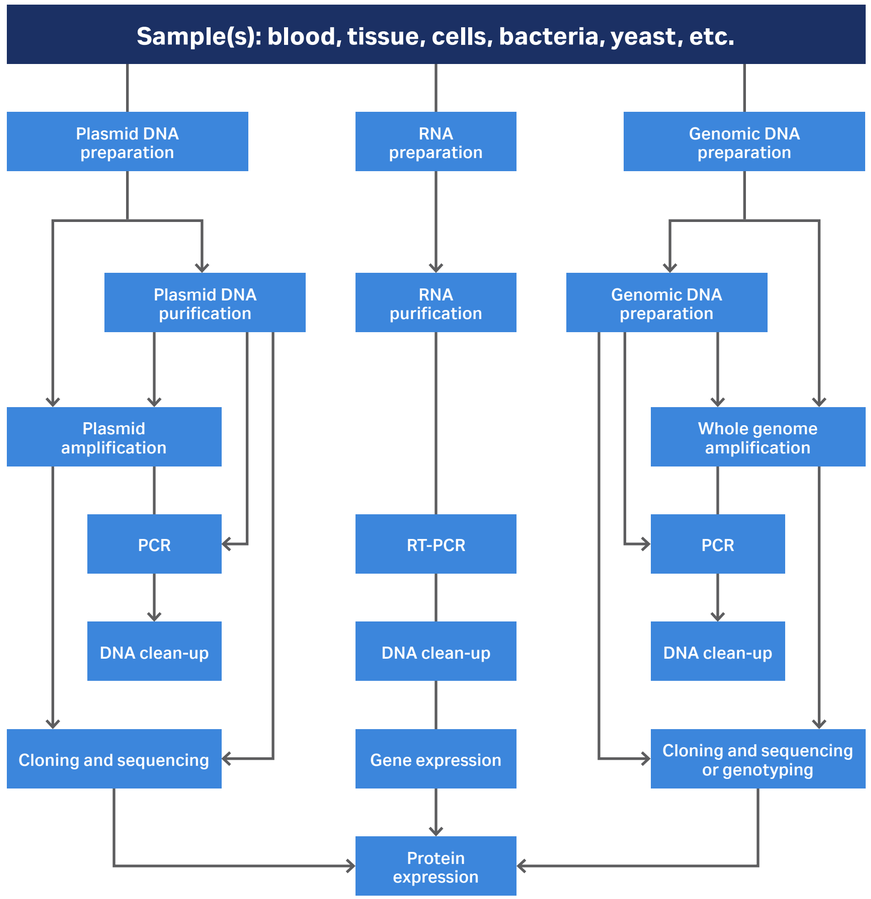
Nucleic acid amplification

Purify. Simplify. Amplify.

cytiva

Your amplification workflow

Nucleic acid amplification is one of the most fundamental techniques used in molecular biology. We support this important step in your workflow with a choice of convenient, ready-to-use kits and high quality reagents to suit your amplification requirements. Whether you want off-the-shelf convenience or the flexibility of independent reagents, our amplification products deliver the consistent quality and reliability your research demands.

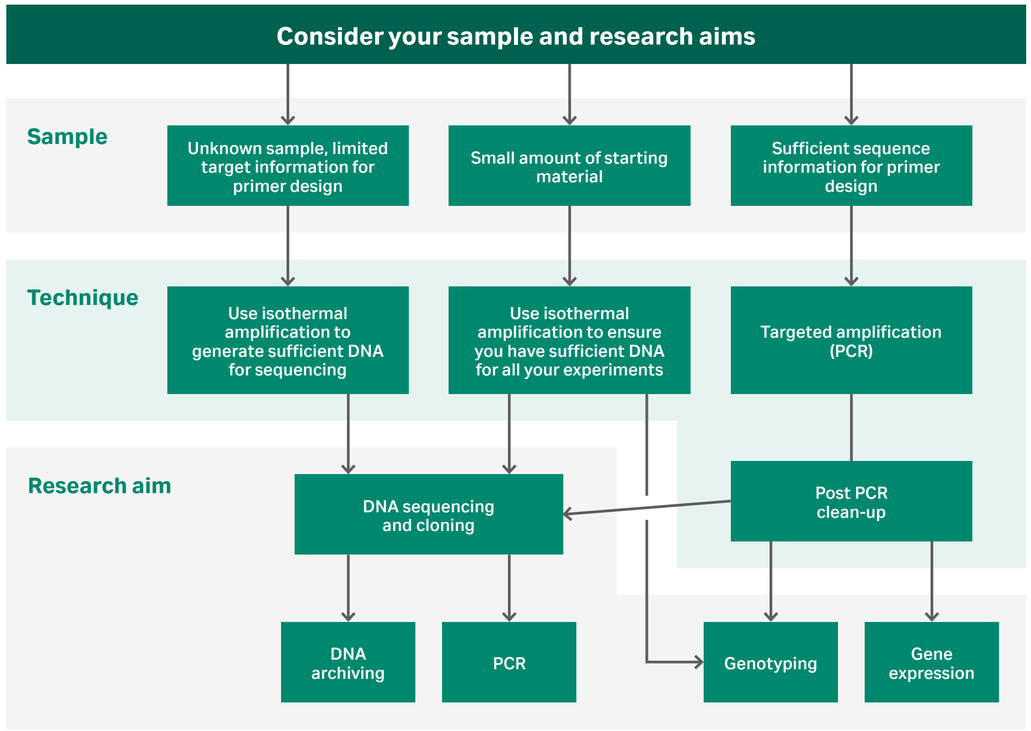


Technology and product selection

DNA amplification takes many forms. The method of choice depends on a number of factors including:

- The availability of sequence-specific primers
- The intended use of the amplified product
- The quantity and quality of source material

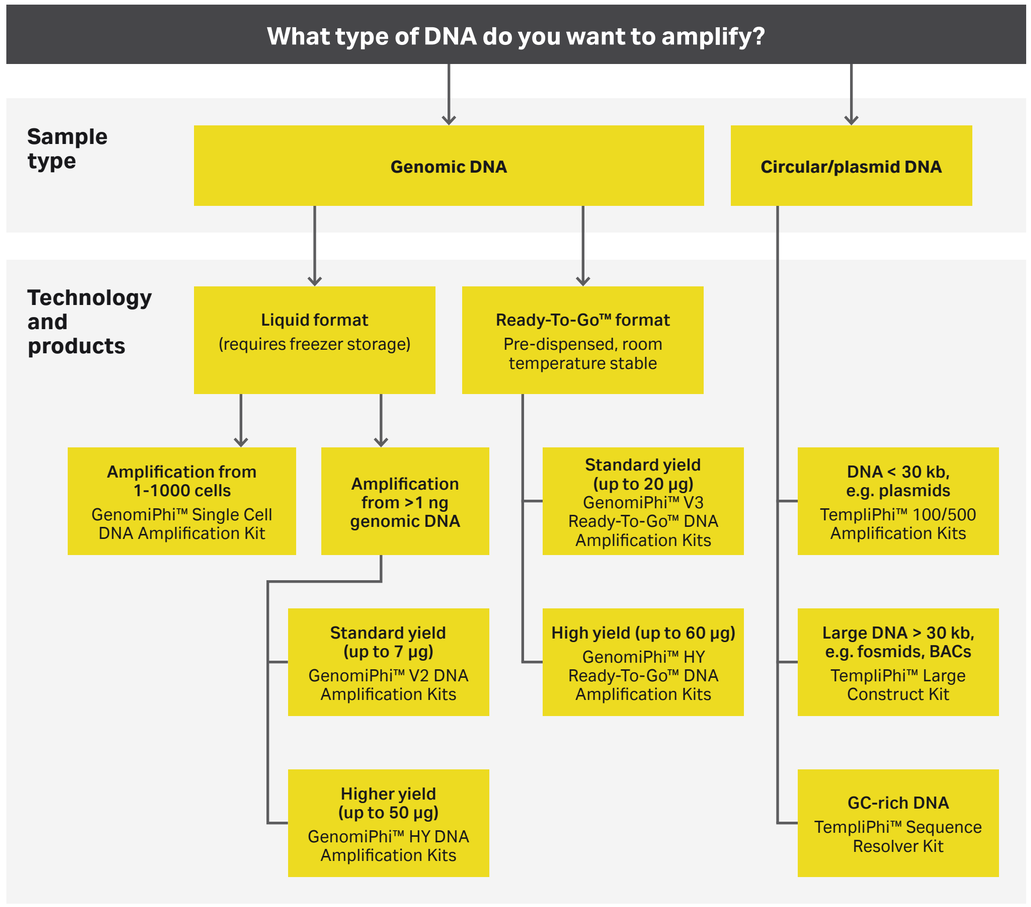
When sequence information is available, a primerspecific DNA amplification (e.g., PCR) can be performed. However, when the amount of input material is small and inadequate sequence information is available to prepare sequence-specific primers, isothermal amplification using phi29 DNA polymerase is an option.



Fast and simple DNA amplification — without thermocycling

Phi29 DNA polymerase based isothermal DNA amplification is a simple, reliable alternative to other DNA amplification procedures. The highly processive Phi29 DNA polymerase elicits strong strand displacement enabling rapid DNA replication from multiple sites. Phi29 also has 3′-5′ exonuclease proofreading activity, resulting in 100-fold higher fidelity compared to Taq DNA polymerase. From very small amounts of starting material, Phi29 DNA polymerase rapidly produces consistent microgram yields of high quality DNA that is ready for direct use in a range of downstream analyses, including sequencing and genotyping. The one-tube, one-temperature format simplifies DNA preparation, facilitating automation for high-throughput sample amplification.

