User-Developed Protocol

Simultaneous WGA and WTA in one tube using REPLI-g® Cell WGA & WTA and purified nucleic acid

This protocol is optimized for simultaneous whole genome amplification (WGA) and whole transcriptome amplification (WTA) in one tube (whole nucleic acid amplification [WNA]) using purified nucleic acid samples and the REPLI-g Cell WGA & WTA Kit (cat. nos. 150052 and 150054). Potential inhibitors present in the starting material may have negative effects on amplification. We recommend upstream nucleic acid purification by the QIAamp® Kits. Use intact nucleic acids for WNA reaction for highest sensitivity and reliability. For amplification of degraded nucleic acid, higher amounts of nucleic acids are necessary. The amount of nucleic acid necessary for WNA increases with the fragmentation degree of nucleic acids.

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

IMPORTANT: Please consult the Safety Information and Important Notes sections in the *REPLI-g Cell WGA & WTA Kit 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.

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)

Note: This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important points before starting

- For best amplification, the DNA and the RNA template should be intact.
- After WNA, it is not possible to differentiate whether amplified DNA is derived from RNA or DNA.
- This protocol 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). The protocol 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).
- An amount of nucleic acids that corresponds to 25–1000 cells is optimal for WTA and 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 (NTC) 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.
- The WNA reaction is not suitable for the amplification of small RNA molecules, such as tRNAs or miRNAs.
- Although all sequences are well represented, the amplified cDNA does not contain full-length cDNAs. The amplification process is started by random-primed cDNA synthesis. Consequently, transcript sequences are amplified in pieces. When oligo dT primed amplification is performed, regions close to the 3' ends of poly A+ transcripts (approximately 700–1500 bp) will be overrepresented in the amplification product. Regions close to the 5' cap of the poly A+ transcript will be under represented for transcripts >1500 nt in length.

Things to do before starting

- The Quantiscript®/WGA ready mix, ligation mix, and REPLI-g SensiPhi® amplification mix described in the protocol must always be prepared fresh. They cannot be stored for later use.
- All buffers and reagents should be vortexed before use to ensure thorough mixing.
- Quantiscript RT enzyme mix, ligase mix, and REPLI-g SensiPhi DNA Polymerase should be thawed on ice. All other components can be thawed at room temperature (15–25°C).

For increased speed and convenience, all incubation steps of the protocol can be preprogrammed on a thermal cycler (Table 1).

Table 1. Thermal cycling parameters

Step*	Time	Temperature	Additional comments
QuantiScript/WGA Ready reaction	60 min	42°C	Add QuantiScript/WGA Ready mix prior to incubation (step 4)
	3 min	95°C	Stops reaction
Ligation	30 min	24°C	Add ligation mix prior to incubation (step 6)
	5 min	95°C	Stops ligation
Whole transcriptome amplification	2 h	30°C	Add REPLI-g SensiPhi amplification mix prior to incubation (step 8)
	5 min	65°C	Inactivates all enzymes.
		4°C	Cools amplified DNA.

*Set the heating lid to 50°C for all steps.

Procedure

1. **Place 7 µl nucleic acid solution (containing RNA and DNA) into a microcentrifuge tube. If using less than 7 µl of nucleic acid solution, add H_20 sc to bring the volume up to 7 µl.**

   Note: The mixture of RNA and DNA from the same sample material must contain both nucleic acids. Both nucleic acids must be represented as in ≥25 cells (~150 pg DNA, 250 pg RNA).

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.**

4. **Prepare Quantiscript/WGA Ready mix (Table 2). Add 10 µl Quantiscript/WGA Ready mix to the lysed cell sample, mix by vortexing, and centrifuge briefly.**

   Note: Quantiscript/WGA Ready mix must be prepared fresh.

   Important: To enrich the WNA amplification product for mRNA poly A+ sequences, omit Random Primer from the WNA mix.

   Important: If performing NGS, be aware that when amplifying total RNA (i.e., without enriching for mRNA poly A+), ribosomal RNA (rRNA) will be represented to the same level it is in the cell and will be detected with high coverage in the subsequent NGS run. Amplification of rRNA does not occur when amplifying mRNA-enriched (poly A+) RNA (by omitting the random primer). Note that the amplified product will be DNA; therefore, subsequent library preparation must be performed using a DNA library prep kit. Refer to Appendix A of the *REPLI-g Cell WGA & WTA Kit Handbook* for more information on using amplified DNA for NGS.

