Spotlight on magnetic beads

cytiva

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Introduction

Magnetic beads are versatile super(paramagnetic) particles well suited to DNA extraction, size selection, and clean-up as alternatives to column-based purification, providing flexibility in scale and handling.

The bead-based approach can greatly simplify extraction and size selection steps while introducing a level of consistency that is otherwise challenging to achieve between different staff and labs. For example, rather than separate reagents for isolation, size selection, and PCR clean-up—key steps in sample and library preparation—you could use magnetic bead-based kits to have a single solution and automatable workflow. Magnetic beads can also simplify library preparation for multiplexed sequencing and are readily automatable.

This eBook provides a range of articles to help you use magnetic beads to optimize your workflow and achieve reproducible results.

Magbeads 101: A guide to choosing and using magnetic beads

What are magnetic beads?

Magnetic beads are made up of tiny (20 to 30 nm) particles of iron oxides, such as magnetite (Fe3O4), which give them superparamagnetic properties.

Superparamagnetic beads are different to more common ferromagnets in that they exhibit magnetic behavior only in the presence of an external magnetic field. This property is dependent on the small size of the particles in the beads, and enables the beads to be separated in suspension, along with anything they are bound to. Since they don't attract each other outside of a magnetic field, they can be used without any concern about unwanted clumping.

There are many types of magnetic beads available. Different surface coatings and chemistries give each type of bead its own binding properties, which can be used for magnetic separation (isolation and purification) of nucleic acids, proteins, or other biomolecules in an easy, effective, and scalable way.

This ease-of-use makes them automation friendly and well suited for a range of applications, including sample preparation for next-generation sequencing (NGS) and polymerase chain reaction (PCR), protein purification, molecular and immunodiagnostics, and even magnetic-activated cell sorting (MACS), among many others. They also ease some of the challenges associated with extracting nucleic acids from different sample types.

What is magnetic separation?

Magnetic separation uses a magnetic field to separate micrometer-sized paramagnetic particles from a suspension. In molecular biology, magnetic beads

provide a simple and reliable method of purifying various types of biomolecule, including genomic DNA, plasmids, mitochondrial DNA, RNA, and proteins.

For example, under optimized conditions, DNA selectively binds to an appropriately-coated bead surface, leaving contaminants in solution. You can then use this purified DNA directly in molecular biology applications.

A key advantage to using magnetic beads is that you can isolate nucleic acids and other biomolecules directly from a crude sample, and from a variety of different types of sample, with minimal processing. This sets magnetic beads apart from other methods of nucleic acid isolation, which might have different protocols for different types of sample, and involve more hands-on time.

How does magnetic bead DNA extraction work?

Magnetic beads have been around in one form or another for decades. Their potential in nucleic acid purification was recognized in the 1990s, as demonstrated by the US patent: "DNA purification and isolation using magnetic particles". The approach, largely unchanged since, relies on using magnetic beads with a coating that can bind nucleic acids reversibly by just adjusting buffer conditions (Fig 1).

After binding DNA, an external magnetic field attracts the beads to the outer edge of the containing tube, immobilizing them. While the beads are immobilized, the bead-bound DNA is retained during the washing steps. Adding elution buffer, and removing the magnetic field then releases the DNA as a purified sample, ready for quantitation and analysis.

This approach removes the need for vacuum or centrifugation, which minimizes stress or shearing forces on the target molecules, requires fewer steps and reagents than other DNA extraction protocols, and is amenable to automation in 24, 96, and 384-well plates.

So, it's no wonder that magnetic beads are gaining in popularity. Indeed, manufacturers have now developed numerous commercial nucleic acid isolation kits based on magnetic beads. They have options for various surface chemistries and a range of applications. The ligand-binding properties can range from being indiscriminate to sequence- or tag-specific, all of which can be a challenge to keep up with.

Table 1 gives an overview of the latest range of magnetic bead types, with their key properties and applications.

Figure 1. Overview of magnetic bead-based DNA extraction using Sera-Mag™ beads.

Table 1. *Comparison of magnetic bead surface chemistries and applications.*

Type	Properties	Applications	Variations
Carboxylate-modified	• Can associate with nucleic acids for direct capture. • Surface suitable for conjugation through covalent bonding. • Can capture molecules containing amino groups.	Conjugation or direct binding applications: • Covalent attachment • Affinity purification and pull-down • Nucleic acid isolation and purification • NGS size selection	High-speed version available
Amine-blocked	• Surface suitable for conjugation through covalent bonding. • Non-surfactant, non-protein-blocked surface. • Low non-specific binding.	Conjugation applications, similar to carboxylate-modified beads.	High-speed version available
Oligo(dT)-coated	• Hybridizes with mRNA poly-A tails. • High colloidal stability.	mRNA binding applications: • mRNA extraction and purification • RT-PCR • cDNA library construction • Subtractive hybridization • NGS (RNA sequencing)	
Streptavidin-coated	• Binds biotinylated ligands such as proteins, nucleic acids, and peptides. • Covalently bound streptavidin coating. • Fast reaction kinetics. • Low non-specific binding. • High throughput and precision.	Immunoassay and molecular biology applications: • Sample preparation and assay development for genomics and proteomics.	High-speed version available Biotin binding ranges: • 2500 to 3500 pmol/mg • 3500 to 4500 pmol/mg • 4500 to 5500 pmol/mg
Streptavidin-blocked	• Binds biotinylated ligands such as proteins, nucleic acids, and peptides. • Non-surfactant, non-protein-blocked surface. • Lower non-specific binding than streptavidin-coated beads via additional blocking of non-specific binding sites.	High-specificity biotin-binding applications • Molecular and immunodiagnostics • NGS library preparation	High-speed version available
NeutrAvidin™-coated	• Binds biotinylated ligands such as proteins, nucleic acids, and peptides. • Fast reaction kinetics. • Low non-specific binding. • High throughput and precision.	Alternative to Streptavidin in immunoassay and molecular biology applications: • Sample preparation and assay development for genomics and proteomics.	High-speed version available Biotin binding range: • 3500 to 4500 pmol/mg
Protein A/G	• Binds IgA and IgG proteins • Coating based on IgA/IgG fusion protein. • Broad binding capabilities.	Antibody isolation applications: • Affinity purification and pull-down • Immunoprecipitation	
Silica-coated	• Reversibly binds nucleic acids based on salt concentration. • Monodisperse particles with narrow size ranges of 400 µm or 700 µm.	Applications with low sample amounts • Nucleic acid extraction for molecular diagnostics applications such as qPCR.	
Mag Sepharose™	• Broad range of ligand options. • Porous, providing greater surface area than other magnetic beads.	Convenient alternative to sepharose columns, with protein purification applications including: • Affinity purification or capture • Immunoprecipitation	

Custom bead conjugation

Even with all these surface chemistry options, it's impossible to cover every need and eventuality. That's where custom conjugation comes in.