Phi29 based DNA Amplification Kits address a range of sample types and downstream applications.



Two methods for Phi29 DNA amplification

Use TempliPhi™ DNA Amplification Kits to prepare DNA directly from plasmid or fosmid glycerol stocks or colonies.
For other sample types, GenomiPhi™ kits are available in a range of formats, including the convenient Ready-To-Go™ format.

Principle of rolling circle amplification

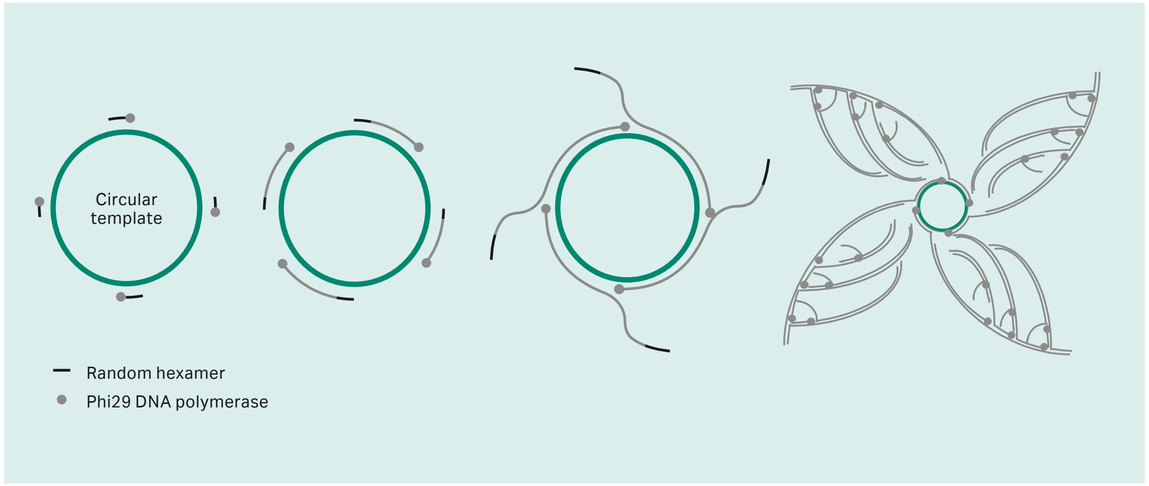


Fig 1. Random hexamer primers anneal to the circular template DNA at multiple sites. Phi29 DNA polymerase extends each of these primers. Strand displacement occurs when the polymerase reaches a downstream-extended primer. Subsequent priming leads to an exponential isothermal amplification.

Principle of Phi29 DNA polymerase-based multiple displacement amplification

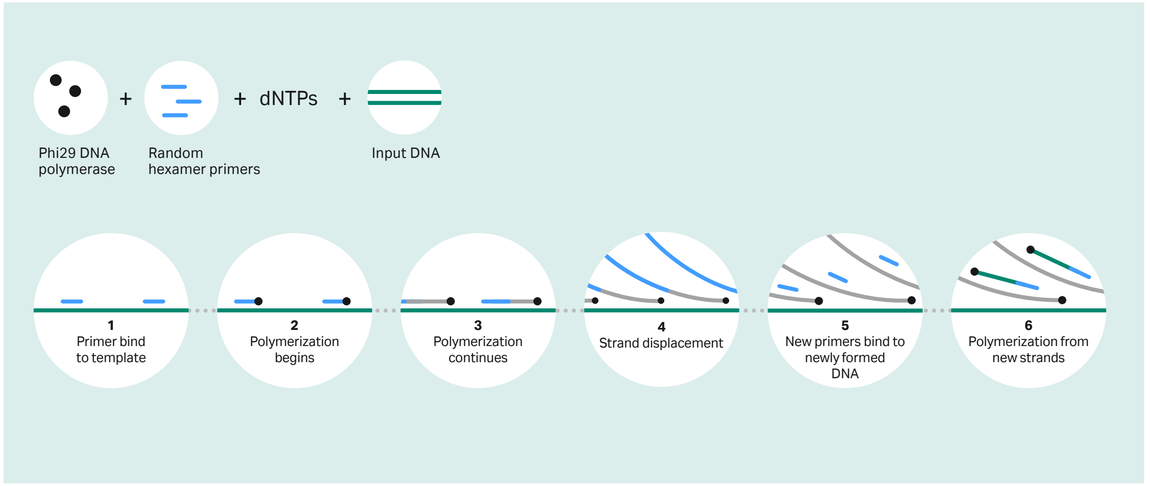


Fig 2. Template DNA is primed at multiple locations with random hexamers. Phi29 DNA polymerase then extends primers, replicating template and displacing any downstream extended primers. Strand displacement and subsequent priming leads to an exponential increase of new template for isothermal amplification.

