96 Well Alkaline Lysis Plasmid Preps

Before you begin: Make sure there are deepwell plates and deepwell plates with glass bead autoclaved. Make sure all necessary reagents are made.

1. INCUBATION: Aliquot 1.2ml of LB AMP broth (with antibiotics) into Backman 96-square-well titre plate (Titre plate should have 1 glass bead per well). Inoculate with desired clones using sterile toothpicks. Remove toothpicks. Cover plate with breakthable sterile film. Grow overnight at 37°C on shaker set at 250 rmp.

2. GLYCEROL STOCKS: Aliquot 40 µl glycerol (microwave 30 seconds to make les viscous) into 400µl nunc tray wells. Add 200 µl of each culture to wells. Cover with plastic sticky cover and store at -80°C.

3. CENTRIFUGE: Centrifuge the remaining culture (covered with sterile paper and plastic seal) at 4000rmp for 10 minutes. Invert Beckman deep well plate to discard supernatant. Blot the plate on paper towels.

4. FREEZE: Freeze pellets for 15 minutes (to several days) at -80°C to aid cell lysis.

5. RNase: Add 100 µl of fresh RNase (from stock) to 10 ml of P1 (100 µl per well)

6. P1 STEP: Resuspend pellets in 100 µl P1 buffer by vortexing 1 minute at speed 8. Plates should be covered with paper and plastic at this step.

7. P2 STEP: Add 100 µl P2 to lyse cells, vortex at speed 3 for 10 seconds. Put on ice for 5 minutes.

8. P3 STEP: Add 100 µl P3 to neutralize solution. Vortex at speed 3.5 for 30 seconds. Put on ice for 20 minutes.

9. REMOVE DEBRIS: Spin down cell lysates at 4000 rpm for 15 minutes. Pellets should be compact if steps 6 and 7 were performed on ice.
    a. Turn on plate sealer

10. ISOPROPANOL: Add 1 volume cold isopropanol (200 µl) to each well of 400 µl nunc plate.

11. TRANSFER: Pipette supernatant (200 µl) to 400 µl titre plate with isopropanol. Avoid entraining pelleted debris.

12. CENTRIFUGE: Cover with paper and plastic lid and spin down for 15 minutes at 4000 rmp.

13. REMOVE SUPERNATANT: Remove supernatant by inverting plate and blot dry.

14. RESUSPEND: Air dry the plates for 20 minutes and resuspend in 40 µl TE or nuclease free water.
    a. Transfer 40 µl to PCR plate and seal.