If you need to conjugate a custom ligand or require a custom particle size, Cytiva allows you to customize all the Sera-Mag magnetic beads can be customized. Our dedicated experts can help you every step of the way, from defining the product specifications to delivery completion.

We provide custom conjugations of enzymes or antibodies, as well as a range of custom ligands that we can develop in parallel with your projects. We also offer lyophilization of the customized microspheres as part of our Lyo-Stable™ services, based on Ready-To-Go™ stabilization technology.

Drawing on our R&D and manufacturing resources, and history of supplying magnetic beads to kit manufacturers, we will provide custom magnetic bead technology that is ready to use with little or no need for further modification. From completing complex conjugations, to performing your quality control tests before the beads leave our factory, we are equipped to meet your needs.

We provide a broad range of magnetic beads for molecular biology applications such as NGS and PCR. For support in any aspect of your workflow, contact our Scientific Support team or your local Cytiva representative.

Written by Andrew Gane
Product Strategy and Technology Manager, Genomics and Diagnostic Solutions, Cytiva

Andrew is the Product Strategy and Technology Manager within the Genomics and Diagnostic Solutions business responsible for building the innovation pipeline in collaboration with the R&D and commercial teams. His knowledge and understanding of emerging trends and new applications have been fundamental to developing the product portfolio into workflow-based solutions, with a particular focus on next-generation sequencing (NGS). Andrew has more than 30 years' experience in immunodiagnostics and molecular diagnostics in both lab-based and product development roles.

Benefits of moving from column to bead-based DNA isolation

Successful extraction and purification of nucleic acids is crucial in many molecular biology studies, including those involving next-generation sequencing (NGS). Successful extraction often determines the quality of the overall data. While spin column-based isolation methods have been a staple in labs for many years, they are increasingly being replaced by magnetic bead-based methods.

Why is there a shift in approach by researchers in academia and industry alike?

Magnetic beads (or superparamagnetic beads) are an effective alternative to columns that can simplify the processes of DNA/RNA extraction. They have several advantages over columns when used in sensitive applications, such as NGS library preparation. These advantages include the ability to isolate longer strand nucleic acids from samples, greater flexibility in fragment size selection, and allowing for normalization of libraries.

Below are eight key considerations to help you decide if magnetic beads will improve your workflow.

1: Simpler, gentler nucleic acid isolation

The first step of sample preparation involves cell lysis, or disruption, usually with a combination of detergent and mechanical force, to release the genetic material.

In column-based methods, you would centrifuge or clarify the lysate, add the supernatant to a silica membrane to bind nucleic acids, wash with buffer via centrifugation or vacuum manifold, then elute the desired nucleic acid in an appropriate volume of buffer.

These steps provide ample opportunity for sample loss and mechanical shearing of the nucleic acids.

Magnetic bead-based methods are gentler and more versatile than columns, require fewer handling steps (and therefore fewer opportunities for shearing), and offer many surface chemistries for different applications.

The result is a simpler workflow that can be easily automated and scaled up to produce more reproducible nucleic acid isolation at greater quantities than spin column-based methods.

2: Easier automation with bead-based methods

There has never been a better time to automate your DNA/RNA isolation procedures, with manufacturers now offering benchtop systems for low, medium, and high-throughput automation of nucleic acid isolation for NGS and PCR applications.

While column-based isolation methods can be partially automated on generic liquid handling machines, full automation requires systems integrating vacuum manifolds or onboard centrifuges.

Several vendors now offer open platforms that can automate magnetic bead-based reagents from various commercially available kits. Magnetic beads-based kits are well suited for high-throughput applications as, unlike spin column methods, they do not require centrifugation or vacuum processing. As a result, these kits can provide high consistency between experiments and are less prone to sample contamination when automated.

3: Consider the learning curve

In academic research labs, processing many samples for nucleic acids may require a considerable amount of manual pipetting when using column-based methods. The pipetting can lead to frustrating variability in DNA/RNA yield between experiments and individuals. Staff and students may need considerable training and practice to achieve reasonably consistent nucleic acid yields.

Even in automated low or medium-throughput processes using columns, there is likely a requirement for centrifugation and manual handling that affect DNA/RNA integrity.

Washing and eluting DNA/RNA bound to magnetic beads in automated systems is simpler than with columns, requiring little to no additional input from the user, and therefore minimizing the amount of training required.

4: Choose the most appropriate method for your application

Isolation of genomic DNA and total RNA can use silica binding in either the column or magnetic bead format.

However, while columns are well suited for basic applications, such as PCR and electrophoresis to check inserts in vector-based cloning, sample preparation in more sophisticated studies involving methods like quantitative polymerase chain reaction (qPCR) and NGS can be simplified by using magnetic beads.

The simplicity of magnetic bead-based protocols means they can be automated easily, making them an excellent option for procedures that require quick turnaround times, including total viral DNA/RNA extraction for qPCR-based diagnosis of diseases and high-throughput sequencing applications.

Magnetic beads can also support specific diagnostic applications that use samples with low DNA amounts, such as liquid biopsies containing circulating cell-free DNA (cfDNA).

5: Find a surface chemistry for your application

While spin columns are limited to silica, cellulose, or ion exchange resins, magnetic beads provide a range of surface chemistries that extend their use beyond that of standard DNA/RNA extraction and purification techniques.

For example, in exome sequencing, streptavidin-coated magnetic beads enable the capture of specific genes or exon fragments hybridized to biotinylated complementary sequences. These magnetic beads are well suited for use with targeted sequencing panels for cancer, enabling the capture of specific disease-associated sequences for downstream NGS and tumor profiling. Similar techniques are also used for microsatellite isolation in population genetics.

6: For unique applications, consider custom bead conjugation

In addition to the standard array of surface chemistries, magnetic beads can be conjugated to enzymes, antibodies, and custom ligands. For example, Protein A/G-conjugated magnetic beads are widely used in immunoprecipitation (IP) and co-IP assays to study protein interactions. As with nucleic acid isolation, protocols with these beads are easily automated, freeing up time and reducing the need for manual handling of samples.

