December 2019

REPLI-g® Advanced DNA Single Cell Kit Handbook

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

Sample to Insight

QIAGEN

Contents

Kit Contents

REPLI-g Advanced DNA Single Cell Kit	**(24)**	**(96)**
Catalog no.	**150363**	**150365**
Number of 50 µl reactions	**24**	**96**
REPLI-g sc DNA Polymerase (blue lid)	48 µl	4 x 48 µl
REPLI-g Advanced sc Reaction Buffer (yellow lid)	700 µl	5 x 700 µl
REPLI-g Advanced Buffer DLB (clear lid)	1 tube	2 tubes
Stop Solution (red lid)	1.8 ml	1.8 ml
REPLI-g Advanced sc Storage Buffer	2 x 1.9 ml	2 x 1.9 ml
DTT, 1 M (lilac lid)	1 ml	1 ml
H_2O sc	1.5 ml	2 x 1.5 ml
Quick-Start Protocol	1	1

Storage

The REPLI-g Advanced DNA Single Cell Kit is shipped on dry ice. The kit, including all reagents and buffers, should be stored immediately upon receipt at –15 to –30°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, unless otherwise indicated on the label. For longer storage, the kit should be stored at –65 to –90°C.

Intended Use

The REPLI-g Advanced DNA 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

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 Advanced DNA Single Cell Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The REPLI-g Advanced DNA Single Cell Kit contains an optimized Phi 29 polymerase formulation, as well as buffers and reagents for whole genome amplification (WGA) from eukaryotic single cells, very small samples or purified genomic DNA using Multiple Displacement Amplification (MDA) (1).

This kit is an improved version of the REPLI-g Single Cell Kit, including optimized reaction chemistry, a new single cell storage buffer and an improved protocol, that increases the uniformity of amplification and reduces potential amplification bias. In addition, the MDA reaction time has been shortened to 2 h.

This handbook contains protocols for amplification of DNA from single eukaryotic cells or purified genomic DNA from various sample materials for use in numerous research areas (Table 1).

Table 1. Range of sample materials 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
Plants*	Stomata research Pollen analysis

* Cells without cell walls or purified genomic DNA.

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

Genotyping and DNA sequence analysis of biological samples can be limited by the small amount of available sample. The REPLI-g Advanced DNA Single Cell Kit allows uniform amplification of whole genomic DNA from limited samples and enables a greater variety and number of analyses to be performed. The average product length of 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 performed (2). DNA amplified with REPLI-g Advanced DNA Single Cell Kit is highly suited for next-generation sequencing (NGS), array CGH genotyping applications, or qPCR analysis.

Typical DNA yields from a REPLI-g Advanced DNA Single Cell Kit reaction are approximately 25–35 µg per 50 µl reaction. Depending on the quality of the input cell and its DNA, the resulting amount of DNA may be less (fragmented DNA or damaged cells should not be used). For best amplification results, we recommend collecting cells in or storing with REPLI-g Advanced sc Storage Buffer (included with kit and available separately).

Principle and procedure

The REPLI-g Advanced DNA Single Cell Kit provides highly uniform amplification across the entire genome, with negligible sequence bias. The method is based on multiple displacement amplification (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. MDA technology is used in the presence of exonuclease-resistant primers to achieve high yields of DNA product from all kinds of eukaryotic tissues.

