

## **User-Developed Protocol:**

### **Whole genome amplification from dried blood spots using the REPLI-g<sup>®</sup> Midi Kit**

This procedure has been adapted by customers and is for whole genome amplification from dried blood spots using the REPLI-g Midi Kit. **The procedure has not been thoroughly tested and optimized by QIAGEN.**

**Note:** This protocol may be adapted for use with the REPLI-g Mini Kit, using the same reaction setup. In rare cases, potential inhibitors present in the starting material may have inhibitory effects on amplification when using the REPLI-g Mini Kit. In these cases, we recommend using the REPLI-g Midi Kit. Alternatively, upstream genomic DNA purification can be performed (e.g., using a QIAamp<sup>®</sup> Kit) with subsequent whole genome amplification of the purified DNA following the standard protocol in the *REPLI-g Mini/Midi Handbook*.

**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
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)

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

- 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 7). All other components can be thawed at room temperature.
- Buffer D2 should not be stored longer than 3 months.
- 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).

### 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>.
- Set a water bath or heating block to 30°C.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.

### Procedure

1. Place a dried blood spot (2–3 mm diameter) into a microcentrifuge tube containing 500 µl TE buffer. Wash the blood spot by inverting the tube several times and incubate at room temperature (15–25°C) for 30 min.
2. Centrifuge the sample for 15 s and discard the supernatant. Rinse the blood spot with a further 500 µl TE buffer, centrifuge briefly, and discard the supernatant.
3. Place 40 µl TE buffer directly onto the blood spot in the microcentrifuge tube.
4. Prepare sufficient Buffer D2 (denaturation buffer) for the total number of whole genome amplification reactions (Table 1).

**Note:** The total volume of Buffer D2 given in Table 1 is suitable for up to 3 reactions.

**Table 1. Preparation of Buffer D2**

Component	Volume*
DTT, 1 M	10 µl
Reconstituted Buffer DLB <sup>†</sup>	110 µl
<b>Total volume</b>	<b>120 µl</b>

\* Volumes given are suitable for up to 3 reactions. Excess Buffer D2 can be stored at -20°C for up to 3 months.

<sup>†</sup> Reconstitution of DLB is described in the “Things to do before starting” section.

5. Add 40 µl Buffer D2 to each microcentrifuge tube containing blood spot in TE buffer. Mix by pipetting up and down 3 times and place on ice for 10 min.
6. Add 40 µl Stop Solution to each microcentrifuge tube containing lysed blood cells and mix by pipetting up and down 3 times.

**Note:** 10 µl lysed blood spot material is used in a 50 µl REPLI-g reaction.

7. 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.

**User-developed  
protocol**

**8. 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 spin down 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 2. Preparation of Master Mix**

<b>Component</b>	<b>Volume/reaction</b>
Nuclease-free water	10 $\mu$ l
REPLI-g Midi Reaction Buffer	29 $\mu$ l
REPLI-g Midi DNA Polymerase	1 $\mu$ l
<b>Total volume</b>	<b>40 <math>\mu</math>l</b>

**9. Add 40  $\mu$ l master mix to 10  $\mu$ l lysed and neutralized blood spot material (step 6). Mix well by vortexing for 10 s and centrifuge briefly.**

**10. 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.

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

**12. 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/ $\mu$ l.

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