LENTIVIRAL BARCODING PLASMID PREPARATION

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The barcode

Order your barcode primer with the following three components in this 5'-3' order:

1) cgaagagtaaccgttgctaggagagacca
2) Random sequence
3) cgatatcgccaccgtggctgaatgagactggt

For example this one:

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cgaagagtaaccgttgctaggagagaccaNNNNTGNNNNCANNNNACNNNNGANNNNGTNNNNAGNNNNATGTCTcgatatcgccacc
gtggctgaatgagactggt
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Other Reagents

- From Addgene: pLARRY vector – empty backbone (maxiprep)
- From NEB:
  - NdeI and BamHI restriction enzymes with CutSmart buffer
  - NEBuilder HiFi Assembly Master Mix (#E2621)
- From Thermofisher Scientific:
  - SLIDE-A-LYZER dialysis membranes (#88404)
  - Electroporation cuvettes (1 mm, #P41050)
- From CORNING/SIGMA
  - 25x25cm Petri dishes (Sigma #CLS431111)
- Programmable cuvette electroporator (i.e. GenePulser from BioRad).
- Electrocompetent bacteria (NEB DB10, Thermofisher Scientific Stbl4 or Lucigen ENDURA DUOs).
- Thermocycler (i.e. BioRad C1000)

PROTOCOL

1) Prepare 20 25x25 cm LB-AGAR Ampicillin plates.
2) Digest 1 ug of the pLARRY empty vector with NdeI and BamHI overnight. Purify and resuspend in water.
3) Resuspend the barcode primer and prepare a 100 nM dilution in water.
4) Thaw the 2x NEBuilder HiFi Assembly Master Mix on ice.
5) Prepare 10 HiFi reactions in a final volume of 20 ul each.
   a. 2 ul of barcode primer
   b. 100 ng of cut and purified pLARRY vector
   c. 10 ul Master mix
   d. Up to 20 ul with dH2O.
6) Incubate the reactions at 50ºC in a thermocycler for 1h.
7) Mix all the reactions (200 ul) and pipette over a dialysis membrane. Dialyze against 50 ml dH2O water for 1h (we use SLIDE-A-LYZER dialysis membranes from Thermofisher Scientific). You should recover at least 100 ul of the original volume added.
8) Thaw the electro-competent cells, and follow manufacturer instructions.

9) Mix 50 ul of electrocompetent cells (ultracompetent DB10 strain from NEB, or Lucigen Endura Duo, or ThermoScientific Stbl4) with 4 ul of the dialyzed ligation at 4ºC.
   ****This step can be scaled according to necessity. For every 200 ul of electrocompetent cells one should expect about $1x10^6$ colonies.
10) Add the mix to a pre-chilled electroporation cuvette (1 mm channel width).
11) Electroporate using Gene Pulser II (Bio-Rad) with the following setup:
    a. 2 kV
    b. 200 Ohm
    c. 25 uF
    The time constant should be 50 miliseconds.
12) Add 1 ml fresh warm SOB buffer to each cuvette and mix all the cuvettes into a single vial. Incubate the cells for 1h at 37ºC with rotation at 200 rpm.
13) Plate the mix in 25x25 cm agar plates (0.5 ml per plate).
14) Grow the plates at room temperature for 24h.
15) Verify that hundreds of thousands of small colonies formed by counting 10 random squares of 1 cm^2, and then multiplying this number by (62.5 x the number of plates).
16) Collect the colonies from each plate with 20 ml of Ampicillin-supplemented LB.
17) Incubate all the LB-cell mix at 37ºC for 1h to allow bacteria to recover. Then collect all the cell product in a flask for centrifugation.
18) Proceed immediately with the maxiprep.