7: Use magbeads for both size selection and clean-up in NGS

NGS sample prep has a series of steps that can influence the quality of the final data: nucleic acid isolation, purification, size selection and clean-up, and library normalization. Using the same type of reagent throughout these steps would be simpler

While some vendors do offer columns for DNA fragment size selection, these kits are often restricted to removal of fragments below a specific length.

Carboxylate-coated magnetic beads greatly simplify the process of DNA fragment size selection for NGS in both manual and high-throughput automated workflows. Simply altering the ratio of buffer to beads to DNA enables reproducible selection of fragments within a very specific size range.

With magnetic beads, clean-up of libraries for NGS workflows is as simple as eluting the DNA from the beads with a buffer.

Watch our webinar comparing approaches to NGS sample prep.

8: The new normal for NGS library normalization

Overlooking the importance of library normalization can lead to wasted capacity and incomplete or unreliable datasets after sequencing. Although columns can be used for some stages of NGS library preparation, unlike magnetic beads, they cannot be used for normalization.

Where your sample is plentiful and time is a factor, magnetic beads remove the need for careful quantitation by qPCR or fluorimetry and provide an equimolar quantity of library fragments across multiple samples.

Magnetic beads are versatile tools for nucleic acid purification, requiring fewer handling steps than columns and offering surface chemistries tailored to different applications. Switching from columns to beads makes automation easier and therefore addresses ongoing high throughput sample demands. For help with making the switch from spin columns to magnetic beads, contact our Scientific Support team.

Written by Michael Murphy
Modality Specialist USCAN/Asia, Genomics and Diagnostic Solutions, Cytiva

Michael is the USCAN/Asia Genomics Product Specialist at Cytiva. His primary focus is building long-term strategic partnerships with customers and developing design-in of customized solutions for NGS library prep, single-cell Omics and cell-free technologies. Michael has 25 years' experience in the diagnostics business environment working in OEM business development and commercial roles serving customers launching novel products into emerging markets.

What's hot in immunodiagnostics, and where we are taking it

Magnetic bead-based approaches in immunodiagnostics have become essential to meet the needs of healthcare today. While ELISAs are still popular, the benefits of magnetic beads—accuracy, speed, and simplicity— are driving us towards precision health.

What is immunodiagnostics?

Immunodiagnostics takes the ability of antibodies to recognize and specifically bind to an antigen of interest and applies it in a clinical setting. For example, you might use an immunodiagnostic assay to detect an antigen presented by a tumor or virus found in a blood sample.

This sensitive, specific, and time-saving methodology plays a vital role in understanding and diagnosing health conditions, from infectious diseases and cancers to metabolic disorders and cardiac health. These insights enable the development of targeted treatments and therapies with a view and trend towards precision health.

Over the years, the immunodiagnostics market has grown to tens of billions of dollars, with the Enzyme-linked Immunosorbent Assay (ELISA) at the forefront.

Improving accuracy, speed, and simplicity in immunoassays

The earliest immunoassays were developed in the 1950s and relied on radiolabeling. This approach gave them sensitivity and accuracy. The technologies evolved rapidly, leading to the development of the ELISAs and enzyme immunoassays (EIAs) we use today for diagnostics.

Both ELISAs and EIAs use an enzymatic reaction between, for example, horseradish peroxidase (HRP) and luminol to generate a color change that is proportional to the concentration of antigen-bound antibody in the assay. Although they were two independently developed techniques, users often use the terms interchangeably.

As well as ELISAs, we now have chemiluminescent immunoassays (CLIAs), florescent immunoassays (FIAs), and lateral flow assays (LFAs) in common use as in vitro diagnostic tests, each providing alternatives in methodology and reporting.

The landscape is constantly changing, however. The big players in the market are pushing assay technology forward, adopting microfluidics and magnetic beads. So, clinical scientists now have access to a dizzying array of immunodiagnostic products, with the list expanding all the time.

Some of these immunodiagnostics target single biomarkers, but recent years have seen a trend towards detecting multiple analytes, demanded by the complexity and needs of making reliable early diagnoses.

Whatever their targets and methodology, new immunodiagnostics need to encompass a few properties for market success:

- Sensitivity and accuracy with reproducible results.
- Rapid workflow and results.
- Simplicity, requiring minimal training.
- Cost efficiency.
- Immunodiagnostics in oncology and infectious disease

Oncology and infectious disease cover a substantial portion of the market for immunodiagnostics, and there are as many potential target biomarkers and combination of biomarkers as there are diseases.

Molecular assays, based on NGS or PCR, can pick up known mutations linked to cancer. These hallmarks can help diagnose and even forecast disease development and response to different therapies.

The immunodiagnostic assays you develop could be designed to complement these molecular assays, providing a similar function for protein biomarkers.

Using immunodiagnostics in oncology

In oncology, an immunodiagnostic can confirm the presence of a solid tumor directly or indirectly by detecting known tumor-associated antigens or antibodies against them. Importantly, this approach might also flag up the resurgence of a tumor or key changes in it that affect the effectiveness of the therapeutic approach.

Finding and developing assays for these biomarkers has become quite the trend in immunodiagnostics development, leading to complex screening tools and new technologies.

In early 2019, OPKO Health Inc. gained approval from the US Food and Drug Administration (FDA) for prostate-specific antigen (PSA) testing to improve the accuracy of prostate cancer diagnosis, ultimately with a view to reduce unnecessary prostate biopsies.

OPKO Health's test uses microfluidics, a recent development in immunodiagnostic approaches, to assay an array of biomarkers in multiplex.

Use of immunodiagnostics in infectious disease

Immunodiagnostics complements molecular assays in the study of infectious diseases as well. While an infection is running its course, it might be relatively straightforward to use NGS or PCR to identify the pathogen. But, if the infection goes dormant, an immunoassay might be the only reliable way of identifying that pathogen.

This is important, as a dormant pathogen may activate later, or react to what seems like a completely unrelated condition, therapy, or change in environment. The stipulations on treatment of multiple sclerosis with the monoclonal antibody Natalizumab™ (Tysabri) makes a good example.

If the patient has a dormant John Cunningham virus infection, they face an increased risk of developing progressive multifocal leukoencephalopathy (PML) when treated with this drug. Therefore an immunodiagnostic test for antibodies against the John Cunningham virus is essential for any clinician considering Tysabri for a patient.

