Linearization of MiniPrep Plasmid DNA

1. Make Linearization Master Mix:

per reaction:

10 uL	10 X NEB Buffer #3
1 uL	BSA 100 X
61 uL	DI H2O
3 uL	NEB Not1 (10 U / uL)
---	---
75 uL	

2. In a sterile 1.5 mL microcentrifuge tube, add 75 uL of Linearization Master Mix to 25 uL of mini prep plasmid DNA (5 – 10 ug). Mix by pipetting.

3. Incubate at 37C overnight (> 14 hr).

4. Run a few representative samples on a 1 % agarose gel (ex: 0.5 g agarose + 1 mL 50 X TAE buffer + ~49 mL DI H2O) to verify linearization. (each sample – 1 uL reaction + 9 uL DI H2O + 1.7 uL 10 X Sample buffer) (Run at 170 V for 5 minutes against a 1 kb ladder)

5. Add 100 uL of phenol / chloroform / isoamyl alcohol (25:24:1) to each tube. Vortex for 30 seconds and then spin at high speed for 5 minutes. Transfer 95 uL of the aqueous phase (top layer) into a sterile 1.5 mL tube. **Make sure no interface or organic phase is removed.**

6. Add 100 uL of chloroform / isoamyl alcohol (24:1) to each tube. Vortex for 30 seconds and then spin at high speed for 5 minutes. Transfer 95 uL of the aqueous phase (top layer) into a sterile 1.5 mL tube. **Make sure no interface or organic phase is removed.**

7. Add 30 uL of 7.5 M NH4OAc (Ammonium acetate) to aqueous layer.

8. Add 130 uL of cold (4C) 100 % isopropanol. Vortex and place at -20C for > 30 min.

9. Spin tubes at high speed (4C) for > 30 min. Aspirate the supernatant with a 27 gauge needle, being careful not to aspirate the DNA pellet.

10. Gently add 500 uL of cold (-20C) 80 % ethanol to DNA pellet. Spin for 10 min. at 4C. Aspirate the supernatant with a 27 gauge needle.

11. Dry in a 37 C heat block for 15 – 20 min. Verify that all the EtOH has evaporated.

12. Resuspend dried pellet in 10 uL of sterile RNase free DI H2O and store at -20C.