

Recovery of shRNA inserts from cells expressing the pRETROSUPER construct.

The identity of the shRNA constructs from infected and selected cells can be determined by recovery of the genomic shRNA insert and subsequent sequence analysis. Genomic DNA is isolated according standard methods (e.g. DNAzol). The genomic insert is recovered by PCR using the primers:

pRS forward: 5'- CCCTTGAACCTCCTCGTTGACC- 3'
pRS reverse: 5'- GAGACGTGCTACTTCCATTTGTC- 3'

PCR protocol:

Components:

Expand Long Template PCR system (Roche Diagnostics GmbH no. 1 681 834)

pRS forward 10 μ M

pRS reverse 10 μ M

Genomic DNA (200 ng/ μ l)

PCR mix:

Assemble PCR mix A and B on ice, keep buffer 3 at RT.

Reaction mix A: 5 μ l buffer 3
 2 μ l MgCl₂ (50 μ M)
 16.5 μ l H₂O
 1.5 μ l enzyme mix

Reaction mix B: 1.5 μ l pRSforward (10 μ M)
 1.5 μ l pRSreverse (10 μ M)
 3.75 μ l dNTPs (10mM each)
 16.25 μ l H₂O
 2 μ l genomic DNA (400ng)

Combine PCR mix A and B in PCR reaction tubes, vortex, spin.

PCR reaction:

Cycle 1:

10' 94°C

Cycle 2:

20" 94°C

30" 62°C

1.5' 68°C

Repeat cycle 2 30 times

Cycle 3:

10' 68°C

END

To check PCR product, analyze 3 μ l of each PCR reaction on a 1.2% 1XTBE/Agarose gel. The fragment should be 643bp.

The PCR product can be digested with EcoRI and XhoI for re-cloning into the backbone of pSUPER or pRETROSUPER. This fragment of approx. 300bp contains the H1 promoter plus the hairpin sequence.