Qiagen miniprep from *Agrobacterium tumefaciens*

Materials

- **Qiagen Miniprep kit (Cat # 27106, QIAGEN, Valencia, CA)**
- **Tabletop centrifuge**
- **50 ml falcon centrifuge tubes**
- **1.5 ml and 2 ml microcentrifuge tubes**
- **28°C incubator with shaker (250 rpm)**
- **YEP liquid and YEP plate with appropriate antibiotics**
- **YEP composition: Yeast extraction: 5g/l; Trptone: 10g/l; NaCl: 5g/l**

Methods

- **Harvest the Agrobacterium cells**

1. Inoculate a loop of freshly grown Agrobacterium into a 20 ml of YEP + antibiotic (typically spectinomycine at final concentration of 100 mg/L) in a 200 ml flask. Place the flask @28°C with 250 rpm shaking overnight. Typically do this step around 4 pm.

2. Harvest the bacterial culture around 8 am the second day, or when the bacterial culture reaches OD_{600}=1.0-1.2. Transfer the overnight bacterial culture to 50 ml centrifuge tube and centrifuge at 5000 rpm in centrifuge (Beckman coulter, rotor JS-5.3) for 10 minutes at room temperature.

3. Discard the supernatant into a waste beaker and place the tube upside down on a paper towel to completely drain all the liquid.

- **Plasmid isolation**

Before start, make sure **1)** provided RNase solution has been added to Buffer P1 and stored at 2–8°C; ethanol (96–100%) has been added to to Buffer PE before use (see bottle label for volume). **2)** Warm the elution buffer at 60°C water-bath/air-bath. **3)** Wear gloves when handling buffers, as Buffers P2, N3, and PB contain irritants.

1. To the pellet, add 650 µl of cold P1 buffer, resuspend the pellet thoroughly by vortexing or pipetting up and down. Transfer the bacteria equally (about 325 µl each) to two newly labeled 2 ml eppendorf tubes.

Note: It is important that the bacteria pellet is completely resuspended.

2. Add 325 µl of P2 buffer to each tube and mix thoroughly but gently by inverting the tube 6-10 times. Incubate at room temperature for 5min.

   Note: The solution should turn blue if Qiagen BlueLyse has been added in P1 buffer.

   Note: It is important the incubation time shouldn't last longer than 5min.

3. Add 445 µl of N3 buffer to each tube and mix immediately by inverting 6-10 times.

   Note: the blue color should disappear as the precipitate forms.

4. Centrifuge the tubes in a microcentrifuge for 15 minutes at 15,000 rpm at room temperature. Meanwhile, label one Qiagen column.
5. Collect the supernatant from one tube and add it into the labeled column, which is placed on a Qiagen provided 2 ml tubes.
6. Centrifuge for 1 min at 14,000 rpm. If only prepare one plasmid, a tube with appropriate weight is needed to balance the centrifugation.
7. Discard flow-through and put the column back in the tubes.
8. Take the supernatant from the second tube and add it into the columns. This is to combine both cell lysates from the same culture.
9. Centrifuge for 1 min at 14,000 rpm.
10. Discard flow-through and put the column back in the tubes.
11. Add 500 µl of PB buffer to the column.
12. Centrifuge for 1 min at 14,000 rpm.
13. Discard flow-through and put the column back in the tubes.
14. Add 750 µl of PE buffer (make sure ethanol is in the PE buffer) to the column. Be careful not to spill on the side of the column or the column's label might be erased.
15. Centrifuge 1 min at 14,000 rpm.
16. Discard flow-through and put the column back in the tubes.
17. Centrifuge additional 2 min at 14,000 rpm.
18. Place the column in a newly labeled 1.5 ml tube.
19. Add 60 µl of pre-warmed elution buffer to the column and let sit on the bench for 2-3 minutes.
20. Centrifuge 1 min at 14,000 rpm.
21. Discard the column and close the tube. DNA should be in the elution.

22. Measure the DNA concentration by Nanodrop. A successful DNA preparation usually give a total of 1.5-2.2 µg of DNA with about 30-40 µg/µl.

References

QIAGEN Plasmid Miniprep Handbook – June 2006
(http://www.qiagen.com/literature/handbooks/literature.aspx?id=1000229&r=1013)