

GenomiPhi™ DNA amplification kits

HIGHLY REPRESENTATIVE AND RELIABLE WHOLE GENOME AMPLIFICATION

Nucleic acid analysis at the genome level has developed at a fast pace in recent years with improved accessibility of next-generation sequencing (NGS) and other modern techniques opening multiple investigative pathways. With so many ways to interrogate the genome, the amount of starting material can often be limiting, requiring prioritization of analyses. GenomiPhi™ DNA amplification technology provides the researcher the ability to generate sufficient high-quality DNA for successful downstream analysis.

Amplification technologies

GenomiPhi™ products, powered by Phi29 polymerase technology, are available in kits that provide an easy-to-use method which delivers highly representative and reliable whole genome amplification (Fig 1). Each kit contains all the components necessary for whole-genome amplification by utilizing isothermal multiple strand displacement amplification, with minimal hands-on time.

The GenomiPhi™ method of whole-genome amplification, multiple displacement amplification (MDA), is a non-PCR based DNA amplification technique (Fig 2). Compared with conventional PCR amplification techniques, MDA generates larger sized products with a lower error frequency.

MDA uses Phi29 DNA polymerase, a highly processive enzyme with excellent strand-displacement activity, in combination with random-sequence hexamer primers to amplify DNA at a constant temperature. Microgram quantities of high molecular weight DNA can be produced from as little as 10 ng of genomic DNA using this simple and robust method.

Amplification with GenomiPhi™ DNA amplification technology can be achieved in just a few simple steps (Fig 3) and is highly uniform over the entire genome, so locus representation remains close to the original DNA sample. This method is carried out with very high fidelity due to Phi29 DNA polymerase proof-reading activity. The average product length is over 10 kb.



Fig 1. GenomiPhi™ DNA amplification kit.

This optimum level of fidelity helps minimize genetic variation between generations.

Phi29 polymerase processing can generate very large DNA products up to 100× longer. Phi29 has 3'–5' exonuclease proofreading activity, resulting in 100-fold higher fidelity compared to Taq DNA polymerase. Random hexamers allow complete coverage across all samples.

This method has been actively used in whole genome amplification (WGA) and is a promising method for applications such as single cell genome sequencing and sequencing-based genetic studies.

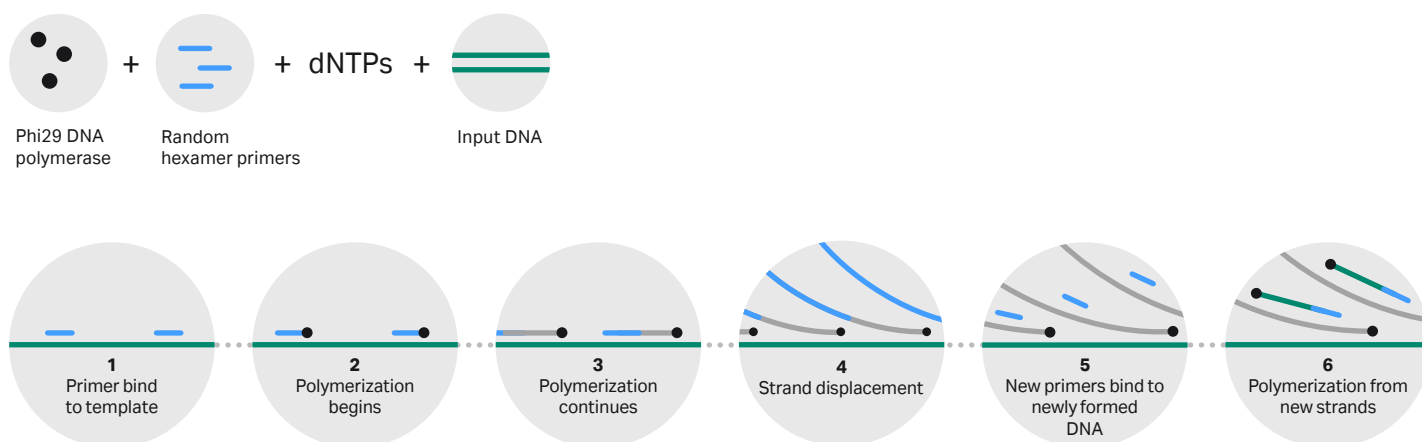


Fig 2. MDA amplification. (1) Random hexamer primers anneal to the linear template DNA at multiple sites. (2) Polymerization: Phi29 DNA polymerase extends each of these primers. (3) Polymerization continues. (4) When the DNA polymerase reaches a downstream extended primer, strand-displacement synthesis occurs. (5) The displaced strand is rendered single-stranded and available to be primed by more hexamer primers. (6) The process continues, resulting in exponential, isothermal amplification.

