April 2024

REPLI-g® Single Cell Handbook

For whole genome amplification from single cells, limited samples, or purified genomic DNA

Sample to Insight

Contents

Kit Contents

REPLI-g Single Cell Kit	**(24)**	**(96)**
Catalog no.	**150343**	**150345**
Number of 50 µL reactions	**24**	**96**
REPLI-g sc DNA Polymerase (blue lid)	48 µL	4 x 48 µL
REPLI-g sc Reaction Buffer (yellow lid)	700 µL	4 x 700 µL
Buffer DLB (clear lid)	1 tube	2 tubes
Stop Solution (red lid)	1.8 mL	1.8 mL
PBS sc 1x (clear lid)	1.5 mL	4 x 1.5 mL
DTT, 1 M (lilac lid)	1 mL	1 mL
H_2O sc	1.5 mL	4 x 1 mL
Quick Start Protocol	1	1

Shipping and Storage

The REPLI-g Single Cell Kit is shipped on dry ice. The kit, including all reagents and buffers, should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. When stored under these conditions and handled correctly, the products can be kept at least 6 months after shipping without showing any reduction in performance. For longer storage, the kit should be stored at –70°C.

Intended Use

The REPLI-g Single Cell Kit is intended for molecular biology applications. This product is 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.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety, where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of REPLI-g Single Cell Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The REPLI-g Single Cell Kit contains an optimized Phi 29 polymerase formulation, as well as buffers and reagents for whole genome amplification (WGA) from single cells, very small samples, or purified genomic DNA using Multiple Displacement Amplification (MDA) (1). This handbook contains protocols for amplification of DNA from single cells, such as isolated tumor cells or bacteria, fresh blood, or purified genomic DNA (Table 1). Supplementary protocols that can also be used with the REPLI-g Single Cell Kit for the amplification of DNA from various other samples that are often analyzed in clinical research, including dried blood cards, buccal cells, tissue, serum, plasma, and laser-microdissected cells are available online at www.qiagen.com/literature, from the REPLI-g Single Cell Kit product page on the QIAGEN website under the "Resources" tab, or from QIAGEN Technical Services. For a complete list of protocols, refer to Table 3, page 12.

Table 1. Range of sample material and research areas

Sample material (cells/DNA)	Research area
Human/animal	Biomarker research (SNPs, mutations, CNVs) Stem cell research Analysis of circulating fetal cells Mosaicism studies Genetic predisposition studies Typing of transgenic animals
Cancer	Somatic genetic variant analysis Tumor progression Tumor stem cells/evolution Analysis of circulating tumor cells
Bacteria	Metagenomic studies Pathogen analysis Microbial genotyping
Plants*	Stomata research Pollen analysis

* Cells without cell walls or purified genomic DNA.

Genotyping and DNA sequence analysis of biological samples can be limited by the small amount of sample available. The REPLI-g Single Cell Kit allows uniform amplification of whole genomic DNA from small samples, enabling a greater variety and number of analyses to be performed. The average product length of REPLI-g Single Cell (SC) amplified DNA is typically more than 10 kb, with a range between 2 kb and 100 kb, enabling all downstream applications such as complex genetic analysis, including long-range copy number variations, to be carried out. REPLI-g SC amplified DNA is highly suited for next-generation sequencing, array CGH genotyping applications, or qPCR analysis.

Typical DNA yields from a REPLI-g Single Cell Kit reaction are approximately 40 µg per 50 µL reaction. Depending on the quality of the input DNA, the resulting amount of DNA may be less (fragmented or damaged DNA should not be used). For best amplification results, a suitable cell sample is necessary that is properly collected and stored.

Principle and procedure

The REPLI-g Single Cell Kit provides highly uniform amplification across the entire genome, with negligible sequence bias (2). The method is based on MDA technology, which carries out isothermal genome amplification utilizing a uniquely processive DNA polymerase capable of replicating up to 100 kb without dissociating from the genomic DNA template. In contrast to PCR-based methods, Phi 29 polymerase has a 3'→5' exonuclease proofreading activity to maintain 1000-fold higher fidelity than Taq Polymerase during replication. Multiple Displacement Amplification (MDA) technology is used in the presence of exonuclease-resistant primers to achieve high yields of DNA product from all kinds of mammalian and bacterial tissues (Table 1).