TempliPhi™ 100/500 Amplification Kits

TempliPhi™ 100/500 Amplification Kits are used to prepare DNA directly from plasmid or fosmid glycerol stocks or colonies, which eliminates overnight culture steps.

The amplified DNA can be used directly for sequencing and library construction without further purification, further simplifying your process and reducing hands-on time, without compromising on sequencing success and read lengths.

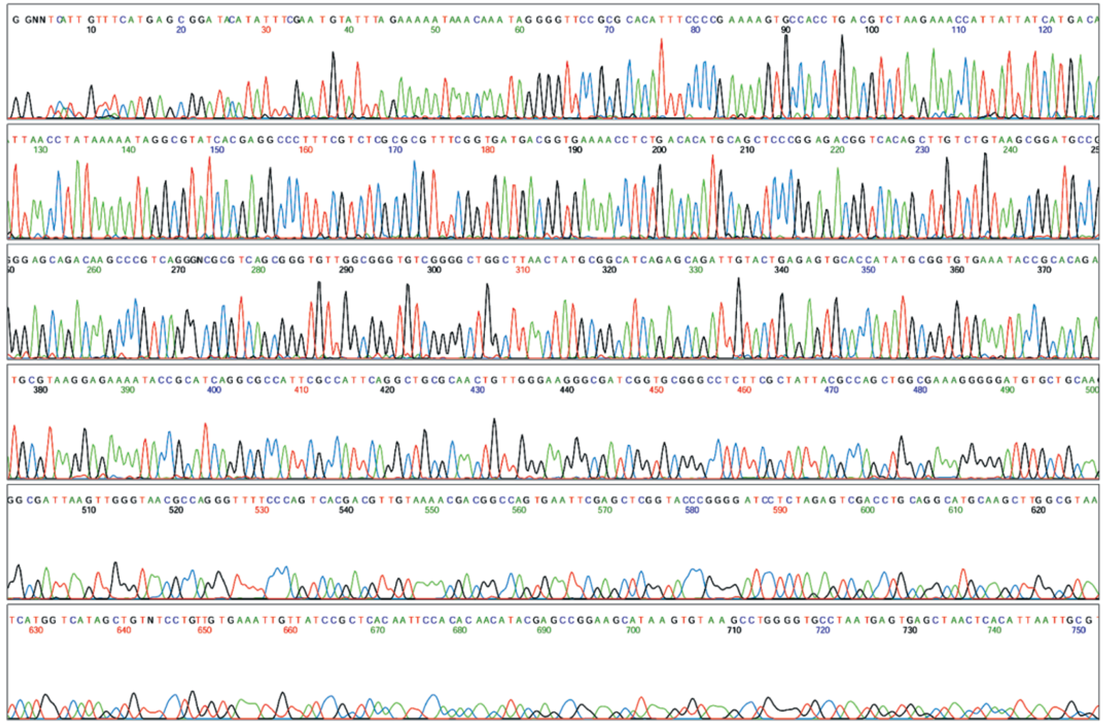


Fig 3. Plasmid DNA template was amplified with the TempliPhi™ 100 Amplification Kit and subsequently sequenced using the DYEnamic Terminator Cycle Sequencing Kit and analyzed on an ABI PRISM 3100 Genetic Analyzer.

GenomiPhi™ DNA Amplification Kits

Genomic DNA preparation is a fundamental step in genetic analysis and obtaining high quality DNA is key for successful downstream analysis. GenomiPhi™ DNA Amplification Kits provide an easy-touse method that delivers highly representative and reliable whole genome amplification.

GenomiPhi™ DNA Amplification Kits come in a range of formats. For amplification from > 1 ng of starting DNA, researchers can choose between the traditional liquid format (V2 or HY) that requires storage at -80°C, and Ready-To-Go™ Amplification Kits (V3 or HY) featuring predispensed, room-temperature stable reaction mixes. The Ready-To-Go™ products provide higher DNA yields while simplifying workflow and handling, significantly reducing overall process time.

For amplification from very small amounts of starting material, or directly from as little as a single cell, the GenomiPhi™ Single Cell DNA Amplification Kit provides greatest sensitivity. By removing DNA contamination using novel methodologies, sensitivity down to 1 fg is achieved, so background is no longer an issue and all that is amplified is template DNA.

GenomiPhi™ Ready-To-Go™ Kits can be used with various kinds of samples, including blood lysates and dried blood spots.

