Protocol for Gel Purification with OMEGA Gel Extraction Kit

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse running buffer as its pH will increase and reduce yields.

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose.

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 mL microcentrifuge tube. Assuming a density of 1 g/mL, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 mL.

4. Add 1 volume Binding Buffer (XP2).

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 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 the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µL 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.

6. Insert a HiBind® DNA Mini Column in a 2 mL Collection Tube.

7. Add no more than 700 µL DNA/agarose solution from Step 5 to the HiBind® DNA Mini Column.

8. Centrifuge at 10,000 x g for 1 minute at room temperature.

9. Discard the filtrate and reuse collection tube.

10. Repeat Steps 7-9 until all of the sample has been transferred to the column.

11. Add 300 µL Binding Buffer (XP2).

12. Centrifuge at maximum speed (≥13,000 x g) for 1 minute at room temperature.

13. Discard the filtrate and reuse collection tube.

14. Add 700 µL SPW Wash Buffer.

Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

15. Centrifuge at maximum speed for 1 minute at room temperature.

16. Discard the filtrate and reuse collection tube.

Optional: Repeat Steps 14-16 for a second SPW Wash Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

17. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

18. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

19. Add 30-50 µL Elution Buffer or deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If eluting DNA with deionized water, make sure that the pH is around 8.5.

20. Let sit at room temperature for 2 minutes.

21. Centrifuge at maximum speed for 1 minute.

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

22. Store DNA at -20°C.