August 2013

REPLI-g® Mitochondrial DNA Kit Handbook

For specific whole genome amplification of human mitochondria from total DNA samples

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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Contents

Kit Contents

REPLI-g Mitochondrial DNA Kit	**(25)**
Catalog no.	**151023**
Number of 50 µl reactions (approximately 3–5 µg yield)	**25**
REPLI-g Midi DNA Polymerase (blue lid)	25
REPLI-g mt Reaction Buffer (yellow lid)	700 µl
REPLI-g Human mt Primer Mix (red lid)	50 µl
RNase-Free Water	1.8 ml
Quick Start Leaflet	1

Shipping and Storage

The REPLI-g Mitochondrial DNA 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, this product can be kept at least 6 months after shipping without showing any reduction in performance. For longer storage, the kit should be stored at –65 to –90°C.

Intended Use

The REPLI-g Mitochondrial DNA 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.

24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:

CHEMTREC

USA & Canada ■ Tel: 1-800-424-9300

Outside USA & Canada ■ Tel: +1-703-527-3887 (collect calls accepted)

Quality Control

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

Introduction

The REPLI-g Mitochondrial DNA Kit contains REPLI-g DNA polymerase, buffers, and primers for whole genome amplification of the human mitochondrial genome from small DNA samples using Multiple Displacement Amplification (MDA).

Genotyping and DNA sequence analysis of samples may be limited by the small amount of sample available. In these cases, analysis of mitochondrial DNA is more difficult as it comprises only a very small fraction of the entire genome. In addition, pure mitochondrial preparations are usually cumbersome to obtain. The REPLI-g Mitochondrial DNA Kit allows uniform amplification of the whole mitochondrial genome with minimal nuclear DNA contamination, enabling a wider variety and number of analyses to be performed. Numerous publications have demonstrated the successful utilization of REPLI-g amplified DNA for next-generation sequencing (NGS) applications. Since the use of whole genome amplified DNA for NGS and array applications has been debated, potential factors that could influence the success of using amplified DNA for these downstream applications were studied. The results suggest that the quality of input material strongly influences the success of downstream NGS experiments. If working with low quality DNA (e.g., degraded DNA) or aged tissue material, the resulting amplified DNA may not give reliable results. However, NGS results comparable to that obtained with purified gDNA are obtained when performing WGA using REPLI-g technology on intact cells or non-degraded purified DNA. Sequence coverage and alignment comparison of the genomic loci sequence indicate lower levels of junk DNA after WGA and comparable error rates between amplified and genomic DNA.

Typical DNA yields from a REPLI-g Mitochondrial DNA Kit reaction are approximately 3–5 µg per 50 µl reaction. The average product length is typically greater than 10 kb.

Principle and procedure

Typical DNA preparations comprise approximately 0.1% mitochondrial DNA (e.g., 10 µg human DNA contains approximately 10 ng mitochondrial DNA). The REPLI-g Mitochondrial DNA Kit provides specific amplification of the whole human mitochondrial genome and yields approximately 3–5 µg amplified mitochondrial DNA per reaction (corresponding to approximately 2 x 10^9 mitochondrial genome copies/µl). This corresponds to an enrichment of mitochondrial DNA of up to 40 million-fold, depending on the starting amount. Residual amounts of amplified genomic DNA are minimized and do not interfere with mitochondrial-specific downstream methods such as qPCR or direct Sanger sequencing.

The sample DNA is denatured by an incubation in REPLI-g mt Reaction Buffer for 5 min at 75°C. After denaturation has been stopped by cooling the solution

to room temperature, REPLI-g Midi DNA Polymerase is added. The isothermal amplification reaction proceeds for at least 8 hours or overnight at 33°C.

The REPLI-g Mitochondrial DNA Kit provides highly uniform amplification across the entire human mitochondria genome. The method is based on MDA technology, which uses a unique processive DNA polymerase for isothermal genome amplification. The DNA polymerase has 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.

If targeting non-human mitochondrial genomes, replace REPLI-g Human mt Primer Mix with a primer mix appropriate for the species under study.

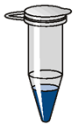
This kit generates high yields of concentrated mitochondrial DNA. Amplified DNA should be diluted 1:1000 prior to use in downstream PCR assays (see note in protocol step 11, page 11 for more details).

