Protocol for VTPHG

1. Oligo design for VTPHG

1.1 Find NGG in upstream -150 to -500 of a target genes TSS.

1.2 Select sgRNA: 20bp length upstream of NGG and do not have TTTT in it. Higher CG number gives more efficient activation

1.3 add overhang to Sense: 5'- ttcg-N20

add overhang to Anti-sense: 5'- aaac-N20

eg: Target: 5'-AGCAATCGA**CATGCAAGCGGCTCGGAGCC*AGG***GCACCTGC- 3'

Sense: ttcg**CATGCAAGCGGCTCGGAGCC**

Anti-sense: aaac**GGCTCCGAGCCGCTTGCATG**

1.4 Annealing: resuspend oligos with water to 20uM

Set up annealing reaction: primer-F 5ul

Primer-R 5ul

*1x annealing buffer 40ul

95℃ 5 min then close the PCR Amplifier and let it cool to RT slowly

*10x annealing buffer: 100mM Tris-HCl (pH 7.5), 10mM EDTA, 1M NaCl

2. Backbone preparation

2.1 Use BbsI to digest VTPHG (see map).

Set up digestion reaction: BbsI(NEB) 1ul

10x Cutsmart buffer (NEB) 5ul

Plasmid Xul (2ug)

Add water to 50ul

37℃ 1.5h

2.2 Run agarose gel, we can see two bands, the small one is about 700bp (this can facilitate us to select correct clone by PCR), the large one is about 14kb, cut the large band and extract the backbone by Qiagen kit. Measure the DNA concentration, shall be around 50ng/ul.

3. Ligation

3.1 clone sgRNA into VTPHG

Set up ligation reaction: sgRNA 4ul

VTPHG 1ul

T4 DNA ligase (Roche) 0.5ul

T4 DNA ligase buffer (Roche) 1ul

Add water to 10ul

16℃ 1h

3.2 Transformation

Add 5ul ligation product into 20ul TOP10 competent cells, following standard transformation, then plate.

PCR select the correct clone

Primers used: U6BP-Fs:TCAACAAACGaacaataggacac

sgRNA2.0-Rs: aAAAAAGCACCGACTCGGTG

The correct clone will give around 300bp PCR product, while 1kb is uncorrect.

3.3 Culture bacteria and miniprep

StandaPurification and injection