	226	FLJ38993	55	hCG27427.2	98	LOC51270	124	SIGIRR	206
AP1S3	117	FLJ40629	94	hCG38053.3	236	LRP5	169	SLC15A4	229
APBB2	232	FLJ43663	192	hCG38403.2	78	LRRCC1	209	SLC16A2	89
ARHGAP5	172	FLJ46041	36	hCT12692.2	162	MAGED4	80	SLC19A1	81
ARHGEF10	147	fs03c07.y1	15	hCT1643271.1	190	MBNL1	6	SLC39A4	151
BCAS3	243	GAL	46	hCT1648454.1	188	MGC:13379	7	SLCO4A1	38
BCL2A1	193	GBP1	145	hCT1784913.1	18	MGC10911	62	SPANXD	41
BEXL1	179	GPC5	189	hCT1833869	8	MGC15416	34	SPANXD	132
BZW1	210	GPR114	69	hCT1844408.1	110	MGC22773	101	SRPUL	71
C10orf11	150	GTPBP4	176	hCT18705.1	115	MGC42174	224	SRPX	88
C21orf6	65	GYG	127	hCT8651.1	70	MGC50722	222	SSB	67
C2orf13	104	GYG	171	HECTD3	235	MGEA5	120	ST7	31
C2orf13	136	hCG1641068.3	155	HNRPK	43	MGMT	10	STMN3	167
C3orf10	40	hCG1641550.3	144	HT011	215	MPZL1	246	SYT11	195
C5orf13	175	hCG1643350.2	194	HTPAP	45	MYO10	109	TACC2	112
CA3	216	hCG1644183.3	214	HYPK; SERF2	20	NANOS1	42	TAF6L	53
CCL20	14	hCG1646764.3	68	IDH3A	4	NDUFAF1	230	TCFL4	180
CCNDBP1	248	hCG1654216.1	207	IDH3G	37	NDUFB3	26	TGFB2	237
CCNG2	73	hCG1659708.3	27	IDS	217	NEDD5	245	THADA	113
CDC14A	204	hCG1731660.2	66	IFITM2	58	NME4	19	THSD1	134
CEBPA	203	hCG1733583.1	199	IFITM3	21	NME4	47	TLOC1	22
CHFR	24	hCG1742145.1	187	IFITM3	49	NSBP1	51	TM4SF10	79
CHKL	208	hCG17581.2	201	IGLJ3	143	NSE1	130	TMSNB	3
CLK3	111	hCG1770437.2	183	IL27RA	86	OPRS1	241	TPM3	240
CLN8	244	hCG1774546.2	99	IMPDH1	97	PCSK5	242	TRAM1	164
COPS7B	142	hCG1776624.1	225	INM01	137	PCYT1A	198	TRAM1L1	223
CREB3L1	148	hCG1783311.2	32	IRAK1BP1	12	PGAM1	83	TRIP6	93
CRIP2	57	hCG1793602.1	166	ITIH3	160	PGAM1	140	TTC11	161
CSE-C	249	hCG1814693.1	87	JAM2	23	PIGB	29	USP4	174
CYP2S1	106	hCG1818302.1	103	KAL1	138	PIGB	56	USP51	76
DKFZP564I122	35	hCG1820368.1	159	KCNN4	168	PIGK	28	VPS13D	107
DRG2	1	hCG1820694.1	202	KCTD8	85	PKE	133	WFDC3	205
EBRP	123	hCG1820813.1	173	KIAA0261	33	POLR3K	116	YWHAG	96
EGF	13	hCG1986460	186	KIAA0690	239	PPP1R2	149	YWHAZ	152
EPS8L3	82	hCG1991368.1	72	KIAA1229	74	PPP1R2	154	ZDHHC15	128
FAM18B	122	hCG2005887	11	KIAA1458	247	PPRC1	165	ZFP36L1	238
FAM35A	219	hCG2009963	105	KPNA4	197	PRKAR1B	39	ZMYND19	170
FBXO33	25	hCG2015269	84	LEF1	108	PTK7	75	ZNF215	121
FHOD3	63	hCG2026048	220	LGALS9	44	PTPN9	59	ZNF44	52
FLJ10357	92	hCG2033356	90	LOC122618	9	PTPRG	60	ZNF462	178
FLJ10377	200	hCG2040332	5	LOC134147	163	PTX3	231	ZNF513	139
FLJ13984	16	hCG2040549	218	LOC134218	131	REPS2	100	ZNF584	234
FLJ13984	61	hCG2040606	211	LOC136306	135	RYBP	196	ZNF586	102

Genes with more than one entry are due to different probes used for the same gene [a]For un-named genes the Celera genomics® gene identifiers (hCG) are used.
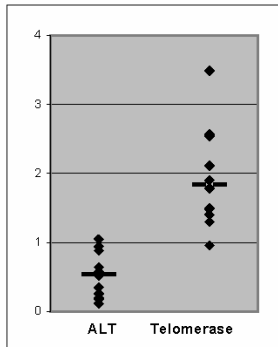
1:   DRG2: developmentally regulated GTP binding protein 2

P-value: 1.1E-07   **Fold change: 0.3**

Chromosome: 17p11.2

Genbank: NM_001388.3   Gene ID: hCG1986077

Biological process: cell growth and/or maintenance; signal transduction

Molecular function: GTP binding

Cellular component:

Expression levels in ALT cell lines   **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.52	0.57	0.64	0.26	0.35	0.19	1.04	0.11	0.88	0.94	0.56	0.17

Expression levels in Telomerase cell lines   **median: 1.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.48	2.12	2.54	2.57	1.50	1.78	3.49	2.11	0.96	1.30	1.90	1.40
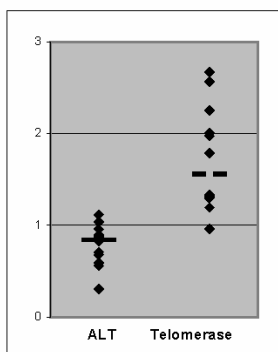
2:   SFXN4: sideroflexin 4

P-value: 1.0E-06   **Fold change: 0.5**

Chromosome: 10q26.13

Genbank: NM_178867.3   Gene ID: hCG37954.2

Biological process: cation transport

Molecular function: cation transporter activity

Cellular component: membrane

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.04	0.89	0.59	0.86	0.56	0.71	0.31	0.68	1.12	0.88	0.83	0.96

Expression levels in Telomerase cell lines   **median: 1.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.19	1.33	1.30	1.78	1.97	0.96	1.31	2.67	2.00	2.56	2.25	0.96
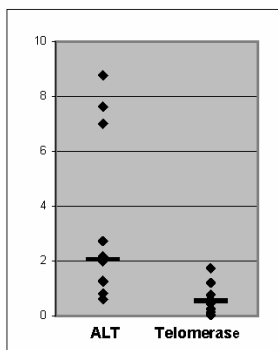
3:   TMSNB: thymosin, beta, identified in neuroblastoma cells

P-value: 1.8E-05   **Fold change: 3.8**

Chromosome: Xq21.33-q22.3

Genbank: NM_021992.1   Gene ID: hCG1748783.2

Biological process: biological_process unknown; cytoskeleton organization and biogenesis

Molecular function: actin binding; molecular_function unknown

Cellular component: cytoskeleton

Expression levels in ALT cell lines   **median: 2.0**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.80	0.60	1.27	1.24	7.64	2.75	2.16	7.01	8.78	1.25	2.10	1.98

Expression levels in Telomerase cell lines   **median: 0.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.20	0.51	0.76	0.24	0.45	0.76	0.03	1.72	0.46	0.13	0.58	0.57
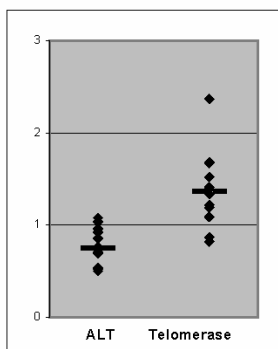
4:   IDH3A:

P-value: 1.8E-05   **Fold change: 0.5**

Chromosome: 15q25.1-q25.2

Genbank: BE546690   Gene ID: hCG2039407.1

Biological process: carbohydrate metabolism;metabolism; tricarboxylic acid cycle

Molecular function: isocitrate dehydrogenase (NAD+) and oxidoreductase activity

Cellular component: mitochondrion

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.53	0.74	1.04	0.71	0.76	0.92	0.85	1.08	0.70	0.96	0.50	0.53

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.52	1.68	1.09	1.19	1.34	1.40	0.87	2.37	1.67	1.41	1.22	0.82
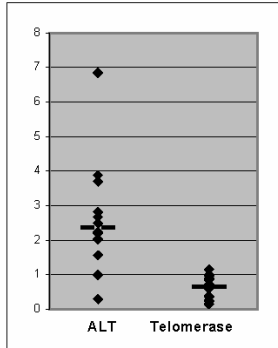
5:   N/A

P-value:	3.5E-05								Fold change:	3.6	

Chromosome:

Genbank: Gene ID: hCG2040332

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 2.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.30	3.88	2.24	2.67	2.03	2.82	2.21	2.49	3.70	1.00	1.57	6.85

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.72	0.24	0.15	1.15	1.00	0.90	0.60	0.97	0.85	0.25	0.36	0.39
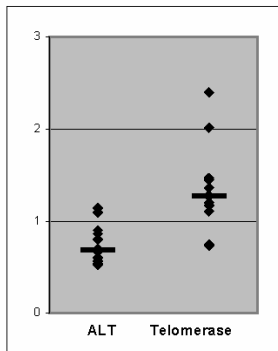
6:   MBNL1: muscleblind-like (Drosophila)

P-value:	4.4E-05					Fold change:	0.5	

Chromosome: 3q25   Genbank: NM_021038.2   Gene ID: hCG28028.3

Biological process: muscle development;myoblast differentiation;embryonic limb morphogenesis;neurogenesis;embryonic development (sensu Mammalia)

Molecular function: double-stranded RNA binding;nucleic acid binding

Cellular component: nucleus;cytoplasm

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.87	1.10	0.54	0.68	0.90	0.52	0.57	0.67	0.81	1.14	0.60	0.70

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.45	2.02	1.11	1.36	1.47	0.75	1.28	1.20	2.40	0.74	1.17	1.27
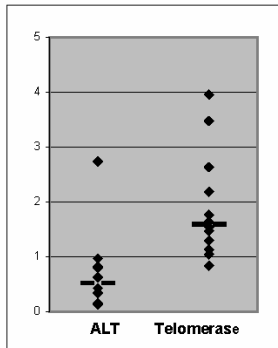
7:   MGC:13379: HSPC244

P-value:	4.4E-05					Fold change:	0.3	

Chromosome: 11q13.1

Genbank: NM_016499.2   Gene ID: hCG40864.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.96	0.42	0.33	0.63	0.33	0.34	0.80	0.14	0.12	2.75	0.81	0.61

Expression levels in Telomerase cell lines   **median: 1.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.64	2.19	1.13	1.78	3.96	1.48	0.83	3.48	1.65	1.55	1.30	1.04

8:   N/A

P-value:	4.4E-05								Fold change:	1.5	

Chromosome:

Genbank: Gene ID: R26_hCT1833869

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 1.1**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	1.12	1.17	1.39	1.23	1.05	1.12	1.65	0.98	1.06	1.31	0.81

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.07	1.01	0.62	0.60	0.91	0.94	0.85	0.71	0.99	0.47	0.39	0.55

9: LOC122618: hypothetical protein BC015003

P-value: 6.1E-05   **Fold change:** 2.2

Chromosome: 14q32.33

Genbank: NM_138790.1   Gene ID: hCG40818.2

Biological process: metabolism

Molecular function: catalytic activity

Cellular component:

Expression levels in ALT cell lines   median: 1.1

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.18	1.73	0.65	1.03	1.85	0.70	1.18	1.10	1.20	2.03	0.83	0.70

Expression levels in Telomerase cell lines   median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.70	0.45	0.25	0.53	0.25	0.93	0.85	0.53	0.53	0.70	0.35	0.25
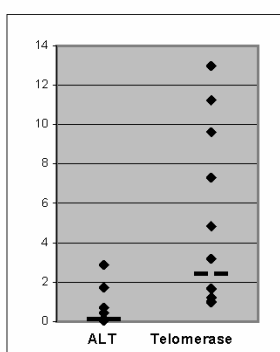
10: MGMT: O-6-methylguanine-DNA methyltransferase

P-value: 6.7E-05   **Fold change:** 0.1

Chromosome: 10q26   Genbank: NM_002412.1   Gene ID: hCG39601.4

Biological process: DNA ligation

Molecular function: DNA-methyltransferase activity; transferase activity; DNA binding; methylated-DNA-[protein]-cysteine S-methyltransferase activity

Cellular component: nucleus

Expression levels in ALT cell lines   median: 0.1

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.72	0.03	0.07	0.11	0.11	0.08	0.15	0.43	2.87	0.15	0.05	0.70

Expression levels in Telomerase cell lines   median: 2.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
9.60	12.96	7.29	0.97	1.21	0.99	11.23	1.01	4.83	3.18	1.68	1.65
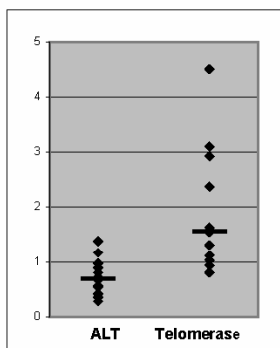
11: : CDNA FLJ13334 fis, clone OVARC1001846

P-value: 8.2E-05   **Fold change:** 0.4

Chromosome: 2

Genbank: AK023396   Gene ID: hCG2005887

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.35	0.42	0.67	0.72	0.80	0.56	0.54	0.97	1.17	1.38	0.28	0.90

Expression levels in Telomerase cell lines   median: 1.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.31	1.55	1.58	1.63	0.80	1.11	1.03	4.51	3.10	2.93	2.38	0.93

12: IRAK1BP1: interleukin-1 receptor-associated kinase 1 binding protein 1

P-value: 1.0E-04   **Fold change:** 1.7

Chromosome: 6q14-q15

Genbank: AK098678   Gene ID: hCG32735.2

Biological process: I-kappaB kinase/NF-kappa B cascade

Molecular function: protein binding

Cellular component:

Expression levels in ALT cell lines   median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.84	2.27	1.80	1.74	1.12	1.36	1.25	1.08	1.26	1.03	0.94	1.19

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.85	0.97	0.36	0.44	0.41	0.97	0.59	2.12	0.78	0.57	0.74	0.72
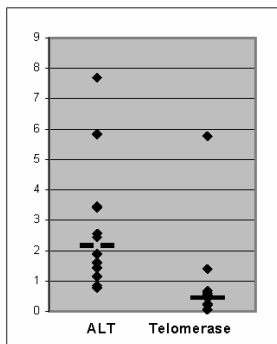
13: EGF: epidermal growth factor (beta-urogastrone)

P-value: **1.0E-04**   **Fold change: 4.8**

Chromosome: 4q25   Genbank: NM_001963.2   Gene ID: hCG19911.2

Biological process: pos. regulatn of cell proliferatn;EGFR signaling pathway;chromosome organization and biogenesis (sensu Eukarya);activatn of MAPK;DNA replication

Molecular function: EGFR activating ligand activity;growth factor activity;calcium ion binding

Cellular component: integral to membrane;extracellular;nucleus;plasma membrane

Expression levels in ALT cell lines   **median: 2.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.42	2.56	0.78	5.83	1.43	1.15	1.89	3.45	1.60	2.44	7.69	0.85

Expression levels in Telomerase cell lines   **median: 0.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.60	0.25	0.68	1.39	0.44	0.06	0.06	5.76	0.23	0.53	0.47	0.21
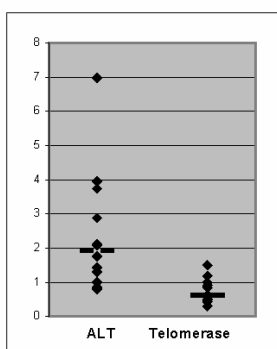
14: CCL20: chemokine (C-C motif) ligand 20

P-value: **1.0E-04**   **Fold change: 3.1**

Chromosome: 2q33-q37   Genbank: NM_004591.1   Gene ID: hCG14841.3

Biological process: signal transduction;inflammatory response;cell-cell signaling;chemotaxis;antimicrobial humoral response(sensu Vertebrata);immune response

Molecular function: chemokine activity

Cellular component: extracellular space

Expression levels in ALT cell lines   **median: 1.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.31	3.74	0.80	2.10	3.95	1.43	6.98	1.00	2.89	0.86	1.77	2.12

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.91	0.85	0.51	0.30	0.49	1.18	0.61	0.59	1.00	1.50	0.63	0.44
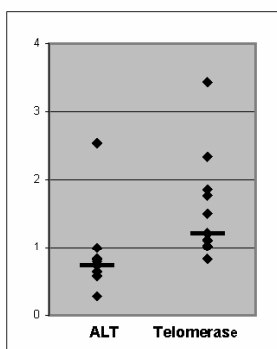
15: fs03c07.y1 Human Lens cDNA (Normalized): fs Homo sapiens cDNA clone fs03c07 5', mRNA sequence.

P-value: **1.0E-04**   **Fold change: 0.6**

Chromosome: 11   Genbank: CD673703   Gene ID: hCG2040534

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.28	0.63	0.57	0.73	0.74	0.99	0.58	0.81	0.83	2.54	0.73	0.78

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.01	1.77	1.85	0.82	1.50	1.10	1.03	2.34	3.43	1.12	1.22	1.20
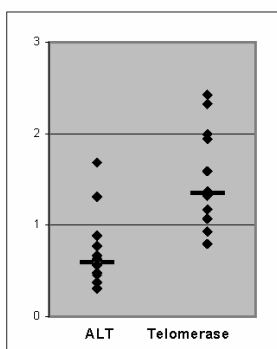
16: FLJ13984: hypothetical protein FLJ13984

P-value: **1.0E-04**   **Fold change: 0.4**

Chromosome: 2q31.1

Genbank: NM_024770.1   Gene ID: hCG2004932

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.47	0.45	0.48	0.63	0.77	0.31	0.37	0.67	1.68	1.31	0.56	0.88

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.93	1.99	1.37	1.07	1.32	0.80	1.34	2.42	2.32	1.94	1.59	1.17
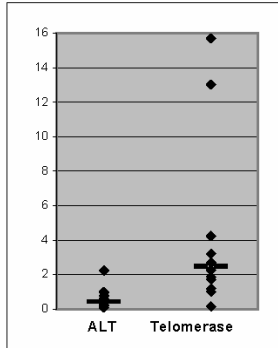
17: N/A

P-value:	1.5E-04								Fold change:	0.2	

Chromosome:

Genbank: Gene ID: hCG2042185

Biological process:

Molecular function:

Cellular component:

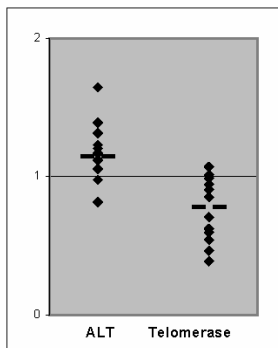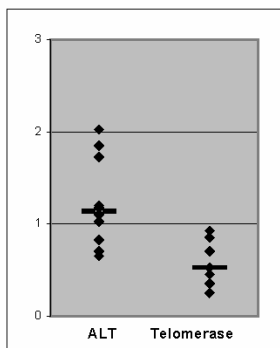**Expression levels in ALT cell lines**   median: 0.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.23	0.60	0.51	0.07	0.09	0.76	0.94	0.37	0.23	0.20	0.18	1.00

Expression levels in Telomerase cell lines   median: 2.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.18	0.14	1.00	13.02	1.85	4.24	15.71	3.20	4.22	1.70	2.68	2.25

18: N/A

P-value:	1.5E-04								Fold change:	1.6	

Chromosome:

Genbank: Gene ID: R26_hCT1784913.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.18	1.07	1.54	0.97	1.27	1.15	1.43	2.10	1.37	1.20	1.32	0.85

Expression levels in Telomerase cell lines   median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.03	0.94	0.81	0.63	0.73	0.85	0.83	0.74	1.75	0.56	0.38	0.51

19: NME4: non-metastatic cells 4, protein expressed in

P-value:	2.1E-04								Fold change:	0.5	

Chromosome: 16p13.3   Genbank: NM_005009.2   Gene ID: hCG1985512.1

Biological process: nucleoside metabolism; GTP, UTP and CTP biosynthesis

Molecular function: transferase activity; nucleoside-diphosphate kinase activity; kinase activity; ATP binding

Cellular component: mitochondrion

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.40	0.51	0.01	0.92	0.84	0.77	0.24	0.05	1.33	0.50	0.94	0.85

Expression levels in Telomerase cell lines   median: 1.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.87	2.08	1.49	1.14	1.63	1.06	2.43	1.49	2.93	4.59	0.75	0.88
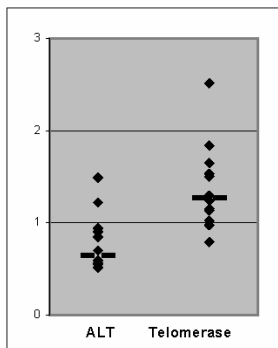
20: HYPK; SERF2: Huntingtin interacting protein K; small EDRK-rich factor 2

P-value:	2.1E-04								Fold change:	0.5	

Chromosome: 15q15.1

Genbank: NM_016400.2   Gene ID: hCG2003792

Biological process:

Molecular function: protein binding

Cellular component:

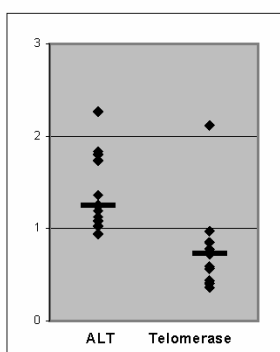**Expression levels in ALT cell lines**   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.70	0.59	1.22	0.91	0.51	0.94	0.85	0.56	0.56	1.49	0.55	0.55

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	1.65	1.30	1.24	1.50	1.15	1.02	2.51	1.84	1.14	1.53	0.79

21: IFITM3: interferon induced transmembrane protein 3 (1-8U)

| P-value: | 2.1E-04 | | | | | | Fold change: | 0.5 | | | |

Chromosome: 11p15.5

Genbank: BG421288   Gene ID: hCG2040233

Biological process: immune response

Molecular function:

Cellular component: Integral to membrane

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.83	0.85	0.72	0.53	0.59	0.90	0.93	0.66	0.73	1.64	0.46	0.62

Expression levels in Telomerase cell lines   median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.16	2.26	1.00	1.06	1.17	1.00	1.79	0.87	3.42	2.42	1.28	3.52
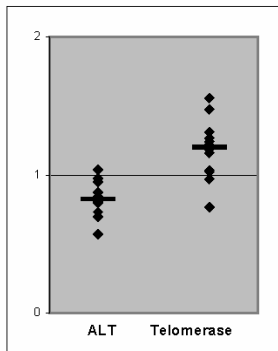
22: FLJ32803; TLOC1: hypothetical protein FLJ32803; translocation protein 1

| P-value: | 2.1E-04 | | | | | | Fold change: | 0.7 | | | |

Chromosome: 3q26.2

Genbank: NM_003262.2   Gene ID: hCG16182.3

Biological process: cotranslational membrane targeting; protein transport

Molecular function: receptor activity; protein transporter activity

Cellular component: endoplasmic reticulum;integral to membrane

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.04	0.80	0.98	0.70	0.81	0.95	0.73	0.57	0.84	1.04	0.70	0.87

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.02	1.22	1.56	1.31	1.48	1.19	0.77	0.97	1.27	1.24	1.16	1.03

23: JAM2: junctional adhesion molecule 2

| P-value: | 3.0E-04 | | | | | | Fold change: | 5.5 | | | |

Chromosome: 21q21.2

Genbank: NM_021219.2   Gene ID: hCG401152.3

Biological process: cell-cell adhesion

Molecular function:

Cellular component: integral to plasma membrane

Expression levels in ALT cell lines   median: 2.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
8.89	0.59	3.09	6.65	2.57	3.38	0.83	2.36	2.03	2.76	1.17	3.86

Expression levels in Telomerase cell lines   median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.77	0.17	0.59	1.42	0.64	0.38	0.20	4.02	0.59	0.30	0.25	0.29
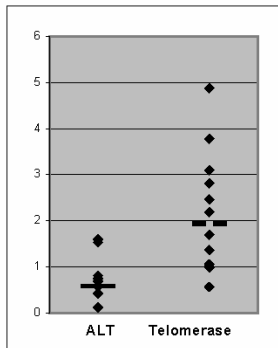
24: CHFR: checkpoint with forkhead and ring finger domains

| P-value: | 3.0E-04 | | | | | | Fold change: | 0.3 | | | |

Chromosome: 12q24.33

Genbank: NM_018223.1   Gene ID: hCG22242.4

Biological process: cell cycle; cell division; mitosis; mitotic check point; ubiquitin cycle

Molecular function: ligase activity; metal ion, protein and zinc ion binding

Cellular component: nucleus

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.74	0.70	0.42	0.59	0.43	0.57	0.58	1.59	0.57	1.53	0.13	0.81

Expression levels in Telomerase cell lines   median: 1.9

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.81	1.06	1.37	3.78	0.57	4.87	3.09	0.99	1.01	1.70	2.19	2.47
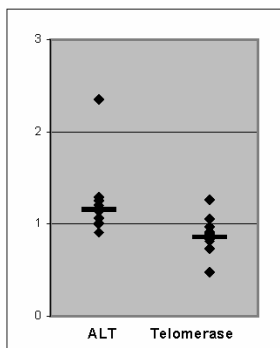
25: FBXO33: F-box only protein 33

P-value: 3.0E-04   Fold change: 1.3

Chromosome: 14q13.3

Genbank: BC042535   Gene ID: hCG21239.2

Biological process: ubiquitin cycle

Molecular function:

Cellular component:

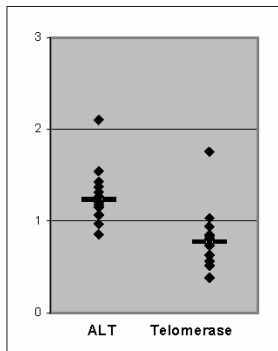**Expression levels in ALT cell lines**   median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.18	1.00	1.25	2.35	1.25	1.01	1.13	1.20	1.06	1.29	1.00	0.91

Expression levels in Telomerase cell lines   median: 0.9

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.85	1.26	0.89	0.81	1.05	0.84	0.73	0.86	0.48	0.47	0.91	0.97

26:

NDUFB3: NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa

P-value: 3.0E-04   Fold change: 0.6

Chromosome: 2q31.3   Genbank: NM_002491.1   Gene ID: hCG1776639.1

Biological process: mtichondrial electron transport

Molecular function: NADH dehydrogenase activity; oxidoreductase activity

Cellular component: mitochondrion

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.31	0.99	0.83	0.38	0.94	0.57	0.37	1.19	0.43	0.86	0.62	0.84

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.32	2.72	1.00	1.00	1.69	1.42	1.33	1.87	1.22	1.94	1.11	0.55
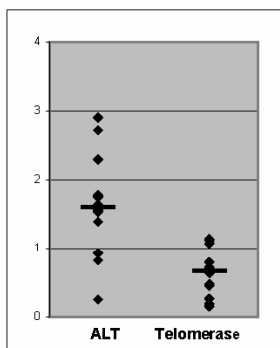
27:   N/A

P-value: 3.0E-04   Fold change: 2.4

Chromosome: 6

Genbank: AI814302   Gene ID: hCG1659708.3

Biological process:

Molecular function:

Cellular component:

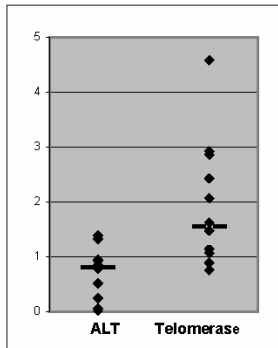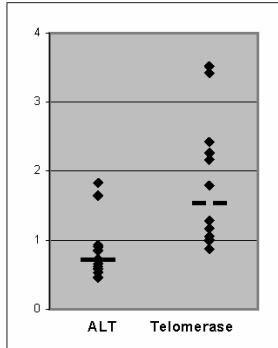**Expression levels in ALT cell lines**   median: 1.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.82	1.39	0.93	1.64	1.54	0.25	2.30	2.91	2.72	1.57	1.75	1.78

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.80	0.63	1.14	0.45	0.70	0.47	0.15	1.07	0.19	0.72	1.12	0.26

28:   PIGK: phosphatidylinositol glycan, class K

P-value: 3.0E-04   Fold change: 1.6

Chromosome: 1p31.1

Genbank: NM_005482.1   Gene ID: hCG22810.4

Biological process: proteolysis and peptidolysis

Molecular function: legumain activity;hydrolase activity

Cellular component: integral to membrane;endoplasmic reticulum

Expression levels in ALT cell lines   median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.25	1.24	1.22	1.55	1.78	1.00	1.08	3.46	1.05	1.24	0.92	1.00