Purified Mitochondrial DNA Procedure

Purified total DNA

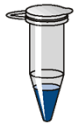
Add REPLI-g mt Reaction Buffer and REPLI-g Human mt Primer Mix
Vortex

5 min at 75°C



Add REPLI-g Midi DNA Polymerase
Vortex

8 h at 33°C
3 min at 65°C



Amplified mitochondrial DNA

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
- Microcentrifuge
- Vortexer
- Water bath or heating block
- Pipets and pipet tips
- Ice

Protocol: Amplification of Human Mitochondrial DNA

Important points before starting

- This protocol is optimized for human mitochondrial genome amplification from purified genomic DNA. If used for amplification of mitochondrial genome from purified genomic DNA of another species, the REPLI-g Human mt Primer Mix should be substituted with an appropriate primer mix for that species. The template DNA should be suspended in TE or water.
- QIAamp® and DNeasy® Kits enable purification of high-quality genomic DNA from a variety of sample types (including blood, tissue, and body fluids). Genomic DNA purified with these kits is ideal for use in REPLI-g Mitochondrial DNA reactions. Alternative extraction methods may selectively enrich for nuclear DNA, reducing the amount of mitochondrial DNA.
- Degraded DNA is not suitable for use in this procedure
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 6). REPLI-g mt Reaction Buffer and REPLI-g Human mt Primer Mix should be thawed at room temperature (15–25°C).
- REPLI-g mt Reaction Buffer should be vortexed for at least 10 s before use to ensure thorough mixing.

Things to do before starting

- Set a water bath or heating block to 75°C for use in step 5
- Set a water bath or heating block to 33°C for use in step 8

Procedure

1. **Place 1 to 10 µl template DNA into a microcentrifuge tube.**
   The amount of template DNA should be >1 ng. Smaller amounts (≥0.1 ng) of starting material can be used if the DNA is of sufficient quality and a high proportion of the mitochondrial DNA has been purified.
2. **Adjust the sample volume to 20 µl with RNase-Free Water (supplied).**
   The volume of water added depends on the volume of DNA template. The total volume of DNA template and water should be 20 µl.
3. **Prepare a fresh amplification mix (see Table 1). Mix by vortexing and centrifuge briefly.**
4. **Add 29 µl amplification mix to the DNA. Mix by vortexing and centrifuge briefly.**
5. **Incubate the sample for 5 min at 75°C. Allow sample to cool down to room temperature (15–25°C).**

Table 1. Amplification mix

Component	Volume/reaction
REPLI-g mt Reaction Buffer	27 µl
REPLI-g Human mt Primer Mix	2 µl
Total volume	**29 µl**

6. **Thaw REPLI-g Midi DNA Polymerase on ice.**
7. **Add 1 µl REPLI-g Midi Polymerase to the DNA (from step 5). Mix and centrifuge briefly.**
8. **Incubate the sample at 33°C for 8 h.**
9. **Inactivate REPLI-g Midi DNA Polymerase by heating the sample for 3 min at 65°C.**
10. **If not using directly, store the amplified DNA at 2–8°C for short-term storage or –15 to –30°C for long-term storage.**

    DNA amplified using the REPLI-g Mitochondrial DNA Kit should be treated as pure DNA with minimal freeze-thaw cycles. We therefore recommend storage of the DNA at a concentration of at least 100 ng/µl.

    Note: Optical density (OD) measurements overestimate REPLI-g amplified DNA. See Appendix B, page 14, for an accurate method of quantifying DNA amplified using the REPLI-g Mitochondrial DNA Kit.

    Note: In no-template controls, high-molecular weight products can be generated by random extension of primer–dimers. This DNA will not affect the quality of actual samples or specific downstream genetic assays.

11. **Amplified DNA can be used in a variety of downstream applications, including next-generation sequencing and quantitative PCR. For downstream applications, use the correct amount of REPLI-g amplified mt DNA in water or TE buffer according to the manufacturer's instructions.**

    Note: If the amplified DNA will be used in next-generation sequencing, use up to 1 µg for shearing into a random library of fragments.

    Note: If the amplified DNA will be analyzed using PCR, dilute the DNA after inactivation 1:1000 in water or TE buffer. Use 2–3 µl diluted DNA for each PCR.

