

7B6, a ubiquitous mRNA: with significant homology to L41 human ribosomal protein RNA

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We report here the nucleotide sequence of a 350 base pair cDNA clone previously designated p7B6 (1) identified originally as a cell cycle-independent transcript in a cDNA library from a patient with chronic lymphocytic leukaemia (1). This clone has been used as an invariant control in Northem blot analysis (1–3) but no DNA sequence was available. The cDNA hybridises to a constantly expressed mRNA that appears to be abundant in human tissue (2) and mammalian cell lines cultured in different physiological conditions (1, 3) thus providing a good control transcript that may be very useful for RNA quantification.

We obtained the cDNA sequence from the original clone (p7B6/pBR322) using a conventional double-stranded sequencing method (4). Fasta (5) searching of the EMBL database revealed that p7B6 shared homology (95.4% identity in a 283bp overlap) to the mRNA for the human ribosomal protein L41 (accession number Z12962, unpublished) which participates in amino-acyl-tRNA binding ensuring fidelity in translation of mRNA. A sequence alignment using ALIEN (6) between p7B6 and the human ribosomal protein L41 is shown in Figure 1.

Sequence similarity was also observed with two expressed sequence tags recently identified in a human HepG2 3′-directed MboI cDNA library (7). Clones Hshmo1e06 and Hshmo2h10 (accession numbers D11773 and D11860) have 95% identity over a 96bp overlap and 94.7% identity over a 95bp overlap respectively with p7B6.

p7B6, with a small transcript size of 0.6kb (1) has a significant advantage over other loading controls e.g. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin whose transcripts are within the range of average mRNA size distribution (1.3kb and 2.1kb respectively) since it allows accurate detection of low abundance class mRNA during repeated re-probing of membranes in Northern hybridisations. Now that the cDNA sequence for p7B6 is available it may also prove useful as an invariant control in quantitative RT-PCR experiments with eukaryotic cells and tissues.

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h7b6      1   GAAGCGCAAAAGAAGAAATA-GAGGCAGAGGTCCAAGTAAACCGCTAGCTTGTTGCACCAGT
HL41      122 GAAGCGCAAAAGAAGAAAGATGAGGCAGAGGTCCAAGTAAACCGCTAGCTTGTTGCACC-GT
              ################## # ######################################## ##

h7b6      63  GGAGGCCACAGGAGCAGAAACATGGAATGCCAGACGCTGGGGATGCTGGTACAA-TTGTGGG
HL41      183 GGAGGCCACAGGAGCAGAAACATGGAATGCCAGACGCTGGGGATGCTGGTACAAGTTGTGGG
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h7b6      125 ACTGCATGCTACTGTCTAGAGCTTGTCTCAATGGATCATAGACTT-ATCGCCCCTCTGTACGC
HL41      245 ACTGCATGCTACTGTCTAGAGCTTGTCTCAATGGATCTAGAACTTCATCGCCCCTCTGATCGC
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h7b6      187 CGATCACCTCTCACACCCACCTTGCTCATAAACAAAATGCCCATGTTGGTCCTCTGCCCTGG
HL41      307 CGATCACCTCTGAGACCCACCTTGCTCATAAACAAAATGCCCATGTTGGTCCTCTGCCCTGG
              ########### # ##################################################

h7b6      249 ACCTGTGACATTCTGGACTATTTCTGTGTTTATTT----------------------------
HL41      369 ACCTGTGACATTCTGGACTATTTCTGTGTTTATTTGTGGCCGAGTGTAACAACCATATAATA
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h7b6          ------------------------------------------------
HL41      431 AATCACCTCTTCCGCTGTTTTAGCTGAAGAATTAAATCAAAAAAAAA
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Figure 1. Comparison of the cDNA sequence of p7B6 and L41 human ribosomal protein. Key; identity #.