Table 2. Preparing Quantiscript/WGA Ready mix*

Component	Volume/reaction
RT/Polymerase Buffer	4 µl
gDNA Wipeout Buffer	2 µl
Random Primer	1 µl
Oligo dT Primer	1 µl
WGA Ready Enzmye	1 µl
Quantiscript RT Enzyme Mix	1 µl
Total volume†	**10 µl**

* Scale up accordingly if performing several reactions at one time.
† Mix by vortexing, and centrifuge briefly.

5. **Incubate at 42°C for 60 min. Stop the reaction by incubating at 95°C for 3 min, then cool on ice.**

6. **Prepare the ligation mix (Table 3). Add 10 µl ligation mix to the RT reaction from step 5. Mix by vortexing, and centrifuge briefly.**

   Important: When preparing the ligation mix, add the components in the order shown in Table 3.

   Note: The ligation mix must be prepared fresh.

Table 3. Preparing ligation mix*

Component	Volume/reaction
Ligase Buffer	8 µl
Ligase Mix	2 µl
Total volume†	**10 µl**

* Scale up accordingly if performing several reactions at one time.
† Mix by vortexing, and centrifuge briefly.

7. **Incubate at 24°C for 30 min. Stop the reaction by incubating at 95°C for 5 min.**
8. **Prepare REPLI-g SensiPhi amplification mix (Table 4). Add 30 µl REPLI-g SensiPhi amplification mix to the ligation reaction from step 7. Mix by vortexing, and centrifuge briefly.**

   Note: REPLI-g SensiPhi amplification mix must be prepared fresh.

Table 4. Preparing REPLI-g SensiPhi amplification mix*

Component	Volume/reaction
REPLI-g sc Reaction Buffer	29 µl
REPLI-g SensiPhi DNA Polymerase	1 µl
Total volume†	**30 µl**

* Scale up accordingly if performing several reactions at one time.
† Mix by vortexing, and centrifuge briefly.

9. **Incubate at 30°C for 2 h.**
10. **Stop the reaction by incubating at 65°C for 5 min.**
11. **If not being used directly, store the amplified DNA at –15°C to –30°C until required for downstream applications.**

    DNA amplified using the REPLI-g Cell WGA & WTA 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 the amplified DNA at a concentration of at least 100 ng/µls.

    Note: If analyzing the amplified DNA by real-time PCR, we recommend diluting the amplified DNA with water or TE buffer after inactivation to 1–3 ng/µl and using 2–3 µl of diluted DNA for real-time PCR.

    Note: NGS library preparation must be performed using a DNA library prep kit. Refer to Appendix A of *REPLI-g Cell WGA & WTA Kit Handbook* for more information on using amplified DNA for NGS. Due to the very high molecular weight of amplified DNA, a heating step (5 min 95°C, cool on ice) of diluted DNA prior to PCR may be beneficial for reproducible real-time PCR.

    Note: The high-molecular-weight DNA generated by random extension of primer-dimers in NTC will not affect the quality of downstream applications.
12. **Amplified DNA behaves like purified genomic DNA and has an approximate length of 2000–70,000 bp. It is highly suited for use in a variety of downstream applications, including NGS, array CGH, and quantitative PCR.**

    Note: Typical DNA yields are approximately 20 µg DNA per 60 µl reaction and should to be diluted appropriately. Optical density (OD) measurements overestimate REPLI-g amplified DNA. Refer to Appendix B, *REPLI-g Cell WGA & WTA Kit Handbook*, for an accurate method of quantifying REPLI-g amplified DNA. Alternatively, purify the reaction with a QIAamp Kit using the protocol “Purification of REPLI-g amplified DNA using the QIAamp

DNA Mini Kit" or purify the reaction with EtOH using the protocol "Purification of DNA amplified using REPLI-g Kits" in the *REPLI-g Cell WGA & WTA Kit Handbook*. Following purification, determine the amount of DNA using a standard OD measurement.