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PCR cleanup

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[1]BYU

1 Works for me dx.doi.org/10.17504/protocols.io.bgaqjsdw

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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

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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.

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