GenomiPhi™ DNA amplification kits

GenomiPhi™ DNA amplification kits simplify the process of genomic DNA preparation and stock replenishment and make it easier to prepare multiple DNA samples simultaneously. The kits come in a range of formats and sizes. For amplification from > 1 ng of starting DNA, researchers can choose between the liquid format (GenomiPhi™ V2 or HY) that requires storage at -80°C, or the Ready-To-Go™ format (GenomiPhi™ V3 or HY) featuring room-temperature stable reaction mixes which yield reproducible results after storage at room temperature for several months. The Ready-To-Go™ reaction mixture is provided as pre-dispensed, single-dose, lyophilized cakes in either strips of 8 tubes, 96-well or 5 × 96-well plates and provides higher DNA yields while simplifying workflow and handling, significantly reducing overall processing time.

GenomiPhi™ HY DNA Amplification Kits for mid-scale whole genome amplification

GenomiPhi™ HY DNA Amplification Kit contains all of the components necessary for mid-scale whole genome amplification by isothermal strand displacement amplification (Phi29). Amplification is highly uniform over the entire genome so that locus representation remains extremely close to the original DNA sample. A typical DNA yield of 40 to 50 µg DNA can be achieved in four hours with little hands-on time.

The kit was verified with DNA from various clinical samples including blood and buccal swabs to deliver a high-quality yield. The average product length is over 10 kb.

GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kits for mid-scale whole genome amplification

GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit supports the same applications as GenomiPhi™ HY DNA Amplification Kit with the added benefit that the reaction mixture is provided as pre-dispensed, single-dose, lyophilized cakes. A typical DNA yield of 40 to 60 µg DNA can be achieved.

GenomiPhi™ V2 DNA Amplification Kits for smaller-scale whole genome amplification

GenomiPhi™ V2 DNA Amplification Kit contains all of the components necessary for smaller scale whole genome amplification by isothermal strand displacement amplification (Phi29). A typical DNA yield of 4 to 7 µg DNA can be achieved in less than two hours with little hands-on time.

GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kits for smaller-scale whole genome amplification

GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit supports the same applications as GenomiPhi™ V2 DNA Amplification Kit with the added benefit that the reaction mixture is provided as pre-dispensed, single-dose, lyophilized cakes. A typical DNA yield of 12 to 20 µg DNA can be achieved in less than two hours from only 10 ng of genomic DNA input.

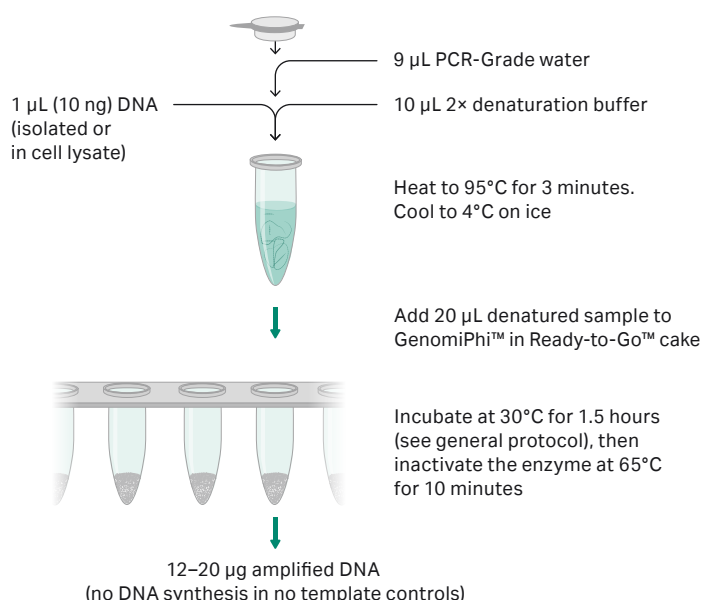


Fig 3. Isothermal DNA amplification with GenomiPhi™ in Ready-to-Go™ cake.

GenomiPhi™ Single Cell DNA Amplification Kits for amplification of genomic DNA from as little as a single cell

GenomiPhi™ Single Cell DNA Amplification Kit has been optimized to wholly amplify genomic DNA from as little as a single cell in just a two-step workflow, generating micrograms of high quality DNA for use in downstream applications. High quality lysis reagents are optimized to fully release genomic DNA from the cell and subsequently denature the DNA to enable optimal amplification and coverage. Background amplification, often associated with multiple displacement amplification (MDA), is suppressed throughout the incubation so that only input DNA is amplified. A typical DNA yield of 4 to 7 µg DNA can be achieved.

