Maxiprep library DNA

1. Thaw 2 vials of Stbl4 electrocompetent cells on ice
2. Spin down library DNA in tubes and transfer 2 µl of each sub library to a new 1.5 ml tube
3. Add 54 µl Stbl4 cells to each tube and incubate 10 minutes on ice
4. Transfer 25 µl cells to a 1 mm gap cuvette and electroporate at EC1 (1.8 kV)
5. Immediately add 1 ml pre-warmed SOC and transfer to new 1.5 ml tube
6. Recover for 1-2 hour2 while shaking at 30 ℃
    a. Repeat transformation for the other half of the library
7. Take 10 µl from each SOC tube and pool per sub set of the library in a new 1.5 ml tube
8. Add 80 µl LB to each pool (total volume 100 µl)
    a. 10 µl of this will give a 10^{-3} dilution of sub library
9. Make 10-fold serial dilutions up to 10^{-6}
10. Plate 10 µl of each dilution on a pre-warmed 10 cm ampicillin plate
11. Incubate all plate O/N at 37 ℃
12. Transfer the remaining recovered cells to a 500 ml flask containing 100 ml amp medium and grow O/N while shaking at 30 ℃
13. Maxiprep each sub library using the Qiagen Plasmid Plus Maxi Kit (according to the manufacturer's instructions) if the transformation efficiency exceeds the library size by 20-fold

Generation library lentivirus

1. Seed 293T cells at 7.5/10/12 x 10^6 per 15 cm plate and grow O/N at 37 ℃ in total 30.2 ml IMDM with 10% FCS without P/S
2. For transfection, choose the plate were confluency of the 293T cells is about 70%
3. Prepare transfection mix (amounts for two 15 cm plates):
    a. 3 ml Optimem containing:
        i. 13.4 µg pMD.G
        ii. 26.8 µg pCMV dR8.91
        iii. 20.2 µg Bassik whole genome library
    b. 3 ml Optimem containing:
        i. 181 µl TransIT293 transfection reagent
4. Mix solution a and b together
5. Incubate 30 min at RT
6. Add transfection mix dropwise to 293T cells
7. Remove supernatant from cells containing the virus 48 hours later
    a. 293T cells should look very sick (membranes bubbly, etc) at this stage
8. Filter supernatant with 0.45 µm filter to remove 293T cells
9. Aliquot virus in 5 ml and 1.5 ml volumes
    a. 5 ml for screen
    b. 1.5 ml for titration of virus
10. Store virus at -80 ℃

Titration lentivirus for MOI = 0.3-0.5

For the screen each cell should only contain one sgRNA sequence. This experiment will determine the amount of virus to add to the target cell to get a multiplicity of infection (MOI) of 0.3-0.5.

1. Thaw 1.5 ml aliquot of virus at RT
2. Add 2.5 x 10^6 HAP1 Cas9 to each well of a 6-well plate in total 5 ml complete IMDM
3. Add 0, 50, 75, 100, 150 or 200 µl virus to a single 6-well
4. Spinfect:
    a. Centrifuge plate 3,900 RPM for 1 hour at 37 ℃
5. Transfer cells to incubator and grow for 48 hours at 37 ℃
6. Wash cells with PBS
7. Add 500 µl trypsin and incubate for 5-10 minutes at 37 ℃
8. Add 4.5 ml complete IMDM
9. Resuspend cells completely and transfer 500 µl to a new 6-well (2x)
10. To one well add 4.5 ml complete IMDM (−puromycin condition)
11. To the other well first add 2 ml complete IMDM and then 2.5 ml complete IMDM containing 2x 3.5 µg/ml puromycin (+puromycin condition)
    a. Repeat for all conditions
12. Grow cells for 48 hours at 37 ℃
13. Wash cells with PBS
14. Add 500 µl trypsin and incubate for 5-10 minutes at 37 ℃
15. Add 4.5 ml complete IMDM
16. Count cells and determine MOI for each condition as follows:

$$\frac{Cell\,count + puro}{Cell\,count - puro}$$

    a. Choose virus amount for actual screen that will give you an MOI of 0.3-0.5

Note 1: when infecting the library for a screen make sure to exactly repeat this protocol to get the same MOI.
Note 2: with each new virus batch or library, repeat this process.