PURIFICATION OF PLASMID DNA USING WIZARD PLUS KIT
by TCH 1/29/02; Updated by JK Park 5/15/04

1. Pellet 2ml of overnight bacterial culture by centrifugation for 1 min. at 14,000rpm in a **2.0ml** microcentrifuge tube, decant/aspirate the supernatant. [**Optional**: to maximize the DNA yield, you can repeat this step by pelleting **another 2ml** culture in the same tube] (**Note**: The 2.0ml tubes are used because they are easy to resuspend the pellets).

2. Resuspend the cell pellet in **200ul** of **Cell Resuspension Solution** (50mM Tris, (pH7.5); 10mM EDTA; 100ug/ml RNase A) by vortexing.

3. Add **200ul** of **Cell Lysis Solution** and mix by shaking the rack. The cell suspension should be clear immediately.

4. Add **200ul** of **Neutralization Solution** (1.32M potassium acetate) and mix by inverting the tube couple of times.

5. Centrifuge the lysate at 14,000rpm in a microcentrifuge for 3 min.

6. Attach the minicolumn to the syringe barrel, pipette **1ml** of resuspended resin into the minicolumn/syringe assembly (**Note**: It is absolutely critical to shake and **mix the resin well**, even between your pipettings).

7. Transfer all of the cleared lysate to the barrel of the minicolumn/syringe assembly containing the resin, mix well by inverting the barrel.

8. Attach the minicolumn/syringe set to the vacuum, turn on the valve, let all of the samples completely pass through the column; break the vacuum at the source.

9. Add **2ml** of **Column Wash Solution** (containing ethanol) to the syringe barrel and reapply the vacuum to draw all of the solution through the minicolumn (**Note**: for the best result, add **1ml** Wash Solution at a time, **x2**).

10. Transfer the minicolumn to a 1.5ml tube with lid cut off, spin out the remaining wash solution in a microcentrifuge @ speed around **9000rpm** for **30 sec**.

11. Set the minicolumn into a new1.5ml tube, add in **100ul** of warm ddH_2O (**pre-heated at 70°C** in a heat block), spin @ top speed for 1 min.

12. Remove and discard the minicolumn. Collect the DNA solution, read the OD_{260}, and store DNA at -20°C.

NOTES:

1. This is a quick way to prepare DNA for the transfection of mammalian cells. But the best quality of DNA is still prepared by CsCl-banding.
2. If you want to recover more DNA, pellet bacterial cells twice in the same 2.0ml tubes, and there is **no need** to double the amount of reagents in this case.