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

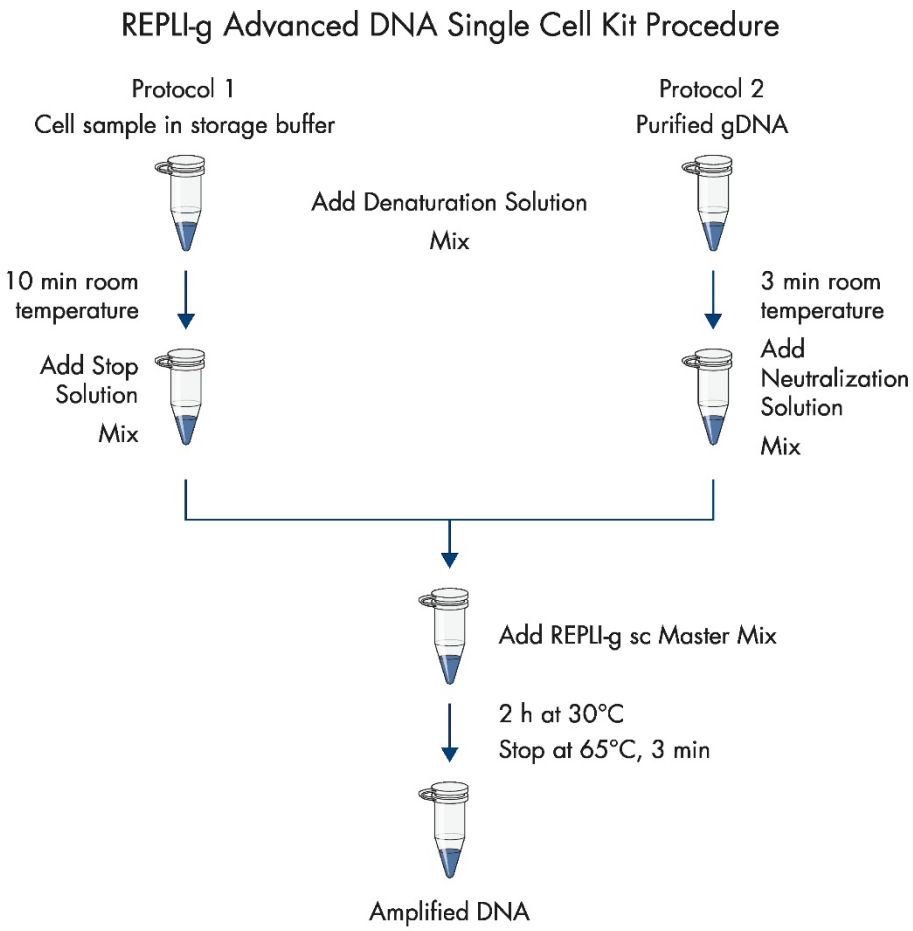
Genetic analyses often require substantial amounts of genomic DNA. Whole genome amplification overcomes the limits of low DNA quantity when starting with 1–1000 cells. The REPLI-g Advanced DNA Single Cell Kit overcomes these limitations by using a simple and reliable method to achieve accurate genome amplification. In addition, all components provided with the REPLI-g Advanced DNA Single Cell Kit are exposed to UV radiation in a standardized procedure to minimize contaminating DNA. Reaction set-up requires only 15 min and 3 steps, making the REPLI-g Advanced DNA Single Cell Kit protocol easy and reliable.

In the first step of the procedure, the cell sample is lysed under isothermal alkaline conditions and the cell is denatured. Cell lysis at room temperature (15–25°C) reduces the number of possible DNA breaks and therefore improves uniformity of whole genome amplification.

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 2 h at 30°C (see flowchart, next page). Because of the shortened MDA reaction time, the REPLI-g Advanced DNA Single Cell Kit enables single cell collection, WGA and even downstream analysis to be performed in a single day.

The amplified DNA can be stored long-term at –20°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**


REPLI-g Advanced DNA Single Cell Kit Procedure
Protocol 1
Cell sample in storage buffer
Protocol 2
Purified gDNA
Add Denaturation Solution
Mix
10 min room temperature
3 min room temperature
Add Stop Solution
Mix
Add Neutralization Solution
Mix
Add REPLI-g sc Master Mix
2 h at 30°C
Stop at 65°C, 3 min
Amplified DNA

Description of protocols

Separate protocols in this handbook provide detailed instructions for using the REPLI-g Advanced DNA Single Cell Kit for single eukaryotic cells or purified genomic DNA (Table 2).

The protocol "Amplification of Genomic DNA from Single Eukaryotic Cells", page 11, is optimized for single cell material from eukaryotic cells (without cell wall) such as sorted cells, tissue culture cells and cells or tissue from biopsies.

The protocol "Amplification of Purified Genomic DNA", page 15, is optimized for whole genome amplification from genomic DNA template.

