Supplementary Protocol November 2018

Purification of REPLI-g® amplified DNA by LiCl/EtOH precipitation

This protocol is designed for the purification of 5–40 µg DNA amplified using the REPLI-g Advanced DNA Single Cell Kit (cat. nos. 150363 and 150365), REPLI-g WTA Single Cell Kit (cat. nos. 150063 and 150065), or the REPLI-g Cell WGA & WTA Kit (cat. nos. 150052 and 150054) by lithium chloride/ethanol precipitation.

Product use limitations

REPLI-g Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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)
- Ethanol (96–100%)
- Ethanol (70%)
- Lithium chloride (7.5 M)
- EDTA (0.5 M, pH 8.0)

Important points before starting

- All centrifugation steps should be performed at 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) for no longer than 10 minutes**.
   Note: If not purifying the complete volume, transfer the amplified DNA to a new 1.5 ml microcentrifuge tube.
2. **Adjust the volume to 60 µl by adding the appropriate volume of TE buffer if necessary.**
3. **Add 30 µl 7.5 M lithium chloride and 6 µl EDTA (0.5 M; pH 8.0) to REPLI-g amplified DNA, mix the sample by flicking the tube several times, centrifuge briefly, and let the sample incubate for 30 minutes at room temperature (15–25°C).**
4. **Add 150 µl ethanol (96–100%). Mix carefully by flicking the tube several times.**
   Note: Do not mix by pipetting up and down.
   Note: A cotton-like precipitate will be visible.
5. **Centrifuge at maximum speed (15000 x g) for 2 minutes. With most centrifuges, 15000 x g corresponds to 13000 rpm**.
   Note: Do not centrifuge for more than 2 minutes. Centrifugation for longer periods will condense the precipitate, making it more difficult to dissolve.
6. **Aspirate supernatant carefully using a pipet and discard the supernatant.**

7. **Add 100 µl ethanol (70%) to the pellet. Flick the tube several times.**
8. **Centrifuge at maximum speed for 1 minute.**

   Note: Do not centrifuge for more than 1 minute. Centrifugation for longer periods will condense the precipitate, making it more difficult to dissolve.
9. **Repeat steps 6 to 8.**
10. **Aspirate supernatant carefully using a pipet and discard the supernatant.**
11. **Centrifuge briefly to collect residual supernatant at the bottom of the tube.**
12. **Aspirate residual supernatant carefully using a pipet and discard the supernatant.**

    Note: Avoid aspiration of the pellet.
13. **Incubate the microcentrifuge tube containing the precipitate of REPLI-g amplified DNA for 10 minutes at room temperature (15–25°C).**
14. **Add 60 µl 1x TE buffer (pH 8.0) to dissolve REPLI-g amplified DNA.**

    Note: If the DNA is not dissolved completely, dissolve the pellet overnight at 4–10°C.

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

    Purified DNA can be directly used for all downstream applications. It does not contain tags or labeled molecules. Concentration of purified DNA/cDNA is typically above 150 ng/µl if DNA/cDNA of a complete reaction is purified.
15. **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. See Table 1 for information on handling of amplified DNA/cDNA.

    Optical density (OD) measurements can accurately determine the concentration of purified REPLI-g 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).

Table 1. Applications and handling

Downstream application	Use of amplified DNA/cDNA	QIAGEN products
NGS	Covaris®: 500 – 2000 ng* Nextera Library prep kit: 50 ng† GeneRead™ DNA Library Prep Kits†: 50–1000 ng fragmented DNA	GeneRead DNA Library Prep Kit
Real-time PCR, PCR	10 ng	QuantiTect®, QuantiFast®, QuantiNova™ Kits
Microarray	See supplier's instructions	–
Sanger sequencing, Pyrosequencing®	PCR has to be performed from the region of interest prior to sequencing. See advice for PCR.	PyroMark® products

* Dependent on sequencing platform used.

† See dedicated supplementary protocols on **www.qiagen.com**.

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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