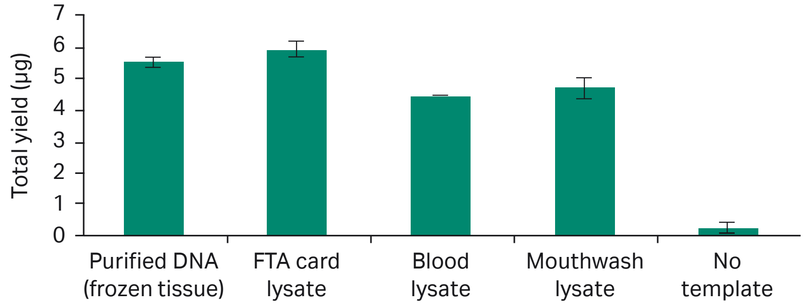


Fig 4. Amplification yields for GenomiPhi™ V2 DNA Amplification Kit using purified DNA (10 ng) or nonpurified cell lysates.

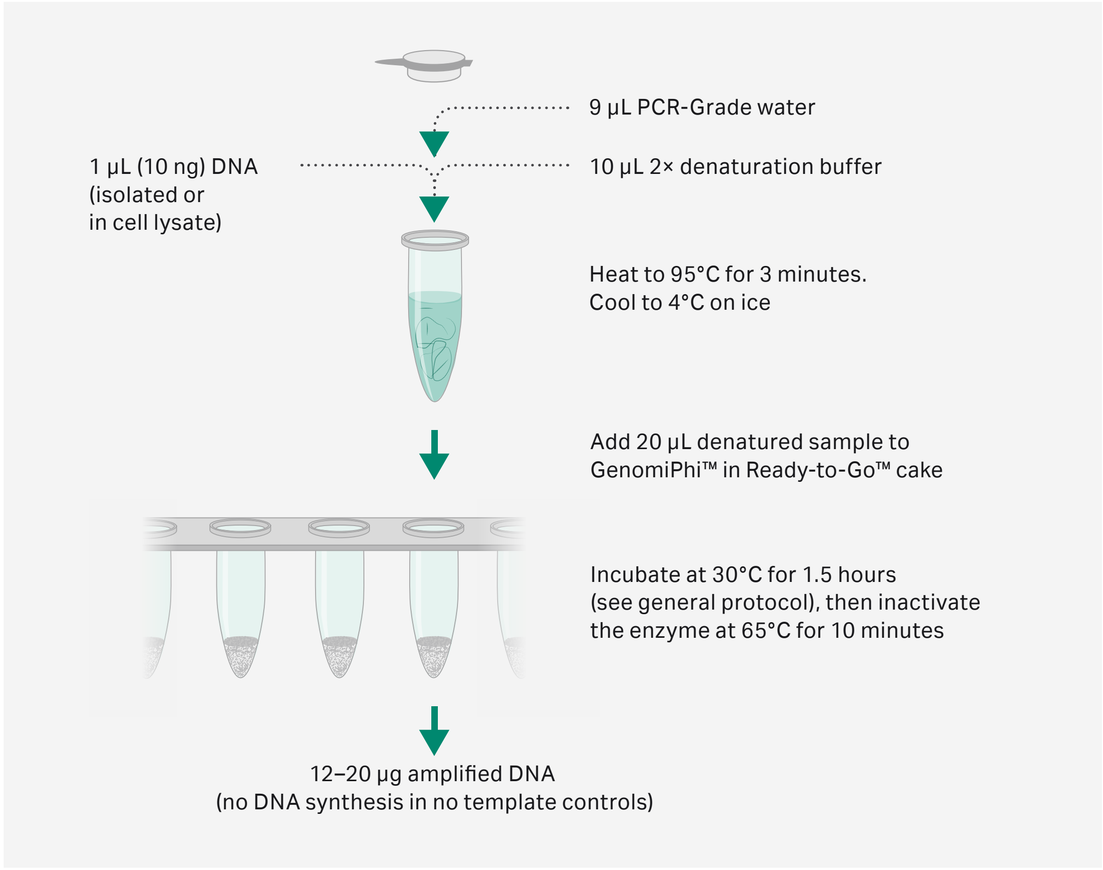


Fig 5. GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit protocol.

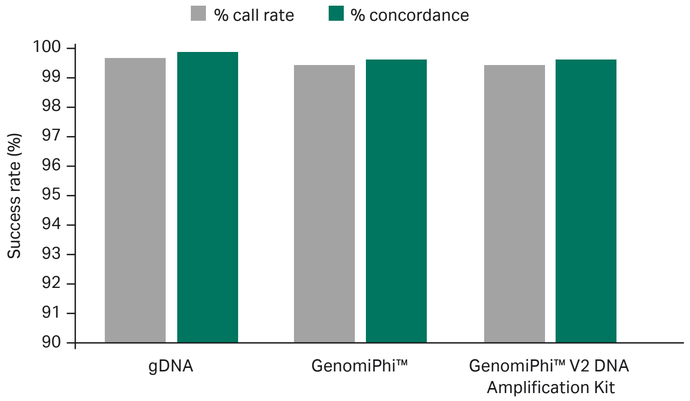


Fig 6. Individual human genomic DNA (gDNA) obtained from Coriell was amplified with GenomiPhi™ V2 DNA Amplification Kits and subjected to analysis on the Affymetrix™ 100K SNP chip.