GenomiPhi™ DNA Amplification Kits and the underlying technologies are well-known and widely used. They have been tested for:

- Fidelity
- Stability
- Uniformity

Fidelity

Amplification of DNA from diverse sources with the GenomiPhi™ HY DNA Amplification Kit

This application note describes the amplification of human genomic DNA from whole blood and buccal swabs using the GenomiPhi™ HY DNA Amplification Kit. The amplified DNA was then used directly in single and multiplex PCR reactions to amplify regions of mitochondrial DNA.

Testing has demonstrated that the GenomiPhi™ HY DNA Amplification Kit generates about 50 µg of amplified DNA in 4 hours. Components of crude blood and buccal cell lysates can inhibit the amplification reaction but purifying the DNA sample prior to amplification or extending the duration of amplification to 5 or 6 hours can overcome this inhibition. Hexamer amplification occurs in the absence of DNA template, but the reaction is

inefficient and no significant amplification product is produced within the first 2 hours of incubation at 30°C. If you use purified DNA as template, stopping the reaction after 2 hours allows for an easy distinction between plus-DNA and minus-DNA amplification reactions while still yielding large quantities of amplified product. The amplified DNA is ready to be used directly in most applications.

 [Read the application note](#)

A comparative performance evaluation of GenomiPhi™ V3 Ready-To-Go™ and GenomiPhi™ V2 DNA amplification kits

The incorporation of a Ready-To-Go™ format into the design of GenomiPhi™ V3 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™ V2 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 GenomiPhi™ V3 Ready-To-Go™ removes these requirements. The addition of denatured DNA directly to the stabilized reaction mix of GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit initiates the amplification reaction, thus eliminating the setup stage involved with GenomiPhi™ V2 DNA Amplification Kit in which the polymerase is exposed in the absence of a template.

DNA amplified with GenomiPhi™ V2 and GenomiPhi™ V3 Ready-To-Go™ DNA amplification kits provided 100% identical allele calls to a single-source human gDNA that had not been previously amplified in short tandem repeat (STR) genotyping (Table 1).

While the DNA products from GenomiPhi™ V2 and GenomiPhi™ V3 Ready-To-Go™ DNA amplification kits produced similar results in downstream applications, the yield from GenomiPhi™ V3 Ready-To-Go™ was significantly greater.

 [Read the application note](#)

Table 1. Allele calls for 16 loci determined by STR profiling of DNA (GenomiPhi™ V2 or V3-amplified and non-amplified control gDNA)

Markers	GenomiPhi™ V2 DNA Amplification Kit		GenomiPhi™ V3 Ready-To-Go™ V3 DNA Amplification Kit		Control gDNA (Not amplified)	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D8S1179	14	15	14	15	14	15
D21S11	29	31.2	29	31.2	29	31.2
D7S820	8	11	8	11	8	11
CSF1PO	12	-	12	-	12	-
D3S1358	17	18	17	18	17	18
TH01	6	9.3	6	9.3	6	9.3
D13S317	9	11	9	11	9	11
D16S539	9	13	9	13	9	13
D2S1338	22	25	22	25	22	25
D19S433	13	14	13	14	13	14
vWA	16	19	16	19	16	19
TPOX	11	-	11	-	11	-
D18S51	16	18	16	18	16	18
AMEL	X	Y	X	Y	X	Y
D5S818	12	-	12	-	12	-
FGA	20	23	20	23	20	23

Stability

Stability of Custom Ready-To-Go™ products at ambient temperature for extended storage periods

The patented Ready-To-Go™ technology utilizes lyophilization to provide long-term room temperature stability to biomolecules. All Ready-To-Go™ products can be shipped and stored without the need for refrigeration.

The GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit offers highly efficient and representative whole-genome amplification with microgram yields from nanogram amounts of DNA sample. Human genomic DNA was amplified using GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit. The DNA yield was determined using Quant-iT PicoGreen™ dsDNA Reagent (Thermo Fisher Scientific). A typical DNA yield of 12 to 20 µg DNA can be achieved in less than 2 hours from only 10 ng of genomic DNA input.