Supporting proteome screening with immunodiagnostics

Proteomics is the large-scale and inclusive study of the proteins produced by our cells. Mass spectrometry is a useful tool in proteomics, profiling the structural diversity of proteins and helping to identify biomarkers, but in the context of specific conditions, it can be too broad by itself.

Clinical scientists can add a level of specificity using immunoassays that effectively enables them to perform targeted proteome screening.

These mass spectrometric immunoassays (MSIAs) combine the selectivity of antibodies with the sensitivity and speed of mass spectrometry. The assays enable

a level of analysis beyond the capabilities of an ELISA, including the identification of alternative structural conformations and single mutations in proteins.

MSIAs also have the potential to serve as standardized and quantitative screening tools for more general screening of the population as they are also fast and easily automatable. These tools could be used to screen an individual's proteome and detect subtle signs of a disease that is not yet presenting any symptoms.

Next-generation immunodiagnostics

With the rapid evolution in immunodiagnostics, two key technologies enabling the next generation are microfluidics and magnetic beads.

Microfluidic assays

Microfluidics is used in a variety of application areas, including antibody characterization. These systems use immobilized antibodies on surface plasmon resonance (SPR) chips to study analyte interaction, but can also be used the other way around to look for biomarkers in samples in real-time.

The OPKO Health PSA screening assay mentioned earlier uses this approach for biomarker identification, and there are many microfluidics-based devices already on the market.

Magnetic immunoassays

Magnetic beads, or superparamagnetic particles, have been around for a while, and gained popularity in many applications, from antibody screening and immunoprecipitation (Mag Sepharose), to sample and library prep in NGS workflows (Sera-Mag).

A key draw towards magnetic beads, in addition to the ease of use, is the array of surface chemistry options they offer, which provide as many functions as you can think of conjugations.

For immunoassays, magnetic particles such as Sera-Mag beads offer a convenient alternative to plate-based ELISAs and, as they rely on broadly the same assay principles, transition from plate to bead is straightforward in most cases.

The same development considerations apply when building assays, with non-specific binding and sensitivity being top of most people's list of concerns. Having the assay on the bead provides a level of flexibility that cannot be achieved by ELISA, and manipulation in high throughput settings becomes far easier.

There are already numerous examples of magnetic beads simplifying workflows and enabling multiplexed magnetic immunoassays.

Enabling precision health

Tying back to precision health, these advances in immunodiagnostics ensure the continued march towards better prevention, diagnostics, and treatments for patients, specific to their needs and leading to improved well-being for us all.

Magnetic bead-based approaches enable diagnostics manufacturers to check off all those properties for an immunoassay's market success. So, it is no surprise that all the big players now have a range of bead-based assays on the market.

We provide a broad range of magnetic beads for molecular biology applications, including a series of surface chemistries well-suited for magnetic immunoassay development.

For support with any aspect of your assay development workflow, contact our Scientific Support team.

Written by Erik Braat
Modality Specialist, Genomics and Diagnostic Solutions, Cytiva

Erik works within the Genomics and Diagnostic Solutions (G&Ds) business as a Modality Specialist covering a dedicated part of the EMEA region. He provides direct support to customers, distributors and the Cytiva commercial organization. Erik joined five years ago as a magnetic bead specialist and quickly moved into the broader role supporting the complete G&Ds portfolio. His 20 years' experience in chromatography and bioprocess purification including antibody affinity enhance his knowledge of magnetic bead-based assays as an ideal solution supporting future breakthroughs in immunodiagnostics.

Unraveling the challenges of nucleic acid isolation

Analyzing nucleic acids is powerful, and provides insight 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.

Complexity remains at the heart of nucleic acid extraction. Therefore it is important to choose the 'right' isolation protocol for your sample or application .

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 you need from other nucleic acids and cellular components in the sample, while also keeping it in good condition for downstream analysis.

There are several approaches towards extraction which vary from gentle to aggressive. The choice resides on factors such as the target DNA, source organism, the type and quality of starting material, and the application. 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 your 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 your DNA in a suitable format (solvent and concentration). Often, this will be just a matter of precipitating your 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 DNA isolation 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 don't 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's 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.

DNA extraction

Magnetic beads provide an excellent alternative to traditional isolation and clean-up methods due to their versatility and ease of use. They don't 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.

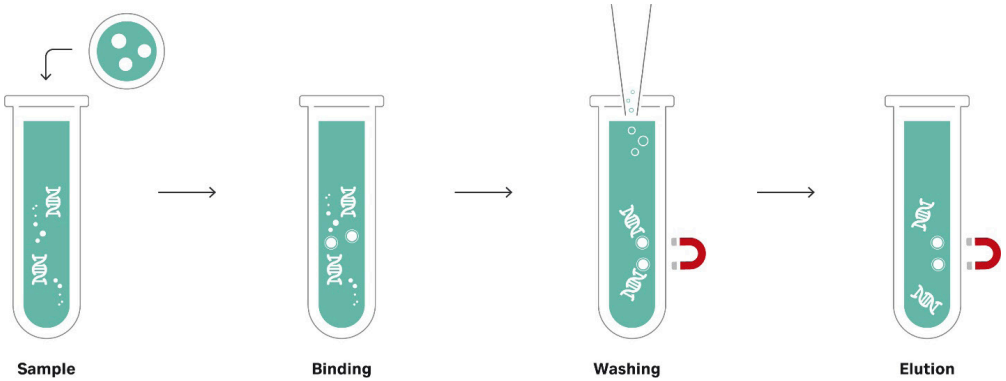


Fig 1. *The principle of magnetic beads for nucleic acid isolation.*

Cytiva's SeraSil-Mag silica coated magnetic beads are an appropriate example. They help address several challenges in DNA extraction and suit a range of applications, including all the sample types described previously, when used with appropriate buffers. Their binding capacity and tight size distribution deliver highly reliable results while being easy to use without centrifugation.

Extract DNA from a variety of samples using SeraSil-Mag beads.

Our genomics experts aim to support you in all your workflows. For support in any aspect of your application, contact our Scientific Support team or your local Cytiva representative.

Written by Chike Ejiofor
Field Application Specialist,
Genomics and Diagnostic Solutions, Cytiva

Chike is a Senior Field Applications Specialist for Genomics and Diagnostic Solutions, focusing on supporting the commercial teams in their understanding of the NGS workflow, DNA sequencing, RNAi and Real-Time PCR. Chike has worked in the healthcare industry for over 15 years, after spending a year as a Research Scientist at the University of Westminster, where he focused on validating ATG-like genes in T. Vaginalis.

