GE Healthcare
Life Sciences

Application note 29-0323-19 AA

A comparative performance evaluation of illustra™ Ready-To-Go™ GenomiPhi™ HY and illustra GenomiPhi HY DNA amplification kits

Abstract

We have produced an ambient-temperature-stable version of GenomiPhi for whole genome amplification (WGA) called illustra Ready-To-Go GenomiPhi HY DNA Amplification Kit to simplify product handling and storage, improve consistency, and reduce the risk of contamination. For comparative performance analysis, we amplified human genomic DNA (gDNA) using Ready-To-Go GenomiPhi HY and GenomiPhi HY DNA amplification kits. The results show that Ready-To-Go GenomiPhi HY produced consistent and improved yields over GenomiPhi HY DNA Amplification Kit. We used WGA products from both kits in downstream applications such as endpoint and quantitative PCR.

Introduction

GenomiPhi uses Phi29 DNA polymerase and random hexamer primers for isothermal WGA, generating microgram quantities of high molecular weight DNA from nanogram quantities of starting material. Phi29 DNA polymerase offers high fidelity during DNA replication due to its proofreading 3'–5' exonuclease activity (1, 2). We designed Ready-To-Go GenomiPhi HY DNA Amplification Kit to offer you high yield whole genome amplification with a simplified workflow plus long-term ambient-temperature stability in a Ready-To-Go format.

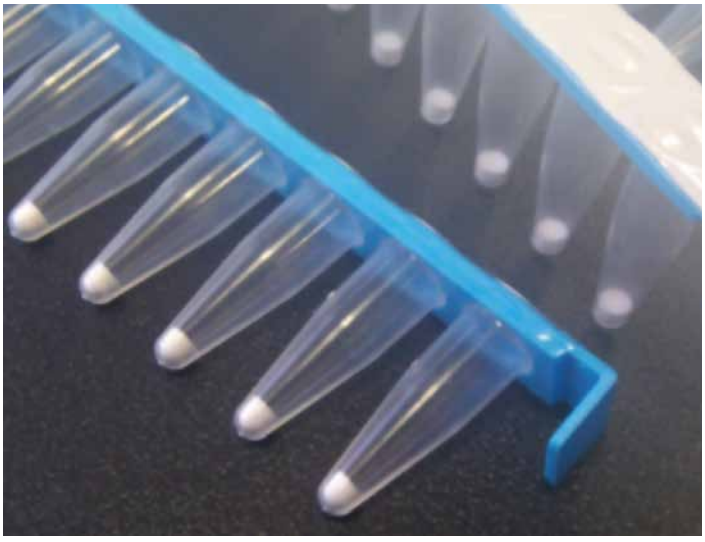

Fig 1. Ready-To-Go format provides reproducible and reliable performance in a simple to use ambient temperature stable format.

Materials and methods

GenomiPhi amplification

Purified human gDNA (10 ng) and Lambda DNA (10 ng) were amplified using the standard protocols for each of the two GenomiPhi HY kits (Tables 1 and 2). DNA yield from each amplification reaction was quantitated using Quant-IT™ PicoGreen™ dsDNA quantitation reagent. The samples were also taken at 30 min intervals and quantitated with PicoGreen assay to monitor amplification yield throughout the recommended 4 h incubation.

Table 1. Overview of standard protocol for GenomiPhi HY DNA Amplification Kit

GenomiPhi HY DNA Amplification Kit	
Remove from freezer and thaw on ice	
Input DNA (10 ng)	2.5 µl
Sample buffer	22.5 µl
Heat to 95°C 3 min, cool to 4°C on ice	
Reaction buffer	22.5 µl
Enzyme mix	2.5 µl
Incubate at 30°C	4 h
Inactivate enzyme at 65°C for 10 min	
Typical product yield	40 to 50 µg

Table 2. Overview of standard protocol for Ready-To-Go GenomiPhi HY DNA Amplification Kit

Ready-To-Go GenomiPhi HY DNA Amplification Kit	
Remove from shelf	
Input DNA (10 ng)	2.5 µl
PCR-grade water	22.5 µl
2x denaturation buffer	25 µl
Heat to 95°C 3 min, cool to 4°C on ice	
Add sample to cake	50 µl
Incubate at 30°C	4 h
Inactivate enzyme at 65°C for 10min	
Typical product yield	40 to 60 µg

Negative [i.e., no template control (NTC)] amplifications in which template DNA was substituted with water were carried out in parallel. A schematic representation of GenomiPhi Ready-To-Go HY DNA Amplification Kit workflow is shown in Figure 1.