Expression levels in Telomerase cell lines   median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.61	1.75	0.67	0.76	0.94	0.81	0.76	1.05	0.93	0.98	0.61	0.75
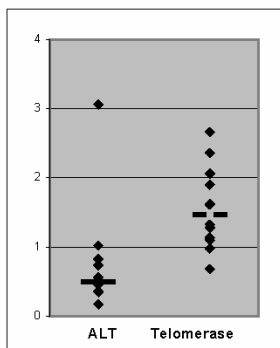
29: PIGB: phosphatidylinositol glycan, class B

P-value: 3.0E-04   Fold change: 0.3

Chromosome: 15q21-q22

Genbank: NM_004855.3   Gene ID: hCG40051.3

Biological process: GPI anchor biosynthesis; protein amino acid glycosylation
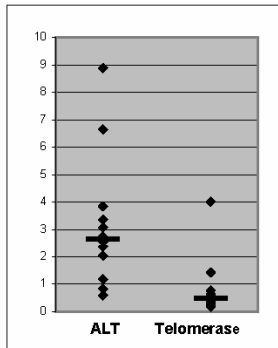
Molecular function: catalytic activity

Cellular component: endoplasmic reticulum membrane

Expression levels in ALT cell lines   median: 0.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.83	0.37	0.74	0.51	0.46	0.49	0.56	0.18	1.02	3.06	0.17	0.35

Expression levels in Telomerase cell lines   median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	1.61	1.33	1.13	2.36	1.10	0.68	2.06	1.90	2.66	1.62	1.28
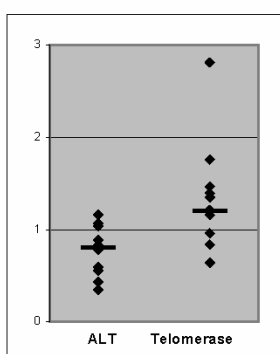
30: N/A

P-value: 3.0E-04   Fold change: 0.7

Chromosome: 13

Genbank: R60865   Gene ID: hCG23704.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.16	0.83	0.43	0.35	0.89	0.56	0.59	0.89	1.07	0.78	0.55	1.04

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.35	0.96	1.39	2.81	1.19	0.64	1.21	1.20	1.76	1.16	1.46	0.84
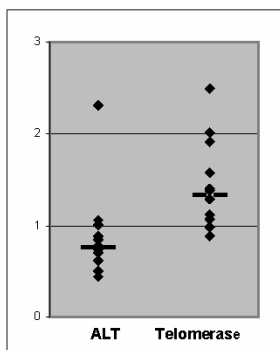
31: ST7: suppression of tumorigenicity 7

P-value: 4.2E-04   Fold change: 0.6

Chromosome: 7q31.1-q31.3

Genbank: NM_018412.2   Gene ID: hCG15955.3

Biological process:

Molecular function: binding

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.85	0.78	0.75	0.89	1.06	0.50	0.43	1.01	0.74	2.31	0.61	0.70

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.12	1.58	1.29	1.38	1.92	1.40	2.50	0.99	0.89	2.02	0.98	1.07
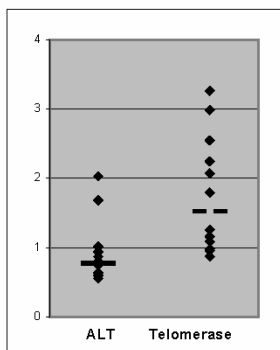
32: N/A

P-value: 4.2E-04   Fold change: 0.5

Chromosome:

Genbank: Gene ID: hCG1783311.2

Biological process:

Molecular function:

Cellular component:
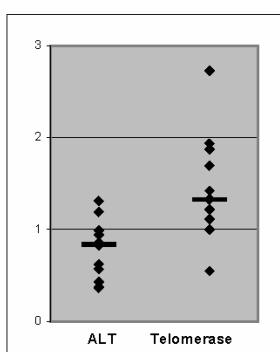
Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.03	1.02	0.80	0.61	0.75	0.87	0.94	0.65	0.64	1.68	0.56	0.60

Expression levels in Telomerase cell lines   median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.55	2.07	1.09	0.96	1.16	0.98	1.80	0.87	3.26	2.25	1.26	2.98
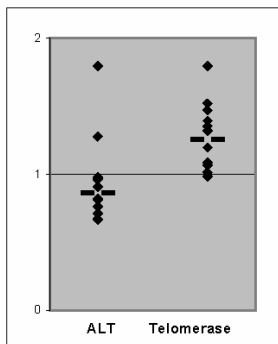
33: KIAA0261: KIAA0261

P-value: 4.2E-04   Fold change: 0.7

Chromosome: 10q23.31

Genbank: BC036507   Gene ID: hCG23249.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.77	1.28	0.71	0.96	0.82	0.67	0.97	0.82	1.80	0.67	0.91	0.98

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.36	1.80	0.98	1.47	1.20	1.09	1.07	1.52	1.39	1.02	1.32	0.98

34: MGC15416: hypothetical protein MGC15416

P-value: 4.2E-04   Fold change: 0.4

Chromosome: 16p13.3

Genbank: NM_138418.2   Gene ID: hCG22438.3

Biological process:

Molecular function:

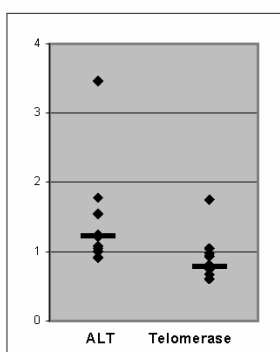Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.65	1.43	0.70	0.74	0.65	0.57	0.57	1.32	0.90	0.90	0.63	0.96

Expression levels in Telomerase cell lines   median: 1.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.62	2.61	0.91	1.00	1.70	1.00	2.32	2.25	3.10	2.84	1.98	0.84

35: DKFZP564I122: DKFZP564I122 protein

P-value: 4.2E-04   Fold change: 1.8

Chromosome: 1p34.1

Genbank: AL080062   Gene ID: hCG1780057.1

Biological process:

Molecular function: Cobalt ion binding

Cellular component:

Expression levels in ALT cell lines   median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.87	2.06	1.01	1.95	1.46	1.22	1.76	1.31	1.28	0.98	0.83	1.55

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.25	0.64	0.67	1.08	0.58	0.97	0.99	1.11	0.60	1.19	0.55	0.74

36: FLJ46041: FLJ46041 protein

P-value: 4.9E-04   Fold change: 0.6

Chromosome: 6q24.3

Genbank: AK127932   Gene ID: hCG1749747.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.30	0.58	0.18	0.68	0.80	0.40	0.86	1.01	0.89	0.20	1.27	1.01

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.80	10.44	1.36	1.42	0.84	1.11	1.22	0.99	1.05	1.05	1.57	1.98

37: KIAA1444; IDH3G: PDZ domain containing 4;isocitrate dehydrogenase 3 (NAD+) gamma

P-value: 5.0E-04   **Fold change:** 0.5
Chromosome: Xq28   Genbank: NM_174869.1   Gene ID: hCG2004980
Biological process:
Molecular function: protein binding
Cellular component: membrane

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.22	0.20	1.27	0.65	0.83	0.53	0.79	0.71	0.30	1.25	0.61	0.79

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.59	0.87	1.25	1.23	1.33	1.13	1.74	1.47	1.58	1.54	1.62	1.15

38:
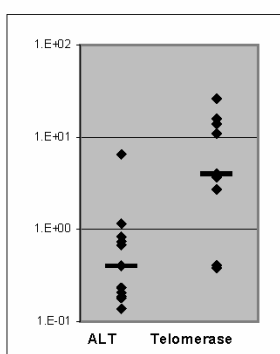
SLCO4A1: solute carrier organic anion transporter family, member 4A1

P-value: 5.0E-04   **Fold change:** 0.1
Chromosome: 20q13.33   Genbank: NM_016354.3   Gene ID: hCG1748044.2
Biological process: ion transport
Molecular function: transporter activity
Cellular component: integral to membrane

Expression levels in ALT cell lines   median: 0.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.84	0.21	0.40	0.24	1.16	0.41	0.19	0.14	6.53	0.68	0.74	0.18

Expression levels in Telomerase cell lines   median: 4.0

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
4.03	3.69	4.04	0.41	4.03	2.73	10.93	15.78	15.92	26.39	13.94	0.38
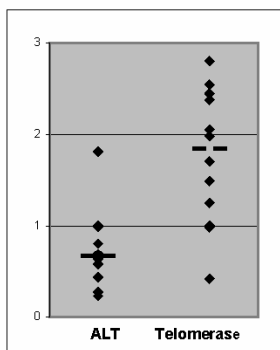
39:

PRKAR1B: protein kinase, cAMP-dependent, regulatory, type I, beta

P-value: 5.0E-04   **Fold change:** 0.4
Chromosome: 7pter-p22   Genbank: NM_002735.1   Gene ID: hCG1993358
Biological process: signal transduction;protein amino acid phosphorylation
Molecular function: cAMP-dependent protein kinase regulator activity;3',5'-cAMP binding
Cellular component: cAMP-dependent protein kinase complex

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.00	0.66	0.67	1.00	1.81	0.23	0.69	0.27	0.63	0.57	0.43	0.81

Expression levels in Telomerase cell lines   median: 1.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.44	1.49	1.00	1.70	2.54	2.80	0.41	0.99	2.05	2.37	1.98	1.25
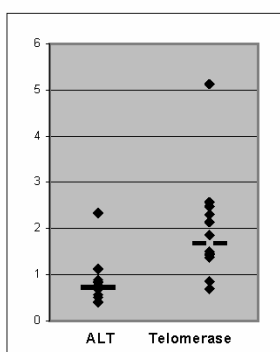
40: chromosome 3 open reading frame 10

P-value: 5.8E-04   **Fold change:** 0.4
Chromosome: 3
Genbank: AF281279   Gene ID: hCG1811444.1
Biological process:
Molecular function:
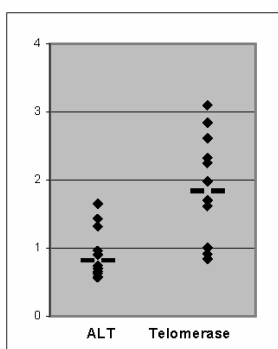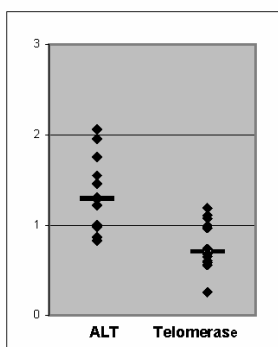Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.50	0.56	0.40	0.72	0.88	0.77	0.73	0.83	1.12	2.34	0.69	0.69

Expression levels in Telomerase cell lines   median: 1.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.50	2.57	2.48	0.69	2.14	0.85	1.43	2.30	5.13	1.85	1.37	1.45
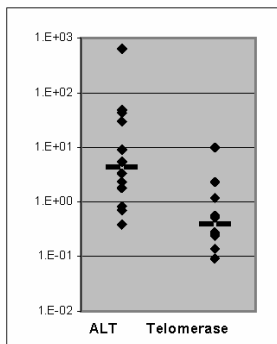
41: SPANXD: SPANX family, member D

P-value: **5.8E-04**   **Fold change:**   **11.1**

Chromosome: Xq27.1

Genbank: BC054023   Gene ID: hCG1756726.2

Biological process: biological_process unknown

Molecular function: molecular_function unknown

Cellular component: nucleus

Expression levels in ALT cell lines   **median: 4.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.33	0.39	3.32	42.90	5.42	0.70	9.01	638	48.04	0.83	29.80	1.79

Expression levels in Telomerase cell lines   **median: 0.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.17	2.32	0.26	0.28	0.24	0.09	0.14	0.51	0.26	0.56	9.94	0.55
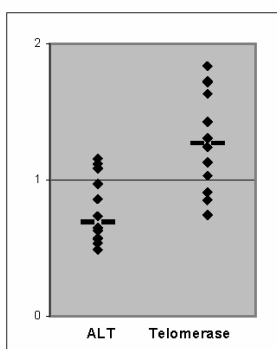
42: NANOS1: nanos homolog 1 (Drosophila)

P-value: **5.9E-04**   **Fold change:**   **0.5**

Chromosome: 10q26.13

Genbank: BI495757   Gene ID: hCG1820523.1

Biological process: regulation of translation

Molecular function: RNA binding; metal ion binding; zinc ion binding

Cellular component: cytoplasm

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.12	0.65	0.63	0.57	0.73	0.54	0.49	1.16	1.08	0.97	0.57	0.86

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.30	1.13	0.91	1.63	1.72	0.85	0.74	1.71	1.42	1.83	1.24	1.03
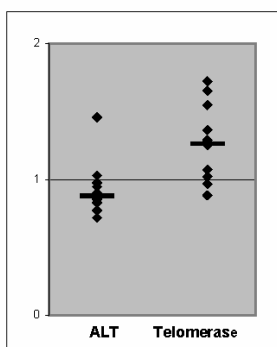
43: HNRPK: heterogeneous nuclear ribonucleoprotein K

P-value: **5.9E-04**   **Fold change:**   **0.7**

Chromosome: 9q21.32-q21.33

Genbank: NM_002140.2   Gene ID: hCG1985922

Biological process:

Molecular function: DNA, RNA and protein binding

Cellular component: nucleus; ribonuclearprotein complex

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.95	0.87	0.90	0.83	0.90	0.77	0.98	1.03	0.86	1.46	0.72	0.85

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.07	1.65	0.89	1.28	1.36	1.02	1.02	1.72	1.29	1.55	1.25	0.97
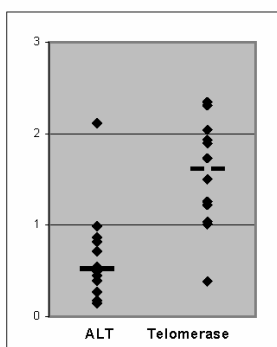
44: LGALS9: lectin, galactoside-binding, soluble, 9 (galectin 9)

P-value: **5.9E-04**   **Fold change:**   **0.3**

Chromosome: 17q11.2

Genbank: NM_002308.2   Gene ID: hCG1749919.3

Biological process: heterophilic cell adhesion

Molecular function: galactose binding; sugar binding

Cellular component:

Expression levels in ALT cell lines   **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.39	0.50	0.17	0.55	0.27	0.14	0.45	0.71	0.87	2.12	0.82	0.99

Expression levels in Telomerase cell lines   **median: 1.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.35	2.04	1.50	1.73	2.31	1.01	0.38	1.93	1.04	1.26	1.89	1.22
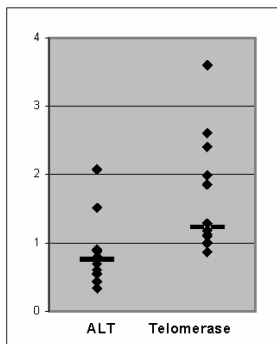
45: HTPAP: HTPAP protein

P-value: 5.9E-04 **Fold change:** 0.6

Chromosome: 8p11.23

Genbank: NM_032483.2 Gene ID: hCG23115.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 0.8

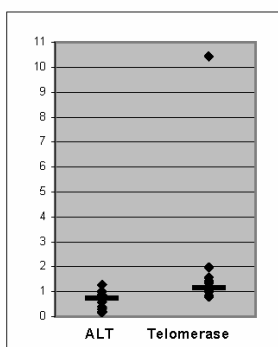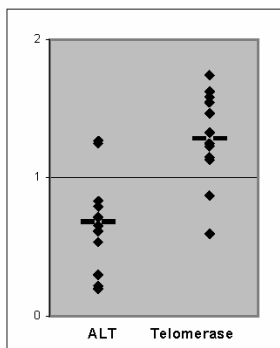| 1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.78	0.90	0.76	0.55	1.51	0.60	0.34	0.44	0.88	2.07	0.80	0.69

Expression levels in Telomerase cell lines median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.87	1.10	1.18	1.29	2.61	1.00	1.00	2.40	1.99	3.60	1.12	1.85

46: GAL: galanin

P-value: 5.9E-04 **Fold change:** 0.5

Chromosome: 11q13.1 Genbank: NM_015973.2 Gene ID: hCG27180.1

Biological process: growth hormone secretion;smooth muscle contraction; neuropeptide signaling pathway;insulin secretion

Molecular function: neuropeptide hormone activity

Cellular component: extracellular; cellular_component unknown

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.85	0.51	1.15	0.74	0.50	0.45	0.64	0.63	0.28	2.81	1.04	0.66

Expression levels in Telomerase cell lines median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.60	3.36	1.28	0.91	6.06	1.03	1.15	35.64	1.61	3.53	0.97	0.73
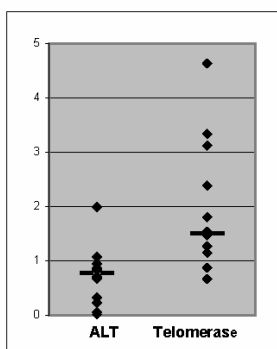
47: NME4: non-metastatic cells 4, protein expressed in

P-value: 5.9E-04 **Fold change:** 0.5

Chromosome: 16p13.3 Genbank: NM_005009.2 Gene ID: hCG1985512.1

Biological process: nucleoside metabolism;UTP, GTP and CTP biosynthesis

Molecular function: transferase activity; nucleoside-diphosphate kinase activity; ATP binding; kinase activity

Cellular component: mitochondrion

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.00	0.67	0.01	1.06	0.84	0.71	0.23	0.06	0.94	0.33	0.87	0.83

Expression levels in Telomerase cell lines median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
3.34	1.81	1.28	1.48	1.55	1.28	2.39	1.14	3.13	4.64	0.66	0.87
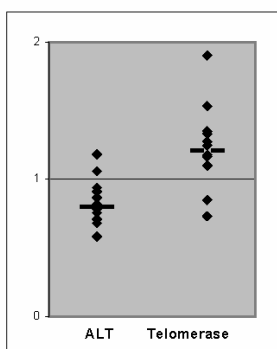
48: FLJ36674; LOC201158: hypothetical protein FLJ36674; similar to CGI-148 protein

P-value: 5.9E-04 **Fold change:** 0.7

Chromosome: 17p11.2 Genbank: NM_173622.1 Gene ID: hCG1820468.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.94	0.91	1.18	0.71	1.06	0.76	0.78	0.82	0.79	0.87	0.68	0.58

Expression levels in Telomerase cell lines median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.85	1.18	1.35	1.10	1.33	0.73	1.16	1.90	1.10	1.53	1.28	1.25

49: IFITM3: interferon induced transmembrane protein 3 (1-8U)

P-value: 5.9E-04 **Fold change:** 0.5

Chromosome: 11p15.5

Genbank: BF768108 Gene ID: hCG2036716

Biological process:

Molecular function:

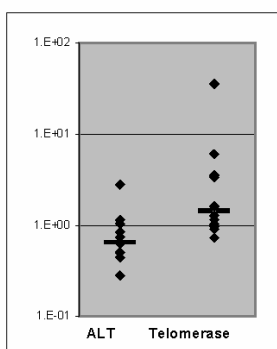Cellular component:

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.56	1.01	0.60	0.58	0.75	0.90	0.86	0.75	0.69	1.69	0.42	0.51

Expression levels in Telomerase cell lines median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.73	2.93	0.99	0.88	1.36	0.91	1.52	1.07	2.86	2.80	1.06	2.93
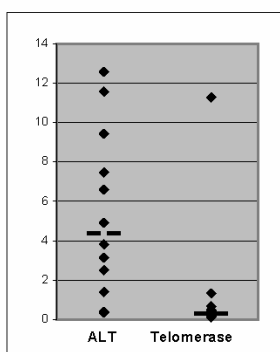
50: SATB1: special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)

P-value: 5.9E-04 **Fold change:** 14.2

Chromosome: 3p23 Genbank: NM_002971.2 Gene ID: hCG26781.3

Biological process: establishment and/or maintenance of chromatin architecture; regulation of transcription, DNA-dependent

Molecular functn: dsDNA binding; transcriptn factor activity. Cellular component: nucleus

Expression levels in ALT cell lines median: 4.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.39	1.40	9.42	3.14	4.91	3.82	7.46	6.59	11.56	12.57	0.35	2.50

Expression levels in Telomerase cell lines median: 0.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.45	0.30	0.17	0.23	0.21	0.11	0.31	0.48	0.29	1.33	11.27	0.67
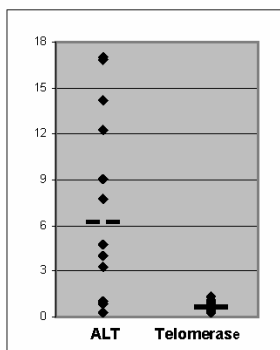
51: NSBP1: nucleosomal binding protein 1

P-value: 6.8E-04 **Fold change:** 9.9

Chromosome: Xq13.3

Genbank: NM_030763.1 Gene ID: hCG1646094.3

Biological process: regulation of transcription, DNA-dependent

Molecular function: transcriptional activator activity; chromatin binding

Cellular component: :nucleus; chromatin

Expression levels in ALT cell lines median: 6.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.27	1.01	16.84	7.76	9.06	4.77	3.97	17.02	12.27	14.21	0.85	3.26

Expression levels in Telomerase cell lines median: 0.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.48	0.47	0.92	1.08	0.99	0.34	0.41	1.32	0.27	0.79	0.86	0.31
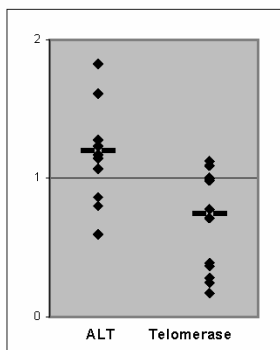
52: ZNF44: zinc finger protein 44 (KOX 7)

P-value: 7.8E-04 **Fold change:** 1.6

Chromosome: 19p13.2

Genbank: NM_016264.1 Gene ID: hCG2002471.1

Biological process: regulation of transcription, DNA-dependent

Molecular function: nucleic acid binding; DNA binding

Cellular component: nucleus;intracellular

Expression levels in ALT cell lines median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.28	0.80	1.28	1.61	1.15	1.28	1.83	1.23	0.59	1.17	1.07	0.86

Expression levels in Telomerase cell lines median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.25	1.12	0.71	0.37	0.98	1.01	0.78	0.99	1.09	0.28	0.17	0.39
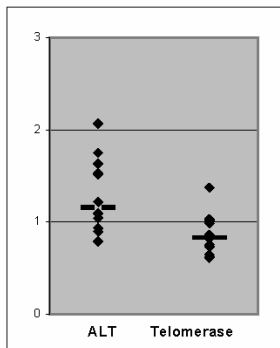
53: TAF6L

P-value: 7.9E-04 **Fold change:** 1.4

Chromosome: 11

Genbank: NM_018336.1 Gene ID: hCG1727076.2

Biological process: chromatin remodelling; regulation and initiation of transcription

Molecular functn: DNA binding; RNA pol II transcriptn and transcriptn initiatn factor activity

Cellular component: histone deacetylase complex; nucleus

Expression levels in ALT cell lines **median: 1.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.93	0.90	2.06	1.04	1.09	1.75	1.53	1.09	1.63	1.22	1.52	0.79

Expression levels in Telomerase cell lines **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.02	1.03	0.65	0.85	0.62	0.86	0.61	0.76	1.37	0.82	0.98	0.73
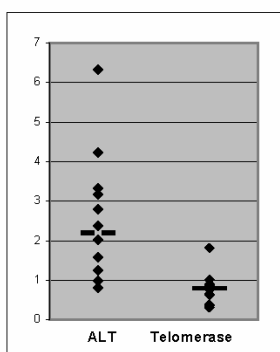
54: FLJ20032: hypothetical protein FLJ20032

P-value: 7.9E-04 **Fold change:** 2.8

Chromosome: 4q24

Genbank: AK000039 Gene ID: hCG1778365.2

Biological process:

Molecular function:

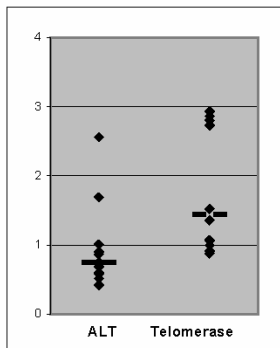Cellular component:

Expression levels in ALT cell lines **median: 2.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.83	6.33	1.59	2.38	0.98	3.33	4.23	1.25	3.17	2.02	2.80	0.81

Expression levels in Telomerase cell lines **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.32	0.39	0.63	0.84	0.36	0.88	1.82	0.65	0.76	1.82	1.02	0.90
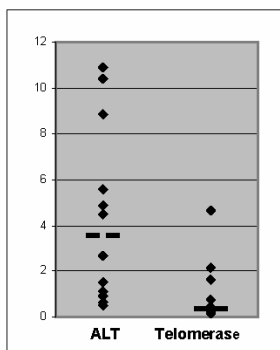
55: FLJ38993: Hypothetical protein FLJ38993; Homo sapiens hypothetical protein FLJ38993, mRNA (cDNA clone MGC:9297 IMAGE:3894832)

P-value: 7.9E-04 **Fold change:** 10.1

Chromosome: 1q42.13 Genbank: NM_152495.1 Gene ID: hCG1641724.2

Biological process: intracellular signaling cascade

Molecular function:

Cellular component: integral to membrane

Expression levels in ALT cell lines **median: 3.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
5.59	0.90	0.62	8.86	10.90	1.10	1.49	0.49	4.51	10.41	2.65	4.89

Expression levels in Telomerase cell lines **median: 0.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.25	0.18	0.23	0.43	1.61	0.34	0.18	0.14	0.37	2.13	4.67	0.73
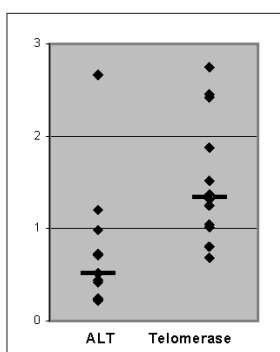
56: PIGB: phosphatidylinositol glycan, class B

P-value: 7.9E-04 **Fold change:** 0.4

Chromosome: 15q21-q22

Genbank: NM_004855.3 Gene ID: hCG40051.3

Biological process: GPI anchor biosynthesis; protein amino acid glycosylation

Molecular function: catalytic activity

Cellular component: endoplasmic reticulum membrane

Expression levels in ALT cell lines **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.98	0.52	0.73	0.42	0.52	0.72	0.23	0.51	1.20	2.66	0.24	0.44

Expression levels in Telomerase cell lines **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.68	1.37	1.25	1.02	2.75	1.04	0.80	2.45	1.88	2.42	1.52	1.32
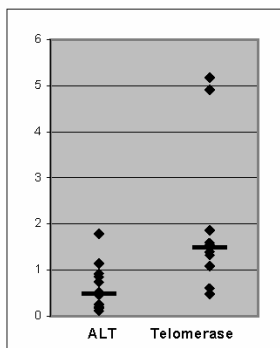
57: CRIP2: cysteine-rich protein 2

P-value: **8.0E-04**   **Fold change:**   **0.3**

Chromosome: 14q32.3

Genbank: NM_001312.2   Gene ID: hCG2029375

Biological process:

Molecular function: zinc ion binding

Cellular component:

Expression levels in ALT cell lines   **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.78	0.52	0.25	0.17	0.19	0.46	0.85	0.92	1.14	0.19	0.11	0.73

Expression levels in Telomerase cell lines   **median: 1.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.86	1.08	0.60	1.47	1.54	1.51	4.91	0.48	5.17	1.32	1.59	1.39

58: IFITM2: interferon induced transmembrane protein 2 (1-8D)

P-value: **8.0E-04**   **Fold change:**   **0.5**

Chromosome: 11p15.5

Genbank: NM_006435.1   Gene ID: hCG1741135.2

Biological process: immune response

Molecular function: protein binding

Cellular component: integral to membrane

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.82	0.91	0.70	0.41	0.63	0.92	0.93	0.57	0.78	1.88	0.39	0.48

Expression levels in Telomerase cell lines   **median: 1.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.81	2.31	0.99	0.88	1.10	1.01	1.68	1.07	3.37	3.54	1.27	3.09
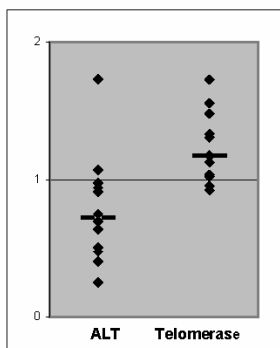
59: PTPN9: protein tyrosine phosphatase, non-receptor type 9

P-value: **8.0E-04**   **Fold change:**   **0.6**

Chromosome: 15q23   Genbank: NM_002833.2   Gene ID: hCG2005449

Biological process: transport;protein amino acid dephosphorylation

Molecular function: hydrolase activity;non-membrane spanning protein tyrosine phosphatase activity;transporter activity

Cellular component: intracellular

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.73	0.94	0.40	0.70	0.98	0.75	0.91	0.25	0.47	1.07	0.64	0.51

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.18	1.33	1.17	1.13	1.73	1.04	0.96	1.56	1.48	1.31	0.93	1.02
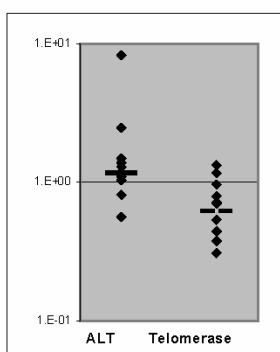
60: PTPRG: protein tyrosine phosphatase, receptor type, G

