

**User-developed
protocol**

User-Developed Protocol:

Whole genome amplification from laser-microdissected cells using the REPLI-g[®] Midi Kit

This procedure has been adapted by customers and is for whole genome amplification from laser-microdissected cells 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 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 CO₂.
- 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. **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 15 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 15 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.

2. **Place 0.5 µl laser-microdissected cell material (>600 cells/µl) into a microcentrifuge tube.**
3. **Add 2.5 µl TE buffer to the sample.**
4. **Add 3.5 µl Buffer D2. Mix by vortexing and centrifuge briefly.**
5. **Incubate for 10 min on ice.**
6. **Add 3.5 µl Stop Solution to the lysed LMD cell material. Mix by vortexing and centrifuge briefly.**
Note: 10 µl lysed and neutralized laser-microdissected cells are used in a 50 µl REPLI-g Midi reaction (Step 9).
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.
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.

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Table 2. Preparation of Master Mix

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

9. Add 40 μ l master mix to 10 μ l laser-microdissected cells (step 6).

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/ μ l.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/Support/MSDS.aspx.

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