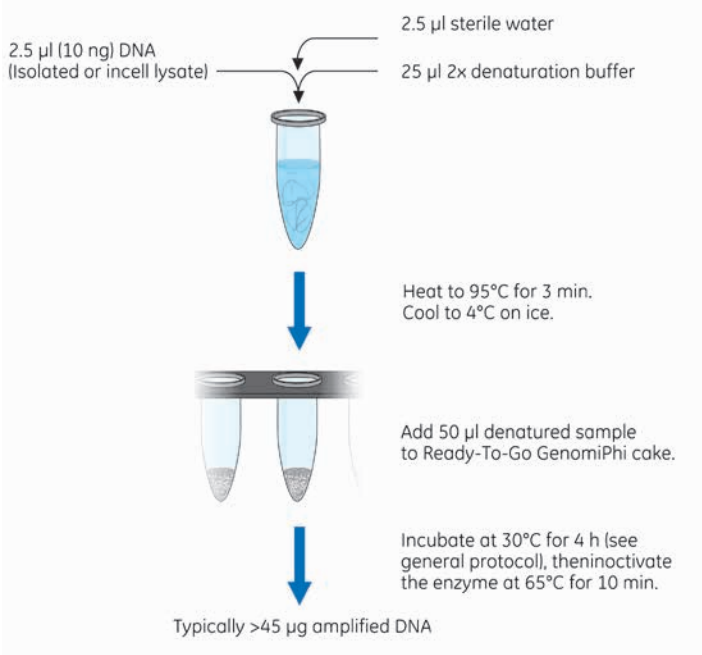

Fig 2. Workflow for Ready-To-Go GenomiPhi HY DNA Amplification kit.

Endpoint PCR analysis

Endpoint PCR amplification of the human p53 gene was performed on amplified human gDNA and corresponding WGA negative control reactions from both GenomiPhi kits. Each GenomiPhi amplification reaction was carried out according to the standard protocol using 10 ng of input DNA. The products were quantitated and diluted prior to PCR amplification. Each PCR was prepared using two illustra PureTaq™ Ready-To-Go PCR beads, 1 µl each of 10 µM forward and reverse p53 primers, 4 ng of DNA, plus PCR-grade water to a total volume of 50 µl. PCR amplifications were performed using a PTC-200 thermal cycler (MJ Research) with the following PCR cycling conditions: 95°C for 3 min, followed by 35 cycles of 95°C for 30 s; 55°C for 1 min; 72°C for 1 min.

Quantitative PCR analysis

Quantitative PCR was performed for individual loci on 5 separate chromosomes for both Ready-To-Go GenomiPhi HY amplified DNA and unamplified human DNA. Reactions were carried out with 5 µl of template at concentrations of 3 ng/µl, 0.3 ng/µl and 0.03 ng/µl for primer set 1 or 3 ng/µl and 0.3 ng/µl for primer set 2, 15, 18 and 22. Ready-To-Go GenomiPhi HY negative (no template) control WGA reactions were also included in the study. Quantitative PCR was performed on an ABI PRISM® 7900HT Sequence Detection System with GelStar nucleic acid stain.

Results and discussion

GenomiPhi DNA yield

DNA quantitation after amplification of human gDNA or Lambda DNA using Ready-To-Go GenomiPhi HY DNA Amplification Kit showed a slightly increased yield compared to GenomiPhi HY DNA Amplification Kit for both human genomic and lambda DNA (Fig 3).

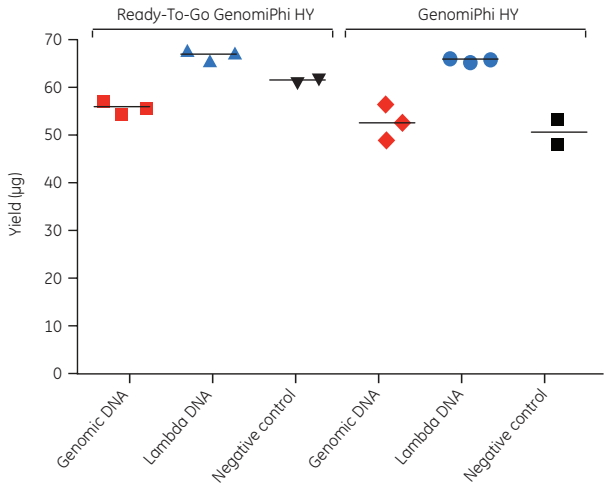

Fig 3. Determination of the yield of human gDNA or Lambda DNA amplified with either Ready-To-Go GenomiPhi HY or GenomiPhi HY DNA amplification kits.

Agarose gel analysis of amplification reactions (Fig 4) confirmed the presence of high molecular weight DNA in all the reactions.