P-value: **8.0E-04**   **Fold change:**   **1.9**

Chromosome: 3p21-p14   Genbank: NM_002841.2   Gene ID: hCG23837.3

Biological process: protein amino acid dephosphorylation; transmembrane receptor protein tyrosine kinase signaling pathway; one-carbon compound metabolism. Molecular functn: carbonate dehydratase, zinc ion binding;transmembrane receptor protein tyrosine phosphatase and hydrolase activity. Cellular component: integral to plasma membrane

Expression levels in ALT cell lines   **median: 1.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.04	0.56	0.81	1.38	8.25	1.10	1.30	1.15	2.48	1.49	1.19	1.13

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.72	1.33	0.80	0.38	0.44	0.44	0.38	0.54	0.31	0.71	1.17	0.96
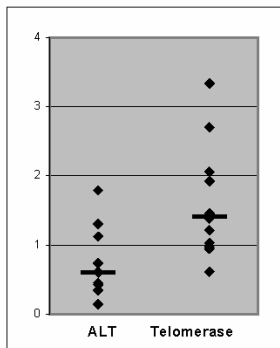
61: FLJ13984: hypothetical protein FLJ13984

P-value: 8.0E-04   **Fold change:** 0.4

Chromosome: 2q31.1

Genbank: NM_024770.1   Gene ID: hCG2004932

Biological process:

Molecular function:

Cellular component:
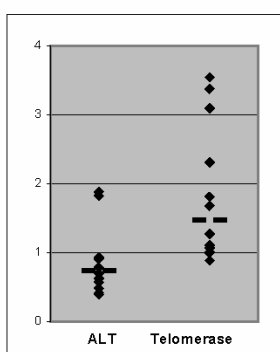
Expression levels in ALT cell lines   **median: 0.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.60	0.43	0.34	0.61	0.73	0.35	0.14	1.12	1.79	1.30	0.45	0.74

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.44	2.70	1.22	1.03	1.92	0.62	0.95	3.33	2.06	1.46	1.39	0.97

62: MGC10911: hypothetical protein MGC10911

P-value: 8.0E-04   **Fold change:** 0.6

Chromosome: 7p22.3

Genbank: NM_032302.1   Gene ID: hCG19463.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.92	0.93	1.01	0.82	0.59	1.28	0.64	0.53	0.89	1.49	0.57	0.93

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.03	0.99	1.12	1.68	1.32	1.92	0.69	1.62	0.95	1.87	1.51	1.04

63: FHOD3

P-value: 8.0E-04   **Fold change:** 0.6

Chromosome: 18q12

Genbank: NM_025135.1   Gene ID: hCG2042959

Biological process: cell and actin organisation and biogenesis

Molecular function: actin binding

Cellular component:

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.68	1.31	0.62	0.64	0.76	0.78	1.04	0.92	1.14	0.89	0.72	0.88

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.78	1.63	1.04	1.50	1.25	0.89	1.27	1.84	1.66	1.85	1.43	0.96
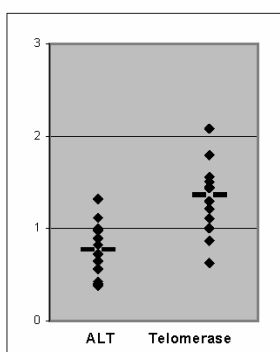
64: ADP-GK: ATP-dependent glucokinase

P-value: 8.0E-04   **Fold change:** 0.6

Chromosome: 15q22.33

Genbank: NM_031284.3   Gene ID: hCG1787419.2

Biological process: glycolysis

Molecular functn: phosphotransferase activity, alcohol group as acceptor, Mg ion bimding

Cellular component:

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.12	0.89	0.56	1.00	0.82	0.73	0.65	0.39	0.98	1.32	0.43	0.40

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.44	1.56	1.21	1.11	1.79	1.30	0.63	2.08	1.51	1.45	1.00	0.87
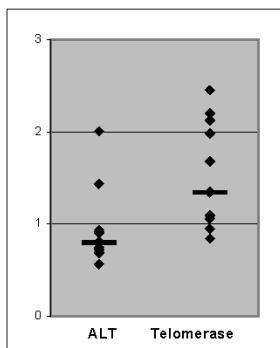
65: C21orf6: chromosome 21 open reading frame 6

P-value: 8.0E-04 **Fold change:** 0.6

Chromosome: 21q22.11

Genbank: NM_016940.1 Gene ID: hCG401159.3

Biological process:

Molecular function: acylphosphatase activity

Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.56	0.79	0.72	0.93	0.74	0.91	0.68	0.90	1.44	2.01	0.80	0.69

Expression levels in Telomerase cell lines median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.94	1.68	1.34	2.12	1.98	2.45	1.09	2.20	1.06	1.35	0.84	1.06
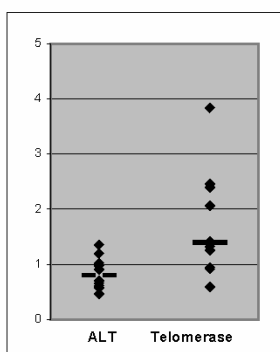
66: N/A

P-value: 8.0E-04 **Fold change:** 0.6

Chromosome:

Genbank: Gene ID: hCG1731660.2

Biological process:

Molecular function:

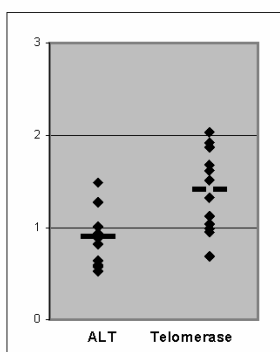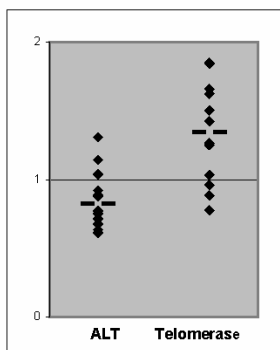Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	0.58	0.46	0.47	0.90	0.66	0.98	0.70	0.61	1.35	1.02	0.91

Expression levels in Telomerase cell lines median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.39	2.06	1.41	1.25	1.32	0.94	2.45	0.59	2.45	0.92	1.38	3.83
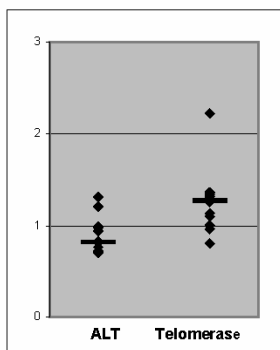
67: SSB:

P-value: 8.0E-04 **Fold change:** 0.6

Chromosome: 2q31.1

Genbank: BU540259 Gene ID: hCG1982987

Biological process: RNA processing and nuclear export; histone mRNA metabolism; tRNA modification; transcription form RNA pol III promoter. Molecular function: mRNA and tRNA binding. Cellular component: cytoplasm; nucleus; ribonucleoprotein complex

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.73	0.77	0.95	1.21	1.00	0.70	0.82	0.83	1.31	0.72	0.73	0.98

Expression levels in Telomerase cell lines median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.00	1.33	1.29	1.10	1.36	0.96	1.14	2.22	1.26	1.32	1.37	0.81
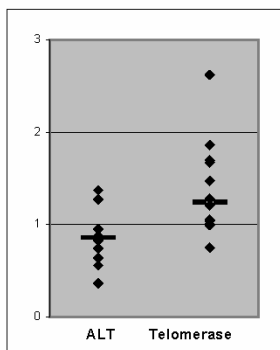
68: N/A

P-value: 8.0E-04 **Fold change:** 0.7

Chromosome:

Genbank: Gene ID: hCG1646764.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.37	0.87	0.74	0.88	1.27	0.56	0.37	0.85	0.95	0.94	0.64	0.83

Expression levels in Telomerase cell lines median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.67	1.86	1.05	1.04	1.21	1.01	1.28	2.62	1.47	1.70	0.99	0.75
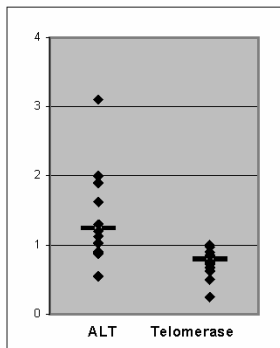
69: GPR114: G protein-coupled receptor 114

P-value:	9.0E-04						Fold change:	1.6		

Chromosome: 16q13

Genbank: NM_153837.1 — Gene ID: hCG1817974.2

Biological process: neuropeptide signaling pathway

Molecular function: receptor activity; G-protein coupled receptor activity

Cellular component: membrane

Expression levels in ALT cell lines — median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.90	0.55	1.63	2.00	1.30	3.10	0.88	1.20	1.13	1.30	1.90	1.03

Expression levels in Telomerase cell lines — median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.85	0.97	0.72	0.68	0.25	1.00	0.63	1.00	0.75	1.00	0.90	0.50
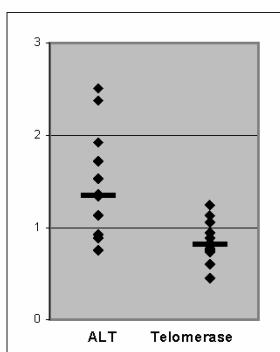
70: N/A

P-value:	1.1E-03						Fold change:	1.6		

Chromosome:

Genbank: — Gene ID: R26_hCT8651.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines — median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.92	1.92	2.38	1.53	0.75	0.89	2.51	1.36	1.13	1.34	1.13	1.72

Expression levels in Telomerase cell lines — median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.25	0.81	0.75	1.06	0.74	0.77	0.94	0.60	1.13	0.89	0.83	0.45

71: SRPUL: sushi-repeat protein

P-value:	1.1E-03						Fold change:	3.3		

Chromosome: Xq21.33-q23

Genbank: NM_014467.1 — Gene ID: hCG20192.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines — median: 1.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.84	3.11	1.85	2.74	2.13	0.80	1.72	2.05	2.67	0.72	1.31	0.96

Expression levels in Telomerase cell lines — median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.19	1.32	1.29	0.14	1.04	0.17	0.08	0.42	0.66	0.19	0.88	1.29

72: : Transcribed locus, highly similar to XP_169258.3 hypothetical LOC219638 [Homo sapiens]

P-value:	1.1E-03						Fold change:	2.2		

Chromosome: 11 — Genbank: AK024304 — Gene ID: hCG1991368.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines — median: 1.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.68	1.78	1.15	3.18	3.05	2.08	0.80	0.63	2.93	1.08	1.08	0.93

Expression levels in Telomerase cell lines — median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.43	0.78	0.70	0.25	0.28	0.63	0.50	1.30	0.83	0.93	0.65	0.58

73:   CCNG2: cyclin G2

P-value: **1.1E-03**   **Fold change: 2.3**

Chromosome: 4q21.22

Genbank: NM_004354.1   Gene ID: hCG22999.3

Biological process: cell cycle checkpoint; mitosis; cytokinesis; cell cycle

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 2.1**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.39	3.51	0.65	1.03	2.76	2.19	0.89	1.77	2.85	2.73	1.98	1.01

Expression levels in Telomerase cell lines   **median: 0.9**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.95	2.04	0.57	0.71	1.23	0.92	0.59	0.61	0.40	0.99	0.88	0.95

74:   KIAA1229: KIAA1229 protein

P-value: **1.1E-03**   **Fold change: 1.7**

Chromosome: 1p22.3

Genbank: BC029420   Gene ID: hCG25236.3

Biological process:

Molecular function:

Cellular component:

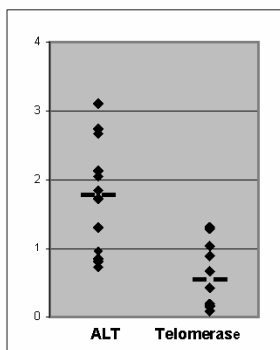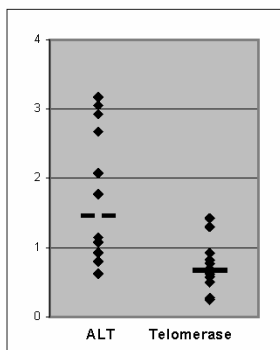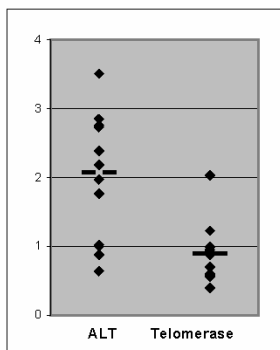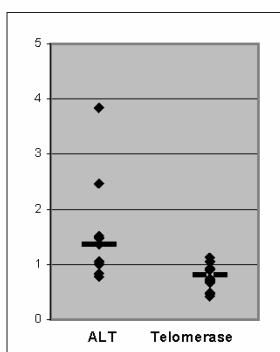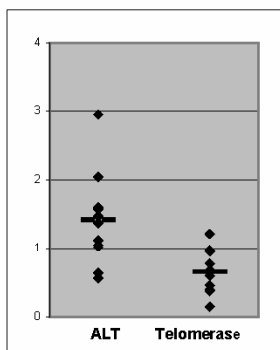**Expression levels in ALT cell lines**   **median: 1.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.37	1.00	1.52	0.84	2.47	1.05	1.48	3.84	1.36	1.48	1.00	0.78

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.67	0.91	0.73	1.05	0.90	0.70	1.12	0.93	0.49	1.04	0.42	0.46

75:   PTK7: PTK7 protein tyrosine kinase 7

P-value: **1.1E-03**   **Fold change: 2.2**

Chromosome: 6p21.1-p12.2   Genbank: NM_002821.3   Gene ID: hCG2001916

Biological process: signal transduction;protein amino acid phosphorylation;cell adhesion

Molecular function: ATP binding; protein binding; protein-tyrosine kinase, transmembrane receptor protein tyrosine kinase, transferase and receptor activity

Cellular component: integral to plasma membrane

Expression levels in ALT cell lines   **median: 1.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.47	1.60	1.61	1.37	2.05	1.58	1.02	2.95	0.64	1.12	1.05	0.56

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	0.60	0.68	0.77	1.22	1.22	0.37	0.95	0.39	0.46	0.14	0.64
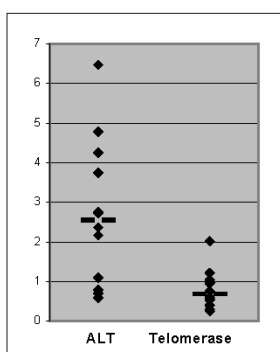
76:   USP51: ubiquitin specific protease 51

P-value: **1.1E-03**   **Fold change: 3.7**

Chromosome: Xp11.22

Genbank: AI368143   Gene ID: hCG1685885.2

Biological process: ubiquitin cycle; ubiquitin-dependent protein catabolism

Molecular function: cysteine type endopeptidase and ubiquitin thioesterase activity; Zn ion binding. Cellular component:

Expression levels in ALT cell lines   **median: 2.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.69	0.78	3.75	1.09	2.73	4.25	0.58	6.47	4.78	2.36	2.16	2.76

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.02	0.76	0.95	0.53	0.40	1.22	0.25	0.55	1.04	0.96	0.29	0.60
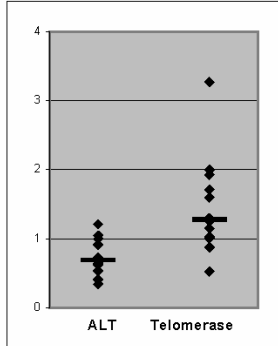
77: FLJ14803: hypothetical protein FLJ14803

P-value: 1.1E-03   **Fold change:** 0.5

Chromosome: 7q32.3

Genbank: NM_032842.1   Gene ID: hCG18972.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.72	1.00	0.91	1.21	0.63	0.34	0.53	0.99	0.62	1.05	0.41	0.66

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.52	1.15	1.25	1.30	1.60	0.87	1.03	2.00	1.71	3.27	1.92	1.00

78: N/A

P-value: 1.1E-03   **Fold change:** 7.3

Chromosome:

Genbank: Gene ID: hCG38403.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 4.0**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.95	4.24	3.69	0.25	10.02	8.57	11.08	7.25	1.39	1.03	15.71	0.12

Expression levels in Telomerase cell lines   **median: 0.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.85	0.08	0.16	0.15	0.97	0.56	0.06	1.79	0.53	0.13	1.37	0.62

79: TM4SF10: transmembrane 4 superfamily member 10

P-value: 1.1E-03   **Fold change:** 18.0

Chromosome: Xp11.4

Genbank: NM_031442.2   Gene ID: hCG1747231.2

Biological process:

Molecular function:

Cellular component: integral to membrane
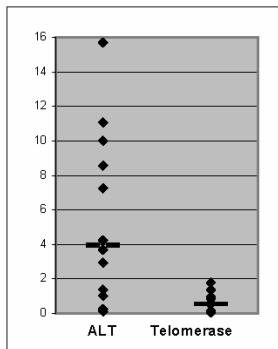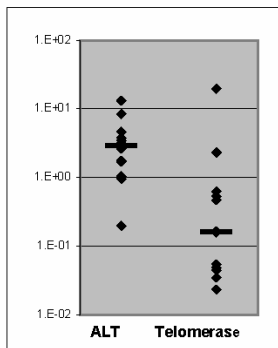
Expression levels in ALT cell lines   **median: 2.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.77	2.64	0.95	4.63	8.43	1.75	3.17	3.46	1.05	0.20	13.11	1.71

Expression levels in Telomerase cell lines   **median: 0.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.05	0.47	0.62	0.05	0.16	0.04	0.03	0.02	2.31	0.16	0.53	19.65

80: MAGED4: melanoma antigen, family D, 4

P-value: 1.1E-03   **Fold change:** 2.4

Chromosome: X

Genbank: NM_177535.1   Gene ID: hCG32481.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 1.3**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.60	1.44	1.37	1.31	1.48	1.17	1.30	3.30	1.98	0.02	0.94	0.57

Expression levels in Telomerase cell lines   **median: 0.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.70	1.14	1.07	0.77	0.73	1.06	0.01	0.39	0.13	0.11	0.10	0.34
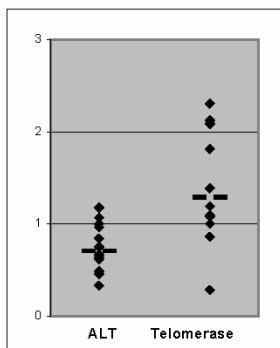
81:   SLC19A1: solute carrier family 19 (folate transporter), member 1

P-value:	**1.1E-03**		**Fold change: 0.5**

Chromosome: 21q22.3

Genbank: NM_194255.1   Gene ID: hCG401276.3

Biological process: transport

Molecular function: folic acid binding;reduced folate carrier activity

Cellular component: membrane

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.96	1.07	0.48	1.00	0.64	0.61	0.75	0.66	0.84	1.18	0.33	0.45

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.08	2.08	1.39	1.00	2.12	1.09	0.86	2.31	2.13	1.81	1.19	0.28

82:   EPS8L3: EPS8-like 3

P-value:	**1.1E-03**		**Fold change: 0.6**

Chromosome: 1p13.2

Genbank: NM_024526.2   Gene ID: hCG40254.3

Biological process:

Molecular function: receptor activity

Cellular component:

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	1.07	0.33	0.59	0.92	0.87	0.58	0.96	0.93	0.86	0.30	0.62

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.14	2.22	1.61	0.81	1.36	1.04	1.26	2.15	2.54	1.34	1.57	0.57
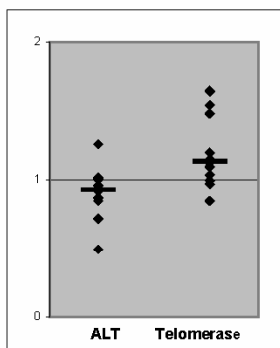
83:   PGAM1: phosphoglycerate mutase 1 (brain)

P-value:	**1.1E-03**		**Fold change: 0.8**

Chromosome: 10q25.3   Genbank: BE827571

Gene ID: hCG2015269; hCG2015269; hCG25778.3; hCG2015138

Biological process: glycolysis

Molecular function: bisphosphoglycerate mutase and phosphatase activity; phosphoglycerate mutase activity. Cellular component: cytosol

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.49	0.72	0.94	0.93	1.01	0.87	0.92	0.96	1.00	0.93	1.26	0.85

Expression levels in Telomerase cell lines   **median: 1.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.20	1.64	0.97	1.54	1.09	1.00	1.12	1.65	1.15	1.48	1.03	0.85

84:   N/A

P-value:	**1.1E-03**		**Fold change: 0.7**

Chromosome: 6

Genbank: AA897384   Gene ID: hCG17797.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.8**
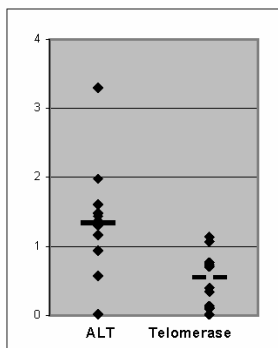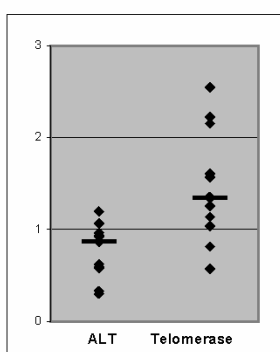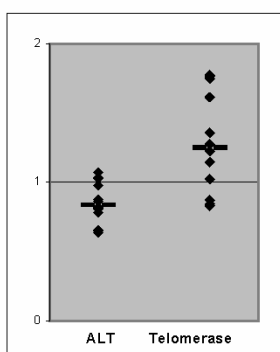
1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.03	0.88	0.64	0.86	0.66	0.78	1.07	0.82	1.03	0.98	0.65	0.81

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.77	1.28	1.02	0.84	1.61	1.23	1.15	0.83	1.76	1.36	1.75	0.87
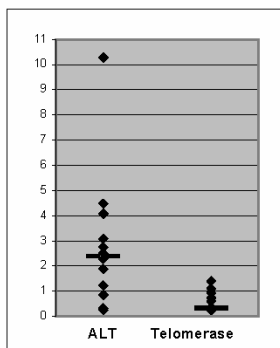
85: KCTD8: potassium channel tetramerisation domain containing 8

P-value:	1.2E-03							Fold change:	7.1		

Chromosome: 4p13

Genbank: H18367   Gene ID: hCG1647805.3

Biological process:

Molecular function:

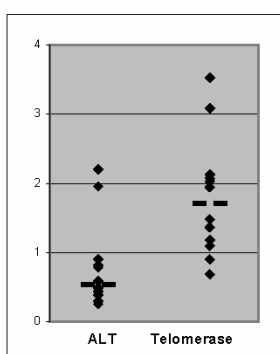Cellular component:

Expression levels in ALT cell lines — median: 2.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.33	3.08	1.88	0.85	1.23	4.48	4.08	2.28	10.28	2.75	0.25	2.50

Expression levels in Telomerase cell lines — median: 0.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.40	0.25	0.72	0.25	0.30	0.33	0.25	0.25	0.60	1.10	0.93	0.35

86: IL27RA: interleukin 27 receptor, alpha

P-value:	1.2E-03							Fold change:	0.3		

Chromosome: 19p13.11

Genbank: NM_004843.2   Gene ID: hCG27523.2

Biological process: cell surface receptor linked signal transduction;immune response

Molecular function: transmembrane receptor activity

Cellular component: integral to plasma membrane
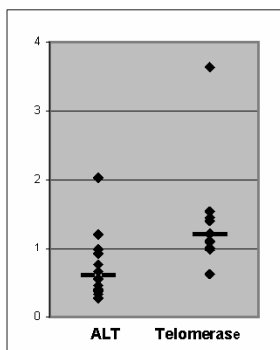
Expression levels in ALT cell lines — median: 0.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.96	2.20	0.26	0.44	0.49	0.78	0.39	0.30	0.90	0.59	0.39	0.82

Expression levels in Telomerase cell lines — median: 1.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.03	1.10	2.08	1.18	1.95	3.09	1.48	0.69	0.90	3.53	2.13	1.37

87: : Clone IMAGE:5311129, mRNA

P-value:	1.4E-03							Fold change:	0.5		

Chromosome: 9

Genbank: BC045702   Gene ID: hCG1814693.1

Biological process:

Molecular function:

Cellular component:
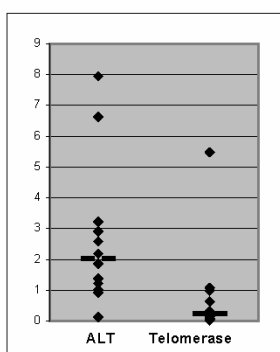
Expression levels in ALT cell lines — median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.91	0.99	0.45	0.37	0.39	0.76	2.03	0.33	1.21	0.55	0.27	0.66

Expression levels in Telomerase cell lines — median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.21	1.21	0.62	1.11	1.10	1.22	1.45	1.54	1.01	3.64	0.97	1.40

88: SRPX: sushi-repeat-containing protein, X-linked

P-value:	1.4E-03							Fold change:	8.2		

Chromosome: Xp21.1

Genbank: NM_006307.2   Gene ID: hCG18713.3

Biological process: cell adhesion

Molecular function: protein binding

Cellular component: membrane

Expression levels in ALT cell lines — median: 2.0

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.59	0.13	1.37	2.90	6.62	1.22	1.01	7.94	2.18	3.22	0.93	1.86

Expression levels in Telomerase cell lines — median: 0.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.32	0.09	1.08	0.63	0.15	0.17	0.02	0.19	0.31	0.05	5.48	0.99
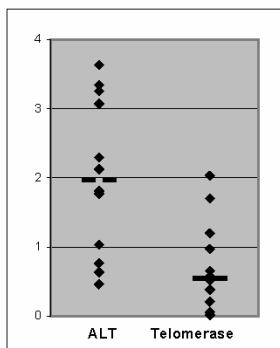
89:   SLC16A2: solute carrier family 16 (monocarboxylic acid transporters), member 2 (putative transporter)

P-value: 1.4E-03   Fold change: 3.6

Chromosome: Xq13.2   Genbank: NM_006517.1   Gene ID: hCG21949.2

Biological process: transport; monocarboxylic acid transport. Molecular function: transporter activity; monocarboxylic acid transporter activity; symporter activity

Cellular component: integral to plasma membrane; membrane fraction

Expression levels in ALT cell lines   median: 2.0

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.76	1.03	3.64	3.34	3.26	2.12	1.77	0.63	0.46	2.30	3.07	1.81

Expression levels in Telomerase cell lines   median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.57	1.20	1.70	0.38	0.51	0.38	0.01	0.05	0.65	0.97	0.21	2.03
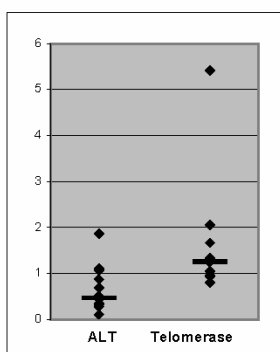
90:   N/A

P-value: 1.4E-03   Fold change: 0.4

Chromosome:

Genbank: Gene ID: hCG2033356

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.5
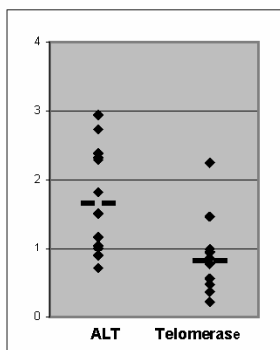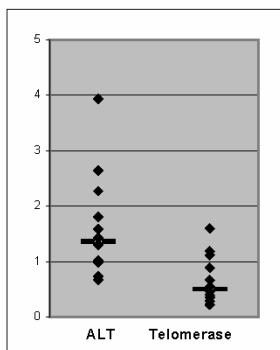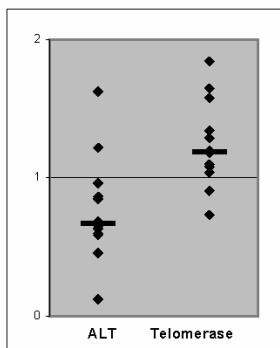
1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.11	0.33	1.11	0.35	0.28	1.07	0.51	0.69	0.87	1.87	0.44	0.32

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.27	0.93	2.06	1.05	0.96	1.04	1.25	1.67	5.41	1.33	1.34	0.80

91:   ACVR2: activin A receptor, type II

P-value: 1.4E-03   Fold change: 2.1

Chromosome: 2q22.2-q23.3   Genbank: NM_001616.2   Gene ID: hCG19691.3

Biological process: protein amino acid phosphorylation; transmembrane receptor protein serine/threonine kinase signaling pathway

Molecular function: ATP binding; receptor, transferase and transforming growth factor beta receptor activity.   Cellular component: integral to plasma membrane

Expression levels in ALT cell lines   median: 1.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.17	2.32	2.94	2.73	2.38	1.82	1.51	0.89	2.29	1.00	0.70	1.04

Expression levels in Telomerase cell lines   median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.00	0.36	0.76	0.78	2.25	0.85	0.47	1.47	0.95	0.22	0.93	0.55

92:   FLJ10357

P-value: 1.4E-03   Fold change: 2.7

Chromosome: 14q11.2

Genbank: NM_018071.2   Gene ID: hCG1812753.2

Biological process: regulation of Rho signal transduction

Molecular function: Rho guanyl-nucleotide exchange factor activity

Cellular component: intracellular

Expression levels in ALT cell lines   median: 1.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.80	0.73	0.67	1.30	2.27	0.99	1.03	3.93	2.64	1.59	1.01	1.42

