Experiment 10.1 – Homemade vs. Commercial Qiagen Buffers

Purpose:

This was a preliminary experiment to investigate the efficiency of each of the homemade buffers we had prepared individually when 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)	(4)	(5)	(6)
All Commercial Buffers	Replace Buffer P1 only	Replace Buffer P2 only	Replace Buffer N3 only	Replace Buffer PB only	Replace Buffer PE only

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)

    - 50 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)	(4)	(5)	(6)
219.6 ng/µL	189.1 ng/µL	221.5 ng/µL	102.8 ng/µL	240.2 ng/µL	239.5 ng/µL

Discussion:

This preliminary experiment showed that most of the homemade buffers performed satisfactorily. Buffer P1 produced somewhat lower yield compared to the commercial buffer, but some variation in the results are expected. Buffer N3 was significantly lower in yield, so we decided to re-make that buffer before conducting a full experiment to compare the effectiveness of the homemade buffers as a whole. In making the buffers the first time, the volume displacement caused by the solid agents was not considered, which led to a decrease in molarity of some of the components. It appears that this only had a negative effect on Buffer N3, which is most likely due to decreased concentration of the binding agent Gu·HCl. After this buffer is reproduced, an additional experiment will be conducted to compare the homemade buffers to the commercial buffers as a complete set.