

Protocol for surrogate construction (Annealing method)

1. Prepare surrogate cloning vector:

Digest 5~10 µg of surrogate expressing vector with *KpnI* and *AgeI*, incubate at 37°C for overnight, then separate the restricted plasmid by electrophoresis using 1% agarose gel. The resulting surrogate cloning vector bearing two sticky ends, as depicted below, (~9.3kb) is then recovered by gel extraction kit. The purified vector is ready for ligation of annealed oligos (or a larger DNA fragment generated by PCR containing *KpnI* / *AgeI* protruding ends):

EGFP CDS - *KpnI* - *PmeI* - *AfeI* - *AgeI* - mCherry CDS

5'-gaagc**ttggtac** cgtttaaacagcgcta **ccggtatggtg** -3'
3'-**cttcgaac** catggcaaat~~ttg~~tcgcgat**ggcc** **ataccac** -5'

2. Prepare annealed oligos (sgRNA targeting sequence) for cloning:

1) Design sgRNA targeting sequence according to the genomic sequence:

To mimic sgRNA targeting to its authentic DNA template, the base-pairing region of sgRNA including PAM sequence (5'-N₂₀-NGG-3'; N=A, C, G or T) are cloned into the *KpnI*-*AgeI* sites in between EGFP and mCherry CDS. **For generation of a reporter to detect CRISPR-mediated indel, please be sure that the mCherry CDS is out of frame relative to the open reading frame (ORF) of EGFP after cloning. Also, avoid potential in-frame STOP codon formation that may be generated after indel reaction in the spacer region of EGFP and mCherry genes.**

To create oligomer pair containing sticky-ends for ligating to the *KpnI* & *AgeI*-digested surrogate cloning vector, add **c** at the 5' end of the sense oligo and put **a** at the 3' end of the sense oligo; in antisense strand oligo, add **ccggt** at the 5' end and put **ggtac** at the 3' end as depicted below:

Sense oligo: 5'-**c**-N₂₀-NGG-**a**-3'

Antisense oligo: 5'-**ccggt**-CCN-N₂₀-**ggtac**-3'

For example, if the target site is GGGGCCACTAGGGACAGGATTGG (5'-N₂₀-NGG-3'), the guide oligos will be:

Sense guide oligo: 5'-**c**-GGGGCCACTAGGGACAGGATT**GG**-**a**-3'

Antisense guide oligo: 5'-**ccggt**-CCAATCCTGTCCCTAGTGGCCCC-**ggtac**-3'

(6) Transform into Stbl3 competent cells (optimal for lentivector).

Note: To generate a reporter for CRISPRi, the inserted DNA fragment should be in-frame relative to EGFP ORF and/or mCherry ORF. (The design strategy is different from the reporter used for detection of CRISPR-mediated indel).