Expression levels in Telomerase cell lines   median: 0.5

| 6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.89	0.67	0.55	0.22	1.12	0.40	0.29	0.36	0.46	1.60	0.36	1.19

93: TRIP6: thyroid hormone receptor interactor 6

P-value: **1.4E-03** **Fold change: 0.6**

Chromosome: 7q22

Genbank: NM_003302.1 Gene ID: hCG20459.3

Biological process:

Molecular function: thyroid hormone receptor binding; protein binding

Cellular component:

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.63	0.59	0.86	1.22	0.96	0.46	0.65	1.62	0.69	0.12	0.85	0.60

Expression levels in Telomerase cell lines median: 1.2

| 6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.19	1.18	1.65	0.91	1.34	1.10	1.08	0.73	1.29	1.84	1.58	1.04
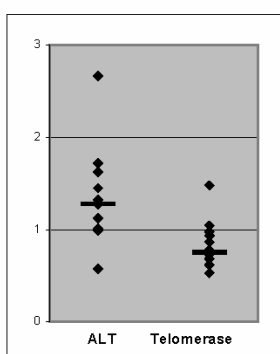
94: FLJ40629: hypothetical protein FLJ40629

P-value: **1.4E-03** **Fold change: 1.7**

Chromosome: 2q14.1

Genbank: NM_152515.2 Gene ID: hCG16261.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.12	2.66	1.32	1.28	1.13	1.45	0.99	0.58	1.63	1.28	1.72	1.01

Expression levels in Telomerase cell lines median: 0.8

| 6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.68	0.73	1.05	0.62	0.62	0.93	0.78	0.53	1.48	0.68	0.98	0.87
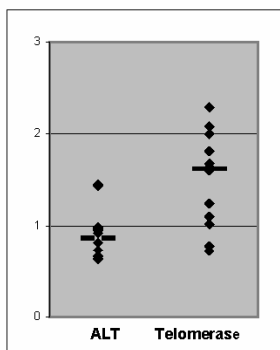
95: SERP1: stress-associated endoplasmic reticulum protein 1

P-value: **1.4E-03** **Fold change: 0.5**

Chromosome: 3q25.1 Genbank: NM_014445.2 Gene ID: hCG21346.3

Biological process: plasma membrane organization and biogenesis;protein amino acid glycosylation;response to stress

Molecular function:

Cellular component: ribosome;endoplasmic reticulum

Expression levels in ALT cell lines median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.96	0.82	0.95	0.92	0.66	0.63	0.63	1.44	0.98	1.45	0.66	0.73

Expression levels in Telomerase cell lines median: 1.6

| 6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.60	2.00	1.24	1.02	2.29	0.78	0.72	1.81	1.68	1.10	2.08	1.64
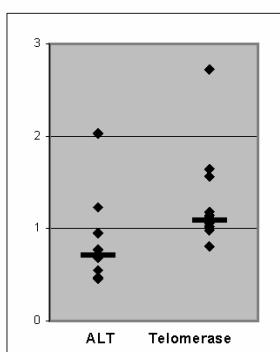
96: YWHAG: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide

P-value: **1.4E-03** **Fold change: 0.7**

Chromosome: 7q11.23 Genbank: NM_012479.2 Gene ID: hCG41357.2

Biologl procss:cytoskeltn organisatn&biogensis; inhibit apoptosis&protnkinase; regulatn mitosis, neuron differentiatn, signal transductn & synaptic plasticity. Molec. functn:actin, insulinlike g.f. receptr & protn kinaseC bindg; protn kinaseC inhibitn. Cell compont:cytoplsm

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.46	0.70	0.73	0.95	0.72	0.47	0.55	0.77	1.23	2.03	0.69	0.69

Expression levels in Telomerase cell lines median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	1.56	0.81	1.03	1.64	1.18	1.12	1.02	1.07	2.72	1.06	1.14
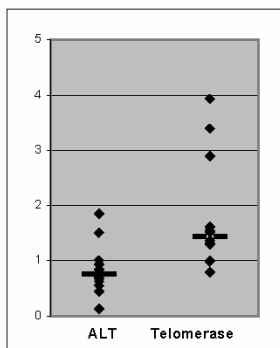
97: IMPDH1: IMP (inosine monophosphate) dehydrogenase 1

P-value: 1.4E-03   Fold change: 0.5

Chromosome: 7q31.3-q32

Genbank: NM_000883.2   Gene ID: hCG1811280.1

Biological process: GMP & GTP biosynthesis; response to stimulus; visual perception

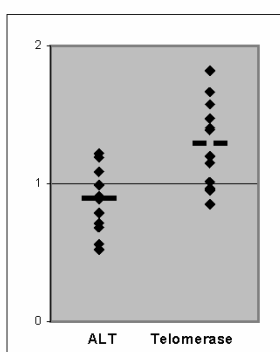Molecular function: IMP dehydrogenase activity;K ion binding; oxidoreductase activity

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.93	0.68	0.13	0.80	1.01	0.55	0.72	1.85	0.83	1.51	0.44	0.62

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.52	3.39	1.30	0.99	3.93	1.36	1.61	2.90	0.98	1.54	0.79	1.32

98: N/A

P-value: 1.4E-03   Fold change: 0.7

Chromosome:

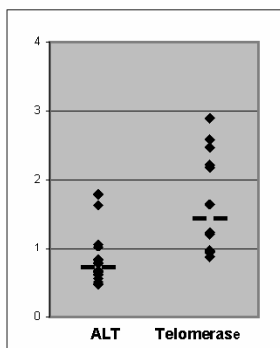Genbank: Gene ID: hCG27427.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.22	0.99	1.09	0.52	0.68	0.56	0.71	0.91	0.79	0.89	1.19	0.90

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.96	1.47	1.01	1.20	1.39	1.66	0.95	1.82	1.40	1.15	1.57	0.85

99: : QV3-CT0192-021099-002-e11 CT0192 Homo sapiens cDNA, mRNA sequence.

P-value: 1.4E-03   Fold change: 0.5

Chromosome: 11   Genbank: AW752159   Gene ID: hCG1774546.2
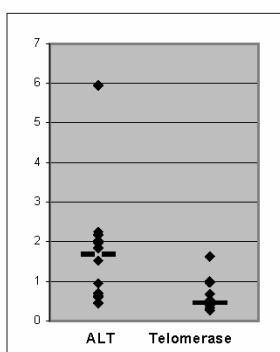
Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.79	0.83	0.77	0.56	0.66	1.02	1.06	0.65	0.61	1.63	0.47	0.50

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.22	2.18	0.98	0.93	1.23	0.87	1.64	0.95	2.59	2.47	1.21	2.90

100: REPS2: RALBP1 associated Eps domain containing 2

P-value: 1.6E-03   Fold change: 3.6

Chromosome: Xp22.2

Genbank: NM_004726.1   Gene ID: hCG1817839.1

Biological process: EGFR signaling pathway; protein complex assembly

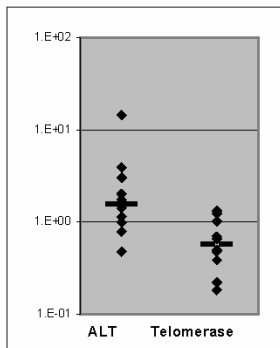Molecular function: Ca ion binding; protein binding

Cellular component:

Expression levels in ALT cell lines   median: 1.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.70	1.85	2.18	5.95	2.25	2.03	1.53	0.45	0.95	0.60	0.63	1.98

Expression levels in Telomerase cell lines   median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.00	0.97	0.40	0.33	0.48	1.63	0.43	0.53	0.28	0.68	0.40	0.45

101: MGC22773: hypothetical protein MGC22773

P-value: **1.6E-03**   **Fold change:** **2.7**

Chromosome: 1p31.1

Genbank: NM_145258.1   Gene ID: hCG1657579.3

Biological process:

Molecular function:

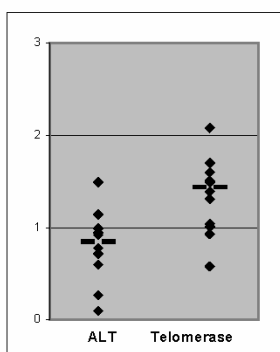Cellular component:

Expression levels in ALT cell lines   **median: 1.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.91	0.79	0.99	0.99	0.48	1.41	2.02	14.40	2.99	1.74	1.14	3.03

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.39	1.32	0.66	0.50	0.70	1.03	1.23	0.22	0.39	1.01	0.48	0.18

102: ZNF586: zinc finger protein 586

P-value: **1.6E-03**   **Fold change:** **0.6**

Chromosome: 19q13.43

Genbank: NM_017652.1   Gene ID: hCG1773387.2

Biological process: regulation of transcription, DNA-dependent

Molecular function: nucleic acid binding

Cellular component: intracellular
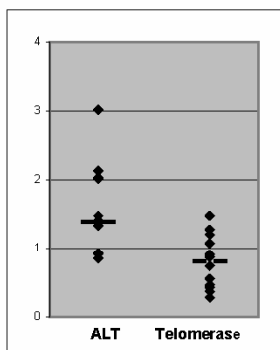
Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.14	1.49	0.92	0.27	0.72	0.78	0.72	0.99	0.10	0.60	0.94	0.95

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.70	1.04	0.93	2.08	1.39	1.49	1.01	1.51	1.31	1.60	1.50	0.58

103: : CDNA: FLJ21778 fis, clone HEP00201

P-value: **1.8E-03**   **Fold change:** **1.7**

Chromosome: 4

Genbank: AK075468   Gene ID: hCG1818302.1

Biological process:

Molecular function:

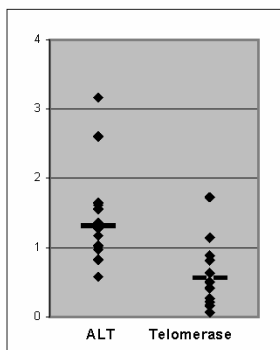Cellular component:

Expression levels in ALT cell lines   **median: 1.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.91	1.48	0.93	0.85	2.02	2.13	1.37	1.41	2.04	0.93	3.02	1.33

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.20	0.43	0.28	0.46	0.56	0.91	1.28	1.48	0.74	1.07	0.87	0.37

104: C2orf13: chromosome 2 open reading frame 13

P-value: **1.9E-03**   **Fold change:** **2.3**

Chromosome: 2p13.3

Genbank: NM_173545.1   Gene ID: hCG1986872.1

Biological process:

Molecular function:

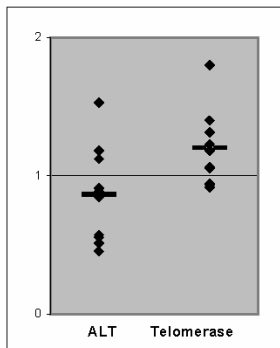Cellular component:

Expression levels in ALT cell lines   **median: 1.3**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.60	1.56	0.58	1.03	1.17	1.28	1.62	1.65	0.83	3.17	0.97	1.36

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.15	0.50	0.89	0.42	0.07	0.22	0.81	0.17	0.64	1.73	0.27	1.15

105: N/A

P-value:	1.9E-03							Fold change:	0.7	

Chromosome:

Genbank: Gene ID: hCG2009963

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.12	0.91	0.85	0.89	0.85	0.57	0.45	0.51	0.55	1.18	0.91	1.53

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.23	1.40	0.94	1.23	0.94	1.18	1.06	1.80	1.32	1.23	1.06	0.92
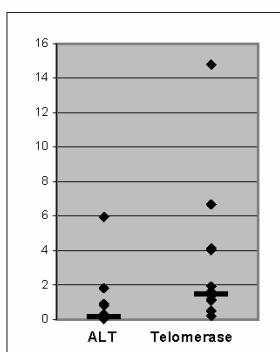
106: CYP2S1: cytochrome P450, family 2, subfamily S, polypeptide 1

P-value:	1.9E-03							Fold change:	0.1	

Chromosome: 19q13.1

Genbank: NM_030622.5   Gene ID: hCG22316.4

Biological process: electron transport

Molecular function: monooxygenase activity

Cellular component: endoplasmic reticulum; membrane; microsome

Expression levels in ALT cell lines   **median: 0.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
5.95	0.19	0.10	0.16	0.03	0.90	0.82	0.03	1.82	0.12	0.04	0.31

Expression levels in Telomerase cell lines   **median: 1.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.38	4.12	1.16	1.62	4.02	1.92	0.21	1.17	6.67	14.78	1.10	0.51
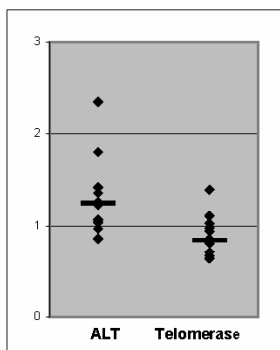
107: VPS13D: vacuolar protein sorting 13D (yeast)

P-value:	1.9E-03							Fold change:	1.5	

Chromosome: 1p36.21

Genbank: AK074350   Gene ID: hCG2005521

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 1.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.23	1.26	1.36	0.85	1.04	0.86	1.80	2.35	1.42	1.80	1.06	0.97

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.94	1.39	0.86	0.83	0.67	0.63	0.83	1.03	1.11	0.97	0.71	0.81
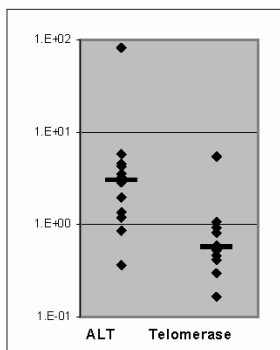
108: LEF1: lymphoid enhancer-binding factor 1

P-value:	1.9E-03							Fold change:	5.3	

Chromosome: 4q23-q25

Genbank: NM_016269.2   Gene ID: hCG21073.3

Biological process: regulation of transcription, DNA-dependent; Wnt receptor signaling pathway

Molecular function: DNA binding

Cellular component: nucleus

Expression levels in ALT cell lines   **median: 3.0**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.36	0.36	4.28	5.77	81.50	1.19	0.86	3.54	3.24	4.56	1.97	2.86

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.53	0.30	0.81	0.60	1.07	0.56	0.17	0.93	0.46	0.93	5.44	0.41
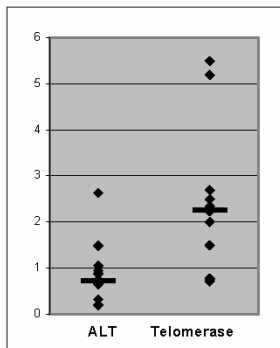
109: MYO10: myosin X

P-value:	**1.9E-03**	**Fold change:**	**0.3**

Chromosome: 5p15.1-p14.3

Genbank: NM_012334.1   Gene ID: hCG37573.2

Biological process: neuropeptide signaling pathway

Molecular function: motor activity; actin binding; ATP binding

Cellular component: cytoskeleton;myosin

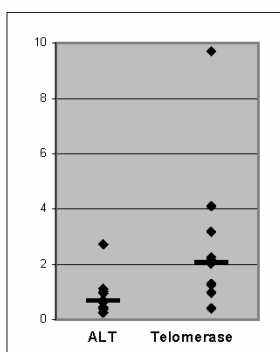**Expression levels in ALT cell lines** median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.20	0.66	0.70	0.64	2.63	0.95	0.32	0.88	1.05	0.70	0.75	1.49

Expression levels in Telomerase cell lines median: 2.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.77	2.49	2.00	2.34	1.49	0.71	0.75	2.23	5.19	5.49	2.69	2.29

110: N/A

P-value:	**1.9E-03**	**Fold change:**	**0.3**

Chromosome:

Genbank: Gene ID: R26_hCT1844408.1

Biological process:

Molecular function:

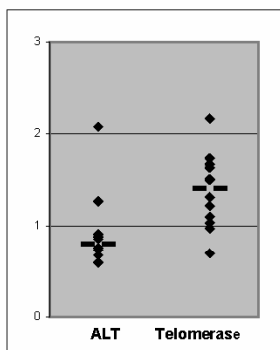Cellular component:

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.41	0.27	0.96	1.01	0.63	0.72	1.13	0.68	0.96	2.73	0.46	0.67

Expression levels in Telomerase cell lines median: 2.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.41	0.98	4.11	1.25	0.99	2.13	2.26	1.30	9.70	2.04	3.18	2.16

111: CLK3: CDC-like kinase 3

P-value:	**1.9E-03**	**Fold change:**	**0.6**

Chromosome: 15q24   Genbank: NM_001292.1   Gene ID: hCG1739025.2

Biological process: protein amino acid phosphorylation

Molecular function: transferase activity; ATP binding; protein serine/threonine kinase activity; protein-tyrosine kinase activity

Cellular component: nucleus

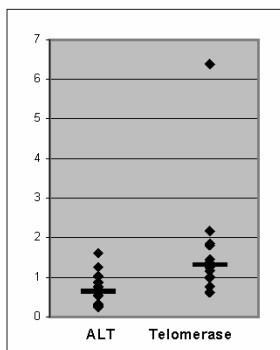**Expression levels in ALT cell lines** median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.85	0.59	0.73	0.85	0.87	0.67	0.90	0.75	1.27	2.08	0.59	0.60

Expression levels in Telomerase cell lines median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.51	1.67	1.22	0.97	1.31	1.10	0.69	2.17	1.74	1.63	1.50	1.03

112: TACC2: transforming, acidic coiled-coil containing protein 2

P-value:	**1.9E-03**	**Fold change:**	**0.5**

Chromosome: 10q26

Genbank: NM_006997.1   Gene ID: hCG41426.3

Biological process:

Molecular function:

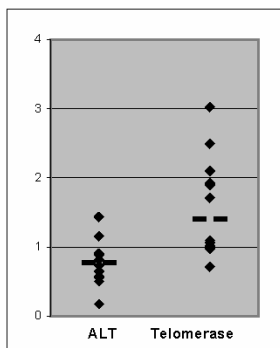Cellular component: nucleus

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.61	1.03	0.25	0.77	0.27	0.28	0.33	0.87	0.63	1.26	0.54	0.68

Expression levels in Telomerase cell lines median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.01	2.17	0.62	1.26	1.38	0.77	1.84	1.81	0.99	6.38	1.45	1.16

113: THADA: thyroid adenoma associated

P-value: **1.9E-03**   **Fold change: 0.5**

Chromosome: 2p22.1

Genbank: BC037990   Gene ID: hCG1643320.3

Biological process:

Molecular function:

Cellular component:
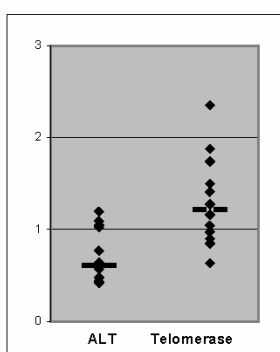
Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.50	1.43	0.91	0.73	1.15	0.56	0.17	0.57	0.81	0.89	1.44	0.64

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.99	1.06	1.90	1.92	1.71	0.97	2.49	2.10	1.09	3.03	1.01	0.71

114: N/A

P-value: **1.9E-03**   **Fold change: 0.5**

Chromosome:

Genbank: Gene ID: hCG24202.3

Biological process:

Molecular function:

Cellular component:

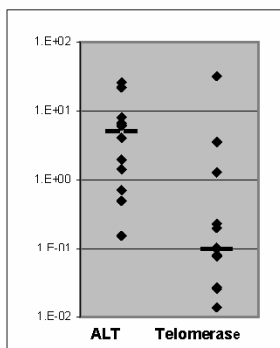**Expression levels in ALT cell lines**   **median: 0.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.05	1.20	0.44	0.48	0.58	0.57	0.64	1.03	0.77	1.09	0.48	0.42

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.85	1.88	0.90	1.28	2.35	1.04	0.97	1.74	1.41	1.16	1.50	0.63

115: N/A

P-value: **1.9E-03**   **Fold change: 51.0**

Chromosome:

Genbank: Gene ID: R26_hCT18705.1

Biological process:

Molecular function:

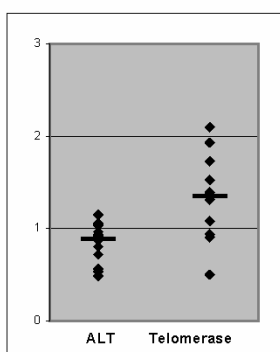Cellular component:

Expression levels in ALT cell lines   **median: 5.1**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
25.87	21.90	8.06	0.50	1.43	1.97	4.10	6.67	6.23	0.71	6.08	0.15

Expression levels in Telomerase cell lines   **median: 0.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
32.03	1.29	0.10	0.10	0.08	0.03	0.23	3.56	0.03	0.08	0.20	0.01

116: POLR3K: polymerase (RNA) III (DNA directed) polypeptide K, 12.3 kDa

P-value: **1.9E-03**   **Fold change: 0.7**

Chromosome: 16p13.3   Genbank: NM_016310.2   Gene ID: hCG1787292.2

Biological process: RNA elongation; transcription; regulation of transcription, DNA-dependent; transcription from Pol III promoter

Molecular function: transferase, DNA-directed RNA polymerase and transcription factor activity. Cellular component: nucleus; DNA-directed RNA polymerase III complex

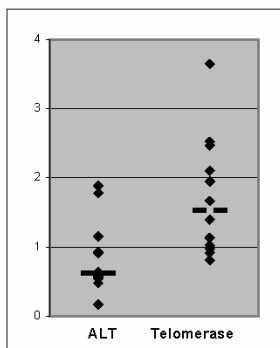**Expression levels in ALT cell lines**   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.05	0.53	1.15	1.03	0.91	0.72	0.49	0.81	0.87	0.94	0.56	0.97

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.09	1.39	0.91	1.31	0.94	1.08	1.73	1.93	1.52	1.34	1.37	0.50

117: AP1S3: adaptor-related protein complex 1, sigma 3 subunit

P-value: 1.9E-03   Fold change: 0.4

Chromosome: 2q36.3

Genbank: NM_178814.2   Gene ID: hCG21436.2

Biological process: endocytosis;intracellular protein transport

Molecular function: protein transporter activity

Cellular component: clathrin vesicle coat;coated pit;AP-1 adaptor complex;Golgi trans face

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.16	1.88	0.91	0.48	0.56	0.55	0.93	0.64	1.78	0.17	0.59	0.61

Expression levels in Telomerase cell lines   median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.02	2.52	0.98	0.81	1.67	1.40	3.64	2.10	1.95	2.47	1.13	0.92
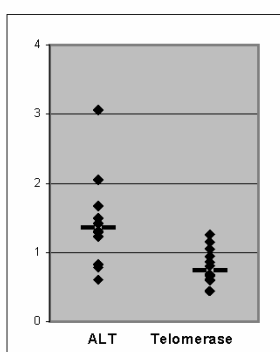
118: SCARB2: scavenger receptor class B, member 2

P-value: 1.9E-03   Fold change: 1.8

Chromosome: 4q21.21-q21.22

Genbank: NM_005506.2   Gene ID: hCG23840.2

Biological process: cell adhesion

Molecular function: receptor activity

Cellular component: integral to plasma membrane; lysosomal membrane; membrane fraction

Expression levels in ALT cell lines   median: 1.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.30	3.06	0.83	0.61	1.68	2.05		1.50	1.43	0.79	1.31	1.42	1.23

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.60	1.05	0.68	0.95	0.86	0.69	1.15	0.66	0.45	0.61	0.81	1.26
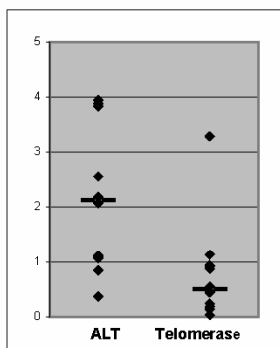
119: FLJ23033: hypothetical protein FLJ23033

P-value: 1.9E-03   Fold change: 4.3

Chromosome: 1p22.3

Genbank: NM_024686.3   Gene ID: hCG24792.3

Biological process: protein modification
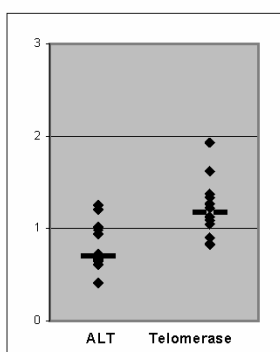
Molecular function: tubulin-tyrosine ligase activity

Cellular component:

Expression levels in ALT cell lines   median: 2.1

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.08	3.95	3.89	3.83	0.84	1.10	2.56	3.88	1.11	1.06	0.37	2.19

Expression levels in Telomerase cell lines   median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.24	0.04	0.94	0.91	0.44	0.14	3.29	0.54	0.18	0.87	0.46	1.13

120: MGEA5: meningioma expressed antigen 5 (hyaluronidase)

P-value: 1.9E-03   Fold change: 0.6

Chromosome: 10q24.1-q24.3

Genbank: NM_012215.1   Gene ID: hCG25645.3

Biological process: glycoprotein catabolism

Molecular function: hyaluronoglucosaminidase activity
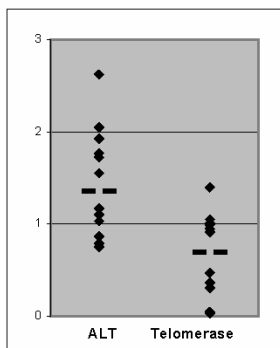
Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.65	0.73	1.25	0.61	0.94	0.67	0.41	1.20	0.68	1.02	0.98	0.65

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.27	1.93	0.83	1.62	1.22	1.13	0.90	1.09	1.05	1.37	0.83	1.34

121: ZNF215: zinc finger protein 215

P-value:	**1.9E-03**	**Fold change: 2.0**

Chromosome: 11p15.4

Genbank: NM_013250.1   Gene ID: hCG24083.4

Biological process: regulation of transcription, DNA-dependent

Molecular function: nucleic acid binding; transcription factor activity; zinc ion binding

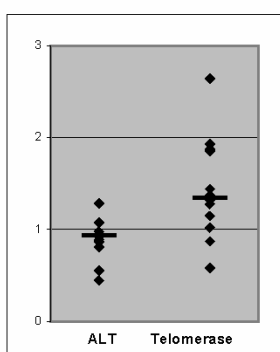Cellular component: nucleus

Expression levels in ALT cell lines   **median: 1.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.86	2.05	1.72	0.79	1.77	1.03	1.55	1.17	2.62	1.93	0.75	1.10

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.40	0.99	0.30	0.36	1.01	0.47	0.03	0.04	0.94	1.05	0.03	0.91

122: FAM18B: myocardin-related transcription factor B

P-value:	**1.9E-03**	**Fold change: 0.7**

Chromosome: 17p11.2

Genbank: NM_016078.1   Gene ID: hCG1997516

Biological process: regulation of transcription, DNA-dependent

Molecular function: DNA binding

Cellular component: nucleus

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.08	0.92	0.98	0.87	1.28	0.98	0.81	0.98	0.95	0.89	0.55	0.45

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.02	1.87	1.44	0.87	1.85	0.58	1.15	2.64	1.32	1.93	1.28	1.37
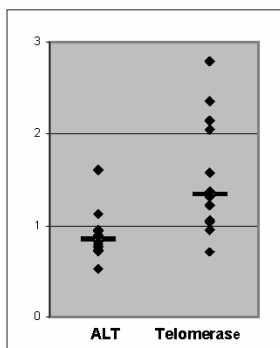
123: EBRP: emopamil binding related protein, delta8-delta7 sterol isomerase related protein

P-value:	**1.9E-03**	**Fold change: 0.6**

Chromosome: 13q12-q13   Genbank: NM_032565.1   Gene ID: hCG1643106.4

Biological process: sterol metabolism

Molecular function: cholestenol delta-isomerase activity

Cellular component: endoplasmic reticulum; integral to membrane

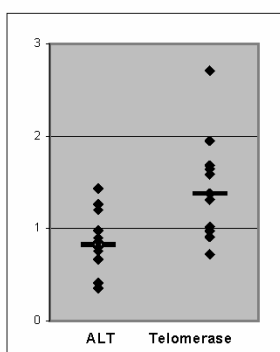**Expression levels in ALT cell lines**   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.95	0.83	0.96	0.77	0.80	0.74	0.71	0.52	0.90	1.61	0.89	1.13

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.95	1.06	1.22	1.32	2.14	0.71	1.37	1.58	2.36	2.79	2.05	1.04

124: LOC51270: E2F-like protein

P-value:	**1.9E-03**	**Fold change: 0.6**

Chromosome: Xq26.2

Genbank: NM_016521.2   Gene ID: hCG1643568.2

Biological process: regulation of transcription, DNA-dependent;regulation of cell cycle

Molecular function: transcription factor activity

Cellular component: transcription factor complex
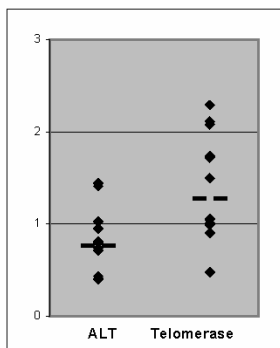
Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.80	0.76	0.41	0.35	0.98	0.66	0.86	1.20	1.26	0.90	0.67	1.43

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.58	0.98	1.37	2.71	1.64	0.72	1.31	1.38	1.95	0.91	1.68	1.02

