QIAGEN Supplementary Protocol:

Isolation of large-construct DNA using the QIAGEN® Plasmid Maxi Kit

This protocol is designed to provide up to 150 µg BAC/PAC/P1 DNA or up to 400 µg cosmid DNA. This protocol is recommended for applications that do not require genomic DNA-free preparations. Genomic DNA contamination levels can vary considerably and may comprise up to 30% of large-construct DNA preparations. If a genomic DNA-free isolate is required, the QIAGEN® Large-Construct Kit (cat. no. 12462) is strongly recommended.

Please be sure to read the *QIAGEN Plasmid Purification Handbook* or the *QIAGEN Large-Construct Kit Handbook* carefully before beginning this procedure.

Additional buffers P1, P2, and P3 are necessary for this protocol since the increased culture volume requires higher amounts than those provided with the kit. The following is the ordering information needed for the recommended kits and additional buffers:

Ordering information

Product	Cat. No.
QIAGEN Plasmid Maxi Kit (10)	12162
QIAGEN Plasmid Maxi Kit (25)	12163
Buffer P1	19051
Buffer P2	19052
Buffer P3	19053
RNase A	19101

Alternatively, all buffers may be prepared according to the compositions provided in Appendix A of the *QIAGEN Plasmid Purification Handbook*. Folded filters may be obtained from suppliers such as Schleicher and Schuell, cat. no. 311651.

Recommended culture volumes

Construct	QIAGEN-tip 500
BAC	500 ml
PAC	500 ml
P1	500 ml
Cosmid	500 ml

Important notes before starting

- Add RNase A solution to Buffer P1 before use to give a final concentration of 100 µg/ml.
- Check Buffer P2 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.
- Chill Buffer P3 to 4°C before use.
- Warm elution Buffer QF to 65°C for use in step 12.

Procedure

1. **Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 ml LB medium containing the appropriate selective antibiotic. Incubate for ~8 hours at 37°C with vigorous shaking (~300 rpm).**
2. **Dilute 0.5–1.0 ml of the starter culture into 500 ml selective LB medium (1/500 to 1/1000 dilution). Grow at 37°C for 12–16 hours with vigorous shaking (~300 rpm).**
3. **Harvest the bacterial cells by centrifugation at 6000 x *g* for 15 min at 4°C.**
4. **Resuspend the bacterial pellet in 20 ml of Buffer P1.**

   For efficient lysis it is important to use a vessel that is large enough to allow complete mixing of the lysis buffers. Ensure that RNase A has been added to Buffer P1. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

   Note: If no centrifugation tubes suitable for 60 ml volumes are at hand, the resuspended bacteria can be split into 2 x 10 ml. The two batches should be processed separately through steps 5 and 6 and then combined after step 7, before the filtration step.
5. **Add 20 ml of Buffer P2, mix gently but thoroughly by inverting 4–6 times, and incubate at room temperature for 5 min.**

   Do not vortex, as this will result in shearing of both genomic and large BAC/PAC/P1/cosmid DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min.
6. **Add 20 ml of chilled Buffer P3, mix immediately but gently by inverting 4–6 times, and incubate on ice for 10 min.**

   Do not vortex, as this will result in shearing of both genomic and large BAC/PAC/P1/cosmid DNA.
7. **Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing BAC/PAC/P1/cosmid DNA promptly.**

   After centrifugation, the supernatant should be clear.
   Note: If the sample has been processed in two batches from step 4, they should be combined now for step 8.
8. **Filter the lysate through a folded filter prewetted with distilled water.**
9. **Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.**
10. **Apply the sample from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow. This will require 2 loading steps due to the large sample volume.**

11. **Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.**

12. **Elute DNA with 15 ml Buffer QF, prewarmed to 65°C.**

    Use of prewarmed Buffer QF will make elution of the large DNA molecules more efficient. Collect the eluate in a 30 ml tube. Use of polycarbonate centrifuge tubes is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

13. **Precipitate DNA by adding 10.5 ml (0.7 volumes) 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 DNA pellet with 5 ml of room-temperature 70% ethanol, and centrifuge at ≥15,000 x *g* for 15 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).**

    Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided.