User-Developed Protocol

Whole genome amplification of purified DNA samples using the REPLI-g® Cell WGA & WTA Kit

This procedure has been adapted by customers and is for parallel whole genome amplification using purified DNA and the REPLI-g Cell WGA & WTA Kit (cat. nos. 150052 and 150054).

It is optimized for use of nucleic acids from all vertebrate species (e.g., human, mouse, rat, sorted cells, tissue culture cells, or cells picked under the microscope) and cannot be used for nucleic acids isolated from cells that have been fixed using formalin or other cross-linking agents (e.g., single cell samples obtained by laser microdissection from formalin-fixed, paraffin-embedded [FFPE] tissues).

This protocol has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *REPLI-g Cell WGA & WTA Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate safety data sheets (SDSs), available from the product supplier. REPLI-g Cell WGA & WTA Kits are intended for research use. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- Water bath, thermo cycler, or heating block
- Vortexer
- Microcentrifuge tubes
- Microcentrifuge
- Ice
- Pipettes and pipette tips
- Nuclease-free water
- TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0)

Important points before starting

- If using REPLI-g Cell WGA & WTA Kits for the first time, read "Important Notes" in the *REPLI-g Cell WGA & WTA Handbook*.
- Potential inhibitors that are present in the starting material may have inhibitory effects on amplification. We recommend upstream nucleic acid purification by the QIAamp® Kits.
- Use intact DNA for whole-genome amplification (WGA) reactions, for highest sensitivity and reliability.
- For amplification of degraded DNA, higher amounts of nucleic acids are necessary.
- The amount of DNA that is necessary for WGA increases with the fragmentation degree of nucleic acids.
- For best amplification, the template should be >10 kb.

- An amount of DNA that corresponds to 25–1000 cells is optimal for WGA reactions using the REPLI-g Cell WGA & WTA Kit.
- Avoid DNA or RNA contamination of reagents, by using separate laboratory equipment (e.g., pipettes, filter pipette tips, reaction vials, etc.). Set up the REPLI-g Cell WGA & WTA Kit reaction in a location free of nucleic acids.
- DNA yields of approximately 10 µg will be present in negative (no template) controls because DNA is generated during REPLI-g reaction by primer multimer formation, which generates high-molecular-weight DNA. This DNA will not affect the quality of the actual sample and will not give a positive result in downstream assays.

Things to do before starting

- Vortex all buffers and reagents before use, to ensure thorough mixing.

Procedure

1. **Place 7 µl isolated DNA (containing > 75 pg DNA) into a microcentrifuge tube. If using less than 7 µl of DNA, add H20 sc to bring the volume up to 7 µl.**
2. **Add 3 µl NA Denaturation Buffer. Mix carefully by flicking the tube and centrifuge briefly.**

   Note: Ensure that no droplets stick to the wall of the tube above the meniscus.
3. **Incubate at 95°C for 3 min. Cool to 4°C.**

   Important: Aliquots cannot be stored at this point. They must be processed immediately using the “Protocol: Amplification of Genomic DNA” in the *REPLI-g Cell WGA & WTA Handbook*.
4. **Immediately perform WGA (see protocol “Amplification of Genomic DNA”, *REPLI-g Cell WGA & WTA Handbook*).**