Amplification of pooled sgRNA library

1

Pooled sgRNA library transformation. Add 50ng of library in prechilled tubes to 25ul Lucigen Endura Competent cells. Electroporate the cells according to the manufacturer's directions. Repeat for a total of 2 electroporations. Immediately add 1ml of warm Lucigen Recovery medium to each reaction and incubate 1h at 37 °C.

2

Prewarm 1 standard LB agar plate (100-mm Petri dish, carbenicillin) for calculating electroporation efficiency at 37 °C.

3

After the 1-h recovery period, pool electroporated cells and mix well by inverting.

4

Prepare a dilution for calculating the transformation efficiency. To prepare the dilution mix, add 10 μl of the pooled electroporated cells to 990 μl of LB medium for a 100-fold dilution and mix well. Then add 100 μl of the 100-fold dilution to 900 μl of LB medium for a 1,000-fold dilution and mix well.

5

Plate 100 μl of the 1,000-fold dilution on a prewarmed standard LB agar plate (100-mm Petri dish, ampicillin from Step 2). This is a 10,000-fold dilution of the full transformation that will be used to estimate the transformation efficiency.

6

To grow pooled electroporated cells, add the remaining cells from Step 3 to 2 x 500ml LB+carbenicillin and grow O/N at 37 °C.

7

Calculate electroporation efficiency. Count the number of colonies on the 10,000-fold dilution plate. Multiply the number of colonies by 10,000 and the number of electroporations to obtain the total number of colonies. Proceed only if the total number of colonies is greater than 20 colonies per sgRNA in the library.

8

Harvest all bacteria from Step 6 and extract plasmid DNA using midi preps.

9

Pool the resulting plasmid DNA and use a NanoDrop UV spectrophotometer to quantify the product.