Genetic analyses often require large amounts of genomic DNA. Whole genome amplification overcomes the limits of low DNA quantity if the genome of a small number of cells, or even individual cells, is being analyzed. The REPLI-g Single Cell Kit uses a simple and reliable method to achieve accurate genome amplification from just single cells or limited samples. All ingredients provided with the kit are uniquely UV exposed by an innovative and standardized

procedure to avoid amplification of contaminating DNA. The easy reaction set-up and very low handling time of approximately 15 minutes makes the REPLI-g Single Cell Kit procedure an easy and reliable method.

In the first step of the procedure, the cell sample is lysed and the DNA is denatured. After denaturation has been stopped by the addition of neutralization buffer, a master mix containing buffer and DNA polymerase is added. The isothermal amplification reaction proceeds for 8 hours at 30°C (see flowchart), and can be preprogrammed in a thermal cycler.

REPLI-g SC amplified DNA can be stored long-term at –15°C to –30°C with no negative effects.

For further information, including special downstream applications that we recommend for cleanup of amplified DNA, please visit our WGA Spotlight page, **www.qiagen.com/wga**

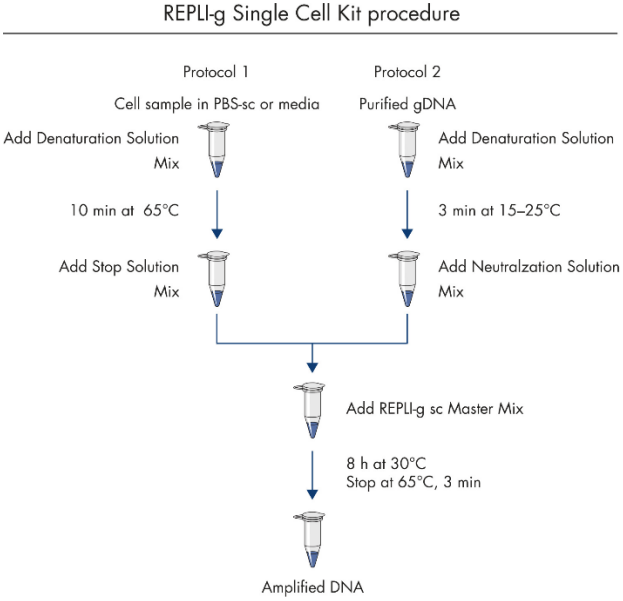

Figure 1. REPLI-g Single Cell Kit procedure.

Description of protocols

Different protocols in this handbook provide detailed instructions for using the REPLI-g Single Cell Kit for purification of single cells or purified genomic DNA. For a complete list of supplementary protocols that can also be used with the REPLI-g Single Cell Kit for the amplification of DNA from dried blood cards, buccal cells, tissue, serum, plasma, and laser-microdissected cells, refer to Appendix A, page 25.

The protocol "Amplification of Genomic DNA from Single Cells", page 14, is optimized for single cell material from all species of, for example, vertebrates, bacteria (gram positive and gram negative), plants (without the cell wall), sorted cells, tissue culture cells, and cells or tissue from biopsies, etc.

The protocol "Amplification of Purified Genomic DNA", page 18, is optimized for whole genome amplification from genomic DNA template.

Table 2. Protocol selection according to starting material

Starting material	Protocol
Single cells, 2–1000 cells	Amplification of Genomic DNA from Single Cells, page 14
Purified genomic DNA (1–10 ng)	Amplification of Purified Genomic DNA, page 18

