1.1 Amplification of pamiR from plasmid stocks and preparation of high-density glycerol stocks

Notes:

To maintain the complexity and distribution of constructs, re-transformation should be performed at a minimum of 30× coverage of the plasmid pool. This corresponds to more than 70,000 colony-forming units (CFUs) during library amplification. Coverage can be verified either by plating a small aliquot of the transformation mixture or by plating the entire transformation on LB-agar plates under antibiotics selection.

Although the plasmid size within the library is uniform, amplification biases can still arise during liquid culture. Therefore, the protocol below minimizes growth in liquid culture and includes a CFU assessment step prior to the preparation of amplified plasmid DNA and glycerol stocks.

It is recommended to transform the starting plasmid material only once, generate sufficient plasmid DNA for most downstream applications, and prepare glycerol stocks directly from this initial transformation. Repeated rounds of transformation and amplification are discouraged.

Transformation can be performed using either chemically competent or electrocompetent cells. Electroporation generally yields higher transformation efficiencies but is more sensitive to residual salts in the DNA sample. Chemical transformation of E. coli is more robust, although typically less efficient. Below, we provide a standard protocol for chemical transformation. Users should adapt the highest-efficiency protocol recommended by the manufacturer of the competent cells.

As a starting point, we recommend performing four parallel transformations and plating them onto four large LB-agar plates (15 cm diameter).

Supplies needed:

- pamiR plasmid stock: use at least 100 ng per transformation
- Highly chemically or electro-competent E. coli cloning strains, we use Takara Stellar
- Sterile 2 ml Eppendorf tubes
- SOC or LB medium
- LB-Agar big petri dishes (15 cm diameter) supplemented with Kanamycin (50 µg/ml) → LB-Agar K
- 500 ml of liquid LB supplemented with Kanamycin (50 µg/ml) in Erlenmeyer flask → LB-K
- Sterile serological pipette
- Sterile centrifugation containers
- Sterile 60% Glycerol in LB-medium
- Liquid nitrogen

Instructions:

1. Pre-heat SOC or LB medium and LB-Agar K at 37°C
2. All steps should be carried out near a flame or under a sterile hood
3. Thaw competent cells on ice (50-100 µl in 1.5 ml Eppendorf tube)

4. Add at least 100 ng but not more than 1 µl plasmid/10 µl of bacteria per transformation
5. Store on ice for 30 min
6. Heat shock at 42°C for 60 sec
7. Immediately place back on ice and store for 3 min
8. Add 1 ml or pre-heated SOC or LB to competent cells to each tube
9. Incubate at 37°C for 60 min
10. Plate content of tube(s) on LB-Agar K and incubate plates over-night (o/N) at 37°C
11. Check plate(s) the next day. When in doubt, count cells on a fraction of the plate. Plate(s) should have more than 70 000 colonies
12. Add approximately 20 ml LB (KAN) directly onto the plate(s) and re-suspend bacteria using a cell spreader or by careful up-and-down pipetting
13. Combine all responded cell suspensions in Erlenmeyer containing the LB-K and incubate at 37°C for 90 min
14. Remove culture for the plasmid preparation and pellet cells (4000*g* for 5 min). These pellets can be stored at -20°C for long term or at 4°C for a few hours. Carry out plasmid preparation according to the manufacturer's instruction. Safe 220 ml for the preparation of high-density glycerol stocks as described below
15. Pellet cells from 200 ml by centrifugation (e.g., 2 x 100 ml in 2 x 50 ml falcon tubes) (4000*g* for 5 min)
16. Resuspend pellet(s) in 7.5 ml cell culture and add 7.5 ml sterile 60% Glycerol in LB-medium
17. Aliquot into 2 ml Eppendorf tubes and flash freeze in liquid nitrogen
18. Store at -80°C
19. Use an entire aliquot of glycerol stock for the amplification