Testing results show that Ready-To-Go™ stabilization technology can be used to produce reagents and assay mixtures that are stable at ambient temperatures for years (Fig 4). The technology

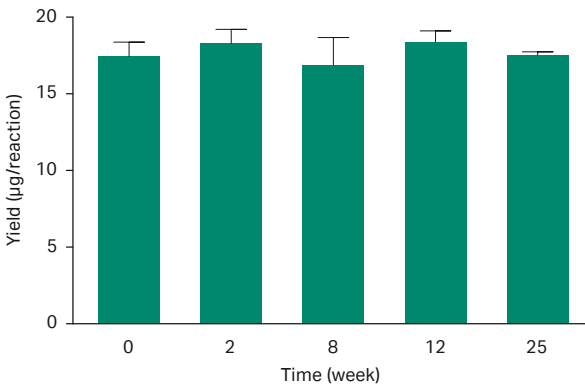


Fig 4. Yield of human genomic DNA amplified using GenomiPhi™ V3 DNA Amplification Kit in Ready-To-Go™ format. DNA yield determined using Quant-iT PicoGreen™ dsDNA Reagent. Mean +/- SD of triplicate determinations.

has been shown to be compatible with various nucleic acid amplification methods, including standard PCR, RT-PCR, and whole genome amplification with Phi29 DNA polymerase. Stabilized products perform the same after prolonged storage at ambient temperatures as they did before stabilization.

Read the application note

From sample collection to whole genome amplification at ambient temperature: a simple solution

This application note describes a simple method for elution of DNA from FTA™ dried blood spot samples in a form that is suitable for whole genome amplification using the GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit. The method allows DNA to be easily recovered from sample collection punched discs. Yields of DNA after amplification with GenomiPhi™ V3 Ready-To-Go™ were consistently between 15 and 18 µg for both 1.2 and 3 mm punched discs. These yields are within the specification range of 12–20 µg (Table 2).

Table 2. Yields of DNA amplified from discs punched from FTA™ dried blood sports were measured by Quant-iT PicoGreen™ dsDNA assay

Disc size (mm)	3	3	3	1.2	1.2	1.2
Replicate #	1	2	3	1	2	3
Yield of GenomiPhi™ amplified DNA (µg)	17.4	15.6	17.4	15.7	16.9	16.0

DNA was eluted from FTA™ dried blood spots following the alkaline denaturation protocol, amplified with GenomiPhi™ V3 in Ready-To-Go™ format and subjected to STR amplification. STR analysis produced the expected allele calls for the full profile of 15 alleles (plus amelogenin) for all replicates tested (Table 3).

This demonstrates that amplified DNA generated with GenomiPhi™ V3 Ready-To-Go™ following alkaline pH elution is suitable for subsequent genetic analyses including STR profiling, which showed full concordance between amplified and unamplified DNA.

Table 3. Allele calls for 16 loci determined by STR profiling

Markers	GenomiPhi™ V3 Ready-To-Go™ amplified DNA from FTA™ card				Unamplified control gDNA			
	Allele 1	Allele 2	Height 1	Height 2	Allele 1	Allele 2	Height 1	Height 2
D8S1179	12	15	1507	1473	12	15	2255	2531
D21S11	30.2	31.2	1129	789	30.2	31.2	644	656
D7S820	10	11	349	242	10	11	482	402
CSF1PO	10	-	319	-	10	-	505	-
D3S1358	15	18	344	351	15	18	442	406
TH01	8	9.3	122	162	8	9.3	1982	2161
D13S317	8	11	219	214	8	11	170	150
D16S539	11	12	854	704	11	12	1376	1161
D2S1338	16	20	391	196	16	20	2151	1769
D19S433	12	15	309	270	12	15	776	754
vWA	16	18	1810	1857	16	18	3113	2439
TPOX	8	-	863	-	8	-	6505	-
D18S51	14	17	266	225	14	17	236	195
AMEL	X	Y	998	2200	X	Y	1709	1694
D5S818	12	13	995	923	12	13	905	798
FGA	22	24	474	439	22	24	322	265

- indicates no allele

Uniformity

Genomic DNA preparation using GenomiPhi™ V2 and HY DNA Amplification Kits

This application note describes the GenomiPhi™ V2 and HY Amplification Kit protocol and testing of the resultant amplified DNA.

Single nucleotide polymorphism (SNP) concordance analysis is often used to assess the accuracy of genotyping assays. To confirm that GenomiPhi™ V2 and HY DNA Amplification kits maintain concordance and call rate of the original genomic DNA, amplification products (obtained from the Coriell Institute) were subjected to SNP concordance analysis. It was found that both the concordance and the call rate of the amplified DNA were very similar to that of the gDNA (Fig 5).

