cDNA Library, *S.pombe*, After + HU, γ, MMS

02-707　　500 ng

This cDNA library (plasmid DNA) is constructed from poly(A)$^+$ RNA derived from *Schizosaccharomyces pombe*, strain h-L972 after treatment with HU(hydroxyurea), γ-radiation, and MMS(methylmethane sulfonate). It is constructed by the Linker-Primer method (Ref.1) by Professor Hiroshi Nojima of Research Institute for Microbial Diseases, Osaka University. This library is unidirectionally cloned by using the oligo $(dT)_{18}$ linker primer which contains the restriction enzyme site 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 *in E. coli*, and express cloned genes not only in *S. pombe* but also in mammalian cells as it contains SV40 promoter. It also contains f1 ori which is necessary for ssDNA synthesis, and bacteriophage T7 and T3 promoter for RNA synthesis (see Figure and Ref.2).

Application

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. It is useful for large-scale protein productions, 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 rate of mRNA of the objective gene.)

2. Cloning the cDNA by functional complementation of the corresponding mutant strains.

Specification

Quantity: 500 ng (40 ng/ul, 13ul) in 10 mM Tris-HCl-1mM EDTA (pH 7.5)

Quality:　1) Number of independent clones: 7.7×10^6

2) Average insert size : longer than 1 kb

Storage:　-20℃

References

1. Kobori,M., Ikeda,Y., Nara,H., Kato,M., Kumegawa,M., Nojima,H., and Kawashima,H. " 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 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 researches of the purchaser. It is not allowed to amplify and transfer the library to a third person.
* Related products: human tissue specific cDNA libraries and cDNA libraries of model organisms (See HP).

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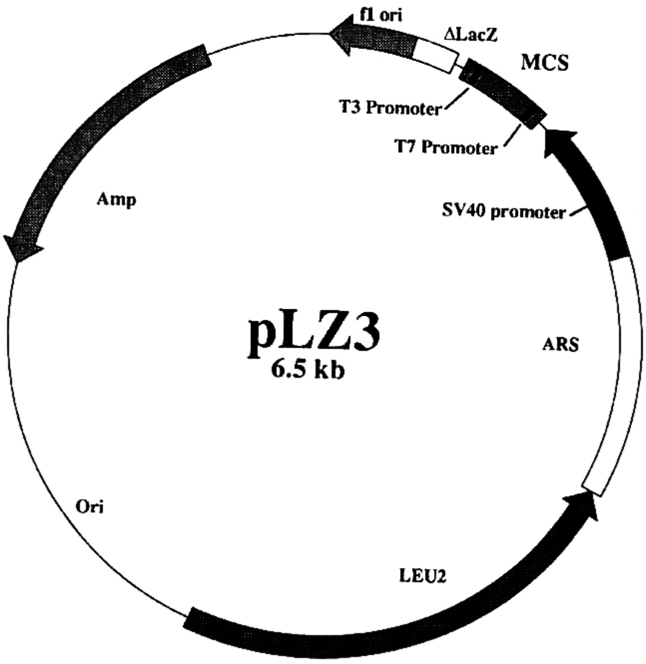

; MCS(pLZ3)

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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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Fig. Structure of pLZ3 and the restriction sites

Ars is the S. pombe region required for replication in *S. pombe*, and **Ori** is an origin required for replication in *E. coli*

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