Amplification of pooled sgRNA library Feng Zhang

Timing 2 d

18 *Pooled sgRNA library transformation*. Electroporate the library at 50–100 ng μl-1 using Endura ElectroCompetent cells according to the manufacturer's directions. If you are amplifying a ready-made genome-scale library from Addgene, repeat for a total of 1 electroporation per 10,000 sgRNAs in the library. If you are amplifying a custom sgRNA library, repeat for a total of 1 electroporation per 5,000 sgRNAs in the library and include an additional electroporation for the control Gibson reaction.

19 Prewarm 1 large LB agar plate (245-mm square bioassay dish, ampicillin) per electroporation of the sgRNA library at 37 °C. Each large LB agar plate can be substituted with 10 standard LB agar plates. Prewarm 1 standard LB agar plate (100-mm Petri dish, ampicillin) for calculating electroporation efficiency at 37 °C. For amplification of a custom sgRNA library, include an additional standard LB agar plate for the control Gibson reaction.

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

21 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.

22 Plate 100 μl of the 1,000-fold dilution on a prewarmed standard LB agar plate (100-mm Petri dish, ampicillin from Step 19). This is a 10,000-fold dilution of the full transformation that will be used to estimate the transformation efficiency.

23 If you are amplifying a custom sgRNA library, repeat Steps 21 and 22 for the control Gibson reaction.

24 To plate pooled electroporated cells, add 1 volume of LB medium to the pooled electroporated cells from Step 20, mix well, and plate the mixture on large LB agar plates (option A) or standard LB agar plates (option B).

A **Plating on large LB agar plates**

I Plate 2 ml of electroporated cells on each of the prewarmed large LB agar plates from Step 19 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.

B **Plating on standard LB agar plates**

I Alternatively, plate 200 μl of electroporated cells on each of the prewarmed standard LB agar plates from Step 19 using the same technique as described in Step 24A(i).

**CRITICAL STEP**

Plating of the electroporated cells evenly is important for preventing intercolony competition that may skew the sgRNA library distribution.

25 Incubate all LB agar plates overnight at 37 °C for 12–14 h.

**CRITICAL STEP**

Limiting the bacterial growth time to 12–14 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.

26 *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 on all plates. If you are amplifying a ready-made sgRNA library from Addgene, proceed only if the total number of colonies is greater than 100 colonies per sgRNA in the library. If you are amplifying a custom sgRNA library, proceed only if there are more than 500 colonies per sgRNA in the library.

**CRITICAL STEP**

Obtaining a sufficient number of colonies per sgRNA is critical to ensuring that the full library representation is preserved and that sgRNAs did not drop out during amplification.

27 In addition, for amplification of a custom sgRNA library, calculate the electroporation efficiency for the control Gibson reaction and proceed only if there are at least 20 times more colonies per electroporation in the sgRNA library condition as compared with the control Gibson reaction.

28 *Harvest colonies from the LB agar plates*. Pipette 10 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.

29 For each LB agar plate, repeat Step 28, for a total of 2 LB medium washes, to capture any remaining bacteria.

30 Calculate the number of maxipreps needed by measuring the OD600 value of the harvested bacterial suspension as follows: number of maxipreps = OD600 value · (total volume of suspension)/1,200. Perform maxipreps of the amplified sgRNA library by using the Macherey-Nagel NucleoBond Xtra Maxi EF Kit according to the manufacturer's directions.

**CRITICAL STEP**

Using an endotoxin-free plasmid purification kit is important for avoiding endotoxicity in virus production and mammalian cell culture. To ensure that the plasmid preparation is endotoxin-free, it is important to dilute the bacterial suspension to an OD600 value within the linear range of the spectrophotometer, typically ∼0.1–0.5, and measure the OD600 value of the dilution. Then multiply the OD600 value by the dilution factor to obtain the OD600 value of the bacterial suspension. Approximately one maxiprep is needed for two densely plated large LB agar plates.

31 Pool the resulting plasmid DNA and use a NanoDrop UV spectrophotometer to quantify the product. Maxiprepped sgRNA library can be aliquotted and stored at −20 °C for at least 1 year.