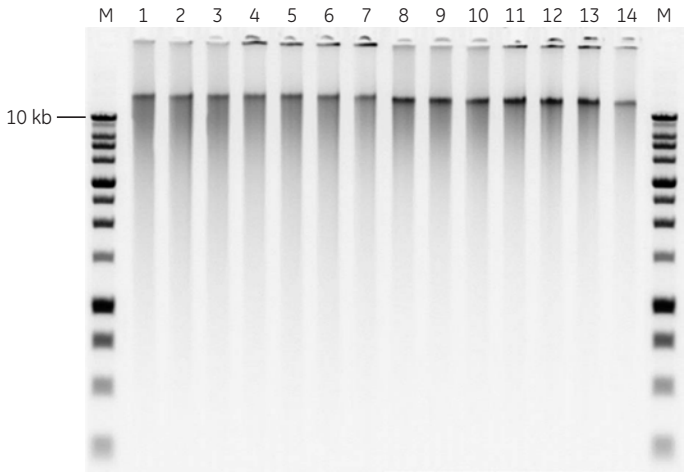

Fig 4. Agarose gel (1% agarose, 1x TAE) showing amplified human gDNA or Lambda DNA using Ready-To-Go GenomiPhi HY (lanes 1to 3 - gDNA; lanes 4 to 6 - Lambda DNA) or liquid GenomiPhi HY (lanes 8 to 10 – gDNA; lanes 11 to 13 - Lambda DNA). Negative (no template) controls were run for Ready-To-Go GenomiPhi HY (lane 7) and liquid GenomiPhi HY (lane 14), M = 1 kb ladder.

The yield from Ready-To-Go GenomiPhi HY reactions was determined at 30 min intervals using a PicoGreen assay. The amplification reaction reached a plateau at the end of the recommended 4 h incubation period with some background amplification in the negative controls (Fig 5).

Note: Template-independent background is a known characteristic of GenomiPhi HY (and Phi29-based WGA in general) under certain conditions. This is also observed in Ready-To-Go GenomiPhi HY where no template DNA is added, but this does not affect performance.

GenomiPhi HY kits are especially formulated at elevated reagent concentrations to deliver very high DNA yields. As a result, GenomiPhi HY kits will generate background in the absence of DNA template due to nonspecific interactions between the polymerase and unoccupied primers. In the presence of denatured DNA template, amplification strongly favors complementary strand synthesis from the added template thus suppressing the generation of any significant background.

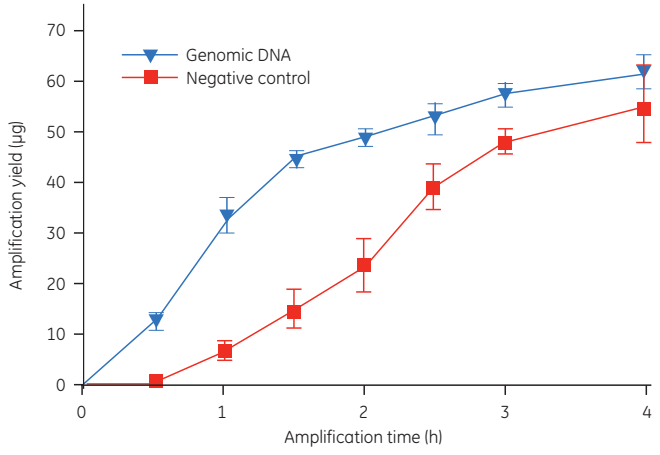

Fig 5. Time course of gDNA (10 ng) amplification with Ready-To-Go GenomiPhi HY Amplification Kit. Water was substituted for template DNA in the negative control reactions.

Endpoint PCR analysis

The expected 1.1kb p53 PCR product was amplified from both the GenomiPhi HY and Ready-To-Go GenomiPhi HY WGA samples (Fig 6). No product was generated in the WGA negative (no template) control reactions.

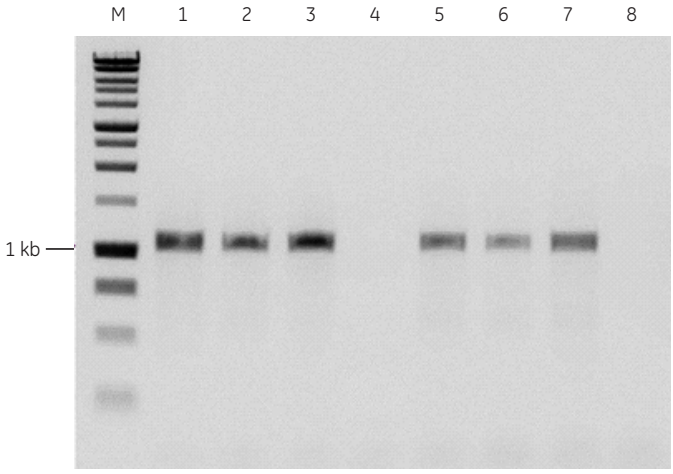

Fig 6. Agarose gel (1% agarose, 1x TAE) showing the expected 1.1 kb p53 PCR product for GenomiPhi HY WGA reactions (lanes 1 to 3) or Ready-To-Go GenomiPhi HY WGA reactions (lanes 5 to 7). WGA negative (no template) controls for GenomiPhi HY and Ready-To-Go GenomiPhi HY showed no PCR product (lanes 4 and 8), M = 1 kb ladder.

