Genomic DNA prep for next generation sequencing, using GenomiPhi™

A method is described for genomic DNA preparation using Ready-To-Go™ GenomiPhi, which is stable at ambient temperature, greatly simplifying sample handling in genomics workflows, such as that for next generation sequencing (NGS).

Introduction

GenomiPhi is highly useful if limited DNA samples are available, or there is a need to simply and rapidly stock up on genomic DNA prior to techniques such as sequencing, genotyping or array CGH. Based on Phi29 DNA polymerase, GenomiPhi is available in Ready-To-Go (RTG™) room-temperature stable format, as well as the classic liquid format. RTG GenomiPhi has higher yield than the liquid format, giving high quality, amplified DNA, representative of the whole genome, in < 2 h.

The DNA can be used not only for NGS, but also for array CGH, high-throughput genotyping, DNA archiving, PCR, restriction digests, hybridisation and cloning. Genomic DNA prep to downstream application result can be achieved in one day, with a one tube, simple protocol for all sample types.

Overview of Ready-To-Go GenomiPhi Phi29 polymerase technology

Genomic DNA from limited samples, such as buccal swabs and FTA™/Guthrie card blood, can be amplified using Phi29 DNA polymerase. In this isothermal technique, random hexamers prime denatured DNA to form complementary strands (Fig 1). These are displaced and become templates for further rounds of amplification (1). This multiple strand displacement reaction results in microgram quantities of genomic DNA from nanogram input. It is amenable to automation and does not require specific primers or a thermal cycler. DNA from FTA or Guthrie card dried blood spots, buccal swabs, and other scarce or precious samples can be amplified by boiling samples in water followed by standard PCR or whole genome amplification (WGA).

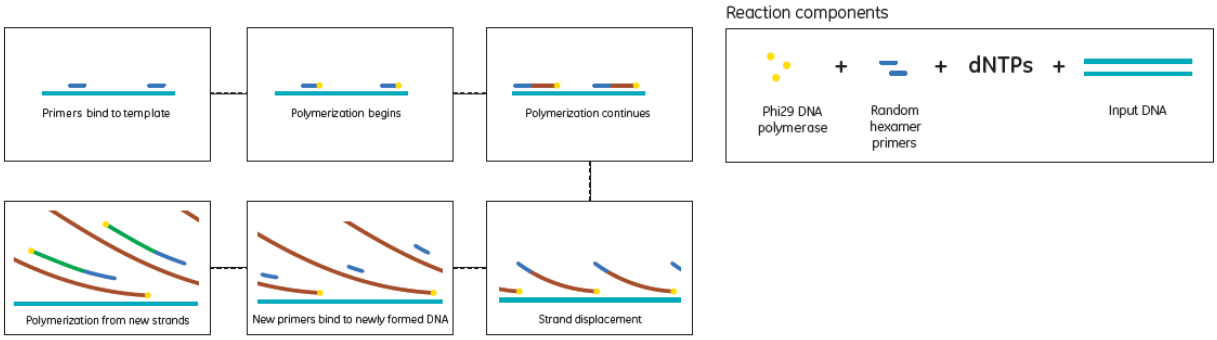

Fig 1 Schematic showing the GenomiPhi method of genomic DNA amplification

DNA amplification results with illustra™ GenomiPhi DNA Amplification Kits

Amplified genomic DNA can be used in the same applications as unamplified genomic DNA. For WGA, common applications include NGS, library construction, forensic analysis (e.g. DNA fingerprinting), genotyping (e.g. SNP analysis), PCR analysis, DNA cloning, CGH, whole genome DNA sequencing, HLA typing, and loss of heterozygosity (LOH). Microgram quantities of genomic DNA can be amplified from small amounts of input DNA using GenomiPhi.

Figure 2 shows an overview of the protocol for whole genome amplification using the Ready-To-Go GenomiPhi V3 DNA Amplification Kit. DNA is briefly heat-denatured in denaturation buffer then cooled. This is added to the freeze-dried cake which contains DNA polymerase, random hexamers, nucleotides, salts and buffers. Isothermal amplification proceeds at 30°C for 1.5 hours. After amplification the enzyme is heat inactivated during a 10 minute incubation at 65°C.

