Quick-Start Protocol

April 2016

REPLI-g® Midi Kits

REPLI-g Midi Kits (cat. nos. 150043 and 150045) should be stored at –30 to –15°C for up to 6 months if not otherwise stated on label. For longer storage, the kits should be stored at –70°C. Reconstituted Buffer DLB can be stored for 6 months at –20°C if not otherwise stated on label. Buffers D1, N1 and D2 should not be stored longer than 3 months if not otherwise stated on label.

Further information

- *REPLI-g Mini/Midi Handbook*: **www.qiagen.com/HB-0469**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Amplification of purified genomic DNA

Notes 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 best results, template DNA should be >2 kb in length with some fragments >10 kb.
- Typical DNA yields from a REPLI-g Midi Kit reaction are approximately 40 µg per 50 µl reaction.
- Add 500 µl nuclease-free water to Buffer DLB, mix well and centrifuge briefly.
- Thaw REPLI-g Midi DNA Polymerase on ice.
- ▲ denotes instructions for 2.5 µl template genomic DNA.
- ● denotes instructions for 5 µl template genomic DNA.

1. Prepare sufficient Buffer D1 and Buffer N1 for the total number of amplification reactions (see Table 1).

Table 1. Preparation of Buffer D1 and Buffer N1

Component	Buffer D1*	Buffer N1*
Reconstituted Buffer DLB	9 µl	–
Stop solution	–	12 µl
Nuclease-free water	32 µl	68 µl
Total volume	**41 µl**	**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 tube. The amount of template DNA should be >10 ng.
3. Add ▲ 2.5 µl or ● 5 µl Buffer D1. Mix by vortexing and centrifuge briefly.
4. Incubate the samples at room temperature (15–25°C) for 3 min.
5. Add ▲ 5 µl or ● 10 µl Buffer N1. Mix by vortexing and centrifuge briefly.
6. Prepare a master mix on ice according to Table 2. Add components in the order listed. Mix and centrifuge briefly.

Table 2. Preparation of Master Mix for genomic DNA

	Volume per reaction	
Component	**▲ 2.5 µl gDNA**	**● 5 µl gDNA**
Nuclease-free water	10 µl	–
REPLI-g Midi Reaction Buffer	29 µl	29 µl
REPLI-g Midi DNA Polymerase	1 µl	1 µl
Total volume	**40 µl**	**30 µl**

7. Add ▲ 40 µl or ● 30 µl master mix to ▲ 10 µl or ● 20 µl denatured gDNA, from step 5.
8. Incubate at 30°C for 8–16 h.
9. Inactivate REPLI-g Midi DNA Polymerase by heating samples for 3 min at 65°C.

10. If performing PCR analysis, dilute amplified DNA 1:100 with TE, and use 3 µl of diluted DNA for each PCR.

    Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A of the *REPLI-g Mini/Midi Handbook* for an accurate method of quantifying REPLI-g amplified DNA.

11. Store amplified DNA at 4°C for short-term storage, or –20°C for long-term storage.

    We recommend storage at a concentration of at least 100 ng/µl.

Amplification of genomic DNA from blood or cells

Notes before starting

- Add 500 µl nuclease-free water to Buffer DLB, mix well and centrifuge briefly.
- Thaw REPLI-g Midi DNA Polymerase on ice.
- The protocol is optimized for 0.5 µl whole blood or cell material.
- Cell concentration should be >600 cells/µl.
- High concentrations of heparin in blood can inhibit the REPLI-g reaction.
- 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**.

1. Prepare sufficient Buffer D2 for the total number of amplification reactions (see Table 3).

Table 3. Preparation of Buffer D2

Component	Volume*
Reconstituted Buffer DLB	55 µl
Dithiothreitol (DTT), 1 M	5 µl
Total volume	**60 µl**

* Volumes given are suitable for up to 15 reactions.

2. Mix 2.5 µl PBS with 0.5 µl cell material or blood in a microcentrifuge tube.
3. Add 3.5 µl Buffer D2. Mix by vortexing and centrifuge briefly.

4. Incubate the samples on ice for 10 min.
5. Add 3.5 µl Stop Solution. Mix by vortexing and centrifuge briefly.
6. Prepare a master mix on ice according to Table 4. Add components in the order listed. Mix and centrifuge briefly.

Table 4. Preparation of Master Mix for blood or cells

Component	Volume per reaction
Nuclease-free water	10 µl
REPLI-g Midi Reaction Buffer	29 µl
REPLI-g Midi DNA Polymerase	1 µl
Total volume	**40 µl**

7. Add 40 µl master mix to 10 µl denatured DNA from blood or cells from step 5.
8. Incubate at 30°C for 8–16 h.
9. Inactivate REPLI-g Midi DNA Polymerase by heating samples for 3 min at 65°C.
10. If performing PCR analysis, dilute amplified DNA 1:100 with TE and use 3 µl of diluted DNA for each PCR.

    Optical density (OD) measurements do not accurately quantify double-stranded DNA. See Appendix A of the *REPLI-g Mini/Midi Handbook* for an accurate method of quantifying REPLI-g amplified DNA.
11. Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.

    We recommend storage at a concentration of at least 100 ng/µl.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

Trademarks: QIAGEN®, Sample to Insight®, REPLI-g® (QIAGEN Group). 1102265 04/2016 HB-0608-002 © 2016 QIAGEN, all rights reserved.