

## **User-Developed Protocol:**

### **Whole genome amplification from genomic DNA using the REPLI-g<sup>®</sup> Midi Kit with increased sample volumes**

This procedure has been adapted by customers and is for whole genome amplification from genomic DNA using the REPLI-g Midi Kit with larger sample volumes than recommended in the *REPLI-g Mini/Midi Handbook*. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

**IMPORTANT:** Please consult the “Safety Information” and “Important Notes” sections in the *REPLI-g Mini/Midi Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

#### **Equipment and reagents to be supplied by user**

- Microcentrifuge tubes
- Microcentrifuge
- Water bath or heating block
- Vortexer
- Pipets and pipet tips
- Ice
- Nuclease-free water

#### **Important points before starting**

- This protocol is optimized for whole genome amplification from >10 ng genomic DNA template. The template DNA should be suspended in TE buffer. Smaller amounts (1–10 ng) of starting material can be used if the DNA is of sufficient quality.
- For best results, the template DNA should be >2 kb in length with some fragments >10 kb.
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 5). All other components can be thawed at room temperature.

## 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<sub>2</sub>.
- 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. Place 15 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).

### 2. Add 2 µl Buffer DLB to the DNA. Mix by vortexing and centrifuge briefly.

### 3. Incubate the samples at room temperature (15–25°C) for 3 min.

### 4. Add 3 µl Stop Solution to the samples. Mix by vortexing and centrifuge briefly.

### 5. Thaw REPLI-g Midi DNA Polymerase on ice. Thaw all other components at room temperature, vortex, and centrifuge briefly.

The REPLI-g Midi Reaction Buffer may form a precipitate after thawing. The precipitate will dissolve by vortexing for 10 s.

### 6. Prepare a master mix on ice according to Table 1. Mix and centrifuge briefly.

**IMPORTANT:** Add the master mix components in the order listed in Table 1. 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 the REPLI-g Midi DNA Polymerase.

**Table 1. Preparation of Master Mix**

Component	Volume/reaction
REPLI-g Midi Reaction Buffer	29 µl
REPLI-g Midi DNA Polymerase	1 µl
<b>Total volume</b>	<b>30 µl</b>

### 7. Add 30 µl of the master mix to 20 µl denatured DNA (step 4).

### 8. 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 11.

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- 9. Inactivate REPLI-g Midi DNA Polymerase by heating the sample at 65°C for 3 min.**
- 10. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.**

DNA amplified using the REPLI-g Midi 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.

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Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from [www.qiagen.com/Support/MSDS.aspx](http://www.qiagen.com/Support/MSDS.aspx).

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