George Lawless

Triton Lysis Cesium Chloride Plasmid Prep.

1. Grow 500ml culture overnight in appropriate antibiotic.

2. Spin down bacteria 5K 10 min.

3. Resuspend pellet in 25ml STET.

4. Add lyzozyme(a little bit at the end of a spatula is fine).

5. Transfer to 125-250ml flask.

6. Boil over flame until solution just begins to boil, the color will change from yellowish to creamy white.

7. Set on ice 5-10 min.

8. Spin viscous mess in JA20.1 or SW27 rotor at 20K for 1hr.

9. Pour supernatent into 50ml tube [blue cap Falcon tube][At this point one can add RNase and incubate for approximately 30 min. at 37º. After incubation phenol extract 1-2 times, chloroform extract 1 time and isopropanol ppt.]

10. Bring up to 30ml with TE. [If isopropanol ppt. step is preformed then one can bring up DNA in smaller volumes, just use similar proportions of TE, CsCl and EtBr.]

11. Add 30gm CsCl.

12. Add 2.80ml ethidium bromide and shake well.

13. Allow purple protein gunk to float to top.

14. Remove DNA solution from underneath and add to large quickseal tube for the Vti50 rotor(other size rotors and tubes are fine).

15. Seal tubes and spin at 45,000 for 12-18 hrs at 20-25 degrees C.

16. Remove bands and reband in either the Vti80 at 80,000 for 4 hrs or Vti65 at 54,000 overnight(can use first spin directly if you are in a hurry).

17. Remove bands extract ethidium with isoamyl or n-butyl alcohol and dialyze to remove CsCL(Alternatively triple volume of the band with TE and ethanol precipitate directly; make sure to wash the pellet with 70% EtOH.).

18. Ethanol precipitate resuspend in H_2O .

Solutions:

STET

		500 ml
8%	Sucrose	40 gms.
5%	Triton	25 ml
50mM	EDTA	50 ml of 0.5 M EDTA
50mM	Tris pH 8.0	25ml of 1.0 M Tris