User-Developed Protocol:

Whole genome amplification of 20 µg from genomic DNA using the REPLI-g® Midi Kit

This procedure has been adapted by customers and is for whole genome amplification from genomic DNA using the REPLI-g Midi Kit with 20 µg yield. **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 (15–25˚C).

User-developed protocol

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).**
   Note: 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 reactions.*

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 reactions.*

2. **Place 2.5 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).
3. **Add 2.5 µl Buffer D1 to the DNA. Mix by vortexing and centrifuge briefly.**
4. **Incubate the samples at room temperature (15–25°C) for 3 min.**
5. **Add 5 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, and 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 3. Mix and centrifuge briefly.**

   Important: Add the master mix components in the order listed in Table 3. 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 3. Preparation of Master Mix

Component	Volume/reaction
REPLI-g Midi Reaction Buffer	14.5 µl
REPLI-g Midi DNA Polymerase	0.5 µl
Total volume	**15 µl**

8. **Add 15 µl of the master mix to 10 µl 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 11.

10. **Inactivate REPLI-g Midi DNA Polymerase by heating the sample at 65°C for 3 min.**
11. **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.