**Agrobacterium Plasmid Mini-prep protocol**

*(Using the Promega Wizard Plus SV Miniprep DNA Purification System)*

This protocol is used for restriction mapping plasmids transformed into *A. tumefaciens* to verify that they are correct before proceeding with plant transformation. The protocol was originally provided to us by Dr. Carolyn Napoli, University of Arizona.

1. Pellet 2 ml of *Agrobacterium* overnight culture and completely resuspend the cell pellet in 200 ul Wizard Cell Resuspension Solution by vortexing.

2. Add 20 ul Lysozyme solution (50mg/ml) and incubate at room temperature for 5 minutes.

3. Add 200 ul Wizard Cell Lysis Solution to each sample, invert to mix.

4. Add 10 ul Alkaline Protease Solution, invert and mix. Incubate 5 minutes at room temperature.

5. Add 350 ul Neutralization Solution, invert to mix.

6. Centrifuge the bacterial lysate at top speed for 10 minutes at room temperature.

7. Insert Spin Column into a 2 ml Collection Tube and transfer the cleared lysate (about 850 ul) into Spin Column.

8. Centrifuge at top speed for 1 minute at room temperature. Discard flow-through and reinsert Column into Collection Tube.


10. Repeat Step 9 with 250 ul Wash Solution and centrifuge for 2 minutes.

11. Transfer Spin Column to a sterile 1.5ml microcentrifuge tube.

12. Add 100 ul of Nuclease-Free Water to the Spin Column. Centrifuge at top speed for 1 minute at room temperature.

13. Discard column and store DNA at -20C or below.