

## **Protocol: Plasmid DNA Purification Using the QIAprep Spin Miniprep Kit and a Microcentrifuge**

### **The Idea**

A miniprep is a way of extracting plasmid DNA from cells. You will be provided with *E. coli* cells expressing the plasmid pSB4A5. pSB4A5 will be the destination plasmid used for all assemblies. It contains an ampicillin resistance gene (hence the "A" in its name).

### **Materials**

- Pellet of appropriate bacterial cells
- QIAprep Buffers
  - P1, P2, N3, PB, PE
- Deionized, sterile H<sub>2</sub>O

### **Procedure**

#### **1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a 1.7ml microcentrifuge tube.**

No cell clumps should be visible after resuspension of the pellet. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

#### **2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.**

Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. *Do not allow the lysis reaction to proceed for more than 5 min.* If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

#### **3. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.**

To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. The solution should become cloudy. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless.

#### **4. Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.** A compact white pellet will form.

#### **5. Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.**

**6. Centrifuge for 30–60 s. Discard the flow-through.**

**7. Recommended: Wash the QIAprep spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**

**8. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**

**9. Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.**

**Important:** Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

**10. Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.**