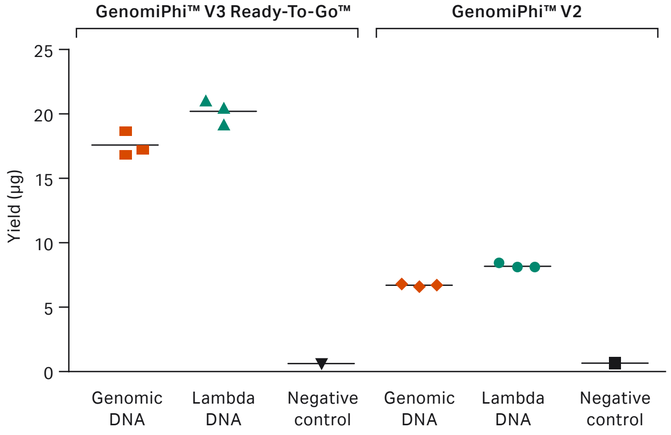


Fig 7. Yield of human genomic DNA (g) or Lambda DNA (l) amplified in GenomiPhi™ V3 Ready-To-Go™ or GenomiPhi™ V2 DNA Amplification Kits, determined by PicoGreen™ assay.

GenomiPhi™ Single Cell DNA Amplification Kit can amplify genomic DNA from as little as 1 to 1000 cells without interference from non-specific amplification. Proprietary reagents and manufacturing techniques ensure background amplification does not interfere with results when working with such minute quantities of starting template, and allow sensitivity down to 1 fg of DNA. Our optimized cell lysis protocol ensures uniform release of DNA from the cell. Amplified gDNA shows high genome coverage, low amplification bias, and a low error rate. The DNA has been successfully used in aCGH, SNP analysis, and Next Generation Sequencing.

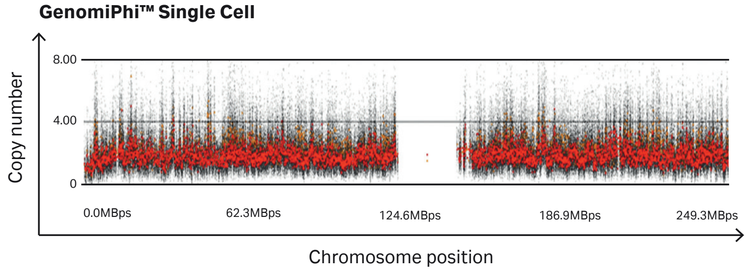


Fig 8. Copy number graph showing WGA gDNA normalized to the unamplified bulk control gDNA samples. Graph represent overlap of at least two separate samples. Colored dots = mean intensity of 30 probes; representative graph of chromosome 1 is shown.

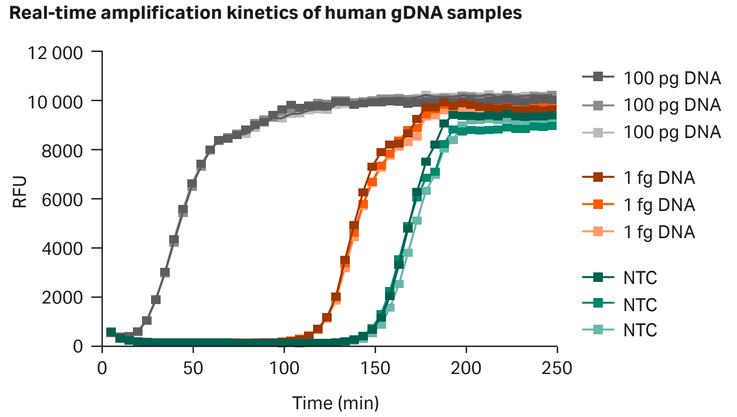


Fig 9. Human gDNA (100 pg and 1 fg) was amplified with GenomiPhi™ Single Cell DNA Amplification Kit. Amplification kinetics were monitored on a Tecan™ plate reader in real time by the addition of SYBR™ Green I. There is clear separation between 1 fg samples and no template control (NTC) samples, demonstrating the sensitivity of the system.

