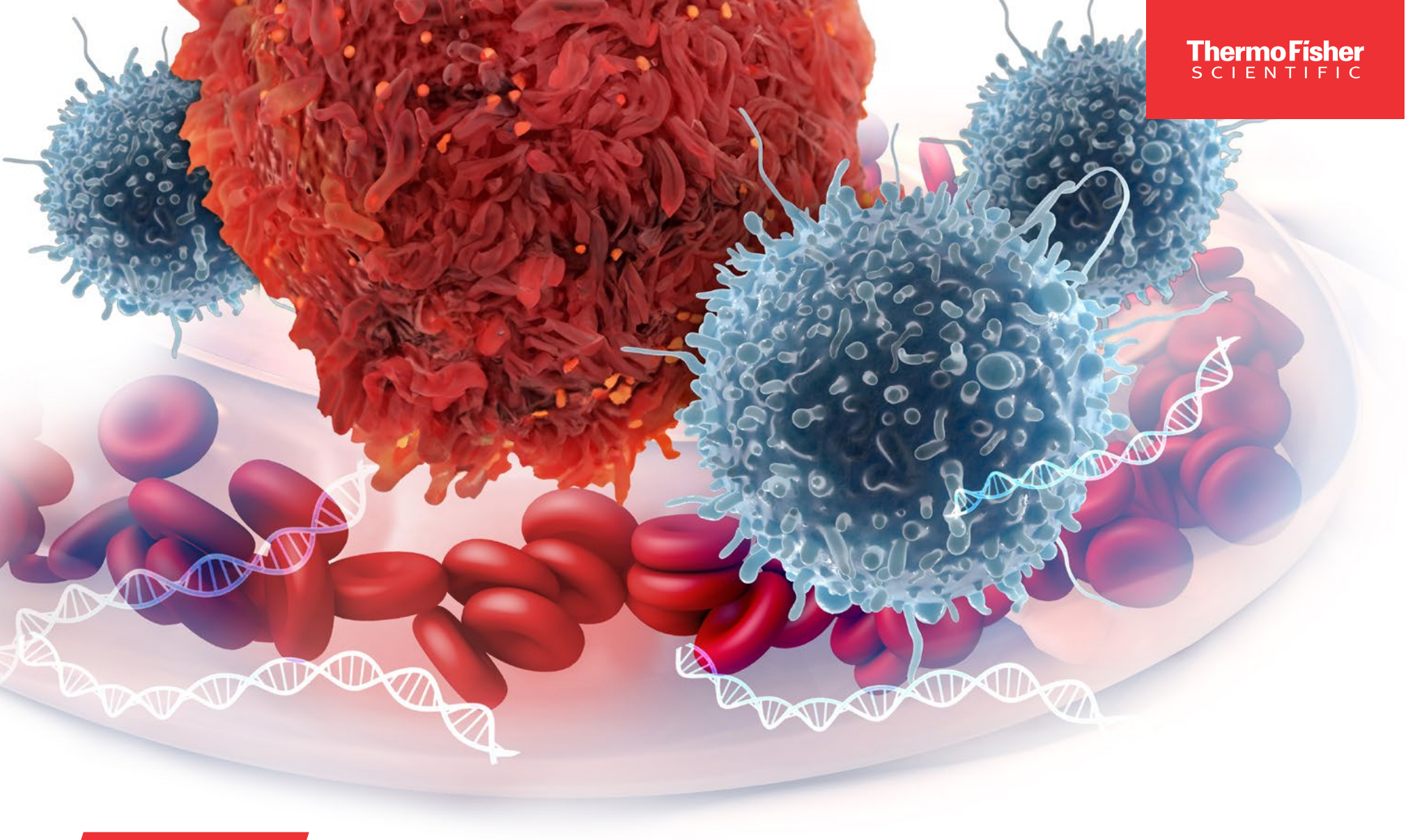

Cancer research

Synergetic biomarkers: Fueling advancements in cancer research

Introduction

New technologies are enabling greater access to genetic and protein biomarkers that are helping revolutionize cancer research, leading to significant advancements in diagnosis, prognosis, and treatment.

Genetic biomarkers can reveal insights into cancer predisposition, response to targeted therapies, and immunotherapy response. Greater amounts of high-quality DNA and RNA can help deliver more robust and sensitive results from advanced genetic analysis methodologies such as real-time quantitative PCR (RT-qPCR) and next-generation sequencing (NGS). More efficient solutions to extracting high quality DNA and RNA from solid tissues and liquid biopsy can both provide improvements for biomarker identification.

