

## Construction of Inducible shRNA Expression Lentiviral Vector

### 1. Designing shRNA sequence and insert into *Bgl*II and *Xba*I sites of pENTR4-H1tetOx1

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 AGGTCACTTCTATCCTGA ACGTGTGCTGTCCGT TCAGGATAGGAAGTGATCT TTTT GGAAAT-3'  
3'-GGG TCCAGTGAAAGATAGA ACT 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-H1tetOx1 digested with *Bgl*II and *Xba*I, and transform DH10B or another suitable *E. coli* strain and select for Kanamycin-resistant clones.

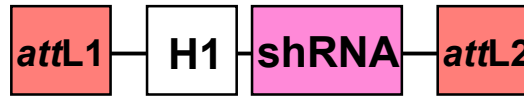
Inserted shRNA sequence should be confirmed by sequencing with the primer pH1up2:CAGGAAGATGGCTGTGAGG (in the H1 promoter). Denature at 98°C with 2% DMSO for sequencing.

### 2. Transfer H1tetOx1-shRNA to lentiviral vector by Gateway technology

pENTR4-H1tetOx1 inserted with shRNA sequence and lentiviral vector (e.g. CS-RfA-ETV) are mixed with Gateway LR Clonase (Invitrogen), and transform DH10B or another suitable *E. coli* strain, and select for Ampicillin-resistant clones.

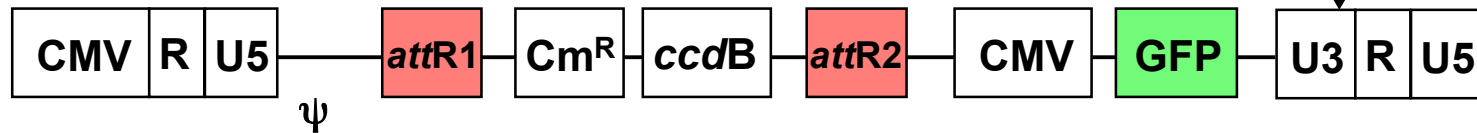
# shRNA Expression by Lentiviral Vectors

## Entry Vector for RNAi

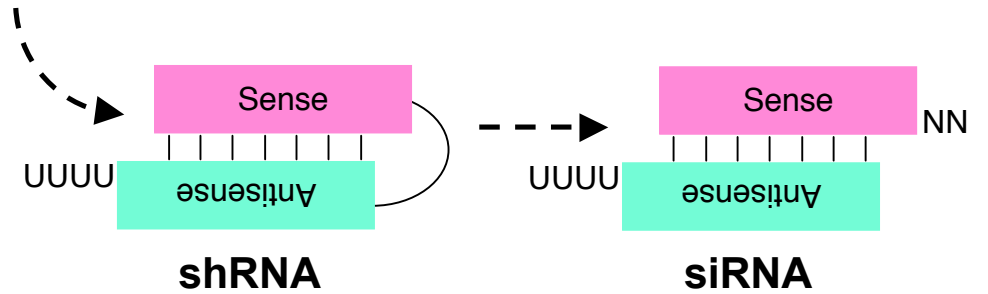
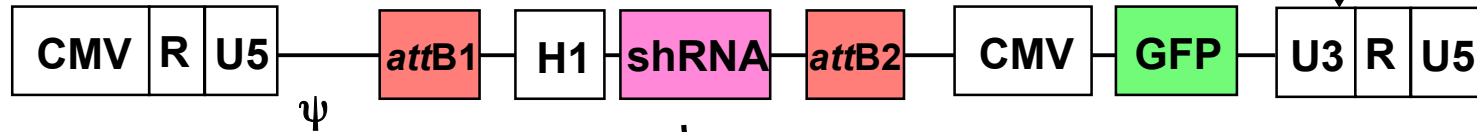


