Miniprep (Omega Kit)

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 500 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.

5. Transfer suspension into a new 2 mL microcentrifuge tube.

6. Add 500 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 CO_2 in the air.

7. Add 700 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 at room temperature. 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.

10. Transfer 700 µL cleared lysate 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. Repeat Steps 10-12 until all cleared lysate has been transferred to the HiBind® DNA Mini Column.

14. Add 500 µL HBC Buffer.

Note:
HBC Buffer must be diluted with 100% isopropanol before use. Please see Page 6 for instructions.

15. Centrifuge at maximum speed for 1 minute.

16. Discard the filtrate and reuse collection tube.

17. 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.

18. Centrifuge at maximum speed for 1 minute.

19. Discard the filtrate and reuse the collection tube.

Optional:
Repeat Steps 17-19 for a second DNA Wash Buffer wash step.

20. 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.

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

22. add 80-100 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.

23. Let sit at room temperature for 1 minute.

24. 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.

25. Store DNA at -20°C.