



Aug 08, 2018

Version 2

Cloning guides to lentiCRISPR v2 V.2

DOI

dx.doi.org/10.17504/protocols.io.sfvebn6

Amit Weiner¹

¹University of Toronto



Amit Weiner

University of Toronto

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.sfvebn6>

Protocol Citation: Amit Weiner 2018. Cloning guides to lentiCRISPR v2. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.sfvebn6>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: August 08, 2018

Last Modified: August 31, 2018

Protocol Integer ID: 14549

Keywords: guides to lenticrispr v2, lenticrispr v2, cloning guide

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