User-Developed Protocol:

Whole genome amplification from flash-frozen tissue sections using the REPLI-g® Midi Kit

This procedure has been adapted by customers and is for whole genome amplification from flash-frozen tissue sections 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® 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 the 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 5). 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).

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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.

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

Procedure

1. **Place flash-frozen tissue section (approximately 2 mm^3) in a microcentrifuge containing 10 µl TE buffer. Incubate at room temperature (15–25°C) for 10 min vortexing occasionally.**
2. **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 6 reactions.

Table 1. Preparation of Buffer D2

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

* Volumes given are suitable for up to 6 reactions. Excess Buffer D2 can be stored at -20°C for up to 3 months.

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

3. **Add 10 µl Buffer D2 to each microcentrifuge tube containing flash-frozen tissue. Mix by vortexing briefly and place on ice for 30 min.**
4. **Add 10 µl Stop Solution to each microcentrifuge tube containing lysed tissue and mix briefly by vortexing. Spin down the tissue debris by pulse centrifugation.**

   Note: 10 µl lysed and neutralized tissue cells are used in a 50 µl REPLI-g Midi reaction.

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 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.

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Table 2. Preparation of Master Mix

Component	Volume/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 lysed and neutralized tissue cells (step 4). Mix well by vortexing for 10 s and centrifuge briefly.**
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 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.