QIAGEN Supplementary Protocol

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 Single Cell Kit (cat. nos. 150343 and 150345), 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 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)
- 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 min.**

   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 min 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 min. With most centrifuges, 15000 x *g* corresponds to 13000 rpm.**

   Note: Do not centrifuge for more than 2 min. 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 min.**

   Note: Do not centrifuge for more than 1 min. Centrifugation for longer periods will condense the precipitate, making it more difficult to dissolve.

9. **Repeat steps 6–8.**
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 min 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.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN 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.

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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