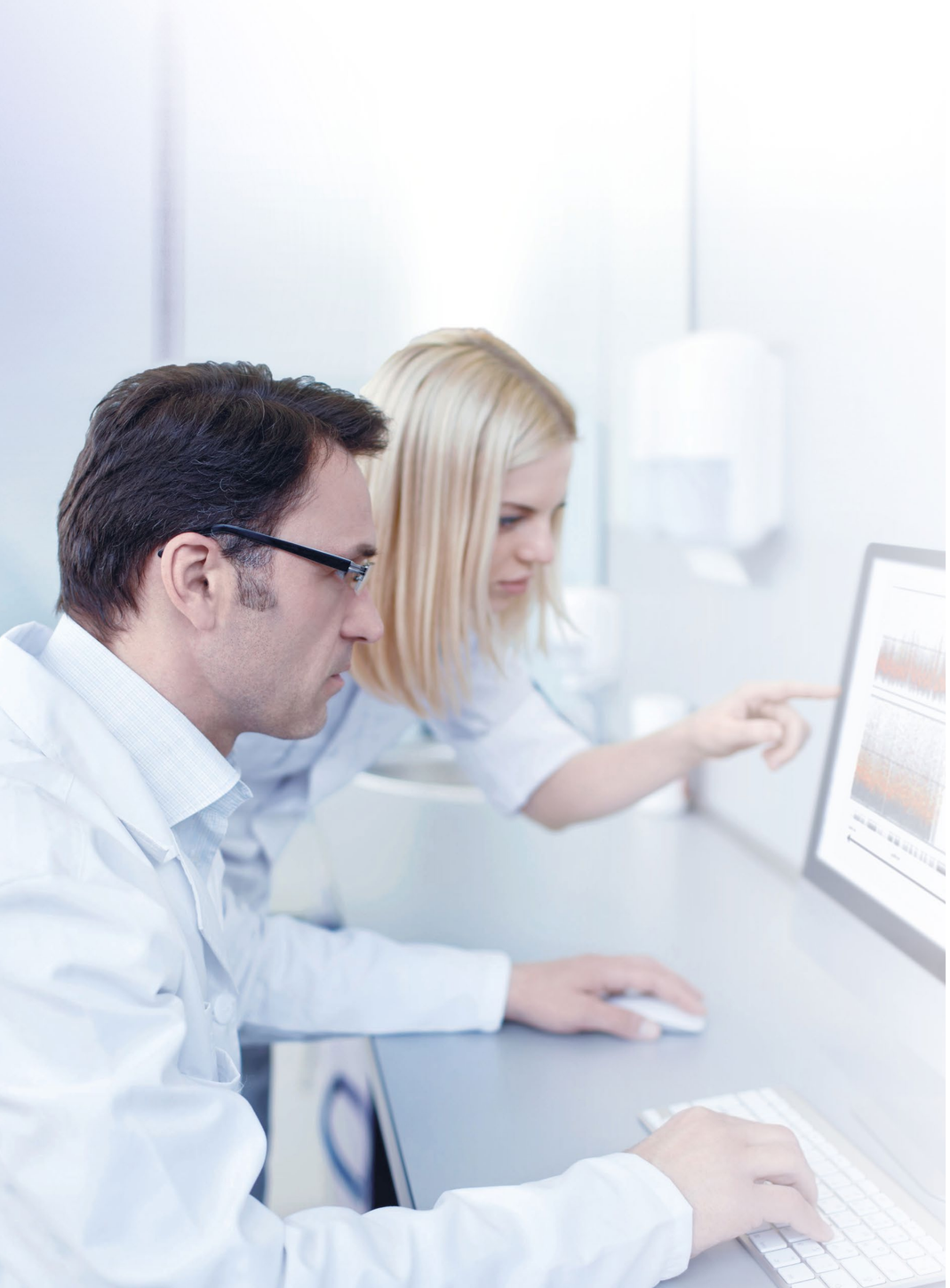
Sequencing and precision diagnostics

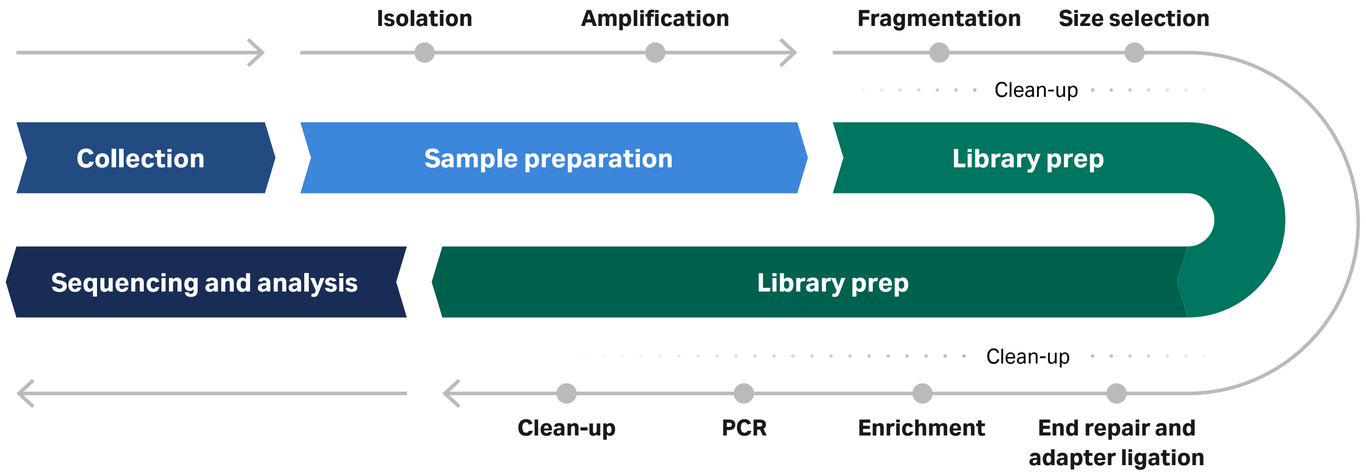
For today and beyond

Enabling next-generation sequencing workflows

cytiva



Sequencing workflow



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Products	FISH	RT QPCR	QPCR	Microarrays	Sanger sequencing	NGS
GenomiPhi™ Single Cell DNA Amplification Kits					•	•
TempliPhi™ 100/500 Amplification Kits					•	•
TempliPhi™ 2000 Reaction Kit					•	•
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GFX™ PCR DNA and Gel Band Purification Kits	•		•	•		•
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Introduction

Developing solutions that support next-generation sequencing (NGS) is an area of key focus for Cytiva.

Nucleic acid sequencing technologies have come a long way since the first Sanger sequencing of the human genome. As the capabilities of sequencing have increased, so too has the range of applications and the questions answered. NGS provides the ability to parallel sequence on a large scale which has had a massive impact and has enabled biological questions at the level of the genome to be addressed. NGS technology now underpins genomics research enabling researchers to sequence an entire genome, transcriptome or exome carrying out experiments and analysing data which until recently would have been considered impossible. Sequencing supports a diverse range of applications including clinical diagnostics and other aspects of medical care ranging from disease risk to therapeutic interventions.

The next steps are to streamline this process and lower the overall cost to make it more accessible to all markets. At Cytiva, we hope to be at the forefront of the simplification and accessibility of the NGS technologies for day-to-day research, clinical diagnostics and much more. If we don't have a standard product to precisely meet your needs, we can develop a customized solution to simplify and streamline your workflow.

What's hot in DNA sequencing and what's next?

DNA sequencing has come a long way. Just four decades after Fredrick Sanger developed the Sanger method, a DNA sequencing technique that won him a Nobel prize, DNA sequencing is a reliable and essential tool not only for scientists, but also clinicians, law enforcement and even educators.

Our appetite for faster, better sequencing is insatiable, and has spurred development of new sequencing technologies from large banks of high-throughput sequencers to portable units that fit in a drawer. While it took 12 years to sequence the human genome, researchers using current sequencing technology, supported and enabled by advances in computing and data analysis, contemplate sequencing the genomes of populations of hundreds of thousands.

Next-generation sequencing: where are we now?

After the completion of the Human Genome Project, a new wave of technologies, called NGS, came onto the market. These sequencers use massively parallel sequencing of short reads for high throughput.

Illumina™ platforms became market leaders, developing a sequencing-by-synthesis approach on a clonally amplified template.

NGS has fundamentally changed genomics research. It enables scientists to sequence an entire genome, transcriptome, or exome, conducting experiments that were previously impossible. To put that into perspective, generating the raw data for the Human Genome Project now would take less than one day with these systems.

But there are some limitations to these platforms. NGS relies on short reads (less than 1 kb), requiring scaling up to millions of fragments sequenced in parallel. The short-read length is not well-suited for *de novo* assembly, as the data isn't easily reconstructed without a reference genome. Deep sequencing partially compensates for this limitation by minimizing errors, but repetitive regions are still a challenge.

Sample preparation for NGS can be labor-intensive. This is being addressed with commercial kits that simplify the amplification and purification of starting DNA.

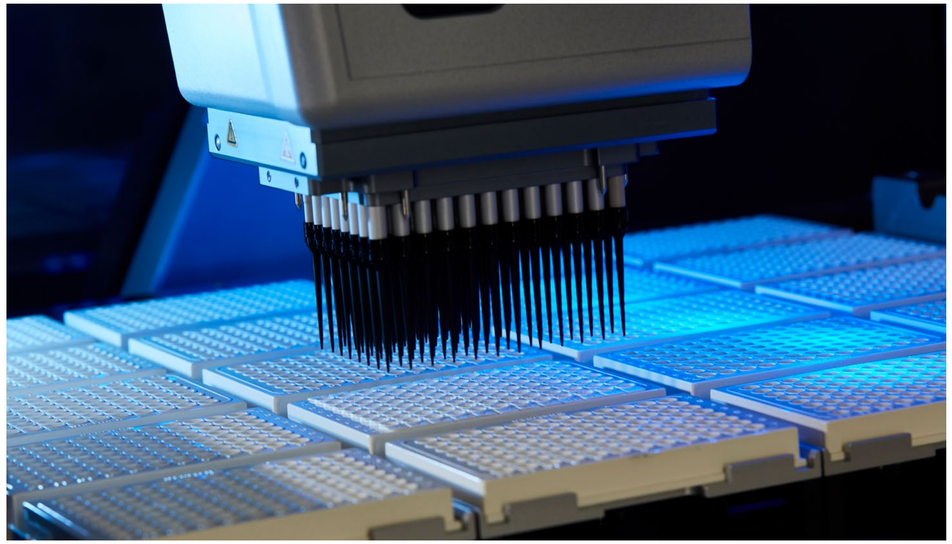
Third-generation sequencing: single-molecule technologies

Enter third-generation sequencers, which provide long-read sequencing (tens of kb) without clonal amplification or the various preparative steps needed for NGS. Long-read sequencing can simplify *de novo* assembly, span repetitive regions and structural variations in one read, and provide lower bias to G-rich/poor regions.

