Supplementary Protocol November 2018

Purification of REPLI-g® amplified DNA using Agencourt® AMPure® XP magnetic beads

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 the Agencourt AMPure XP system (Beckman Coulter, Inc., cat. no. A63880).

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 (70%)
- Agencourt AMPure XP Beads (Beckman Coulter, Inc., cat. no. A63880)
- Magnetic Particle Concentrator (e.g., Dyna Mag., Life Technologies; cat. no. 123.21)

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. **Vortex the Agencourt AMPure XP bottle to resuspend any magnetic particles and add 108 µl of resuspended Agencourt AMPure XP beads to 60 µl of REPLI-g amplified DNA.**
4. **Mix REPLI-g amplified DNA and Agencourt AMPure XP beads thoroughly by pipetting up and down (>10x).**
5. **Incubate at room temperature (15–25°C) for 5 minutes to allow binding of REPLI-g amplified DNA to Agencourt AMPure XP beads.**
6. **Place the tube into the Magnetic Particle Concentrator for 2 minutes to separate beads from the solution.**

   Note: Extend the time if necessary until the solution becomes clear.
7. **Remove cleared supernatant with a pipet and discard.**

   Note: This step has to be performed while the tube is situated on the Magnetic Particle Concentrator. Avoid any pipetting of magnetic beads.

8. **Add 200 µl of ethanol (70%) to each tube comprising separated magnetic beads and incubate for 30 s at room temperature (15–25°C).**

   Note: This step has to be performed while the tube is situated on the Magnetic Particle Concentrator. Avoid any pipetting of magnetic beads.

9. **Carefully remove the cleared supernatant.**

10. **Repeat steps 8-9 twice.**

    Note: Ensure that ethanol is completely removed from the bottom of the tube.

11. **Incubate the microcentrifuge tube for 5 minutes at room temperature (15–25°C).**

    Note: Do not over dry the magnetic beads as this will significantly decrease elution efficiency.

12. **Remove tubes from the Magnetic Particle Concentrator, add 60 µl of 1x TE buffer (pH 8.0) to dissolve REPLI-g amplified DNA, and pipet 10 times to mix.**

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

13. **Place the reaction onto the Magnetic Particle Concentrator for 1 minute to separate the beads from the solution.**

14. **Transfer 55 µl of the eluate to a new tube.**

    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 the 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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