CHEM 584--Cloning sgRNA Sequences into lentiCRISPRv2

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protocol .

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This protocol describes how to clone your chosen sgRNA sequence into the lentiCRISPRv2 plasmid. A method to validate your cloning is also included here.

Ken Christensen 2021. CHEM 584--Cloning sgRNA Sequences into lentiCRISPRv2. **protocols.io** https://protocols.io/view/chem-584-cloning-sgrna-sequences-into-lenticrisprv-bznyp5fw

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Design and Order Guide Sequences

1 Design and order sgRNA sequence oligonucleotides based on your previously identified target sequences.

Oligo 1: 5'-CACCGNNNNNNNNNNNNNNNNNNN
Oligo 2: 3'- CNNNNNNNNNNNNNNNNNNNCAAA

Important Note: Do not include the NGG PAM in your designed oligonucleotides

lentiCRISPRv2 Digestion

2 Digest 2 µg of lentiCRISPRv2 plasmid with BsmBI overnight at 55°C:

Set up the following reaction:
2.5 µl 10X NEB r3.1 Buffer

x µl ddH_2O to make up 25 µl final volume
1 µl BsmBI
2 µg lentiCRISPRv2 plasmid*

*Add the plasmid last, into the reaction mixture, pipette gently to mix and spin briefly.

Gel Purify the Digested Plasmid

3 Use the Zymoclean Gel DNA Recovery kit to gel purify the digested plasmid.

A ~2 kb filler piece should be present on the gel. **Only purify the larger band.** Leave the ~2 kb band.

Anneal the oligonucleotides

4 Suspend the oligonucleotides at 100 µM in autoclaved ddH_2O

5 Set up annealing reaction:

2 µl Oligo 1
2 µL Oligo 2
2 µl 10X T4 Ligation Buffer (NEB)
14 µl ddH_2O

6 Anneal the oligonucleotides in a thermocycler:

95°C for 5 min
Ramp temperature down to 25°C at 5°C/min

7 Dilute annealed oligonucleotides 1:200 with autoclaved ddH_2O (1 µl annealed oligo pair + 199 µl water).

Ligation into lentiCRISPRv2

8 Set up the ligation reaction and incubate at 16°C overnight (with the 2X Instant Stick End Master Mix, you may be able to significantly shorten this incubation).

x µl digested plasmid (50 ng)
1 µl diluted oligonucleotide duplex
6 µl 2X Instant Sticky End Master Mix
x µl ddH2O to make a final volume of 12 µl

Transform into NEB STBL competent cells

9 Add up to 5 µl of your ligation reaction into NEB STBL Mix & Go component cells following the

Mix & Go Competent Cell protocol. Plate your cells on Amp/Carb plates.

Use Colony PCR to screen for the presence of cloned oligo pairs

10 Use the Colony PCR protocol to screen for the presence of cloned oligo pairs using the 2X TaqDog Master Mix.

Run 10-20 µl of your PCR products on a 0.8% agarose gel. Use the 100 bp ladder for your gel.

Primers (provided):

lentiCRISPRv2 For: GTGGAAAGGACGAAACACCG
lentiCRISPRv2 Rev: CTAGGCACCGGATCAATTGC

Expected amplicon for positive clones = 248 bp

Isolate plasmid for positive clones

11 Prepare overnight cultures for positive clones.

Purify plasmid from overnight cultures using the Zymo Plasmid Miniprep-Classic protocol.

Sequence positive plasmids

12 Send plasmid for Sanger Sequencing (Eton Biotechnology).

Sequencing primer:
hU6-F: GAGGGCCTATTTCCCATGATT