Single-molecule sequencing struggles with accuracy, but error rates are improving as the technology matures. It is already possible to reconstruct a genome with long-read sequencing as easily as by NGS.

The future of next-generation sequencing

As next-generation sequencing technologies continue to develop, expect a step change in speed and convenience, doing away with the need for technical preparation steps, and simplifying workflows. These new technologies will inevitably become routine in research, in clinical settings, and far beyond.



01

Sample preparation

Isolation

Unraveling the challenges of nucleic acid isolation

Analyzing nucleic acids is enormously powerful, providing us with insights into a variety of biological processes for basic research and clinical applications. DNA isolation (and RNA isolation) is the first step for many modern genomics techniques and applications, which require high-quality starting material free of contaminants.

How to extract DNA (or RNA)

Genomic DNA extraction is the first step in many molecular biology studies, and all recombinant DNA techniques. Protocols involve breaking open the cells and separating the DNA from other nucleic acids and cellular components in the sample, while also keeping it in good condition for downstream analysis.

The choice of approach depends on several factors, including the target DNA, source organism, the type and quality of your starting material, and the application. They generally all share three common steps: lysis, contaminant removal and DNA recovery.

Step 1: Lysis

Cell lysis involves chemical, mechanical, or enzymatic disruption of cell membranes and denaturation of proteins. The exact method depends on the starting material. Bacteria, mammalian cells, plant cells, and human tissues all might require a slightly different approach.

'Gentle' lysis might involve using a detergent, such as sodium dodecyl sulfate (SDS), or enzymes to break up cell membranes; aggressive lysis might take the form of homogenization to physically break open cell walls.

Step 2: Removing contaminants

You can use both solution-based and solid-phase methods to separate DNA from unwanted lysis debris and potential contaminants. Phenol chloroform DNA extraction, for example, separates water-soluble DNA and denatured proteins into different phases. This is cheap, but slow, and risks carryover of phenol that can affect downstream applications.

Solid-phase extraction binds DNA to a column or bead surface. Silica resins or silica-coated magnetic beads, for example, use chaotropic salts to disrupt hydrogen bonds and bind nucleic acids, enabling contaminants to be washed away. Oligonucleotide-coated resins can also add a level of specificity, but column kits can quickly add up in cost.

Step 3: Recovering the target nucleic acid

Downstream applications require DNA in a suitable format (solvent and concentration). Often, this will be just a matter of precipitating DNA with ethanol, washing, and resuspending in an appropriate buffer. For solid-phase methods, it will first require adjusting the pH or salt concentration of the buffer to release the nucleic acids.

Sample-specific challenges

Cultured mammalian cells and tissues

Cultured cells are relatively easy to lyse with osmotic shock or detergent treatments, while isolating DNA from tissue requires breaking down the extracellular matrix, not just cell membranes. This often requires homogenization followed by silica column (e.g., genomicPrep kits) or mag bead-based (e.g., SeraSil-Mag™) purification, or less favorable phenol-chloroform extraction.

Using formalin-fixed, paraffin-embedded (FFPE) tissue is common in clinical applications and some research studies. It's excellent for preserving tissue structures, but can introduce all sorts of DNA damage with profound effects. That is, as the quality of the DNA isolated directly affects the assay results, positive samples might be overlooked simply because of poor extraction.

Blood

A challenge of DNA extraction from blood is the variability in DNA quantity depending on blood fraction. Red blood cells do not contain DNA, so there's much less per cell in whole blood compared to buffy coat or bone marrow-derived fractions.

Blood coagulation also presents challenges: clotting can prevent effective sample digestion, and some anticoagulants can interfere with PCR amplification.

Bacteria

There are differences between gram-positive and gram-negative samples in DNA extraction from bacteria. Gram-positive samples usually require lysozyme treatment to digest the higher levels of peptidoglycan in the cell wall, whereas for gram-negative samples, a simple osmotic shock might be enough.

DNA is unlikely to be scarce with either type, and it is common to use fast methods, like alkaline extraction and diatomaceous earth, to extract the DNA. Both methods are reliable, but alkaline extraction might not provide the highest purity by itself, and diatomaceous earth can be high cost.

Plant material

Plant cells can be embedded in a tough matrix and have cell walls consisting of glycans and cellulose that are difficult to break. The solvent-based cetyltrimethylammonium bromide (CTAB) extraction method is common for plant material, but it is an aggressive approach. It uses harsh chemicals, is laborious, and often requires further clean-up and optimization for different samples and applications.

Why use magnetic beads for DNA extraction

Magnetic beads provide an excellent alternative to traditional isolation and clean-up methods due to their versatility and ease of use. They do not require additional centrifugation of a potentially already agitated sample, improving the likelihood of recovering larger fragments, and can be scaled up to have a higher binding capacity than columns.

Using magnetic beads is straightforward, needing no hazardous solvents, and releasing the DNA or RNA is just a matter of adjusting the buffer properties (Fig 1). This simplicity also makes magnetic beads well suited to automation in high-throughput applications.

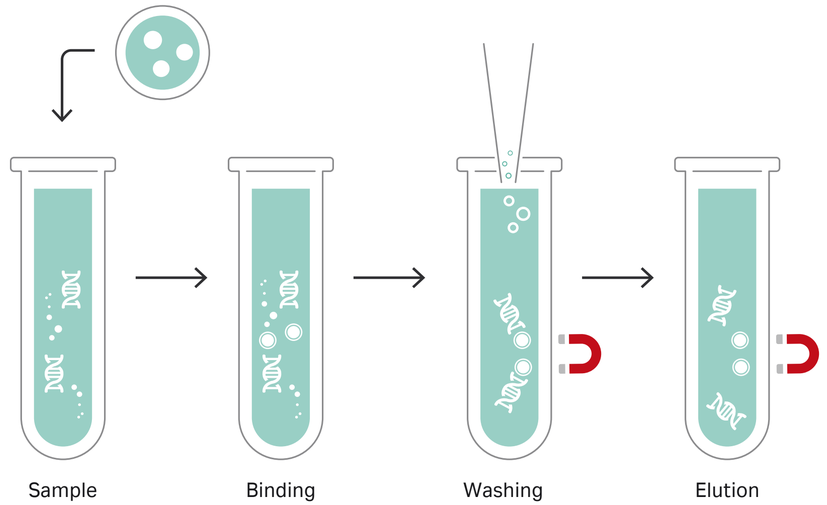


Fig 1. The principle of magnetic beads for nucleic acid isolation.

Nucleon™ extraction systems

Nucleon™ extraction systems rapidly extract high molecular weight from whole blood and cultured cells. The Nucleon™ proprietary resin is added following cell lysis, deproteinization with sodium perchlorate, and a single chloroform extraction.

Nucleon™ BACC

Nucleon™ BACC Genomic DNA Extraction Kits are designed for rapid extraction of high-quality, high molecular weight genomic DNA from blood and cell cultures and feature the proprietary Nucleon™ resin that binds protein while forming a semi-solid stratum during partitioning, which facilitates removal of the aqueous phase and ensures excellent recovery of high quality DNA.

Features and benefits

- Cost effective: non-column format makes scaling up to large sample volumes easy
- High recovery: size of recovered DNA ranges from 23 bp to 250 kbp
- Low-risk and fast: phenol-free protocol and only 30 minutes to complete

Product code	Quantity	Description
RPN8501	25	Nucleon™ BACC1
RPN8502	25	Nucleon™ BACC2
RPN8512	50	Nucleon™ BACC3

Find out more

Nucleon™ PhytoPure

Polysaccharides are common contaminants in plant DNA extracts and can inhibit further enzymatic analysis of DNA. Nucleon™ PhytoPure DNA extraction system has been developed specifically to solve this problem and has been used successfully on a wide range of fresh, frozen or freeze-dried plant material.

Features and benefits

- Fast: enables extraction of DNA in less than 1 hour
- Low-risk: eliminates the need to use phenol
- Simple: easy-to-use protocol requires only one centrifugation step prior to DNA precipitation
- Pure: DNA is of high quality and suitable for RFLP, RAPD and AFLP analyses

Product code	Quantity	Description
RPN8510	50 preparations of 0.1 g	Nucleon™ PhytoPure
RPN8511	50 preparations of 0.1 g	Nucleon™ PhytoPure

Find out more

genomicPrep Mini Kits

The genomicPrep Mini Kits deliver high quality intact high molecular weight gDNA from a variety of samples using a convenient and efficient protocol. The kit delivers high yields of highly pure gDNA suitable for use in a variety of downstream molecular biology workflows including enzyme digests, cloning, electrophoresis, qPCR, genotyping and sequencing.

Blood genomicPrep Mini Spin Kits

Blood genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of high molecular weight genomic DNA (gDNA) from whole blood, buffy coat, bone marrow, and nucleated red blood cells. Uses chaotropic agents to extract DNA from blood cells, denature protein components, and promote the selective binding of DNA to a column-based, novel silica membrane.

Features and benefits

- Fast results: streamlined workflow reduces the number of pipetting volume changes and the overall number of steps to deliver sample to gDNA results in 15 minutes
- Minimal shearing: resulting in the production of 5 to 10 µg of good quality, intact genomic DNA from a 200 µL sample. One kit handles a wide range of blood sample types and volumes from 50 µL to 1 mL.
- High quality: gentle room temperature lysis conditions deliver high-quality, > 97% intact DNA with an average size of > 20 kb

Product code	Quantity	Description
28904263	10	Blood genomicPrep Mini Spin Kit
28904264	50	Blood genomicPrep Mini Spin Kit
28904265	250	Blood genomicPrep Mini Spin Kit

Find out more

Tissue and Cells genomicPrep Mini Spin Kits

Genomic DNA purifications performed with the Tissue and Cells genomicPrep Mini Spin Kit yield consistent results and are highly robust across different sample types.

