

Restriction map and multiple cloning site of pEXP-LIB Vector. Unique restriction sites are in bold.

Description

pEXP-Lib Vector contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA (1,2). After selection with puromycin, nearly all surviving colonies will stably express the gene of interest, thus decreasing the need to screen large numbers of colonies to find functional clones. The expression cassette of pEXP-Lib contains the human cytomegalovirus (CMV) major immediate early promoter/enhancer followed by a multiple cloning site (MCS), the ECMV IRES followed by the gene encoding puromycin resistance (puromycin-N-acetyltransferase, or pac), and the polyadenylation signal of the bovine growth hormone. Ribosomes can enter the bicistronic mRNA either at the 5' end to translate the gene of interest or at the ECMV IRES to translate the antibiotic resistance marker. Sfi IA and Sfi IB indicate two distinct Sfi I sites that differ in their interpalindromic sequences. This arrangement permits cloning of libraries such that all inserts are in the proper orientation for protein translation. pEXP-Lib can be used as an alternative vector in the construction of an expression library using the SMART™ cDNA Library Construction Kit (Cat. No. 634901). Restriction digestion with Sfi I will excise the Stuffer fragment prior to cloning. If you purchased a library in this vector, the inserts will be between the Sfi IA and Sfi IB sites in the vector.

Use

When using the pEXP-Lib Vector, the antibiotic exerts selective pressure on the whole expression cassette; thus, a high dose of antibiotic will select only cells expressing a high level of the gene of interest. This selective pressure also ensures that the expression of the gene of interest will be stable over time in culture. Unless your expression experiments require a pure population of cells, you can use the pool of cells surviving selection instead of isolating and characterizing clonal cell lines. We recommend selecting mammalian clones in 10–100 μ g/ml of puromycin (Cat. No. 631305), depending on the cell line (be sure to establish a kill curve for each lot of puromycin to determine optimal effective dose).

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Location of features:

• $P_{\text{CMV IE}}$ promoter: 232–820

Cloning site for Mammalian Expression cDNA Libraries (Sfi IA and Sfi IB): 952–1172

Internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV): 1220–1820

Puromycin resistance gene: 1839–2438

Fragment containing the bovine growth hormone poly-A signal: 2555–2832

 Ampicillin resistance gene: 4951–4091 pBR322 origin of replication: 3247–3921

Primer locations:

pEXP-Lib Primers

5' primer (848-876): 5'-CTGGCTTATCGAAATTAATACGACTCACT-3' 3' primer (1212-1185): 5'-TTGGCCGCCCTAGATGCATGCTCGACCC-3'

Propagation in *E. coli*:

- Suitable host strains: DH5 α , and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to E. coli hosts.

References:

- 1. Jackson, R.J., et al. (1990) Trends Biochem. Sci. 15:477-483.
- 2. Jang, S.K., et al. (1988) J. Virol. 62:2636-2643.

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