



Description

The pRETROSUPER vector is derived from the Murine Embryonic Stem Cell virus (pMSCV). The pRETROSUPER vector contains the pSUPER shRNA expression cassette. The hairpin oligonucleotides for the SUPER RNAi™ library are cloned downstream of the polymerase III Histone H1-RNA promoter (H1). Upon transfection into a packaging cell line, pRETROSUPER expresses a transcript containing the viral packaging signal, the H1-shRNA cassette and the puromycin resistance gene. The pRETROSUPER has a specifically designed 3'LTR that has a deletion in the LTR promoter elements. This deletion results in inactivation of the LTR mediated transcription upon retroviral integration. The phosphoglycerate kinase promoter (PGK) drives the expression of the puromycin resistance gene (puro) for selection in eukaryotic cells. The pRETROSUPER plasmid can be propagated in E.Coli under ampicillin (AMP) selection.

pRETROSUPER vector information cont'd

Important features:

- 5'LTR: 1-515
- ψ (packaging signal): 516-1404
- H1 promoter (H1): 1506-1721
- PGK promoter (PGK): 1721-2229
- Puromycin resistance gene (puro): 2255-2854
- 3' Δ LTR: 2984-3277
- Ampicilin resistance gene (AMP): 5468-4611

PCR and sequencing primer locations:

- pRSforward (1333-1355): 5'- CCCTTGAACCTCCTCGTTTCGACC- 3'
- pRSseq (1697-1716): 5'- GCTGACGTCATCAACCCGCT-3'
- pRSReverse (1953-1975): 5'- GAGACGTGCTACTTCCATTTGTC- 3'

Propagation in E.Coli:

- Plasmid can be maintained in suitable host strains such as DH5 α , HB101 and others.
- Plasmid confers resistance to ampicilin (100 μ g/ml) and has a high copy number.