↕ Gateway LR Clonase

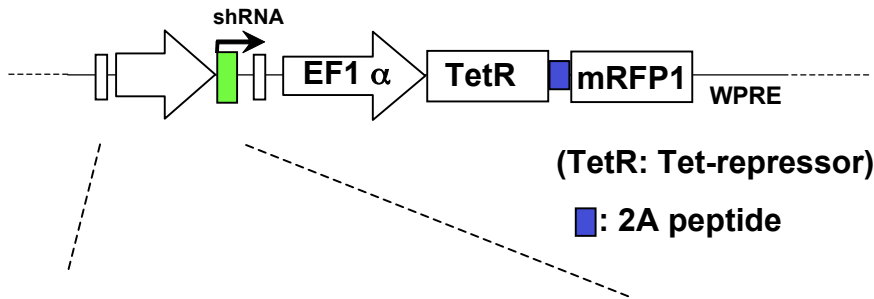
## Lentiviral Vector for RNAi



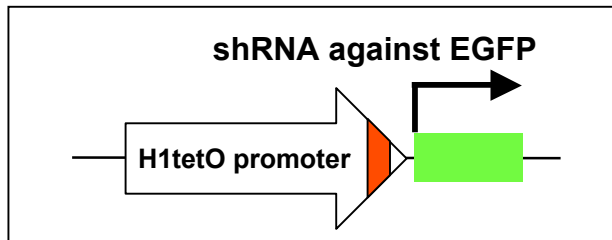
## shRNA Expression Lentiviral Vector



# Inducible shRNA Expression Lentiviral Vector



## LV-H1tetO-EGFP-ETRP



### Tet-inducible H1 promoter

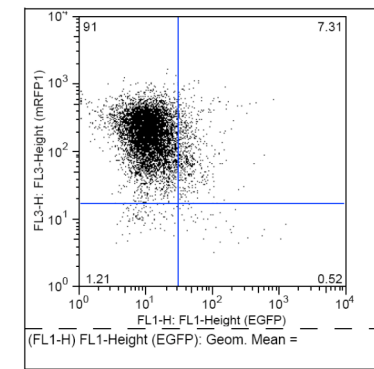
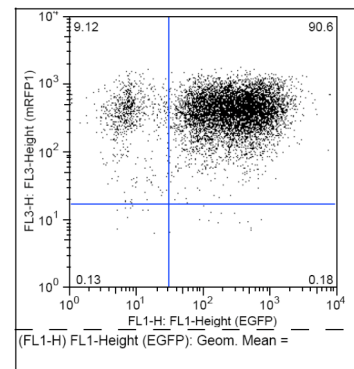
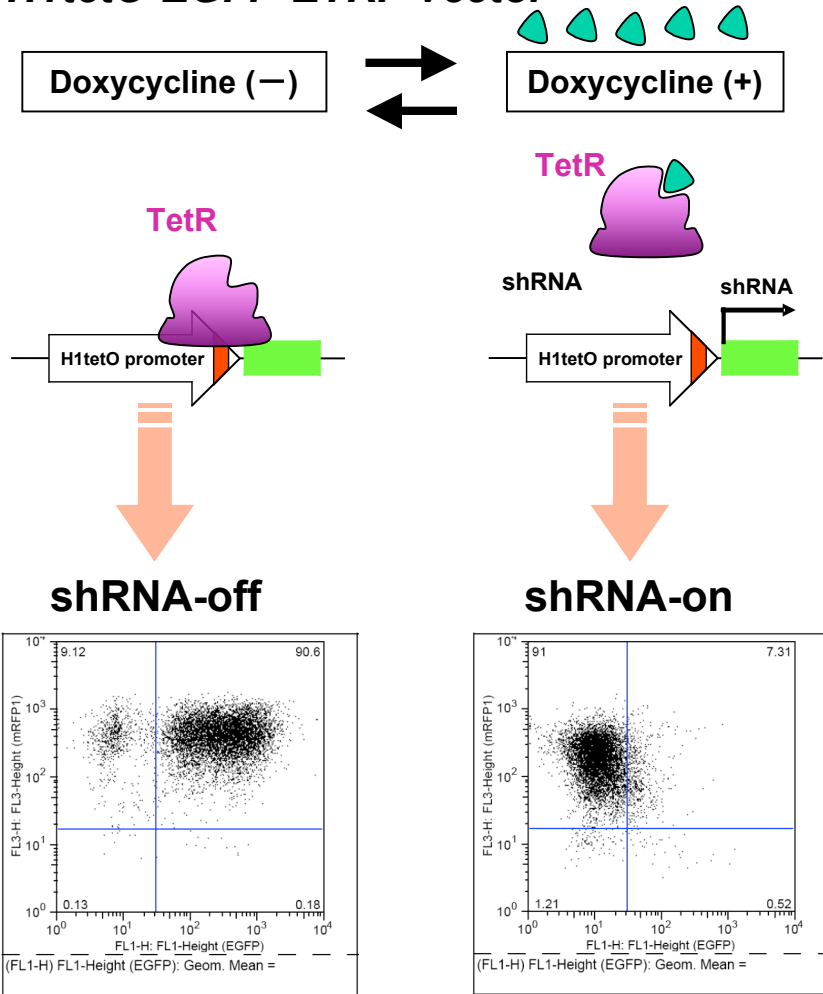
Insert the tet operator sequences between the TATA box in H1 promoter and transcription start site.



**tet Operon (forward direction)**

**TCCCTATCAGTGATAGAGA (19 mer)**

## HeLa(EGFP) cells transduced with LV-H1tetO-EGFP-ETRP vector



→ GFP