TRITON X-100 Plasmid Prep

1. Grow 500mls of bacteria O/N in TB or LB
2. Spin 10min at 5K in GSA (Big Sorvall Rotor)
3. Resuspend in 16mls GT (10%Glucose, 50mM TRIS pH8.0)
4. Transfer to 35ml polypropylene tubes.
5. Ad 2mls 10mg/ml Lysozyme and incubate on ice for 10min.
6. Add 4mls of .5M EDTA
7. Add 8mls 1% Triton X-100
8. Incubate at 37°C for 30-60min.
9. Transfer to swing bucket (SW28) rotor.
10. Spin 30min at 25k at 4°C in SW28. (Nito's lab has two)
11. Remove supernatant into 35ml polypropylene tube carefully measuring volume of supernatant.
12. Bring volume up to 30mls with TE or H_2O.
13. Add 32g CsCl and Spin 30min at 12K at 25°C SA600(Small Sorvall Rotor)
14. A pellet will float to top of tube. Remove supernatant into OPTI Seal tube with 800µl EtBr (10mg/ml).
15. Spin O/N (18+ hrs) at 45K in VTi50 rotor (Nito's lab has two)
16. Pull band in <5mls and transfer to 5ml quick seal tube. Fill up tube with 1g/1ml CsCl TE.
17. Spin 5.5-6hrs in VTi65.2 at 60K. (Peggy's lab has VTi65.2)
18. Pull band in 1-2mls and transfer to 15ml conical tubes.
19. Bring Vol. up to 3-4mls with TE and extract multiple times with H_2O saturated Butanol. (Aspirate of Top layer)
20. Dialyze O/N or EtOH precipatate with 1/10 vol 3M NaOAc twice.
21. Resuspend in TE.