Features and benefits

- Fast results: reduces time from tissue sample to gDNA and produces high-quality product in just 90 minutes
- Simpler purification: color-coded caps and bottles with matching protocol steps minimize the chance for error
- High quality and purity: optimized tissue protocol produces intact, RNA-free gDNA that is > 20 kb in size with a purity of 1.8 (A_{260}/A_{280})

Product code	Quantity	Description
28904274	10	Tissue and Cells genomicPrep Mini Spin Kit
28904275	50	Tissue and Cells genomicPrep Mini Spin Kit
28904276	250	Tissue and Cells genomicPrep Mini Spin Kit

Find out more

Bacteria genomicPrep Mini Spin Kits

Bacteria genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of high molecular weight genomic DNA (gDNA) from Gram-negative (G-ve) and Gram-positive (G+ve) bacteria. The procedure for G-ve bacteria can be completed in about 40 minutes (sample to gDNA).

Features and benefits

- Fast results: streamlined workflow reduces the number of pipetting volume changes and the overall number of steps
- Optimized kit: dedicated kit optimized for bacterial gDNA with separate protocols for G-ve and G+ve bacteria
- Ease of use: color-coded caps and bottles with matching protocol steps minimize the chance for error
- High quality and purity: optimized protocol produces intact, RNA-free gDNA that is > 20 kb in size with a purity > 1.8 (A_{260}/A_{280})

Product code	Quantity	Description
28904257	10	Bacteria genomicPrep Mini Spin Kit
28904258	50	Bacteria genomicPrep Mini Spin Kit
28904259	250	Bacteria genomicPrep Mini Spin Kit

Find out more

Amersham™ RNAspin Kits

Our Amersham™ RNA purification kits are designed to ensure that you get the reproducibility, yield and purity you need, with minimal degradation in every experiment. Amersham™ kits accommodate a diverse range of application requirements and sample types, delivering RNA that can be used for downstream applications, such as qRT-PCR and microarray analysis.

Amersham™ RNAspin Mini Kits

By identifying key elements that affect the quality of preparation the Amersham™ RNAspin Mini Kit allows total RNA isolation from diverse sample types, resulting in the extraction of RNA suitable for use in sensitive downstream applications such as microarray analysis and quantitative or endpoint RT-PCR. Our on-column DNase I digest improves purities by addressing issues of gDNA contamination.

Features and benefits

- Maximized yields: the inclusion of prefilters and a unique lysis buffer makes it less susceptible to foaming
- Efficient: column-binding capacity of 100 µg and elution volumes as low as 40 µL eliminate the need to concentrate sample
- Reliable: well-established silica-membrane technology

Product code	Quantity	Description
25050087	10	Amersham™ RNAspin Mini Kits
25050070	20	Amersham™ RNAspin Mini Kits
25050071	50	Amersham™ RNAspin Mini Kits
25050072	250	Amersham™ RNAspin Mini Kits

Find out more

Amersham™ RNAspin 96 Kit

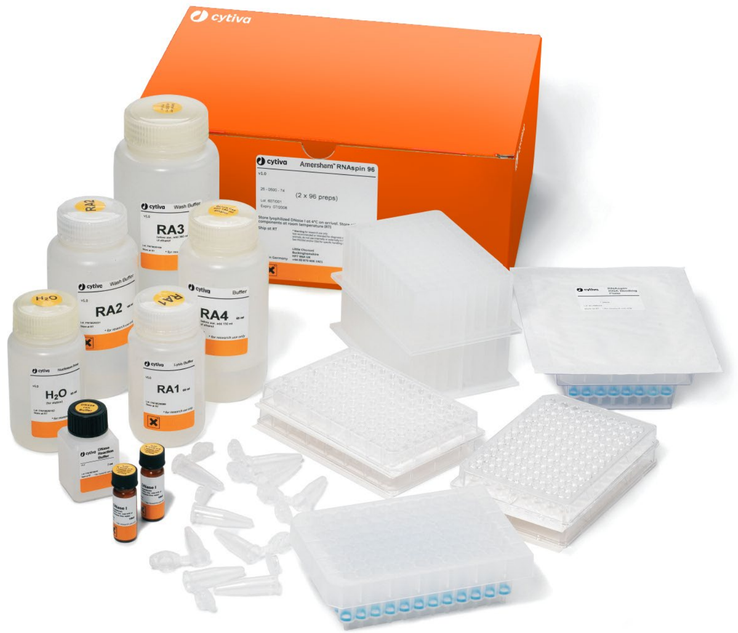
With protocol run times that have been optimized to be as short as possible, Amersham™ RNAspin 96 kits support a high throughput approach, whether samples are processed under vacuum, using centrifugation, manually, or with automation. The on-column lysis for small amounts of sample improves efficiency by avoiding mechanical homogenization.

Features and benefits

- Fast and efficient: purification in a 96-well format with high reproducibility in less than 70 minutes
- Supports automation: integrated wash plate eliminates risk of cross contamination and compatible with common liquid handling instruments for fast integration
- Convenient: DNase I included for convenient on-column gDNA removal, leading to pure total RNA
- Flexible and scalable: includes prefilter accessory plate

Find out more

Product code	Quantity	Description
25050075	4 × 96 preps	Amersham™ RNAspin 96 Kit



SeraSil-Mag™ Silica Coated Magnetic Particles

SeraSil-Mag™ silica coated superparamagnetic particles deliver a high purity extraction solution for highly sensitive applications when sample is scarce. The beads provide an optimal binding surface, with regular morphology, to optimize binding efficiency and reduce variability, simplifying the transition from column purification to bead-based purification.

Features and benefits

- High iron oxide content (60 emu/g): fast magnetic response (~ 5 s) shortens time of magnetic steps during isolation
- Uniformity: particles are uniform in size (submicroscale diameter 700 nm and 400 nm [monodispersed]), providing narrow size distribution
- Low sedimentation rate: good buoyancy enhances ease of handling, automation, and reproducibility
- Purity: Used to isolate and purify genomic DNA from whole human blood providing A_{260}/A_{280} ratios between 1.70–1.90 and A_{260}/A_{230} ratios as high as 2

Product code	Quantity	Description
29357369	5 mL	SeraSil-Mag™ 400 Silica Coated Magnetic Particles
29357371	60 mL	SeraSil-Mag™ 400 Silica Coated Magnetic Particles
29357372	450 mL	SeraSil-Mag™ 400 Silica Coated Magnetic Particles
29357373	5 mL	SeraSil-Mag™ 700 Silica Coated Magnetic Particles
29357374	60 mL	SeraSil-Mag™ 700 Silica Coated Magnetic Particles
29357375	450 mL	SeraSil-Mag™ 700 Silica Coated Magnetic Particles

Find out more

Sera-Xtracta™ Cell-Free DNA Kit

Designed to select for small-fragment cell-free DNA (cfDNA) while minimizing genomic DNA contamination, Sera-Xtracta™ Cell-Free DNA Kit* offers efficient extraction and purification of cfDNA from plasma. High yield and sensitivity make the kit well-suited for applications such as cancer diagnosis and monitoring where sample is precious.

Features and benefits

- High yield and sensitivity: High recovery of small-fragment cfDNA in the size range 50–300 bp which allows more cfDNA to be captured and sensitivity levels to be increased. Minimal co-purification of higher molecular weight genomic DNA is required due to active selection of smaller fragments which further improves sensitivity in downstream applications.
- Scalability: One kit to cover 0.5 mL to 4 mL input of plasma.
- Efficient: Procedure can be completed in less than 120 minutes.
- Ease of use: Compatible with molecular biology techniques, including next-generation sequencing (NGS), qPCR, ddPCR, BEAMing and other amplification and genotyping applications.

Application note

Detection of cancer-associated mutations in liquid biopsies for the identification of therapeutic targets

*For research use only

Product code	Quantity	Description
29437807	96 purifications	Sera-Xtracta™ Cell-Free DNA Kit

Find out more

Request a sample

cytiva.com/DNA-RNA-isolation

Sera-Xtracta™ Genomic DNA Kit

Sera-Xtracta™ Genomic DNA Kit* offers magnetic bead based extraction and purification of genomic DNA from whole blood. The simplified protocol minimizes the co-purification of RNA, removing the need for RNAse treatment in most applications. The kit yields genomic DNA with the high purity and quality required by today's molecular biology techniques.

Features and benefits

- High yield: Recovery rates of 4–8 µg from 200 µL of whole blood.
- High purity: Genomic DNA with a purity ratio (A260/A280) greater than 1.7.
- Simplified protocol: Minimizes the co-purification of RNA removing the need for RNAse treatment in most applications.
- Ease of use: Compatible with molecular biology techniques, including next generation sequencing (NGS), cloning, restriction enzyme digestion, PCR amplification and genotyping applications.

*For research use only

Product code	Quantity	Description
29429140	96 purifications	Sera-Xtracta™ Genomic DNA Kit

Request a sample
cytiva.com/DNA-RNA-isolation

Find out more

Sera-Xtracta™ Virus/Pathogen Kit

The Sera-Xtracta™ Virus/Pathogen Kit* is designed for high-throughput total nucleic acid (DNA/RNA) isolation from bacteria and viruses including Adenovirus (Type 14), Influenza A (H3N2) and COVID-19. For use with respiratory biological matrices, blood and universal transport media, it provides a simple and rapid method to optimize the workflow for sensitive detection of viruses and other pathogens found in low concentrations.

Features and benefits

- Reproducible yields: Offers the advantages of solid phase extraction (using magnetic beads).
- Simplified protocol: Can be adapted for both manual and automated high-throughput processing.
- Scalability: Up to 400 µL of sample.
- Rapid: Extraction procedure can be completed in less than 30 minutes.
- Ease of use: Compatible with molecular biology techniques, including quantitative polymerase chain reaction (qPCR, RT-qPCR), droplet digital PCR (ddPCR), and next-generation sequencing (NGS).

Product code	Quantity	Description
29506009	96 purifications	Sera-Xtracta™ Virus/Pathogen Kit
29514201	1000 purifications	Sera-Xtracta™ Virus/Pathogen Kit

Find out more

Application note ▶
Use of the Sera-Xtracta™ Virus/Pathogen Kit for purification of SARS-CoV-2 samples collected on Whatman™ 903 Proteinsaver cards

Application note ▶
Sensitive detection of total nucleic acid (RNA and DNA) from swabs, blood and transport media using Sera-Xtracta™ Virus/Pathogen Kit

Request a sample ▶
cytiva.com/DNA-RNA-isolation

*For research use only

Amersham™ NAP™ Kits

Amersham™ NAP™ Columns are disposable columns pre-packed with Sephadex™ G-25 DNA Grade resin. NAP™ Columns allow DNA purification by the process of gel filtration using desalting and buffer exchange, and the removal of unincorporated nucleotides from end-labeled oligonucleotides.

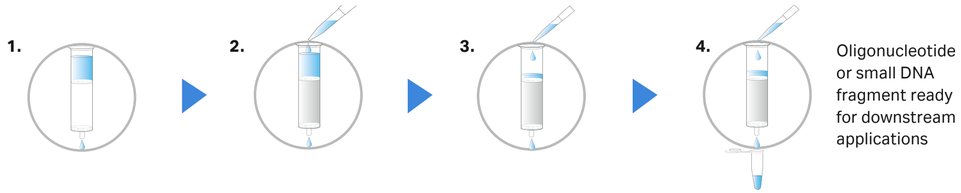
Features and benefits

- Simple: require only gravity to run
- Versatile: used for any DNA greater than 10 bases in length and ideal for the purification of oligonucleotides or very small DNA fragments following synthesis or end-labeling reaction

Available in three sizes depending on input sample volume

Amersham™ NAP™-5	Up to 0.5 mL
Amersham™ NAP™-10	Up to 1 mL
Amersham™ NAP™-25	Up to 2.5 mL

Column purification process



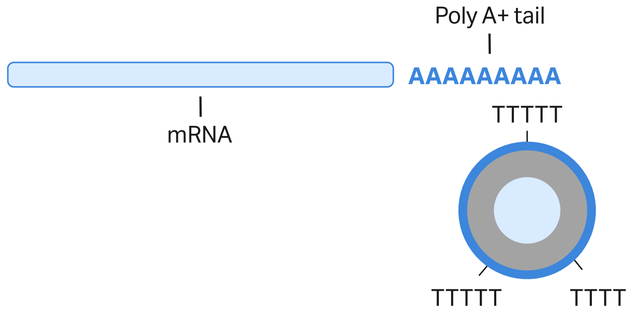
Product code	Quantity	Description
17085301	20	Amersham™ NAP™-5
17085302	50	Amersham™ NAP™-5
17085401	20	Amersham™ NAP™-10
17085402	50	Amersham™ NAP™-10
17085201	20	Amersham™ NAP™-25
17085202	50	Amersham™ NAP™-25

Find out more

Sera-Mag™ Oligo (dT) Coated Magnetic particles

Colloidally stable Sera-Mag™ Oligo (dT) magnetic particles contain covalently bound oligo(dT)14 and will remain in suspension for extended periods of time in the absence of a magnetic field, making them well suited for capturing or isolating mRNA from a variety of sources.

Oligo (dT) particles can also be used as a universal base particle for coupling unique oligo sequences. Simply synthesize the oligo with a poly-A tail for easy attachment to the oligo (dT) particles.



Features and benefits

- Versatile: once isolated, selective purification of mRNA from total RNA for NGS, RT-PCR, cDNA library construction, or subtractive hybridization can be performed
- Performance: the approximate mRNA binding-capacity is 11 µg of mRNA per mg of particles (dependent upon sample and message length)

Product code	Quantity	Description
38152103011150	1 mL	Sera-Mag™ Oligo (dT) Coated Magnetic particles
38152103010150	5 mL	Sera-Mag™ Oligo (dT) Coated Magnetic particles
38152103010350	100 mL	Sera-Mag™ Oligo (dT) Coated Magnetic particles

Find out more

Fundamentals of NGS sample preparation

NGS has enabled us to extract genetic information from samples faster, more reliably, and at lower cost than ever before. Getting your DNA ready for sequencing requires the preparation of a sequencing library as well as a few other steps that depend on the type of sample and the NGS platform.

DNA extraction protocol

The first step in every sample prep protocol is extracting the genetic material — DNA or RNA — from cells and tissues. Other molecules, such as RNA and proteins, interfere with the sequencing process and must be removed before doing anything else. The specific tissue type and storage conditions determine the details of this extraction process.

The traditional gold standard in DNA isolation is phenol-based extraction. Phenol is a hydrophobic solvent that denatures and dissolves proteins, removing them from the DNA-containing aqueous phase. However, it can be tricky to work with, and users need to be careful not to contaminate the aqueous phase with phenol.

Spin columns that specifically bind DNA provide an alternative and are an easy-to-use, but more expensive, method to wash away the debris. Chloroform-based extraction, another alternative, enables you to isolate high-quality DNA without phenol, and commercial kits can include a resin that minimizes the risk of contamination.

Amplification methods

Amplification after extraction is optional, depending on your application and sample size. For example, whole genome sequencing (WGA) with 2 µg of starting material does not necessarily require further amplification. But, with nanograms — or even picograms — of starting material, amplification becomes essential to obtain sufficient coverage for reliable sequence calls.

Isothermal amplification and polymerase chain reaction (PCR) are two common methods to increase the amount of input DNA. PCR uses generic primers to amplify the starting material in a highly uniform manner, but tends to be more error-prone than multiple displacement amplification (MDA).

MDA is an isothermal method, often based on Phi29 polymerase, and excels in accuracy with low rates of false-positives and false-negatives. MDA's main drawback is overrepresentation of some regions of the genome.

More recently developed hybrid methods, such as MALBAC, aim to correct this issue with MDA, but these methods also rely on PCR, and have some of the same associated drawbacks.

The different advantages and disadvantages of these methods mean that each is better suited to detect some features over others. For example, MDA outperforms the other two methods in detecting single-nucleotide variants (SNVs), whereas PCR and MALBAC are better for studying copy number variation (CNV), as described in this *Nature review article*[1].

[1] *Single-cell genome sequencing: current state of the science*. Charles Gawad, Winston Koh, and Stephen R. Quake, *Nature Reviews Genetics volume* **17**, pages 175–188 (2016) (https://www.nature.com/articles/nrg.2015.16).

DNA library preparation for next-generation sequencing

Most NGS platforms analyze DNA in uniform, bite-size pieces, created by DNA fragmentation. This process generates a 'library' of fragments with a narrow length distribution that is optimal for the sequencing platform.

DNA fragmentation

Both mechanical fragmentation (shearing) and enzymatic methods are suitable for NGS. Mechanical methods enable random shearing to produce a variety of overlapping fragments for any given region of the genome. This is ideal for *de novo* assembly.

Enzymatic methods are relatively fast and require less investment upfront but have some 'bias', cleaving some sites preferentially, making *de novo* assembly more challenging without the variety of overlapping fragments.

DNA end-repair

The fragments generated have single-stranded, 'sticky' ends. The next step, end-repair, fills in these sticky ends to create blunt ends, ready for adaptor ligation.

Adaptors

Adaptors are then bound to both the 5' and the 3' ends of the library fragments. They are specific to the sequencing platform, but ultimately all serve to enable in-platform clonal amplification, i.e., Illumina's bridge amplification or BGI's rolling circle amplification.

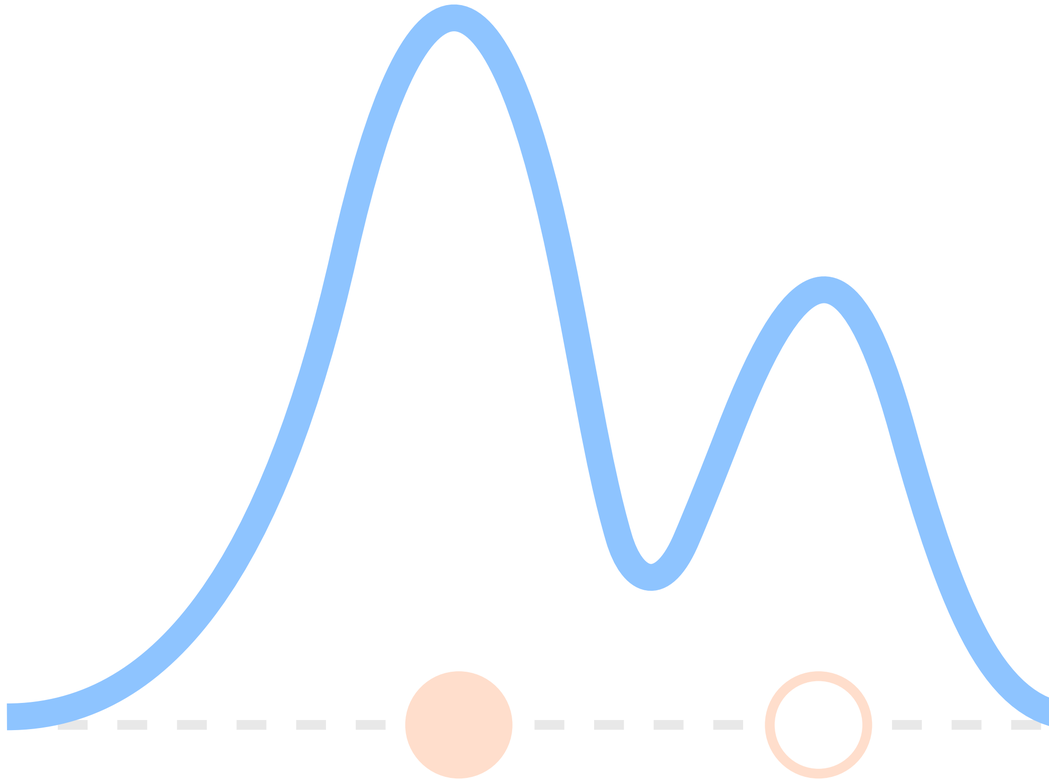
The adaptors are designed to bind to the sequencer-specific substrate, such as a patterned flow cell, contain sequences to enable amplification, and can have barcodes for fragment identification.

Targeted sequencing

These library preparation steps are generally applicable to whole genome sequencing but may differ for targeted sequencing.

In amplicon-based target enrichment, the fragmentation and end-repair steps tend to be unnecessary. Pulling the targeted regions out as amplicon fragments with blunt ends enables you to go directly to adaptor ligation.

Hybridization-based enrichment does require fragmentation. The hybridization probes pull out the regions of interest from the library of overlapping fragments, ready for end-repair.



DNA sequencing: size selection and purification

To speed up the workflow, it might be necessary to ‘clean up’ the library before sequencing by removing fragments that won’t produce relevant data. For NGS workflows that have narrow size requirements, discarding fragments that are either too large or too small to produce useful results can improve sequencing efficiency.

There are different protocols for size selection, which might involve gel electrophoresis or magnetic bead-based selection. Magnetic beads also provide a quick and easy method for final clean-up.

DNA quality control

A final step before proceeding to sequencing is to confirm the quality and quantity of the DNA. Both parameters contribute to the confidence in your sequencing data. You can measure the quantity of your DNA using fluorescence- or qPCR-based methods.

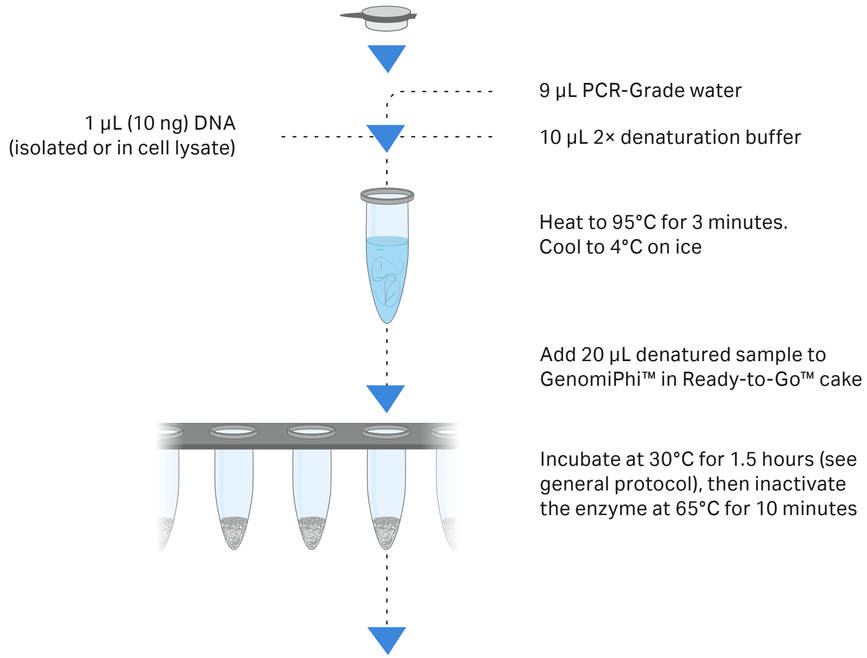
Amplification

Phi29 DNA Polymerase

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. Its high fidelity results in low rates of false positives and negatives in analysis, making it well-suited for identifying single nucleotide polymorphisms (SNPs) and other mutations. The one-tube, one-temperature format simplifies the DNA preparation process, facilitating automation for high-throughput sample amplification.

Our manufacturing processes, including UV and enzymatic reagent cleanup, help to ensure that all kits are free from any detectable DNA contamination and enable sensitivity of amplification down to 1 fg of gDNA.



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

GenomiPhi™ DNA Amplification and Ready-To-Go™ Amplification Kits

GenomiPhi™ DNA Amplification Kits are fundamental in genomic DNA preparation for genetic analysis and obtaining high quality DNA for successful downstream analysis. The kits provide an easy to use method that delivers highly representative and reliable whole genome amplification.

GenomiPhi™ HY DNA Amplification Kits

GenomiPhi™ HY DNA Amplification Kit contains all of the components necessary for midi-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. 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.

GenomiPhi™ amplified DNA is suitable for various applications such as genotyping (SNP, STR, array CGH), cloning, sequencing, and DNA archiving and outperforms PCR-based whole genome amplification techniques. The average product length is over 10 kb.

Features and benefits

- Quick procedure: high yield can be achieved with little hands-on time and a thermal cycler is not required
- Easy to use: the starting material for GenomiPhi™ reactions can be purified DNA from any commercial kit or homebrew method, or a non-purified cell lysate may be used

Product code	Quantity	Description
25660022	25	GenomiPhi™ HY DNA Amplification Kit
25660020	100	GenomiPhi™ HY DNA Amplification Kit
25660025	1000	GenomiPhi™ HY DNA Amplification Kit

Find out more

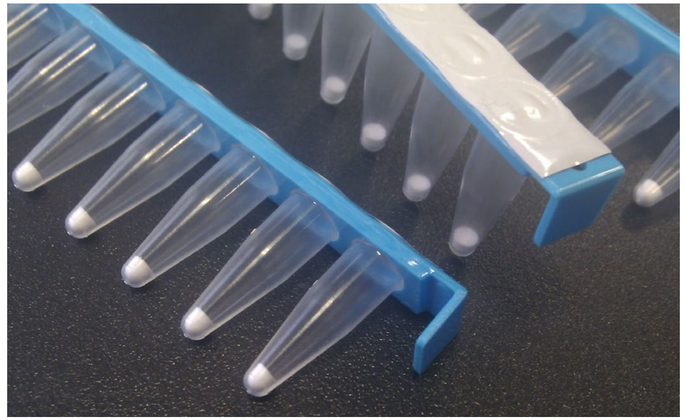
GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kits

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 in either strips of 8 tubes, 96-well or 480-well plates.

Features and benefits

- Reproducibility: preformulated, pre-dispensed, single dose for rapid reaction set up
- Automation-friendly protocol: quick and simple with no thermal cycler required
- Convenient: shipping and storage at room temperature

Product code	Quantity	Description
25660324	24	GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit
25660396	96	GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit
25660397	480	GenomiPhi™ HY Ready-To-Go™ DNA Amplification Kit



Find out more

GenomiPhi™ V2 DNA Amplification Kits

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.

Product code	Quantity	Description
25660030	25	GenomiPhi™ V2 DNA Amplification Kit
25660031	100	GenomiPhi™ V2 DNA Amplification Kit
25660032	500	GenomiPhi™ V2 DNA Amplification Kit

Find out more

GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kits

GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit supports the same applications as GenomiPhi™ V3 DNA Amplification Kit with the added benefit that the reaction mixture is provided as pre-dispensed, single-dose, lyophilized cakes in either strips of 8 tubes, 96-well or 480-well plates.

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.

Product code	Quantity	Description
25660124	24	GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit
25660196	96	GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit
25660197	480	GenomiPhi™ V3 Ready-To-Go™ DNA Amplification Kit

Find out more

GenomiPhi™ Single Cell DNA Amplification Kits

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.

Features and benefits

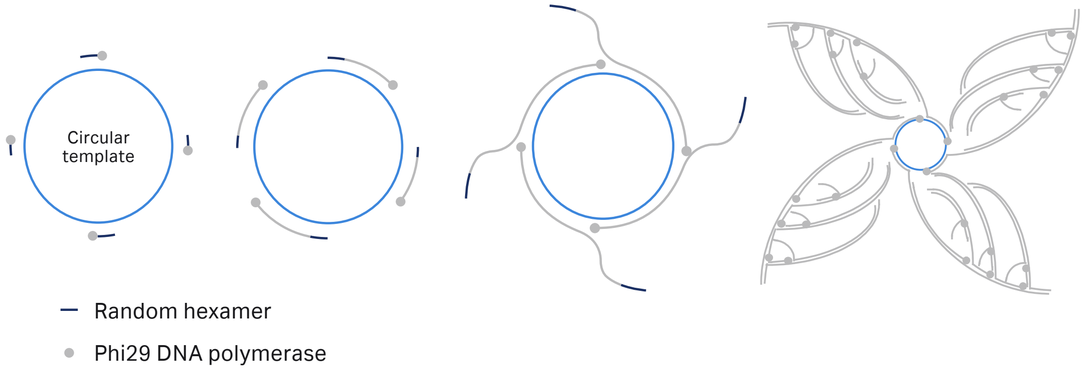
- High purity: high quality reagents and an optional, proprietary enzymatic clean-up step in the protocol ensure that any potential DNA contaminants introduced during set-up are removed before each individual reaction
- Fast: the amplification process is two to four hours depending on the amount of starting DNA
- High yields: 4 to 7 µg can be achieved

Product code	Quantity	Description
29108107	25	GenomiPhi™ Single Cell DNA Amplification Kits
29108039	100	GenomiPhi™ Single Cell DNA Amplification Kits

Find out more

TempliPhi™ DNA Amplification Kits

TempliPhi™ DNA Amplification Kits are used to prepare DNA directly from plasmid or fosmid glycerol stocks or colonies, which eliminates overnight culture steps. TempliPhi™ kits use isothermal rolling circle amplification (RCA) for the exponential amplification of circular DNA using bacteriophage Phi29 DNA polymerase. Phi29 DNA polymerase is active at 30°C, and isothermal amplification is performed at this temperature without the need for thermal cycling.



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 from bacterial or M13 liquid cultures, colonies, plaques, glycerol stocks, or purified circular (plasmid or M13) DNA can be used directly for sequencing and library construction without further purification, simplifying the process and reducing hands-on time without compromising on sequencing success and read lengths. It can also be used for enrichment of small circular viral genomes in complex samples for genotyping by sequencing.

Features and benefits

- Efficient: prepares templates for cycle sequencing, cloning, and transformation from circular DNA starting material without the need for purification
- Simple protocol: reduces time, labour and consumables needed for template preparation and workflow allows for easy automation
- Quick: the TempliPhi™ protocol allows amplification of 96 samples from bacterial colonies with less than 20 minutes of hands-on time

TempliPhi™ 100/500 Amplification Kits

TempliPhi™ 100/500 Amplification Kits are for low to medium throughput laboratories preparing circular DNA constructs.

Product code	Quantity	Description
25640010	100	TempliPhi™ 100 Amplification Kit
25640050	500	TempliPhi™ 100 Amplification Kit

Find out more

TempliPhi™ 2000 Reaction Kit

TempliPhi™ DNA Amplification Kit for 2000 reactions is specially formulated to minimize the number of components, thus simplifying setup on automated liquid handling platforms for high throughput applications.

Product code	Quantity	Description
28964286	2000	TempliPhi™ 2000 Reaction Kit

Find out more

TempliPhi™ Large Construct Kit

TempliPhi™ Large Construct DNA Amplification Kit was developed specifically to prepare templates for BAC or fosmid DNA sequencing for high-throughput users.

