Experiment 11.2 – Homemade vs. Commercial Qiagen Buffers

Purpose:

This experiment was conducted to test the efficiency of homemade Qiagen buffers compared to the commercial buffers using the Qiagen miniprep system. Each test was run with new Qiagen mini-columns and the concentration of DNA was measured using the Nanodrop.

Setup:

	(1)	(2)	(3)
Commercial Buffers	Pellet 3 mL	Pellet 3 mL	Pellet 3 mL
Homemade Buffers	Pellet 3 mL	Pellet 3 mL	Pellet 3 mL

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

Homemade Buffer Compositions:

- Buffer P1 (pH 8.07)
    - 50 mL Tris·HCl solution (50 mM)
    - 0.186 g EDTA (10 mM)
    - Aliquot and spike w/ RNase A before each use (100 µg/mL)
- Buffer P2
    - 45 mL DI water
    - 0.4 g NaOH (200 mM)
    - 5 mL 10% SDS solution (1% w/v)
- Buffer N3 (pH 4.82)
    - 32.3 mL DI water

    - 20.06 g Gu·HCl (4.2 M)
    - 4.42 g KAc (0.9 M)
- Buffer PB
    - 35 mL DI water
    - 15.0 mL 100% isopropanol (30% v/v)
    - 23.88 g Gu·HCl (<5 M)
- Buffer PE
    - 10 mL Tris·HCl solution (10 mM)
    - 40 mL 100% EtOH (80% v/v)

Results:

	(1)	(2)	(3)	Avg.
Commercial	265.6 ng/µL	286.8 ng/µL	304.2 ng/µL	285.5 ng/µL
Homemade	216.1 ng/µL	199.0 ng/µL	187.6 ng/µL	174.2 ng/µL

Discussion:

This experiment shows that the homemade buffers are only about 61% as effective as the commercial buffers. A previous experiment showed that buffers P1 and N3 lead to the decrease in efficiency. Buffer N3 was re-made to Qiagens specifications, but still seems to be less effective. Our stock of Gu·HCl is old, and we suspect that it may have some level of hydration that is increasing the weight of the reagent and therefore decreasing the molarity in the final solution. The homemade buffers are still effective and represent a significant decrease in cost of minipreps. More careful preparation may lead to increased efficiency.