Experiment 11.1 – Mini-column Recycling Protocol

Purpose:

This experiment was designed to see whether or not our mini-column recycling procedure is able to remove all DNA from the mini-column binding matrix. This was performed using the Qiagen miniprep system and the corresponding Qiagen mini-columns and each set was done in triplicate for to ensure repeatability. All measurements of DNA concentration were obtained using the Nanodrop.

Setup:

	(1)	(2)	(3)	Avg.
RFP	219.6 ng/µL	221.5 ng/µL	240.2 ng/µL	202.1 ng/µL
RFP	189.1 ng/µL	102.8 ng/µL	239.5 ng/µL	
RC w/ amilCP	86.9 ng/µL	88.6 ng/µL	94.7 ng/µL	90.1 ng/µL
UC w/ amilCP	110.2 ng/µL	119.8 ng/µL	107.5 ng/µL	112.5 ng/µL

RC – recycled mini-column / UC – used mini-column

Transform from purified DNA samples to see if RFP is still present in eluted products.

Procedure:

Miniprep protocol:

1. Grow up (4x) 5 mL overnight cultures in LB
2. Combine overnights into 1 stock
3. Pellet (6x) 3 mL of overnight culture
    a. 2x - 1.5 mL increments
    b. Centrifuge @ 13,200 rpm for 30 s., discard flow-through
4. Re-suspend each cell pellet w/ 250 µL (ice-cold) Buffer P1
5. Add 250 µL lysis Buffer P2, invert tube gently ~6 times to mix (yellow color will form - proceed to step 6 quickly)
6. Add 350 µL neutralization Buffer N3, invert tube gently until thoroughly mixed (yellow color will disappear)
7. Centrifuge @ 13,200 rpm for 12 min.
8. Transfer supernatant to mini-column
9. Centrifuge @ 13,200 rpm for 1 min., discard flow-through
10. Add 500 µL Buffer PB
11. Centrifuge @ 13,200 rpm for 1 min., discard flow-through
12. Add 750 µL Buffer PE
13. Centrifuge @ 13,200 rpm for 1 min., discard flow-through
14. Centrifuge @ 13,200 rpm for additional 1 min., discard flow-through
15. Transfer mini-column to sterile 1.5 mL Eppendorf tube
16. Add 40 µL elution Buffer EB directly to mini-column matrix, let stand for ~1 min.
17. Centrifuge @ 13,200 rpm for 1 min., remove minicolumn
18. Measure concentration with Nanodrop
19. Store @ -20° C

Mini-column recycling protocol

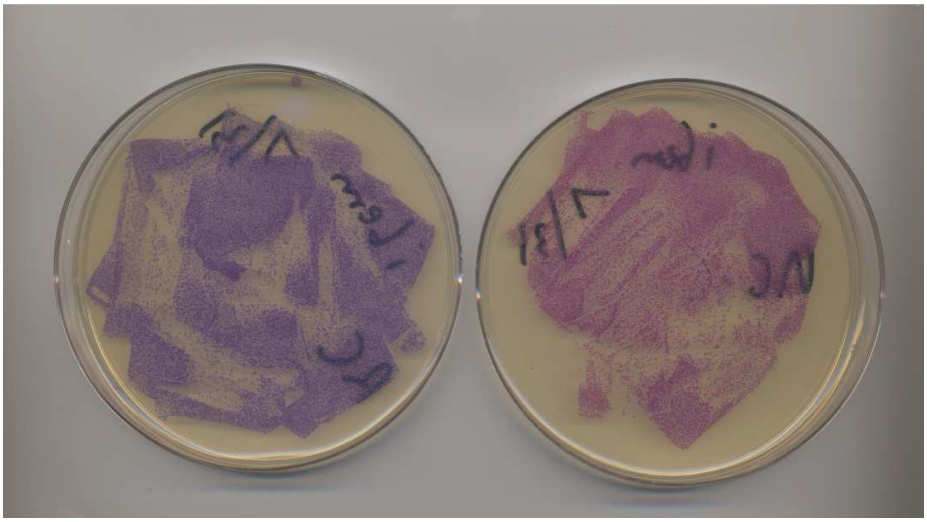
1. Submerge used mini-columns (and collection tubes) in 1M HCl for 24+ hours (this was done over the weekend)
2. Remove mini-columns and collection tubes from 1M HCl bath

3. Centrifuge @ 13,200 rpm for 30 s., discard flow-through
4. Add 700 µL ddH_2O
5. Centrifuge @ 13,200 rpm for 30 s., discard flow-through
6. Add 700 µL ddH_2O
7. Centrifuge @ 13,200 rpm for 30 s., discard flow-through
8. Add 700 µL equilibrium Buffer QBT
9. Centrifuge @ 13,200 rpm for 30 s., discard flow-through
10. Store recycled mini-columns in a sterile, sealed container (zip-lock bag)

Transformation protocol

1. Thaw frozen cells on ice for ~15 min.
2. Aliquot 1 µL DNA plasmid into 1.5 mL Eppendorf tubes
3. Add 40 µL chemically competent cells to DNA aliquots, aspirate w/ pipet to mix
4. Incubate on ice for ~30 min.
5. Heat shock samples in water bath for 60 s. @ 42° C
6. Incubate on ice for ~5 min.
7. Add 200 µL SOC, recover in shaker for ~2 hr.
8. Plate 20 µL transformed cells on chloramphenicol resistant plates.

Results:



Discussion:

This experiment showed definitively that the mini-column recycling procedure effectively removes all residual DNA from the mini-columns. Each of the 3 plates from the used mini-columns had a significant number of red colonies, whereas the recycled mini-columns appeared to have only blue colonies present (an example of each shown above). This shows that the recycling protocol was effective at removing all of the red carry-through from the earlier miniprep. Another experiment will be performed to test whether the recycling process reduces the effectiveness of the mini-column.