Protein biomarker research and analysis can help enable early detection of some cancers, greater understanding of mechanisms of action, or the development of protein monitoring strategies for therapeutic response. For some cancers, these insights can allow for earlier intervention or more personalized treatment.

The combined analyses of genetic and protein biomarkers provides a more comprehensive picture, which can allow for potential future advancements in cancer research. This may offer opportunities to improve diagnostic accuracy, refine risk stratification, or develop even more targeted therapies based on a patient's unique molecular profile.

Contents

Where it began: Solid tumors

Solid tumor classifications

There are three **classifications of solid tumors**: benign, pre-malignant with potential to become malignant, and malignant.[1]

Benign tumors are non-cancerous solid tumors that can occur anywhere in the body. They do not invade other organs or tissues to develop secondary malignant growths (metastasis), but can cause damage by compressing nearby tissues or organs. In rare cases, certain benign tumors can transition to a malignant state.

Pre-malignant tumors have the potential to transform into cancerous tumors, and therefore require monitoring. They exhibit increased production of matrix-remodeling proteases and also release of pro-angiogenic, proliferation, and survival factors in the tumor's microenvironment.

Malignant tumors can invade nearby tissues and also spread to other parts of the body through the blood and lymph systems. They are characterized by alterations in cell physiology, including increased growth signals, intensified anti-growth signals, cell and tissue apoptosis or necrosis, limitless replicative and proliferative potential, prolonged angiogenesis, and metastasis.

Solid malignant tumors can be classified based on the type of cell from which they originate:

- **Invasive and metastatic carcinomas** are malignancies of epithelial origin that can develop in the skin or tissues lining internal organs. Subtypes include adenocarcinoma, basal cell carcinoma, squamous cell carcinoma, transitional cell carcinoma, and ductal carcinoma.
- **Sarcoma and undifferentiated tumors** arise from connective or supporting tissues and can transform into soft or synovial sarcoma. They can originate in bone, cartilage, fat, muscle, or blood vessels. Subtypes include angiosarcoma, bone sarcoma, fibroblastic sarcoma, and rhabdomyosarcoma.
- **Lymphoma** affects lymphocytes, which are cells of the immune system. It begins in infection-fighting lymphocytes found in lymph nodes and spleen. Examples include Hodgkin's disease and non-Hodgkin's lymphomas.

- **Blastoma, brain, and spinal cord cancers** are solid tumors that can arise in the brain, central nervous system, or eyes. Blastomas develop primarily in pediatric populations.
- **Melanomas** are skin malignancies that can also occur in the eyes and, rarely, in internal organs.
- **Germ cell tumors** typically originate in the ovaries and testes but can also occur in the brain, abdomen, or chest.
- **Carcinosarcoma** are rare malignant solid tumors that can arise in different organs. They consist of two types of cancerous cells (biphasic) with the ability to metastasize: carcinoma (epithelial cancer) and sarcoma (connective or mesenchymal tissue cancer). Carcinosarcomas are highly aggressive and often appear to arise *de novo*.

Sample types and research challenges

Solid tumor samples for research may be provided as fresh frozen (FF) tissues or formalin-fixed paraffin-embedded (FFPE) tissue. Biofluid samples such as urine, whole blood, serum, or plasma may also be used for biomarker analysis. Analysis of this wide variety of samples requires a wide range of methodologies that may include immunohistochemistry (IHC), fluorescence *in situ* hybridization (FISH), RT-qPCR, or next-generation sequencing (NGS).

With FFPE tissue researchers can investigate and analyze excised biopsies directly. Immuno-histological or molecular profiling techniques can be used to gain a better understanding of the proteins or study the morphology of the tissue samples. FISH and IHC are more traditional methods used to identify genetic mutations or molecular changes. RT-qPCR and NGS molecular profiling approaches have become more prevalent in recent years because they enable multiplexed analysis of multiple loci, whereas, traditional methods focus on a single target.[1]