125: ADP-GK: ATP-dependent glucokinase

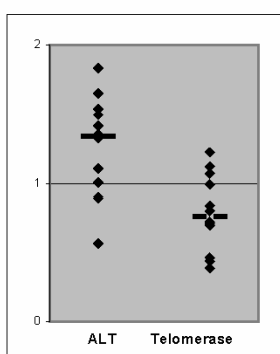| P-value: | 1.9E-03 | | | | | | Fold change: | 0.6 | | | |

Chromosome: 15q22.33
Genbank: NM_031284.3   Gene ID: hCG1787419.2
Biological process: glycolysis
Molecular functn: phosphotransferase activity, alcohol group as acceptor, Mg ion bimding
Cellular component:

Expression levels in ALT cell lines — median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.41	0.74	0.43	0.81	0.95	0.80	0.71	0.41	1.03	1.44	0.40	0.40

Expression levels in Telomerase cell lines — median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.72	2.08	1.06	0.90	2.29	1.01	0.48	2.11	1.50	1.73	1.05	0.99

126: LOC338645: hypothetical protein LOC338645

| P-value: | 1.9E-03 | | | | | | Fold change: | 1.8 | | | |

Chromosome: 11p14.3
Genbank: AY164460   Gene ID: hCG1991034
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines — median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.89	0.90	1.33	0.57	1.83	1.65	1.54	1.50	1.11	1.42	1.35	1.01

Expression levels in Telomerase cell lines — median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.39	1.23	1.12	0.70	0.80	0.84	0.71	0.46	1.07	0.44	0.99	0.72
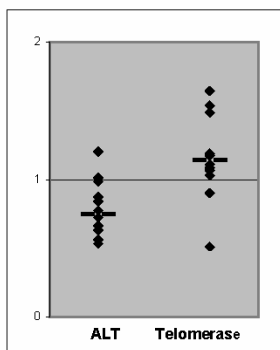
127: GYG:

| P-value: | 1.9E-03 | | | | | | Fold change: | 0.7 | | | |

Chromosome: 3q24-q25.1
Genbank: BF174009   Gene ID: hCG1757059.2
Biological process: glycogen biosynthesis
Molecular function: glycogenin glucosyltransferase activity
Cellular component:

Expression levels in ALT cell lines — median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.88	1.21	0.99	0.64	0.73	0.63	0.78	0.57	0.54	1.01	0.67	0.84

Expression levels in Telomerase cell lines — median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.03	1.49	1.09	1.11	1.54	1.19	0.51	1.19	1.64	1.18	1.07	0.90
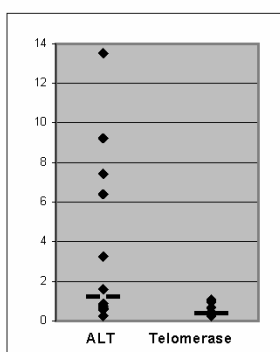
128: ZDHHC15: zinc finger, DHHC domain containing 15
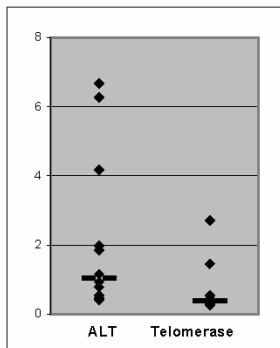
| P-value: | 2.0E-03 | | | | | | Fold change: | 3.1 | | | |

Chromosome: Xq13.2
Genbank: NM_144969.1   Gene ID: hCG20331.3
Biological process: regulation of transcription, DNA-dependent
Molecular function: transcription factor activity; zinc ion binding
Cellular component: nucleus; integral to membrane

Expression levels in ALT cell lines — median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.68	0.88	13.53	0.78	0.60	7.43	3.25	9.22	6.40	0.58	0.25	1.60

Expression levels in Telomerase cell lines — median: 0.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.97	0.45	0.25	0.40	0.30	0.25	0.25	0.25	0.68	1.08	0.40	0.45

129: FLJ30046: hypothetical protein FLJ30046

P-value:	2.1E-03	Fold change: 2.8

Chromosome: 13q22.1
Genbank: NM_144595.2   Gene ID: hCG32998.2
Biological process:
Molecular function:
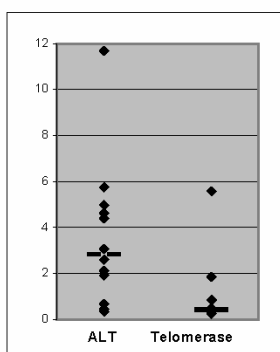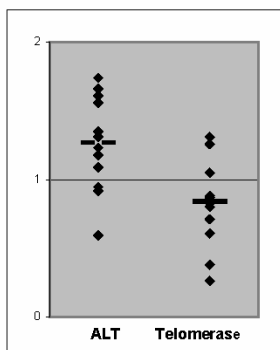Cellular component:

Expression levels in ALT cell lines   median: 1.0

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
4.17	0.93	6.67	0.45	0.40	6.28	0.78	0.53	1.15	1.98	0.43	1.85

Expression levels in Telomerase cell lines   median: 0.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.70	0.25	0.25	0.38	0.43	0.43	0.30	0.53	0.38	0.33	1.45	0.25

130: NSE1: NSE1

P-value:	2.1E-03	Fold change: 6.7

Chromosome: 2p25.1
Genbank: NM_145175.1   Gene ID: hCG1644052.4
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines   median: 2.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.08	4.40	0.45	1.93	11.68	5.75	2.13	2.60	4.63	0.35	4.98	0.68

Expression levels in Telomerase cell lines   median: 0.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.33	0.28	0.28	0.45	0.33	0.25	0.50	0.40	0.50	0.85	5.57	1.85

131: DNAJA5

P-value:	2.1E-03	Fold change: 1.5

Chromosome: 5p13.3
Genbank: NM_194283.1   Gene ID: hCG37729.3
Biological process: protein folding
Molecular function: heat shock protein, unfolded protein, nucleic acid and zinc ion binding
Cellular component: nucleus

Expression levels in ALT cell lines   median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.95	1.56	1.23	1.74	0.60	1.18	1.61	1.35	1.09	0.92	1.66	1.31

Expression levels in Telomerase cell lines   median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.87	0.26	0.81	0.83	0.61	0.88	1.31	0.71	0.38	1.05	0.86	1.26
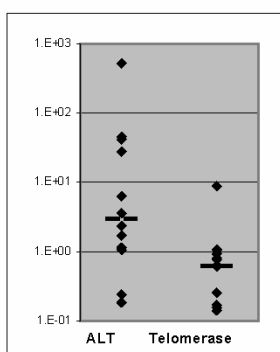
132: SPANXD: SPANX family, member D

P-value:	2.1E-03	Fold change: 4.8

Chromosome: Xq27.1
Genbank: BC054023   Gene ID: hCG1756726.2
Biological process:
Molecular function:
Cellular component: nucleus
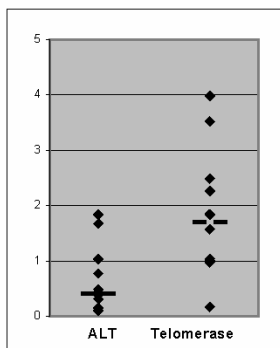
Expression levels in ALT cell lines   median: 3.0

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.70	0.18	2.34	45.18	3.59	1.06	6.30	517	41.42	0.24	27.56	1.15

Expression levels in Telomerase cell lines   median: 0.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.94	1.06	0.16	0.77	0.14	0.17	0.14	0.61	0.26	0.64	8.82	0.81

133: PKE: PKE protein kinase

P-value:	2.1E-03							Fold change:	0.2		

Chromosome: 10q26.3

Genbank: BC045760 Gene ID: hCG2023112.1

Biological process: protein amino acid phosphorylation

Molecular function: protein serine/threonine kinase activity; ATP binding

Cellular component:
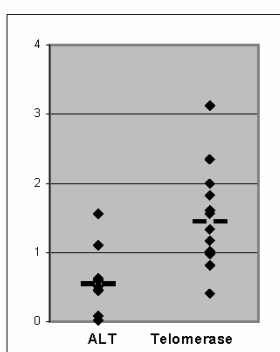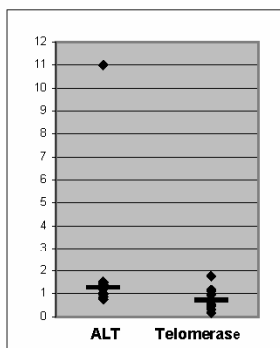
Expression levels in ALT cell lines median: 0.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.03	0.48	0.39	0.10	0.43	0.15	0.31	1.83	0.31	1.67	0.15	0.77

Expression levels in Telomerase cell lines median: 1.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.48	2.26	1.57	1.04	0.17	0.99	3.52	1.83	1.85	3.98	0.98	1.01

134: THSD1; C13orf9: thrombospondin, type I, domain 1; chromosome 13 open reading frame 9

P-value:	2.1E-03					Fold change:	0.4	

Chromosome: 13q14.2 Genbank: NM_016075.1 Gene ID: hCG29569.3

Biological process:

Molecular function:

Cellular component: cell surface

Expression levels in ALT cell lines median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.56	0.60	0.08	0.61	0.63	1.56	0.46	0.51	0.46	0.09	0.03	1.11

Expression levels in Telomerase cell lines median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.61	1.83	1.17	2.00	3.12	1.34	0.41	0.98	0.82	2.35	1.02	1.56

135: LOC136306: hypothetical protein LOC136306

P-value:	2.1E-03							Fold change:	1.8		

Chromosome: 7q34

Genbank: NM_174959.1 Gene ID: hCG2042772

Biological process: transport

Molecular function: transporter activity

Cellular component: integral to membrane

Expression levels in ALT cell lines median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.29	1.20	1.27	0.97	1.53	1.46	0.85	1.46	0.76	11.00	1.39	1.03

Expression levels in Telomerase cell lines median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.76	0.46	0.64	0.69	1.17	0.32	0.95	0.76	1.12	0.54	0.17	0.78
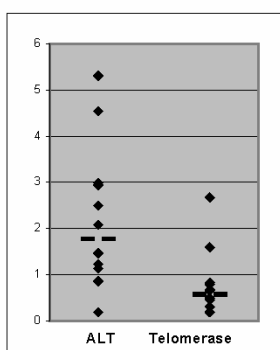
136: C2orf13: chromosome 2 open reading frame 13

P-value:	2.4E-03							Fold change:	3.1		

Chromosome: 2p13.3

Genbank: NM_173545.1 Gene ID: hCG1986872.1

Biological process:

Molecular function:

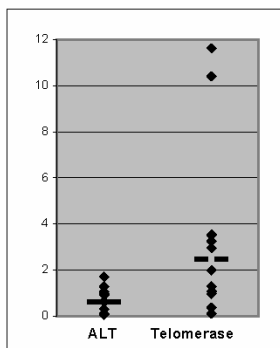Cellular component:

Expression levels in ALT cell lines median: 1.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
5.31	2.99	0.19	1.47	2.50	0.86	1.23	2.95	1.14	4.54	1.46	2.08

Expression levels in Telomerase cell lines median: 0.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.68	1.59	0.82	0.31	0.66	0.31	0.46	0.50	0.19	2.68	0.19	0.80

137: INM01: hypothetical protein INM01

P-value: 2.4E-03   Fold change: 0.2

Chromosome: 8p23.3

Genbank: BC033779   Gene ID: hCG1736809.2

Biological process:

Molecular function:

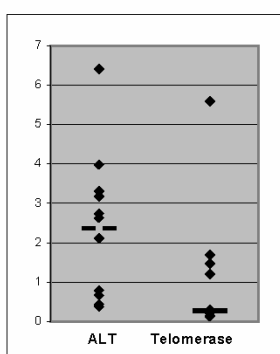Cellular component:

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.27	1.70	0.29	0.29	0.59	0.90	0.95	0.05	1.04	0.10	0.05	0.63

Expression levels in Telomerase cell lines   median: 2.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.96	3.54	1.28	2.95	3.24	3.50	1.06	10.41	0.36	11.63	1.98	0.09

138: KAL1: Kallmann syndrome 1 sequence

P-value: 2.4E-03   Fold change: 8.5

Chromosome: Xp22.32   Genbank: NM_000216.1   Gene ID: hCG401093.3

Biological process: axon guidance;chemotaxis;cell motility;cell adhesion

Molecular function: extracellular matrix structural constituent;protein binding;serine-type endopeptidase inhibitor activity

Cellular component: extracellular space;extracellular matrix

Expression levels in ALT cell lines   median: 2.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.17	2.11	0.79	2.74	0.43	2.63	2.11	6.40	3.98	3.30	0.38	0.66

Expression levels in Telomerase cell lines   median: 0.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.21	1.68	0.27	0.13	0.15	0.22	0.26	0.28	0.30	1.47	5.59	0.25
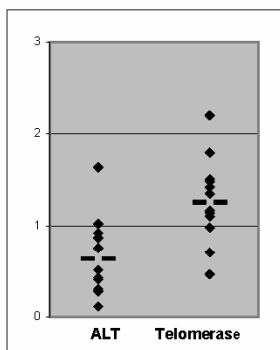
139: ZNF513: zinc finger protein 513

P-value: 2.4E-03   Fold change: 0.5

Chromosome: 2p23.3

Genbank: NM_144631.3   Gene ID: hCG1784441.2

Biological process: regulation of transcription, DNA dependent

Molecular function: DNA binding; zinc ion binding

Cellular component: nucleus

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.51	1.64	0.92	0.30	1.02	0.75	1.02	0.41	0.28	0.87	0.11	0.42

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.14	1.16	1.48	2.20	1.10	1.35	1.50	0.71	0.46	1.42	0.98	1.80
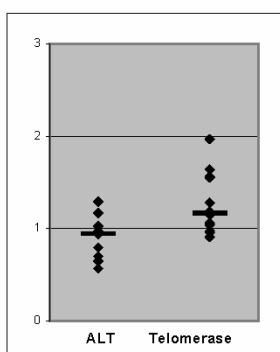
140: PGAM1: phosphoglycerate mutase 1 (brain)

P-value: 2.4E-03   Fold change: 0.8

Chromosome: 10q25.3   Genbank: NM_002629.2

Gene ID: hCG25778.3; hCG2015269; hCG2015138; hCG25778.3

Biological process: metabolism; glycolysis.   Cellular component: cytosol

Molecular function: bisphosphoglycerate phosphatase, isomerase, hydrolase, phosphoglycerate mutase and bisphosphoglycerate mutase activity
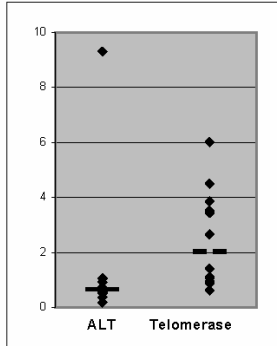
Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.57	0.70	0.65	0.79	1.17	0.64	0.94	0.96	1.29	0.97	1.03	0.95

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.28	1.64	0.97	1.56	1.15	0.96	1.06	1.55	1.19	1.97	1.04	0.91

141: LOC339803: hypothetical protein LOC339803

P-value: **2.4E-03**   **Fold change: 0.3**

Chromosome: 2p16.1

Genbank: AK023856   Gene ID: hCG1783888.2

Biological process:

Molecular function:

Cellular component:

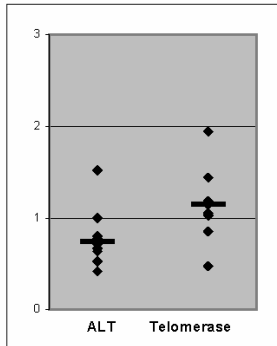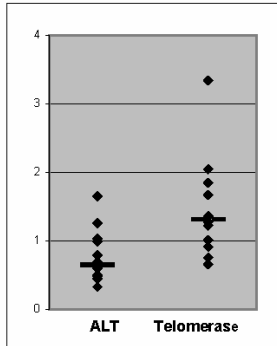**Expression levels in ALT cell lines**   **median: 0.7**
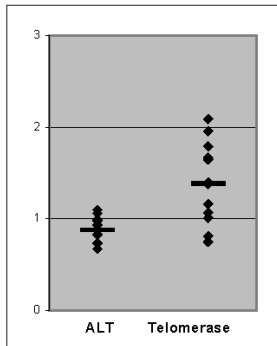
1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.62	0.73	0.92	0.71	0.52	1.05	1.05	0.37	9.30	0.58	0.19	0.58

Expression levels in Telomerase cell lines   **median: 2.0**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.95	1.41	3.85	0.89	0.62	4.50	1.07	2.66	1.12	6.01	3.52	3.44

142: COPS7B: COP9 constitutive photomorphogenic homolog subunit 7B (Arabidopsis)

P-value: **2.4E-03**   **Fold change: 0.6**

Chromosome: 2q37.1   Genbank: NM_022730.1   Gene ID: hCG33982.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.00	0.77	1.52	1.00	0.67	0.80	0.42	0.64	0.53	0.80	0.53	0.72

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.05	1.03	0.85	1.94	1.14	1.16	1.19	1.16	1.44	1.18	1.05	0.48

143: IGLJ3: immunoglobulin lambda joining 3

P-value: **2.4E-03**   **Fold change: 0.5**

Chromosome: 22q11.1-q11.2

Genbank: AA622375   Gene ID: hCG1780806.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.33	0.50	0.44	0.49	1.03	0.60	0.61	0.79	0.99	1.65	1.26	0.68

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.66	1.22	1.85	0.75	1.01	0.91	1.36	2.05	3.34	1.35	1.67	1.28

144: N/A

P-value: **2.4E-03**   **Fold change: 0.6**

Chromosome: 9

Genbank: BG205746   Gene ID: hCG1641550.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.88	0.67	0.93	0.74	0.99	0.98	0.87	1.06	0.88	1.10	0.83	0.82

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.01	1.67	1.40	1.07	1.64	1.16	0.75	2.09	1.79	1.38	1.95	0.81
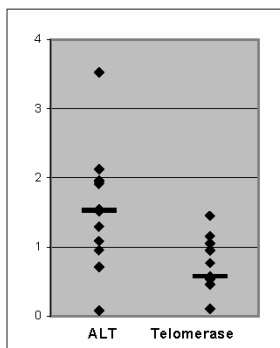
145: GBP1: guanylate binding protein 1, interferon-inducible, 67kDa

P-value:	2.4E-03	Fold change:	2.7

Chromosome: 1p22.2

Genbank: NM_002053.1   Gene ID: hCG1774285.4

Biological process: immune response

Molecular function: GTP binding; GTPase activity

Cellular component: membrane

Expression levels in ALT cell lines   median: 1.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.54	1.91	1.96	1.29	1.08	0.95	1.52	3.53	0.07	2.13	1.95	0.71

Expression levels in Telomerase cell lines   median: 0.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.16	0.95	1.05	0.53	0.46	0.46	0.77	0.53	0.58	0.10	0.57	1.45
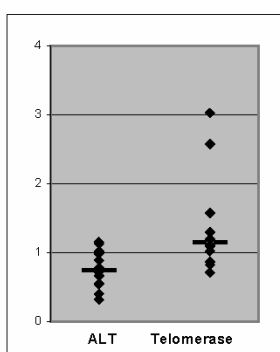
146: SH3MD2: SH3 multiple domains 2

P-value:	2.4E-03	Fold change:	0.6

Chromosome: 4q32.3

Genbank: NM_020870.1   Gene ID: hCG1811386.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.02	1.15	0.32	0.40	0.66	0.55	1.12	0.54	0.77	0.72	0.89	0.98

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.29	3.02	0.82	1.57	1.11	1.19	1.02	1.09	0.71	1.30	0.86	2.57
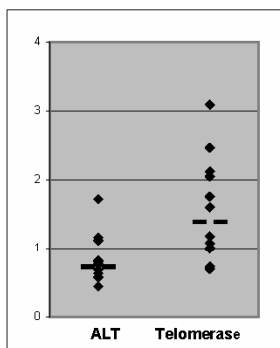
147: ARHGEF10: Rho guanine nucleotide exchange factor (GEF) 10

P-value:	2.4E-03	Fold change:	0.5

Chromosome: 8p23

Genbank: NM_014629.1   Gene ID: hCG2005257.1

Biological process: regulation of Rho protein signal transduction

Molecular function: Rho guanyl-nucleotide exchange factor activity

Cellular component: intracellular

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.81	0.76	0.80	0.63	1.16	0.57	0.68	0.57	0.68	1.72	0.44	1.12

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.01	1.60	0.73	1.76	2.12	1.18	1.08	2.05	2.47	3.09	0.69	0.99
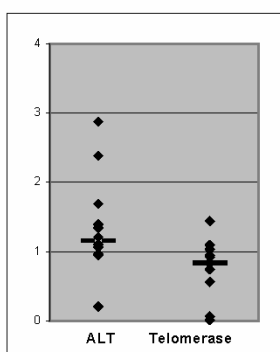
148: CREB3L1: cAMP responsive element binding protein 3-like 1

P-value:	2.4E-03	Fold change:	1.4

Chromosome: 11p11.2

Genbank: NM_052854.1   Gene ID: hCG25775.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.34	1.07	1.69	1.10	2.38	1.39	0.97	2.87	0.21	0.95	1.11	1.21

Expression levels in Telomerase cell lines   median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.92	1.03	0.95	0.57	1.10	0.83	0.02	0.74	0.85	0.01	0.07	1.44
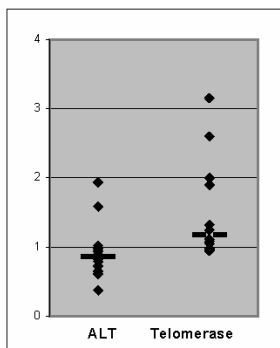
149: PPP1R2: protein phosphatase 1, regulatory (inhibitor) subunit 2

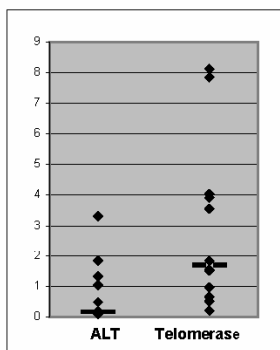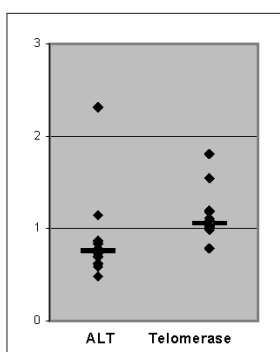| P-value:	2.4E-03							Fold change:	0.7		

Chromosome: 3q29

Genbank: NM_006241.3   Gene ID: hCG22103.3

Biological process: glycogen metabolism; generation of precursor metabolites and energy

Molecular function: protein binding; type 1 serine/threonine specific protein phosphatase activity.

Cellular component:

Expression levels in ALT cell lines median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.94	0.72	1.02	0.38	0.94	0.83	0.98	1.59	0.90	0.79	0.61	0.65

Expression levels in Telomerase cell lines median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
3.15	1.90	1.24	1.05	2.60	1.11	1.08	2.00	0.98	1.32	0.95	0.96
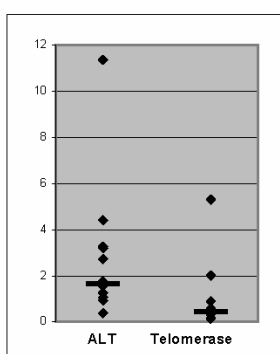
150: C10orf11: chromosome 10 open reading frame 11

P-value:	2.4E-03							Fold change:	3.6		

Chromosome: 10q22.3

Genbank: NM_032024.2   Gene ID: hCG22507.4

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 1.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.21	0.38	1.76	2.72	11.36	1.26	3.27	4.42	0.94	1.56	1.57	1.06

Expression levels in Telomerase cell lines median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.01	0.16	0.42	0.33	0.58	0.17	0.49	0.50	0.89	5.30	0.36	0.14

151: SLC39A4: solute carrier family 39 (zinc transporter), member 4

P-value:	2.4E-03							Fold change:	0.1		

Chromosome: 8q24.3

Genbank: NM_017767.1   Gene ID: hCG1992198.1

Biological process: metal ion transport

Molecular function: metal ion transporter activity

Cellular component: membrane

Expression levels in ALT cell lines median: 0.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.13	1.84	0.48	0.15	0.14	0.15	0.17	3.31	1.33	1.04	0.11	0.10

Expression levels in Telomerase cell lines median: 1.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
4.04	7.85	3.92	1.83	0.20	0.51	3.56	1.50	1.54	8.13	0.65	0.96

152: YWHAZ: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide

P-value:	2.4E-03							Fold change:	0.7		

Chromosome: 8q23.1   Genbank: NM_003406.2

Gene ID: hCG2009146; hCG2009146; hCG21543.2; hCG1820813.1

Biological process:

Molecular function: protein domain specific binding   Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.48	0.87	1.15	0.62	0.84	0.78	0.70	0.73	0.84	2.31	0.74	0.59

Expression levels in Telomerase cell lines median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.79	1.55	1.08	1.03	1.03	1.20	0.99	1.01	1.18	1.81	1.11	1.02
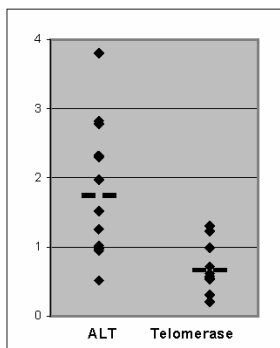
153: ABCB4: ATP-binding cassette, sub-family B (MDR/TAP), member 4

P-value:	2.4E-03		Fold change:	2.6

Chromosome: 7q21.1   Genbank: NM_000443.2   Gene ID: hCG1743207.2

Biological process: response to xenobiotic stimulus;lipid metabolism;transport;response to drug

Molecular function: ATP binding;nucleotide binding;ATP-binding cassette (ABC) transporter activity. Cellular component: membrane fraction;integral to plasma membrane

Expression levels in ALT cell lines median: 1.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.97	2.78	2.32	3.81	1.97	2.30	2.82	1.52	1.02	0.94	0.51	1.26

Expression levels in Telomerase cell lines median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.71	0.98	0.20	0.61	0.57	0.53	0.54	1.23	0.71	1.31	1.30	0.30
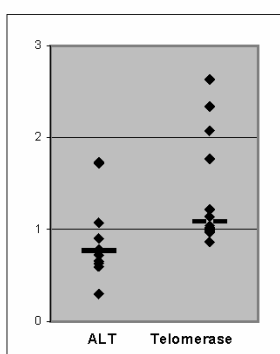
154: PPP1R2: protein phosphatase 1, regulatory (inhibitor) subunit 2

P-value:	2.4E-03	Fold change:	0.7

Chromosome: 3q29

Genbank: NM_006241.3   Gene ID: hCG22103.3

Biological process: glycogen metabolism; generation of precursor metabolites and energy

Molecular function: protein binding; type 1 serine/threonine specific protein phosphatase activity. Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.73	0.78	0.78	0.30	1.07	0.65	0.72	1.72	0.90	0.63	0.60	0.77

Expression levels in Telomerase cell lines median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.34	1.77	1.02	1.01	2.63	1.22	0.97	2.07	0.99	0.86	1.04	1.14
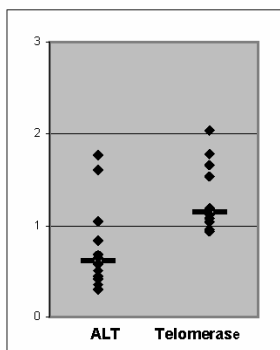
155: : Transcribed sequence with moderate similarity to protein sp:P47755 (H.sapiens) CAZ2_HUMAN F-actin capping protein alpha-2 subunit

P-value:	2.4E-03	Fold change:	0.5

Chromosome: 7   Genbank: BU728167   Gene ID: hCG1641068.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.64	0.44	0.41	0.30	1.05	0.35	0.50	1.77	0.68	1.61	0.57	0.84

Expression levels in Telomerase cell lines median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.96	1.54	1.12	0.94	1.78	1.19	1.04	2.04	1.08	1.66	0.94	1.19
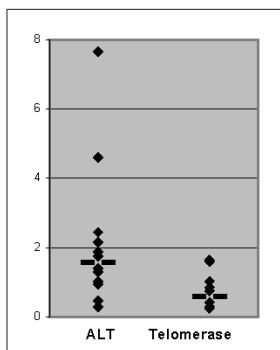
156: FLJ32685: hypothetical protein FLJ32685

P-value:	2.7E-03	Fold change:	2.7

Chromosome: 3p24.1

Genbank: NM_152534.1   Gene ID: hCG1995993; hCG2039986; hCG1995993

Biological process: protein amino acid phosphorylation

Molecular function: ATP binding; protein kinase activity; transferase activity

Cellular component:

Expression levels in ALT cell lines median: 1.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.48	1.88	4.60	1.40	1.75	2.45	2.15	1.30	7.65	0.30	0.95	1.03

Expression levels in Telomerase cell lines median: 0.6

| 6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.60	0.25	0.75	0.30	0.25	0.25	0.30	1.65	0.75	1.03	0.43	0.85
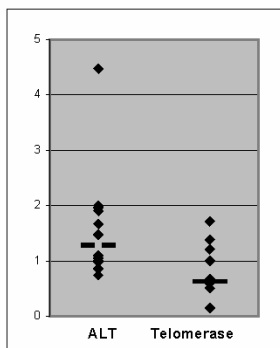
157: ALDH6A1: aldehyde dehydrogenase 6 family, member A1

P-value: 2.7E-03 **Fold change:** 2.0

Chromosome: 14q24.3 Genbank: NM_005589.2 Gene ID: hCG21723.2

Biological process: valine metabolism;metabolism;pyrimidine nucleotide metabolism

Molecular function: methylmalonate-semialdehyde dehydrogenase (acylating) activity;oxidoreductase activity

Cellular component: mitochondrion

Expression levels in ALT cell lines **median: 1.3**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.05	4.47	0.86	1.47	1.96	0.74	1.00	1.09	2.00	1.67	1.90	0.98

Expression levels in Telomerase cell lines **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.61	1.21	0.60	0.51	1.38	1.72	0.67	1.00	0.60	0.65	0.15	0.61
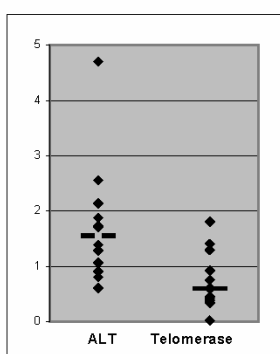
158: FLJ21159: hypothetical protein FLJ21159

P-value: 2.7E-03 **Fold change:** 2.6

Chromosome: 4q31.3

Genbank: NM_024826.1 Gene ID: hCG26928.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines **median: 1.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.61	1.29	0.91	1.07	4.70	1.74	1.72	1.40	2.56	2.14	0.81	1.88

Expression levels in Telomerase cell lines **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.34	1.41	0.76	0.61	0.59	0.45	0.03	0.42	1.81	0.93	1.30	0.39
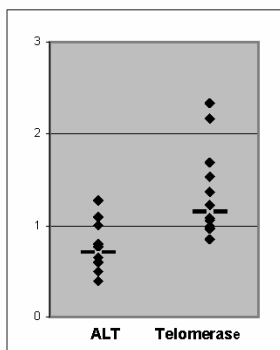
159: : ty30g12.x1 NCI_CGAP_Ut2 Homo sapiens cDNA clone IMAGE:2280646 3', mRNA sequence.

