

User-Developed Protocol:

Whole genome amplification from genomic DNA using the REPLI-g® 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 CO₂.
- 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
Total volume	30 µl

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.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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

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