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REPLI-g® Screening Handbook

REPLI-g Screening Kit

For whole genome amplification from purified genomic DNA, blood, and cells

Sample to Insight

QIAGEN

Contents

Kit Contents

REPLI-g Screening Kit **Catalog no.** **No. of 40 µl reactions (up to 8 µg yield)**	**150126** **200**
REPLI-g Mini DNA Polymerase (blue lid)	2 x 100 µl
Buffer SB1 (white lid)	2 x 1.7 ml
Buffer SB2 (yellow lid)	2 x 1.7 ml

Shipping and Storage

The REPLI-g Screening Kit is shipped on dry ice. The kit, including all reagents and buffers, should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. For longer storage the kit should be stored at –70°C.

Under these conditions, the components are stable for 6 months post manufacture without showing any reduction in performance and quality.

Intended Use

The REPLI-g Screening 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 Screening Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The REPLI-g Screening Kit contains DNA polymerase, buffers, and reagents for whole genome amplification from small samples using multiple displacement amplification (MDA) (1). This handbook contains protocols for amplification of DNA from various research samples, including purified DNA, whole blood, and cultured cells.

Genotyping and DNA sequence analysis of research samples can be limited by the small amount of sample available. The REPLI-g Kit allows uniform amplification of whole genomic DNA from small samples, enabling a greater variety and number of analyses to be performed.

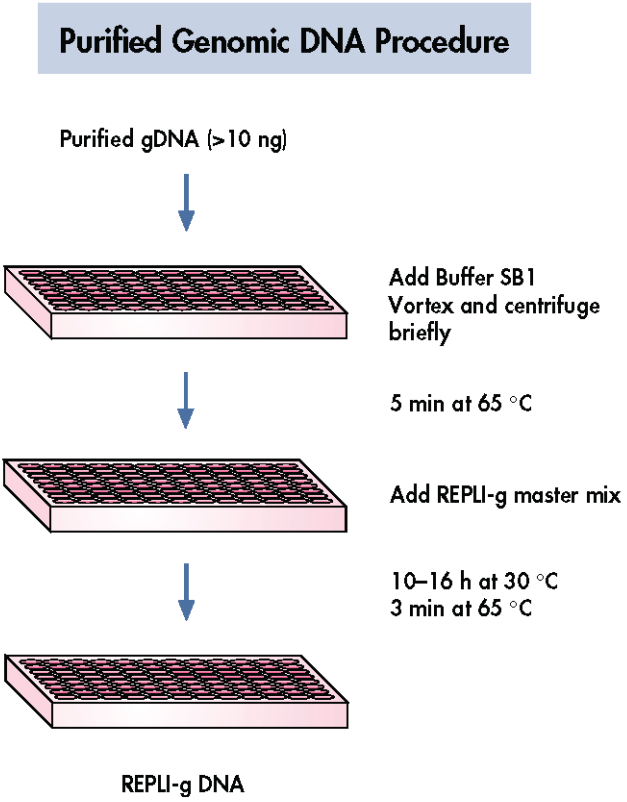
Typical DNA yields from a REPLI-g Screening Kit reaction are up to 8 µg per 40 µl reaction. The yield may depend on the quality of the sample. The average product length is typically greater than 10 kb, with a range between 2 and 100 kb.

Traditional methods of genomic DNA amplification include the time-consuming process of creating EBV-transformed cell lines followed by whole genome amplification using random or degenerate oligonucleotide-primed PCR. However, all PCR-based methods can generate nonspecific amplification artifacts, give incomplete coverage of loci, and generate DNA less than 1 kb long that cannot be used in many downstream applications. This amplification bias results in an unreliable template for diagnostic testing.

Principle and procedure

The sample material is lysed and the DNA is denatured by incubating in Buffer SB1 at 65°C. After denaturation has been stopped by cooling the solution down to room temperature, Buffer SB2 and DNA polymerase are added. The isothermal amplification reaction proceeds for at least 10 hours or overnight at 30°C.

REPLI-g Kits provide 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. The DNA polymerase has a 3′→5′ exonuclease proofreading activity to maintain high fidelity during replication and is used in the presence of exonuclease-resistant primers to achieve high yields of DNA product.



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
- Pipets and pipet tips
- Ice

Protocol: Amplification of Purified Genomic DNA Using the REPLI-g Screening Kit

Important points before starting

- This protocol is optimized for whole genome amplification from >10 ng genomic DNA template. The template DNA should be suspended in Buffer TE.* Smaller amounts (1–10 ng) of starting material can be used if the DNA is of sufficient quality.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
  REPLI-g Mini DNA Polymerase should be thawed on ice (see step 4). Buffer SB1 and Buffer SB2 should be thawed at room temperature (15–25°C).