Table 3. Supplementary protocols for use with the REPLI-g Single Cell Kit*

Starting material	Amount	Protocol
Dried blood spots	Blood card punch (2-3 mm)	Whole genome amplification from dried blood spots using the REPLI-g Single Cell Kit
Buccal cells	One swab	Whole genome amplification from buccal cells using the REPLI-g Single Cell Kit
Laser-microdissected cells	<3 µL dissected cells (from frozen tissue without DNA fragmentation)	Whole genome amplification from laser-microdissected cells using the REPLI-g Single Cell Kit
Biopsies	Tissue sample of approximately 2 mm^3	Whole genome amplification from flash-frozen tissues using the REPLI-g Single Cell Kit
Plasma and serum		Whole genome amplification from plasma and serum using the REPLI-g Single Cell Kit
Flash-frozen tissue	Tissue sample of approximately 2 mm^3	Whole genome amplification from flash-frozen tissue using the REPLI-g Single Cell Kit
High-throughput amplification of genomic DNA in 96-well format	>10 ng purified DNA	Whole genome amplification from genomic DNA in a 96-well format using the REPLI-g Single Cell Kit
Amplification of low concentrations of genomic DNA	>10 ng purified DNA	Whole genome amplification from genomic DNA using the REPLI-g Single Cell Kit with increased sample volumes

* Available online at www.qiagen.com/literature, from the REPLI-g Single Cell Kit product page on the QIAGEN website under the "Resources" tab, or from QIAGEN Technical Services.

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.

- Microcentrifuge tubes
- Water bath or heating block
- Microcentrifuge
- Vortexer
- Pipettes and pipette tips
- Ice

Protocol: Amplification of Genomic DNA from Single Cells

Important points before starting

- This protocol is optimized for single cell material from all species of vertebrates, bacteria (gram positive and gram negative), plants (without the cell wall), sorted cells, tissue culture cells, and cells. The protocol cannot be used with fixated cells that are treated with formalin or other cross-linking agents (e.g., single cell samples obtained by laser microdissection from formalin-fixed, paraffin-embedded tissues).
- Samples of 1–1000 intact cells (e.g., human or bacterial cells) are optimal for whole genome amplification reactions using the REPLI-g Single Cell Kit.
- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Single Cell reaction in a location free of DNA.
- For the amplification of purified genomic DNA, refer to page 18.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 6). All other components can be thawed at room temperature (15–25°C).
- Buffer D2 (denaturation buffer) should not be stored longer than 3 months.
- DNA yields of approximately 40 µg will be present in negative (no-template) controls because DNA is generated during the REPLI-g Single Cell reaction by random extension of primer dimers, generating high-molecular-weight product. This DNA will not affect the quality of the actual samples and will not give a positive result in downstream assays.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL H_2O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.

  Note: Reconstituted Buffer DLB can be stored for 6 months at –15°C to –30°C. Buffer DLB is pH-labile.

- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Set a water bath, heating block, or a programmable thermal cycler to 30°C.
- If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

Procedure

1. Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 4).

   Note: The total volume of Buffer D2 given in Table 4 is sufficient for 12 reactions. If performing fewer reactions, store residual Buffer D2 at –15°C to –30°C. Buffer D2 should not be stored longer than 3 months.

Table 4. Preparation of Buffer D2

Component	Volume (µL)*
DTT, 1 M	3
Buffer DLB (reconstituted)†	33
Total volume	**36**

* Volumes given are sufficient for 12 reactions.
† Reconstitution of Buffer DLB is described in "Things to do before starting", page 15.

2. Place 4 µL cell material (supplied with PBS) into a microcentrifuge tube. If using less than 4 µL of cell material, add PBS sc to bring the volume up to 4 µL.

Note: The amount of PBS sc supplied with the REPLI-g Single Cell Kit is insufficient to prepare serial dilutions of cell material.

Alternatively, 0.5 µL whole blood can be used.

3. Add 3 µL Buffer D2. Mix carefully by flicking the tube and centrifuge briefly.

   Note: Ensure that the cell material does not stick to the tube wall above the buffer line.

4. Incubate for 10 min at 65°C.
5. Add 3 µL Stop Solution. Mix carefully by flicking the tube and centrifuge briefly. Store on ice.
6. Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly.

   The REPLI-g sc Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.

7. Prepare a master mix according to Table 5. Mix and centrifuge briefly.

   Important: Add the master mix components in the order listed in Table 4. After the addition of water and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge the mixture before adding REPLI-g sc DNA Polymerase.

