Maxi Plasmid Preparation

1. Grow 500 ml of culture O/N on shaking platform at 37°C. Generally we grow the bacteria in LB with 50-100 µg/ml Ampicillan (100mg/ml stock in –20°C).

2. Spin cultures in 500 ml bottles 10 min @ 5K, 4C (GSA rotor) to pellet.

3. Vortex hard to break up pellet. Resuspend the pellet in 20 ml solution I. Add 2 mg/ml (40 mg) lysozyme. Let sit at RT for 10 minutes.

4. Add 40 ml solution II. Invert gently to mix and put on ice for 5 min.

5. Add 30 ml solution III, mix gently and put on ice for 10 min.

6. Spin 15 min, 5K @ 4°C.

7. Filter supernatant through kimwipe towels into fresh bottle.

8. Add 0.6 volumes (54 ml) of isopropanol, invert to mix, and let sit @ RT for 20 min.

9. Spin 15 min., 5K @ 4°C.

10. Remove liquid, invert bottle on paper towel and remove excess liquid with a kimwipe.

11. Dissolve pellet in 2 to 3 ml of ddH₂O, transfer to 15 ml tube and bring volume up to 4 ml with ddH₂O.

12. Add 1.1 g/ml CsCl (i.e. 4.4 g) and 75 µl Ethidium Bromide (100 µg/ml). **Discard the tips with EtBr in the EtBr waste.**

13. Spin @ 3K for 10 min to pellet the RNA.

14. Transfer to ultracentrifuge tube and spin 65,000 rpm for a **minimum** of 4.5 hours or O/N @ 55,000 rpm. **Always balance tubes carefully.** Use balancing solution to top off tubes and/or to fill balancing tube.

15. Pull the bands with an 18 gauge needle and transfer to a new ultracentrifuge tube. **Throw all EtBr contaminated solutions and plastic in the EtBr waste containers.**

16. Fill tube with CsCl balancing solution (110 g CsCl + 100 ml ddH₂O).

17. Spin in ultracentrifuge 65,000 rpm for a **minimum** of 4.5 hours, or O/N at 55,000 rpm. **Always balance tubes carefully.**
18. Pull bands with an 18 gauge needle and transfer to 15 ml tube. **Throw all EtBr contaminated solutions and plastic in the EtBr waste containers.**

19. Add 2 volumes of ddH$_2$O. i.e. if your band is 1 ml then add 2 ml of ddH$_2$O.

20. Extract with an equivalent volume of water saturated butanol. 3-4X or until clear. **Do the butanol extractions in the fume hood** and discard the extracted material in the butanol/EtBr waste.

21. Add 2 new volumes of 100 % EtOH and put @ -20°C for 20-30 min.

22. Spin in centrifuge for 30 min. 3K @ 4°C (usually there is so much DNA that spinning @ RT is fine).

23. Wash with 10 ml ice cold 70% EtOH.

24. Spin in centrifuge 5 min. 3K @ 4°C.

25. Dissolve the pellet in 500 µl- 1 ml of TE or ddH$_2$O leave 30 min. and read O.D.$_{260/280}$.

**Solution I:**

36g Dextrose
100 ml 1M Tris pH 8.0
80 ml 0.5M EDTA
bring to 4 L with ddH$_2$O

**Solution II:**

1% SDS
0.2N NaOH

**Solution III:**

294.45g KOAC
115 ml Acetic Acid
to 1L with ddH$_2$O