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# PCR cleanup

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Alex Zegarra[1]

[1]BYU

Alex Zegarra

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**Protocol status:** Working
**We use this protocol and it's working**

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1 Column Equilibration

2 A. Insert DNA Mini Column into a 2 mL Collection Tube

3 Add 100 uL 3M NaOH to the DNA Mini Column

4 Centrifuge at maximum speed for 30-60 seconds

5 Discard the filtrate and reuse the collection tube

6 Rinse with 200 uL of Neutralization Buffer (P3) or 3M sodium acetate pH=4.5

7 2. Save 2 microliters of your PCR reaction mix in a separate tube for a pre-cleanup sample in your gel. Dilute the rest of your PCR reaction mixture 20 fold (22 uL of PCR reaction to 440 uL) in Mini-Prep PB buffer. Transfer the entire diluted PCR reaction into the DNA Mini Column

8 Centrifuge at maximum speed for 1 minute

9 Save the filtrate in a separate tube and reuse the collection tube

10 Add 500 uL PB Buffer

11 Centrifuge at maximum speed for 1 min

12 Save the filtrate in the tube from step 9 and reuse collection tube

13 Add 700 uL PE

**NOTE: (ENSURE YOU ADDED THE 100% ETHANOL TO THE PE WHEN YOU MADE IT)**

14 Centrifuge at maximum speed for 1 min

15 Discard the filtrate and reuse the collection tube

**OPTIONAL: (REPEAT STEP 13-15 FOR A SECOND DNA WASH BUFFER (PE)**

16 Centrifuge the empty DNA Mini Column for 2 minutes at maximum speed to dry the column matrix

**note: ( It's important to dry the DNA Mini column matrix before elution. Residual ethanol may interfere with elution and**

17 Transfer the DNA Mini Column to a clean 1.5 mL microcentrifuge tube

18 Add 40 uL Elution Buffer or sterile deionized water directly to the center of the column membrane

**Note: (Efficiency of eluting DNA from the DNA Mini Column is dependent on pH. If using sterile deionized water, makes sure that the pH is around 8.5)**

19 Let sit at room temperature for 1 min

20 Centrifuge at maximum speed for 1 min.

**Note: (This represents approx 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration) (Concentration decreases as you yield more-start with enough for your disired yield)**

21 Store DNA at -20 C, or use immediatly in a gel. If you see your PCR amplicacon in the gel, throw away the wash you saved. If you know it amplified (because of the 2 microliters you saved) but don't see it in your elution, then it may be in the wash. You can add more HBC buffer to the wash and try 1 more time.