   Note: Scale up accordingly if performing several reactions at once by preparing a master mix sufficient for the total number of reactions.

   The master mix should be kept on ice and used immediately upon addition of REPLI-g sc DNA Polymerase.

Table 5. Preparation of master mix

Component	Volume/reaction (µL)
H_2O sc	9
REPLI-g sc Reaction Buffer	29
REPLI-g sc DNA Polymerase	2
Total volume	**40**

8. For each reaction, add 40 µL master mix to 10 µL denatured DNA (from step 5).

9. Incubate at 30°C for 8 h.

   After incubation at 30°C, heat the water bath or heating block up to 65°C if the same water bath or heating block will be used in step 10.

   Note: If a thermal cycler is used with a heated lid, the temperature of the lid should be set to 70°C.

10. Inactivate REPLI-g sc DNA Polymerase at 65°C for 3 min.

11. If not being used directly, store amplified DNA at 4°C for short-term storage or –15°C to –30°C for long-term storage.

    DNA amplified using the REPLI-g Single Cell Kit should be treated as genomic DNA with minimal freeze-thaw cycles. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µL.

12. Use the correct amount of REPLI-g amplified DNA diluted in water or TE according to the manufacturer's instructions. If performing PCR analysis, dilute an aliquot of amplified DNA 1:100 and use 2 µL of diluted DNA for each PCR reaction.

    Note: If bacterial cells have been used as target material for REPLI-g Single Cell Kit, dilute the amplified DNA at least 1:10,000.

13. Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing, array CGH, and quantitative PCR.

    Note: Typical DNA yields are approximately 40 µg per 50 µL reaction and need to be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix A, page 25, for an accurate method of quantifying REPLI-g amplified DNA.

Protocol: Amplification of Purified Genomic DNA

Important points before starting

- This protocol is optimized for whole genome amplification from >10 ng of purified genomic DNA template. The template DNA should be suspended in TE. If the DNA is of sufficient quality (e.g., high-molecular-weight DNA with no inhibitors [e.g., detergents or organic solvents]), smaller amounts (1–10 ng for eukaryotic DNA or 10–100 pg for bacterial DNA) may be used.
- Avoid DNA contamination of reagents by using separate laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Single Cell reaction in a location free of DNA.
- For direct amplification of DNA from cell material, see page 14.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 6). All other components can be thawed at room temperature (15–25°C).
- Buffer D1 (denaturation buffer) and Buffer N1 (neutralization buffer) should not be stored longer than 3 months.
- DNA yields of approximately 40 µg will be present in negative (no-template) controls because DNA is generated during the REPLI-g Single Cell reaction by random extension of primer dimers, generating high-molecular-weight product. This DNA will not affect the quality of the actual samples and will not give a positive result in downstream assays.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL H_2O sc to the tube provided. Mix thoroughly and centrifuge briefly to dissolve.
- Note: Reconstituted Buffer DLB can be stored for 6 months at –15°C to –30°C. Buffer DLB is pH-labile.

- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Set a water bath or heating block to 30°C.

Procedure

1. Prepare sufficient Buffer D1 (denaturation buffer) and Buffer N1 (neutralization buffer) for the total number of whole genome amplification reactions (Tables 6 and 7).

   Note: The total volumes of Buffer D1 and Buffer N1 given in Tables 6 and 7 are sufficient for 12 reactions. If performing fewer reactions, store residual Buffer D1 and Buffer N1 at –15°C to –30°C. Buffer D1 and Buffer N1 should not be stored longer than 3 months.

Table 6. Preparation of Buffer D1

Component	Volume (µL)*
Reconstituted Buffer DLB†	7
Nuclease-free water	25
Total volume	**32**

* Volumes given are sufficient for 12 reactions.
† Reconstitution of Buffer DLB is described in "Things to do before starting", page 15.

Table 7. Preparation of Buffer N1

Component	Volume (µL)*
Stop Solution	9
Nuclease-free water	51
Total volume	**60**

* Volumes given are sufficient for 12 reactions.