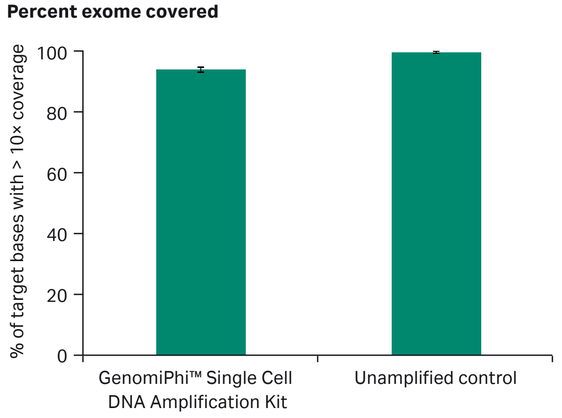


Fig 10. gDNA amplified with GenomiPhi™ Single Cell DNA Amplification Kit results in a high percentage of exome sequence coverage when run in whole exome sequencing (10x coverage).

Ordering information

Product	Pack size	Product code
GenomiPhi™ Single Cell DNA Amplification Kit	25 reactions	29108107
	100 reactions	29108039
GenomiPhi™ V2 DNA Amplification Kit	25 reactions	25660030
	100 reactions	25660031
	500 reactions	25660032
GenomiPhi™ HY DNA Amplification Kit	25 reactions	25660022
	100 reactions	25660020
	1000 reactions	25660025
GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit	24 reactions	25660124
	96 reactions	25660196
	480 reactions	25660197
TempliPhi™ 100/500 DNA Amplification Kit	100 reactions	25640010
	500 reactions	25640050
TempliPhi™ 2000 Reaction Kit*	2000 reactions	28964286
TempliPhi™ Large Construct Kit	1000 reactions	25640080
TempliPhi™ Sequence Resolver Kit	20 reactions	28903529
	200 reactions	28903531

* Supplied with premixed components for high-throughput convenience.

Instant and reliable PCR mixes with the convenience of Ready-To-Go™ Beads

Preformulated Ready-To-Go™ Beads give you the assurance of reliable PCR assays in a convenient bead format. Each bead contains all the reagents for PCR or RT-PCR, stabilized for storage at room temperature. Just add template and primers to the bead, and then follow your normal thermocycling method.

Ready-To-Go™ Beads save time on reagent preparation, while ensuring reproducibility from operator to operator. Because they can be stored at room temperature, you also save on freezer space, and they are ready to use as soon as you are.

Ready-To-Go™ Beads are provided in either 0.5 or 0.2 mL tubes that are compatible with most thermocyclers. The 0.2 mL tubes are supplied assembled in a 96-well (8 × 12) format that allows strips of eight wells to be easily removed, giving you the flexibility of using individual tubes, strips of eight, or a 96-well format.

PuReTaq™ Ready-To-Go™ PCR Beads, for standard PCR

- Optimized for standard PCR, when reconstituted, each bead contains:
  - ~ 2.5 units of PuReTaq™ DNA polymerase, 10 mM TrisHCl (pH9), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, stabilizers, and BSA
- Use high purity reagents, including recombinant Taq DNA polymerase, to ensure each bead is free of contaminating DNA.
- Each batch is functionally tested.

Amersham™ Hot Start Mix in Ready-To-Go™ format, for high-specificity amplification

- Novel hot start method (primer sequestration), inhibits primer-dimer formation to maximize target amplification efficiency.
- Mix does not contain antibodies, eliminating risk of mammalian-source contamination.
- No chemical inactivation, eliminates need for extensive heating step and reduces the chance of heat-induced depurination of DNA.
- Highest specificity in sequence amplification for greater confidence in downstream applications.

A Hot Start Master Mix is also available, for applications requiring additional optimization.

Amersham™ Ready-To-Go™ RT-PCR Beads, for amplification from RNA templates

- Optimized one-tube, one-step reactions for both cDNA synthesis and PCR means no need to open the tube or change conditions between steps.
- A reduced number of pipetting steps minimizes risk of contamination and RNA degradation, improving assay reproducibility.
- Each lot is functionally tested for the ability to generate highly specific PCR products to ensure lot-to-lot reproducibility.
- When reconstituted, each bead contains:
    - ~ 2 units of PuReTaq™ DNA polymerase, M-MuLV Reverse Transcriptase, RNase inhibitor, 10 mM TrisHCl (pH9), 60 mM KCl, 1.5 mM MgCl2, 200 µM of each dNTP, stabilizers, and BSA
- Includes control beads, containing rabbit globin mRNA and specific primers.

Amersham™ Ready-To-Go™ RAPD Analysis Beads, for rapid detection of genomic polymorphisms

- Sensitive, simple technique for the detection of polymorphisms, using nanogram quantities of DNA.
- RAPD Analysis Ready-To-Go™ Beads are flexible for use with a wide variety of organisms.
- Each lot is functionally tested to ensure its ability to generate a differential banding pattern between two control strains of *E. coli*.
- Analysis Beads include a single RAPD primer whereas the Analysis kit includes 6 commonly used RAPD primers.
- Ready-To-Go™ Beads for PCR are available in tube or multiwell plate formats.