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 or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
Reduced or no high-molecular weight DNA visible in agarose gel in some samples but DNA yield in other samples (e.g., positive control) is approximately 3–5 µg	
Reaction failed. Possible inhibitor in the genomic DNA template	Clean up or dilute the genomic DNA and re-amplify.
DNA yields of up to 3 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 DNA can be generated by random extension of primer–dimers. This DNA will not affect sample quality or specific downstream genetic assays.
DNA yields of approximately 3–5 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.
Downstream application results not optimum	
Sensitive downstream applications may require DNA cleanup after REPLI-g reaction	Contact QIAGEN Technical Services for DNA cleanup recommendations suitable for your application.

Comments and suggestions	
Reduced or no locus representation in real-time PCR analysis but DNA yield is approximately 3–5 µg	
a) DNA template is degraded	Use intact DNA template. Use larger amount of genomic DNA.
b) Amplified DNA is not diluted	Use a 1:1000 dilution of amplified DNA for downstream PCR analysis.
c) Low amount of mitochondrial DNA	The amount of mitochondrial DNA in a total DNA fraction depends on the purification method. We recommend using DNeasy or QIAamp Kits for purification of total DNA.

Appendix A: Determination of DNA Concentration and Yield

Quantification of DNA yield

A 50 µl REPLI-g Mitochondrial DNA Kit reaction typically yields approximately 5 µg DNA regardless of the amount of template DNA, allowing direct use of the amplified DNA in most downstream genotyping experiments. However, if more accurate quantification of DNA is required, it is important to use 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. A protocol for the quantification of REPLI-g amplified DNA can be found in Appendix B.

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 (Molecular Probes, cat. no. P-7581)
- TE buffer (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 TE buffer. Each quantification requires 20 µl. Cover the microcentrifuge tube with aluminum foil or place it in the dark to avoid photodegradation of the PicoGreen.**

   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 dsDNA 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 dsDNA with TE buffer.**
4. **Transfer 20 µl of each DNA standard in duplicate into a 96-well plate labeled A (see Figure 1 below).**

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

Figure 1. 96-Well plate. setup

	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

Grey squares - genomic DNA standard (µg/ml)

5. **Place 2 µl of each REPLI-g amplified DNA sample for quantification into a new 96-well plate and add 298 µl TE buffer to make a 1/150 dilution. Store the remaining REPLI-g amplified DNA at –15 to –30°C**
6. **Place 20 µl diluted REPLI-g DNA (from step 5) into an unused well 96-well plate A.**

   The 1/150 dilutions can be stored at –15 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 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 150 to show the concentration of undiluted sample DNA (as the sample DNA measured by PicoGreen fluorescence had been diluted 1 in 150).**
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 50 µl reaction, multiply by 0.05).**

Ordering Information

Product	Contents	Cat. no.
REPLI-g Mitochondrial DNA Kit — for highly uniform whole genome amplification from mitochondria		
REPLI-g Mitochondrial DNA Kit (25)	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions	151023
REPLI-g UltraFast Kit — for ultrafast, highly uniform whole genome amplification from purified genomic DNA, blood, and cells		
REPLI-g UltraFast Mini Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 20 µl whole genome amplification reactions	150033
REPLI-g Mini and Midi Kits — for highly uniform whole genome amplification from small or precious samples		
REPLI-g Mini Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions	150023
REPLI-g Midi Kit (25)*	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions	150043
REPLI-g Single Cell Kit — for whole genome amplification from single cells, limited samples, or purified genomic DNA		
REPLI-g Single Cell Kit (24)	REPLI-g sc Polymerase, Buffers, and Reagents for 24 x 50 µl whole genome amplification reactions	150343
REPLI-g Single Cell Kit (96)	REPLI-g sc Polymerase, Buffers, and Reagents for 96 x 50 µl whole genome amplification reactions	150345
REPLI-g Screening Kit — for high-throughput manual or automated whole genome amplification from small or precious samples		
REPLI-g Screening Kit (200)*	DNA Polymerase, Buffers, and Reagents for 200 x 40 µl whole genome amplification reactions	150126

* Larger kit sizes available; please inquire

Product	Contents	Cat. no.
REPLI-g Service — large scale highly uniform whole genome amplification and quality assessment from limited or precious samples. See http://www.qiagen.com/REPLIg-Service		
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

* Larger kit sizes available; please inquire

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