2. Place 2.5 µL template DNA into a microcentrifuge tube.

   The amount of template DNA should be >10 ng. A DNA control reaction can be set up using 10 ng (1 µL) control genomic DNA (e.g., REPLI-g Human Control Kit, cat. no. 150090). Adjust the volume by adding PBS sc (provided) to the starting volume of your sample.

3. Add 2.5 µL Buffer D1 to the DNA. Mix by vortexing and centrifuge briefly.
4. Incubate at room temperature for 3 min.
5. Add 5.0 µL Buffer N1. Mix by vortexing and centrifuge briefly. Store on ice.
6. Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly.

   The REPLI-g sc Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
7. Prepare a master mix according Table 8. Mix and centrifuge briefly.

   Important: Add the master mix components in the order listed in Table 8. After the addition of water and REPLI-g sc Reaction Buffer, briefly vortex and centrifuge the mixture before the addition of REPLI-g sc DNA Polymerase.

   Note: Scale up accordingly if performing several reactions at once by preparing a master mix sufficient for the total number of reactions.

   The master mix should be kept on ice and used immediately upon addition of REPLI-g sc DNA Polymerase.

Table 8. Preparation of Master Mix

Component	Volume/reaction (µL)
H2O sc	9
REPLI-g sc Reaction Buffer	29
REPLI-g sc DNA Polymerase	2
Total volume	**40**

8. Add 40 µL master mix to 10 µL denatured DNA (from step 5).
9. Incubate at 30°C for 8 h.

   Maximum DNA yield is achieved using an incubation time of 16 h.

   After incubation at 30°C, heat the water bath or heating block up to 65°C if the same water bath or heating block will be used in step 10.
10. Inactivate REPLI-g sc DNA Polymerase for 3 min at 65°C.

11. If not being used directly, store amplified DNA at 4°C for short-term storage or –15 to –30°C for long-term storage.

    DNA amplified using the REPLI-g Single Cell Kit should be treated as genomic DNA with minimal freeze-thaw cycles. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µL.

12. Use the correct amount of REPLI-g amplified DNA diluted in water or TE according to the manufacturer's instructions. If performing PCR analysis, dilute an aliquot of amplified DNA 1:100 and use 2 µL of diluted DNA for each PCR reaction.

    Note: If bacterial cells have been used as target material for REPLI-g Single Cell amplification, dilute the amplified DNA at least 1:10,000.

13. Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing, array CGH, and quantitative PCR.

    Note: Typical DNA yields are approximately 40 µg per 50 µL reaction and need to be diluted appropriately. Optical density (OD) measurements do overestimate REPLI-g amplified DNA. Refer to Appendix A, page 25, for an accurate method of quantifying REPLI-g amplified DNA.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, visit **www.qiagen.com**).

		Comments and suggestions
All protocols		
Reduced or no high-molecular-weight product in agarose gel in some samples but DNA yield in other samples is approximately 10 µg to 40 µg		
a)	Reaction failed — possible inhibitor in the genomic DNA template	Clean up or dilute the purified genomic DNA and re-amplify.
b)	Reaction temperature is too high	Check the incubator for correct reaction temperature (30°C) during the REPLI-g reaction. If cycler with heated lid is used, set temperature to 70°C. As a control, the REPLI-g reaction can be performed at a lower temperature (e.g., 25–28°C), which should give the appropriate yield.
c)	Carryover of alcohol in isolated DNA sample	Residual alcohol in the DNA sample may reduce the yield of REPLI-g reactions. When using column-based purification procedures, ensure the duration of the drying step prior elution of DNA from the column is sufficient to evaporate residual ethanol.
The negative (no-template) controls have DNA yields of approximately 10 µg to 40 µg but no positive result in downstream assay (e.g., PCR)		
a)	DNA is generated during REPLI-g reaction by random extension of primer-dimers	High-molecular-weight product can be generated by random extension of primer- dimers. This DNA will not affect the quality of actual samples or specific downstream genetic assays.
The negative (no-template) controls have DNA yields of approximately 10 µg to 40 µg and a positive result in downstream assay (e.g., PCR)		
a)	DNA is generated during REPLI-g reaction by contaminating DNA templates	Decontaminate all laboratory equipment and take all necessary precautions to avoid contamination of reagents and samples with extraneous DNA.

