User-Developed Protocol:

Whole genome amplification from genomic DNA in 96-well format using the REPLI-g® Midi Kit

This procedure has been adapted by customers and is for whole genome amplification from genomic DNA in 96-well format using the REPLI-g Midi Kit. **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
- Ice
- Nuclease-free water
- 96-well plates
- Pipets and pipet tips; multichannel with variable tip spacing are recommended for efficient sample processing. The pipet requires a minimum capacity of 30 µl (step 3) and a maximum capacity of 650 µl per tip (step 6).
- Recommended: reservoirs for use with multichannel pipets

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).
- Buffer D3 should not be stored longer than 3 months.

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 D3 (denaturation buffer) for the total number of whole genome amplification reactions (see Table 1).**

   Note: The total volume of Buffer D3 given in Table 1 is suitable for 96 REPLI-g Midi reactions.

Table 1. Preparation of Buffer D3

Component	Volume*
Reconstituted Buffer DLB†	110 µl
Nuclease-free water	290 µl
Total volume	**400 µl**

** Volumes given are suitable for up to 96 reactions.*

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

2. **Place 3 µl template DNA into each individual well of a 96-well plate.**

   The amount of template DNA should be >10 ng.

3. **Add 2 µl Buffer D3 to the DNA. Seal the 96-well plate, mix, and centrifuge briefly.**
4. **Incubate the samples at room temperature (15–25°C) for 5 min.**
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 2. Mix and centrifuge briefly.**

   Important: Add the master mix components in the order listed in Table 2. 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
Stop Solution	75 µl
Nuclease-free water	1525 µl
REPLI-g Midi Reaction Buffer*	2900 µl
REPLI-g Midi DNA Polymerase	100 µl
Total volume	**4600 µl**

** After addition of REPLI-g Midi Reaction Buffer, briefly vortex and centrifuge the mixture before addition of REPLI-g Midi DNA Polymerase.*

7. **Add 44 µl of the master mix to 5 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 9.

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 Mini 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.