THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Protocol for Mini-human library amplification (Ver 1.0)

Materials:

- MegaX DH10B™ T1R Electrocomp™ Cells (Life Technologies, Cat. no. C6400-03)
- 4 electroporation cuvettes (0.1 cm gap, Bio-Rad, 165-2089)
- Qiagen plasmid maxi kit (Qiagen, cat. no. 12163)
- Library DNA supplied in TE or water (400ng)
- 15-ml round-bottomed tube (BD, cat. no. 352059)
- Bacteria spreader (Fisher Scientific, cat. no. 12908140)
- MicroPulser Electroporator(Bio-Rad, cat. no. 1652100)
- 500 mL Terrific Broth medium with 100 μg/mL carbenicillin (ampicillin)
- 1x10mm carbenicillin (ampicillin) bacteria agar plate

Procedure

Day 1 (1.5~2 hours)

1. Add 400ng Mini-human library in 1 vial (100 μL) MegaX DH10B™ T1R Electrocomp™ Cells, mix well by gently flicking the tube. To reduce the risk of arcing, the DNA should be dissolved with TE or ultrapure water (preferred), with least possible volume (<10 μL).

2. Divide the cell/DNA mixture into 4 cuvettes. For each cuvette, perform electroporation per competent cell's manufacturer's protocol with 2.0kV shock, immediately add 1mL pre-warmed recovery medium supplied by the competent cell kit, mix well and transfer to round-bottom 14 mL tube. Shake for 1 hour at 30˚C.

3. Pool 4 transformants together, mix well (4 mL in total). Take 20 μL out and dilute it in 1000 μL recovery medium, mix well. Plate 20 μL diluted sample on 1x10mm carbenicillin (ampicillin) bacteria agar plate, culture overnight at 30˚C.

4. Inoculate the remaining (~3980 μL) in 500 mL Terrific Broth medium with 100 μg/mL carbenicillin (ampicillin), shake overnight at 30˚C.

Day2 (1.5~2 hours)

5. Count the colony number on the agar plate. If it is over 100 (which means over 50x coverage of the original library), maxi-prep the 500 mL culture with Qiagen Maxi-prep kit with the manufacturer's manual. Otherwise, discard the 500 mL culture and restart from step 1.