DNA purification through amplification: Use of Phi29 DNA polymerase to prepare DNA for genomic analyses

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Multiple-primed rolling circle amplification (RCA) and linear amplification using Phi29 DNA polymerase is a simple, reliable alternative to traditional DNA isolation methods. The enzyme, available in TempliPhi™ and GenomiPhi™ DNA Amplification Kits, produces high-quality DNA, from small amounts of starting material, that can be used directly, without purification, in downstream genomic analysis applications. The method is simple to automate, and as the yield is consistent, downstream applications can be easily optimized.

Introduction
DNA isolation is a fundamental step in genetic analysis, and obtaining high-quality DNA is vital for success. Purification of genomic DNA can be labor intensive and involve hazardous organic chemicals. Limited amounts of sample add to the difficulties in performing extensive genetic studies. Despite advances in other molecular biology techniques, the most common method for purification of plasmid DNA from crude lysates is still alkaline lysis (1). The method is simple but is not easily automated, and DNA yields can be highly variable.

An alternative approach to DNA isolation is to use multiple-primed rolling circle amplification (RCA) and linear amplification (2). The methods use a highly processive, strand displacing enzyme, Phi29 DNA polymerase, to amplify DNA. The enzyme, from the bacteriophage Phi29, can incorporate 70 000 nucleotides in a single binding event (3).

Rolling circle and linear amplification are isothermal reactions and do not require thermal cycling to denature the DNA strands between rounds of amplification as in PCR®. When Phi29 DNA polymerase encounters the synthesized strand, it simply displaces it, generating single stranded DNA that is available for further primer annealing. Phi29 DNA polymerase has a 3’—5’ exonuclease activity giving it an error rate of only 1 in 10⁶–10⁷ (4), approximately 100 times better than Taq DNA polymerase (4).

Phi29 DNA polymerase is available in TempliPhi and GenomiPhi DNA Amplification Kits. Large and small plasmid DNA can be amplified with TempliPhi DNA Amplification Kits directly from bacterial colonies or glycerol stocks, eliminating the need for overnight culture. Genomic DNA can be amplified with the GenomiPhi DNA Amplification Kit from whole blood, leaves or a few cells following a simple lysis procedure.

The kits are quick and simple to use, eliminating time-consuming cell culture steps and hazardous chemicals used in traditional DNA isolation techniques. The methods only involve a few liquid handling steps, making them easy to automate.

High-quality, high-molecular weight DNA
The product of a Phi29 amplification reaction is high-molecular weight, double-stranded copies of the input DNA. Amplification of high-quality genomic DNA gives an average fragment size of 30–40 kb (Fig 1), much larger than can be obtained by genome amplification techniques such as degenerative oligonucleotide primed PCR (DOP-PCR) (data not shown). Approximately 80% of the amplified DNA can be digested with restriction endonucleases (5).

Amplification is an end point reaction, terminating when all the nucleotides in the reaction are exhausted. Every reaction contains the same product yield, making optimization of downstream
Innovations Forum: Phi29 DNA amplification applications much simpler than with other DNA isolation methods. Average yields are shown in Figure 2.

**Direct use**

The product of TempliPhi DNA Amplification Kits is suitable for direct use in sequencing reactions. No additional purification is required. The amplification product is compatible with DYEnamic™ ET Terminator (Fig 3) and BigDye™ v3.1 Terminator Cycle Sequencing Kits. The amplification product is also suitable for cloning (6) and library construction following digestion and ligation.

The product of a GenomiPhi DNA amplification reaction can be directly used in most downstream applications without further purification. The high fidelity of Phi29 DNA polymerase ensures that the amplification product is representative of the starting material. GenomiPhi amplified DNA has been successfully used in many applications including PCR (simple, multiplex and real-time), SNP genotyping (Third Wave Invader™ assay, MegaBACE™ S NuPe™ genotyping kit, Affymetrix™ GeneChip™ HuSNP™ chip, Pyrosequencing™), STR and SSR genotyping, comparative genomic hybridization (CGH), cloning and library construction, heteroduplex analysis, slot and dot blots, yeast-2-hybrid systems, and microarray analysis (Fig 4).

**Summary**

TempliPhi and GenomiPhi DNA Amplification Kits produce high-quality DNA from small amounts of starting material. The amplification product can be used directly in downstream genomic analysis applications, eliminating the need for traditional DNA isolation methods.

**References**


**Ordering Information**

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