March 2024

REPLI-g® Mini/Midi Kit Handbook

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

Sample to Insight

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Kit Contents

REPLI-g Mini Kit	**(25)**	**(100)**
Catalog no.	**150023**	**150025**
Number of 50 µL reactions (approximately 10 µg yield)	**25**	**100**
REPLI-g Mini DNA Polymerase (blue lid)	25 µL	100 µL
REPLI-g Mini Reaction Buffer (yellow lid)	725 µL	2 x 1.45 mL
Buffer DLB (clear lid)	1 tube	2 tubes
Stop Solution (red lid)	1.8 mL	1.8 mL
PBS, 1x (clear lid)	1.8 mL	1.8 mL
DTT, 1 M (lilac lid)	1 mL	1 mL
Quick-Start Protocol	1	1

REPLI-g Midi Kit	**(25)**	**(100)**
Catalog no.	**150043**	**150045**
Number of 50 µL reactions (approximately 40 µg yield)	**25**	**100**
REPLI-g Midi DNA Polymerase (blue lid)	25 µL	100 µL
REPLI-g Midi Reaction Buffer (yellow lid)	725 µL	2 x 1.45 mL
Buffer DLB (clear lid)	1 tube	2 tubes
Stop Solution (red lid)	1.8 mL	1.8 mL
PBS, 1x (clear lid)	1.8 mL	1.8 mL
DTT, 1 M (lilac lid)	1 mL	1 mL
Quick-Start Protocol	1	1

Shipping and Storage

The REPLI-g Kits are shipped on dry ice. The kits, including all reagents and buffers, should be stored immediately upon receipt at –20°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 storage more than 6 months, the kit should be stored at –70°C.

The expiration date for the product is provided on the label and will vary based on the date of manufacture of the kit.

Intended Use

REPLI-g Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

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.

Emergency information

CHEMTREC
USA & Canada: 1-800-424-9300
Outside USA & Canada: +1 703-527-3887

Quality Control

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

Introduction

The REPLI-g 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 samples, including purified DNA, whole blood, and tissue culture cells. Supplementary protocols for the amplification of DNA from dried blood cards, buccal cells, tissue, serum, plasma, and laser-microdissected cells are available from QIAGEN Technical Services or online at **www.qiagen.com/literature**

Genotyping and DNA sequence analysis of biological 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 Mini Kit reaction are approximately 10 µg per 50 µL reaction. Typical DNA yields from a REPLI-g Midi Kit reaction are approximately 40 µg per 50 µL reaction. The average product length is typically greater than 10 kb, with a range between 2 kb and 100 kb.

Principle and procedure

The sample material is lysed, and the DNA is denatured by adding denaturation buffer. After denaturation has been stopped by addition of neutralization buffer, a master mix containing buffer and DNA polymerase is added. The isothermal amplification reaction proceeds for at least 8 (Midi kit) or 10 (Mini kit) hours or overnight at 30°C.

The REPLI-g 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. 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.

For further information, please visit our WGA Resource page, application page and WGA tutorial online at **www.qiagen.com/wga**

For special downstream applications, we recommend to clean up the amplified DNA. Please visit **www.qiagen.com/wga**, WGA tutorial, cleanup recommendations.

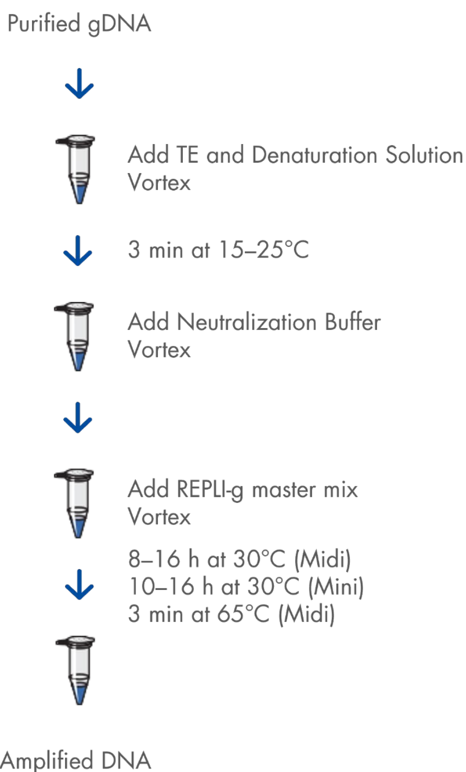

Figure 1. Purified genomic DNA procedure.