 [Read the application note](#)

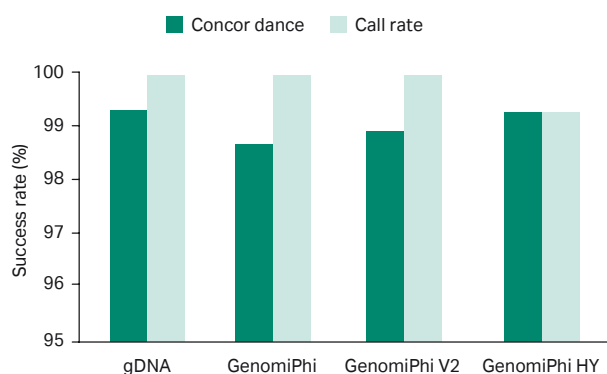


Fig 5. SNP concordance analysis of amplification products of individual gDNA using the Affymetrix™ 10K human genome SNP chip.

Uniform amplification of genomic DNA from 1 to 1000 cells using GenomiPhi™ Single Cell DNA Amplification Kit

The GenomiPhi™ Single Cell DNA Amplification Kit uniformly amplifies genomic DNA from 1 to 1000 cells via a multiple strand displacement amplification mechanism. Background amplification, often associated with multiple displacement amplification (MDA), is suppressed throughout the incubation so that only input DNA is amplified.

The kit is sensitive enough to consistently amplify gDNA from as little as 1 fg of starting template. Yields of 4 to 7 µg of gDNA are obtained from 1 pg templates in less than 2 h and from 1 fg templates in less than 4 h. The amplified gDNA showed high genome coverage, low amplification bias, and a low error rate. The DNA was successfully used in aCGH, SNP analysis, and next-generation sequencing.

Studies show that the percentage of sequencing data matching the human genome from human cell samples amplified using the GenomiPhi™ Single Cell DNA Amplification Kit is equivalent to the percentage produced from unamplified gDNA control samples. This indicates that no contaminants are introduced and amplified during the workflow. Contaminating DNA, which is a common problem in single cell experiments, is effectively eliminated.

Genomic DNA (gDNA) samples amplified from human cells with the GenomiPhi™ Single Cell DNA Amplification Kit show greater than 99% genome coverage in a genome-wide SNP array. The GenomiPhi™ Single Cell DNA Amplification Kit produces minimal errors during amplification of human cell samples resulting in SNP calls that are highly concordant with the control gDNA (Fig 6).

 [Read the application note](#)

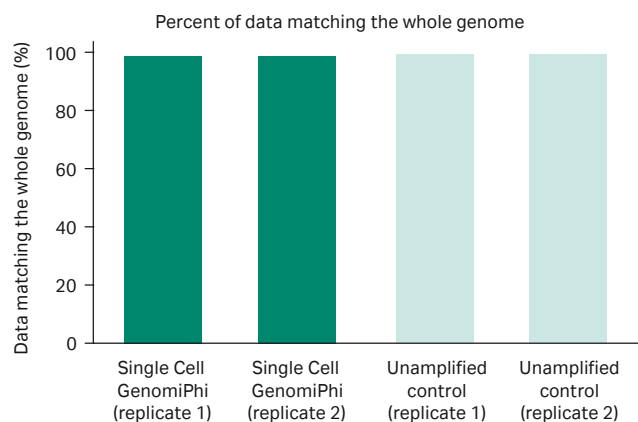


Fig 6. Greater than 98% of gDNA amplified from human cell samples using GenomiPhi™ Single Cell DNA Amplification Kit matches the human genome. This is equivalent to unamplified control samples.

Ordering information

Product	Pack size (reactions)	Product code
GenomiPhi™ HY DNA Amplification Kit	25	25660022
GenomiPhi™ HY DNA Amplification Kit	100	25660020
GenomiPhi™ HY DNA Amplification Kit	1000	25660025
GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit	Sample pack	25660308
GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit	24	25660324
GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit	96	25660396
GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit	280	25660397
GenomiPhi™ V2 DNA Amplification Kit	25	25660030
GenomiPhi™ V2 DNA Amplification Kit	100	25660031
GenomiPhi™ V2 DNA Amplification Kit	500	25660032
GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit	Sample pack	25660108
GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit	24	25660124
GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit	96	25660196
GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit	480	25660197
GenomiPhi™ Single Cell DNA Amplification Kit	25	29108107
GenomiPhi™ Single Cell DNA Amplification Kit	100	29108039

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