Experiment 3.1 – Promega Miniprep Protocol

Purpose:

This experiment was performed to observe the effects of several parameters of the Promega miniprep protocol on plasmid recovery. Several alterations were made to the protocol and run in parallel with the original method, and DNA concentrations of the products were measured with the Nanodrop in order to compare the results. Each trial was performed using the same overnight culture for each of several different constructs.

Setup:

Trial	Re-suspended in TE Buffer	Lysis to Neutralization Duration	Elution Agent Used
1	Y	~ 1 min.	Elution Buffer
2	N	~ 2 min.	ddH_2O

Procedure:

Promega miniprep protocol:

1. Grow 1.5 mL overnight cultures
2. Transfer 600 µL of each overnight culture to a 1.5 mL Eppendorf tube
3. Pellet and re-suspend in TE buffer (if necessary)
    a. Centrifuge for 30 s. @ 13,200 rpm, discard supernatant
    b. Re-suspend with 600 µL TE buffer by aspirating with pipet
4. Add 100 µL cell lysis buffer, invert tube ~6 times to mix
    a. Either proceed immediately or wait for ~2 min. to neutralization step
5. Add 350 µL of ice cold neutralization buffer, invert tube until thoroughly mixed
6. Centrifuge for 3 min. @ 13,200 rpm
7. Transfer supernatant (~900 µL) to a minicolumn w/ collection tube
8. Centrifuge for 30 s. @ 13,200 rpm, discard flow-through
9. Add 200 µL endotoxin removal wash solution
10. Centrifuge for 30 s. @ 13,200 rpm, discard flow-through
11. Add 400 µL column wash solution
12. Centrifuge for 30 s. @ 13,200 rpm, discard flow-through
13. Centrifuge for 1 min. @ 13,200 rpm to remove residual column wash
14. Transfer minicolumn to 1.5 mL Eppendorf tube
15. Add 30 µL of elution agent directly to the minicolumn matrix, let stand for ~1 min.
    a. Either elution buffer or ddH_2O
16. Centrifuge for 30 s. @ 13,200 rpm
17. Use Nanodrop to measure DNA concentration
18. Store @ -20° C

Results:

Trial	J23100+RFP	J23108+RFP	J23110	J23114
1	58.1 ng/µL	43.3 ng/µL	98.4 ng/µL	95.9 ng/µL
2	38.0 ng/µL	30.5 ng/µL	47.4 ng/µL	49.1 ng/µL

Discussion:

The results of this experiment showed that variation 1 of the protocol was ~50-100% more effective than variation 2. This experiment was not planned in advanced and therefore the controls were not well designed, so the direct effect of each change to the protocol was not evident in the results. However, since method 1 was much more effective we will continue to re-suspend in TE buffer, proceed immediately to neutralization, and elute using the elution buffer moving forward.