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
- Nuclease-free water

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

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. Smaller amounts (1–10 ng) of starting material can be used if the DNA is of sufficient quality.
- For direct amplification from blood see pages 14–16.
- 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 6). All other components can be thawed at room temperature (15–25°C).
- Buffer D1 and Buffer N1 should not be stored longer than 3 months.
- Blue text (marked with a ▲) denotes prep volumes if 2.5 µL template DNA is used; red text (marked with a ●) denotes prep volumes if 5 µL template DNA is used.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL nuclease-free water to the tube. Mix thoroughly and centrifuge briefly.
  Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile. Avoid neutralization with CO_2.
- 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 (see Tables 1 and 2, below).

   Note: The total volumes of Buffer D1 and Buffer N1 given in Tables 1 and 2 are suitable for up to ▲ 15 or ● 7 reactions. Buffer D1 and Buffer N1 should not be stored longer than 3 months.

Table 1. Preparation of Buffer D1

Component	Volume (µL)*
Reconstituted Buffer DLB†	9
Nuclease-free water	32
Total volume	**41**

* Volumes given are suitable for up to ▲ 15 or ● 7 reactions.
† Reconstitution of DLB is described in the "Things to do before starting" section, previous page.

Table 2. Preparation of Buffer N1

Component	Volume (µL)‡
Stop solution	12
Nuclease-free water	68
Total volume	**80**

‡ Volumes given are suitable for up to ▲ 15 or ● 7 reactions.

2. Place ▲ 2.5 µL or ● 5 µL template DNA into a microcentrifuge. 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 TE to the starting volume of your sample.
3. Add ▲ 2.5 µL or ● 5 µL Buffer D1 to the DNA. Mix by vortexing and centrifuge briefly.
4. Incubate the samples at room temperature for 3 min.
5. Add ▲ 5 µL or ● 10 µL Buffer N1 to the samples. Mix by vortexing and centrifuge briefly.

6. Thaw REPLI-g Mini DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly. The REPLI-g Mini Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
7. Prepare a master mix on ice according to Table 3. Mix and centrifuge briefly.

   Important: Add the master mix components in the order listed in Table 3. After the addition of water and REPLI-g Mini Reaction Buffer, briefly vortex and centrifuge the mixture before the addition of REPLI-g Mini DNA Polymerase. The master mix should be kept on ice and used immediately upon addition of the REPLI-g Mini DNA Polymerase.

Table 3. Preparation of Master Mix

Component	Volume/reaction (µL)
Nuclease-free water*	▲10; ● 0
REPLI-g Mini Reaction Buffer	29
REPLI-g Mini DNA Polymerase	1
Total volume	**▲ 40; ● 30**

* Add 10 µL nuclease-free water to the master mix if the template DNA in step 2 has a volume of ▲ 2.5 µL. If the volume of the template DNA in step 2 is ● 5 µL, no nuclease-free water should be added.

8. Add ▲ 40 µL or ● 30 µL of the master mix to ▲ 10 µL or ● 20 µL of denatured DNA (step 5).
9. 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 10.

   Note: If a thermal cycler is used with a heated lid, temperature of the lid should be set to 70°C.
10. Inactivate REPLI-g Mini DNA Polymerase by heating the sample for 3 min at 65°C.
11. If performing PCR analysis, dilute the amplified DNA 1:20 and use 3 µL of diluted DNA for each PCR.

