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Cloning guides to lentiCRISPR v2 V.2

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Protocol status: Working
We use this protocol and it's working

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Troubleshooting

Vector preparation:

1 Digest the lentiCRISPRv2 vector:
3µg vector
2µl 10X Tango buffer (Thermo Fisher)
1µl 20mM DTT
1-1.5µl Esp3I (Thermo Fisher)
Water to 20µl

In a thermocycler:
37°C for 4 hours, inactivate at 65°C for 20 mins, keep at 4°C.
SKIP the alkaline phosphatase step.

2 Use a gel purification/PCR cleanup kit (Qiagen) **WITHOUT** running on a gel.
Optional: run ~200ng of the purified vector on a gel to verify digestion.

Guide insert preparation:

3 Anneal and phosphorylate gRNA oligos pair:
1µl of each oligo (100µM stock)
1µl 10X T4 ligation buffer (not PNK buffer)
0.5µl T4 PNK
Water to 10µl

In a thermocycler: 37ºC for 30 mins, 95ºC for 5 mins, ramp down to 25ºC at 0.1ºC/sec (or 5-6ºC/min).
Optional: keep at 4ºC.

4 Serially dilute the annealed oligos to 1:500

Ligation:

5 Out of ligation at a vector:insert molar ratios of 1:5, 1:10, 1:20, I found that 1:5 works best.

50ng vector
1.5µl 10X T4 ligation buffer (NEB)
1µl T4 ligase (NEB)
2µl diluted oligos (1:500)
Water to 15µl

Incubate at RT for 1-2 hours.

Transformation:

6 Transform 5µl of the ligation reaction to 50µl Stbl3 chemically competent cells.

If you incubate the Stbl3 cells at 30°C the colonies will be VERY small so look for them carefully. Incubating them at 37°C didn't result in LTR recombination in my hands.