The starting material for amplification can be a small amount of bacterial cells containing a BAC or fosmid DNA, or any circular DNA sample. Bacterial colonies can be picked from agar plates and added directly to the TempliPhi™ Large Construct reaction. Alternatively, microliter quantities of a saturated bacterial culture or a glycerol stock can serve as starting material. The product of the TempliPhi™ Large Construct reaction is high molecular weight, double-stranded concatemers of the circular template. The TempliPhi™ Large Construct product is double-stranded DNA that can be sequenced with forward and reverse primers.

Amplification is completed in 18 hours and generates 5 µg of total DNA.

Product code	Quantity	Description
25640080	1000	TempliPhi™ Large Construct Kit

Find out more

TempliPhi™ Sequence Resolver Kits

The TempliPhi™ Sequence Resolver Kit produces exceptional sequencing results from difficult templates such as those with high GC content and secondary structures. The kit resolves most of the sequencing gaps that routinely elude other commonly used finishing methods. The use of this kit eliminates the need to use dGTP chemistry, which can affect the accuracy of a sequence due to the appearance of sequence compression. In addition, it eliminates the need for additives in the sequencing reaction. The TempliPhi™ Sequence Resolver Kit provides a convenient, time-saving, and economical solution to the problem of sequencing difficult templates and can be used to amplify fosmid and BAC templates for downstream sequencing with slight modifications to the protocol.

Product code	Quantity	Description
28903529	20	TempliPhi™ Sequence Resolver Kit
28903530	50	TempliPhi™ Sequence Resolver Kit
28903531	200	TempliPhi™ Sequence Resolver Kit

Find out more

Nucleotides

Amersham™ dNTPs are high purity deoxynucleotides for amplification, dideoxy sequencing, labeling, mutagenesis, cDNA synthesis, and expression profiling. They are free from DNase, RNase, and nicking enzyme activity.

Features and benefits

- Performance: greater than 99% triphosphate purity (by HPLC) for consistency
- Convenient: buffer-free, ready-to-use solutions at a variety of concentrations
- Tested: functionally tested to produce a 20.7 kb PCR amplification product from λ DNA

Product code	Quantity	Description
28406501	25 µmol	Amersham™ dATP, Solution, 100 mM
28406502	100 mmol	Amersham™ dATP, Solution, 100 mM
28406503	500 µmol	Amersham™ dATP, Solution, 100 mM
28406512	100 mmol	Amersham™ dCTP, Solution, 100 mM
28406522	100 mmol	Amersham™ dGTP, Solution, 100 mM
28406531	25 mmol	Amersham™ dTTP, Solution, 100 mM
28406532	100 mmol	Amersham™ dTTP, Solution, 100 mM
28406541	25 µmol	Amersham™ dUTP, Solution, 100 mM
28406542	100 mmol	Amersham™ dUTP, Solution, 100 mM
28406551	4 × 25 µmol	Amersham™ dNTP Set (100 mM each A,C,G,T)
28406552	4 × 100 µmol	Amersham™ dNTP Set (100 mM each A,C,G,T)
28406553	4 × 500 µmol	Amersham™ dNTP Set (100 mM each A,C,G,T)
28406557	10 mmol	Amersham™ dNTP Set (20 mM each A,C,G,T)
28406558	4 × 10 µmol	Amersham™ dNTP Set (100 mM each A,C,G,T)

Find out more

Also available: high purity rNTPs providing easy to use solutions which save time for *in vitro* transcription. rNTPs are all lot tested for ribonuclease contamination.

Three main challenges and solutions in NGS sample preparation

DNA for sequencing might come from a variety of sources, including fresh tissue, formalin-fixed paraffin-embedded (FFPE) tissue, cultured cells, and liquid biopsies. Each source comes with its own challenges for maximizing the three key aspects: yield, integrity, and purity.

Challenge 1: Yield

Different workflows and kits vary significantly in the amount of starting material required. Your workflow might require you to use a specific type of kit, and therefore starting DNA, or vice versa.

It is important to understand which workflows and kits suit your application and the typical amounts of starting material they need. If the two don't match up, can you try another approach?

If your sample is insufficient, what can we learn from those studying at the single-cell level? Commercially available whole genome amplification (WGA) kits provide the opportunity to expand your starting material from nanograms to micrograms in a matter of hours. This technique provides improved coverage compared to PCR-based amplification and is associated with fewer amplification errors.

Your fragmentation method can also affect your final DNA yield. Physical fragmentation can result in unexpectedly small DNA fragments which can be lost, reducing the amount of DNA available for sequencing. If you have the option, enzymatic fragmentation can provide better predictability and control over fragmentation.

Challenge 2: Integrity

Having enough DNA won't make for accurate sequencing if your DNA is degraded. Degradation can affect all kinds of samples, but long-term storage and exposure to fixatives, as you might find in FFPE samples, can exacerbate the damage.

A DNA integrity number (DIN) measurement can indicate the level of DNA damage. Although not a perfect predictor of usability, or parameters such as library complexity, DIN measurement is an easy method to check DNA integrity.

Extracting a little more DNA can compensate for low quality to some extent. However, there's not much you can do about previous storage conditions, unless you can choose newer samples or those that haven't gone through such harsh processing.

If you can not acquire better samples, DNA repair might improve your outcomes. Several commercial kits can, for example, modify blocked 3' ends or fix DNA nicks. These simple repairs help make more fragments suitable for sequencing.

Challenge 3: Purity

Producing reliable results in sequencing requires samples free of proteins, organic solvents and surfactants. You might also have tissue-specific contaminants to consider.

Researchers often measure DNA purity by looking at the 260:280 nm absorbance ratio. A high-purity sample should have a 260:280 ratio of 1.8 to 2.0. Nucleic acids have an absorbance maximum at 260 nm and finding a ratio below 1.8 can indicate contamination.

As a secondary check, measure the 260:230 ratio, which will detect the presence of commonly used solvents and surfactants, such as phenol and EDTA. Values between 2.0 and 2.2 indicate high purity.

- Remove hemoglobin by preferential lysis of red blood cells early in your workflow
- Remove heparin by washing
- Do a phenol-chloroform extraction to reduce protein contamination
- Use a phenol-free extraction kit to remove phenol contamination

02

Library preparation

Fragmentation/ size selection

Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent

Sera-Mag™ Select PCR clean-up and size selection reagent is based on well-known solid phase reversible immobilization technology, for selective binding of DNA fragments for applications such as NGS and PCR clean-up. It combines the convenience of magnetic bead technology, using the exceptional binding characteristics of Sera-Mag™ Carboxyl SpeedBeads with an optimized binding solution in a ready-to-use formulation.

Features and benefits

- Reliability: high yield in the recovery of specific fragment sizes for optimal sequencing efficiency
- Simplicity: one product for size selection and PCR clean-up instead of two separate products
- Minimal disruption: follows standard protocols for size selection and PCR clean-up, allowing for direct integration into existing workflows

Product code	Quantity	Description
29343045	5 mL	Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent
29343052	60 mL	Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent
29343057	450 mL	Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent

Find out more

Free sample

Review the datafile and request a free sample: cytiva.com/sera-mag-select

Size selection brings better data to NGS workflows

Maximizing the usable data from any given sequencing run by optimizing upstream library construction and sample preparation steps can lead to additional savings. These processes are relatively inexpensive and have substantial influence on final data quality.

The significance of size selection

Genomic sequencing relies on having high quality libraries. Part of this is making sure library fragment sizes are within the optimum range for a given instrument, typically 200–500 bp for illumina™ systems. This range is a consequence of the effect of fragment length on cluster generation and the efficiency of the sequencing process itself.

Small fragments tend to cluster more efficiently on the flow cell than larger fragments. A bias towards smaller fragments leaves much of the sequencing capacity unused. Selecting fragment sizes below 150 bp can risk carryover of unwanted adaptor and primer dimers, the sequencing of which leads to a lot of unusable data and further wasting of capacity.

Fragments larger than optimum pose the opposite challenge. Although it is possible to sequence fragments > 1 kb in length, this is inefficient and prone to errors — an issue that third generation sequencers attempt to solve.

Individual samples might also have different shearing profiles, with narrow to wide distributions. Setting an instrument up for 600 bp fragments when there is a 200–1000 bp distribution, for example, means that many of the sequencing templates will not be viable or read to sufficient depth. This produces little useful data and low uniformity of coverage.

A size selection step enables a randomly fragmented library to be taken and only the fragments fitting the optimal/target range for the instrument and application to be pulled out. This saves time and cost by maximizing the efficiency of sequencing runs.

A note on DNA fragmentation methods

There are various options for fragmentation, some of which attempt to bypass the need for size selection altogether. The choice of method may depend on the application, starting material, and equipment available.

Enzymatic methods tend not to be completely random, but provide some control over fragment sizes through varied incubation times. However, these are less well suited for *de novo* assembly due to the likelihood of making fewer overlapping fragments.

There are various options for mechanical shearing, which use sonication or focused acoustic technologies. These are random, and can be tuned to produce predictable shearing profiles.

Size selection methods

The approaches to size selection include enzymatic, gel-based, and magnetic bead-based methods, the suitability of each depending on the needs of the experiment. These also provide an opportunity to clean up adaptor dimers and any other leftover reagents.

Enzymatic approach

Illumina's Nextera kits produce libraries for various applications compatible with Illumina technology in one step.

When launched, they attempted to get around the need for size selection by using transposon-based fragmentation and tagging, known as 'tagmentation', saving several workflow steps. However, library profiles tended to be broad, leaving users often reverting to a separate size selection step.

Nextera kits now include magnetic bead-based size selection reagents.

Gel-based approach

Gels have long been used for nucleic acid purification, enabling you to physically remove the chosen fragment size. Gel-based systems, such as Sage's Pippin Prep, help automate this process, but have inherently limited throughput. A typical 96-sample batch requires close to 10 hours to process.

Magnetic bead-based approach

The introduction of magnetic beads for convenient and high throughput size selection and clean-up has transformed NGS workflows, with Cytiva Sera-Mag™ particles integral to this success.

Originally developed for the isolation of PCR products, these beads have polystyrene cores covered in magnetite and a layer of carboxyl molecules. Nucleic acids bind to them reversibly in the presence of polyethene glycol (PEG) and salt; a process known as solid phase reversible immobilization.

The beads are otherwise inert and have high binding capacities, due to large surface areas. The size of fragment bound can be adjusted by simply altering the volumetric ratio of PEG/salt/beads to DNA. From a practical point of view, this bead chemistry makes it straightforward to size select a very specific range of fragments consistently and reproducibly.

The magnetic bead-based approach is well suited for high throughput applications with automation, and the cost of reagents is also low compared to other approaches. These properties make magnetic beads a simple solution for optimizing NGS sample prep.

End repair and adapter ligation

T4 DNA Ligase

T4 DNA Ligase affords a quick and efficient method for performing DNA ligation reactions and can be used for a variety of ligation procedures such as conventional vector cloning, TA cloning, linker or adaptor ligation, and library construction.

Features and benefits

- Convenient: ready-to-use format and room temperature incubation
- Flexible: ligation products can be used directly for transformation

Find out more

Product code	Quantity	Description
27036101	50	T4 DNA Ligase, reaction beads

Enrichment/ hybrid capture

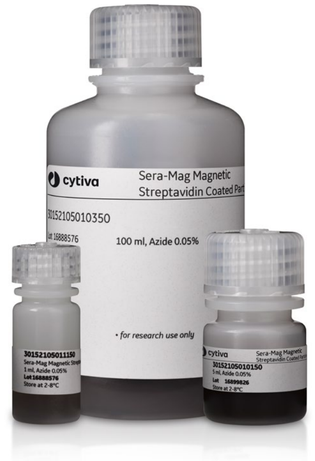
Sera-Mag™ Streptavidin-Coated Magnetic Particles

Sera-Mag™ Streptavidin-coated magnetic beads provide solid phase support in immunoassays and molecular biology applications.

The magnetic streptavidin particles can also be used as a universal base particle for coating biotinylated proteins, oligos or other ligands to the particle surface.

Features and benefits

- Optimized application: contain covalently bound streptavidin with low (2500 to 3500 pmol/mg), medium (3500 to 4500 pmol/mg) or high (4500 to 5500 pmol/mg) biotin binding capacities providing a choice of biotin-binding capacity
- High capacity and precision: for enrichment and targeted sequencing applications



Product code	Quantity	Description
30152103011150	1 mL	Sera-Mag™ Streptavidin-Coated - 2500 to 3500 (low) pmol per mg
30152103010150	5 mL	Sera-Mag™ Streptavidin-Coated - 2500 to 3500 (low) pmol per mg
30152103010350	100 mL	Sera-Mag™ Streptavidin-Coated - 2500 to 3500 (low) pmol per mg
30152104011150	1 mL	Sera-Mag™ Streptavidin-Coated - 3500 to 4500 (med) pmol per mg
30152104010150	5 mL	Sera-Mag™ Streptavidin-Coated - 3500 to 4500 (med) pmol per mg
30152104010350	100 mL	Sera-Mag™ Streptavidin-Coated - 3500 to 4500 (med) pmol per mg
30152105011150	1 mL	Sera-Mag™ Streptavidin-Coated - 4500 to 5500 (high) pmol per mg
30152105010150	5 mL	Sera-Mag™ Streptavidin-Coated - 4500 to 5500 (high) pmol per mg
30152105010350	100 mL	Sera-Mag™ Streptavidin-Coated - 4500 to 5500 (high) pmol per mg

Find out more

Sera-Mag™ SpeedBeads Streptavidin-Blocked Magnetic Particles

Sera-Mag™ SpeedBeads Streptavidin-blocked provide high biotin-binding capacity along with a strong affinity for targeted, biotin-labeled molecules such as nucleic acids, proteins and peptides with very low nonspecific binding.

When the beads are combined with any of these molecules the strong non-covalent associate between the Streptavidin and biotin ensures efficient capture of the target molecule.

Features and benefits

- Increased throughput and precision: the SpeedBead particles combine fast reaction kinetics and low, non-specific binding
- Optimized for demanding applications: including bead-in PCR and enrichment

Product code	Quantity	Description
21152104011150	1 mL	Sera-Mag™ SpeedBeads Streptavidin-blocked
21152104010150	5 mL	Sera-Mag™ SpeedBeads Streptavidin-blocked
21152104010350	100 mL	Sera-Mag™ SpeedBeads Streptavidin-blocked
21152104010450	1000 mL	Sera-Mag™ SpeedBeads Streptavidin-blocked

Find out more

PCR clean-up

Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent

Sera-Mag™ Select PCR clean-up and size selection reagent is based on well-known solid phase reversible immobilization technology, for selective binding of DNA fragments for applications such as NGS and PCR clean-up. It combines the convenience of magnetic bead technology, using the exceptional binding characteristics of Sera-Mag™ Carboxyl SpeedBeads with an optimized binding solution in a ready-to-use formulation.

Features and benefits

- Reliability: high yield in the recovery of specific fragment sizes for optimal sequencing efficiency
- Simplicity: one product for size selection and PCR clean-up instead of two separate products
- Minimal disruption: follows standard protocols for size selection and PCR clean-up, allowing for direct integration into existing workflows

Product code	Quantity	Description
29343045	5 mL	Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent
29343052	60 mL	Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent
29343057	450 mL	Sera-Mag™ Select PCR Clean-Up and Size Selection Reagent

Find out more

Sera-Mag™ Carboxylate and SpeedBead Carboxylate

Carboxylic groups on the surface of Sera-Mag™ SpeedBeads and Sera-Mag™ Carboxylate-Modified Magnetic Beads permit easy covalent coupling to target biomolecules of interest, such as proteins and nucleic acids, using convenient carbodiimide chemistry.

The cauliflower-shaped surface paired with proprietary Sera-Mag™ and SpeedBead chemistry, provides a large surface area and offers excellent sensitivity and low non-specific binding for greater accuracy. This can maximize sample retention or reduce the amount of beads required.

Find out more

Sera-Mag™ Carboxylate-Modified Magnetic Particles

Sera-Mag™ Carboxylate-Modified Magnetic Beads combine a fast magnetic response time and high binding capacity, sensitivity, stability and physical integrity.

Features and benefits

- Ease of use: covalent coupling of proteins, nucleic acids, etc. to carboxyl groups on the surface using standard coupling technologies
- Convenient: isolation, selection and clean-up of nucleic acids or direct conjugation of specific oligos and enzymes

Product code	Quantity	Description
24152105050250	15 mL	Sera-Mag™ Carboxylate-Modified [E7] Magnetic Particles
24152105050350	100 mL	Sera-Mag™ Carboxylate-Modified [E7] Magnetic Particles
24152105050450	1000 mL	Sera-Mag™ Carboxylate-Modified [E7] Magnetic Particles
44152105050250	15 mL	Sera-Mag™ Carboxylate-Modified [E3] Magnetic Particles
44152105050350	100 mL	Sera-Mag™ Carboxylate-Modified [E3] Magnetic Particles
44152105050450	1000 mL	Sera-Mag™ Carboxylate-Modified [E3] Magnetic Particles

Find out more

What is the difference between E3 and E7 bead?

E3 and E7 refer to the different manufacturing process for the beads. E3 and E7 beads behave similarly and we continue to provide both bead types to our customers for test and validation in their chosen application.

Request a free sample
cytiva.com/solutions/genomics/sequencing/sera-mag

Sera-Mag™ SpeedBead Carboxylate-Modified Magnetic Particles

Sera-Mag™ Speedbeads have a second layer of magnetite applied through the same core shell design process, allowing a reaction twice as fast as the Sera-Mag™ Carboxylate-Modified beads when in the presence of a magnetic field. Speedbeads are especially useful where the reaction medium is highly viscous, and in clinical assays requiring a faster magnetic response time.

Features and benefits

- Convenient: isolation, selection and clean-up of nucleic acids or direct conjugation of specific oligos and enzymes
- Reliable: fast, precise and high binding capacity for sample preparation, nucleic acid isolation, proteomics and immunoassay applications

Product code	Quantity	Description
45152105050250	15 mL	Sera-Mag™ SpeedBead Carboxylate-Modified [E7] Magnetic Particles
45152105050350	100 mL	Sera-Mag™ SpeedBead Carboxylate-Modified [E7] Magnetic Particles
65152105050250	15 mL	Sera-Mag™ SpeedBead Carboxylate-Modified [E3] Magnetic Particles
65152105050350	100 mL	Sera-Mag™ SpeedBead Carboxylate-Modified [E3] Magnetic Particles
65152105050450	1000 mL	Sera-Mag™ SpeedBead Carboxylate-Modified [E3] Magnetic Particles

Find out more

GFX™ Purification Kits

GFX™ Purification kits utilize proprietary glass fiber technology and optimized buffer solutions to efficiently capture DNA with high recovery and purity for use in a range of downstream applications.

GFX™ PCR DNA and Gel Band Purification Kits

Designed for the rapid purification and concentration of PCR products or DNA fragments ranging in size from 50 bp to 10 Kbp. This kit can be used to purify DNA from reaction volumes up to 100 µL or agarose gel slices up to 900 mg. Includes a capture buffer that contains a visual color indicator to ensure optimal pH for maximum DNA binding.

Features and benefits

- Versatile: with a choice of two different input samples — either DNA in solution or DNA-containing agarose gel bands — and the flexibility to use two different elution buffers and an elution volume range of 10 to 50 µL to suit the requirements of any downstream application
- High purity: typical recoveries range between 60% and 80% for DNA fragments from agarose gel to as high as 95% for PCR products from solution. All recoveries have exceptional purity with 99.5% of contaminants removed
- Reliability: consistent and successful removal of dNTPs and primers from PCR mixtures

Product code	Quantity	Description
28903466	10	GFX™ PCR DNA and Gel Band Purification Kit
28903470	100	GFX™ PCR DNA and Gel Band Purification Kit
28903471	250	GFX™ PCR DNA and Gel Band Purification Kit

Find out more

GFX™ 96 PCR Purification Kit

The GFX™ 96 PCR Purification Kit uses a glass fibre matrix technology in a 96-well format for highly efficient purification of PCR products. DNA fragments from PCR are captured by the matrix in presence of a chaotropic salt and contaminants are removed by washing the matrix with a buffered ethanol solution. Purified DNA is ready for use in most applications, including fluorescent sequencing, microarrays, labeling, hybridization, ligation, and transformation.

Features and benefits

- Fast: purification of up to 96 PCR products (0.1 to 10 kb) simultaneously in as little as 15 min
- High yield: pure DNA recovered in a small volume of water or a low ionic strength buffer. Typical recoveries are > 85% for PCR products 100 bp to 10 kb in length; salt removal typically ≥ 99%
- Solvent-free: avoids ethanol precipitations and hazardous organic extractions

Product code	Quantity	Description
28903445	96 Well Plates	GFX™ 96 PCR Purification Kit

Find out more

ExoProStar™ PCR Clean-Up Technology

ExoProStar™ PCR Clean-Up Technology, ExoProStar™ S, and ExoProStar™ 1-Step

ExoProStar™ PCR Clean-Up Technology is optimized to purify PCR reactions in a fast and efficient way prior to Sanger sequencing reaction setup.

