QIAGEN Supplementary Protocol

Purification of DNA amplified using REPLI-g® Kits

This protocol is optimized for the purification of 2–60 μg REPLI-g amplified DNA using a simple centrifugation step. In general, recovery of at least 80% of REPLI-g amplified DNA can be achieved using this protocol. Purification with silica-column-based cleanup methods are not recommended due to recovery loss of up to 50%, which is caused by the large fragment size (2000–70000 bp) of the amplified DNA.

Purification of REPLI-g amplified DNA is necessary if residual primers or nucleotides interfere with downstream analysis (e.g., direct labeling of REPLI-g amplified DNA using labeled nucleotides).

IMPORTANT: Please refer to the handbooks supplied with the respective REPLI-g Kits for general information on the handling and storage of kit components.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- 1.5 or 2 ml microcentrifuge tubes
- Microcentrifuge
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)
- 96–100% ethanol
- 70% ethanol

Important points before starting

- All centrifugation steps should be performed at room temperature (15–25°C).
- Equilibrate REPLI-g amplified DNA to room temperature (15–25°C).
- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

Procedure

1. **After the REPLI-g reaction, equilibrate REPLI-g amplified DNA to room temperature (15–25°C).**

   This protocol is suitable for use immediately following amplification using REPLI-g Kits, or with REPLI-g amplified DNA that has been stored at –20°C.

   Note: If necessary, add the reaction mixture to a new 1.5 ml microcentrifuge tube.

2. **Add 150 µl ethanol (96–100%). Mix carefully by flicking the tube.**

   Note: Do not mix by pipetting up and down.

3. **Centrifuge at maximum speed for 1–2 min.**

   Note: Do not centrifuge for longer than 2 min. Centrifugation for longer periods would condense the precipitate, making it more difficult to dissolve.

4. **Aspirate the supernatant carefully using a pipet and discard the supernatant.**
5. **Add 100 µl of 70% ethanol to the pellet.**
6. **Centrifuge at maximum speed for 1–2 min.**

   Note: Do not centrifuge for longer than 2 min. Centrifugation for longer periods will cause the precipitate to condense, making it more difficult to dissolve.

7. **Aspirate the supernatant carefully using a pipet and discard the supernatant.**
8. **Centrifuge briefly to collect residual supernatant at the bottom of the tube.**
9. **Aspirate the residual supernatant carefully using a pipet and discard the supernatant.**
10. **Incubate the microcentrifuge tube containing the precipitate of REPLI-g amplified DNA for 5 min at room temperature (15–25°C).**
11. **Add 50 µl 1x TE buffer (pH 8.0) to dissolve REPLI-g amplified DNA.**

    Note: Mix carefully to avoid shearing of REPLI-g amplified DNA.

12. **Purified DNA can be directly used for all downstream applications.**

    Optical density (OD) measurements can be performed to accurately determine the concentration of purified REPLI-g amplified DNA.

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For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Selected handbooks can be downloaded from www.qiagen.com/literature. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

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