User-developed protocol

User-Developed Protocol:

Amplification of human mitochondrial DNA from cultured cells using the REPLI-g® Mitochondrial DNA Kit

This procedure has been adapted by customers and is for amplification of human mitochondrial DNA from cultivated cells using the REPLI-g Mitochondrial DNA 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 Mitochondrial DNA 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

Important points before starting

- This protocol is optimized for human mitochondrial genome amplification from >100 cells. The cells should be freshly collected in PBS or media. Fewer number of cell results in yields lower than 2 µg of amplified human mitochondrial DNA.
- REPLI-g Midi DNA Polymerase should be thawed on ice (see step 5). REPLI-g mt Reaction Mix should be thawed at room temperature.
- REPLI-mt Reaction Mix should be vortexed for at least 10 s before use to ensure thorough mixing.

Things to do before starting

- Set a water bath or heating block to 75°C
- Set a water bath or heating block to 33°C

Procedure

1. **Place 5 µl of cell sample into a microcentrifuge tube.**
   The number of cells should be >100. The cells should be collected in PBS or media.
2. **Add 15 µl Water (supplied) to the sample.**
3. **Add 29 µl REPLI-g mt Reaction Mix to the DNA. Mix by vortexing and centrifuge briefly.**
4. **Incubate the sample for 5 min at 75°C. Allow sample to cool down to room temperature (15–25°C).**
5. **Thaw REPLI-g Midi DNA Polymerase on ice.**
6. **Add 1 µl of REPLI-g Midi DNA Polymerase to the denatured DNA (from step 4). Mix and centrifuge briefly.**
7. **Incubate the sample at 33°C for 8–16 h.**
8. **Inactivate REPLI-g Midi DNA Polymerase by heating the sample for 3 min at 65°C.**
   Note: If the amplified DNA is to be analyzed using PCR, dilute the DNA after inactivation 1:1000 in water or TE buffer. Use 2–3 µl of the diluted DNA for each PCR. Optical density (OD) measurements do not accurately quantify double-stranded DNA. See REPLI-g Mitochondrial DNA Handbook, Appendix B, for an accurate way of quantifying REPLI-g amplified DNA.
9. **Store amplified DNA at 4°C for short-term storage or –20°C for long-term storage.**
   DNA amplified using the REPLI-g Mitochondrial DNA Kit should be treated as pure DNA with minimal freeze-thaw cycles.