Avoid bottlenecks in high-throughput NGS sample preparation

Traditional next-generation sequencing (NGS) sample preparation methods can be labor-intensive, requiring careful sample handling by experienced laboratory personnel to produce efficient and reliable sequencing results. Recent years have seen the development and adaptation of several approaches to sample preparation, aiming to improve speed, throughput, and reproducibility.

Many of these approaches are also compatible with automation to greater or lesser extents, providing the opportunity to minimize human error and variation, and further improve consistency between samples from different batches.

The need for sample throughput in NGS workflows

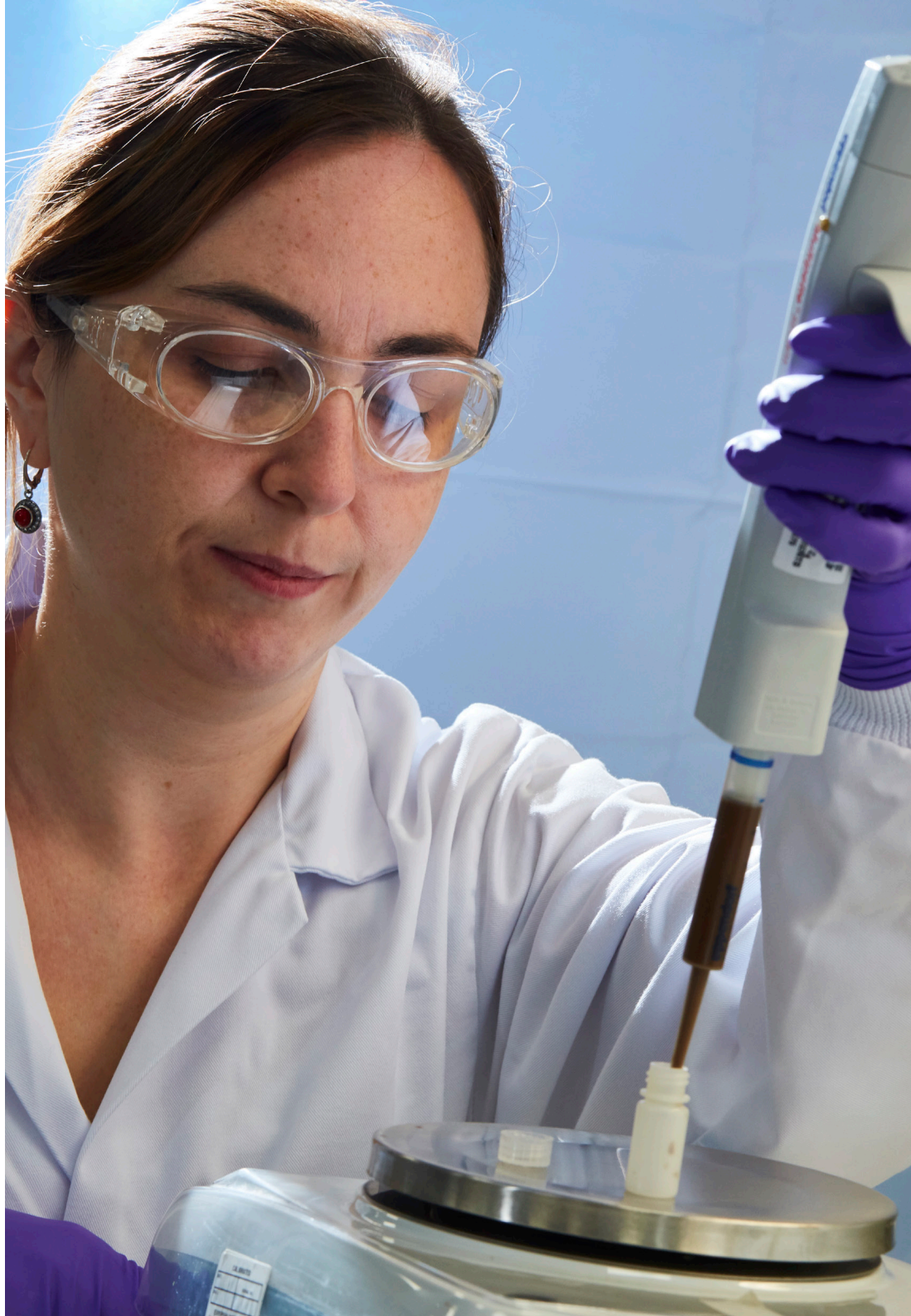
Sample throughput is a key factor in sample preparation for high-throughput sequencing labs, having a direct impact on productivity and cost-effectiveness.

What is sample throughput?

Sample throughput refers to the number of samples processed simultaneously (parallel processing) or in a given timeframe (per day, week, or longer).

NGS applications have varying throughput requirements. Small-scale/low-throughput sample preparation, such as that in small academic research projects, is usually manageable by manual processing, while higher throughput applications can require a level of automation, dependent on format, scale, and equipment options available.

Liquid handling robotics of various scales can automate steps of NGS sample preparation as well as NGS library preparation, providing the speed, throughput,



and reproducibility necessary while minimizing the risk of human error. In critical applications, such as clinical diagnostics, this reliability can reduce the risk of low-quality data and the need to repeat preparation and sequencing on precious samples.

The importance of addressing sample throughput as a potential bottleneck in the workflow is increasing with the accelerating adoption of sequencing in clinical applications, such as cancer diagnostics where early and reliable diagnosis can impact long-term patient outcomes.

Genomic sequencing companies now offer both public and private NGS services for genetic testing. To remain cost-efficient and cost-effective, these companies need to maintain a high sample throughput, processing large numbers of NGS samples per day and keeping their sequencing systems busy churning out data.

The difference between NGS sample prep and library prep

Between sample collection and sequencing in an NGS workflow, there are two key phases: sample preparation and library preparation, each containing a series of steps (Fig 1).