Note: For dilution, add 2 µL amplified to 38 µL water or TE. Use 3 µL of the diluted DNA for each PCR. Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A, page 27, for an accurate method of quantifying REPLI-g amplified DNA.

12. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage. DNA amplified using the REPLI-g kit 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 Mini Kit

Important points before starting

- The protocol is optimized for 0.5 µL whole blood or cell material (e.g., sorted cells, tissue cultured cells, etc.). The cell concentration should be >600 cells/µL. Additional protocols for the amplification of DNA from dried blood cards, buccal cells, tissue, serum, plasma, and laser-microdissected cells are available from QIAGEN Technical Services or online at **www.qiagen.com/literature**
- For the amplification of purified DNA, see "Protocol: Amplification of Purified Genomic DNA Using the REPLI-g Mini Kit", page 10.
- High concentrations of heparin in blood samples can inhibit the REPLI-g reaction (see Troubleshooting Guide, page 25). Blood stabilized in EDTA or citrate may yield better results.
- REPLI-g DNA Polymerase Mini should be thawed on ice (see step 7). All other components can be thawed at room temperature (15–25°C).
- Buffer D2 should not be store longer than 3 months.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL nuclease-free water to the tube. Mix thoroughly and centrifuge briefly.
  Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile. Avoid neutralization with CO_2.
- 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 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 suitable for up to 15 reactions. Buffer D2 should not be stored longer than 3 months.

Table 4. Preparation of Buffer D2

Component	Volume (µl)*
DTT, 1 M	5
Reconstituted Buffer DLB†	55
Total volume	**60**

* Volumes given are suitable for up to 15 reactions.
† Reconstitution of DLB is described in the "Things to do before starting" section, previous page.

2. Place 2.5 µl PBS into a microcentrifuge tube.
3. Add 0.5 µl cell material (>600 cells/µl) or 0.5 µl blood to the PBS. 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 TE to the starting volume of your sample.
4. Add 3.5 µl Buffer D2. Mix by vortexing and centrifuge briefly.
5. Incubate for 10 min on ice.
6. Add 3.5 µl Stop Solution. Mix by vortexing and centrifuge briefly.
7. Thaw REPLI-g Mini DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly. The REPLI-g Mini Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
8. Prepare a master mix according to Table 5 (next page). Mix and centrifuge briefly.

   Important: Add the master mix components in the order listed in Table 5. After the addition of water and REPLI-g Mini Reaction Buffer, briefly vortex and centrifuge the

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

Table 5. Preparation of Master Mix

Component	Volume/reaction (µL)
Nuclease-free water	10
REPLI-g Mini Reaction Buffer	29
REPLI-g Mini DNA Polymerase	1
Total volume	**40**

9. Add 40 µL of the master mix to 10 µL of denatured DNA (step 6).
10. 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 11.

    Note: If a thermal cycler is used with a heated lid, temperature of the lid should be set to 70°C.
11. Inactivate REPLI-g Mini DNA Polymerase by at 65°C for 3 min.
12. If performing PCR analysis, dilute the amplified DNA 1:20 and use 3 µL of diluted DNA for each PCR.

    Note: For dilution, add 2 µL amplified to 38 µL water or TE. Use 3 µL of the diluted DNA for each PCR. Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A, page 27, for an accurate method of quantifying REPLI-g amplified DNA.
13. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage. DNA amplified using the REPLI-g kit 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. Supplementary protocols not contained in this Handbook are available online. Visit **www.qiagen.com/literature**

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

Important points before starting

- This protocol is optimized for whole genome amplification from >10 ng of genomic DNA template. The template DNA should be suspended in TE. Smaller amounts (1–10 ng) of starting material can be used if the DNA is of sufficient quality.
- For direct amplification from cells or blood, see "Protocol: Amplification of Genomic DNA from Blood or Cells Using the REPLI-g Midi Kit", page 21.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 6). All other components can be thawed at room temperature (15–25°C).
- Buffer D1 and Buffer N1 should not be stored longer than 3 months.
- Blue text (marked with a ▲) denotes prep volumes if 2.5 µL template DNA is used; red text (marked with a ●) denotes prep volumes if 5 µL template DNA is used.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL nuclease-free water to the tube. Mix thoroughly and centrifuge briefly.
  Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile. Avoid neutralization with CO_2.
- 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 (see Tables 6 and 7).

