



Restriction Map and Multiple Cloning Site (MCS) of pYEX4T-1. Unique restriction sites are in bold.

Description:

pYEX 4T-1 is based on the pYEULC plasmid (1). The Cu⁺⁺-inducible *CUP1* promoter from the yeast metallothionein gene drives expression of the fusion cassette (2). The GST gene is derived from the parasitic helminth *Schistosoma japonicum* (3). The GST coding region encodes a protein of approximately 27.5 kDa (3). There is a cleavage site for the protease thrombin immediately upstream of the multiple cloning site. The thrombin recognition sequence consists of 18 nucleotides and spans the *BamH I* recognition sequence in the MCS. Treatment of the recombinant fusion protein with thrombin will release the cloned protein from the GST moiety. The MCS includes a stop codon in each reading frame.

pYEX 4T-1 includes the *E. coli* Amp^r gene and the yeast selectable markers *leu2-d* (a *LEU2* gene with a truncated, but functional promoter) and *URA3*. pYEX 4T-1 is maintained at high copy number to provide enough gene product from the inefficient *leu2-d* promoter for cell survival during growth selection on media lacking leucine (4).

Use:

pYEX 4T-1 is a dual-host expression vector designed for high-level expression of glutathione S-transferase (GST) fusion proteins in yeast. Researchers using one- and two-hybrid systems to study protein-DNA and protein-protein interactions can express identified genes in yeast for *in vitro* studies. pYEX 4T-1 allows for the production of GST fusion proteins which are easily tracked using GST antibody and purified by single-step affinity chromatography on glutathione sepharose. Yields of purified fusion protein using the yeast pYEX 4T system have been reported to be from 0.5–6 mg/L (5).

Location of features

- Yeast *CUP1* promoter
Transcription start point: 4850–4856
- Glutathione S-transferase: 5012–5680
GST start codon (ATG): 5012–5014
- Multiple cloning site: 5703–5744
- Ampicillin resistance (β -lactamase) gene
Promoter: 2631–2659
Start codon (ATG): 2701–2703
Stop codon (TAA): 3559–3561
- pUC plasmid replication region
Site of replication initiation: 4319
Region necessary for replication: 3626–4322
- *URA3* coding sequence
Start codon (ATG): 2264–2262
Stop codon (TAA): 1463–1461
- *leu2-d* coding sequence
Start codon (ATG): 7479–7477
Stop codon (TAA): 6389–6387

Sequencing primer locations

- pYEX 4T Forward Sequencing Primer: 5615–5631
5' GCATGGCCTTTGCAGGG
- pYEX 4T Reverse Sequencing Primer: 5803–5787
5' TTTGCAGCTACCCACATT

pYEX-S1 Insertion Primer locations

- 5' PCR Primer: 5012–5030
- 3' PCR Primer: 6209–6191

Propagation in *E. coli*

- Suitable host strains: DH5 α , HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: 400–500

Propagation in yeast

- Nutritional markers: plasmid confers ability to synthesize leucine and uracil
- Yeast replication origin: 2 μ
- *S. cerevisiae* strains suitable for transformation and copper-inducible expression of foreign proteins include:
DY150 (included in kit; *MATa*, *ura3-52*, *leu2-3*, *112*, *trp1-1*, *ade2-1*, *his3-11*, *can1-100*)
6657-4D (*leu2-3*, *112*, *his3-11*, *15*, *CUP1^R*)
DBY745 (*leu2-3*, *112*, *ade1-100*, *ura3-52*, *CUP1^R*)
IMY21 (diploid of the above two strains, 6657-4D and DBY745)
DBY747 (*his3-11*, *leu2-3*, *112*, *trp1-289*, *ura3-52*, *CUP1^R*)

Note: All procedures described in here and in User Manual have been tested using the DY150 yeast strain.

References:

1. Macreadie, I. G., *et al.* (1991) *Gene* **104**:107–111.
2. Macreadie, I. G., *et al.* (1989) *Plasmid* **21**:147–150.
3. Smith, D. B. & Johnson, K. S. (1988) *Gene* **67**:31–40.
4. Gietz, R. D. & Sugino, A. (1989) *Gene* **74**:527–534.
5. Ward, A. C., *et al.* (1994) *Yeast* **10**:441–449.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.