		Comments and suggestions
		If possible, work in a laminar-flow hood. Use sterile equipment and barrier pipet tips only and keep amplification chemistry and DNA templates in separate storage locations.
Downstream application results not optimum		
a)	Some sensitive downstream applications (e.g., labeling reactions) may require DNA cleanup after REPLI-g reaction	Contact QIAGEN Technical Services for DNA cleanup recommendations suitable for your application, or visit **www.qiagen.com**

Single cell protocol

Reduced/no locus representation or allele dropout in downstream analysis, but DNA yield is approximately 10 µg to 40 µg		
a)	Cells are not suitable for whole genome amplification	DNA within the cells is degraded (e.g., inappropriate storage of cells, use of fixed or apoptotic cells).
b)	DNA degraded after cell lysis	Perform cell lysis carefully and avoid vigorous vortexing. Do not store DNA after cell lysis.
Genome is not amplified at all, but DNA yield is approximately 10 µg to 40 µg		
a)	Cells were not lysed	Additional cell envelope break-down is necessary for cells that have strong cell walls (e.g., plant cells, and cells in dormant stages, such as spores and cysts).

Genomic DNA protocol

Reduced/no locus representation or allele dropout in downstream analysis, but DNA yield is approximately 10 µg to 40 µg		
a)	Genomic DNA template is degraded	Use intact genomic DNA template. Use a larger amount of genomic DNA.

Contact Information

For technical assistance and more information, please see our Technical Support Center at **www.qiagen.com/Support**, call 00800-22-44-6000, or contact one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit **www.qiagen.com**).

Appendix A: Determination of DNA Concentration and Yield

Quantification of DNA yield

A 50 µL REPLI-g reaction typically yields approximately 40 µg of DNA, regardless of the amount of template DNA, allowing direct use of the amplified DNA in most downstream genotyping experiments. Depending on the quality of the input DNA, the resulting amount of DNA may be less (fragmented or damaged DNA should not be used). However, if a more accurate quantification of DNA is required, it is important to utilize a DNA quantification method that is specific for double-stranded DNA, since REPLI-g Kit amplification products contain unused reaction primers. PicoGreen® reagent displays enhanced binding to double-stranded DNA and may be used, in conjunction with a fluorometer, to quantify the double-stranded DNA product. For best results, the sample should be diluted with 2 volumes of water and thoroughly mixed prior to addition of PicoGreen. A protocol for the quantification of REPLI-g amplified DNA can be found in Appendix B, page 26.

Quantification of locus representation

Locus representation for each sample can be quantified by real-time PCR (3). Contact QIAGEN Technical Services or visit our website at www.qiagen.com for a protocol.

Appendix B: PicoGreen® Quantification of REPLI-g Amplified DNA

This protocol is designed for quantification of double stranded REPLI-g amplified DNA using PicoGreen reagent.

Important: When working with hazardous chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs), available from the product supplier.

Equipment and reagents to be supplied by user

- Quant-iT™ PicoGreen dsDNA reagent (Invitrogen, cat. no. P7581)
- TE buffer (10 mM TrisCl; 1mM EDTA, pH 8.0)
- Human genomic DNA (e.g., Promega, cat. no. G3041)
- 2 mL microcentrifuge tube
- 96-well plates (suitable for use in a fluorescence microplate reader)
- Fluorescence microplate reader (e.g., TECAN® Ultra)

Procedure

1. In a 2 mL microcentrifuge tube, make a 1:150 dilution of PicoGreen stock solution in TE buffer. Each quantification reaction requires 20 µL. Cover the microcentrifuge tube in aluminum foil or place it in the dark to avoid photodegradation of the PicoGreen reagent.

   For example, to prepare enough PicoGreen working solution for 100 samples, add 13.3 µL PicoGreen to 1986.7 µL TE buffer.

   Important: Prepare the PicoGreen/TE solution in a plastic container as the PicoGreen reagent may adsorb to glass surfaces.

