**Amplification of pooled sgRNA library**

**1. sgRNA library containing plasmid**. Dilute the library containing plasmid to 50–100 ng/ul. Electroporate 1-2 ul plasmid using Endura ElectroCompetent cells according to the manufacturer’s directions. Usually, one electroporation reaction is enough for the this small library that contains only ~278 sgRNAs.

**2. Electroperation** Prewarm 1 large LB agar plate (245-mm square bioassay dish, ampicillin) per electroporation of the sgRNA library at 37°C. Prewarm 1 standard LB agar plate (100-mm Petri dish, ampicillin) for calculating electroporation efficiency at 37°C.

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

**4.** **Bacteria dilution**. Prepare a dilution for calculating the transformation efficiency. To prepare the dilution mix, add 10 ul of the pooled electroporated cells to 990 ul of LB medium for a 100-fold dilution and mix well.

**5.** **Bacteria Plating.** Plate 100 ul of the 100 fold dilution on a prewarmed standard LB agar plate. This is a 1,000-fold dilution of the full transformation that will be used to estimate the transformation efficiency. Plate 2 ml of electroporated cells on each of the prewarmed large LB agar plates from Step 3 using a cell spreader. Spread the liquid culture until it is largely absorbed into the agar and does not drip when the plate is inverted. At the same time, make sure that the liquid culture does not completely dry out, as this will lead to poor survival rates.

**6.** **Bacteria culture.** Limiting the bacterial growth time to ~ 12-18 h ensures that there is sufficient growth for sgRNA library amplification without potentially biasing the sgRNA library distribution through intercolony competition or differences in colony growth rates.

**7. Calculate electroporation efficiency.** Count the number of colonies on the 1,000-fold dilution plate. Multiply the number of colonies by 1,000 and the number of electroporations to obtain the total number of colonies on all plates. Proceed only if the total number of colonies is greater than 100 colonies per sgRNA in the library.

**8. Harvest colonies from the LB agar plates**. Pipette 10-20 ml of LB medium onto each large LB agar plate or 1 ml of LB medium onto each standard LB agar plate. Gently scrape the colonies off with a cell spreader, and transfer the liquid with scraped colonies to a 50-ml Falcon tube. Repeat the washing twice with LB medium to capture any remaining bacteria.

**9. Maxiprep.** Using an endotoxin-free plasmid purification kit is important for avoiding endotoxicity in virus production and mammalian cell culture. Approximately one maxiprep is needed for two densely plated large LB agar plates. Pool the resulting plasmid DNA and use a NanoDrop UV spectrophotometer to quantify the product.

**PCR for NGS is performed in two steps:**

For PCR1: 98C 30s, (98C 1s, 65C 5s, 72C, 15s)\*15-20 cycle, 72C 2 min

->PCR purification of PCR1, then used for PCR2.

For PCR2: 98C 30s, (98C 1s, 60C 5s, 72C, 10s)\*15-20 cycle, 72C 2 min

**PCR primers:**

|  |  |  |
| --- | --- | --- |
| Name | Sequence |  |
| PCR1\_F | aaagtggcaccgagtcggtgcTTTTTTtctagaagagggc |
| PCR1\_R | acgattctgtgatttgtattcagcccatatcgtttcatag |
|  |  |  |
| PCR2\_F1 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAAGTAGAGTCTTGTGGAAAGGACGAAACACCG |
| PCR2\_F2 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATACACGATCTCTTGTGGAAAGGACGAAACACCG |
| PCR2\_R1 | CAAGCAGAAGACGGCATACGAGATGTAACGCGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgactcggtgccactttttcaagttg |
| PCR2\_R2 | CAAGCAGAAGACGGCATACGAGATGAAGGTCTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgactcggtgccactttttcaagttg |