



# illustra™ nucleic acid amplification

Purify. Simplify. Amplify.

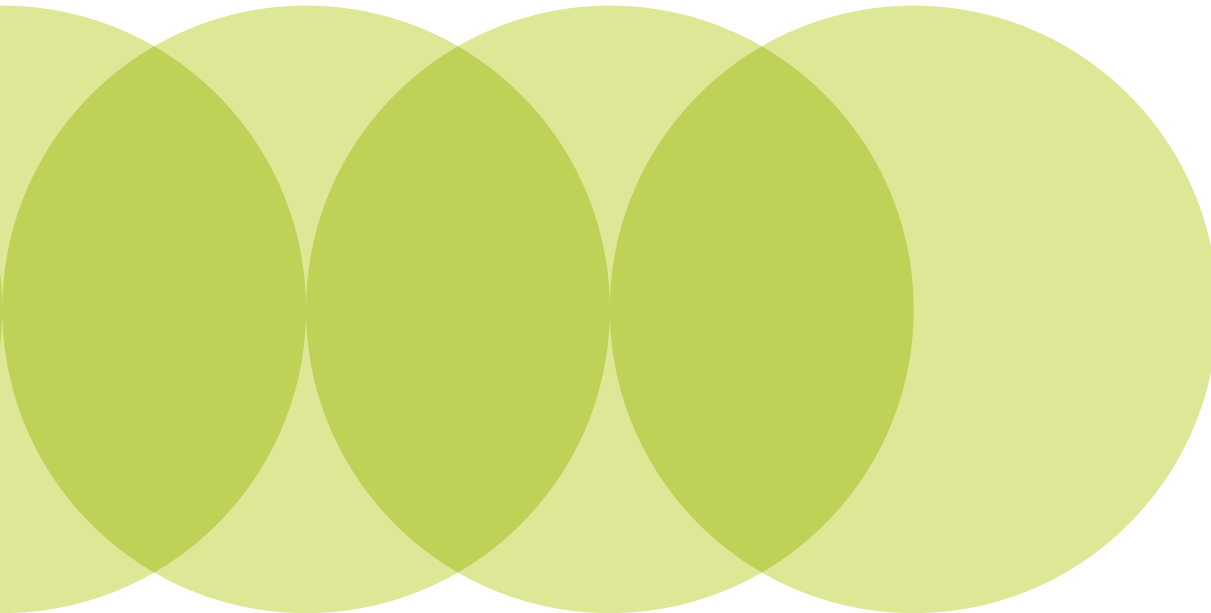


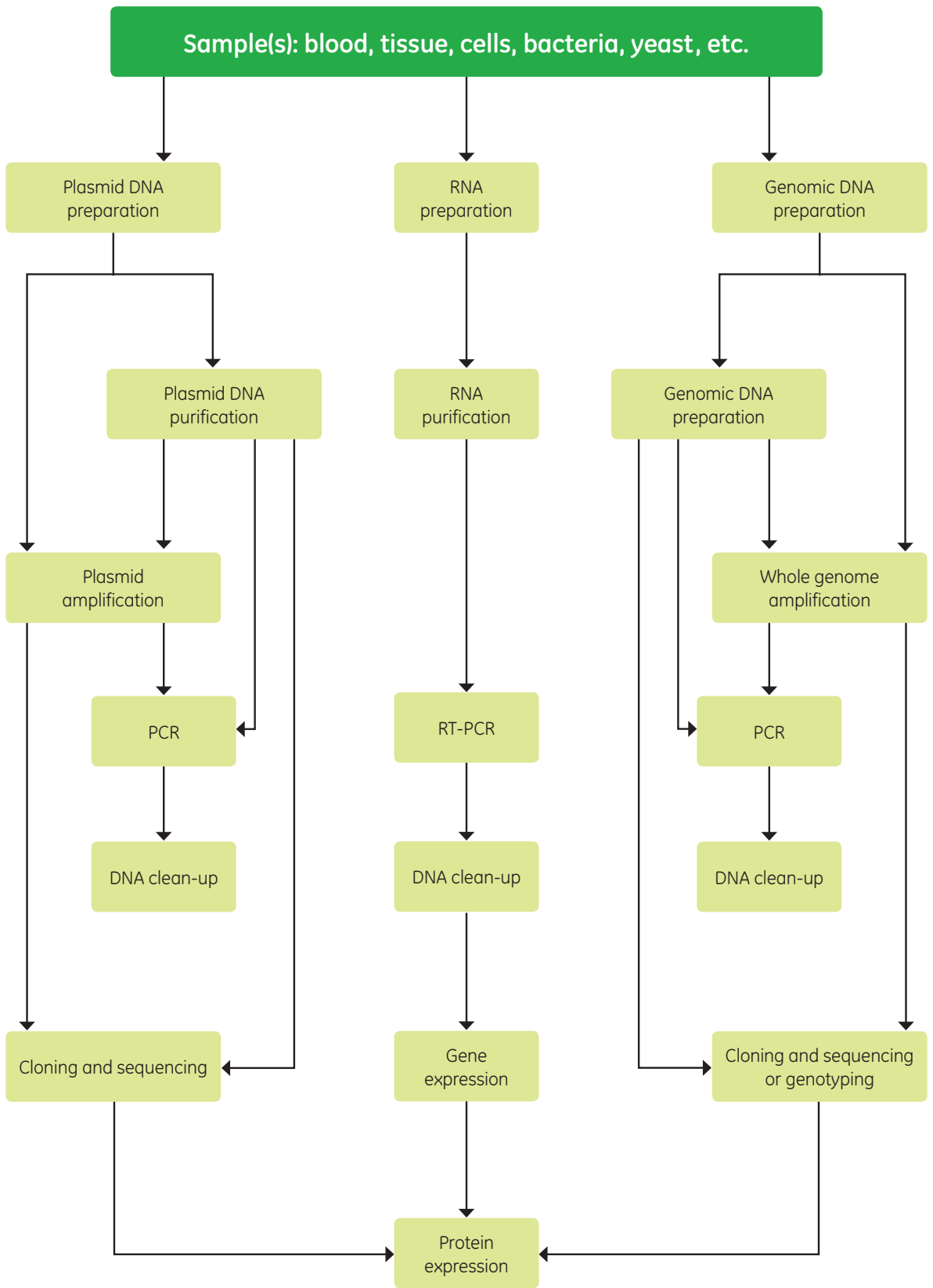
# Meeting your needs

Nucleic acid amplification is one of the most fundamental techniques used in molecular biology. Our illustra range of products supports this important step in your workflow with a choice of convenient, ready-to-use kits and other high quality reagents to suit your amplification requirements.

Whether you want off-the-shelf convenience or the flexibility of independent reagents, illustra amplification products deliver the consistent quality and reliability your research demands.

In addition to illustra amplification products, the illustra range includes products for nucleic acid purification and post-PCR clean-up. For more information, visit our Web site at [www.gelifesciences.com/sampleprep](http://www.gelifesciences.com/sampleprep).





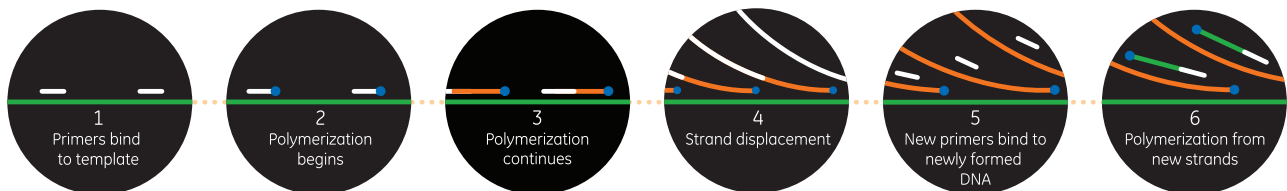
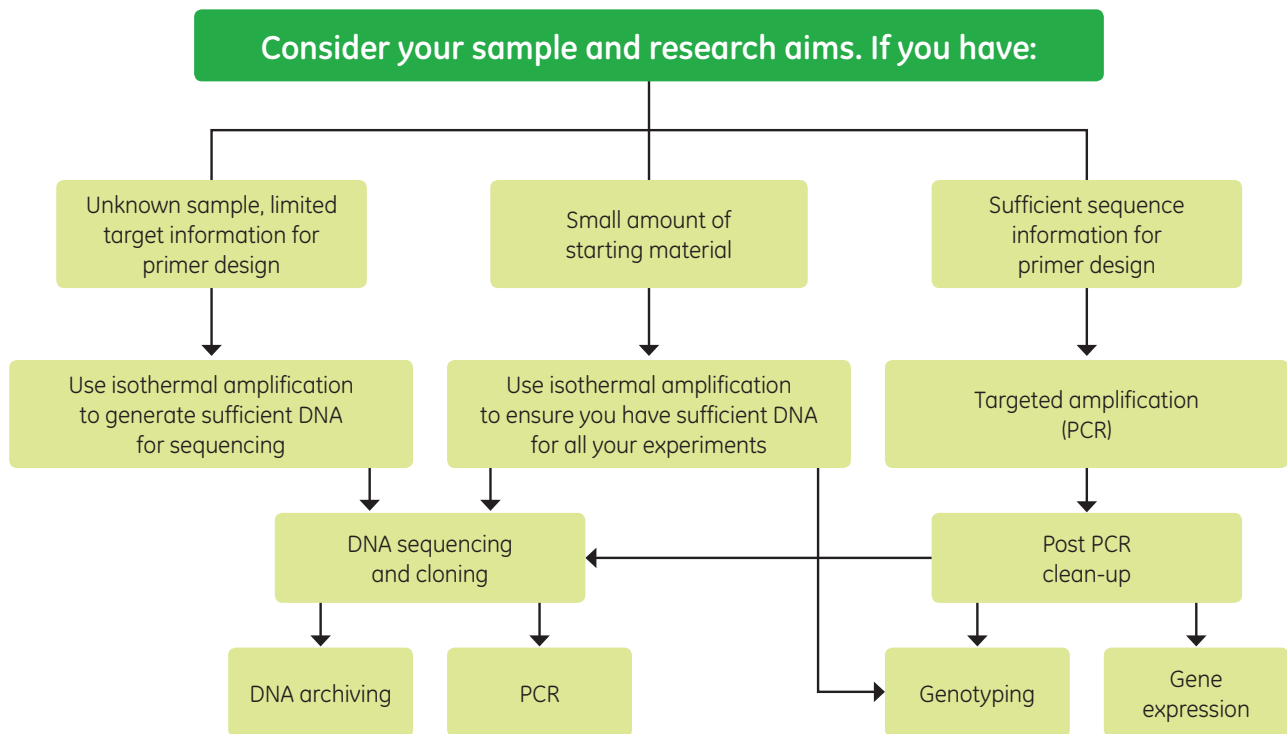
# illustra nucleic acid amplification

## 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 primer-specific 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.



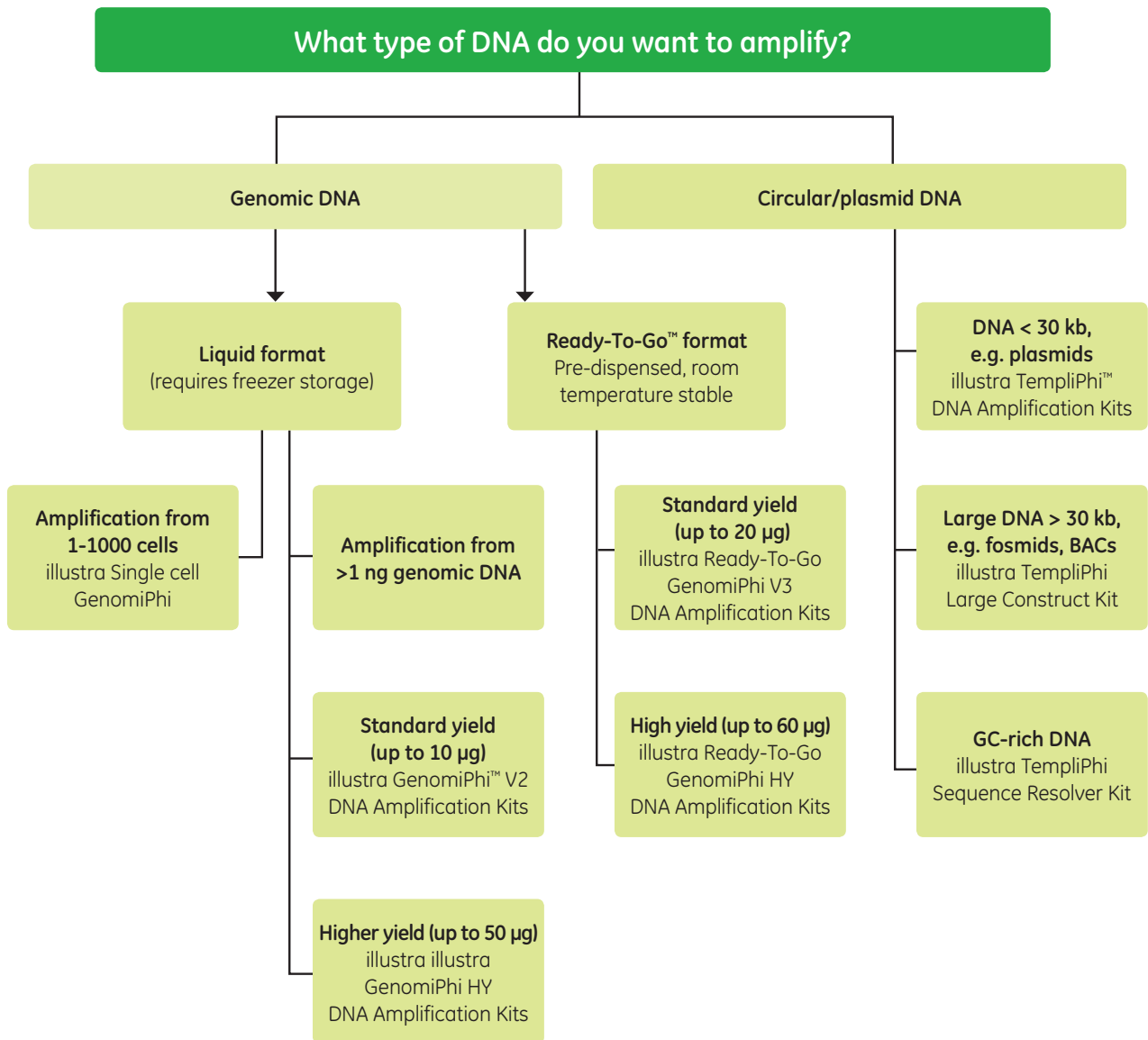
Schematic diagram of the amplification process with the GenomiPhi amplification method. Random hexamer primers anneal to the template DNA at multiple sites. Phi29 DNA polymerase initiates replication at multiple sites on the denatured linear DNA simultaneously. As synthesis proceeds, strand displacement of complementary DNA generates new single-stranded DNA. The subsequent priming and strand displacement replication of this DNA results in the formation of double-stranded DNA.