   Note: The total volumes of Buffer D1 and Buffer N1 given in Tables 6 and 7 are suitable for up to ▲ 15 or ● 7 reactions. 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†	9
Nuclease-free water	32
Total volume	**41**

* Volumes given are suitable for up to ▲ 15 or ● 7 reactions.
† Reconstitution of DLB is described in the "Things to do before starting" section, previous page.

Table 7. Preparation of Buffer N1

Component	Volume (μL)‡
Stop solution	12
Nuclease-free water	68
Total volume	**80**

‡ Volumes given are suitable for up to ▲ 15 or ● 7 reactions.

2. Place ▲ 2.5 μL or ● 5.0 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 with TE to the starting volume of your sample.
3. Add ▲ 2.5 μL or ● 5.0 μL Buffer D1 to the DNA. Mix by vortexing and centrifuge briefly.
4. Incubate the samples at room temperature for 3 min.

5. Add ▲ 5.0 µL or ● 10 µL Buffer N1 to the samples. Mix by vortexing and centrifuge briefly.
6. Thaw REPLI-g Midi DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly. The REPLI-g Midi Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
7. Prepare a master mix on ice according to 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 Midi Reaction Buffer, briefly vortex and centrifuge the mixture before the addition of REPLI-g Midi DNA Polymerase. The master mix should be kept on ice and used immediately upon addition of the REPLI-g Midi DNA Polymerase.

Table 8. Preparation of Master Mix

Component	Volume/reaction (µL)
Nuclease-free water*	▲ 10; ● 0
REPLI-g Mini Reaction Buffer	29
REPLI-g Mini DNA Polymerase	1
Total volume	**▲ 40; ● 30**

* Add 10 µL nuclease-free water to the master mix if the template DNA in step 2 has a volume of ▲ 2.5 µL. If the volume of the template DNA in step 2 is ● 5 µL, no nuclease-free water should be added.

8. Add ▲ 40 µL or ● 30 µL of the master mix to ▲ 10 µL or ● 20 µL of denatured DNA (step 5).
9. Incubate at 30°C for 8–16 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 10.
10. Inactivate REPLI-g Midi DNA Polymerase by heating the sample for 3 min at 65°C.

    Note: If a thermal cycler is used with a heated lid, temperature of the lid should be set to 70°C.
11. If performing PCR analysis, dilute the amplified DNA 1:100 and use 3 µL of diluted DNA for each PCR.

Note: For dilution, add 2 µL of amplified DNA to 198 µL water or TE. Use 2–3 µL of the diluted DNA for each PCR. Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A, page 27, for an accurate method of quantifying REPLI-g amplified DNA.

12. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage. DNA amplified using the REPLI-g kit 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 Midi Kit

Important points before starting

- The protocol is optimized for 0.5–1.0 µL whole blood or cell material (e.g., sorted cells, tissue cultured cells, etc.). The cell concentration should be >600 cells/µL. Additional protocols for the amplification of DNA from dried blood cards, buccal cells, tissue, serum, plasma, and laser-microdissected cells are available from QIAGEN Technical Services or online on **www.qiagen.com/literature**
- For the amplification of purified DNA, see protocol starting on page 17.
- High concentrations of heparin in blood samples can inhibit the REPLI-g reaction (see Troubleshooting Guide, page 25). Blood stabilized in EDTA or citrate may yield better results.
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 7). All other components can be thawed at room temperature (15–25°C).
- Buffer D2 should not be stored longer than 3 months.
- Blue text (marked with a ▲) denotes prep volumes if 0.5 µL blood or cell material is used; red text (marked with a ●) denotes prep volumes if 1 µL blood or cell material is used.

