Protocol: Large Scale Cesium chloride Plasmid Prep

1. Starter culture: Inoculate 5 ml LB + Antibiotic with a single colony (or 25 µL of frozen glycerol stock).

2. Grow for 6 hours (or overnight) until the LB is cloudy.

3. Inoculate 500 mL LB + Antibiotic with 2 ml of starter culture. Grow overnight at 37 °C with vigorous shaking.

4. Transfer cells to 500 mL centrifuge bottles and spin at 5000 RPM for 20 minutes using J10.500 rotor. Decant the supernatent, save the cell pellet. (The cell pellet can be stored at –80 °C, if necessary).

5. Resuspend the cell pellet in **40 mL GTE buffer**. Vortex vigorously with pipet on ice. Leave on ice for 10 minutes.

6. Add **80 ml of Solution II**. Mix gently until solution clears. Leave on ice for 15 minutes.

7. Add **40 ml of Solution III**. Mix gently, but thoroughly. Leave on ice for 10 minutes.

8. Spin at 9000 RPM for 20 minutes at 4 °C.

9. Pour supernatent through cheese cloth in to fresh clean centrifuge bottles.

10. Add **90 ml of HPLC grade Isopropanol**. Mix well. Leave at room temperature for 10 minuts.

11. Spin at 5000 RPM for 20 minutes at 15 °C.

12. Carefully discard the supernatent. Gently add 40 mL of 100% ethanol.

13. Spin at 9000 RPM for 5 minutes at 4 °C. Carefully remove the supernatent. Dry the crude DNA pellet very well using the Speed-Vac system.

14. Add 4 mL TE buffer to DNA pellet. Dissolve the pellet by vortexing.

15. Weigh out **4.5 grams of Ultrapure Cesium chloride** into 50 mL Falcon tubes . Add above DNA solution to the tube and shake until everything dissolves. The solution looks murky, but its OK.

16. Add **100 µL of Ethidium bromide** (stock 10 mg/ml), mix plus **51 µL of Triton-X100** (1:100 stock). Transfer into Ultracentrifuge tubes (1/2 X 2 inch). Use TE to bring volume to just below the neck (Quick-Seal tubes Cat#342412 or OptiSeal Cat# 362185).

Note: Quick-Seal tubes need to be heat sealed. OptiSeal tubes have tube plugs and are not heat sealed.

17. Spin overnight at 65,000 RPM in Ultracentrifuge rotor NVT90 or VTi80 at **20 °C.** Alternatively, spin for 4 hours at 78,000 RPM at 20 °C. ***CAUTION: Do not spin below 20 °C and always balance the tubes!***

18. Carefully remove the centrifuge tubes from the rotor. Place it in a clamp stand. A pink band is visible in the middle of the gradient. Sometimes, two pink band close to each other are visible. The Top band is chromosomal DNA and the Bottom band is the plasmid DNA that you want.

19. Carefully insert a pink needle at the top of the tube to allow air to get in. Insert a second needle attached to a 1 mL syringe just below the plasmid band and carefully draw out the plasmid DNA. ***Note: If using OptiSeal tubes, remove the tube plugs and insert the needle from the top into the tube. No need to pierce the tube.***

20. Transfer the plasmid DNA from syringe to 15 mL Falcon tube. Add **10 mL of NaCl + Water saturated n-Butanol**. Vortex. Spin in clinical centrifuge for 3 minutes. Remove top organic phase that contains ethidium bromide and discard it appropriately. Repeat butanol extraction 3 to 5 times until the organic phase becomes clear (no more ethidium bromide).

21. Transfer aqueous phase to clean JA17 centrifuge tube. Add 2 mL TE buffer and 7.5 mL of 100% ethanol. Precipitate at <u>room temperature</u> for 20 minutes.

22. Spin at 10,000 RPM for 20 minutes in JA17 rotor at <u>room temperature</u>.

23. Discard supernatent. Resuspend pellet in 500 µL of **0.3 M Na Ac pH 5.2**. Transfer the DNA to 1.5 mL eppendorf tube. Add 1 mL of 100% ethanol. Precipitate DNA at –20 °C for 20min. Spin in microfuge at top speed for 25 minutes.

24. Wash the pellet carefully with 100 µl of 70% ethanol. Dry the pellet using Speed-Vac.

25. Resuspend the pellet in 200 µl of TE by vortexing.
Measure absorbance at A260 of 1: 250 diluted sample. Store at –20 °C.

Buffers:

GTE
Glucose = 1.8 Grams
(1M) Tris pH 8.0 = 5.0 mL
(0.5M) EDTA = 4.0 mL
Water = x mL
Final Volume = 200 mL

Autoclave! Otherwise stuff will grow in it.

Solution II
(10N) NaOH = 8 mL
(10%) SDS = 80 mL
Water = 712 mL
Final Volume = 800 mL

Solution III
Dissolve 245.5 Grams of Pottasium acetate in 300 mL water. Add 57.5 mL of Glacial acetic acid. Add water to make final volume of 500 mL.
Important: Volumes have to be exact!

NaCl/Water saturated n-Butanol
Mix equal volume of (0.2 M) NaCl and n-Butanol. Allow the phases to separate. Use the upper organic phase for extractions.

Triton-X100 (1:100 stock)
Mix 10 **µL** of Triton-X100 (straight from the bottle) + 990 **µL** of water in eppendorf tube. Store at –20 °C.