

QIAGEN Supplementary Protocol

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 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 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 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 (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 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. 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 min to allow binding of REPLI-g amplified DNA to Agencourt AMPure XP beads.**
- 6. Place the tube into the Magnetic Particle Concentrator for 2 min 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 min 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 min to separate the beads from the solution.**
- 14. Transfer 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 Quanti-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.

Trademarks: QIAGEN®, GeneRead™, PyroMark®, Pyrosequencing®, QuantiFast®, QuantiNova™, QuantiTect®, REPLI-g®, (QIAGEN Group); Agencourt®, AMPure® (Beckman Coulter, Inc.); Covaris® (Covaris, Inc.); PicoGreen®, Quant-iT™ Qubit® (Life Technologies Corporation).
RG34 June-14 © 2014 QIAGEN, all rights reserved.

www.qiagen.com

Australia ■ 1-800-243-800

Austria ■ 0800/281010

Belgium ■ 0800-79612

Canada ■ 800-572-9613

China ■ 021-51345678

Denmark ■ 80-885945

Finland ■ 0800-914416

France ■ 01-60-920-930

Germany ■ 02103-29-12000

Hong Kong ■ 800 933 965

Ireland ■ 1800 555 049

Italy ■ 800 787980

Japan ■ 03-5547-0811

Korea (South) ■ 1544 7145

Luxembourg ■ 8002 2076

The Netherlands ■ 0800 0229592

Norway ■ 800-18859

Singapore ■ 65-67775366

Spain ■ 91-630-7050

Sweden ■ 020-790282

Switzerland ■ 055-254-22-11

UK ■ 01293-422-911

USA ■ 800-426-8157



Sample & Assay Technologies