E.Z.N.A. Gel Extraction Kit Spin Protocol

Preperation

- Set water bath to 60°C

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1. Perform agarose gel electrophoresis to fractionate DNA fragments. Excise the DNA fragment under UV using a scalpel in a clean 1.5 mL microcentrifuge tube.
2. Add 1 volume XP2 Binding Buffer.
   Assuming a density of 1 g/mL.
3. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.
   The color of the Gel/Binding Buffer mixture should be light yellow.
4. Insert a HiBind® DNA Mini Column in a 2 mL Collection Tube.
5. Add no more than 700 µL DNA/agarose solution from Step 3 to the HiBind® DNA Mini Column.
6. Centrifuge at 10,000g for 1 minute at room temperature.
7. Discard the filtrate and reuse collection tube.
8. Repeat Steps 5-7 until all of the sample has been transferred to the column.
9. Add an equal volume of XP2 Binding Buffer as in Step 2.
10. Centrifuge at maximum speed (≥13,000g) for 1 minute at room temperature.
11. Discard the filtrate and reuse collection tube.
12. Add 700 µL SPW Buffer.
    Note: SPW Buffer must be diluted with 100% ethanol prior to use.
13. Centrifuge at maximum speed for 1 minute at room temperature.
14. Discard the filtrate and reuse collection tube
    Optional: Repeat Steps 14-16 for a second SPW Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.
15. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.
    Residual ethanol may interfere with downstream applications.
16. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.
17. Add 30-50 µL Elution Buffer or deionized water directly to the center of the column
18. membrane.
19. Let sit at room temperature for 2 minutes.
20. Centrifuge at maximum speed for 1 minute.

Optional: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

21. Store DNA at -20°C