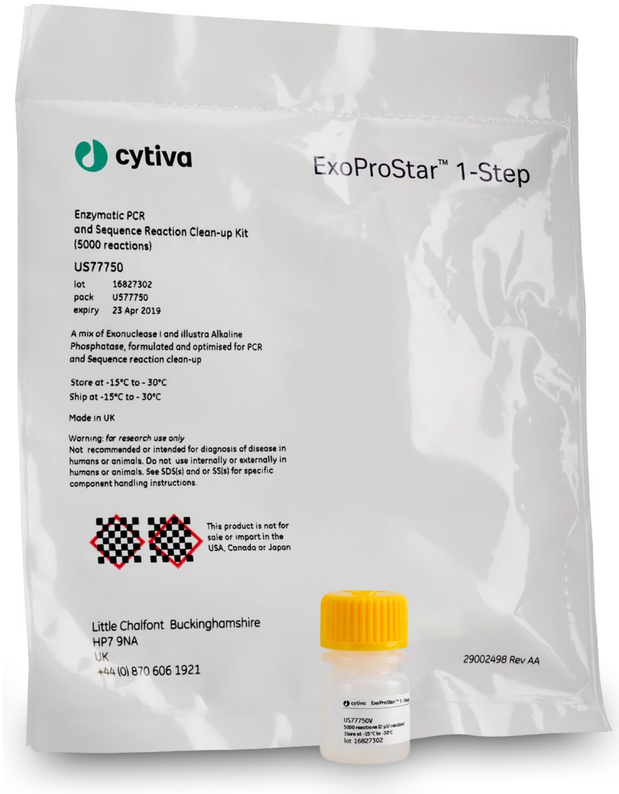
ExoProStar™ PCR Clean-Up Technology contains Alkaline Phosphatase and Exonuclease A, formulated to work together to remove unincorporated primers and nucleotides from amplification reactions in preparation for sequencing, cloning, genotyping or further DNA modifications.

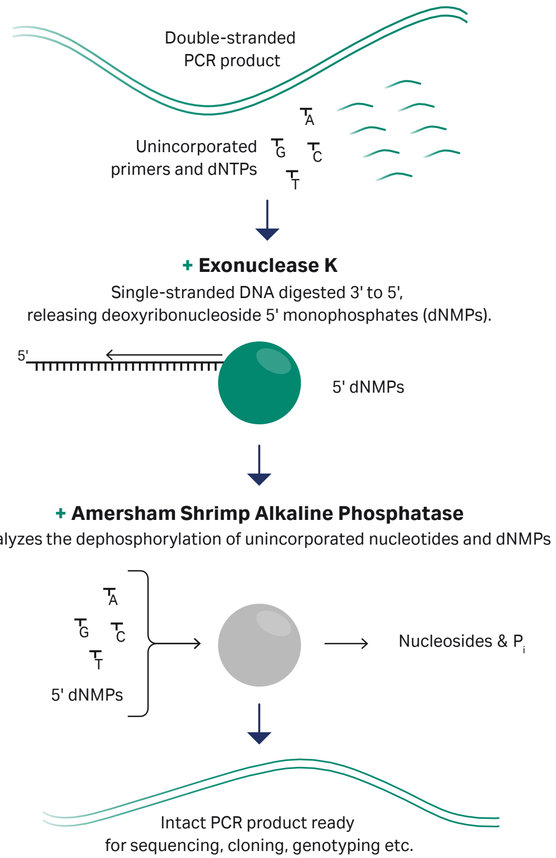
ExoProStar™ 1-Step combines Exonuclease I and Alkaline Phosphatase in one single tube for a single-step method. This 1-step version is not available in Canada and Japan.

Features and benefits

- Simple: enzymes provided in two separate tubes and just two simple pipetting steps are needed to prepare the reaction
- Fast: 30 min protocol or 15 min protocol with ExoProStar™ S and complete heat inactivation of the enzymes within 15 min
- Scalable: suitable for different reaction sizes with no loss of PCR product

Product code	Quantity	Description
US78220	20	ExoProStar™ PCR Clean-Up Technology
US78210	100	ExoProStar™ PCR Clean-Up Technology
US78211	500	ExoProStar™ PCR Clean-Up Technology
US78212	2000	ExoProStar™ PCR Clean-Up Technology
US78225	5000	ExoProStar™ PCR Clean-Up Technology
US79002	20	ExoProStar™ S PCR Clean-Up Technology
US79010	100	ExoProStar™ S PCR Clean-Up Technology
US79050	500	ExoProStar™ S PCR Clean-Up Technology
US79200	2000	ExoProStar™ S PCR Clean-Up Technology
US79500	5000	ExoProStar™ S PCR Clean-Up Technology
US77701	20	ExoProStar™ 1-Step PCR Clean-Up Technology
US77702	100	ExoProStar™ 1-Step PCR Clean-Up Technology
US77705	500	ExoProStar™ 1-Step PCR Clean-Up Technology
US77720	2000	ExoProStar™ 1-Step PCR Clean-Up Technology
US77750	5000	ExoProStar™ 1-Step PCR Clean-Up Technology





Find out more

03

Sequencing and analysis

NGS into the future

The key reason for choosing NGS for clinical diagnostics is its combination of high throughput, speed, and resolution. NGS assays can efficiently analyze the entire genome or exome or focus on a specified number of targeted locations in the genome.

NGS's single-nucleotide resolution also enables it to detect even the smallest possible mutations (SNPs) without necessarily requiring knowledge of the mutation in advance. As the technology improves, the combined detection of SNP and larger abnormalities is becoming easier, providing an all-in-one solution for detecting multiple types of mutations.

The technology also requires a fair bit of expertise to run assays and interpret data, but guidelines from the US Food and Drug Administration and "game-changing" FDA cancer panel approvals are removing these barriers and leading to increased investment and clinical adoption, continuing innovation and decreases in the cost-per-base.

Platforms

The main commercial platforms are those offered by Illumina, Thermo Fisher and Pacific Biosciences. Several companies, such as Oxford Nanopore, are developing advanced technologies based on nanopores.

Although the gigabases of data may be lower and the read lengths may be shorter, benchtop sequencers and even handheld sequencers have been well accepted by lower-throughput laboratories who may face space constraints and are looking for a lower priced option.

Since NGS platforms can sequence entire genomic regions or even entire genomes, a single test can examine hundreds or thousands of clinically important genetic variations. This means that one test can replace multiple conventional single-gene tests, providing an advantage in price and in the amount of precious sample needed for the test itself. Maximizing the efficiency of each sequencing run and ensuring highly reliable data is generated remain the two main objectives of the majority of laboratories.

Key clinical applications of DNA sequencing

The biggest market for NGS is currently in reproductive health, more specifically non-invasive prenatal testing (NIPT), where it's gradually replacing array-based techniques such as array comparative genomic hybridization (aCGH). NIPT provides a lower risk alternative to invasive tests as it analyzes fetal cell-free DNA (cfDNA) from the mother's circulation, making detection of, for example, Down syndrome easier.

NGS is also growing in oncology, Mendelian diseases, complex diseases, and infectious diseases. Clinical scientists can use NGS assays either for diagnosis or for decisions on treatment by studying both small mutations (e.g., SNPs and indels) and larger abnormalities (e.g., CNV) at the same time.

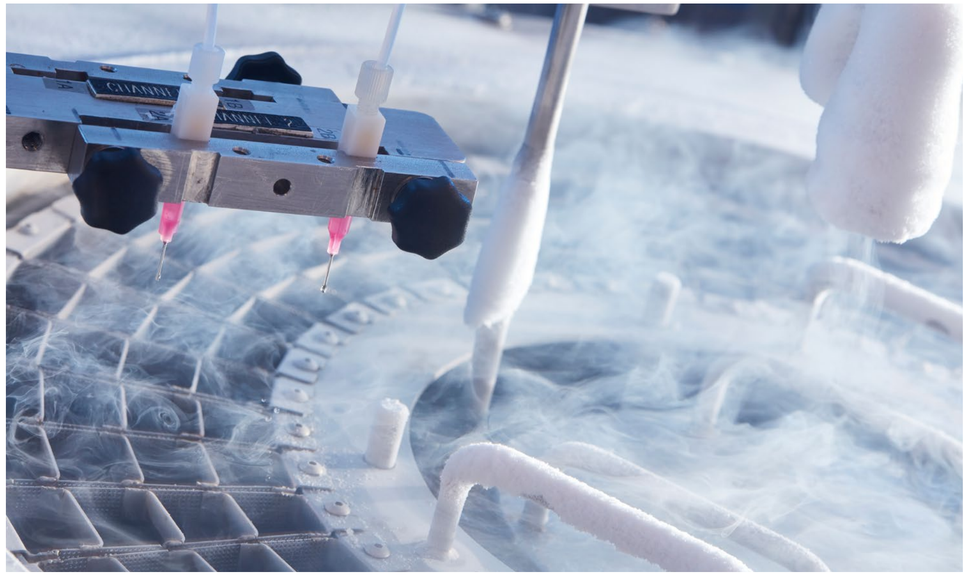
Refining cancer diagnoses is a particular growth area for NGS. As treatments become more personalized, there's a need for classifying cancers in terms of their underlying mutations to help direct treatment options in the clinic. NGS plays a key role in this trend towards precision health, helping to minimize the human and financial costs of ineffective cancer treatments.

The future of NGS

The speed at which NGS will grow in different clinical markets will probably depend on pushing sequencing costs down further, as well as developments in the regulatory landscape. As clinical scientists get more familiar with the possibilities with NGS, they can also contribute to higher growth through demand.

04

Customized solutions



At Cytiva, while we provide catalog products to accommodate a wide range of applications, we also recognize that there are times when products would be more effective if the parameters were slightly different. If this is the case please contact our designated customized solutions team who can help develop a product that simplifies your NGS workflow. From lyophilization and conjugation to custom biology and contract manufacturing, Cytiva can help.

Find out more

cytiva.com/genomics-diagnostics

For local office contact information, visit cytiva.com/contact

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