

**SOP: RP006**

**Small- Scale Purification of Plasmid DNA Using QIAprep Kit**

**Materials and Reagents:**

1. QIAprep Kit (note 1), including:
  - a. Buffer P1 (note 2)
  - b. Buffer P2
  - c. Buffer N3
  - d. Buffer PB
  - e. Buffer PE (note 3)
  - f. Buffer EB
  - g. QIAprep spin columns
  - h. RNase (note 2)
2. Ethanol (note 3)
3. Microcentrifuge

**Protocol for High copy plasmids** (for low copy plasmids see note 4):

1. \_\_\_\_ Scrape a small aliquot (eg 10 µl) of cells from frozen stock or scrape 2-3 colonies from agar surface with a one ml disposable pipet or sterile pipetman tip.
2. \_\_\_\_ Transfer cells into 5.0 – 10.0 ml LB broth supplemented with 100 - 130 µg/ml ampicillin in a 50 ml centrifuge tube (note 5).
3. \_\_\_\_ Incubate overnight at 37°C, 150-300 rpm
4. \_\_\_\_ Centrifuge cell culture at 3,000 x g for 15 minutes.
5. \_\_\_\_ Pour off supernatant, and resuspend pellet in 250 µl of Buffer P1 (note 6).
6. \_\_\_\_ Transfer suspension to a 0.65ml microcentrifuge tube, and add 250 µl of Buffer P2, gently invert tube immediately 4- 6 times.
7. \_\_\_\_ Add 350 µl of Buffer N3 and gently invert 4-6 times. Place in microcentrifuge and spin at maximum speed for 15 minutes.
8. \_\_\_\_ Apply the supernatant from step 4 to the QIAprep spin column by decanting or pipetting.
9. \_\_\_\_ Centrifuge for 30- 60 seconds at max speed. Discard the flow-through.
10. \_\_\_\_ Wash the QIAprep spin column with 500 µl of Buffer PB. Centrifuge for 30- 60 seconds at max speed and discard flow through.
11. \_\_\_\_ Wash QIAprep spin column with 750 µl of Buffer PE. Centrifuge for 30- 60 seconds at max speed and discard flow through.
12. \_\_\_\_ Repeat above centrifugation for 1 min to remove residual wash buffer.
13. \_\_\_\_ Place QIAprep column over a clean 1.7 ml microcentrifuge tube.
14. \_\_\_\_ To elute DNA add 50 µl of Buffer EB (10mM Tris, pH 7.5), or sterile Milli-Q H<sub>2</sub>O to the center of the QIAprep column. Let stand for 15-30 min at RT, and centrifuge for 1 min.
15. \_\_\_\_ Discard QIAprep spin column, cap microcentrifuge tube and store for further applications.

**Notes:**

1. All buffers and spin columns come within the kit, but can be ordered separately upon need.
2. Buffer P1 requires the addition of RNase prior to use. Also, it is stored at 4°C.
3. Buffer PE requires the addition of 90- 100% ethanol, check label on buffer for proper amount.
4. For the purification of Low copy plasmids, refer to SOP: RP002 and use of Qiagen Maxi-Kit
5. Supplement LB broth with ampicillin at a 1:1000 dilution(diluting 100 mg/ml to 100 µg/ml final concentration).  
eg. 0.002 ml (2.0 µl) stock ampicillin in 2.0 ml broth or 1.1ml stock ampicillin in 1 liter broth.
6. For a 10 ml culture, double the amount of buffer volumes used. eg. For a 5ml culture, add 250ul of Buffer P1; for a 10ml culture add 500ul Buffer P1

**References:**

QIAprep Miniprep Handbook, March 2003, pg 22-23, 35.