P-value: 3.1E-03 **Fold change:** 0.6

Chromosome: 22 Genbank: AI669018 Gene ID: hCG1820368.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.10	1.28	0.39	0.49	0.49	0.49	1.01	0.77	1.28	0.65	0.80	0.59

Expression levels in Telomerase cell lines **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.17	2.34	1.37	0.99	0.98	0.97	1.23	1.08	1.06	1.69	1.54	0.85
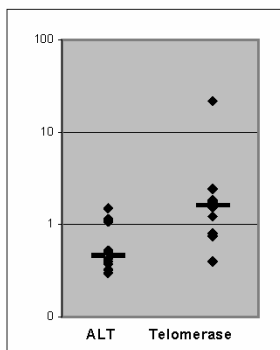
160: ITIH3: inter-alpha (globulin) inhibitor H3

P-value: 3.1E-03 **Fold change:** 0.3

Chromosome: 3p21.2-p21.1

Genbank: NM_002217.1 Gene ID: hCG17557.3

Biological process:

Molecular function: serine-type endopeptidase inhibitor activity

Cellular component: extracellular

Expression levels in ALT cell lines **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.40	1.08	1.13	1.50	0.50	1.15	0.38	0.43	0.30	0.38	0.33	0.53

Expression levels in Telomerase cell lines **median: 1.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
21.65	1.55	1.23	1.75	2.43	1.83	0.40	1.85	0.75	0.80	0.40	1.70
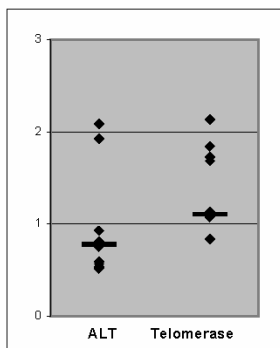
161: TTC11: tetratricopeptide repeat domain 11

P-value:	3.1E-03					Fold change:	0.7

Chromosome: 7q22.1

Genbank: NM_016068.1   Gene ID: hCG17349.2

Biological process:

Molecular function:

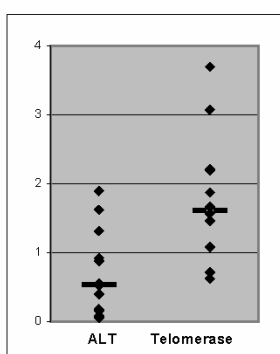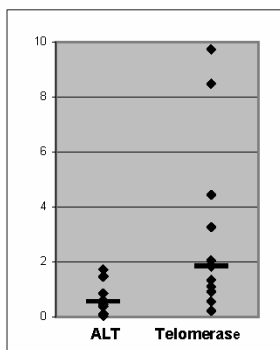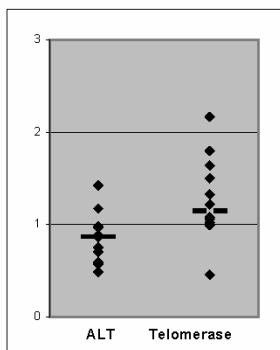Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.59	0.56	0.77	0.80	0.78	0.53	0.75	1.92	0.80	2.09	0.93	0.52

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.09	1.84	1.08	0.83	1.73	1.07	0.83	1.68	1.10	2.13	1.13	1.10

162: : thrombospondin, type I domain containing 6

P-value:	3.1E-03					Fold change:	0.3

Chromosome:19p13.3

Genbank: Gene ID: R26_hCT12692.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.52	0.92	0.88	0.18	1.62	0.08	0.40	0.06	1.89	1.31	0.55	0.16

Expression levels in Telomerase cell lines   median: 1.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.21	3.69	1.08	1.66	3.07	1.56	1.46	0.71	0.62	2.19	0.72	1.87

163: LOC134147: hypothetical protein BC001573

P-value:	3.1E-03					Fold change:	0.3

Chromosome: 5p15.31

Genbank: NM_138809.2   Gene ID: hCG1758400.1

Biological process:

Molecular function: hydrolase activity

Cellular component:

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.51	0.08	0.60	0.44	1.44	0.85	0.36	0.04	1.47	0.11	0.61	1.71

Expression levels in Telomerase cell lines   median: 1.9

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.04	0.21	1.82	0.91	1.09	1.33	0.56	3.28	8.49	9.73	4.46	1.88

164: TRAM1: translocation associated membrane protein 1

P-value:	3.1E-03					Fold change:	0.8

Chromosome: 8q13.2

Genbank: NM_014294.3   Gene ID: hCG18447.3

Biological process: cotranslational protein targeting to membrane

Molecular function: receptor activity

Cellular component: endoplasmic reticulum; integral to membrane

Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.49	0.88	1.17	0.59	0.71	0.75	1.42	0.57	0.97	0.98	0.87	0.87

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.08	1.80	1.33	1.01	1.50	0.99	1.22	1.06	2.17	1.02	0.46	1.64
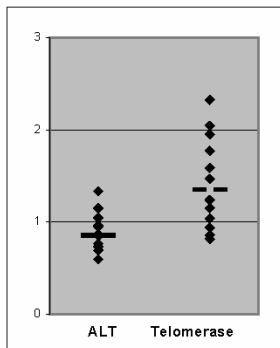
165: PPRC1: peroxisome proliferative activated receptor, gamma, coactivator-related 1

P-value: 3.1E-03   Fold change: 0.6

Chromosome: 10q24.32   Genbank: NM_015062.3   Gene ID: hCG25641.3

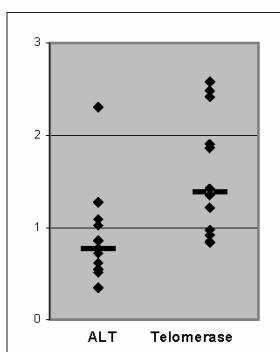Biological process:

Molecular function: nucleic acid binding

Cellular component:

Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.60	1.33	0.95	1.15	0.86	0.77	0.69	0.85	0.85	0.73	0.96	1.04

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.24	1.15	1.04	2.32	1.47	0.86	0.81	1.95	1.77	2.04	1.58	0.94

166: N/A

P-value: 3.1E-03   Fold change: 0.6

Chromosome:

Genbank: Gene ID: hCG1793602.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.55	0.78	0.77	0.35	0.72	0.62	1.03	1.27	0.86	2.31	1.09	0.51

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.92	2.48	1.35	1.22	1.86	1.42	0.84	1.91	2.58	2.42	0.84	0.97
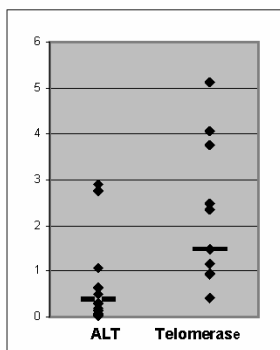
167: STMN3: BTB (POZ) domain containing 4

P-value: 3.1E-03   Fold change: 0.3

Chromosome: 20q13.3

Genbank: NM_015894.2   Gene ID: hCG2018447; hCG2018447; hCG1643090.3

Biological process: regulation of transcription, DNA-dependent

Molecular function: zinc ion binding;protein binding;nucleic acid binding

Cellular component: nucleus

Expression levels in ALT cell lines   median: 0.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.64	0.49	0.29	0.06	0.01	2.76	2.90	0.02	1.06	0.14	0.18	0.64

Expression levels in Telomerase cell lines   median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.46	1.15	0.92	2.35	5.13	1.49	3.76	1.47	2.49	4.07	0.94	0.41
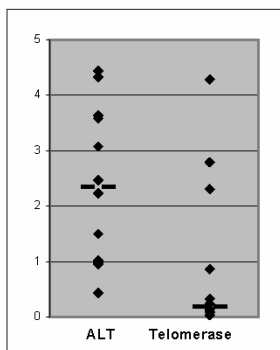
168: KCNN4: potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4

P-value: 3.1E-03   Fold change: 12.2

Chromosome: 19q13.2   Genbank: NM_002250.2   Gene ID: hCG33372.3

Biologl process: ion transport; defense response; potassium ion transport. Molecr functn: ion channel and small conductance Ca+2-activated K+ channel activity; calmodulin binding

Cellr compnt: voltage-gated K+ channel complex; membrane fractn; integral to membrane

Expression levels in ALT cell lines   median: 2.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.07	0.95	3.64	4.44	1.50	3.58	0.44	4.32	2.23	2.47	0.98	1.02

Expression levels in Telomerase cell lines   median: 0.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.31	0.03	0.33	0.14	0.10	0.08	0.03	0.04	0.24	2.79	4.28	0.86
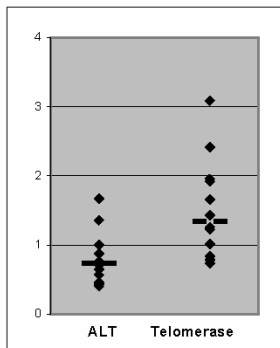
## 169: LRP5: low density lipoprotein receptor-related protein 5

P-value: 3.1E-03   Fold change: 0.5

Chromosome: 11q13.4   Genbank: NM_002335.1   Gene ID: hCG27192.2

Biological process: positive regulation of cell proliferation; lipid metabolism; signal transduction
Molecular function: receptor activity
Cellular component: integral to membrane; membrane

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.67	0.76	1.00	0.56	0.46	0.40	0.70	0.87	0.65	0.43	1.00	1.36

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.73	1.92	1.66	0.84	1.43	1.01	1.26	3.09	1.22	2.41	0.78	1.96
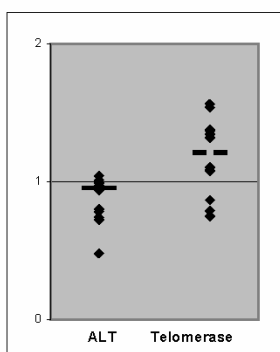
170: ZMYND19: zinc finger, MYND domain containing 19

P-value: 3.1E-03   Fold change: 0.8

Chromosome: 9q34.3

Genbank: NM_138462.2   Gene ID: hCG28444.3

Biological process:
Molecular function: zinc ion binding
Cellular component:

Expression levels in ALT cell lines   median: 1.0

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.01	1.04	1.01	0.72	0.74	0.80	0.97	0.99	0.94	0.97	0.48	0.78

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.37	0.79	0.87	1.37	1.08	1.34	1.10	1.54	1.32	1.56	1.08	0.75
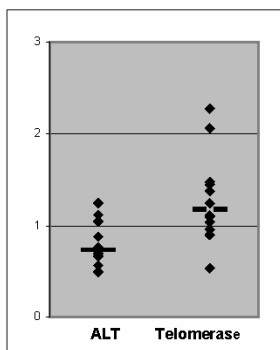
171: GYG: glycogenin

P-value: 3.1E-03   Fold change: 0.6

Chromosome: 3q24-q25.1

Genbank: NM_004130.2   Gene ID: hCG21210.3

Biological process: glycogen biosynthesis
Molecular function: glycogenin glucosyltransferase activity
Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.05	1.25	0.88	0.66	0.75	0.56	0.71	0.76	0.49	1.12	0.69	0.68

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.45	2.06	0.96	0.90	2.27	1.04	0.53	1.38	1.48	1.25	1.10	1.11
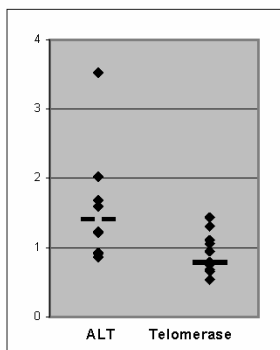
172: ARHGAP5: Rho GTPase activating protein 5

P-value: 3.1E-03   Fold change: 1.8

Chromosome: 14q12

Genbank: NM_001173.1   Gene ID: hCG2014162

Biological process: Rho protein signal transduction;cell adhesion
Molecular function: GTPase activity;Rho GTPase activator activity
Cellular component: membrane;cytoplasm

Expression levels in ALT cell lines   median: 1.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.86	3.53	0.86	1.60	1.23	0.91	1.21	2.02	1.69	1.69	1.60	0.93

Expression levels in Telomerase cell lines   median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.54	1.31	0.68	0.75	1.44	0.95	1.11	1.05	0.66	0.78	0.80	0.78
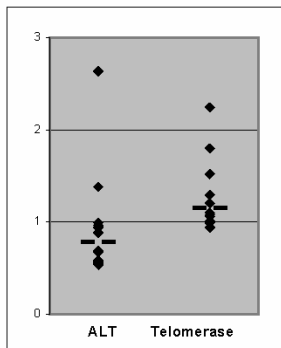
173: N/A

P-value:	3.1E-03	Fold change:	0.7

Chromosome:
Genbank: Gene ID: hCG1820813.1; hCG2009146; hCG1820813.1
Biological process:
Molecular function:
Cellular component:

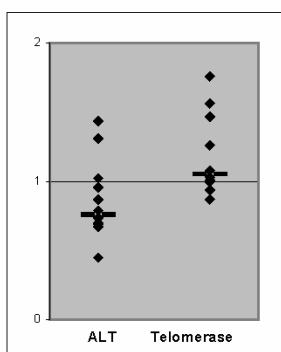**Expression levels in ALT cell lines**   **median: 0.8**
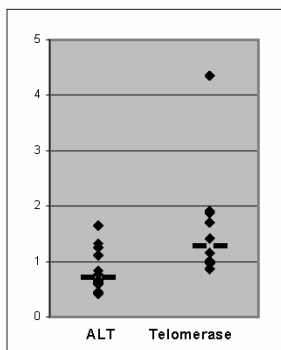
1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.58	0.94	0.99	0.55	0.96	0.67	0.54	0.88	1.38	2.63	0.69	0.57

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.01	1.80	1.06	1.10	1.29	1.20	0.99	1.20	1.52	2.24	1.08	0.94

174: USP4:

P-value:	3.1E-03	Fold change:	0.7

Chromosome: 3p21.3
Genbank: AW444548   Gene ID: hCG1993742
Biological process: ubiquitin-dependant protein catabolism; ubiquitin cycle
Molecular function: cysteine-type endopeptidase activity; ubiquitin thiolesterase activity
Cellular component: nucleus; lysosome

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.43	1.31	1.02	0.70	0.73	0.96	0.73	0.68	0.69	0.87	0.45	0.79

Expression levels in Telomerase cell lines   **median: 1.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.08	0.94	0.87	1.00	1.56	1.47	1.00	1.26	1.47	1.76	1.03	1.01
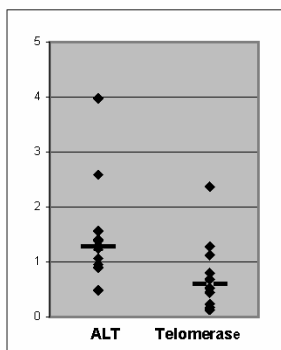
175: C5orf13: chromosome 5 open reading frame 13

P-value:	3.1E-03	Fold change:	2.2

Chromosome: 5q22.2
Genbank: AA331666   Gene ID: hCG2009910; hCG2028192; hCG2009910
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines   **median: 1.3**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.48	0.94	1.42	1.40	3.98	1.31	2.59	1.06	0.89	1.23	1.58	1.28

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.12	0.52	1.29	0.79	2.37	0.23	0.67	0.68	0.45	0.43	0.16	1.12

176: GTPBP4: GTP binding protein 4

P-value:	3.1E-03	Fold change:	0.6

Chromosome: 10p15-p14
Genbank: NM_012341.1   Gene ID: hCG24698.3
Biological process:
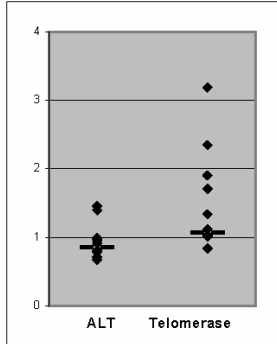Molecular function: GTP binding
Cellular component: nucleus

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.65	1.33	0.84	0.61	1.25	0.45	0.77	1.11	0.42	1.64	0.64	0.59

Expression levels in Telomerase cell lines   **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	1.87	0.86	1.16	1.91	1.02	0.97	4.35	1.70	1.87	1.42	0.87
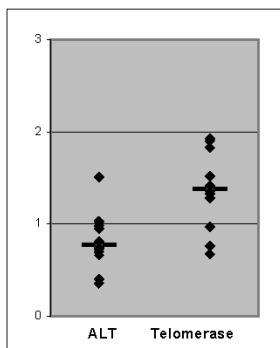
177: SCAMP2: secretory carrier membrane protein 2

P-value:	3.1E-03						Fold change:	0.6

Chromosome: 15q23-q25

Genbank: NM_005697.3 — Gene ID: hCG40805.3

Biological process: post-Golgi transport; protein transport

Molecular function:

Cellular component: integral to membrane; membrane fraction

Expression levels in ALT cell lines — median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.51	1.03	0.98	0.95	0.72	0.81	1.02	0.40	0.66	0.70	0.74	0.35

Expression levels in Telomerase cell lines — median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.35	0.67	1.52	1.28	1.90	1.83	1.41	1.42	1.92	0.76	0.97	1.33
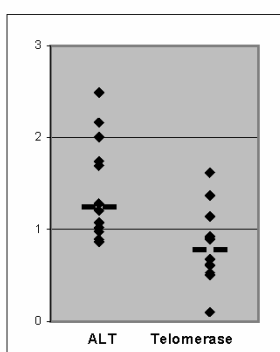
178: ZNF462: zinc finger protein 462

P-value:	3.1E-03						Fold change:	1.6

Chromosome: 9q32

Genbank: NM_021224.2 — Gene ID: hCG15653.3

Biological process: regulation of transcription, DNA-dependent

Molecular function: DNA binding;zinc ion binding

Cellular component: nucleus

Expression levels in ALT cell lines — median: 1.2

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	2.00	0.87	2.16	1.02	0.89	0.98	1.74	2.49	1.69	1.28	1.07

Expression levels in Telomerase cell lines — median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.90	0.89	0.92	1.14	0.68	0.53	0.61	1.62	0.51	0.10	1.37	0.62
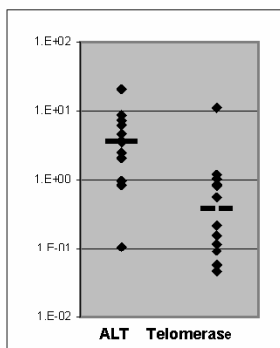
179: BEXL1: brain expressed X-linked-like 1

P-value:	3.1E-03						Fold change:	9.4

Chromosome: Xq22.1-q22.3

Genbank: BC015794 — Gene ID: hCG17757.3

Biological process:

Molecular function:

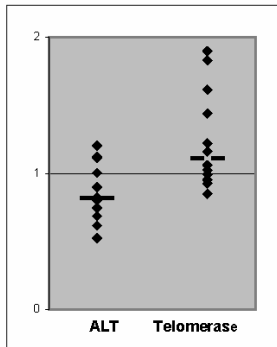Cellular component:

Expression levels in ALT cell lines — median: 3.6
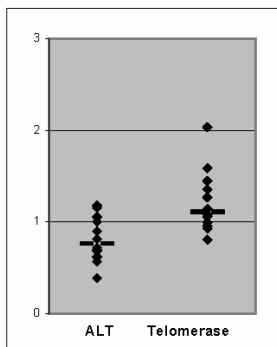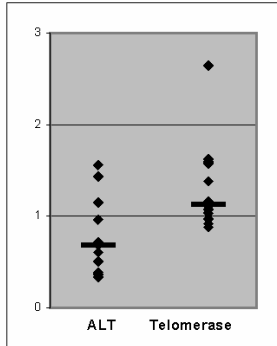
1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.07	0.83	20.83	4.65	0.97	3.75	6.21	0.10	8.69	2.50	7.30	3.53

Expression levels in Telomerase cell lines — median: 0.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
11.18	1.19	1.03	0.56	0.85	0.22	0.09	0.83	0.16	0.12	0.05	0.06
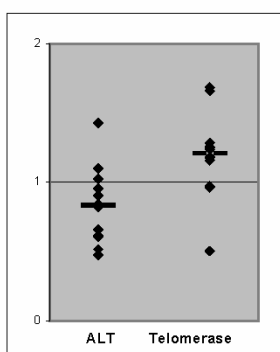
180: TCFL4: transcription factor-like 4

P-value:	3.1E-03						Fold change:	0.7

Chromosome: 17q21.1

Genbank: NM_013383.2 — Gene ID: hCG16944.3

Biological process: regulation of transcription, DNA-dependent

Molecular function: transcription factor activity

Cellular component: cytoplasm; nucleus

Expression levels in ALT cell lines — median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.43	1.10	0.91	0.84	0.96	0.66	0.61	0.48	0.61	1.03	0.52	0.82

Expression levels in Telomerase cell lines — median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.24	1.28	1.16	1.66	1.18	1.18	0.50	0.97	1.25	1.69	1.25	0.96

181: LOC51234: hypothetical protein LOC51234

P-value:	3.1E-03								Fold change:	0.8

Chromosome: 15q13.2
Genbank: NM_016454.2   Gene ID: hCG38789.3
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.40	0.81	0.95	0.91	0.98	0.78	0.78	0.68	0.99	1.46	0.72	0.79

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.71	2.35	1.03	1.12	1.90	1.01	0.84	3.19	1.01	1.34	1.02	0.84
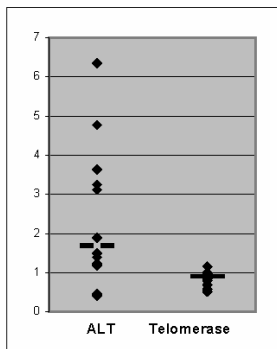
182: LOC284475: hypothetical protein LOC284475

P-value:	3.1E-03								Fold change:	1.9

Chromosome: 1p12
Genbank: AL832882   Gene ID: hCG1999407
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines   median: 1.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.42	4.77	3.12	6.36	1.89	3.25	3.63	1.50	1.24	1.18	0.46	1.40

Expression levels in Telomerase cell lines   median: 0.9

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.58	1.16	0.69	0.95	0.79	0.84	0.91	1.01	0.97	0.51	0.92	0.99

183: N/A

P-value:	3.1E-03								Fold change:	0.7

Chromosome:
Genbank: Gene ID: hCG1770437.2
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.82	1.13	0.75	0.69	1.12	0.53	0.62	1.00	0.90	1.21	0.81	0.82

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.93	1.90	1.03	1.00	1.44	0.96	0.85	1.62	1.83	1.16	1.22	1.06

184: N/A

P-value:	3.1E-03								Fold change:	0.7

Chromosome:
Genbank: Gene ID: hCG22106.2
Biological process:
Molecular function:
Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.00	0.90	0.70	0.72	1.05	0.62	0.39	0.68	0.81	1.15	1.18	0.57

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.06	1.59	1.36	0.96	1.27	1.08	0.81	2.03	1.00	1.14	1.45	0.93

185: N/A

P-value: 3.1E-03   **Fold change:** 0.6

Chromosome:

Genbank: Gene ID: hCG26523.2

Biological process:

Molecular function:

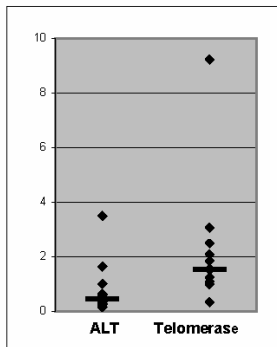Cellular component:
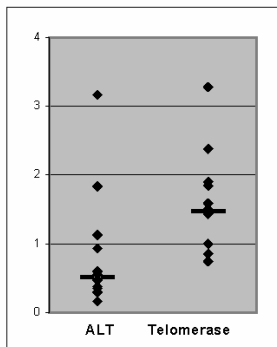
Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.60	0.71	0.36	0.33	1.43	0.38	0.50	1.15	0.68	1.56	0.96	0.69

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.03	1.58	0.92	1.10	0.88	1.38	1.07	2.65	1.16	1.59	1.62	0.97
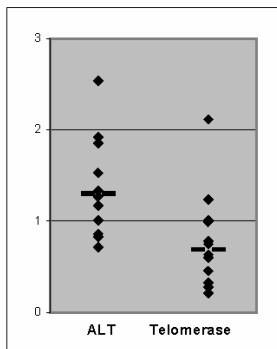
186: : ym33h07.r1 Soares infant brain 1NIB Homo sapiens cDNA clone IMAGE:20072 5'

P-value: 3.5E-03   **Fold change:** 1.9

Chromosome: 19   Genbank: H28867   Gene ID: hCG1986460

Biological process:

Molecular function:

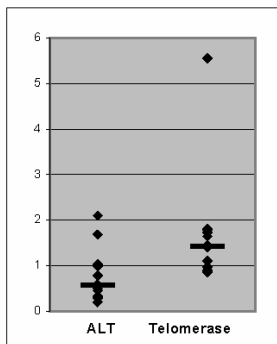Cellular component:

Expression levels in ALT cell lines   median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.72	1.53	0.83	1.01	1.33	1.53	1.85	0.86	2.54	1.92	1.27	1.17

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.11	0.21	0.99	0.75	0.33	1.24	0.78	0.63	0.28	0.46	0.60	1.01
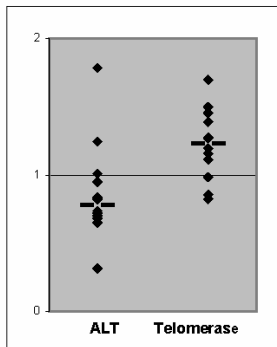
187: N/A

P-value: 3.5E-03   **Fold change:** 0.6

Chromosome: 13   Genbank: AW182500

Gene ID: hCG1742145.1; hCG2030722; hCG1992242; hCG1983637; hCG1742145.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.84	1.25	1.01	0.31	0.83	0.74	0.69	0.72	0.95	1.79	0.65	0.71

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.50	1.39	0.99	1.20	1.16	1.27	0.83	1.70	1.28	1.46	0.86	1.12
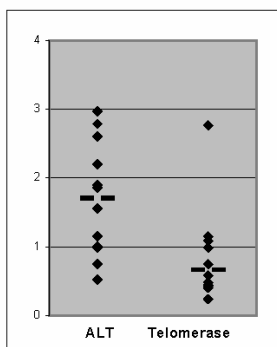
188: N/A

P-value: 3.9E-03   **Fold change:** 2.6

Chromosome:

Genbank: Gene ID: R26_hCT1648454.1
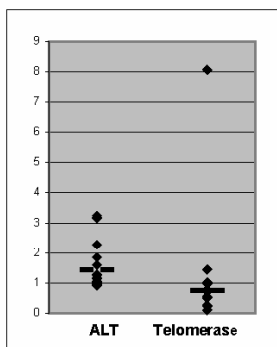
Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 1.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.20	2.79	0.99	1.90	0.53	1.01	1.86	2.61	2.97	1.56	1.15	0.75

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.75	0.75	1.09	0.99	0.24	0.48	1.15	0.40	0.44	0.59	2.77	0.42
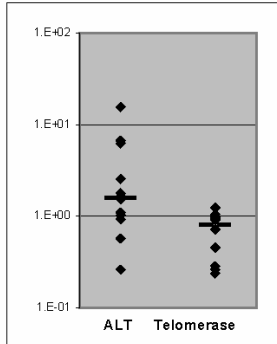
189: GPC5: glypican 5