Molecular applications such as qPCR and NGS are utilized as driving forces behind molecular profiling of solid tumors. With these applications there is a need for high-quality nucleic acid techniques that streamline isolation workflows enabling sample-to-answer results quickly and efficiently. Before extracting DNA or RNA from FFPE samples, deparaffinization must be conducted to dewax and de-crosslink the fixed embedded tissue. Conventional methods of deparaffinization can require extensive hands-on time, may involve hazardous materials, and can result in significant tissue loss. Solutions such as Applied Biosystems™ AutoLys™ M Tubes and Caps paired with Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit, deliver high-quality DNA and RNA from FFPE tissues using safe and convenient magnetic-bead-based isolation (Figure 1). This method saves researchers' hands-on time, eliminates the need for hazardous chemicals, and minimizes tissue loss due to pelleting techniques.[2-6]

Explore this workflow

A convenient, solvent-free deparaffinization method for FFPE sample preparation

Keep exploring solid tumor samples

Application note: Comparison of DNA and RNA from fresh-frozen vs. FFPE tissue samples

Application note: Mutation detection sensitivity in matched FFPE tissue and liquid biopsy samples

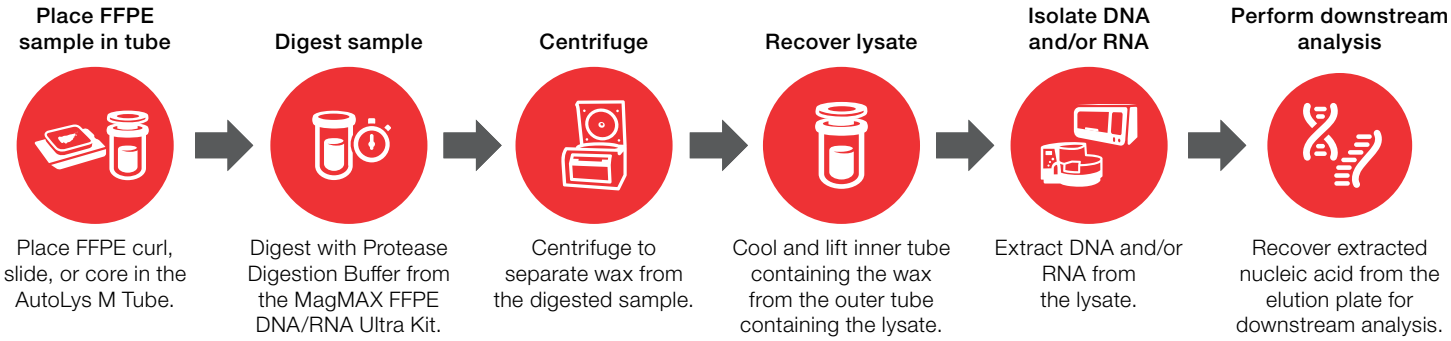

Figure 1. Workflow for deparaffinization and nucleic acid isolation using AutoLys M Tube system and MagMAX FFPE DNA/RNA Ultra Kit.

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The emerging potential of liquid biopsy

A new opportunity for less invasive testing

Tissue biopsies remain the standard method in molecular analysis research of genetic abnormalities linked to cancer. However, solid tissue sample collection is invasive and occurs at a single timepoint, providing only a single snapshot to analyze the tumor and assess tumor heterogeneity. In contrast, **liquid biopsies** are taken from bodily fluids such as urine, whole blood, serum, or plasma. Circulating biomarkers such as circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and exosomes that reflect the presence and progression of disease can be investigated with these samples. Liquid biopsy is emerging as a promising, less-invasive companion to solid tumor testing in cases where invasive testing is not practical or to monitor and study cancer progression over time. Continued advancements in the way we study these biomarkers and the techniques we use can allow **liquid biopsies** to significantly impact the future of the cancer research field.[1-7]

Oncology research and less invasive prenatal testing have been revolutionized by liquid biopsy studies in recent years. As a minimally invasive complementary or alternative approach to tissue biopsies, liquid biopsies can be less risky, painful, and costly, and are increasingly being used to analyze biomarkers in liquid samples. Recent studies have shown the utility of liquid biopsies for:

- Enhancing understanding of tumorigenesis, metastasis, and therapy resistance
- Detecting cancer at early stages when treatment may be most successful
- Monitoring of cancer development, disease progression, and recurrence
- Tracking response or resistance during and after treatment to allow for real-time adjustments to treatments
- Assessing fetal chromosomal anomalies

The present and future of biomarkers

Cell-free DNA (cfDNA) is the total extracellular DNA that is released from a variety of cells in the body, including normal cells, dying cells, and tumor cells. It can originate from various tissues and organs, reflecting the overall genetic makeup of an individual.

