User-Developed Protocol:

Isolation of BAC DNA 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.**

This procedure has been used successfully for isolation of 150–250 kb BAC DNA from a mouse-BAC library cloned in pBeloBAC11 from *Escherichia coli* strain HB101/r. The yield of BAC DNA from 100 ml culture was typically 20–40 µg.

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. **Pick a single BAC colony and inoculate a starter culture of 5 ml LB medium containing the appropriate antibiotic.**
2. **Inoculate 0.5 ml pre-culture into 100 ml selective LB medium. Grow at 37°C for 14 hours with vigorous shaking (~250 rpm).**
3. **Divide the cells into two 50 ml tubes, and harvest the cells by centrifugation at 4500 x *g* for 20 min.**
4. **Resuspend each bacterial pellet in 10 ml Buffer P1.**
   Ensure that RNase A (100 µg/ml) has been added to Buffer P1.
5. **Add 10 ml Buffer P2 to each tube. Mix thoroughly and gently by inverting 4–6 times, and incubate at room temperature for 5 min.**
   Check Buffer P2 before use for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
6. **Add 10 ml chilled Buffer P3 to each tube. Immediately mix by gently inverting 4–6 times, and incubate on ice for 15 min.**
7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing plasmid DNA promptly.**
8. **Centrifuge the supernatant again at ≥20,000 x *g* for 15 min at 4°C. Remove supernatant containing plasmid DNA promptly.**
9. **Equilibrate a QIAGEN-tip 100 by applying 4 ml Buffer QBT, and allow the column to empty by gravity flow.**
10. **Pool the two supernatants from step 8. Apply the sample to the QIAGEN-tip and allow it to enter the resin by gravity flow.**
11. **Wash the QIAGEN-tip with 2 x 10 ml Buffer QC.**

12. **Elute DNA with 5 x 1 ml Buffer QF, prewarmed to 65°C.**
    Prewarming the elution buffer may help to increase yields. Eluting in 5 aliquots of 1 ml instead of 1 aliquot of 5 ml prevents cooling of the elution buffer.

13. **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.**

14. **Wash the DNA pellet with 2 ml of room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 10 min. Carefully decant the supernatant without disturbing the pellet.**

15. **Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris·Cl, pH 8.5).**

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Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.

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