Things to do before starting

- Prepare Buffer DLB by adding 500 µL nuclease-free water to the tube. Mix thoroughly and centrifuge briefly.

  Note: Reconstituted Buffer DLB can be stored for 6 months at –20°C. Buffer DLB is pH-labile. Avoid neutralization with CO_2.
- 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 D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 9, page 21).

   Note: The total volume of Buffer D2 given in Table 9 is suitable for up to 15 reactions. Buffer D2 should not be stored longer than 3 months.

Table 9. Preparation of Buffer D2

Component	Volume (µL)*
DTT, 1 M	5
Reconstituted Buffer DLB†	55
Total volume	**60**

* Volumes given are suitable for up to 15 reactions.
† Reconstitution of DLB is described in the "Things to do before starting" section, previous page.

2. Place ▲ 2.5 µL or ● 2.0 µL PBS into a microcentrifuge tube.
3. Add ▲ 0.5 µL or ● 1.0 µL cell material (>600 cells/µL) or ▲ 0.5 µL or ● 1.0 µL blood to the PBS. 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 TE to the starting volume of your sample.
4. Add 3.5 µL Buffer D2. Mix by vortexing and centrifuge briefly.
5. Incubate for 10 min on ice.
6. Add 3.5 µL Stop Solution. Mix by vortexing and centrifuge briefly.
7. Thaw REPLI-g Midi DNA Polymerase on ice. Thaw all other components at room temperature, vortex, then centrifuge briefly. The REPLI-g Midi Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.
8. Prepare a master mix according to Table 10 (next page). Mix and centrifuge briefly.

   Important: Add the master mix components in the order listed in Table 10. After addition of water and REPLI-g Midi Reaction Buffer briefly vortex and centrifuge the mixture before

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

Table 10. Preparation of Master Mix

Component	Volume/reaction (µL)
Nuclease-free water	10
REPLI-g Midi Reaction Buffer	29
REPLI-g Midi DNA Polymerase	1
Total volume	**40**

9. Add 40 µL of the master mix to 10 µL of denatured DNA (step 6). 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. Incubate at 30°C for 8–16 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 10.

    Note: If a thermal cycler is used with a heated lid, temperature of the lid should be set to 70°C.
11. Inactivate REPLI-g Midi DNA Polymerase by at 65°C for 3 min.
12. If performing PCR analysis, dilute the amplified DNA 1:100 and use 3 µL of diluted DNA for each PCR.

    Note: For dilution, add 2 µL amplified to 198 µL water or TE. Use 2-3 µL of the diluted DNA for each PCR. Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A, page 27, for an accurate method of quantifying REPLI-g amplified DNA.
13. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage. DNA amplified using the REPLI-g kit 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.

Note: Cleanup of REPLI-g amplified DNA is not required for most common downstream applications. However, the amplified DNA should be diluted as recommended above prior to use downstream.

If cleanup after WGA is required for your downstream application, please visit our webpage **www.qiagen.com/wga**, select WGA tutorial and then cleanup recommendations. A supplementary protocol for REPLI-g reaction cleanup is also available online, see "Purification of REPLI-g amplified DNA using the QIAamp DNA Mini Kit". Supplementary protocols not contained in this Handbook are available online. Visit **www.qiagen.com/literature**

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 in 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
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 (Mini) or 40 µg (Midi)	
a) Reaction failed. Possible inhibitor in the genomic DNA template	Clean up or dilute the genomic DNA and reamplify.
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 (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 the column based 96-well format purification procedures, ensure the duration of the drying step prior elution of DNA from the column is sufficient to evaporate residual ethanol
DNA yields of approximately 10 µg (Mini) or 40 µg (Midi) 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.

	Comments and suggestions
DNA yields of approximately 10 µg (Mini) or 40 µg (Midi) 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.