Circulating tumor DNA (ctDNA) is the fraction of cfDNA that originates from tumor cells. It carries genetic alterations or mutations present in the tumor cells, providing valuable information about the genetic characteristics of the tumor. ctDNA can be isolated from samples such as plasma and serum to be used as a less invasive biomarker for monitoring tumor dynamics, assessing treatment response, detecting minimal residual disease, or detecting the emergence of resistance mutations.

Although liquid biopsy can provide valuable access to ctDNA, cfDNA, and fetal DNA, these molecules are often scarce within a large volume of liquid sample, particularly compared to normal or maternal free-floating DNA. Extracting these rare molecules in sufficient quantities is crucial for downstream analyses. A key step in studying these targets is efficient isolation of the fragmented DNA while leaving the larger genomic DNA molecules behind. This aspect of cfDNA enrichment ensures that the shorter, fragmented DNA is concentrated and ready for sensitive downstream analysis. High resolution size-based recovery can help to increase the concentration of ctDNA to improve detection.[8]

Explore an end-to-end workflow for cell-free DNA analysis

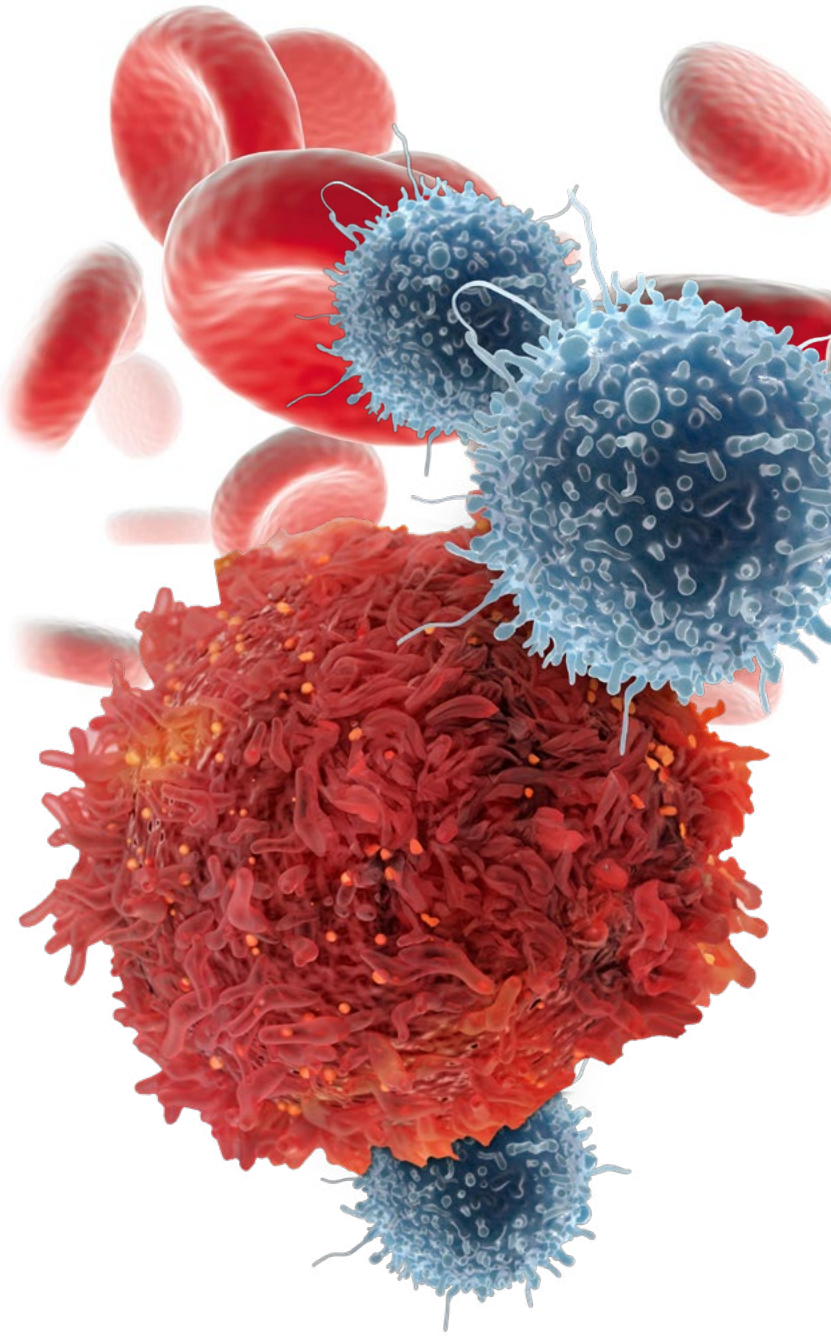
Application note: A complete next-generation sequencing workflow for circulating cell-free DNA isolation and analysis

Analyzing total RNA including cell-free RNA (cfRNA), microRNAs, and RNA in gene fusions can also serve as valuable biomarker targets and help to provide important insights to oncology research.

Circulating tumor cells (CTCs): Intact tumor cells can break free of their parent tumors and circulate in the bloodstream. CTCs can yield additional valuable information about the composition and behavior of tumors. However, CTCs are less abundant than ctDNA. Highly sensitive methods are required to capture and detect them. CTCs enable investigation of tumor genetics as well as physical structure, protein composition, and potential sites for immune system action. In some cases, CTCs are agents of metastasis by anchoring in a new location where they begin to multiply. The intact cellular structure of CTCs may help identify the original source of metastatic cancer.[9]

Learn about CTC isolation using Dynabeads™

Application note: Isolation of circulating tumor cells using Dynabeads magnetic beads



Exosomes: Distinct from the nucleic acid and cellular tumor debris, **exosomes** are a type of small (30–150 nm) extracellular vesicle (EV) that are secreted by cells both *in vitro* and *in vivo* into many body fluids. They carry a cargo of nucleic acid and protein that can yield valuable biomarkers to assess the genetic, physiological, and pathological status of their parent cells. Although they are released from cells under both pathological and normal conditions, cancer cells secrete more exosomes than non-cancerous cells. Exosomes carry signals between cells as part of an intricate intercellular communications network of physiological and pathological processes that can cross biological barriers. In cancer, they carry their cargo between primary and secondary tumors elsewhere in the body, potentially influencing growth, invasion, and drug resistance. Exosomes are often more abundant than CTCs and can be another less invasive source of valuable information about metastatic cancers.[10,11]

Driving discovery in liquid biopsy workflows

The key to identifying tumor biomarkers and uncovering valuable insights begins with high quality sample preparation.

Explore resources to inspire your cancer research

Sample preparation solutions for cancer research

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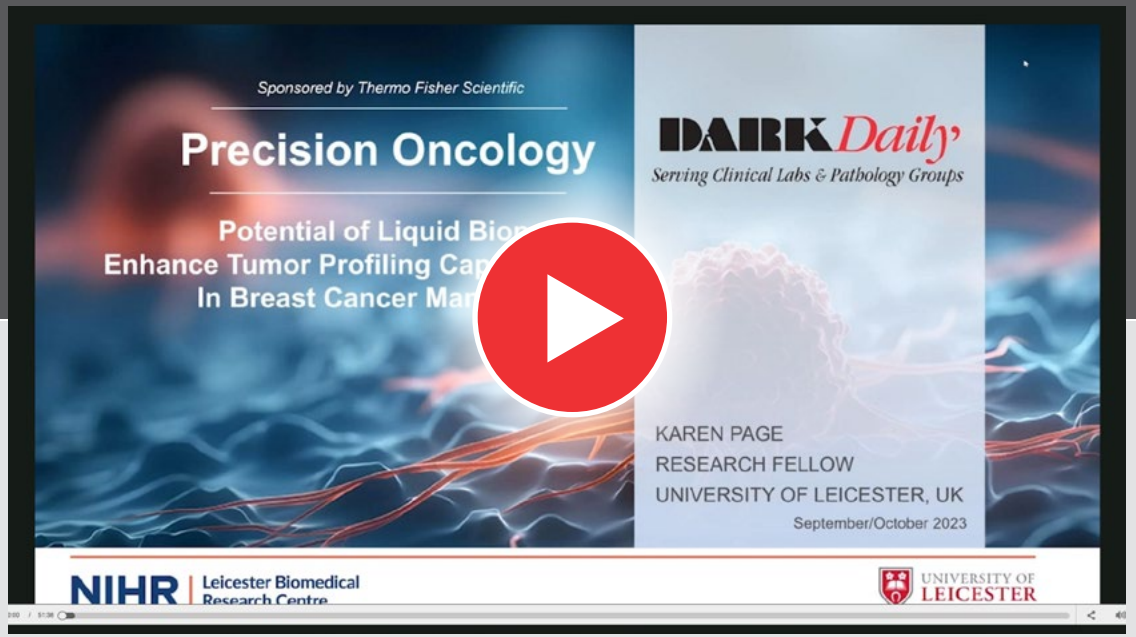
Cell-free DNA

Precision oncology:

Potential of liquid biopsy to enhance tumor profiling capabilities in breast cancer management



Learn more about the work of research fellow Dr. Karen Page of The University of Leicester, Leicester Cancer Research Center, using liquid biopsy with automation enablement and subsequent molecular analysis for research into the monitoring of breast cancer.