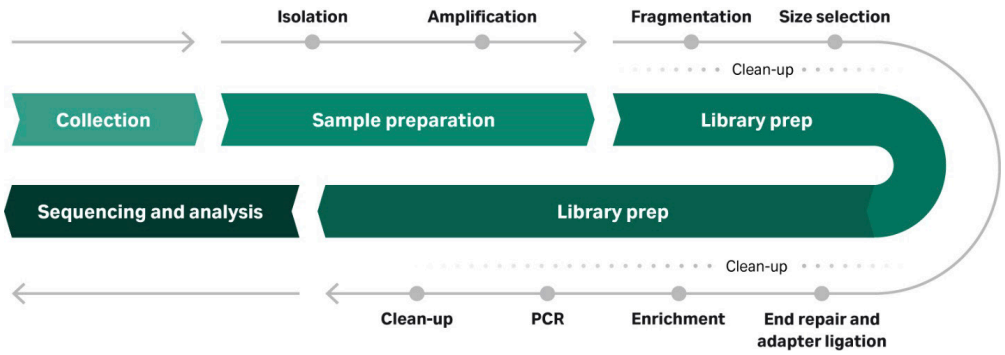


Fig 1: *Typical NGS workflow from sample collection to sequencing and analysis. Sample preparation approach can vary considerably, dependent on the source, quantity, and quality of starting material.*

NGS sample preparation involves sample-specific steps for extracting nucleic acids, such as genomic DNA from formalin-fixed, paraffin-embedded (FFPE) human tissue samples. These FFPE samples, for example, might require an extra step to repair damaged DNA to make them suitable for sequencing.

Sample preparation can also include an amplification step to increase the amount of nucleic acid fragments to a level sufficient for library preparation and sequencing instruments.

Library preparation takes the purified and amplified nucleic acid (DNA or RNA) from sample prep and processes them to be of optimum length by adding sequencing barcodes and adaptors. In some cases, a hybridization- or PCR-based enrichment step further optimizes the library for sequencing by enriching the specific regions of interest.

The different approaches to NGS sample preparation

There are various technologies and approaches enabling sample preparation for NGS. These range from traditional solution-based protocols, through solid-phase and membrane chemistries, to magnetic bead-based approaches.

Magnetic bead-based approaches are arguably a subcategory of solid-phase extraction, but there are numerous chemistries possible, and the workflow is distinct enough to consider as a separate type altogether.

Each of these approaches follows the three basic steps to DNA (or RNA) isolation:

- Lysis of cells in sample, varying from gentle detergents to aggressive homogenization.
- Removal of contaminants, including unwanted nucleic acids and denatured proteins.
- Recovery of nucleic acids into an appropriate buffer for any downstream application.

Table 1 lists several of these technologies and approaches to NGS sample prep.

Table 1: *Overview of technologies and approaches to NGS sample preparation.*

Type	Example method or chemistry	Key points
Solution-based	Phenol-chloroform (organic solvent)	• Produces high yield and quality DNA. • Relies on hazardous chemicals requiring careful handling.
	Phi29-based amplification	• Amplifies DNA without the need for thermal cycling. • Used for Whole Genome Amplification. • Highly processive and proofreading polymerase.
Solid-phase	Silica spin column	• Uses chaotropic buffers to bind positively charged silica membrane with negatively charged DNA. • Robust and can be automated.
Magnetic bead-based	Silica coated magnetic beads	• High-throughput isolation of nucleic acids from a range of sample types. • No need for centrifugation or vacuum systems. • Robust and amenable to automation.
	Oligo(dT) surface chemistry	• High-throughput purification of mRNA. • Specific sequence capture using intermediate oligonucleotide with complementary target sequence. • No need for centrifugation or vacuum systems. • Robust and amenable to automation.

Solution-based sample preparation methods

Probably the most well-known and widely used solution-based method for nucleic acid isolation is phenol-chloroform DNA extraction. Although this method uses hazardous chemicals, when handled correctly it yields high-quality DNA at a low cost. The approach is common in research laboratories, where students and post-doctoral researchers perform phenol-chloroform extractions as part of daily routine.

However, the approach is not so amenable to high-throughput applications. It requires careful handling and could have phenol (an inhibitor) carryover. Also, although modern liquid handling robots are quite sophisticated, they cannot judge and achieve a balance between yield and purity on a sample-to-sample basis that becomes second nature to researchers.

As such, phenol-chloroform-based sample preparation is better suited for low-throughput, high-yield needs, rather than high-purity or throughput required by sequencing labs.

An alternative solution-based method that is more suited to automation involves Phi29 DNA polymerase-based DNA amplification. This approach faithfully amplifies limited samples, such as those from single cells, using a one-tube, one-temperature format.

The high-fidelity Phi29 DNA polymerase is active at 30°C, does not require thermal cycling, and can produce micrograms of DNA from picograms of starting material. Phi29 relies on isothermal amplification, and can be used for both circular (or circularized) DNA template and linear DNA template (Fig 2).

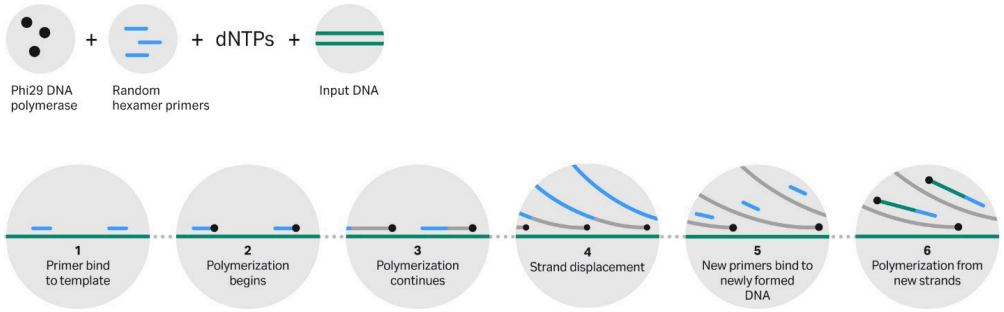


Fig 2: *Principle of Phi29 DNA polymerase-based amplification. Template DNA is primed at multiple locations with random hexamers. Phi29 DNA polymerase then extends primers, replicating template and displacing any downstream extended primers. Strand displacement and subsequent priming leads to an exponential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

Commercial kits using Phi29 polymerase, such as Genomiphi™ and TempliPhi™ DNA amplification kits, are designed for minimal hands-on time using simple automation-friendly protocols. These types of kits can also provide options for pre-dispensed reagents in single tubes, or multi-well plates, making them suitable for a range of throughput needs and liquid handling systems.

Solid-phase DNA isolation

Solid-phase approaches to DNA extraction rely on the dependable chemistry between positively-charged silica and negatively-charged DNA. A typical protocol uses chaotropic salts to disrupt hydrogen bonds between strands, facilitating the adsorption of the nucleic acid phosphate residues to the silica membrane.