Genomic DNA protocol

Reduced or no locus representation in real-time PCR analysis but DNA yield is approximately 10 µg (Mini) or 40 µg (Midi)	
Genomic DNA template is degraded	Use intact genomic DNA template. Use larger amount of genomic DNA.
Allele dropout observed in genotyping assay but DNA yield is approximately 10 µg (Mini) or 40 µg (Midi)	
Genomic DNA template is degraded	Use intact genomic DNA template. Use larger amount of genomic DNA.

Blood and cell protocol

Reduced or no locus representation in real-time PCR analysis but DNA yield is approximately 10 µg (Mini) or 40 µg (Midi)	
Higher than normal concentration of heparin used as blood coagulant	Dilute the heparin-treated blood up to 5-fold using 1x PBS
Allele dropout observed in genotyping assay but DNA yield is approximately 10 µg (Mini) or 40 µg (Midi)	
Higher than normal concentration of heparin used as blood anticoagulant	Dilute the heparin-treated blood up to 5-fold using 1x PBS.

Appendix A: Determination of Concentration and Yield

Quantification of DNA yield

A 50 µL REPLI-g reaction typically yields approximately 10 µg (Mini) or 40 µg (Midi) 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

Quantification of 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 material safety data sheets (MSDSs), 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 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 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

Figure 2. 96-well plate. Grey squares: genomic DNA standard (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 –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 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 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 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 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.

For a complete list of references, visit the QIAGEN Reference Database online at **www.qiagen.com/RefDB/search.asp** or contact QIAGEN Technical Services or your local distributor.

1. Dean, F. B., Hosono, S., Fang, L., Wu, X., Faruqi, A. F., Bray-Ward, P., ... & Lasken, R. S. (2002). Comprehensive human genome amplification using multiple displacement amplification. *Proceedings of the National Academy of Sciences*, 99(8), 5261-5266.
2. Hosono, S., Faruqi, A. F., Dean, F. B., Du, Y., Sun, Z., Wu, X., ... & Lasken, R. S. (2003). Unbiased whole-genome amplification directly from clinical samples. *Genome research*, 13(5), 954-964.
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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 amplification reactions: REPLI-g UltraFast DNA Polymerase, Buffers	150033
REPLI-g UltraFast Mini Kit (100)	For 25 whole genome amplification reactions: REPLI-g UltraFast DNA Polymerase, Buffers	150035
REPLI-g FFPE Kit (25)	DNA Polymerase, Buffers, and Reagents for 25 × 50 µL whole genome amplification reactions	150243

Product	Contents	Cat. no.
REPLI-g Screening Kit — for high-throughput manual or automated whole genome amplification from small or precious samples		
REPLI-g Screening Kit (200)	For 200 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers	150126
Related products		
REPLI-g Human Control Kit (25)	Human control DNA for 25 × 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 MinElute Columns, Proteinase K, Carrier RNA, 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
QuantiTect® Probe PCR Kit (200)*	For 200 x 50 µL reactions: 3 × 1.7 mL QuantiTect Probe PCR Master Mix, 2 x 2.0 mL RNase-free water	204343
QuantiTect Multiplex PCR Kit (200)*	For 200 x 50 µL reactions: 3 x 1.7 mL QuantiTect Multiplex PCR Master Mix (contains ROX™ dye), 2 × 2 mL RNase-Free Water	204543

Product	Contents	Cat. no.
QIAGEN Multiplex PCR Kit (100)*	For 100 × 50 µL multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 × 0.85 mL), 5x Q-Solution (1 × 2.0 mL), distilled water (2 × 1.7 mL)	206143

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Document Revision History

Date	Changes
07/2011	Disclaimer for other products under the Ordering Information has been removed. Updated disclaimer for the Phi 29 DNA polymerase.
08/2023	Updated the handbook according to QIAGEN's modern branding. Shipping and Storage section now mentions that the expiration date is provided on the label and will vary according to date of manufacture. Omitted products in Ordering Information.
03/2024	Corrected volume of Buffer N1 on page 11 and volume of Template DNA in Table 3.

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