Supplementary Protocol November 2018

Purification of DNA amplified using REPLI-g® Advanced DNA Single Cell Kits

This protocol is optimized for the purification of 2–60 µg REPLI-g advanced amplified DNA using a simple centrifugation step. In general, recovery of at least 80% of REPLI-g advanced 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 advanced amplified DNA is necessary if residual primers or nucleotides interfere with downstream analysis (e.g., direct labeling of REPLI-g advanced 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

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 advanced 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 advanced amplified DNA to room temperature (15–25°C).**

   This protocol is suitable for use immediately following amplification using REPLI-g Advanced DNA Single Cell Kits, or with REPLI-g advanced 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 minutes.**

   Note: Do not centrifuge for longer than 2 minutes. 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 minutes.**

   Note: Do not centrifuge for longer than 2 minutes. 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 advanced amplified DNA for 10 minutes at room temperature (15–25°C).**
11. **Add 50 µl 1x TE buffer (pH 8.0) to dissolve REPLI-g advanced amplified DNA.**

    Note: Mix carefully to avoid shearing of REPLI-g advanced 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 advanced amplified DNA.
13. **If not being used directly, store the amplified DNA/cDNA at –15 to –30°C until required for downstream applications. We recommend storage of the amplified DNA/cDNA at a concentration of at least 100 ng/µl.**

    Amplified DNA/cDNA behaves like purified genomic DNA and has an approximate length of 2000 bp up to 70,000 bp. It is highly suited for use in a variety of downstream applications, particularly next-generation sequencing and quantitative PCR.

    OD measurements can accurately determine the concentration of purified REPLI-g advanced amplified DNA, for example, using the QIAxpert (QIAGEN, cat. no. 9002340). For applications where accurate quantification of double-strand DNA is especially important, such as using the Nextera library prep protocol to prepare an NGS library, concentration determination has to be performed using Quant-iT™ PicoGreen® dsDNA reagent (Life Technologies, cat no. P7581) or the Qubit® dsDNA BR Assay system (Life Technologies, cat. no. Q32850).

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.

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