qPCR analysis

Quantitative PCR analysis across five randomly chosen genomic loci on different human chromosomes was carried out to compare representation between Ready-To-Go GenomiPhi HY amplified DNA and unamplified DNA. The loci on all five chromosomes were successfully amplified from both templates (Fig 7). The Ct values for corresponding targets were very similar ($\bar{x}$ = 0.8, σ = 0.5). We did not observe any PCR amplification from the negative (no template) control WGA reactions.

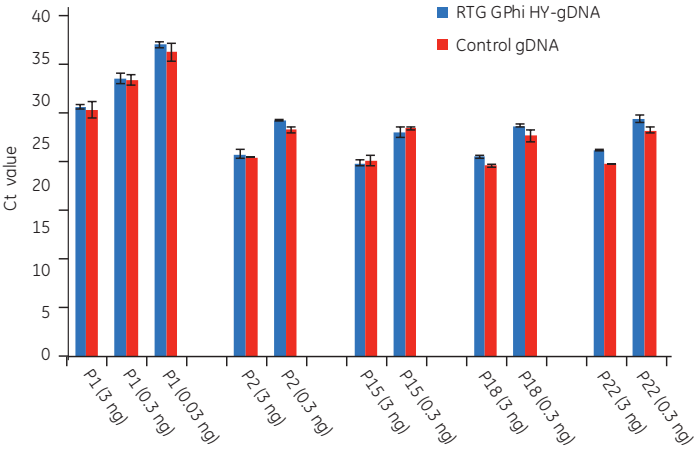

Fig 7. Comparison of Ct values between Ready-To-Go GenomiPhi HY DNA and unamplified DNA control from qPCR of individual loci on five different chromosomes (1, 2, 15, 18, and 22). We included template dilutions to show the response to changes in copy number.

Conclusions

The incorporation of a Ready-To-Go format into the design of Ready-To-Go GenomiPhi HY DNA Amplification Kit has resulted in several advantages with respect to workflow and handling, and a significant reduction in the overall process time. The drawback of GenomiPhi HY and indeed other DNA amplification kits in liquid format is the need to keep them frozen to maintain performance. Apart from the inconvenience of freezer storage, the reagents must be thawed carefully on ice, mixed for use, and returned promptly to storage. The ambient-temperature stability of Ready-To-Go GenomiPhi HY removes these requirements.

The addition of denatured DNA directly to the stabilized reaction mix of Ready-To-Go GenomiPhi HY DNA Amplification Kit initiates the amplification reaction thus eliminating the setup stage involved with GenomiPhi HY DNA Amplification Kit.

In summary:

- We have used GenomiPhi HY and Ready-To-Go GenomiPhi HY DNA amplification kits to successfully amplify human gDNA for typical downstream applications
- Using PCR primers specific for the p53 tumor suppressor and the amplified product from GenomiPhi HY and Ready-To-Go GenomiPhi HY DNA amplification kits as templates, we obtained the expected 1.1 Kb product in a PCR
- Comparisons using qPCR showed similar representation of loci between amplified DNA from Ready-To-Go GenomiPhi HY and nonamplified genomic DNA

References

1. Estaban, J.A. *et al.*, Fidelity of phi29 DNA polymerase, *J. Biol. Chem.* **268,** 2719–2726 (1993).
2. Nelson, J.R. *et al;* TempliPhi, Phi29 DNA polymerase based rolling circle amplification of templates for DNA Sequencing, *BioTechniques* **32**, S44-S47 (2002).

Ordering information

Product	Quantity	Code number
illustra Ready-To-Go GenomiPhi HY DNA Amplification Kit	24 reactions	25-6603-24
illustra Ready-To-Go GenomiPhi HY DNA Amplification Kit	96 reactions	25-6603-96
illustra Ready-To-Go GenomiPhi HY DNA Amplification Kit	5 × 96 reactions	25-6603-97
illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit	24 reactions	25-6601-24
illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit	96 reactions	25-6601-96
illustra Ready-To-Go GenomiPhi V3 DNA Amplification Kit	5 × 96 reactions	25-6601-97

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/genomiphi

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

imagination at work

29-0223-19 AA 11/2012