Protocol for mini-prep with OMEGA E.Z.N.A.® Plasmid DNA Mini Kit I.

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for 12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.

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

3. Decant or aspirate and discard the culture media.

4. Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 6.

5. Transfer suspension into a new 1.5 mL microcentrifuge tube.

6. Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid

acidification from CO2 in the air.

7. Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms.

Note: It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

8. Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

9. Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube. Optional Protocol for Column Equilibration:

1) Add 100 µL 3M NaOH to the HiBind® DNA Mini Column.

2) Centrifuge at maximum speed for 30-60 seconds.

3) Discard the filtrate and reuse the collection tube.

10. Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

11. Centrifuge at maximum speed for 1 minute.

12. Discard the filtrate and reuse the collection tube.

13. Add 500 µL HBC Buffer.

Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

14. Centrifuge at maximum speed for 1 minute.

15. Discard the filtrate and reuse collection tube.

16. Add 700 µL DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 6 for instructions.

17. Centrifuge at maximum speed for 1 minute.

18. Discard the filtrate and reuse the collection tube.

Optional: Repeat Steps 16-18 for a second DNA Wash Buffer wash step.

19. 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.

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

21. Add 30-100 µL Elution Buffer or sterile 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 using sterile deionized water, make sure that the pH is around 8.5.

22. Let sit at room temperature for 1 minute.

23. 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.

24. Store DNA at -20°C

Reference: OMEGA E.Z.N.A.® Plasmid DNA Mini Kit I