Gel Extraction of DNA

E.Z.N.A.™ Gel Extraction Kit

1. **Excise gel slice containing DNA fragment of interest.**
   a. Gel electrophoresis fractionates DNA fragments.
   b. The gel is exposed to UV to find the DNA fragments (stained by Ethidium bromide).
   c. The goal DNA band is identified.
   d. Physically remove the slice of gel contains the goal DNA with clean surgical blade.
2. **DNA Purification**
   e. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml microcentrifuge tube.
   f. Add an equal volume of Binding Buffer (XP2).
   g. Incubate the mixture at 55°C-60°C for 7 min or until the gel has completely melted.
   h. Mix by shaking or vortexing the tube in increments of 2-3 minutes.
   (IMPORTANT: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when pH >8.0. If the color of the mixture becomes orange or red, add 5 µl of 5M Sodium Acetate, pH 5.2 to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.)
   i. Place a HiBind® DNA column in a provided 2 ml collection tube.
   j. Apply 700 µl of the DNA/agarose solution to the HiBind® DNA column, and centrifuge at 10, 000 x g for 1 min at room temperature.
   k. Discard liquid and place the HiBind® DNA column back into the same collection tube. For volumes greater than 700 µl, load the column and centrifuge successively, 700 µl at a time. Each HiBind® DNA column has a total capacity of 25 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.
   l. Add 300 µl of Binding Buffer (XP2) into the HiBind® DNA column. Centrifuge at 10,000 x g for 1 min at room temperature to wash the column. Discard the flow-through and re-use the collection tube.
   m. Wash the HiBind ® DNA column by adding 700µl of SPW Wash Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temp.
   (Note: SPW Wash Buffer Concentrate must be diluted with absolute ethanol before use. See label for directions. If refrigerated, SPW Wash Buffer must be brought to room temperature before use.)
   n. Repeat step 8 with another 700µl of SPW Wash Buffer diluted with absolute ethanol.

o. Discard liquid and centrifuge the empty HiBind® DNA column for 2 min at maximal speed ($13,000 x g) to dry the column matrix. Do not skip this step, it is critical for the removal of ethanol from the HiBind® DNA column.
p. Place a HiBind® DNA column into a clean 1.5 ml microcentrifuge tube. Add 30-50µl (depending on desired concentration of final product) of Elution Buffer (10 mM Tris-HCl, pH 8.5) directly onto the column matrix and incubate at room temperature for 1 minute. Centrifuge for 1 min at maximal speed ($13,000 x g) to elute DNA. This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.