Quick-Start Protocol March 2016

QIAGEN® Large-Construct Kit

The QIAGEN Large-Construct Kit (cat. no. 12462) can be stored at room temperature (15–25°C) for up to 2 years if not otherwise stated on label.

Further information

- *QIAGEN Large-Construct Handbook*: **www.qiagen.com/HB-1157**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Add RNase A solution to Buffer P1 to a final concentration of 100 µg/ml.
- Resuspend 1 vial of ATP-Dependent Exonuclease per preparation in 225 µl Exonuclease Solvent. Mix by tapping and let stand for 15 minutes.
- Dissolve precipitates in Buffer P2 for SDS by warming to 37°C.
- Prechill Buffer P3 to 4°C.
- Prewarm elution Buffer QF to 65°C.
- Do not exceed 500 ml culture for preparing BAC, PAC, P1 or cosmid DNA.
- Prepare a 100 mM ATP solution with 2.75 g ATP (dehydrated disodium salt not provided) in 40 ml distilled water. Adjust the pH to 7.5 with 10 M NaOH (~1 ml). Adjust the volume to 50 ml with distilled water.

1. Pellet bacterial overnight culture by centrifuging at 6000 x *g* for 15 min at 4°C.
2. Completely resuspend the bacterial pellet in 20 ml Buffer P1.
3. Add 20 ml Buffer P2, mix gently but thoroughly by inverting 4–6 times and incubate at room temperature (15–25°C) for 5 min. Do not vortex.
4. Add 20 ml chilled Buffer P3, mix immediately but gently by inverting 4–6 times and incubate on ice for 10 min.

5. Centrifuge at ≥20,000 x *g* for 30 min at 4°C. Remove supernatant containing BAC/PAC/P1/cosmid DNA promptly.
6. Filter the lysate through a folded filter premoistened with distilled water.
7. Precipitate DNA by adding 0.6 volumes room-temperature isopropanol to the cleared lysate. Mix and centrifuge immediately at ≥15,000 x *g* for 30 min at 4°C. Carefully decant the supernatant.
8. Wash DNA pellet with 5 ml room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.
9. Place the tube containing the DNA pellet upside down on a paper towel and allow the DNA to air-dry for 2–3 min. Carefully remove any additional liquid visible on the tube opening and carefully redissolve the DNA in 9.5 ml Buffer EX, until the DNA is completely dissolved.
10. Add 200 µl ATP-Dependent Exonuclease and 300 µl ATP solution to the dissolved DNA, mix gently but thoroughly and incubate in a water bath or heating block at 37°C for 60 min.
11. Equilibrate a QIAGEN-tip 500 by applying 10 ml Buffer QBT, and allow the column to empty by gravity flow.
12. Add 10 ml Buffer QS to the DNA sample from step 12, apply the whole sample to the QIAGEN-tip, and allow it to enter the resin by gravity flow.
13. Wash the QIAGEN-tip with 2 x 30 ml Buffer QC.
14. Elute DNA with 15 ml Buffer QF, prewarmed to 65°C.
15. 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.
16. Wash DNA pellet with 5 ml room-temperature 70% ethanol and centrifuge at ≥15,000 x *g* for 15 min. Carefully decant the supernatant without disturbing the pellet.
17. Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE buffer, pH 8.0, or 10 mM Tris·Cl, pH 8.5).

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