Experiment 11.3 – Mini-column Recycling Protocol

Purpose:

This experiment investigated the effectiveness of our recycled mini-columns in comparison to new mini-columns. The Qiagen miniprep system was used to conduct this experiment.

Setup:

	(1)	(2)	(3)
NC	Pellet 3 mL	Pellet 3 mL	Pellet 3 mL
RC	Pellet 3 mL	Pellet 3 mL	Pellet 3 mL

NC – new mini-column / RC – recycled mini-column

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)

Results:

	(1)	(2)	(3)	Avg.
NC	181.2 ng/µL	164.8 ng/µL	181.4 ng/µL	175.8 ng/µL
RC	163.6 ng/µL	183.1 ng/µL	189.4 ng/µL	178.7 ng/µL

Discussion:

This experiment showed that the recycled mini-columns are just as effective as new mini-columns. Coupled with the previous experiment that showed that the recycling process effectively removes residual DNA from the mini-column matrix, we have shown experimentally that this is an effective method of reducing consumable lab waste. In conjunction with the homemade buffers, this can also provide significant savings over commercial miniprep kits.