Table 2. Protocol selection according to starting material

Sample material	Protocol
1–1000 eukaryotic cells	Amplification of Genomic DNA from Single Eukaryotic Cells, page 11
Purified genomic DNA (>1 ng)	Amplification of Purified Genomic DNA, page 15

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.

- Pipettes and pipette tips
- Microcentrifuge tubes
- Thermal cycler or heating block
- Microcentrifuge
- Vortexer
- Ice

Protocol: Amplification of Genomic DNA from Single Eukaryotic Cells

Important points before starting

- The REPLI-g Advanced DNA Single Cell Kit is optimized for single cell material from eukaryotic cells (without cell wall) such as sorted cells, tissue culture cells and cells or tissue from biopsies.
- The protocol cannot be used with fixed 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 eukaryotic cells are optimal for whole genome amplification reactions using the REPLI-g Advanced DNA Single Cell Kit.
- Avoid DNA contamination of reagents by using dedicated laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Advanced Single Cell reaction in a location free of DNA.
- For the amplification of purified genomic DNA, refer to page 15.
- REPLI-g sc DNA Polymerase should be thawed on ice (see step 6). All other components can be thawed at room temperature.
- Buffer D2 (denaturation buffer) should not be stored longer than 3 months.
- DNA yields up to 20 µg can be present in negative (no-template) controls because DNA is generated during the REPLI-g Advanced 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 Advanced Buffer DLB by adding 250 µl H_2O sc to the tube provided. Mix thoroughly and pulse-centrifuge to dissolve.

  Note: Reconstituted Advanced Buffer DLB can be stored for 6 months at –20°C. Advanced Buffer DLB is pH-labile.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Set a heating block or a programmable thermal cycler to 30°C.
- If a thermal cycler with a heated lid is used, 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 3).

   Note: The total volume of Buffer D2 shown in Table 3 is sufficient for 12 reactions. If performing fewer reactions, store residual Buffer D2 at –20°C. Buffer D2 should not be stored longer than 3 months.

Table 3. Preparation of Buffer D2

Component	Volume* (µl)
DTT, 1 M	3
REPLI-g Advanced Buffer DLB (reconstituted)†	33
Total volume	**36**

* Component volumes are sufficient for 12 reactions.

† Reconstitution of REPLI-g Advanced Buffer DLB is described above in "Things to do before starting".

2. Place cell material in 4 µl REPLI-g Advanced sc Storage Buffer in a 0.2 ml reaction tube. The final volume after releasing the cell into the buffer must be 4 µl. Try to minimize any liquid volume that is transported with the cell into the tube (e.g., when isolating the single cell by pipetting).

Note: The amount of REPLI-g Advanced sc Storage Buffer supplied with the REPLI-g Advanced DNA Single Cell Kit is insufficient to prepare serial dilutions of cell material. If required, REPLI-g Advanced sc Storage Buffer can be ordered separately (REPLI-g Single Cell Cryo-Protect (15 ml), cat. no. 150370).

Note: If cells are to be stored or transported before WGA we recommend snap-freezing single cells in 4 µl REPLI-g Advanced sc Storage Buffer. Snap-frozen cells should be stored at –20°C.

3. Add 3 µl Buffer D2. Mix the tube by vortexing and pulse-centrifuge.

   Note: Make sure the cell material does not stick to the tube wall above the buffer line.

4. Incubate at room temperature for 10 min.
5. Add 3 µl Stop Solution. Mix the tube by vortexing and pulse-centrifuge. Store on ice.
6. Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex and then pulse-centrifuge. The REPLI-g Advanced sc Reaction Buffer may form a precipitate after thawing. Vortex for 10 s to dissolve the precipitate.
7. Prepare a master mix according Table 4. Mix and pulse-centrifuge.

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

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

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

Table 4. Preparation of master mix

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

* Scale up accordingly if performing several reactions simultaneously.

8. For each reaction, add 40 µl master mix to 10 µl denatured DNA (from step 5) and continue with step 9.
9. Incubate at 30°C for 2 h.

