Cloning guides to lentiCRISPR v2 Version 2

Amit Weiner

Abstract

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Protocol

Vector preparation:

Step 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.

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.

ANNOTATIONS

Hi,

I just wondering that Qiagen pcr clean up kit jut purify DNA up to 10kb, so how you get the digested lentiCRISPRv2 (after digested is around 12.8 kb)?

Guide insert preparation:

Step 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.

Step 4.

Serially dilute the annealed oligos to 1:500

Ligation:

Step 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:

Step 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.