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pMEV

Catalog# P1000
Lot# Labeled on vial

Materials Provided

1. pMEV plasmid: 20 μg in 40μl TE (pH7.5), 0.5 mg/ml.
2. N-terminal Sequencing primer (5'-ccgtcagatcactagaagctt-3'). 50 pmole at 10 pmol/ml (50 ul).
3. Product Information Sheet.

Receiving and Storage:

Upon receiving, spin the vials briefly in a microcentrifuge to collect the contents. Store the products at 2-8^{0}C if used immediately and store at –20^{0}C for extended storage.

Prokaryotic/Eukaryotic Selection:

Kan/Neo (2228-3022): Confers kanamycin resistance in bacteria; It confers G418 resistance in mammalian cell.

Other Features:

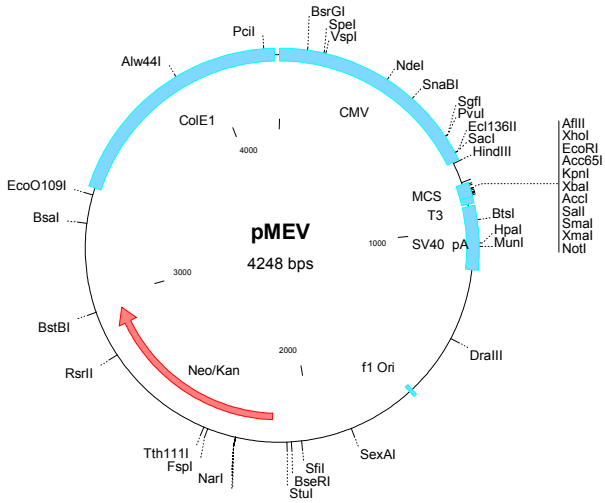
Name	Start	End
CMV Promoter:	1	750
Multiple Cloning Site (MCS):	835	886
T3 RNA Polymerase Promoter:	886	907
SV40 Polyadenylation signal:	915	1137
f1 Replication Origin:	1613	1625
Neo/Kan-Resistance Gene:	2150	2944
ColE1 Replication Origin:	3400	4232

Maintenance of pMEV Vectors

For help with general plasmid DNA manipulation techniques like restriction digestion, ligation and transformation, see *Molecular Cloning: A Laboratory Manual (*Sambrook *et al., 1989*) or *Current Protocols in Molecular Biology* (Ausubel et al., 1994). We recommend that you propagate the vectors in *E. coli* strains that are recombinant deficient (*rec*A) and endonuclease A-deficient (*end*A) (e.g. DH5α or XL1-Blue).

Circular Map of pMEV:

The full-length sequence of pMEV can be downloaded from www.biomyx.net



About pMEV Vectors

pMEV mammalian expression vectors are designed for high level protein expression and detection in mammalian cells. The human cytomegalovirus immediate-early (CMV) promoter provides high-level expression in a wide range of mammalian cells. The sequences in between the CMV promoter and the MCS are optimized to eliminate potential secondary structures. In addition, pMEV is less than 4.3 kilo-base pairs long, owing to the use of the Neo/Kan coding sequence in the vectors for both bacterial selection with kanamycin and mammalian cell selection with G418. The small size makes cloning process more efficient and higher yield for plasmid purification. Stable mammalian cell lines can be established by selecting clones with G418 after transfection with pMEV vectors.

Note: *pMEV is not a fusion vector. If an affinity tag is desirable, another vector set (pMEV-2HA, Cat#P1001) can be used.*

Cloning into pMEV Vectors

The coding sequence for the gene of interest (GOI) can be inserted into pMEV vector using any of the restriction sites (or their combinations) in the MCS region shown below. **Because pMEV is not a fusion vector, an initiation ATG (start codon) must be included with the gene or interest.** The resulting clone will express the gene of interest only.

Sequencing of resulting clones:

After cloning of the gene of interest into pMEV vector, the resulting clones could be verified by DNA sequencing using one of the following primers (not provided).

N-terminal primer (nt 741-761):
5'-CCGTCAGATCACTAGAAGCTT-3'

C-terminal primer (complementary to nt 928-908):
5'-CTTATCATGTCTGCTCGAAGC-3'

Note*: If the Hind III site was used to clone the gene of interest, a different N-terminal primer (CMV-F1: 5'-GGTAGGCGTGTACGGTGG-3') should be used.*

Multiple cloning site of pMEV

Hind III
5′-(751)ACTAGAAGCTTTATTGCGGTAGTTTATCACAGTTAAATTGCT
AACGCAGTCAGTGCTTTGACACAACAGTCTCGAACTTAAGCCTAGC

Xho I **Xba I** **Sma I**
EcoR I **Kpn I** **Sal I** **Not I**
CTCGAGAATTCACGCGTGGTACCTCTAGAGTCGACCCGGGCGGCCGCTTCC(891)-3′

General References:

Sambrook, J. *et al,* 1989, **Molecular Cloning: A Laboratory manual**. Second Edition. (Plainview, New York: Cold Spring Harbor Laboratory Press).
Ausubel F. M. *et al*, 1994, **Current Protocols in Molecular Biology** (New York: Greene Publishing Associates and Wiley-Interscience).