PEG Maxiprep Protocol

1. Grow 250ml E.coli containing your plasmid in LB-Amp o/n at 37 °C
2. Spin down @5000 rpm 5 min
3. Pour off supernatant, allow pellet to drain to remove all media
4. Resuspend pellet in 10ml Qiagen Buffer P1 (Solution I)
5. Add 10ml Qiagen buffer P2 (Solution II)
6. Once mixture clears, add 10ml Qiagen Buffer P3 (Solution III)
7. Either pass through Qiagen filter, or spin in SS34 15000rpm 10min then filter through whatman #1 paper
8. Add 0.6 vol isopropanol, spin in 50ml conical 4000rpm 5 min
9. Wash with 70% ethanol. Resuspend pellet in 5ml Solution I, add 5µl 10mg/ml RNAse A.
10. Incubate at 37 °C for 20min
11. Add 5ml Solution II
12. Add 5ml Solution III
13. Spin 5 min 4000 rpm, or filter
14. Add 0.6 vol isopropanol to supernatant.
15. Spin 4000 rpm 10 min
16. Wash pellet in 70% ethanol, resuspend in 400µl T.E.
17. Add 400µl phenol/chloroform. Vortex and spin
18. Take aqueous phase to fresh tube, add 40µl 3M NaOAc, 1ml Ethanol
19. Spin 1 min
20. Wash pellet in 70% ethanol
21. Resuspend in 1ml TE. Add 400µl PEG solution and incubate on ice for 1hr
22. Spin 10 min.
23. Resuspend pellet in 1-200µl TE, measure OD26