Mombo Broth Plasmid Prep

1. Grow cells 24-30 hrs at 37 C in 1x Mombo broth with appropriate selection (100-500 ml of culture will give one a large cell number).

2. Pellet cells at 5,000 rpm/4 C/ 15 minutes.

3. Wash cell with 25 mls of saline solution, and pellet as in step 2.

4. Resuspend cells in 6 mls of freshly prepared lysozyme buffer by pipetting up and down with a 10 ml pipette.

5. incubate in an ice/water bath for 20 minutes.

6. Add 12 mls of freshly prepared 0.2M NaOH/1% SDS. Mix carefully, but thoroughly by inversion.

7. Incubate on an ice/water bath for 10 minutes.


9. Incubate in an ice/water bath for 20 minutes.

10. Pellet mixture at 15,000rpm/4 C/15 minutes.

11. Collect supernatant and add 50ul of 1mg/ml RNase A, and incubate at 37 C for 20 minutes.

12. Add 50 ul more of RNase A and incubate for an additional 10 minutes at 37 C.


14. Add two volumes of 100% EtOH, and place tubes at -70 C for 30' - 1 hour or -20 C overnight.

15. Pellect nucleic acid at 10,000 rpm/4 C/ 15 minutes.
16. Dissolve pellet in 1.5 ml of dH2O.

17. Add 0.4 ml of 4M NaCl and mix.

18. Add 2 ml of 13% PEG (6000-8000) and mix.

19. Place at 4 C overnight or on ice/water bath for 1-2 hours.

20. Pellet plasmid at 10,000 rpm/4 C/ 10 minutes.

21. Wash once with 75% EtOH.

22. Dissolve in dH2O or TE.

23. For cesium chloride gradient: resuspend plasmid in TE pH 7.6.

24. Add 1g CsCl/ ml solution.

25. Add 0.8 ml 10 mg/ml EtBr per 10 ml of ultracentrifuge tube volume.

26. Add 10ul of 1:100 Triton X-100:dH2O (0.01%) per ml of tube volume.

27. Transfer to an ultracentrifuge tube, balance opposing tubes within 0.01g, seal and spin at appropriate conditions:
   for Beckman NYT rotors:
   NVT65  65,000 rpm/20 C/4 hours
   NVT90  78,000 rpm/20 C/4 hours
   TLN100 100,000 rpm/20 C/4 hours

28. For a second column purification, extract plasmid band with a syringe or pierce tube and allow plasmid to drip into a clean tube, then introduce plasmid to a second ultracentrifuge tube and adjust volume with CsCl/TE solution, balance, seal and spin as done previously.

29. Extract plasmid band form tube with a syring or by allowing plasmid to drip into a clean tube, and extract 4 times with water saturated butanol to remove EtBr.