2. Prepare a 16 µg/mL stock solution of genomic DNA in TE buffer.
3. Make 200 µL of 1.6, 0.8, 0.4, 0.2, and 0.1 µg/mL DNA standards by further diluting the 16 µg/mL genomic DNA with TE buffer.
4. Transfer 20 µL of each DNA standard in duplicate into a 96-well plate labeled A (see figure below).

   Note: the 96-well plate must be suitable for use in a fluorescent microplate reader.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H			1.6	0.8	0.4	0.2	0.1	1.6	0.8	0.4	0.2	0.1

Gray squares: genomic DNA standards (µg/µL)

5. Place 2 µL of each REPLI-g amplified DNA sample for quantification into a new 96-well plate and add 198 µL TE buffer to make a 1:100 dilution. Store the remaining REPLI-g amplified DNA at –15°C to –30°C.
6. Place 2 µL diluted REPLI-g DNA (from step 5) into an unused well of 96-well plate A and add 18 µL TE to make a 1:1000 dilution.

   The 1:100 dilutions from step 5 can be stored at –15°C to –30°C and used for future downstream sample analysis.
7. Add 20 µL PicoGreen working solution (from step 1) to each sample (amplified DNA and DNA standards) in 96-well plate A. Gently shake the plate on the bench top to mix the samples and reagent.
8. Centrifuge the 96-well plate briefly to collect residual liquid from the walls of the wells.

9. Measure the sample fluorescence using a fluorescence microplate reader and standard fluorescence filters (excitation approximately 480 nm; emission approximately 520 nm).

   To ensure that the sample readings remain in the detection range of the microplate reader, adjust the instrument's gain so that the sample with the highest DNA concentration yields fluorescence intensity near the fluorometer's maximum.

Calculation of DNA concentration and yield

10. Generate a standard curve by plotting the concentration of DNA standards (µg/mL) (x-axis) against the fluorescence reading generated by the microplate reader (y-axis). Plot an average of the fluorescence recorded for each DNA standard of the same concentration.
11. Use the standard curve to determine the concentration (µg/mL) of the diluted REPLI-g amplified DNA sample. This is achieved by plotting the fluorescence reading of the sample against the standard curve and reading the DNA concentration on the x-axis.

    Note: The calculation of DNA concentration depends on the standard curve and the determination of the slope. For accurate results, the standard curve should be a straight line. Any deviation from this may cause inaccuracies in the measurement of REPLI-g amplified DNA concentrations.
12. Multiply the value determined in step 11 by 1000 to show the concentration of undiluted sample DNA (since the sample DNA measured by PicoGreen fluorescence had been diluted 1 in 1000).
13. To determine the total amount of DNA in your sample, multiply the concentration of undiluted sample DNA (µg/mL) (determined in step B12) by the reaction volume in milliliters (i.e., for a 50 µL reaction, multiply by 0.05).

References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

1. Dean, F.B. et al (2002) Comprehensive human genome amplification using multiple displacement amplification. Proc. Natl. Acad. Sci. USA 99, 5261.

2. Hosono, S. et al (2003) Unbiased whole-genome amplification directly from clinical samples. Genome Res. 13, 954.

3. Yan, J., Feng, J., Hosono, S., Sommer, S.S. (2004) Assessment of multiple displacement amplification in molecular epidemiology. Biotechniques 37, 136.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Single Cell Kit (24)	REPLI-g sc Polymerase, Buffers, and Reagents for 24 whole genome amplification reactions (yields up to 40 µg/reaction)	150343
REPLI-g Single Cell Kit (96)	REPLI-g sc Polymerase, Buffers, and Reagents for 96 whole genome amplification reactions (yields up to 40 µg/reaction)	150345
Related products		
REPLI-g Mini Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µL whole genome amplification reactions (typical yield 10 µg per reaction)	150023
REPLI-g Midi Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µL whole genome amplification reactions (typical yield 40 µg per reaction)	150043

* Additional sizes available.

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.

Document Revision History

Revision	Description
April 2024	Removed “To prepare a master mix for multiple reactions, scale up according to the number of reactions and add 10%” footnote from Tables 5 and Table 8.

Limited License Agreement for REPLI-g Single Cell Kit

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