A45: High Speed Plasmid Mini Kit (from MIDSCI) Protocol
TCH, 12/01/08 (Revised 12/19/08)

MIDSCI: Reorder Number: IB47101

Step 1

- Transfer 2ml of bacterial culture to a microcentrifuge tube.
- Microcentrifuge for 1 minute and discard the supernatant.
- Repeat step 1 once with same microcentrifuge tube: total 4ml bacterial culture used.

Step 2

- Add 200ul of **PD1 Buffer** (RNase A added) to the tube and resuspend the cell pellet by vortex.
- Add 200ul of **PD2 Buffer** and mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA.
- Let stand at room temperature foe 2 minutes or until the lysate is homologous.
- Add 300ul of **PD3 Buffer** and mix immediately by inverting the tube 10 times. Do not vortex.
- Microcentrifuge for 3 minutes.

Step 3

- Place a **PD Column** in a **Collection Tube**.
- Add the supernatant from Step 2 to the **PD Column** and microcentrifuge for 30 seconds. Discard the following-through and place the PD Column back in the 2ml Collection Tube.
- Add **400ul** of **W1 Buffer** into the center of the **PD Column**, centrifuge for 30 seconds. Discard the following-through and place the **PD Column** back in the 2ml Collection Tube.
- Add 600ul of **Wash Buffer** (ethanol added) into the center of the **PD Column**, centrifuge for 30 seconds.
- Discard the following-through and place the **PD Column** back in the 2ml **Collection Tube**.
- Centrifuge for again for 3 minutes to dry the column matrix.

Step 4

- Transfer the dried **PD Column** to a new microcentrifuge tube (1.7ml tube is recommended).
- Add 50ul of **Elution Buffer** or water into the center of the column matrix, let stand for 2 minutes or until the **Elution Buffer** or water is absorbed by the matrix.
- Centrifuge for 2 minutes to elute the DNA.

NOTE: This is the replacement of Promega's Wizard Prep Kit.