Preparation of Plasmid DNA using the Wizard

1. Innoculate 3 mL of 2XYT (with appropriate antibiotic) with desired number of independent transformants. Grow 2 hr. to overnight at 37C (300 rpm).

2. Pour cells into a sterile 2 mL tube and spin at high speed for 2 min. Use a cut-off 21 gauge needle to remove as much supernatant as possible.

3. Add 200 uL of Cell Resuspension Solution and resuspend pellet by pipetting.

4. Add 200 uL of Cell Lysis Solution and mix by inverting the tube 3 times. DO NOT vortex or chromosomal DNA will shear and detach from cell membrane and will contaminate the plasmid DNA prep.

5. Add 200 uL of Neutralization Solution and mix by inverting the tube 3 times. DO NOT vortex.

6. Spin at high speed for 5 min. and pour supernatant into a 2 mL tube.

7. Add 1 mL of the DNA Purification Resin to the supernatant and mix by inverting. Shake the Purification Resin vigorously before use.

8. Attach a 3 mL disposable syringe barrel to each minicolumn and insert the tip of the minicolumn into the vacuum manifold.

9. Pour the Resin / DNA mix into the syringe barrel. Apply vacuum to pull mix completely into minicolumn. Once entire mix has entered the mini column, break the vacuum by pulling on the vacuum tubing. DO NOT break the vacuum by closing the stopcock.

10. Add 2 – 3 mL of Column Wash Solution to the syringe barrel (Make sure EtOH has already been added to the Column Wash Solution). Reapply the vacuum to draw the wash solution through the minicolumn. Continue to draw a vacuum for 1 – 2 min. after the wash solution has completely passed through the column.

11. Transfer the minicolumn to a 1.5 mL microcentrifuge tube. Spin the tube at high speed for 20 – 40 sec. to remove any residual wash solution.

12. Transfer minicolumn to a new sterile 1.5 mL microcentrifuge tube. Add 55 uL of pre-heated (65C) sterile water to the minicolumn. Wait 1 min.

13. Spin minicolumn at high speed for 40 sec. to elute DNA.

14. Take A260 and A280 readings to determine concentration and purity of plasmid DNA. Store the plasmid DNA at -20C.