**Propagation of genome-wide lentiviral CRISPR-guide RNA libraries**

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**MATERIALS**

**REAGENTS**

• Library DNA (50 µl at 20 ng/µl in TE buffer provided by Addgene 67988-Mouse/67989-Human)

• NEB 10-beta Electrocompetent E. coli (NEB, cat. no. C3020K)

• Qiagen plasmid maxi kit (Qiagen, cat. no. 12163)

• Yeast extract (Oxoid, cat. no. LP0021)

• Trypton (Sigma-Aldrich, cat. no. T7293)

• Sodium chloride (Sigma-Aldrich, cat. no. S7653)

• Agar (BD, cat. no. 214040)

• Ampicillin (Sigma-Aldrich, cat. no. A9518-5G)

**EQUIPMENT**

• BioRad GenePulser Xcell (BioRad, cat. no. 165-2662)

• Electroporation cuvette, 0.1 cm gap (BioRad, cat. no. 165-2083)

• 100-mm bacterial dish (Sterilin, cat. no. 101Vr20)

• 15-ml round-bottomed tube (BD, cat. no. 352059)

• 50-ml Falcon tube (BD, cat.no. 352098)

• Bacteria spreader (Fisher Scientific, cat. no. 12908140)

**REAGENT SETUP**

**Ampicillin stock solution (50 mg/ml, 1000x concentrated)** Dissolve 2 g in 20 ml distilled water. Add 20 ml 100% ethanol and store at -20 °C.

**LB+amp plate** Dissolve 5 g of NaCl, 5g of Yeast extract and 10 g of tryptone in 1 L distilled water and adjust pH to 7.5. Add 20 g of agar and autoclave. When it cools down to 55 °C, add 1 ml of ampicillin (50 mg/ml), pour 20 ml per 100-mm petri dish or 66 ml to 140-mm dish. Let plates sit on the bench for overnight. Store the plates at 4 °C.

**2xTY medium** Dissolve 5 g of sodium chloride, 10 g of yeast extract and 16 g of tryptone to 1 L of distilled water. Autoclave and store at room temperature.

**PROCEDURE**

**Electroporation**

**1|** Pre-warm 500 ml 2xYT+amp medium in a 1000-ml flask and SOC recovery medium (supplied with the competent cells) at 37 °C for 1 h.

**2|** Place four electroporation cuvettes and four 1.5-ml tubes on ice.

**3|** Dilute the library DNA (obtained from Addgene) by mixing 5 µl of the DNA and 5 µl of sterile water to the final concentration of 10 ng/µl. Add 1 µl to each of three pre-chilled 1.5-ml tubes and keep the tubes on ice.

**4|** As a positive control of electroporation, dilute the control pUC19 DNA (provided with the competent cells) by 1:5 to a final concentration of 10 pg/µl using sterile water. Add 1 µl of the diluted DNA to the last pre-chilled 1.5-ml tube and keep the tube on ice.

**5|** Thaw one viral of the frozen electrocompetent cells on ice. This will take approximately 5 min. Mix the cells by flicking gently. Keep the cells on ice.

**6|** Set the electroporator: 2.0 kV, 200 Omega and 25 µF.

**7|** Bring the ice box containing the DNA tubes, the competent cells and the electroporation cuvettes, and the pre-warmed SOC recovery medium next to the electroporator.

**Note:** Once cells are electroporated, SOC must be added immediately. Make sure that all reagents, pipettes (P200 and P1000), tips and a 50-ml tube and a 15-ml tube are around the electroporator.

**8|** Add 25 µl of the cells to the first tube containing the library DNA, mix gently by pipetting up and down 2-3 times and transfer them to a pre-chilled cuvette without making bubbles. Deposit cells across the bottom of the cuvette by gently hitting the bench a few times. Wipe out water and ice around the cuvette completely.

**9|** Electroporate.

**10|** Immediately add 1000 µl of the pre-warmed SOC recovery medium to the cuvette, gently mix up and down trice, then transfer the cells to a 50-ml tube.

**11|** Repeat Step 7-8 for the next two tubes with the library DNA. Add all electroporated cells into the same 50-ml tube and place the tube in bacterial shakers at 37 °C.

**12|** Perform the fourth electroporation for the positive control and add the cells into a 15-ml tube.

**13|** Shake at 37 °C for 1 h.

**Electroporation efficiency measurement**

**14|** Add 180 µl of pre-warmed SOC into 10 wells in a 96-well PCR plate (Wells A1-A5 and B1-B5).

**15|** Add 20 µl of the bacteria electroporated with the library into Well A1. Then add 20 µl of the bacteria electroporated with the positive control into Well B1.

**16|** Using multi-channel pipette, mix the bacteria in Wells A1 and B1, and then transfer 20 µl from Column 1 to 2.

**17|** Dilute the bacteria serially from Column 2 to 3, 3 to 4 and 4 to 5 in the same way.

**18|** Plate 50 µl from each well in Columns 2-5 to a LB+amp plate and incubate overnight at 37 °C.

**19|** In the following morning, count colonies. From pUC19 transformation, calculate the transformation efficiency in colony forming unit (cfu)/µg. This is usually higher than 1.0 x 1010 cfu/µg. From library DNA transformation, when *X* number of colonies are obtained on the plate derived from Column *Y* and there were *Z* µl of bacteria/SOC solution at Step 11 (*Z* is 3000 when this protocol is used), the total number of colony forming unit is given in *X* x 10^*Y* x 1/50 x *Z.* This should be >5 x 107 cfu for the faithful library replication.

**Culture bacteria transformed with the library DNA** (Continued from Step 15)

**20|** Add the remaining bacteria electroporated with the library (step **15**) to the pre-warmed 500 ml 2xTY+amp (ampicillin, 50 µg/ml) medium and incubate at 37 °C overnight with shaking.

**Note**. The volume of bacteria culture is very flexible. If a small amount of DNA is required, this volume can be 200 ml, which are enough for one Maxi-prep column. Or, if more plasmid is required, 1-2 L culture can also be performed although it may require a slightly longer culture time.

**Plasmid purification**

**21|** Use 2-3 columns from the Qiagen plasmid maxi kit to purify plasmid from 500 ml culture. Follow the manufacturer’s instruction. Change the number of columns if the bacteria have been cultured in a different volume appropriately.