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pUEX, a bacterial expression vector related to pEX with universal host specificity

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The pUEX vectors were designed as relatives of the pEX family of bacterial expression vectors (Stanley and Luzio, 1984), giving controlled expression in all *E. coli* strains as a result of the cI857 gene located in the plasmid sequence. The construction was designed so that the cI gene and cro gene fragment were located about the Pr and Prm promoters in the same way as is found in bacteriophage lambda. The unique restriction enzyme sites and reading frames through the cloning linker are identical to those of the corresponding pEX vector (Fig. 1). As with the pEX plasmids, all transformation and amplification procedures must be carried out at <34°C (we normally use 30°C). Logarithmically growing cells are maintained at 42°C for 2h to express fusion proteins, but are never used for DNA extraction after this step.

The major goal of generating the pUEX vectors was to gain the freedom to use high efficiency bacterial strains for making large cDNA libraries. The pUEX vectors transformed into DH5 or MC1061 cells gave a similar transformation efficiency compared with other plasmids (e.g. pBR322) and were capable of expressing cro'-β-galactosidase fusion proteins at high levels (about 30% of total protein) in a controlled manner.

When DNA was isolated from cells harbouring the pUEX plasmids it was noticed that the yield was several fold higher than for cultures harbouring the pEX plasmids so the copy number of plasmid in the cells was measured by counting the percentage of ampicillin-resistant colonies after transfering cultures to 42°C in the absence of ampicillin. For pEX1 transformed cells, ampicillin resistance is lost almost immediately giving rise to a 50% loss after about 1 division of the cells. This suggests that the pEX plasmids are maintained at a single copy per cell and are not replicated when expression is switched on at 42°C. For pUEX the 50% level of resistance to ampicillin was reached after a further 2 to 3 division cycles depending on the host strain. Assuming that plasmid replication is inhibited at 42°C as found for pEX, this gives a copy number of about 4. The higher copy number facilitates epitope mapping in the vector (Stanley and Herz, 1987) since DNA preparation for double strand sequencing is made easier. A full assembled sequence has been submitted to the EMBL nucleotide sequence data bank. The pUEX plasmids may be obtained from Amersham International plc, Lincoln Place Green End, Aylesbury, Buckinghamshire, England, HP20 2TP.

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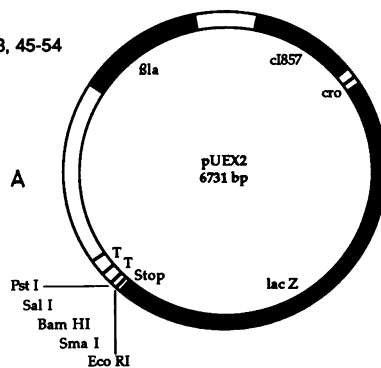

B

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             SmaI BamHI  SalI             PstI
pUEX1   GCC CGG GGA TCC GTC GAC CTG CAG CCA AGC TTG CTG ATT GAT TGA
        Ala Arg Gly Ser Val Asp Leu Gln Pro Ser Leu Leu Ile Asp ***

        EcoRI   SmaI BamHI   SalI           PstI
pUEX2   GAA TTC CCG GGG ATC CGT CGA CCT GCA GCC AAG CTT GCT GAT TGA
        Glu Phe Pro Gly Ile Arg Arg Pro Ala Ala Lys Leu Ala Asp ***

                    SmaI BamHI  SalI            PstI
pUEX3   GAA TTA ATT CCC GGG GAT CCG TCG ACC TGC AGC CAA GCT TGC TGA
        Glu Leu Ile Pro Gly Asp Pro Ser Thr Cys Ser Gln Ala Cys ***
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Figure 1 Construction of pUEX plasmids

A: The pUEX vectors were constructed by cloning a *Pst* I - *Bgl* II fragment containing the cI857 gene, Pr promoter and cro gene from pOU110 (Larsen et al., 1984) into M13mp9 and deleting 36bp of the cro gene by site directed mutagenesis. This fragment was then excised and ligated into *Pst* I and *Bam* HI cut pLK915 (Stanley and Luzio, 1984). The pUEX vectors were generated from this intermediate by cloning a *Bam* HI fragment containing the lac Z gene into the vector and deleting one of the *Bam* HI sites so as to make a vector essentially identical to pEX2, but containing the cI857 gene. pUEX1 and pUEX3 were constructed from pUEX2 by exchanging restiction enzyme fragments with the appropriate pEX plasmids. B: Reading frames and unique restriction enzyme sites of pUEX1-3

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