User-Developed Protocol:

Isolation of bacteriophage-P1-derived constructs using the QIAGEN® Plasmid Midi Kit

This procedure has been adapted by customers from the QIAGEN® Plasmid Midi Kit Protocol. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully for isolation of 110 kb P1 DNA (pAdsacBII with an 80 kb insert) from *Escherichia coli* strain NS3529. Yield of P1 DNA was typically 10–50 µg from 500 ml culture.

Please be sure to read the *QIAGEN Plasmid Purification Handbook* and the detailed QIAGEN Plasmid Midi Kit Protocol carefully before beginning this procedure.

Procedure

1. **Inoculate 3 ml of 2x YT containing 25 µg/ml kanamycin with a single colony from a selective plate and grow overnight, shaking at 37°C.**

2. **Inoculate 500 ml of 2x YT containing 25 µg/ml kanamycin with 0.5 ml of the overnight culture. Shake at 37°C for approximately 3 h until an A_{550} of 0.15 is reached.**
   Be sure to measure cell density and follow recommended A_{550} readings to obtain optimal yields.

3. **Add 5 ml of freshly made, filter-sterilized 0.1 M IPTG to the 500 ml culture. Shake for a further 3 h at 37°C or until an A_{550} of 1.3–1.5 is reached.**
   IPTG is added to induce P1 replication.

4. **Harvest cells by centrifugation at 5000 x *g* for 10 min.**
   Cell pellets can be stored overnight at –20°C following complete removal of the supernatant.

5. **Resuspend the bacterial pellet in 8 ml Buffer P1.**
   Note: QIAGEN recommends using 20 ml of Buffer P1 for optimal lysis.
   Ensure that RNase A (100 µg/ml) has been added to Buffer P1.

6. **Add 8 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature for 5 min.**
   Note: QIAGEN recommends using 20 ml of Buffer P2 for optimal lysis.
   Check Buffer P2 before use for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.

7. **Add 8 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 15 min.**
   Note: QIAGEN recommends using 20 ml of Buffer P3 for optimal lysis.

8. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**
9. **Centrifuge again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**
10. **Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.**
11. **Apply the supernatant from step 9 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**
12. **Wash the QIAGEN-tip with 2 x 10 ml Buffer QC.**
13. **Elute DNA with 5 ml Buffer QF.**
    Prewarming Buffer QF to 50°C before elution may increase yield.
14. **Precipitate DNA by adding 3.5 ml room-temperature isopropanol to the eluted DNA. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.**
15. **Wash the DNA pellet with 2 ml of room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min.**
16. **Carefully decant the supernatant. Air-dry the DNA pellet for 5–10 min.**
    Caution: Do not overdry DNA pellet after washing with 70% ethanol.
17. **Cut off the bottom 0.5 cm of a 1 ml pipette tip and resuspend the DNA pellet in 500 µl of TE, pH 8.0.**
    This step avoids shearing the high-molecular-weight DNA.