   Note: If a heating block is used, heat the heating block up to 65°C immediately after incubation at 30°C. If a thermal cycler is used, set up the cycler program for steps 9 and 10.

   Note: If a thermal cycler with a heated lid is used, 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 using amplified DNA immediately, store at 4°C for short-term storage or –20°C for long-term storage.

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

    Note: If amplified DNA has been stored at –20°C, we recommend heating the diluted DNA at 65°C for 3 min and then cooling on ice prior to real-time PCR to ensure reproducible results.
12. Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing (NGS), array CGH and quantitative PCR. 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: Typical DNA yields are approximately 25–35 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 21, 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 >1 ng of purified genomic eukaryotic DNA template. It is recommend to use template DNA suspended in TE. DNA of low quality or contaminated with detergents or organic solvents may compromise the amplification reaction.
- Avoid DNA contamination of reagents by using dedicated laboratory equipment (e.g., pipets, filter pipet tips, reaction vials, etc.). Set up the REPLI-g Advanced Single Cell reaction in a location free of DNA.
- For direct amplification of DNA from cell material, see page 11.
- For best results, 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.
- Buffer D1 (denaturation buffer) and Buffer N1 (neutralization buffer) should not be stored longer than 3 months.
- DNA yields up to 20 µg can be present in negative (no-template) controls because DNA is generated during the REPLI-g Advanced 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 Advanced Buffer DLB by adding 250 µl H_2O sc to the provided tube. Mix thoroughly and pulse-centrifuge to dissolve.

  Note: Reconstituted Advanced Buffer DLB can be stored for 6 months at –20°C. Advanced Buffer DLB is pH-labile.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Set a heating block or a programmable thermal cycler 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 (Table 5 and Table 6).

   Note: The total volumes of Buffer D1 and Buffer N1 given in Table 5 and Table 6 are sufficient for 24 reactions. If performing fewer reactions, store residual Buffer D1 and Buffer N1 at –20°C. Buffer D1 and Buffer N1 should not be stored longer than 3 months.

Table 5. Preparation of Buffer D1

Component	Volume* (µl)
Reconstituted REPLI-g Advanced Buffer DLB†	7
Nuclease-free water	57
Total volume	**64**

* Component volumes are sufficient for 24 reactions.

† Reconstitution of REPLI-g Advanced Buffer DLB is described above in "Things to do before starting".

Table 6. Preparation of Buffer N1

Component	Volume* (µl)
Stop Solution	18 µl
Nuclease-free water	102 µl
Total volume	**120**

* Component volumes are sufficient for 24 reactions.

2. Place 2.5 µl template DNA into a 0.2 ml reaction tube. The amount of template DNA should be >1 ng. Adjust the volume by adding TE buffer or water to the starting volume of your sample. 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).
3. Add 2.5 µl Buffer D1 to the DNA. Mix by vortexing and pulse-centrifuge.

   Note: Make sure that no drops stick to the tube wall above the buffer line.
4. Incubate at room temperature for 3 min.
5. Add 5.0 µl Buffer N1. Mix by vortexing and pulse-centrifuge. Store on ice.

6. Thaw REPLI-g sc DNA Polymerase on ice. Thaw all other components at room temperature, vortex and then pulse-centrifuge. The REPLI-g Advanced sc Reaction Buffer may form a precipitate after thawing. Vortex for 10 s to dissolve the precipitate.
7. Prepare a master mix according Table 7. Mix and pulse-centrifuge.

   Important: Add the master mix components in the order listed in Table 7. After the addition of water and REPLI-g Advanced sc Reaction Buffer, briefly vortex and pulse-centrifuge the mixture before the addition of REPLI-g sc DNA Polymerase.

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

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

Table 7. Preparation of master mix

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

* Scale up accordingly if performing several reactions simultaneously.

8. For each reaction, add 40 µl master mix to 10 µl denatured DNA (from step 5) and continue with step 9.
9. Incubate at 30°C for 2 h.

   Note: If a heating block is used, heat the heating block up to 65°C immediately after incubation at 30°C. If a thermal cycler is used, set up cycler program for steps 9 and 10.

