Design of shRNA inserts for pSHAG-1

HAIRPIN PROTOCOL v2.1

Oligo design for insertion into *Bse*RI *Bam*HI cut pSHAG vectors.

Using sense, coding sequence of any gene....

(N1, N2, N30, N31, are numbered positions)

1) Find 5'-N1 NNNNN….NNNNNNNNNN C N30 N31-3'

The oligo must end in a "C" so that pol III, which initiates at a "G" in the U6 promoter, will initiate precisely at the first base of the antisense strand

2) Get reverse complement of (1)

5'- N31' N30' G N'N'N'N'N'N'…..N'N'N'N'N'N'N'N'N'N1'-3'

3) remove N30' N31' to get

5'- G N'N'N'N'N'N'N'N'…..N'N'N'N'N'N'N'N1'-3'

4) Add 5'-GAAGCTTG-3' to 3'END to get

5'- G N'N'N'N'N'N'N'N'N'…..N'N'N'N'N'N'N1'GAAGCTTG -3'

5) **OPTIONAL** *(to reduce hairpin formation by DNA oligos prior to ligation)*

From (1)

in positions N2 through N28

convert every <u>third</u> base possible

from A to G <u>or</u> from C to T

such that
A) adjacent bases are not changed
B) more than 4 bases are not changed
C) homopolymeric runs greater than 6 bases in length do not result

6) To the 3'END of (4) ADD (5) to get

5'- G N’N’N’N’N’N’N’N’N’….N’N’N’N’N’N’N1’GAAGCTTG N1 NNNNNNN…..NNNNNNNN C N30 N31

8) Add pol III terminator TTTTTT to get

5'- G N’N’N’N’N’N’N’N’N’….N’N’N’N’N’N’N1’GAAGCTTG N1 NNNNNNN…..NNNNNNNN C N30 N31 TTTTTT

The inclusion of the HindIII site allows for rapid identification of clones containing a hairpin.

9) Drop G to get

5'- N’N’N’N’N’N’N’N’N’….N’N’N’N’N’N’N1’GAAGCTTG N1 NNNNNNNN….NNNNNNN C N30 N31 TTTTTT = oligo A

10) Get reverse complement of (9)

11) to (10) Add GATC to 5’ end

12) to (11) Add CG to 3’ end = oligo B

Ligate annealed, kinased oligos into *Bse*RI *Bam*HI cut pSHAG vectors.

The resulting vectors can be used in transient transfections.

Alternatively, the U6 hairpin region can be transferred to a variety of mammalian vectors using LR clonase enzyme and appropriate Gateway acceptor vectors.