

Jan 05, 2022

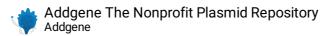
Cloning shRNA Oligos into pLKO.1 V.3

Addgene The Nonprofit Plasmid Repository¹

¹Addgene

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dx.doi.org/10.17504/protocols.io.b3hxqj7n



This is the protocol accompanying the "pLKO.1 – TRC Cloning Vector". For information about the PLKO.1-TRC cloning vector and tips on designing shRNA oligos for pLKO.1 see Addgene's website: http://www.addgene.org/tools/protocols/plko/

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STEP MATERIALS

Biolabs Catalog #B7003S

SNEBuffer 1 - 5.0 ml New England

Biolabs Catalog #B7001S Step 10

Step 10

Agel - 300 units New England

Biolabs Catalog #R0552S Step 11

Ecorl - 10,000 units New England

Biolabs Catalog #R0101S In 2 steps

Ecorl - 10,000 units New England

Biolabs Catalog #R0101S In 2 steps

T4 DNA Ligase Reaction Buffer - 6.0 ml New England

Biolabs Catalog #B0202S Step 26

Annealing Oligos

- 1 Resuspend oligos in ddH20 to a concentration of 20 μ M.
- 2 Add 5ul Forward oligo

■5 μL

3 Add 5ul Reverse oligo

■5 μL

4 Add 5ul 10X NEB Buffer 2

□5 μL

5 Add 35 μL ddH20

⊒35 μL

6 Incubate for 4 minutes at 95°C in a PCR machine or in a beaker of boiling water.

© 00:04:00

7 Incubate the sample at 70°C for 10 minutes in a PCR machine.

© 00:10:00

8 Slowly cool to room temperature over the period of several hours.

© 03:00:00

This will take a few hours, but it is important for the cooling to occur slowly for the oligos to anneal.

If using a beaker of water, remove the beaker from the flame, and allow the water to cool to room temperature.

Digesting pLKO.1 TRC Cloning Vector

9 Mix: 6 μg pLKO.1 TRC-cloning vector (maxiprep or miniprep DNA)

■6 µg

10 with 5 μ L 10x NEB buffer 1

□5 μL

■ NEBuffer 1 - 5.0 ml New England

Biolabs Catalog #B7001S

11 with 1 μL Agel

□1 μL

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12 Bring up to 50 μl with ddH20

13 Incubate at 37°C for 2 hours.

© 02:00:00



1	4	Purify with	Qiaquick gel	extraction k	cit elutina	in 30 ப (of ddH20
- 1	4	I UIIIV WILLI	Claudick del	CALIGULIOII r	nı. Ciutiilu	III JU HL 1	JI UUI IZO.

15 Digest eluate with EcoRI by mixing: 30 μ L pLKO.1 TRC-cloning vector digested with AgeI

16 with 5 μL 10x NEB buffer for EcoRI

■5 μL

⊠ EcoRI - 10,000 units **New England**

Biolabs Catalog #R0101S

17 with 1 μL EcoRI

□1 μL

⊠ EcoRI - 10,000 units New England

Biolabs Catalog #R0101S

18 and 14 μL ddH20

■14 μL

19 Incubate at 37°C for 2 hours.

© 02:00:00

20 Run digested DNA on 0.8% low melting point agarose gel until you can distinctly see 2 bands, one 7kb and one 1.9kb.

When visualizing DNA fragments to be used for ligation, use only long-wavelength UV light. Short wavelength UV light will increase the chance of damaging the DNA.

- 21 Cut out the 7kb band and place in a sterile microcentrifuge tube.
- Purify the DNA using a Qiaquick gel extraction kit. Elute in 30 μL of ddH20.

23 Measure the DNA concentration.

Ligating and Transforming into Bacteria

Use your ligation method of choice. For a standard T4 ligation, mix: $2\,\mu\text{L}$ annealed oligo from "Annealing Oligos" section above.

■2 μL

With 20 ng digested pLKO.1 TRC-cloning vector from the "Digesting pLKO.1 TRC Cloning Vector" section above.

⊒20 ng

If you were unable to measure the DNA concentration, use 1 μ L

26 With 2 μL 10x NEB T4 DNA ligase buffer

■2 μL

⊠T4 DNA Ligase Reaction Buffer - 6.0 ml **New England**

Biolabs Catalog #B0202S

27 With 1 μ L NEB T4 DNA ligase

□1 μL

⊠T4 DNA Ligase - 20,000 units **New England**

Biolabs Catalog #M0202S

- 28 Bring up to 20ul with ddH20
- 29 Incubate at 16°C for 4-20 hours.

© 04:00:00

30 Transform 2 μL of ligation mix into 25 μL competent cells, following manufacturer's protocol.

Due to the long terminal repeats found in lentiviral plasmids, we recommend using a

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strain that reduces the frequency of homologous recombination of unstable regions, such as Invitrogen Stbl3™ or NEB Stable cells. This will ensure that the repeats will be maintained and often results in a greater yield of DNA.

31 Plate on LB agar plates containing 100 µg/mL ampicillin or carbenicillin (an ampicillin analog).