Our convenient Ready-To-Go™ format streamlines your workflows while delivering consistent, reliable results. We can also prepare custom Ready-To-Go™ formulations to meet your specific needs. Please contact us for further information.

Ordering information

Product	Pack size	Product code
Ready-To-Go™ RT-PCR Beads	0.2 mL hinged tube with cap, 96 reactions	27925901
	0.5 mL tubes, 100 reactions	27926601
	0.2 mL tubes/plate, 96 reactions	27926701
Ready-To-Go™ PCR Beads	0.2 mL tubes/plate, 96 reactions	27955701
	0.2 mL tubes/plate, 5 × 96 reactions	27955702
	0.5 mL tubes, 100 reactions	27955801
	0.2 mL hinged tube with cap, 96 reactions	27955901
Hot Start Mix Ready-To-Go™ format	0.5 mL tubes, 100 reactions	28900646
	0.2 mL tubes/plate, 96 reactions	28900653
	0.2 mL tubes/plate, 5 × 96 reactions	28900654
Ready-To-Go™ RAPD Analysis Beads	Analysis Beads, 100 reactions	27950001
	Analysis Kit, 100 reactions and 6 primers	27950201

Related product		
Amersham™ Hot Start Master Mix	100 reactions	25150001

Independent reagents for flexible method development

Developing and refining PCR methods for your application demands high quality reagents you can rely on. We functionally test our *Taq* DNA polymerase and nucleotides in typical PCR applications to ensure they meet the high standards you expect. We also test our enzyme preparations to ensure they are free of detectable non-specific nuclease activities.

We offer a range of pack sizes, facilitating economical assay development and scale-up. If you don't see a suitable pack size listed, please contact us for a custom quotation.

Taq DNA polymerase (cloned)

Taq DNA polymerase is a proprietary formulation of purified, recombinant *Taq* DNA polymerase. It has an optimum temperature of 75ºC and survives repeated incubations at 95ºC. *Taq* DNA polymerase is supplied at a concentration of 5000 units/mL, with a 10× reaction buffer and additional 25 mM $MgCl_2$ to facilitate assay optimization.

- Highly purified, with excellent batch-to-batch reproducibility for reliable PCR.
- Free from detectable non-specific nuclease activities.
- One unit incorporates 10 nmol of total nucleotides into acid-insoluble material in 30 min at 70ºC, in a total volume of 50 µL.

PCR grade nucleotides

- High purity dNTPs for amplification, dideoxy sequencing, labeling, mutagenesis, cDNA synthesis, and expression profiling
- Free from DNase, RNase, and nicking enzyme activity
- Greater than 99% triphosphate purity (by HPLC) for consistent, high performance
- Buffer-free, ready-to-use solutions at a variety of concentrations
- Functionally tested to produce a 20.7 kb PCR amplification product from λ DNA

Product specifications

Test	Purpose	dNTP specifications
Concentration		100 ± 3 mM
pH	Stability and functionality	7.5 ± 0.2
Triphosphate purity	HPLC (Mono Q™ columns). Separates di- and monophosphates	≥ 99.0% triphosphate
Base purity	Alkaline phosphatase digestion followed by HPLC (C18). Separates and quantitates contaminating bases	≥ 99.5% correct nucleoside
Functional test for PCR	Long PCR. Synthesis of 20.7 kb lambda DNA fragment	Pass
DNases	DNases interfere with DNA amplification and sequencing	Free from DNases
RNases	RNases interfere with reverse transcription	Free from RNases
Nicking activity	Nicking interferes with DNA amplification and sequencing	Free from nicking activity
Sodium content	Ion chromatography	≥ 20 mM Cl^- ion
Bacterial level	CFU/mL before and after filtration	Not tested. Replaced by nuclease testing
Shelf life		24 months

Ordering information

Product	Pack size	Product code
Taq DNA polymerase (cloned)*	250 units	27079804
	10 × 250 units	27079806
Amersham™ dNTP Set (100 mM each A,C,G,T)	4 × 10 µmol	28406558
	4 × 25 µmol	28406551
	4 × 100 µmol	28406552
	4 × 500 µmol	28406553
Amersham™ PCR Nucleotide Mix dNTP Set (25 mM each A,C,G,T)	500 µmol	28406560
Amersham™ PCR Nucleotide Mix dNTP Set (10 mM each A,C,G,T)	500 µmol	28406564

* Source: *E. coli*, in which the gene for *Taq* DNA polymerase from *Thermus aquaticus* has been inserted.

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