July 2011

# REPLI-g® Mini/Midi Handbook

REPLI-g Mini Kit

REPLI-g Midi Kit

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

QIAGEN®

Sample & Assay Technologies

# QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

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- Nucleic acid and protein assays
- microRNA research and RNAi
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# Contents

# 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 |
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| **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 |
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## Storage

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 longer storage, the kit should be stored at –70°C.

## Product Use Limitations

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.

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

## Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding REPLI-g Screening Kit or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

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

## Safety Information

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

The following risk and safety phrases apply to the components of the REPLI-g Mini and Midi Kits:

**Buffer DLB**

Contains potassium hydroxide: corrosive, harmful. Risk and safety phrases:* R22–35. S26–36/37/39–45

**24-hour emergency information**

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

* R22: Harmful if swallowed; R35: Causes severe burns. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; S36/37/39: Wear suitable protective clothing, gloves and eye/face protection; S45; In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

## 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 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

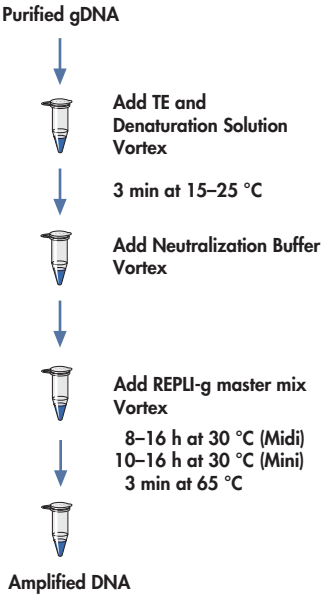
The sample material is lysed and the DNA is denatured by adding denaturation buffer. After denaturation has been stopped by addition of neutralization buffer, a master mix containing buffer and DNA polymerase is added. The isothermal amplification reaction proceeds for at least 8 (Midi kit) or 10 (Mini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

## 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 material safety data sheets (MSDSs), available from the product supplier.

- Microcentrifuge tubes
- Water bath or heating block
- Microcentrifuge
- Vortexer
- Pipets and pipet 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 30ºC.

### 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, page 12).**

   **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* |
|---|---|
| Reconstituted Buffer DLB† | 9 µl |
| Nuclease-free water | 32 µl |
| **Total volume** | **41 µl** |

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

† Reconstitution of DLB is described in the "Things to do before starting" section, page 11.

**Table 2. Preparation of Buffer N1**

| Component | Volume‡ |
|---|---|
| Stop solution | 12 µl |
| Nuclease-free water | 68 µl |
| **Total volume** | **80 µl** |

‡ 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 on page 13. 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 |
|---|---|
| Nuclease-free water* | ▲ 10 µl; ● 0 µl |
| REPLI-g Mini Reaction Buffer | 29 µl |
| REPLI-g Mini DNA Polymerase | 1 µl |
| **Total volume** | **▲ 40 µl; ● 30 µl** |

* 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 25, 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 pages 11–13.
- High concentrations of heparin in blood samples can inhibit the REPLI-g reaction (see Troubleshooting Guide, page 23). 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 stored 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* |
|---|---|
| DTT, 1 M | 5 µl |
| Reconstituted Buffer DLB† | 55 µl |
| **Total volume** | **60 µl** |

* Volumes given are suitable for up to 15 reactions.

† Reconstitution of DLB is described in the "Things to do before starting" section (above).

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 Table 5. 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 |
|---|---|
| Nuclease-free water | 10 µl |
| REPLI-g Mini Reaction Buffer | 29 µl |
| REPLI-g Mini DNA Polymerase | 1µl |
| **Total volume** | **40 µl** |

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 25, 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 pages 20-22.
- 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* |
|---|---|
| Reconstituted Buffer DLB† | 9 µl |
| Nuclease-free water | 32 µl |
| **Total volume** | **41 µl** |

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

† Reconstitution of DLB is described in the "Things to do before starting" section, page 17.

**Table 7. Preparation of Buffer N1**

| Component | Volume‡ |
|---|---|
| Stop solution | 12 µl |
| Nuclease-free water | 68 µl |
| **Total volume** | **80 µl** |

‡ 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 |
|---|---|
| Nuclease-free water* | ▲ 10 µl; ● 0 µl |
| REPLI-g Midi Reaction Buffer | 29 µl |
| REPLI-g Midi DNA Polymerase | 1 µl |
| **Total volume** | **▲ 40 µl; ● 30 µl** |

* 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 25, 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 pages 17-19.
- High concentrations of heparin in blood samples can inhibit the REPLI-g reaction (see Troubleshooting Guide, page 23). 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* |
|---|---|
| DTT, 1 M | 5 µl |
| Reconstituted Buffer DLB† | 55 µl |
| **Total volume** | **60 µl** |

* Volumes given are suitable for up to 15 reactions.

† Reconstitution of DLB is described in the "Things to do before starting" section (above).

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 Table 10. 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 |
|---|---|
| Nuclease-free water | 10 µl |
| REPLI-g Midi Reaction Buffer | 29 µl |
| REPLI-g Midi DNA Polymerase | 1µl |
| **Total volume** | **40 µl** |

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 25, 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 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 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 re-amplify. |
| b) | Reaction temperature is too high | Check the incubator for correct reaction temperature (30°C) during the REPLI-g reaction. If cycler with heated lid is used set temperature to 70°C. As a control, the REPLI-g reaction can be performed at a lower temperature (e.g. 25-28°C) which should give the appropriate yield. |
| c) | Carryover of alcohol in isolated DNA sample | Residual alcohol in the DNA sample may reduce the yield of REPLI-g reactions. When using 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. |

**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.<br>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**

| | |
|---|---|
| Sensitive downstream applications 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. |

## 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 anticoagulant | 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

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.

**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 (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.

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### Cited 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.

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|---|---|---|
| **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 x 50 µl whole genome amplification reactions | 150243 |
| REPLI-g FFPE Kit (100) | DNA Polymerase, Buffers, and Reagents for 100 x 50 µl whole genome amplification reactions | 150245 |
| **REPLI-g Screening Kit — for high-throughput manual or automated whole genome amplification from small or precious samples** | | |
| REPLI-g Screening Kit (1000) | For 1000 whole genome amplification reactions: REPLI-g Mini DNA Polymerase, Buffers | 150127 |

# Ordering Information

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| **REPLI-g Service — large scale highly uniform whole genome amplification and quality assessment from limited or precious samples** | | |
| REPLI-g Service, Single Tube (100 µg) | Whole Genome Amplification Service from single tubes or <84 samples in microplates, 100 µg scale | 805923 |
| REPLI-g Service, Single Tube (500 µg) | Whole Genome Amplification Service from single tubes or <84 samples in microplates, 500 µg scale | 805925 |
| REPLI-g Service (100 µg) | Whole Genome Amplification Service from microplates, 100 µg scale | 805943 |
| REPLI-g Service (500 µg) | Whole Genome Amplification Service from microplates, 500 µg scale | 805945 |
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| 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 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 x 1.7 ml QuantiTect Probe PCR Master Mix, 2 x 2.0 ml RNase-free water | 204343 |

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|---|---|---|
| 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 x 2 ml RNase-Free Water | 204543 |
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## Notes

**Notes**

## Notes

Trademarks: QIAGEN®, QIAamp®, QuantiTect®, REPLI-g® (QIAGEN Group); PicoGreen®, Quant-iT™ (Molecular Probes); ROX™ (Applera Corporation); TECAN® (Tecan Group AG).

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