This silica-DNA association is predictable and easily modulated, enabling debris and potential contaminants to be washed away and purified DNA eluted with a high yield and purity (Fig 3).

Compared to the equivalent solution-based approach, phenol-chloroform extraction, silica spin-columns require fewer pipetting steps and all can be performed in the same vessel. These features make using silica spin-columns for NGS sample prep highly automatable with robotics and liquid handling systems, and so both suitable and in common use for high-throughput applications.

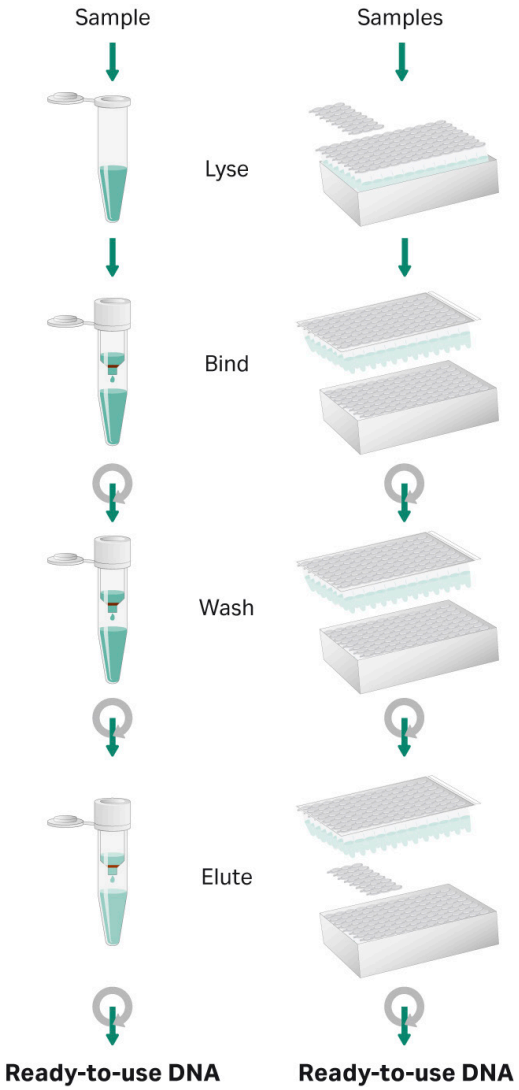


Fig 3: *Solid-phase nucleic acid extraction workflow using a silica membrane spin column.*

Magnetic beads-based nucleic acid isolation

Magnetic beads are a form of solid-phase nucleic acid isolation (DNA extraction and RNA extraction) technology, though there are also surface chemistries that enable capture of other substrates, such as proteins. These beads are composed of particles of iron oxides, such as magnetite, which give them superparamagnetic properties. That is, they exhibit magnetic properties only in the presence of an external magnetic field.

The protocols for using beads are scalable, simple to follow, and generally involve just three key steps that result in high-purity nucleic acid extractions (Fig 4), with no need for centrifugation or vacuum systems:

- Reversibly bind target molecules to magnetic beads added to any of a range of sample types.
- Apply a magnetic field to immobilize the beads while washing away the remainder of the sample.
- Adjust buffer conditions to release the purified target molecules.

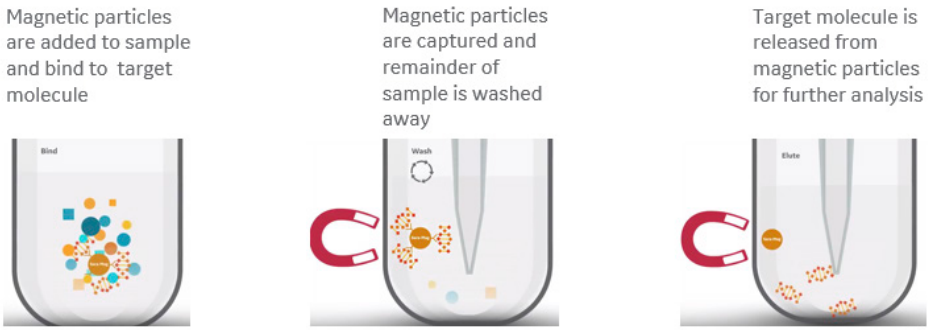


Fig 4: *Overview of magnetic bead-based nucleic acid isolation. (A) Target nucleic acid in the sample binds to beads based on buffer conditions. (B) Magnetic field immobilizes magnetic beads while unwanted debris and contaminants are washed away. (C) Beads release target nucleic acid by changing buffer conditions.*

The range of surface chemistry options for magnetic beads covers a full spectrum of sample types, providing a level of versatility that is challenging to achieve with other approaches to NGS sample prep.

Silica-coated beads, for example, are well suited for the capture of high molecular weight genomic DNA from blood, tissue, and cell samples, even when the sample is scarce. For mRNA, oligo(dT)-coated beads provide the ability to purify directly from cell samples.

For applications where the sample is scarce, such as circulating cell-free DNA (cfDNA) sequencing, a combination of robust silica coatings and efficient buffer chemistry can provide the sensitivity needed to isolate the low molecular weight target molecules.

In each case, binding and releasing target molecules simply involves adjusting buffer conditions, making the workflow readily automatable with liquid handling robotics.

Magnetic bead-based DNA and RNA isolation, therefore, provides a means to avoid bottlenecks in sample preparation for a range of sample types while delivering reliable results and reproducibility in high-throughput environments.

These beads are straightforward to incorporate into sample preparation workflows, removing the need for hazardous solvents in the case of existing phenol-chloroform methods, and providing high binding capacity and scalability cost-effectively.

The magnetic bead-based sample preparation workflow is also simpler than that of solution-based methods or spin columns, with no requirement for centrifugation, vacuum systems, or indeed substantial experience or training to achieve high-purity DNA and RNA isolation.

Magnetic beads can also support NGS library prep in size selection and clean-up, supporting low- to high-throughput, consistency, and time and cost savings throughout the NGS workflow.

Closed vs open systems for NGS workflows

There are many commercial products for NGS sample prep. Most involve a degree of centrifugation, though some rely on vacuum manifolds. Some vendors offer these as part of automated “closed” systems, which provide both advantages and disadvantages to the user.

