PLASMID MINIPREP PROTOCOL

Protocol:

The following procedure is performed at room temperature. Ensure that buffers have been prepared according to the instructions on *page 3*.

1. Add 5 ml of bacterial culture grown in LB medium to a 15 ml conical tube. Spin tube at max speed for 10 min and discard supernatant. Then *add 600 µl of TE to the bacterial cell pellet and resuspend completely.*

2. Add 100 µl of **7X Lysis Buffer (Blue)** and mix by inverting the tube 4-6 times. Proceed to step 3 within 2 minutes.

After addition of 7X Lysis Buffer the solution should change from opaque to clear blue, indicating complete lysis.

3. Add 350 µl of cold **Neutralization Buffer (Yellow)** and mix thoroughly. *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.* **Invert the sample an additional 2-3 times** to ensure complete neutralization.

4. Centrifuge at 11,000 – 16,000 x *g* for 2-4 minutes. 5. Transfer the supernatant (~900 µl) into the provided **Zymo-SpinTM IIN** column.
Avoid disturbing the cell debris pellet.

6. Place the column into a **Collection Tube** and centrifuge for 15 seconds.

7. Discard the flow-through and place the column back into the same **Collection Tube**.

8. Add 200 µl of **Endo-Wash Buffer** to the column. Centrifuge for 30 seconds. *It is not necessary to empty the collection tube.*

9. Add 400 µl of **ZyppyTM Wash Buffer** to the column. Centrifuge for 1 minute.

10. Transfer the column into a clean 1.5 ml microcentrifuge tube then add 30 µl of **ZyppyTM Elution Buffer**2 directly to the column matrix and let stand for one minute at room temperature.

11. Centrifuge for 30 seconds to elute the plasmid DNA.