Construction of shRNA Expression Lentiviral Vector

1. Designing shRNA sequence and insert into *Bgl*II and *Xba*I sites of pENTR4-H1

Target sequence length would be 19 bp starting with A or G. It would be better to introduce mismatch mutation disperse at 3 sites with C to T or A to G in the sense strand (Avoid making TTTT or TTATT sequence).

Example:

Target sequence: AGATCACTTCCTATCCTGA

5′-GATCCCC AGGTCACTTTCTATCTTGA ACGTGTGCTGTCCGT TCAGGATAGGAAGTGATCT TTTTT GGAAAT-3′

3′-GGG TCCAGTGAAAGATAGAACT TGCACACGACAGGCA AGTCCTATCCTTCACTAGA AAAAA CTTTAGATC-5′

(ligated to *Bgl*II) (sense) (linker) (antisense) (termination) (ligated to *Xba*I)

(With this design, ligated shRNA sequence cannot be recleaved by *Bgl*II.)

Synthetic oligonucleotides are annealed and ligated into pENTR4-H1 digested with *Bgl*II and *Xba*I, and transform DH10B or another suitable *E. coli* strain and select for Kanamycin-resistant clones.

Inserted shRNA sequence should be confirmed by sequencing with the primer pH1up2:CAGGAAGATGGCTGTGAGG (in the H1 promoter). Denature at 98°C with 2% DMSO for sequencing.

2. Transfer H1-shRNA to lentiviral vector by Gateway technology

pENTR4-H1 inserted with shRNA sequence and lentiviral vector（e.g. CS-RfA-CG）are mixed with Gateway LR Clonase (Invitrogen), and transform DH10B or another suitable *E. coli* strain, and select for Ampicillin-resistant clones.

shRNA Expression by Lentiviral Vectors