Things to do before starting

- Set a water bath or heating block to 30°C.
- Thaw Buffer SB1 and Buffer SB2 at room temperature.
- Buffer SB1 and Buffer SB2 should be vortexed for at least 10 s before use to ensure thorough mixing.

Procedure

1. Place 2.5–5 µl template DNA into a well of a 96-well plate or 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 with Buffer TE to the starting volume of your sample.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate SDSs, available from the product supplier.

2. Add 17 µl Buffer SB1 to the DNA. Mix by vortexing and centrifuge briefly.

   If using a 96-well plate, seal the plate using a tape sheet before vortexing.

3. Incubate the sample for 5 min at 65°C. Allow samples to cool down to room temperature (15–25°C).

4. Thaw REPLI-g Mini DNA Polymerase on ice.

5. Prepare a master mix according to Table 1. Mix and centrifuge briefly.

Table 1. Preparation of Master Mix

Component	Volume/reaction	Volume/100 reactions
Buffer SB2	17 µl	1700 µl
REPLI-g Mini DNA Polymerase	1 µl	100 µl
Total volume	**18 µl**	**1800 µl**

6. Add 18 µl of the master mix to 19.5–22 µl of denatured DNA (step 3).

7. Incubate at 30°C for 10–16 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 8.

8. Inactivate REPLI-g Mini DNA Polymerase by heating the sample for 3 min at 65°C.

9. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.

   DNA amplified using REPLI-g Kits should be treated as genomic DNA with minimal freeze–thaw cycles. Storage of nucleic acids at low concentration over a long period of time may result in acid hydrolysis. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.

Protocol: Amplification of Genomic DNA from Blood or Cells Using the REPLI-g Screening Kit

Important points before starting

- The protocol is optimized for 0.5 µl whole blood or cell material (e.g., sorted cells, cultured cells, etc.). The cell concentration should be >600 cells/µl. High concentrations of heparin in blood samples can inhibit the REPLI-g reaction (see Troubleshooting Guide, page 13). Blood stabilized in EDTA or citrate may yield better results.
- REPLI-g Mini DNA Polymerase should be thawed on ice (see step 4). All other components can be thawed at room temperature.

Things to do before starting

- Set a water bath or heating block to 30°C.
- Thaw Buffer SB1 and Buffer SB2 at room temperature.
- Buffer SB1 and Buffer SB2 should be vortexed for at least 10 s before use to ensure thorough mixing.

Procedure

1. Add 0.5 µl cell material (>600 cells/µl) or 0.5 µl blood to each well of a 96-well plate or a microcentrifuge tube.

   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).

2. Add 17 µl Buffer SB to each sample. Mix by vortexing and centrifuge briefly.

   If using a 96-well plate, seal the plate using a tape sheet before vortexing.

3. Incubate the sample for 5 min at 65°C. Allow samples to cool down to room temperature (15–25°C).

4. Thaw REPLI-g Mini DNA Polymerase on ice.
5. Prepare a master mix according Table 2. Mix and centrifuge briefly.

Table 2. Preparation of Master Mix

Component	Volume/reaction	Volume/100 reactions
Buffer SB2	17 µl	1700 µl
REPLI-g Mini DNA Polymerase	1 µl	100 µl
Total volume	**18 µl**	**1800 µl**

6. Add 18 µl of the master mix to 17.5 µl of denatured DNA (step 3).
7. Incubate at 30°C for 10–16 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 8.
8. Inactivate REPLI-g Mini DNA Polymerase by heating the sample at 65°C for 3 min.
9. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.

   DNA amplified using REPLI-g Kits should be treated as genomic DNA with minimal freeze–thaw cycles. Storage of nucleic acids at low concentration over a long period of time may result in acid hydrolysis. We therefore recommend storage of nucleic acids at a concentration of at least 100 ng/µl.

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 or protocols in this handbook (for contact information, visit **support.qiagen.com**).

	Comments and suggestions
Reduced or no high-molecular-weight product in agarose gel in some samples, but DNA yield in other samples is approximately 5 µg	
Reaction failed. Possible inhibitor (e.g., salts, heparin, etc.) in the sample	Clean up or dilute the genomic DNA or blood/cells and re-amplify.
DNA yields of approx. 5 µg in negative (no-template) controls 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.
DNA yields of approx. 5 µg in negative (no-template) controls and 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 pipette tips only, and keep amplification chemistry and DNA templates in separate storage locations.