P-value: 3.9E-03 **Fold change:** 2.0

Chromosome: 13q32

Genbank: NM_004466.3 Gene ID: hCG32231.4

Biological process:

Molecular function:

Cellular component: extracellular matrix; integral to plasma membrane

Expression levels in ALT cell lines **median: 1.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
6.24	1.64	6.67	1.52	15.52	1.10	1.79	0.57	2.55	0.93	0.26	1.02

Expression levels in Telomerase cell lines **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.24	0.24	0.29	0.93	0.98	0.24	0.90	1.05	0.26	0.71	1.05	0.45
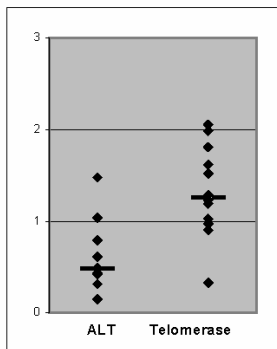
190: N/A

P-value: 3.9E-03 **Fold change:** 0.4

Chromosome:

Genbank: Gene ID: R26_hCT1643271.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.48	0.49	0.62	0.32	0.48	0.44	0.42	0.15	1.48	1.48	1.04	0.79

Expression levels in Telomerase cell lines **median: 1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.19	0.90	1.52	1.99	1.03	0.97	1.29	0.33	2.05	1.81	1.62	1.23

191: N/A

P-value: 3.9E-03 **Fold change:** 0.3

Chromosome:

Genbank: Gene ID: hCG2041462

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines **median: 0.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.63	0.29	0.27	0.41	0.24	1.00	0.48	0.58	1.63	3.52	0.33	0.15

Expression levels in Telomerase cell lines **median: 1.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.51	0.33	3.09	1.08	0.99	1.84	2.08	1.00	9.23	1.24	2.48	1.54

192: FLJ43663: Hypothetical protein FLJ43663, mRNA (cDNA clone IMAGE:5314992), partial cds

P-value: 3.9E-03 **Fold change:** 0.3

Chromosome: 7 Genbank: AK125651 Gene ID: hCG1645276.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines **median: 0.5**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.93	0.38	0.48	0.60	0.17	0.56	0.48	1.83	0.36	3.17	0.30	1.13

Expression levels in Telomerase cell lines **median: 1.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.38	1.59	1.00	0.74	1.90	1.43	0.86	1.00	1.50	3.28	1.46	1.84

193: BCL2A1: BCL2-related protein A1

P-value: 3.9E-03   **Fold change: 0.4**

Chromosome: 15q24.3

Genbank: NM_004049.2   Gene ID: hCG201186.3

Biological process: regulation of apoptosis; anti-apoptosis

Molecular function:

Cellular component: intracellular

Expression levels in ALT cell lines   **median: 0.6**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.68	0.98	0.78	1.02	0.58	0.55	0.28	0.49	0.33	2.10	0.19	0.45

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.40	1.72	0.89	1.44	1.80	1.10	0.85	1.40	1.64	1.78	5.56	0.97
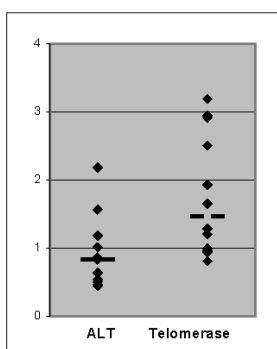
194: N/A

P-value: 4.0E-03   **Fold change: 0.6**

Chromosome:

Genbank: BI004197   Gene ID: hCG1643350.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.18	1.18	0.64	0.46	1.01	0.83	0.86	0.83	0.50	1.56	0.45	0.54

Expression levels in Telomerase cell lines   **median: 1.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.50	2.91	0.99	0.95	1.20	0.81	1.65	0.94	2.94	1.93	1.28	3.18

195: SYT11: synaptotagmin XI

P-value: 4.0E-03   **Fold change: 1.9**

Chromosome: 1q21.2

Genbank: NM_152280.2   Gene ID: hCG17193.3

Biological process: transport

Molecular function: transporter activity

Cellular component: synapse; integral to membrane; synaptic vesicle
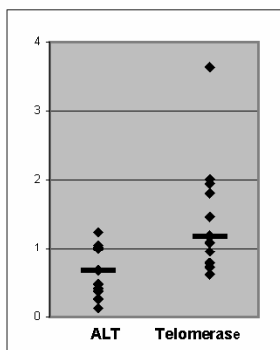
Expression levels in ALT cell lines   **median: 1.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.15	3.24	0.92	1.26	0.99	0.92	1.59	1.85	3.17	1.04	1.15	2.27

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.09	0.23	0.97	1.44	0.50	0.54	0.71	0.77	8.08	0.26	0.97	1.01
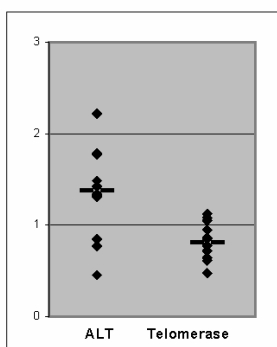
196: RYBP: RING1 and YY1 binding protein

P-value: 4.0E-03   **Fold change: 1.7**

Chromosome: 3p14.2

Genbank: NM_012234.3   Gene ID: hCG16333.3

Biological process: development; negative regulation of transcription from Pol II promoter

Molecular function: transcription corepressor activity

Cellular component:

Expression levels in ALT cell lines   **median: 1.4**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.85	1.32	2.22	1.79	1.43	1.77	1.43	0.45	1.34	1.31	1.49	0.77

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.08	0.85	0.64	0.95	0.72	0.72	0.87	1.05	0.78	1.12	0.48	0.61
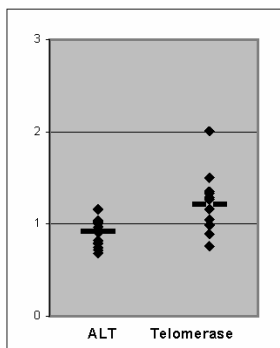
197: KPNA4: karyopherin alpha 4 (importin alpha 3)

P-value: 4.0E-03   **Fold change:** 0.8

Chromosome: 3q25.33

Genbank: NM_002268.3   Gene ID: hCG17225.4

Biological process: NLS-bearing substrate-nucleus import;intracellular protein transport

Molecular function: protein transporter activity

Cellular component: nucleus

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.94	1.04	0.97	0.74	0.82	0.79	0.72	1.01	1.02	1.16	0.91	0.68

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.29	2.01	1.16	1.33	1.50	0.99	0.76	0.89	1.27	0.99	1.35	1.05
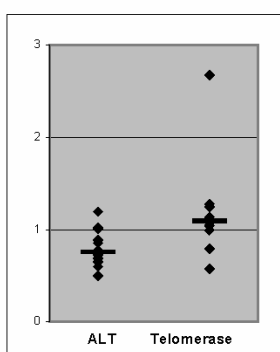
198: PCYT1A; LOC116211: hypothetical protein BC013113;phosphate cytidylyltransferase 1, choline, alpha isoform;hypothetical protein

P-value: 4.0E-03   **Fold change:** 0.7

Chromosome: 3q29   Genbank: NM_138461.1   Gene ID: hCG2002711

Biol procss: defens respnse;biosynthesis;phospholipid biosynthess. Cellr compnt: soluble fractn;integral to membrne;membrne;outer membrne (sensu Gram-ve Bact.). Molr functn: nucleotidyltransferase,choline-phosphate cytidylyltransferase & transferase activity

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	0.65	1.01	0.78	0.74	0.85	0.60	0.89	0.73	1.02	0.69	0.50

Expression levels in Telomerase cell lines   **median: 1.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.08	1.28	1.04	1.11	2.67	1.09	1.14	0.57	1.10	0.79	1.25	0.99
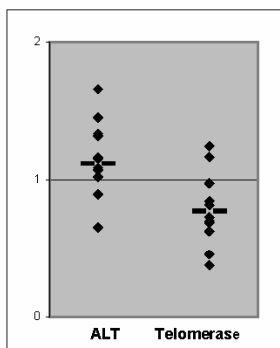
199: : ym61g12.r1 Soares infant brain 1NIB Homo sapiens cDNA clone IMAGE:52876 5', mRNA sequence.

P-value: 4.0E-03   **Fold change:** 1.4

Chromosome: 11   Genbank: H29806   Gene ID: hCG1733583.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 1.1**

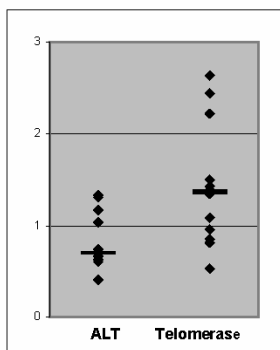| 1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.34	1.45	1.15	1.66	0.89	1.02	1.32	0.65	1.16	1.07	1.02	1.09

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	1.24	0.73	0.37	1.17	0.97	0.63	0.82	0.84	0.70	0.69	0.46
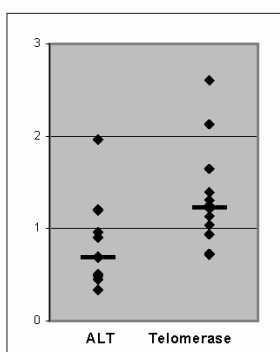
200: FLJ10377: hypothetical protein FLJ10377

P-value: 4.0E-03   **Fold change:** 0.6

Chromosome: 7q32.2

Genbank: NM_018077.1   Gene ID: hCG33003.3

Biological process:

Molecular function: nucleic acid binding

Cellular component:

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.48	0.45	0.68	0.96	1.19	0.34	0.50	1.21	0.91	1.96	0.49	0.69

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.13	2.60	1.25	1.04	1.65	1.21	0.94	2.13	0.72	1.39	1.31	0.73
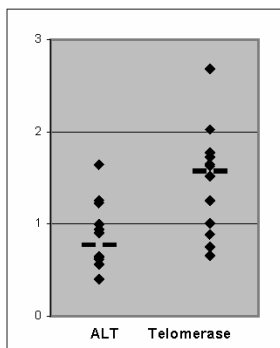
201: : processed pseudogene 1; Homo sapiens PSI1TOM20 pseudogene, complete sequence.

P-value: 4.0E-03   Fold change: 0.5

Chromosome: Xq13.1   Genbank: BM931952   Gene ID: hCG17581.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.90	1.26	0.94	0.40	1.23	0.61	0.64	0.56	0.63	1.64	0.56	1.00

Expression levels in Telomerase cell lines median: 1.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.88	2.02	1.25	1.52	1.73	0.65	0.75	2.68	1.77	1.63	1.65	1.00
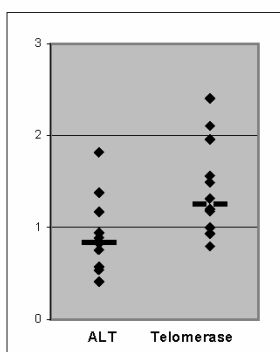
202: N/A

P-value: 4.0E-03   Fold change: 0.7

Chromosome:

Genbank: Gene ID: hCG1820694.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.82	0.94	0.85	0.54	1.17	0.83	0.57	1.38	0.41	0.89	0.57	0.76

Expression levels in Telomerase cell lines median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.49	2.10	1.00	1.56	1.96	1.20	1.00	2.40	1.18	1.32	0.93	0.79

203: CEBPA: CCAAT/enhancer binding protein (C/EBP), alpha

P-value: 4.0E-03   Fold change: 0.6

Chromosome: 19q13.1   Genbank: NM_004364.2   Gene ID: hCG20142.2

Biological process: regulation of transcription, DNA-dependent; transcription

Molecular function: RNA polymerase II transcription factor activity, enhancer binding; DNA binding

Cellular component: nucleus

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.05	1.01	0.37	0.41	0.12	0.47	0.26	1.24	0.99	1.01	0.67	0.68

Expression levels in Telomerase cell lines median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.80	1.95	0.78	0.95	1.09	0.72	1.46	2.01	1.19	3.64	1.17	0.61
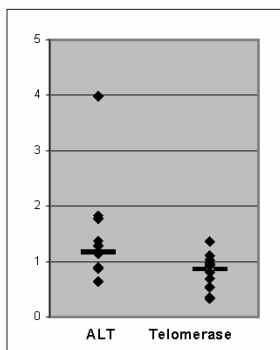
204: CDC14A: CDC14 cell division cycle 14 homolog A (S. cerevisiae)

P-value: 4.0E-03   Fold change: 1.4

Chromosome: 1p21   Genbank: NM_033312.1   Gene ID: hCG32850.3

Biologicl procss: cell proliferatn; protein amino acid dephosphorylatn; regulatn of cell cycle

Molecular function: hydrolase, protein tyrosine phosphatase & protein tyrosine/serine/threonine phosphatase activity

Cellular component: nucleus

Expression levels in ALT cell lines median: 1.2

| 1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.37	0.89	1.29	1.83	1.18	0.64	1.15	3.98	1.77	1.14	0.88	1.17

Expression levels in Telomerase cell lines median: 0.9

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.03	1.11	0.81	0.95	0.53	0.69	0.93	0.97	0.33	1.36	0.35	0.54
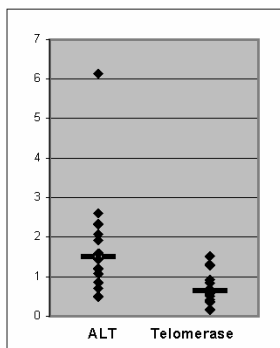
205: WFDC3: WAP four-disulfide core domain 3

P-value: **4.0E-03** **Fold change:** **2.3**

Chromosome: 20q13.12

Genbank: NM_080614.1 Gene ID: hCG38384.3

Biological process:

Molecular function: serine-type endopeptidase inhibitor activity

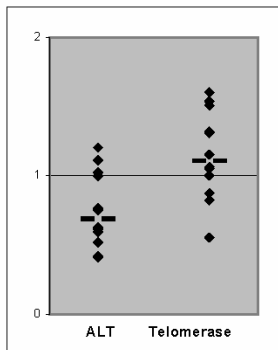Cellular component:

Expression levels in ALT cell lines **median: 1.5**

1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.43	2.60	2.07	0.86	0.50	1.08	1.21	6.13	2.33	1.92	1.59	0.71

Expression levels in Telomerase cell lines **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.31	0.61	0.52	0.41	0.92	0.57	0.69	0.17	1.52	0.84	1.29	0.36
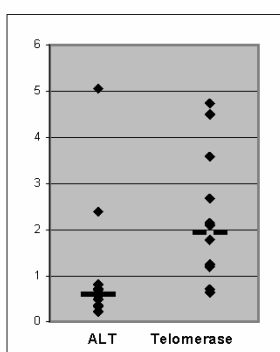
206: SIGIRR: single Ig IL-1R-related molecule

P-value: **4.0E-03** **Fold change:** **0.3**

Chromosome: 11p15.5

Genbank: AY358342 Gene ID: hCG19080.4

Biological process:

Molecular function:

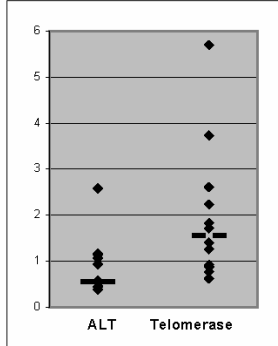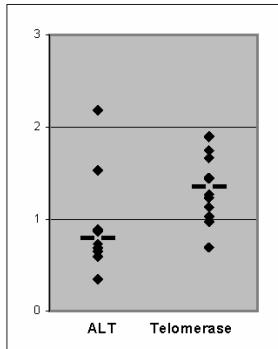Cellular component:

Expression levels in ALT cell lines **median: 0.6**

1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
5.06	2.39	0.49	0.34	0.23	0.81	0.59	0.61	0.73	0.57	0.69	0.36

Expression levels in Telomerase cell lines **median: 1.9**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.78	2.67	1.24	2.10	1.23	0.71	4.74	1.19	4.50	3.58	0.64	2.14

207: N/A

P-value: **4.0E-03** **Fold change:** **0.5**

Chromosome: 18q21.32

Genbank: BX358447 Gene ID: hCG1654216.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines **median: 0.7**
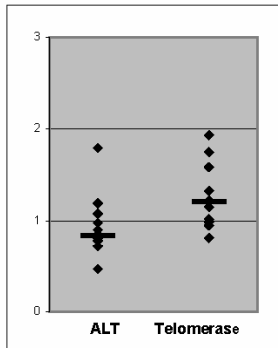
1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.31	1.33	0.62	0.74	0.59	0.66	0.70	1.17	0.70	1.04	0.40	0.40

Expression levels in Telomerase cell lines **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.09	2.22	0.81	1.50	2.64	0.96	0.86	2.44	1.35	1.43	1.39	0.52
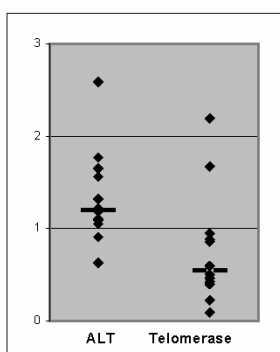
208: CHKL; CPT1B: carnitine palmitoyltransferase 1B (muscle);choline kinase beta

P-value: **4.0E-03** **Fold change:** **2.2**

Chromosome: 22q13.33 Genbank: NM_005198.3 Gene ID: hCG16873.3

Biologicl procss: transport;fatty acid beta-oxidation;fatty acid metabolism. Moleculr functn: acyltransferse,ethanolamine kinase,transferse & carnitine O-palmitoyltransferase activity

Cellular component: mitochondrion;integral to membrane;outer membrane

Expression levels in ALT cell lines **median: 1.2**

1.3C	1R	V2	2RA	GM847	AT1	MET4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.19	2.59	1.21	1.56	1.05	1.65	1.11	1.09	1.77	1.32	0.91	0.63

Expression levels in Telomerase cell lines **median: 0.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.60	0.50	0.42	0.22	0.40	0.95	0.86	0.09	0.88	2.19	1.67	0.46
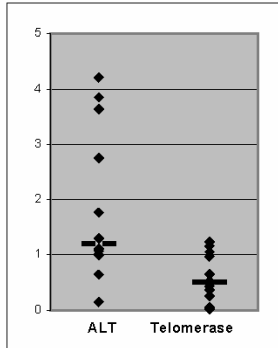
209: LRRCC1

P-value:	4.0E-03		Fold change:	2.4

Chromosome: 8

Genbank: NM_033402.1   Gene ID: hCG20885.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: **1.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.99	0.64	2.75	1.10	3.85	1.76	1.07	1.30	3.63	4.21	0.15	1.01

Expression levels in Telomerase cell lines   median: **0.5**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.97	0.43	0.53	0.65	0.37	0.25	0.01	1.05	1.23	0.04	1.16	0.49
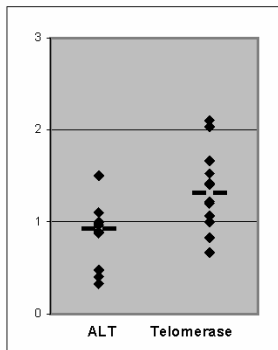
210: BZW1: basic leucine zipper and W2 domains 1

P-value:	4.0E-03		Fold change:	0.7

Chromosome: 2q33

Genbank: NM_014670.2   Gene ID: hCG2012145

Biological process: regulation of translational initiation

Molecular function: translation initiation factor activity

Cellular component:

Expression levels in ALT cell lines   median: **0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.00	0.88	0.41	0.48	0.99	0.48	0.33	0.89	0.96	1.50	0.97	1.10

Expression levels in Telomerase cell lines   median: **1.3**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.06	2.10	0.83	1.41	1.41	1.22	0.67	1.66	1.00	2.03	1.53	1.20
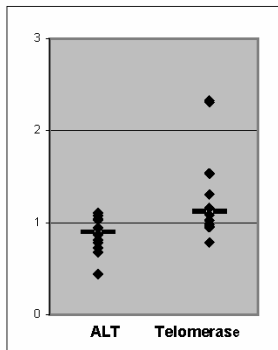
211: : yq69e10.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone IMAGE:201066 5'

P-value:	4.0E-03		Fold change:	0.8

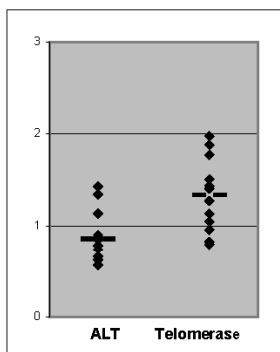Chromosome: 6   Genbank: R99836   Gene ID: hCG2040606

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: **0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.94	0.87	0.78	0.81	1.03	0.73	0.68	0.44	0.95	1.10	1.04	1.08

Expression levels in Telomerase cell lines   median: **1.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.08	2.33	1.16	0.98	1.53	1.30	0.78	0.95	2.31	1.02	1.54	0.95

212: : Clone PP1195 unknown mRNA

P-value:	4.0E-03		Fold change:	0.6

Chromosome: 8

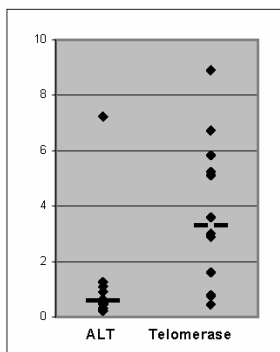Genbank: BG990708   Gene ID: hCG2042587

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: **0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	1.03	0.41	0.75	1.00	0.63	0.60	0.42	0.62	1.11	0.52	0.77

Expression levels in Telomerase cell lines   median: **1.1**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.05	1.54	0.88	1.06	0.82	0.55	1.31	1.15	1.60	1.51	1.00	1.32

213: LOC149134: hypothetical protein LOC149134

P-value:	4.0E-03								Fold change:	0.4	

Chromosome: 1q44

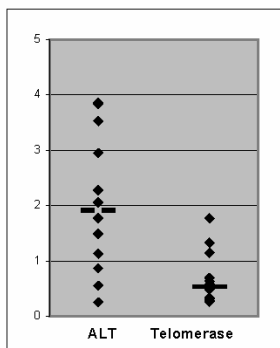Genbank: AK125177   Gene ID: hCG1982549

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.58	1.07	0.44	0.45	0.93	0.54	0.40	1.17	1.14	2.58	0.39	0.46

Expression levels in Telomerase cell lines   median: 1.6

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.83	2.61	1.41	0.62	2.23	0.92	0.77	3.73	5.69	1.71	1.26	0.88

214: N/A

P-value:	4.0E-03	Fold change:	0.6

Chromosome:

Genbank: Gene ID: hCG1644183.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.89	0.69	0.60	0.60	0.88	0.65	0.35	0.73	1.53	2.18	0.86	0.87

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.69	1.67	1.03	1.90	1.45	1.44	1.13	1.75	1.27	1.89	1.23	0.97

215: HT011: uncharacterized hypothalamus protein HT011

P-value:	4.0E-03	Fold change:	0.7

Chromosome: Xq26.1

Genbank: AK027029   Gene ID: hCG1756073.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.79	0.82	0.85	0.78	0.71	0.81	0.46	0.90	1.08	1.19	0.78	0.98

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.58	1.15	0.98	1.75	1.22	1.20	1.02	0.95	1.33	1.93	0.81	1.22
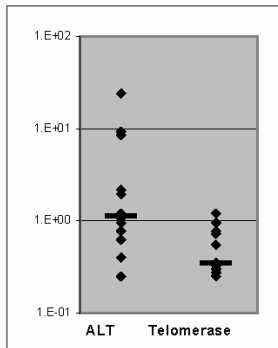
216: CA3: carbonic anhydrase III, muscle specific

P-value:	4.4E-03	Fold change:	3.2

Chromosome: 8q13-q22

Genbank: Gene ID: hCG1640519.3

Biological process: one carbon compound metabolism

Molecular function: carbonate dehydratase activity; lysase activity; zinc ion binding

Cellular component: cytoplasm

Expression levels in ALT cell lines   median: 1.1

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.20	0.25	9.25	1.08	23.95	8.50	2.18	1.95	0.95	0.78	0.40	0.63

Expression levels in Telomerase cell lines   median: 0.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.25	0.35	0.55	0.28	0.30	0.78	0.35	1.20	0.72	0.95	0.25	0.33
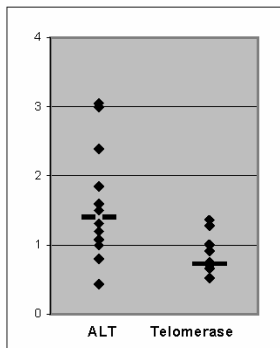
217: IDS: iduronate 2-sulfatase (Hunter syndrome)

P-value: 4.4E-03   **Fold change:** 1.9

Chromosome: Xq28

Genbank: NM_000202.2   Gene ID: hCG1993933

Biological process: metabolism;glycosaminoglycan metabolism

Molecular function: hydrolase, sulfuric ester hydrolase & iduronate-2-sulfatase activity

Cellular component: lysosome

Expression levels in ALT cell lines   median: 1.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.50	3.00	0.79	2.39	1.85	1.31	1.07	1.19	3.05	0.43	0.99	1.59

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.65	1.36	0.71	0.75	0.91	1.28	0.52	0.66	0.52	0.70	0.99	1.01
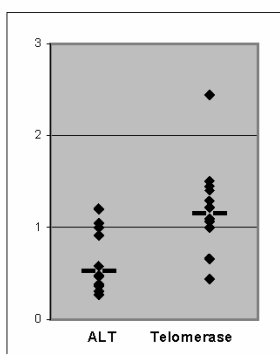
218: N/A

P-value: 4.4E-03   **Fold change:** 0.5

Chromosome:

Genbank: Gene ID: hCG2040549

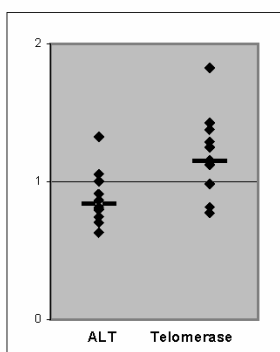Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.38	1.05	0.31	0.92	1.00	0.48	0.47	0.27	1.21	0.36	0.58	1.20

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.44	1.45	0.44	1.29	1.21	0.65	1.50	1.40	1.07	0.66	1.00	1.09

219: FAM35A: unnamed protein product; Sequence 14910 from Patent EP1074617.

P-value: 4.4E-03   **Fold change:** 0.6

Chromosome: 10q23.31   Genbank: AW062518   Gene ID: hCG1817851.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.78	1.43	0.62	0.66	0.90	0.85	1.34	0.87	1.13	0.87	0.56	0.74

Expression levels in Telomerase cell lines   median: 1.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.13	1.77	0.79	1.44	1.27	0.95	1.05	1.98	1.40	1.88	1.51	0.82

220: N/A

P-value: 4.4E-03   **Fold change:** 0.2

Chromosome: 4q32.3

Genbank: BE673692   Gene ID: hCG2026048
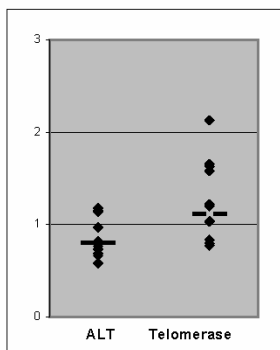
Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.6

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.47	0.59	0.22	0.62	0.51	0.45	7.23	0.91	1.27	0.65	0.32	1.09

Expression levels in Telomerase cell lines   median: 3.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.75	8.90	3.60	3.01	6.73	5.84	2.89	0.79	5.23	0.45	5.10	1.61

221: LOC284014: Similar to RIKEN cDNA C730027E14 (LOC388323), mRNA

P-value: 4.4E-03 **Fold change:** 3.6

Chromosome: 17p13.3

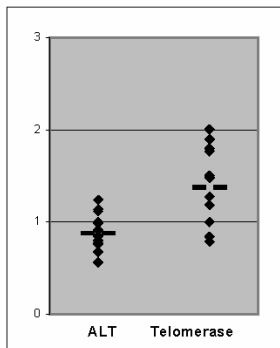Genbank: AK095567 Gene ID: hCG1986016

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 1.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.25	3.84	1.49	3.85	2.28	2.95	1.77	2.06	0.87	0.55	1.13	3.53

Expression levels in Telomerase cell lines median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.49	1.14	0.31	0.50	0.69	1.33	0.58	1.77	0.27	0.64	0.28	0.34
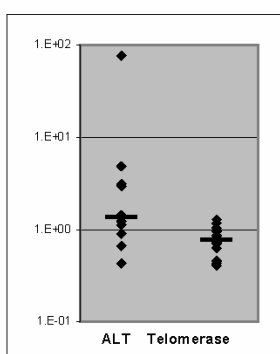
222: MGC50722: hypothetical MGC50722

P-value: 4.4E-03 **Fold change:** 1.8

Chromosome: 9

Genbank: BI831046 Gene ID: hCG2022366

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 1.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.67	0.90	1.37	2.96	1.23	1.38	1.43	76.78	4.88	1.12	3.11	0.43

Expression levels in Telomerase cell lines median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.96	1.04	0.74	0.43	0.70	0.81	0.41	1.17	0.46	0.63	1.28	0.86
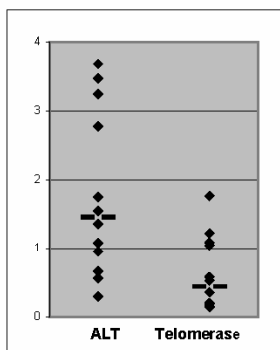
223: TRAM1L1: translocation associated membrane protein 1-like 1

P-value: 5.0E-03 **Fold change:** 3.3

Chromosome: 4q26

Genbank: NM_152402.1 Gene ID: hCG1642808.2

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines median: 1.5

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.66	1.08	0.30	0.95	1.55	1.36	3.48	3.24	3.68	2.78	0.56	1.75

Expression levels in Telomerase cell lines median: 0.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.58	0.14	1.09	1.22	0.15	0.36	0.18	1.05	0.52	1.77	0.18	0.20
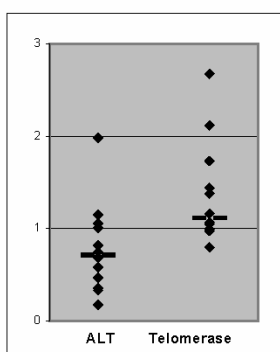
224: MGC42174: hypothetical protein MGC42174

P-value: 5.0E-03 **Fold change:** 0.6

Chromosome: 2q37.1

Genbank: AK094293 Gene ID: hCG2039371; hCG2039371; hCG2013107

Biological process:

Molecular function: RNA binding; ribonuclease activity

Cellular component:

Expression levels in ALT cell lines median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.18	1.01	1.06	0.68	0.82	0.35	0.75	1.15	1.98	0.47	0.33	0.58

Expression levels in Telomerase cell lines median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.80	1.05	1.44	1.73	0.99	1.38	1.16	2.67	0.97	2.11	0.97	1.07
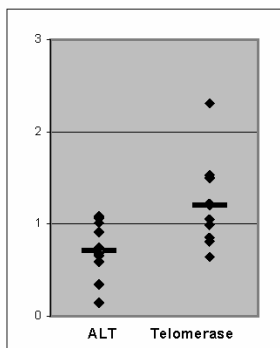
225: : CHR220333 Chromosome 22 exon Homo sapiens cDNA clone C22_426 5', mRNA sequence.

