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AS ONE

cDNA Library, S. pombe, Log Phase

02-703 500 ng

This cDNA library (plasmid DNA) is constructed from *Schizosaccharomyces pombe* strain h-L972-derived poly(A)+ RNA at the log phase by the Linker-Primer method (Ref.1) by Prof. H. Nojima of Research Institute for Microbial Diseases, Osaka University. cDNAs in this library are unidirectionally cloned by using the oligo $(dT)_{18}$ linker primer which contains the restriction enzyme sites of *Not* I, and *Bam*HI (*Bgl* II)-*Sma* I adaptor.

The pLZ3 vector used in this library can replicate both in *S. pombe* and *E. coli*, and express the *S. pombe* genes in mammalian cells as it contains SV40 promoter as well as in *S. pombe* (see Figure and Ref.2).

Applications

1. PCR screening of known or unknown gene: Prepare the primers for the known or unknown gene (cDNA) and amplify the gene by PCR from this library followed by cloning to an appropriate vector (Ref 3). The cloned cDNAs are useful for identifying the coding region, large-scale protein production, and preparation of probes, etc. Standard amplifying conditions: 35 cycles of PCR reactions using 10-100 ng of cDNA as a template. (Change the quantity of template and the number of cycles depending on the expression levels of mRNA of the gene of interest.)

2. Cloning the cDNA by functional complementation of the corresponding *S. pombe* mutants.

Specifications

Quantity: 500 ng (40 ng/ul, 13ul) in 10 mM Tris-HCl-1mM EDTA (pH 7.5)
Quality: Number of independent clones: 28 x 106
Average insert size: longer than 1 kb
Storage: -20°C

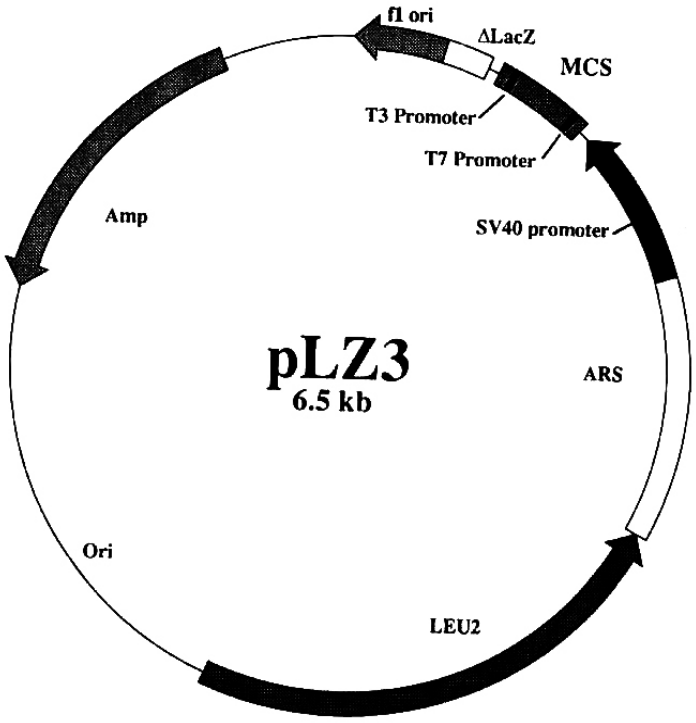
References

1. Kobori M et al “Large scale isolation of osteoclast-specific genes by an improved method involving the preparation of a subtracted cDNA library.” Genes Cells 3: 459-475 (1998) PMID: 9753427
2. Tanaka S and Nojima H ”Nik1: a Nim1-like protein kinase of S. cerevisiae interacts with the Cdc28 complex and regulates cell cycle progression.” Genes to Cells 1, 905-921 (1996) PMID: 9077450
3. Sambrook J and Russell DW Molecular Cloning Chapter 11 ”Preparation of cDNA libraries and gene identification.” CSHL Press (2001)

Note

* This library is to be used only by the purchaser. It is not allowed to amplify and transfer the library to a third person.

Figure 1: Structure of pLZ3 and the restriction sites. Ars is the S. pombe region required for replication in S. pombe, and Ori is a plasmid origin for replication in E. coli.



; MCS(pLZ3)

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                    CpoI(3)   SauI(b) MluI(5)                    AatII(3) BglII(5)  AscI(5)       BalI(b)
            PstI(3)SacI(3)    ApaI(3)   ------                      ------ ------   --------       ------
             SseI(3)------      -------------     T7  Promoter   EcoRI(5)  XbaI(5)AflII(5)      BstXI(5)
            --------   -------   -------      ----------------- ------   ------ ------     ------------ --
NNNCTGCA  CCTGCAGGAGCTCGGACCGGGCCCTTAGGACGCGTAATACGACTCACTATAGGGAATTCGACGTCTAGATCTTAAGGCGCGCCAAGGGGTTGGCCA
NNNG  ACGTGGACGTCCTCGAGCCTGGCCCGGGAATCCTGCGCATTATGCTGAGTGATATCCCTTAAGCTGCAGATCTAGAATTCCGCGCGGTTCCCCAACCGGT

     BstEII(5)
      -------        NheI(5)                                                  SwaI(3)       NruI(b)       SacII(3)
SnaBI(b)    DraIII(3) ------    SceI(3)          NotI(5)  T3 promoter --------SplI(5)------ PacI(3)------  SacI(3)
 ------     ----------      ------------------      -------------------      ------      --------      ------
   CGTGGTAACCACGGGGTGGCTAGCTAGGGATAACAGGGTAATATAGCGGCCGCCCTTTAGTGAGGGTTAATTTAAATCGTACGTCGCGATTAATTAACCGCGGTGGAGCT  CAAT
   GCACCATTGGTGCCCCACCGATCGATCCCTATTGTCCCATTATATCGCCGGCGGGAAATCACTCCCAATTAAATTTAGCATGCAGCGCTAATTAATTGGCGCCACC  TCGACTTA

   TCGCCCTATAGTGAGTCGTATTA -3'
   AGCGGGATATCACTCAGCATAAT -5'
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