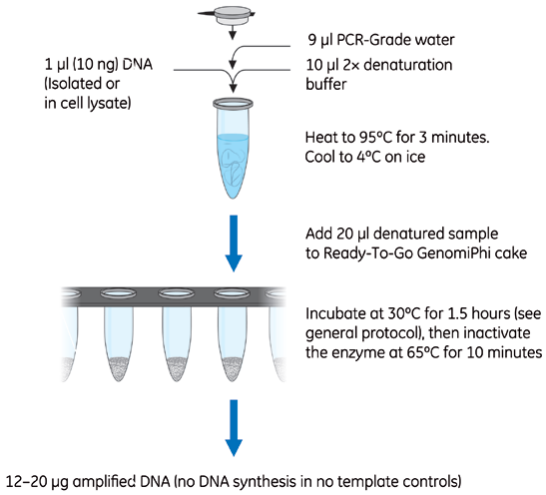

Fig 2 Ready-To-Go GenomiPhi V3 protocol for genomic DNA amplification

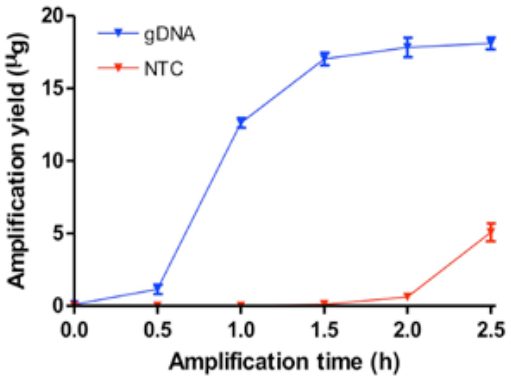

Fig 3 – Typical amplification kinetics with Ready-To-Go GenomiPhi V3 DNA Amplification Kit

Typical amplification kinetics with Ready-To-Go GenomiPhi V3 DNA Amplification Kit are shown in Figure 3. Microgram quantities of DNA are generated from nanogram amounts of starting material in 1.5 hours. Typical DNA yields from a Ready-To-Go GenomiPhi V3 DNA Amplification Kit reaction are >12–20 µg per 20 µl reaction when starting with 10 ng of purified DNA. Kinetics will vary if crude or un-quantified samples are amplified. Increased reaction times (2 hours) may be helpful for samples such as crude blood or buccal swabs. Control reactions without added template DNA do not produce any product during 1.5 h reactions. The average product length is greater than 10 kb. DNA replication is extremely accurate due to the proofreading 3'–5' exonuclease activity of the DNA polymerase.

The negative control reactions did not show any DNA synthesis during the 90 min incubation period for both kits. Agarose gel analysis of amplification reactions confirmed the presence of high molecular weight DNA in all the reactions containing template DNA (Fig 4a). The expected 1.1kb p53 PCR product was amplified from both GenomiPhi V2 and Ready-To-Go GenomiPhi V3 WGA samples (Fig 4b).

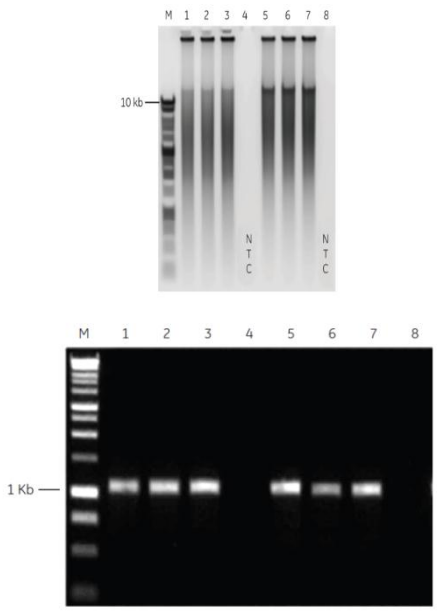

Figure 4 (a) High molecular weight DNA was confirmed in all reactions containing template DNA (GenomiPhi V2: lanes 1-3; RTG GenomiPhi V3: lanes 5-7, NTC: no template control). (b) Agarose gel of PCR results on GenomiPhi V2 (1-3) and RTG GenomiPhi V3 (5-7). Lanes. No product was generated in the WGA negative (no template) control reactions (4,8).

The GenomiPhi DNA can then be effectively used to simplify the preparation of genomic DNA for NGS, and the DNA can be used directly in the protocol.
GenomiPhi V2 is routinely used to provide sufficient genomic DNA to be used in protocols for NGS platforms including those of illumina™ and Roche™.
More details, hints and tips on how to use GenomiPhi can be found in the Handbook: Nucleic Acid Sample Preparation for Downstream Analysis 28-9624-00, downloadable from www.gelifesciences.com.

Product Description	Cat. No.
RTG GenomiPhi V3 Kit 24 rxns	25-6601-24
RTG GenomiPhi V3 Kit 96 rxns	25-6601-96
RTG GenomiPhi V3 Kit 480 rxns	25-6601-97
RTG GenomiPhi HY Kit 24 rxns	25-6603-24
RTG GenomiPhi HY Kit 96 rxns	25-6603-96
RTG GenomiPhi HY Kit 480 rxns	25-6603-97
GenomiPhi V2 DNA Amplification Kit 25 rxns	25-6600-30
GenomiPhi V2 DNA Amplification Kit 100 rxns	25-6600-31
GenomiPhi V2 DNA Amplification Kit 500 rxns	25-6600-32
GenomiPhi HY DNA Amplification Kit 25 rxns	25-6600-22
GenomiPhi HY DNA Amplification Kit 100 rxns	25-6600-20
GenomiPhi HY DNA Amplification Kit 1000 rxns	25-6600-25

Bibliography

Detter, J. C. *et al*. Isothermal strand-displacement amplification applications for highthroughput genomics. *Genomics* **80**, 691–698 (2002).