SLC library pooled electroporation, plating, determination of transformation efficiency and maxi prep

Protocol adapted from the GeCKO library amplification protocol (Zhang's lab)

1. Dilute the library to 50 ng/uL in water or TE (if not already diluted).

2. Electroporate the library

    a. Add 2 uL of 50 ng/uL library to 25 uL of electrocompetent cells with an efficiency of ≥10^9 cfu/ug. We generally use Lucigen Endura cells (cat # 60242)

    b. Electroporate using the manufacturer's suggested parameters/protocol.

    c. Recover in 975 uL recovery media (i.e. media provided with cells) and transfer to a loosely capped tube with an additional 1 mL of recovery media.

    d. Repeat for a total of 4 electroporations and rotate at 250 rpm for 1 hour at 37 C

3. Plate a dilution to calculate transformation efficiency

    a. Pool all 8 mL of electroporated cells. Mix well.
    b. Remove 10 uL and add to 1 mL of recovery media, mix well, and plate 20uL onto a pre-warmed 10cm petri dish (ampicillin). This is a 40,000-fold dilution of the full transformation and will enable you to estimate transformation efficiency to ensure that full library representation is preserved.

4. Plate the transformations

Follow Step a) if your lab has 24.5 cm^2 bioassay plates for large-scale bacteria culture; otherwise follow Step b), which substitutes 20 standard (10 cm round) petri dishes.

    a. Plate 4mL of transformation on each of 2 pre-warmed 24.5 cm_2 bioassay plates (ampicillin) using a spreader. Spread the liquid culture until it is largely absorbed into the agar and won't drip when turned upside down.
    b. Alternatively, spread 400 uL of transformation mix per petri onto 20 pre-warmed petri dishes.

5. Grow all plates inverted for **14 hours** at **30-32 C**. Growth at this lower temperature reduces recombination between the lentiviral long-terminal repeats. (Growth at 37 C is also acceptable if 32 C is not possible.)

6. Calculate transformation efficiency

    a. Count the number of colonies on the dilution plate.
    b. Multiple this number of colonies by 40,000 for the total number of colonies on all plates.
    c. Proceed if the total number of colonies is at least 3 x 10^6. This efficiency is equivalent to ~1000X colonies per construct.

7. Harvest colonies

    a. Pipette 10 mL of LB onto each 24.5 cm^2 bioassay plate (or, 500 uL per 10 cm petri dish)
    b. Scrape the colonies off with a cell spreader/scraper.
    c. Pipette off the liquid plus scraped colonies into a tube and repeat the procedure a second time on the same plate with additional 5-10 mL. *Note: Weigh this tube prior to adding any liquid to it.*

8. Weigh the bacterial pellet to determine the proper number of maxiprep columns to use

    a. Spin down all liquid to pellet the bacteria and then discard the supernatant.
    b. Weigh the bacterial pellet and subtract the weight of the tube

9. Maxi-prep for downstream virus production and future amplification

    a. Using a maxi scale plasmid prep, each column can handle approximately 0.45 g of bacterial pellet.
    b. Perform a sufficient number of maxi preps so as to not overload a column.