## 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 proof-reading activity, resulting in 100-fold higher fidelity compared to *Taq* DNA polymerase. From very small amounts of starting material, Phi29 DNA polymerase

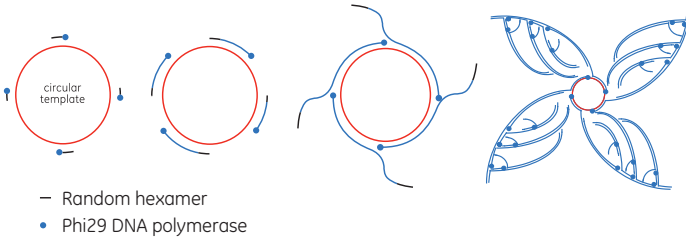
rapidly produces consistent microgram yields of high quality DNA that is ready for direct use in a range of downstream analyses, including sequencing and genotyping. The one-tube, one-temperature format simplifies the DNA preparation process, facilitating automation for high-throughput sample amplification

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



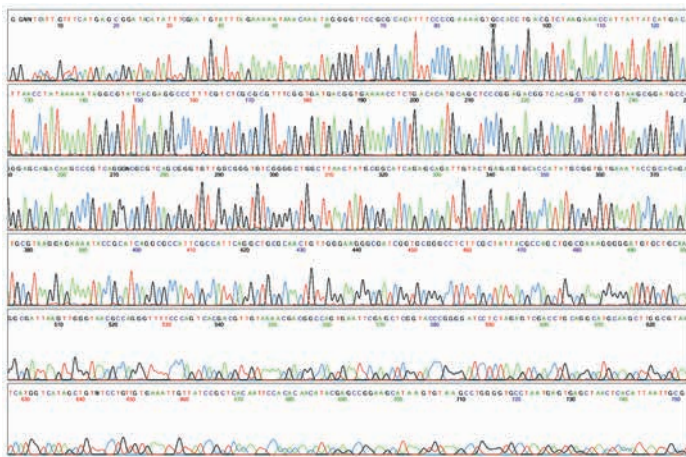
## illustra TempliPhi DNA Amplification Kits

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



Random hexamer primers anneal to the circular template DNA at multiple sites. Phi29 DNA polymerase extends each of these primers. When the DNA polymerase reaches a downstream extended primer, strand displacement synthesis occurs. The displaced strand is rendered single-stranded and available to be primed by more hexamer primer. The process continues, resulting in exponential, isothermal amplification.

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.



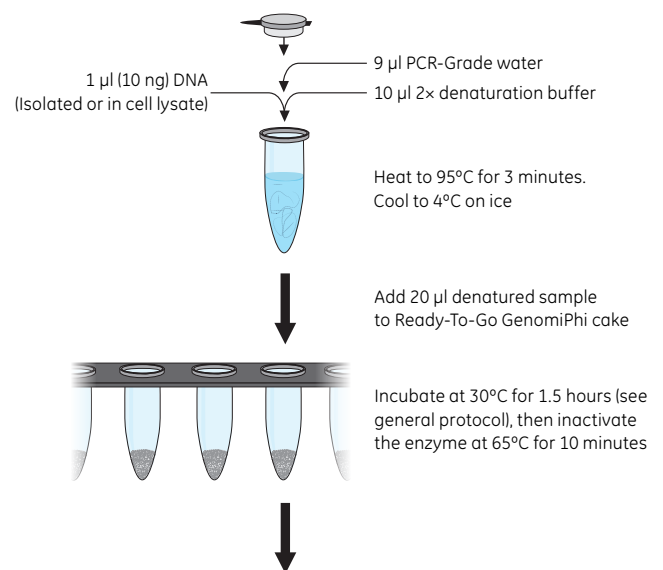
Plasmid DNA template was amplified with the TempliPhi 100 Amplification Kit and subsequently sequenced using the DYEnamic™ Terminator Cycle Sequencing Kit and analyzed on an ABI PRISM™ 3100 Genetic Analyzer.

## illustra 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. illustra GenomiPhi DNA Amplification Kits provide an easy-to-use method that delivers highly representative and reliable whole genome amplification.

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

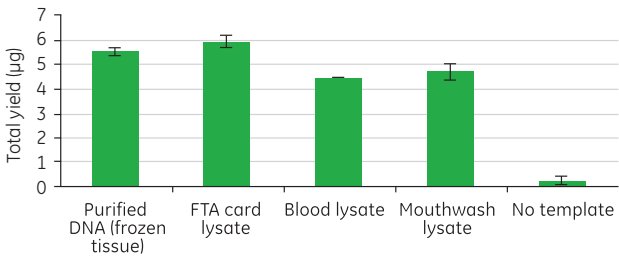
For amplification from very small amounts of starting material, or directly from as little as a single cell, the illustra Single Cell GenomiPhi DNA Amplification kit provides greatest sensitivity. By removing DNA contamination using novel methodologies, sensitivity down to 1 fg is achieved meaning background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



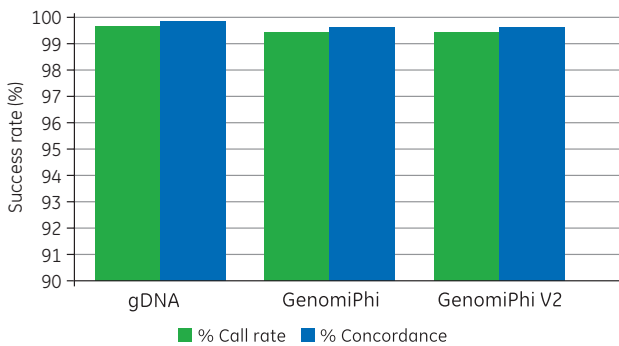
12-20 µg amplified DNA (no DNA synthesis in no template controls)

illustra GenomiPhi V3 DNA Amplification Kit protocol.

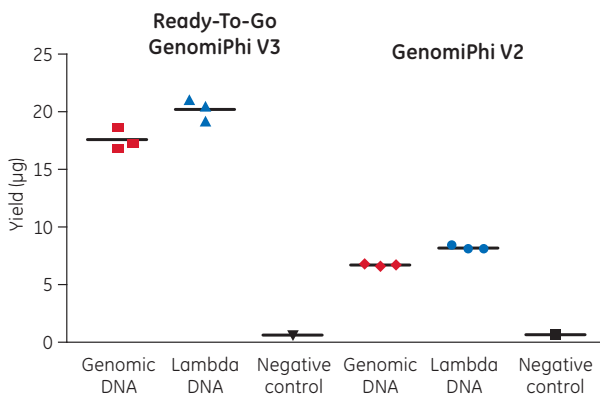
illustra Ready-To-Go GenomiPhi kits can be used with various kinds of samples, including blood lysates and dried blood spots. A protocol has been developed for amplification from Whatman™ FTA card punches with minimal handling, thus enabling a streamlined, efficient workflow for sample collection and analysis.



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

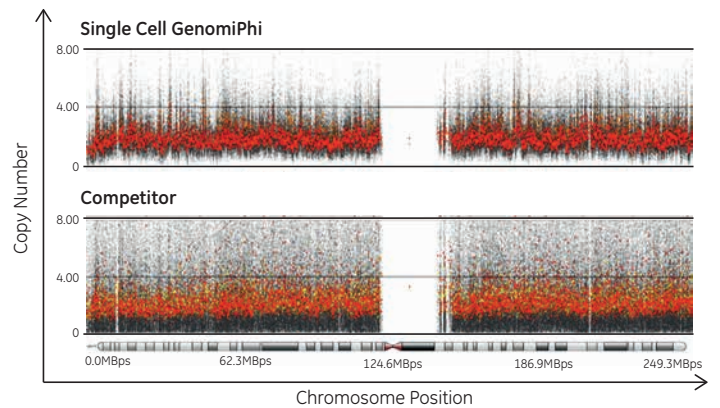


Individual human genomic DNA (gDNA) obtained from Coriell was amplified with illustra GenomiPhi V2 DNA Amplification Kits and subjected to analysis on the Affymetrix™ 100K SNP chip.

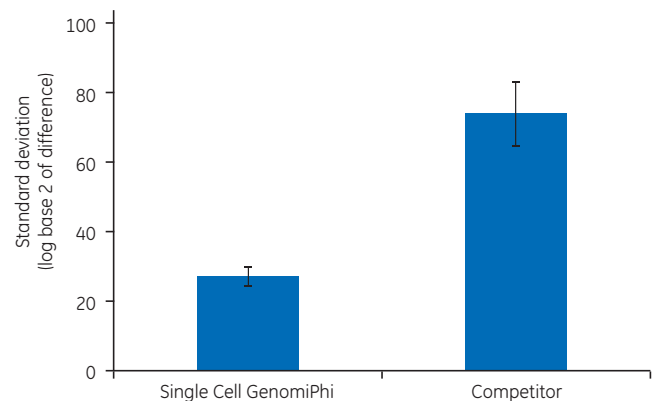


Yield of human genomic DNA (g) or Lambda DNA (l) amplified in Ready-To-Go GenomiPhi V3 or GenomiPhi V2 DNA amplification kits, determined by PicoGreen assay.

illustra Single Cell GenomiPhi DNA amplification kit further develops GenomiPhi technology to robustly amplify genomic DNA from as little as 1 to 1000 cells without interference from non specific amplification. Specially developed reagents and essentially DNA free manufacture ensure background amplification does not interfere with results when working with such minute quantities of starting template, and allows sensitivity down to 1 fg of DNA. Cell lysis protocol has been optimized to ensure 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.

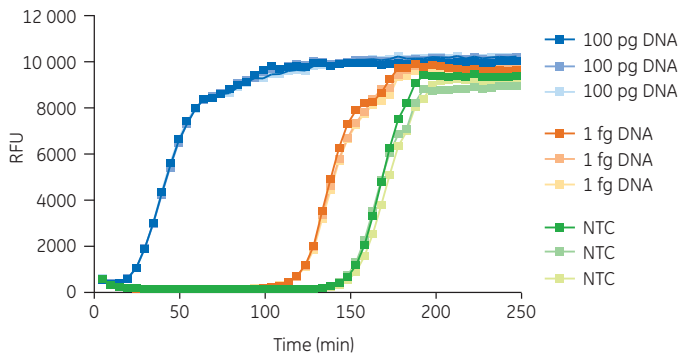


Copy number graph showing WGA gDNA normalized to the unamplified bulk control gDNA samples. Graphs represent overlap of at least two separate samples. Colored dots = mean intensity of 30 probes; representative graph of chromosome 1 is shown.



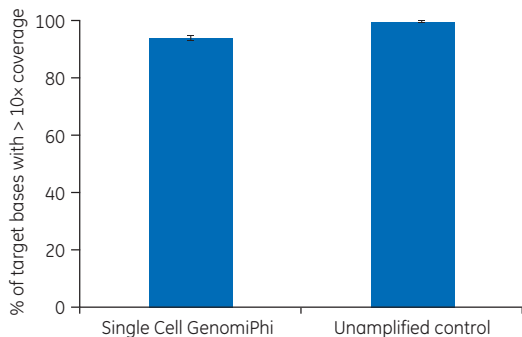
Graph showing amplification bias of Single Cell GenomiPhi DNA Amplification Kit and a PCR-based WGA competitor (Sigma GenomePlex Single Cell Whole Genome Amplification Kit). Each spot intensity was compared with that same spot on one of the control slides. The standard deviation of each slide compared with that control is shown. No statistical comparison between Single Cell GenomiPhi DNA Amplification Kit and the competitor was performed due to low sample numbers.

**Real-time amplification kinetics of human gDNA samples**



Human gDNA (100 pg and 1 fg) was amplified with Single Cell GenomiPhi 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.

**Percent exome covered**

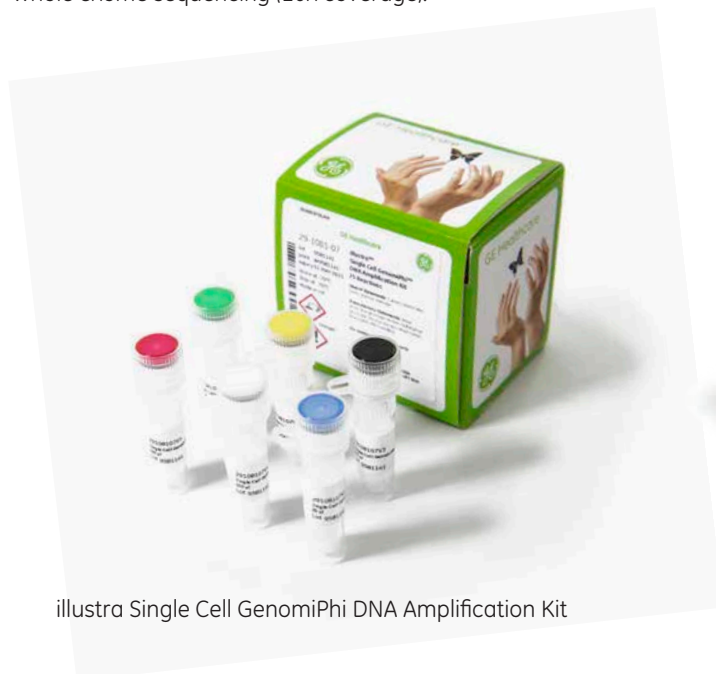


gDNA amplified with Single Cell GenomiPhi DNA Amplification Kit results in a high percentage of exome sequence coverage when run in whole exome sequencing (10x coverage).

**Ordering information**

Product	Pack size	Code number
<b>illustra Single Cell GenomiPhi</b>		
DNA Amplification Kit	25 reactions	29-1081-07
DNA Amplification Kit	100 reactions	29-1080-39
<b>illustra GenomiPhi V2</b>		
DNA Amplification Kit	25 reactions	25-6600-30
DNA Amplification Kit	100 reactions	25-6600-31
DNA Amplification Kit	500 reactions	25-6600-32
<b>illustra GenomiPhi HY</b>		
DNA Amplification Kit	25 reactions	25-6600-22
DNA Amplification Kit	100 reactions	25-6600-20
DNA Amplification Kit	1000 reactions	25-6600-25
<b>illustra Ready-To-Go GenomiPhi V3</b>		
DNA Amplification Kit	24 reactions	25-6601-24
DNA Amplification Kit	96 reactions	25-6601-96
DNA Amplification Kit	5 × 96 reactions	25-6601-97
<b>illustra Ready-To-Go GenomiPhi HY</b>		
DNA Amplification Kit	24 reactions	25-6603-24
DNA Amplification Kit	96 reactions	25-6603-96
DNA Amplification Kit	5 × 96 reactions	25-6603-97
<b>illustra TempliPhi</b>		
100 DNA Amplification Kit	100 reactions	25-6400-10
100 DNA Amplification Kit	500 reactions	25-6400-50
2000 DNA Amplification Kit*	2000 reactions	28-9642-86
Large Construct Kit	1000 reactions	25-6400-80
Sequence Resolver Kit	20 reactions	28-9035-29
Sequence Resolver Kit	50 reactions	28-9035-30
Sequence Resolver Kit	200 reactions	28-9035-31

\* Supplied with premixed components for high-throughput convenience.

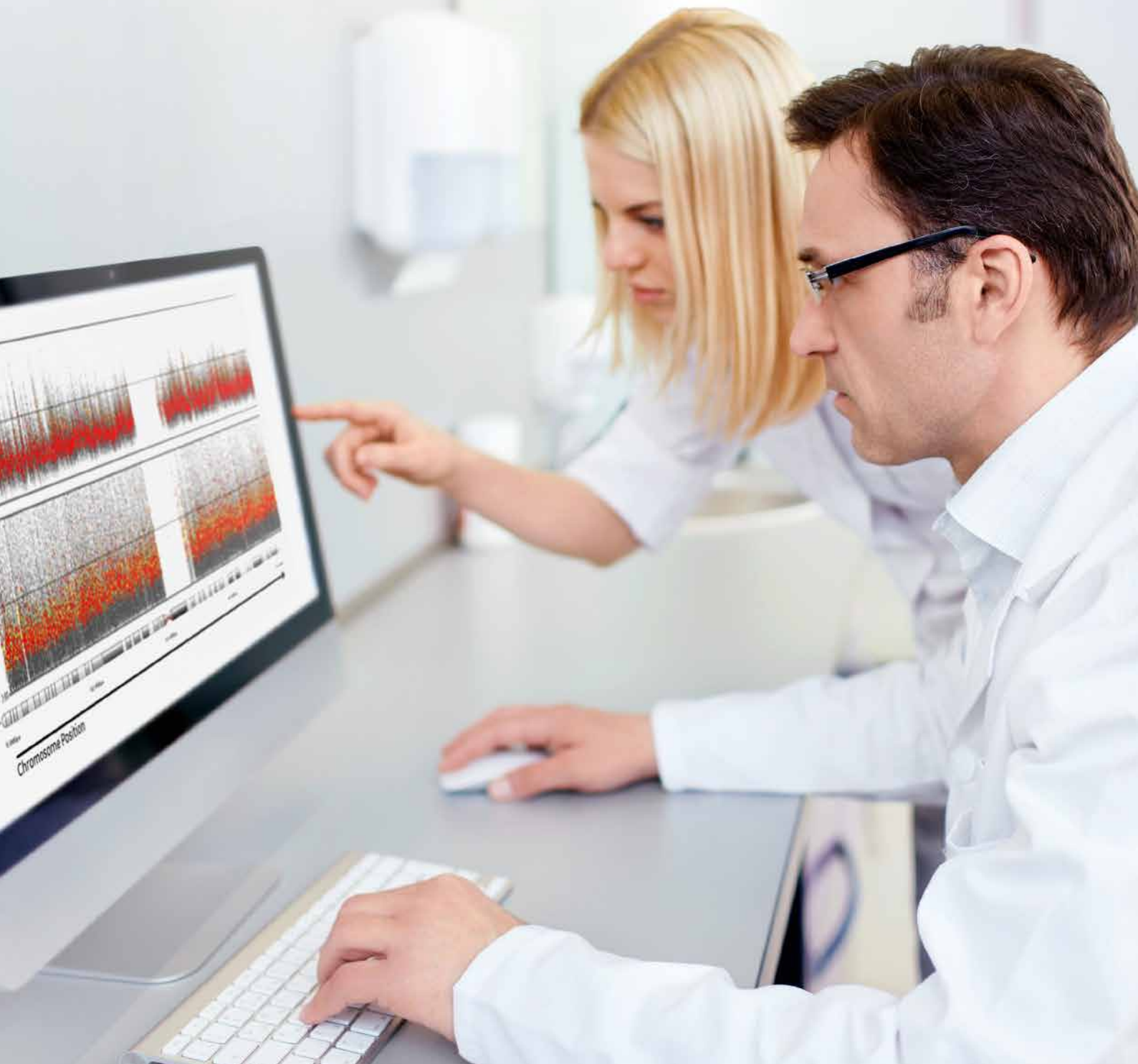
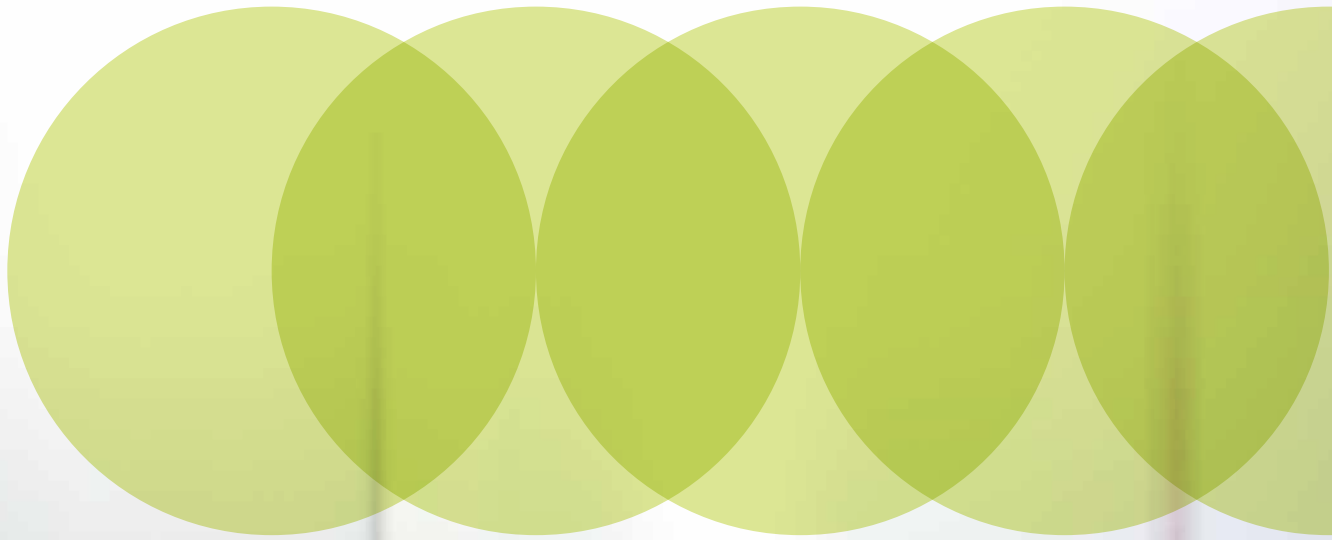


illustra Single Cell GenomiPhi DNA Amplification Kit



illustra GenomiPhi V3 DNA Amplification Kit





## 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.

illustra 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.

Ready-To-Go Beads are available for a range of other molecular biology applications; see our Products for Life Sciences catalog for details. Custom Ready-To-Go Bead formulations can also be prepared, please contact us for further information.

### **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<sub>2</sub>, 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

### **Hot Start Mix Ready-To-Go PCR Beads, 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.

### **Ready-To-Go RT-PCR Beads, for amplification from RNA templates**

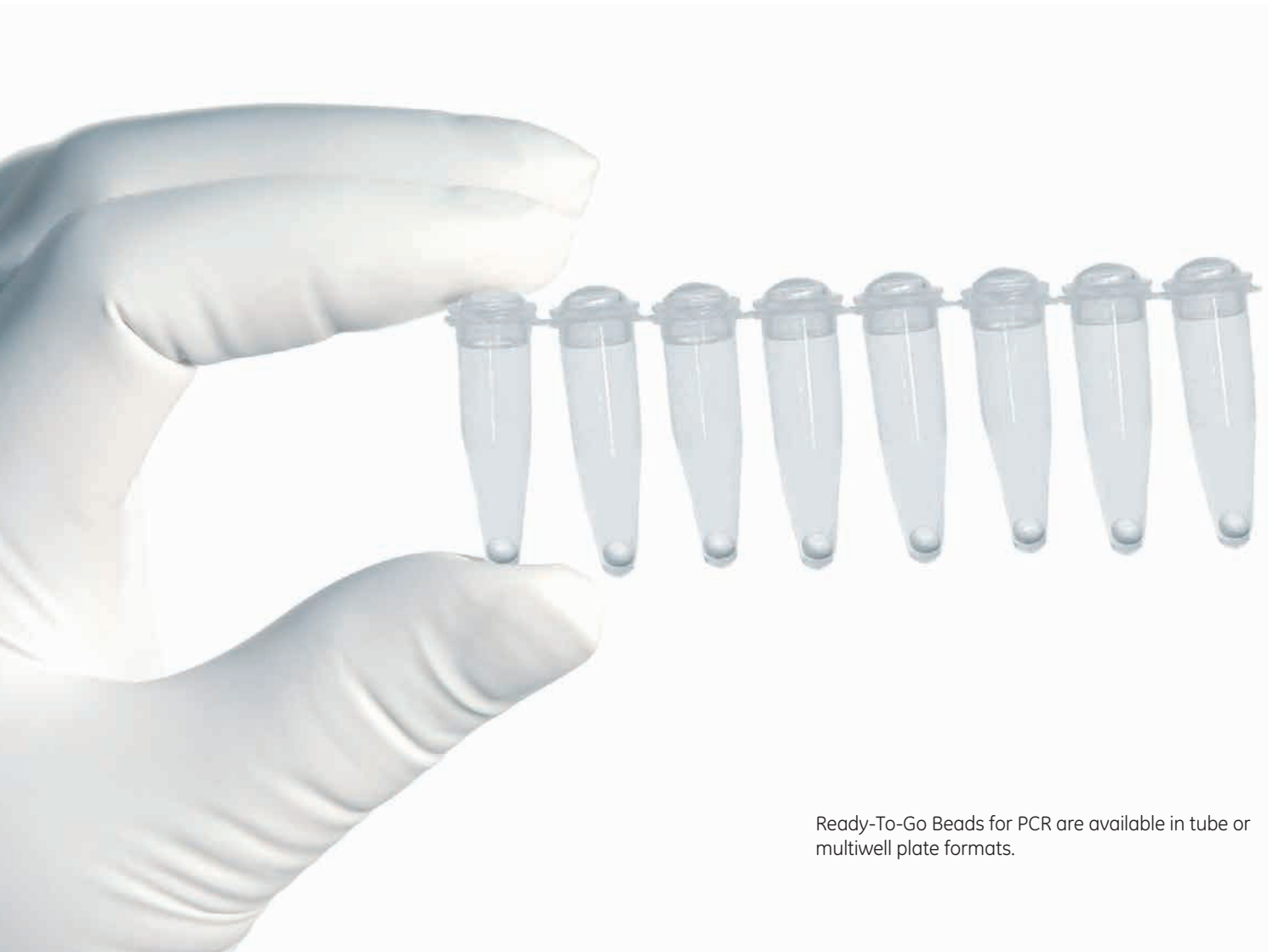
- Optimized one-tube, one-step reactions for both cDNA synthesis and PCR means no need to open the tube or change conditions between steps
- A reduced number of pipetting steps minimizes risk of contamination and RNA degradation, improving assay reproducibility
- Each lot is functionally tested for the 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 MgCl<sub>2</sub>, 200 μM of each dNTP, stabilizers, and BSA
- Includes control beads, containing rabbit globin mRNA and specific primers

### RAPD Analysis Ready-To-Go 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*
- Available with or without six random primers

### Ordering information

Product	Pack size	Code number
<b>Ready-To-Go Beads illustra Ready-To-Go RT-PCR Beads</b>		
0.2 ml hinged tube with cap	96 reactions	27-9259-01
0.5 ml tubes	100 reactions	27-9266-01
0.2 ml tubes/plate	96 reactions	27-9267-01
<b>illustra PuReTaq Ready-To-Go PCR Beads</b>		
0.2 ml tubes/plate	96 reactions	27-9557-01
0.2 ml tubes/plate	5 × 96 reactions	27-9557-02
0.5 ml tubes	100 reactions	27-9558-01
0.2 ml hinged tube with cap	96 reactions	27-9559-01
<b>illustra Hot Start Mix RTG</b>		
0.5 ml tubes	100 reactions	28-9006-46
0.2 ml tubes/plate	96 reactions	28-9006-53
0.2 ml tubes/plate	5 × 96 reactions	28-9006-54
<b>illustra Ready-To-Go RAPD</b>		
Analysis Beads	100 reactions	27-9500-01
Analysis Kit	100 reactions and 6 primers	27-9502-01
<b>Other Master Mix products</b>		
illustra Hot Start Master Mix	100 reactions	25-1500-01



Ready-To-Go Beads for PCR are available in tube or multiwell plate formats.

## 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. Enzyme preparations are also tested 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)

PuReTaq DNA polymerase is a proprietary formulation of purified, recombinant Taq DNA polymerase. It has an optimum temperature of 75°C and survives repeated incubations at 95°C. PuReTaq is supplied at a concentration of 5000 units/ml, with a 10× reaction buffer and additional 25 mM MgCl<sub>2</sub> to facilitate assay optimization. This preparation is also used in our Ready-To-Go PCR and RT-PCR Beads.

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

### illustra 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

## Ordering information

Product	Pack size	Code number
<b>Taq DNA polymerase (cloned)*</b>		
	250 units	27-0798-04
	4 × 250 units	27-0798-05
	10 × 250 units	27-0798-06
	1000 units	28-9373-45
	5000 units	28-9373-46
	10 000 units	28-9373-47
	25 000 units	28-9373-48
<b>dNTP Set (100 mM each A,C,G,T)</b>		
	4 × 25 µmol	28-4065-51
	4 × 100 µmol	28-4065-52
	4 × 500 µmol	28-4065-53
<b>PCR Nucleotide Mix dNTP Set</b>		
25 mM each A,C,G,T	500 µl	28-4065-60
2 mM each A,C,G,T	1 ml	28-4065-62
10 mM each A,C,G,T	500 µl	28-4065-64

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

# illustra post-PCR clean-up

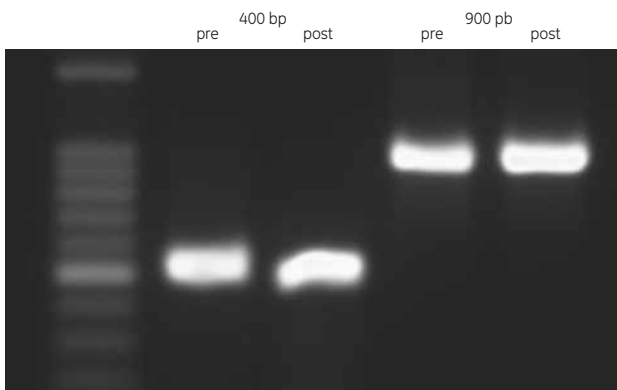
Our range of nucleic acid purification products for post-amplification DNA clean-up is optimized for high performance in your specific application. The products are supplied with prepacked columns for added convenience and speed as well as quick reference protocol cards for ease of use.

## illustra ExoProStar™ and illustra ExoProStar 1-Step

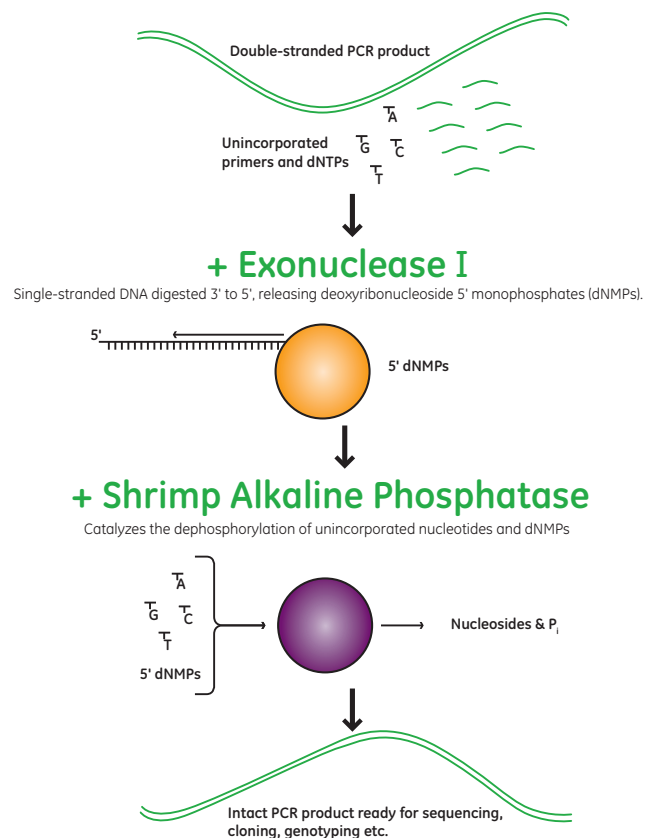
Our illustra ExoProStar and illustra ExoProStar 1-Step products are optimised to purify PCR and sequencing set up reactions quickly, efficiently and reliably.

These enzymatic PCR clean up kits contain illustra Alkaline Phosphatase and Exonuclease f, formulated to work together to remove unincorporated primers and nucleotides from amplification reactions in preparation for sequencing, cloning, genotyping or further DNA modification reactions.

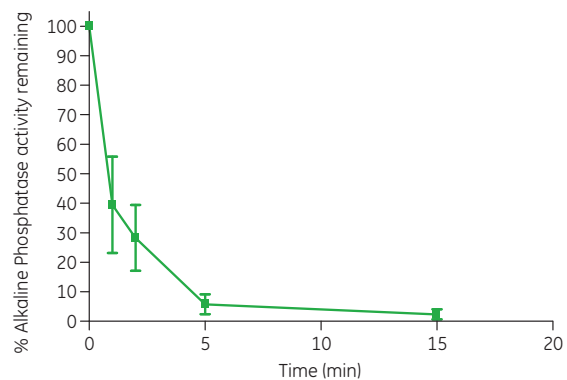
- Enzymes optimised to work together for high efficiency removal of unincorporated primers and nucleotides
- Enzymes provided either as a pre-prepared single tube mix for maximum simplicity or in separate tubes for maximum flexibility
- Fast 30 minute protocol
- Scalable for different reaction sizes
- No loss of PCR product
- Easy to automate
- Complete heat inactivation of both enzymes within 15 min



No Loss of PCR Product: Agarose gel electrophoresis of different size PCR products pre and post digestion with illustra ExoProStar 1-Step. Samples were digested for 15 minutes at 37°C followed by denaturation of the illustra ExoProStar 1-Step enzymes at 80°C for 15 min as per the recommended operating protocol. No loss of PCR product was detected in any of the samples.



PCR Clean-Up workflow with illustra ExoProStar and illustra ExoProStar 1-Step.

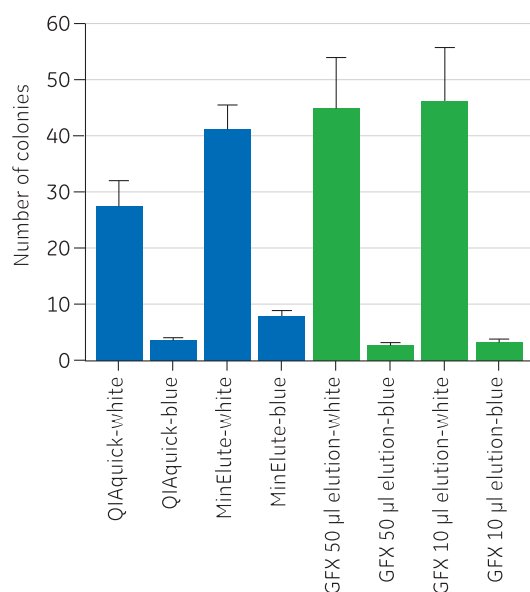


Complete inactivation of Alkaline Phosphatase in 15 min.

Our illustra ExoProStar products build on our long history and expertise in providing DNA Clean-Up products and expands on our original patents for enzymatic sample clean-up using Exonuclease f and Alkaline Phosphatase. With illustra ExoProStar we have improved on existing products to give you enhanced PCR and Sequence reaction clean-up.

## illustra™ GFX™ PCR DNA and Gel Band Purification Kit, for flexible, fast purification

- Fast isolation and concentration of high quality DNA from PCR mixtures
- Fast, easy-to-use method with less than 10 min hands-on time
- Flexible elution volume (10 to 50 µl) for different DNA concentration needs
- Choice of elution buffers to suit your downstream application
- Highly purified DNA is ready for use in sequencing, labeling, restriction enzyme digestion, and cloning
- Can also be used for DNA-containing agarose gel bands, enzyme based DNA modification mixes, and restriction digests



illustra GFX PCR DNA and Gel Band Purification Kit performs equivalently to the four Qiagen kits (QIAquick™ and MinElute™ PCR Purification Kits, and QIAquick and MinElute Gel Extraction Kits) required to obtain the same results. DNA purified from PCR mixtures was used in cloning experiments. White colonies indicate successful cloning. See GE Healthcare data file 28-9075-94 for comparison details.

## illustra GFX 96 PCR Purification Kit, for rapid purification of multiple samples

- Purification of up to 96 PCR products (0.1 to 10 kb) simultaneously in as little as 15 min
- High yields of pure DNA in small volumes of water or low ionic strength buffer
- Typical recoveries are > 85% for PCR products 0.1 to 10 kb with salt removal generally > 99%
- No need for ethanol precipitation or use of hazardous organic solvents
- Purified DNA is ready for use in most applications, including: fluorescent sequencing, microarrays, labeling, hybridization, ligation, and transformation

## Ordering information

Product	Pack size	Code number
<b>illustra ExoProStar PCR Purification Kit</b>		
<b>ExoProStar</b>		
	20 reactions	US77701
	100 reactions	US77702
	500 reactions	US77705
	2000 reactions	US77720
	5000 reactions	US77750
<b>ExoProStar 1-step</b>		
	20 reactions	US78220
	100 reactions	US78210
	500 reactions	US78211
	2000 reactions	US78212
	5000 reactions	US78225
<b>illustra GFX PCR DNA and Gel Band Purification Kit</b>		
	100 purifications	28-9034-70
	250 purifications	28-9034-71
<b>illustra GFX 96 PCR Purification Kit</b>		
	10 × 96 well plates	28-9034-45

In addition to our illustra amplification and post-PCR clean-up products, we offer a range of products for sample preparation and downstream applications. A selection of the most popular are shown in the following *Related products* table and visit [www.gelifesciences.com](http://www.gelifesciences.com) for the complete range.





[www.gelifesciences.com/illustra](http://www.gelifesciences.com/illustra)

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