   Note: If a thermal cycler with a heated lid is used, 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 –20°C for long-term storage.

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

    Note: If amplified DNA has been stored at –20°C, we recommend heating the diluted DNA at 65°C for 3 min and then cooling on ice prior to real-time PCR to ensure reproducible results.

12. Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing (NGS), array CGH and quantitative PCR. 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: Typical DNA yields are approximately 25–35 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 21, 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, see back cover or 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 25–35 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 heating block/thermal cycler 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 up to 20 µg but no positive result in downstream assay (e.g., PCR)	
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.

	Comments and suggestions
The negative (no-template) controls have DNA yields up to 20 µg but no positive result in downstream assay (e.g., PCR)	
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. 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	
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 25–35 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 up to 20 µg	
Cells were not lysed	Additional cell envelope break-down is necessary for cells that have 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 up to 20 µg	
Genomic DNA template is degraded	Use intact genomic DNA template. Use a larger amount of genomic DNA.

Appendix A: Determination of DNA Concentration and Yield

Quantification of DNA yield

A 50 µl REPLI-g reaction typically yields approximately 25–35 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 because REPLI-g Advanced DNA Single Cell 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. A recommended protocol for the quantification of REPLI-g amplified DNA can be found in Appendix B, page 22.

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.

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

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 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 (Thermo Fisher Scientific, 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 1:200 dilution of PicoGreen stock solution in TE buffer. Each quantification reaction requires 40 µl. Cover the microcentrifuge tube with aluminum foil or place it in the dark to avoid photodegradation of the PicoGreen reagent.

   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 40 µl of each DNA standard in duplicate into a 96-well plate (Figure 1).

   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	1.6	1.6										
B	0.8	0.8										
C	0.4	0.4										
D	0.2	0.2										
E	0.1	0.1										
F												
G												
H												

Figure 1. DNA standards (µg/ml), in duplicate, in columns 1 and 2, rows A through E.

5. Make a 1:100 dilution of each REPLI-g amplified DNA sample by mixing 2 µl DNA and 198 µl TE buffer using a new 96-well plate. Store the remaining REPLI-g amplified DNA at –20°C.
6. Place 4 µl diluted REPLI-g DNA (from step 5) into an unused well of the DNA standards 96-well plate (from step 4) and add 36 µl TE to make a 1:1000 dilution.

   Note: The 1:100 dilutions from step 5 can be stored at –20°C and used for future downstream sample analysis.
7. Add 40 µl PicoGreen working solution (from step 1) to each sample (amplified DNA and DNA standards) in the 96-well plate (from step 6). Carefully 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.

   Note: Make sure that no drops stick to the wall above the buffer line.

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

   Note: 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 12) by the reaction volume in milliliters (i.e., for a 50 µl reaction, multiply by 0.05).

Ordering Information

Product	Contents	Cat. no.
REPLI-g Advanced DNA Single Cell Kit (24)	REPLI-g sc Polymerase, Buffers, and Reagents for 24 x 50 µl whole genome amplification reactions (typical yield 30 µg per reaction)	150363
REPLI-g Advanced DNA Single Cell Kit (96)	REPLI-g sc Polymerase, Buffers, and Reagents for 96 x 50 µl whole genome amplification reactions (typical yield 30 µg per reaction)	150365
Related Products		
REPLI-g Single Cell Cryo-Protect (15 ml)	REPLI-g Advanced sc Storage Buffer (8 tubes)	150370
REPLI g Human Control Kit (25)	Human control DNA for 25 x 50 µl whole genome amplification reactions	150090
QIAseq FX DNA Library Kit (24)	Buffers and reagents for DNA fragmentation, ligation and library amplification; for use with Illumina® instruments; includes a plate containing 24 adapters with different sample bar codes	180473

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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Document Revision History

Date	Changes
06/2018	Initial release
12/2019	Increased number of tubes of REPLI-g Advanced sc Reaction Buffer (yellow lid) from 4 to 5 in REPLI-g Advanced DNA Single Cell Kit (96), cat. no. 150365.

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HB-2516-002 12/2019