GuEST-List Library Amplification Protocol

Materials

- 4 x 245mm LB Agar Plates with Ampicillin (eg, Teknova L6010 or homemade)
- 4 x 100mm LB Agar Plates with Ampicillin (eg. Teknova L1004 or homemade)
- LB Broth (eg. prepared from VWR 97064-112)
- Electrocompetent Cells (eg. Lucigen 60242-1)
- Electroporation Cuvettes (eg. Bio-Rad 1652089)
- 14 mL round-bottom loosely capped tubes (eg. Corning 352057)
- Bacterial cell spreader (eg. Bio Plas 6405)
- Maxi-prep plasmid isolation kit (eg. IBI Scientific IB47121)

Procedure

1. Add 2 uL of 50 ng/uL GuEST-List plasmid library to 25 uL electrocompetent cells. Repeat 3x to set up a total of 4 electroporation reactions per library.
2. Per the manufacturer's protocol, transfer each reaction to an appropriate cuvette and electroporate.
3. Immediately after electroporation, wash the cells out of the cuvette with 975 uL of recovery media. Transfer to a 14 mL round-bottom tube, and repeat with all reactions, pooling into one 14 mL tube.
4. Fill the culture tube up to 10 mL with warm LB broth, and split the culture between two 14 mL tubes with 5 mL volume each. Shake for 1 hour at 37 °C.
5. In the meantime, warm the 245mm and 100mm LB Agar with Ampicillin Plates to 37 °C.
6. Once the incubation has finished, prepare a serial dilution as indicated below, and spread each dilution onto a 100mm plate. These plates will enable you to estimate the transformation efficiency.

Final Dilution Factor	LB Broth Volume	Transformation Volume
1:1,000	190 uL	10 uL from transformation
1:10,000	180 uL	20 uL from 1:1,000
1:100,000	180 uL	20 uL from 1:10,000
1:1,000,000	180 uL	20 uL from 1:100,000

7. Plate the remaining transformed material across the four 245mm plates, adding 2.5 mL of transformed material to each and spreading the culture evenly across the plate with the spreader until it has absorbed into the agar.
8. Allow all the plates to incubate overnight at 32 °C.
9. In the morning, image the 100 mm plates and count the number of colonies. Aim for at least 50x colonies per construct in the library (ie, minimum 3 million).
10. Harvest the colonies from the 245 mm plates by adding 10 mL cold LB broth to the plates, using the bacterial spreader to scrape off the colonies, pipetting the liquid with the bacteria into a 50 mL conical on ice, and repeating with an additional 10 mL LB, then once more with an additional 5 mL LB. You will harvest a total of 2 x 50 mL tubes from the four plates.
11. Spin down the tubes, decant the supernatant, and weigh the pellet (being sure to tare with an empty 50 mL conical). Proceed with isolation of the plasmid according to the maxiprep kit instructions.
12. Verify the plasmid quality with whole-plasmid sequencing and/or Illumina sequencing of the protospacer-barcode insert. The library can now be used to make lentivirus via co-transfection into HEK293-T cells with packaging plasmids.