P-value:	5.0E-03		Fold change:	0.6

Chromosome: 22   Genbank: H55394   Gene ID: hCG1776624.1

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.14	1.06	0.34	0.65	0.91	1.01	0.68	0.59	0.66	1.07	1.08	0.74

Expression levels in Telomerase cell lines   median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.22	0.81	1.53	2.31	1.50	1.50	1.20	0.99	0.64	1.21	1.05	0.85

226: ANXA2: annexin A2

P-value:	5.0E-03		Fold change:	0.7

Chromosome: 15q21-q22   Genbank: NM_004039.1   Gene ID: hCG2004404

Biological process: skeletal development

Molecular function: calcium ion binding; calcium-dependent phospholipid binding; cytoskeletal protein binding; phospholipase inhibitor activity

Cellular component: plasma membrane; soluble fraction

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.05	0.79	1.32	0.70	0.81	0.80	1.00	0.74	1.00	0.86	0.63	0.91

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.38	1.82	0.77	1.15	1.43	1.25	0.98	1.12	1.15	1.29	0.81	1.12
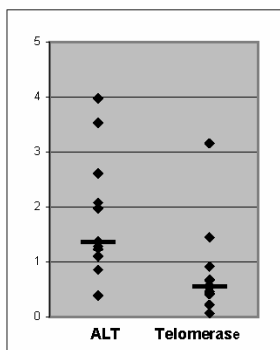
227: LOC375449: similar to microtubule associated testis specific serine/threonine protein kinase

P-value:	5.0E-03		Fold change:	2.5

Chromosome: 5q13.1   Genbank: AI431842   Gene ID: hCG2040209

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 1.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
2.09	1.98	2.61	3.98	1.30	1.09	1.23	0.85	1.36	3.54	0.39	1.38

Expression levels in Telomerase cell lines   median: 0.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.91	0.46	0.66	0.41	0.22	0.06	0.42	0.53	0.57	1.46	3.16	0.67

228: AMOTL2: angiomotin like 2

P-value:	5.0E-03		Fold change:	0.7

Chromosome: 3q21-q22

Genbank: NM_016201.2   Gene ID: hCG14791.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.15	0.79	1.18	0.66	0.97	0.82	0.69	1.14	0.82	0.77	0.73	0.58

Expression levels in Telomerase cell lines   median: 1.1

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
2.13	1.63	0.83	1.58	1.03	1.04	1.04	0.77	1.20	0.80	1.66	1.22

229: SLC15A4: solute carrier family 15, member 4

| P-value: | 5.0E-03 | | | | | | Fold change: | 0.6 |

Chromosome: 12q24.32

Genbank: NM_145648.1　　　　　　　　　　　　　　Gene ID: hCG21380.4

Biological process: oligopeptide transport

Molecular function: transporter activity

Cellular component: membrane

Expression levels in ALT cell lines　　　　　　　　　　　median: 0.9

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.24	0.76	1.12	0.85	1.00	0.80	0.98	0.77	1.14	0.56	0.68	0.91

Expression levels in Telomerase cell lines　　　　　　　median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.48	2.00	0.84	0.84	1.18	1.00	0.79	1.80	1.76	1.89	1.28	1.50
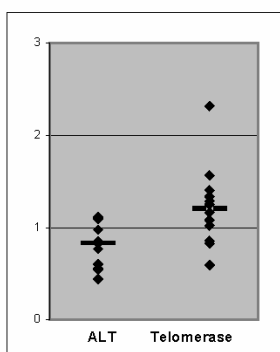
230: NDUFAF1: NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 1

| P-value: | 5.0E-03 | | | | | | Fold change: | 0.7 |

Chromosome: 15q11.2-q21.3　　　Genbank: NM_016013.1　　　Gene ID: hCG1786788.2

Biological process:

Molecular function: chaperone activity

Cellular component: mitochondrion

Expression levels in ALT cell lines　　　　　　　　　　　median: 0.8

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.10	0.77	0.84	0.85	0.98	0.83	1.12	0.44	0.55	1.11	0.56	0.60

Expression levels in Telomerase cell lines　　　　　　　median: 1.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.86	2.32	1.16	0.59	1.34	1.41	1.29	1.57	1.08	1.25	1.02	0.83
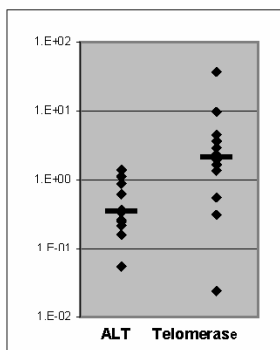
231: PTX3: pentaxin-related gene, rapidly induced by IL-1 beta

| P-value: | 5.0E-03 | | | | | | Fold change: | 0.2 |

Chromosome: 3q25

Genbank: NM_002852.2　　　　　　　　　　　　　　Gene ID: hCG26914.2

Biological process: inflammatory response

Molecular function:

Cellular component: extracellular

Expression levels in ALT cell lines　　　　　　　　　　　median: 0.4

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.88	0.22	0.26	1.39	0.25	0.37	1.40	0.05	0.16	1.12	0.62	0.34

Expression levels in Telomerase cell lines　　　　　　　median: 2.2

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
4.54	9.72	1.97	0.55	1.37	2.33	2.91	1.66	0.31	0.02	3.64	37.07
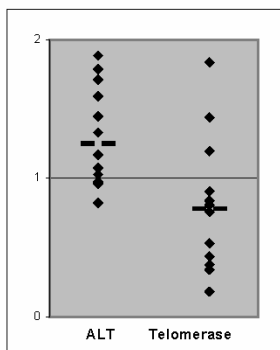
232: APBB2: amyloid beta (A4) precursor protein-binding, family B, member 2

| P-value: | 5.0E-03 | | | | | Fold change: | 1.6 |

Chromosome: 4p14　　　　　　Genbank: NM_173075.1　　　Gene ID: hCG33089.3

Biological process: regulation of transcriptn; -ve regulatn of S phase of cellcycle; actin filament-based movemnt; intracell signal cascde; protn stabilizatn; axonogenesis

Molecular functn: beta-amyloid binding; histone acetyltransfrase binding; transcriptn factor binding. Cellular compont: nucleus; lamellipodium; growth cone; membrne; synapse

Expression levels in ALT cell lines　　　　　　　　　　　median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.82	1.45	1.59	1.79	1.03	0.96	1.71	1.08	1.89	0.97	1.17	1.33

Expression levels in Telomerase cell lines　　　　　　　median: 0.8

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.20	0.53	0.84	0.38	0.81	0.34	1.84	0.91	0.18	0.44	1.44	0.76
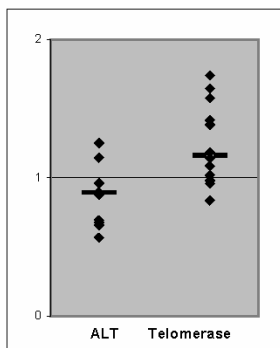
233: SHOC2: soc-2 suppressor of clear homolog (C. elegans)

P-value: 5.0E-03   **Fold change:** 0.8

Chromosome: 10q25   Genbank: NM_007373.2   Gene ID: hCG40725.4

Biological process: fibroblast growth factor receptor signaling pathway; RAS protein signal transduction

Molecular function: 0005554:molecular_function unknown

Cellular component: cytoplasm

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.69	0.88	0.90	0.89	0.96	0.57	0.68	1.25	1.15	0.66	1.15	0.96

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.14	1.65	0.84	1.74	1.18	1.09	0.98	1.58	1.02	1.42	0.96	1.38
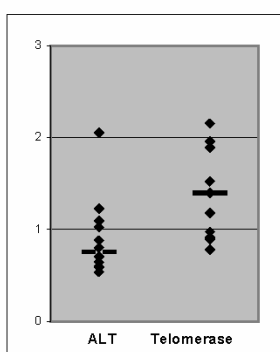
234: ZNF584: zinc finger protein 584

P-value: 5.0E-03   **Fold change:** 0.5

Chromosome: 19q13.43

Genbank: NM_173548.1   Gene ID: hCG1811578.1

Biological process: regulation of transcription, DNA-dependent
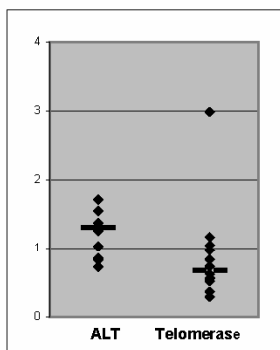
Molecular function: nucleic acid binding

Cellular component: intracellular

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.09	0.71	0.60	1.03	0.71	0.88	0.64	0.59	0.54	2.05	0.80	1.23

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.96	1.89	0.97	1.52	1.95	0.78	0.91	1.39	2.15	1.40	1.18	0.89
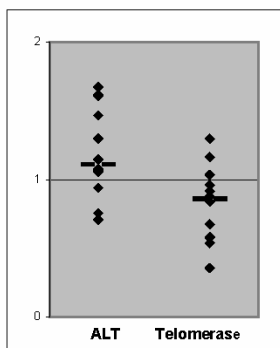
235: HECTD3

P-value: 5.0E-03   **Fold change:** 1.3

Chromosome: 1p34.1

Genbank: NM_024602.3   Gene ID: hCG1815097.1

Biological process: regulation of mitotic metaphase/anaphase transition; ubiquitin cycle

Molecular function: protein binding; ubiquitin protein ligase

Cellular component: anaphase-promoting complex; intracellular

Expression levels in ALT cell lines   **median: 1.1**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.06	1.62	1.61	1.30	1.68	1.07	1.15	1.47	0.94	1.08	0.76	0.71

Expression levels in Telomerase cell lines   **median: 0.9**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.88	1.30	0.68	0.84	0.92	0.96	0.54	1.04	0.59	1.17	0.35	0.58
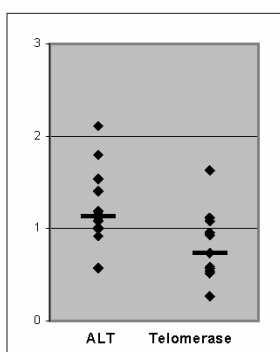
236: N/A

P-value: 5.0E-03   **Fold change:** 1.5

Chromosome:

Genbank: Gene ID: hCG38053.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 1.1**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.80	1.08	1.53	1.01	1.41	1.12	1.15	2.11	0.99	0.92	1.19	0.57

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.63	1.12	0.58	0.54	0.96	1.08	0.73	0.74	0.93	0.57	0.27	0.52
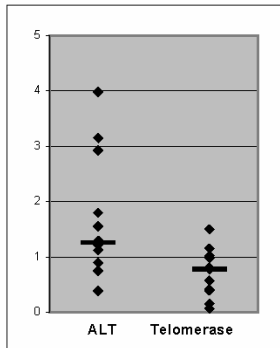
237: TGFB2: transforming growth factor, beta 3

P-value:	5.0E-03		Fold change:	1.6

Chromosome: 1q41   Genbank: NM_003238.1   Gene ID: hCG24906.4

Biological process: cell growth; cell proliferation; cell-cell signaling; organogenesis; regulation of cell cycle; signal transduction

Molecular functn: growth factor activity; transforming growth factor beta receptor binding

Cellular component:

Expression levels in ALT cell lines   **median: 1.3**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
3.98	1.23	3.15	1.22	0.75	0.89	1.12	1.55	2.92	1.29	1.80	0.39

Expression levels in Telomerase cell lines   **median: 0.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.98	1.50	1.02	0.79	0.81	0.78	1.15	0.41	0.07	0.57	0.15	0.39
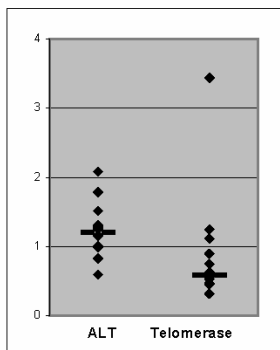
238: ZFP36L1: zinc finger protein 36, C3H type-like 1

P-value:	5.0E-03		Fold change:	2.0

Chromosome: 14q22-q24

Genbank: NM_004926.2   Gene ID: hCG20766.3

Biological process:

Molecular function: transcription factor activity

Cellular component: nucleus

Expression levels in ALT cell lines   **median: 1.2**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.16	0.99	0.60	1.26	1.52	1.01	0.83	1.31	1.79	2.08	1.15	1.28

Expression levels in Telomerase cell lines   **median: 0.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.32	1.25	0.46	0.56	1.12	0.53	0.47	0.46	0.75	3.44	0.63	0.90
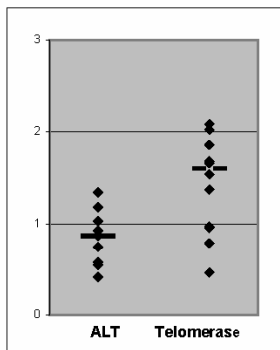
239: KIAA0690: KIAA0690

P-value:	5.0E-03		Fold change:	0.5

Chromosome: 10q24.2

Genbank: NM_015179.2   Gene ID: hCG25780.3

Biological process:

Molecular function: binding

Cellular component: integral to membrane; nucleus

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.57	0.88	1.18	1.03	0.86	0.54	0.41	1.03	1.34	0.75	0.74	0.92

Expression levels in Telomerase cell lines   **median: 1.6**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.37	2.09	0.97	1.68	1.66	0.95	0.46	1.68	1.54	2.03	1.86	0.79
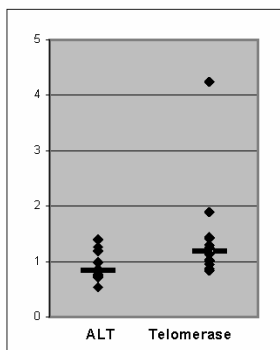
240: TPM3: tropomyosin 3

P-value:	5.0E-03		Fold change:	0.7

Chromosome: 1q21.2

Genbank: BE747618   Gene ID: hCG1817631.1

Biological process: regulation of muscle contraction

Molecular function: actin binding

Cellular component: cytoskeleton; muscle thin filament tropomyosin

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.39	0.99	0.83	0.76	1.19	0.74	0.71	1.26	0.85	0.87	0.54	0.72

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.42	1.44	0.84	1.03	1.25	1.01	0.95	4.24	1.30	1.89	1.13	0.87
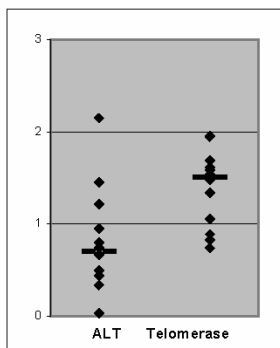
241: OPRS1: opioid receptor, sigma 1

P-value: 5.0E-03   Fold change: 0.5

Chromosome: 9p13.2

Genbank: NM_005866.2   Gene ID: hCG20471.4

Biological process: ergosterol biosynthesis

Molecular function: receptor activity; drug binding; C-8 sterol isomerase activity

Cellular component: integral to plasma membrane; endoplasmic reticulum

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.22	2.15	1.45	0.80	0.74	0.44	0.67	0.49	0.34	0.43	0.03	0.95

Expression levels in Telomerase cell lines   median: 1.5

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.74	0.89	1.61	1.69	1.95	1.34	1.48	0.83	1.54	1.05	1.96	1.59
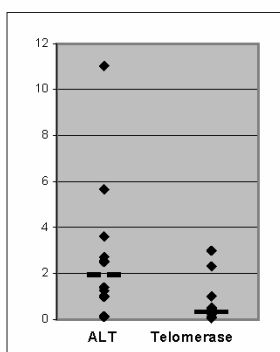
242: PCSK5: proprotein convertase subtilisin/kexin type 5

P-value: 5.0E-03   Fold change: 5.7

Chromosome: 9q21.3

Genbank: NM_006200.2   Gene ID: hCG2036726.1

Biological process: cell-cell signaling; proteolysis and peptidolysis

Molecular function: peptidase activity; hydrolase activity;subtilase activity

Cellular component: extracellular space

Expression levels in ALT cell lines   median: 2.0

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.40	1.02	2.50	0.99	0.12	1.26	5.66	2.55	11.02	2.71	3.61	0.15

Expression levels in Telomerase cell lines   median: 0.3

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.01	0.47	0.10	0.10	0.44	0.08	0.11	2.99	0.52	0.24	2.31	0.06

243: BCAS3: breast carcinoma amplified sequence 3

P-value: 5.0E-03   Fold change: 1.9

Chromosome: 17q23

Genbank: NM_017679.2   Gene ID: hCG1811950.1

Biological process: cell growth and/or maintenance

Molecular function:

Cellular component: nucleus

Expression levels in ALT cell lines   median: 1.3

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.37	1.26	0.83	1.29	1.33	1.55	1.34	1.55	1.03	1.71	0.86	0.73

Expression levels in Telomerase cell lines   median: 0.7

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.83	0.97	0.55	0.62	0.56	1.04	0.52	0.36	1.17	2.99	0.29	0.73
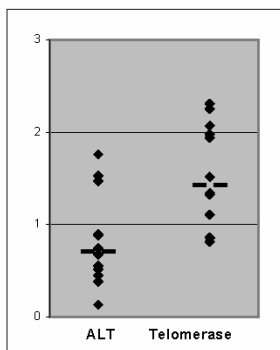
244: CLN8: ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)

P-value: 5.0E-03   Fold change: 0.5

Chromosome: 8p23   Genbank: NM_018941.2   Gene ID: hCG15110.2

Biological process: neurogenesis   Molecular function:

Cellular component: endoplasmic reticulum; integral to membrane; ER-Golgi intermediate compartment

Expression levels in ALT cell lines   median: 0.7

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.52	1.47	0.89	0.68	0.55	0.45	0.13	0.51	0.38	1.76	0.74	0.88

Expression levels in Telomerase cell lines   median: 1.4

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.86	1.11	0.86	1.52	2.25	1.34	0.81	1.94	2.07	2.31	1.98	1.33
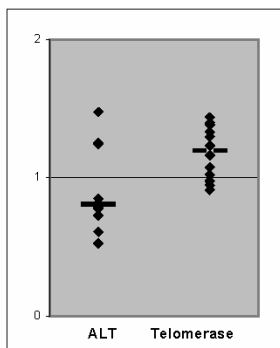
245: NEDD5: neural precursor cell expressed, developmentally down-regulated 5

P-value:	5.0E-03			Fold change:	0.7

Chromosome: 2q37   Genbank: AA346067   Gene ID: hCG2040455

Biological process: cell cycle; cytokinesis

Molecular function: GTP, nucleotide and protein binding; GTPase activity

Cellular component: contractile ring, cytoplasm, nucleus

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.48	1.25	0.82	0.61	0.81	0.78	0.53	1.25	0.73	0.81	0.79	0.85

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.23	1.33	0.95	1.44	1.30	1.16	0.98	1.40	1.38	1.02	0.91	1.08
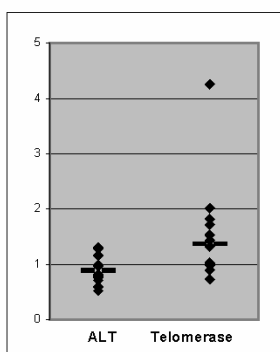
246: MPZL1; FLJ21047: Myelin protein zero-like 1; immunoglobulin superfamily member; Homo sapiens protein zero related protein (PZR) mRNA,

P-value:	5.0E-03			Fold change:	0.7

Chromosome: 1q23.3   Genbank: NM_024569.2   Gene ID: hCG42390.4

Biological process: cell-cell signaling; transmembrane receptor protein tyrosine kinase signaling pathway. Molecular function: protein binding; structural molecule activity

Cellular component: integral to plasma membrane

Expression levels in ALT cell lines   **median: 0.9**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.29	0.98	1.16	0.53	0.72	0.78	0.82	1.31	0.77	0.99	0.59	0.99

Expression levels in Telomerase cell lines   **median: 1.4**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.99	1.72	0.90	1.32	1.43	1.03	1.01	1.82	2.02	4.25	0.73	1.53
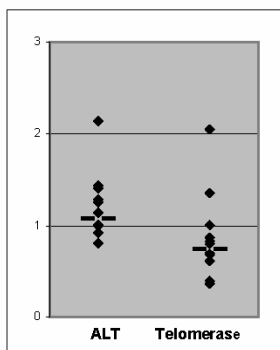
247: KIAA1458: KIAA1458 protein

P-value:	5.0E-03			Fold change:	1.4

Chromosome: 4p12

Genbank: BC031691   Gene ID: hCG17415.3

Biological process:

Molecular function:

Cellular component:

Expression levels in ALT cell lines   **median: 1.1**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.14	0.81	1.00	1.26	1.41	0.92	1.01	1.29	2.14	1.43	1.00	1.02

Expression levels in Telomerase cell lines   **median: 0.7**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.36	0.61	0.83	0.80	1.01	0.61	0.39	2.05	0.87	0.68	0.69	0.36
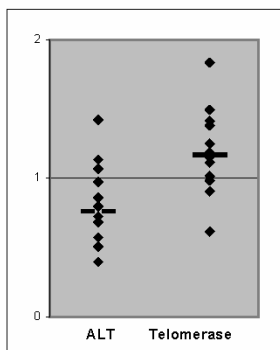
248: CCNDBP1: cyclin D-type binding-protein 1

P-value:	5.0E-03			Fold change:	0.7

Chromosome: 15q14-q15

Genbank: NM_012142.2   Gene ID: hCG38719.2

Biological process:

Molecular function:

Cellular component: nucleus

Expression levels in ALT cell lines   **median: 0.8**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.14	0.73	0.97	1.07	0.40	0.86	0.69	0.69	0.80	1.42	0.57	0.51

Expression levels in Telomerase cell lines   **median: 1.2**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
1.19	1.84	0.98	1.02	1.38	1.11	0.62	1.49	1.15	1.25	0.91	1.41
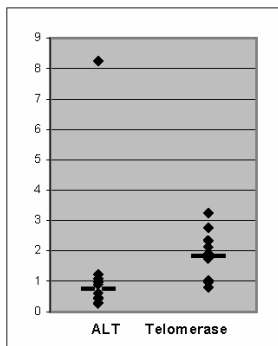
249: CSE-C: cytosolic sialic acid 9-O-acetylesterase homolog

P-value: 5.0E-03   **Fold change:** 0.4

Chromosome: 11q24

Genbank: NM_170601.1   Gene ID: hCG39813.2

Biological process:

Molecular function: hydrolase, serine esterase and sialate O-acetylesterase activity

Cellular component: lysosme

Expression levels in ALT cell lines   **median: 0.7**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
0.61	0.30	0.98	0.95	0.29	1.08	0.42	0.46	0.89	8.26	0.26	1.22

Expression levels in Telomerase cell lines   **median: 1.8**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.80	1.00	1.91	1.00	2.12	1.02	2.77	2.33	1.74	2.35	0.96	3.24
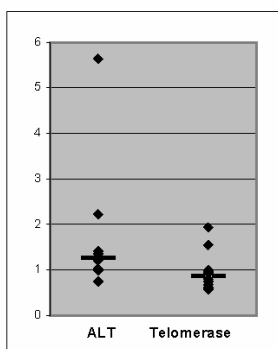
250: ABHD2: abhydrolase domain containing 2

P-value: 5.0E-03   **Fold change:** 1.5

Chromosome: 15q26.1

Genbank: NM_152924.2   Gene ID: hCG28637.2

Biological process: biological_process unknown

Molecular function: catalytic activity; molecular_function unknown

Cellular component: integral to membrane

Expression levels in ALT cell lines   **median: 1.3**

1.3C	1R	V2	2RA	GM847	AT1	MeT4A	SAOS2	U2OS	SKLU1	SUSM1	IIICFc
1.21	2.22	0.75	1.27	1.26	1.28	1.41	1.35	1.00	5.64	1.03	0.99

Expression levels in Telomerase cell lines   **median: 0.9**

6G	1P	V1	BFT3K	GM639	AT22	6TGR	TE85	MG63	A2182	HT1080	F80
0.74	0.94	1.00	0.61	0.96	0.58	0.80	0.61	1.93	1.55	0.97	0.68

B. Quantitation of Western blot analysis for DRG2, AGT and SATB1

	DRG2	Dlower	DRG2:Dlower	AGT	SATB1
Mortal					
JFCF-6	0.58	0.82	0.71	1.14	0.20
HFF5	0.26	0.20	1.32	1.48	0.13
WI-38	0.21	0.44	0.48	0.59	0.13
MRC5	0.56	0.74	0.76	1.31	0.38
IIICF	0.54	0.77	0.71	1.23	0.28
BF-10	0.80	0.82	0.97	0.86	0.20
Median	**0.55**	**0.75**	**0.73**	**1.19**	**0.20**
ALT					
JFCF-6/T.1R	0.47	0.88	0.53	0.00	0.73
GM847	0.16	0.48	0.33	0.00	0.22
WI38-VA13/2RA	0.17	0.58	0.30	0.00	0.73
MRC5-V2	0.38	0.46	0.82	0.00	0.59
IIICF/c	0.58	1.20	0.49	0.25	0.85
BET-3M	1.08	1.17	0.93	1.77	0.64
GM637	0.39	0.58	0.68	1.16	N/A
KMST6	0.17	0.55	0.31	1.21	N/A
SUSM-1	0.33	0.73	0.46	0.00	N/A
AT1BR44neo	0.19	0.38	0.50	0.00	N/A
MeT-4A	0.25	0.31	0.81	0.00	N/A
Saos-2	0.14	0.20	0.72	0.26	N/A
G292	0.75	0.18	4.06	3.58	N/A
U-2 OS	0.72	0.67	1.08	1.13	N/A
SK-LU-1	0.79	1.10	0.72	0.08	N/A
Median	**0.38**	**0.58**	**0.68**	**0.08**	N/A
Median; gel1	0.42	0.73	0.51	0.00	**0.68**
Median; gel2	0.33	0.55	0.72	0.26	N/A
Telomerase					
JFCF-6/T.1P	0.53	0.39	1.36	1.64	0.40
GM639	0.42	0.39	1.08	0.20	0.25
BFT-3K	0.37	0.51	0.72	0.13	0.39
MRC5-V1	0.73	0.74	1.00	1.96	0.49
HT1080	1.00	0.82	1.22	0.63	1.36
BET-3K	1.16	1.12	1.04	2.67	0.36
A549	0.78	0.57	1.37	1.40	N/A
HeLa	0.67	0.13	5.05	1.82	N/A
F80-TERT/K1-1	0.45	0.67	0.67	0.25	N/A
AT22IJE-T	0.42	0.42	1.00	0.70	N/A
MeT-5A/6TGR-B	0.35	0.50	0.70	2.63	N/A
TE-85	0.75	0.49	1.54	0.33	N/A
SJSA-1	0.49	0.36	1.38	0.55	N/A
MG-63	0.64	0.53	1.22	1.35	N/A
A2182	0.66	0.41	1.61	0.69	0.11
Median	**0.64**	**0.50**	**1.22**	**0.70**	N/A
Median; gel1	0.63[a]	0.62[a]	1.06[a]	1.14[a]	**0.40**[a]
Median; gel2	0.64	0.49	1.37	0.70	N/A
MeT-5A[b]	0.12	0.16	0.73	2.39	N/A

[a]Unmatched A2182 omitted from the median of gel 1. [b]MeT-5A was not included in the telomerase analysis as it has some evidence of ALT activity (section 3.1).

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