Compared to so-called “open” systems, where a user can select components from multiple manufacturers to develop a cost-effective protocol for their own purpose, closed systems limit the user to products from the same manufacturer or its partners. Manufacturers take this approach to ensure full compatibility between components and provide a level of reassurance that yield and quality will be consistent.

Large integrated systems can be coupled with other equipment to perform other NGS sample preparation steps, such as amplification. Although these integrated systems are well suited to high-throughput applications, they require a relatively large investment.

This level of investment might only be justifiable in certain situations, for example, high-throughput sequencing labs running clinical NGS panels. For these types of labs, having a single supplier means the entire workflow is already tried and tested, and a single point of contact for support minimizes the risk of delays during any troubleshooting.

Supporting high-throughput sequencing providers

We have many years of magnetic beads, with an R&D team experienced in developing products that help maximize operational efficiency in high-throughput environments.

In addition to a range of existing nucleic acid isolation kits designed for use with a full spectrum of sample types including cultured cells, tissue, blood, plant cells, and bacteria, the team supports customers with customized solutions.

Written by Martina Kahl
Modality Specialist Genomics and Diagnostic Solutions, Cytiva

Martina is a Modality Specialist at Cytiva, responsible for Central and Eastern Europe. With her passion for genomics, molecular diagnostics and immunodiagnostics, she supports diagnostic kit developers and end-users with genomics and diagnostic technologies and services. Martina has more than 20 years’ experience in the diagnostics business environment working in product management and commercial roles, looking at emerging trends, new applications such as next-generation sequencing and providing insights and expertise that has enabled new product portfolio developments.

Size selection brings better data to NGS workflows

The introduction of next-generation sequencing (NGS) technology resulted in a fundamental shift in our approach to genomics. NGS refers to the deep, high-throughput, in-parallel DNA sequencing technologies developed a few decades after the Sanger DNA sequencing method first emerged in 1977 and then dominated for three decades. Even now, more than a decade after second generation sequencers arrived, the market continues to grow.

This is partly because of the constant drive to reduce the cost of sequencing and open up the technology to more researchers and applications. Despite these year-on-year cost reductions, individual sequencing runs remain expensive. Maximizing the usable data from any given run, which can be achieved by optimizing upstream library construction and sample preparation steps, can lead to additional savings.

These processes are inexpensive and have a substantial influence on final data quality. Here's why library fragment size selection is a key step towards data quality, and recommendations on the main methods for carrying out size selection, their advantages and disadvantages.

What does a typical NGS sample prep look like?

Although there are multiple approaches to sequencing, Illumina's sequencing-by-synthesis approach continues to be the most widespread. We've previously discussed the fundamentals of NGS sample prep, which has several common steps for library construction, including:

- Fragmentation through enzymatic or mechanical means.
- End-repair and processing to homogenize the heterogeneous fragment ends.
- Adapter ligation for cluster generation and in-cell clonal amplification.
- Size selection to remove suboptimal fragment sizes and any adaptor dimers.

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 Illuminasystems. 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's possible to sequence fragments > 1 kb in length, this is inefficient and prone to errors—an issue that third generation sequences 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 won't be viable or read to sufficient depth. This produces little useful data and low uniformity of coverage.

A size selection step enables you to take a randomly fragmented library and pull out only the fragments fitting the optimal/target range for the instrument and application (Fig 1). 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 your 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.

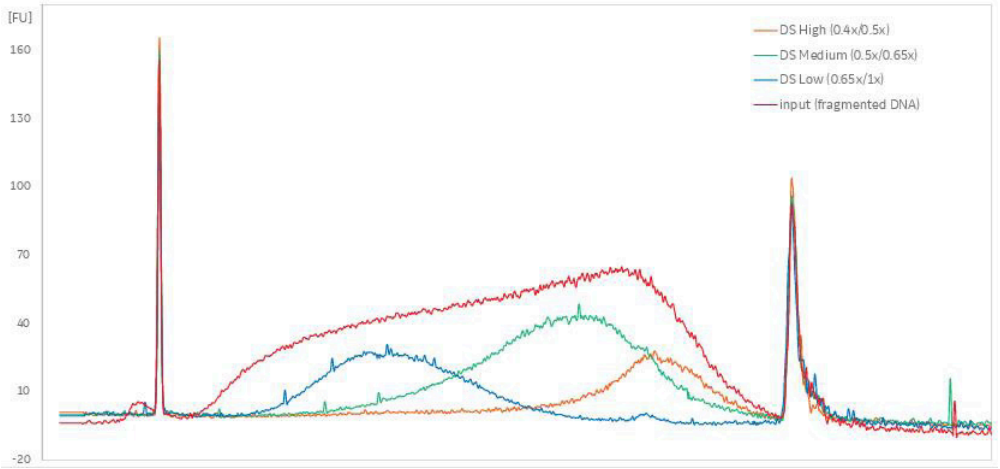


Fig 1. *Enzyme fragmented DNA with dual size selection.*

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's 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 polyethylene 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.

Our genomics experts can support you in optimizing your NGS workflow. To find out more about using magnetic bead-based size selection, or for help and advice on any aspect of your workflow, contact Cytiva's Scientific Support.

Written by Andrew Gane
Genomics & Diagnostics Strategy & Technology Manager, Cytiva

Andrew is the Product Strategy and Technology Manager within the Genomics and Diagnostic Solutions business responsible for building the innovation pipeline in collaboration with the R&D and commercial teams. His knowledge and understanding of emerging trends and new applications have been fundamental to developing the product portfolio into workflow-based solutions, with a particular focus on next-generation sequencing (NGS). Andrew has more than 30 years' experience in immunodiagnostics and molecular diagnostics in both lab-based and product development roles.

Resources

Scientists Guide to Magnetic Beads

https://www.cytivalifesciences.com/solutions/genomics/knowledge-center/magnetic-bead-guide/magnetic-bead-basics

Reproducible protein and peptide cleanup for mass spectrometry

https://www.cytivalifesciences.com/solutions/genomics/knowledge-center/cleanup-for-mass-spectrometry

Magnetic separation with Sera-Mag technology

https://www.cytivalifesciences.com/solutions/genomics/sequencing/sera-mag

Selective binding and PCR clean-up with Sera-Mag Select

https://www.cytivalifesciences.com/Solutions/Genomics/Sequencing/Sera-Mag-Select

Optimize nucleic acid isolation in molecular biology applications with magnetic bead kits

https://www.cytivalifesciences.com/solutions/genomics/sequencing/DNA-isolation

Custom services for genomics

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