	Comments and suggestions
Genomic DNA protocol	
Reduced or no locus representation (allele dropout) in real-time PCR analysis	
Genomic DNA template is degraded	Use intact genomic DNA template. Use larger amount of gDNA.
Blood and cell protocol	
Reduced or no locus representation (allele dropout) in real-time PCR analysis	
Higher than normal concentration of heparin used as blood	Dilute the heparin-treated blood up to 5-fold using 1x PBS.*

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate SDSs, available from the product supplier.

References

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. et al. (2004) Assessment of multiple displacement amplification in molecular epidemiology. Biotechniques **37**, 136.

Appendix A: Determination of DNA Concentration and Yield

Quantification of DNA yield

A 40 µl REPLI-g reaction typically yields approximately 5 µg of DNA regardless of the amount of template DNA, allowing direct use of the amplified DNA in most downstream genotyping experiments. 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.

Quantification of locus representation

Locus representation for each sample can be quantified by real-time PCR (3). Contact QIAGEN Technical Services or visit our Web site at **www.qiagen.com** for more information.

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.

Note: Degraded or old PicoGreen reagent may result in inaccurate DNA quantification. DNA yields in excess of 20 µg should be ignored and, if necessary, quantification should be repeated using fresh 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 SDSs, available from the product supplier.

Equipment and reagents to be supplied by user

- Quant-iT™ PicoGreen dsDNA reagent (Invitrogen, cat. no. P7581)
- Buffer TE (10 mM Tris·Cl; 1 mM 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 Buffer TE. A total of 20 µl is required for each quantification reaction. 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 Buffer TE.

Important: Prepare the PicoGreen/Buffer 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 Buffer TE.
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 Buffer TE.
4. Transfer 20 µl of each DNA standard in duplicate into a 96-well plate labeled A (see the figure below).

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

96-Well Plate

	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 standard (ng/µl).

5. Place 2 µl of each REPLI-g amplified DNA sample for quantification into a new 96-well plate and add 198 µl Buffer TE to make a 1/100 dilution. Store the remaining REPLI-g amplified DNA at –20°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 Buffer TE to make a 1/1000 dilution.

   The 1/100 dilutions can be stored at –20°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, approx. 480 nm; emission, approx. 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 fluorimeter'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 (as 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) (step 12) by the reaction volume in milliliters (i.e., for a 40 µl reaction, multiply by 0.04).

Ordering Information

Product	Contents	Cat. no.
REPLI-g Mini and Midi Kits — for highly uniform whole genome amplification from small or precious samples		
REPLI-g Mini Kit (25)	For 25 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers	150023
REPLI-g Mini Kit (100)	For 100 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers	150025
REPLI-g Midi Kit (25)	For 25 whole genome amplification reactions: REPLI-g Midi DNA Polymerase, Buffers	150043
REPLI-g Midi Kit (100)	For 100 whole genome amplification reactions: REPLI-g Midi DNA Polymerase, Buffers	150045
REPLI-g UltraFast Mini Kit (25)	For 25 whole genome amplifications: REPLI-g DNA Polymerase, Buffers, and Reagents	150033
REPLI-g UltraFast Mini Kit (100)	For 100 whole genome amplifications: REPLI-g DNA Polymerase, Buffers, and Reagents	150035
REPLI-g Advanced DNA Single Cell Kit (24)	For 24 whole genome amplifications from single cells or low input genomic DNA: REPLI-g single cell DNA Polymerase, Buffers, Enzymes, and single-cell storage solution	150363

Product	Contents	Cat. no.
Related products		
REPLI-g Human Control Kit (25)	Human control DNA for 25 x 50 µl whole genome amplification reactions	150090
QIAamp® DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp DNA Micro Kit (50)	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	56304
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Mini Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304

*Larger sizes are available. Please visit **qiagen.com** for details.

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

Date	Changes
03/2021	Updated the Kit Contents, Shipping and Storage, and Ordering Information sections. Changed all instances of precise storage temperature (from "–20°C" to "–30 to –15°C").

Limited License Agreement for REPLI-g Screening Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. QIAGEN grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.qiagen.com. Some of these additional protocols have been provided by QIAGEN users for QIAGEN users. These protocols have not been thoroughly tested or optimized by QIAGEN. QIAGEN neither guarantees them nor warrants that they do not infringe the rights of third-parties.
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