Karen Page, PhD, is a Research Fellow at the University of Leicester. She has worked in the field of cfDNA and liquid biopsy for over 20 years, with a primary research interest in the role of liquid biopsies and their utility in breast cancer, focussing on early-stage disease, minimal residual disease, acquired resistance to endocrine therapy, and relapse. Dr. Page was a member of the working group that was the first to describe whole genome analysis of cfDNA. This work was recommended by Faculty 1000 (F1000) as "an example of bench to bedside science that might be useful in the risk assessment and the monitoring of cancer." As the lead in the laboratory for next-generation sequencing, Dr. Page works closely with both clinicians and commercial partners to organise, carry out, and deliver projects that involve testing new products, reagents, equipment, and methodologies.

Advancements in cancer biomarker isolation: CTCs and exosomes

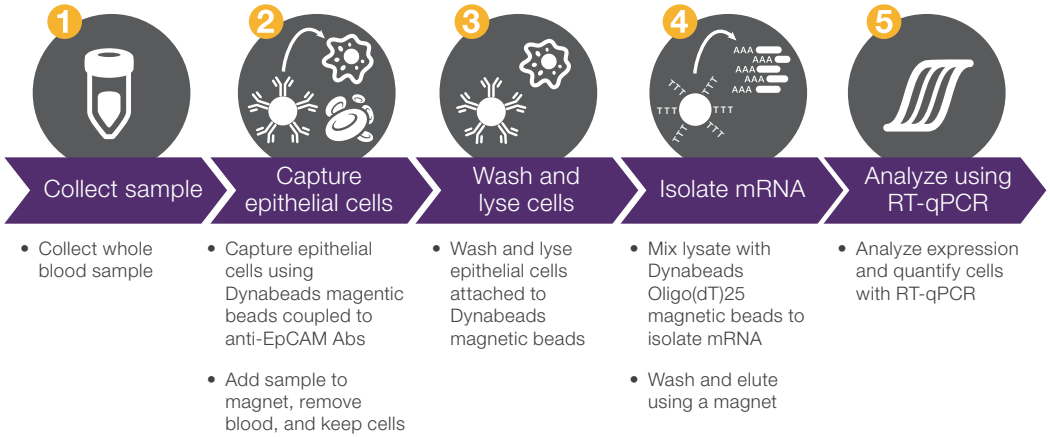


Figure 2. General workflow proposed for positive isolation to capture epithelial cells from whole blood samples.

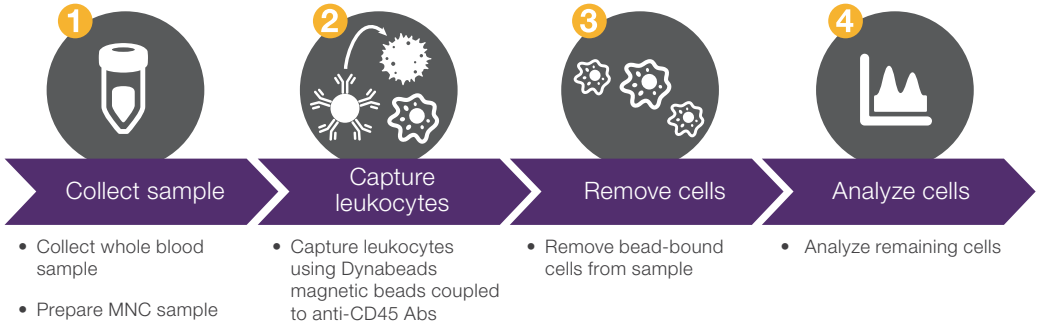


Figure 3. General workflow proposed for depletion to capture and remove leukocytes using Invitrogen™ Dynabeads™ MyOne™ CD45 Leukocyte Depletion beads.

Harnessing magnetic bead technology for CTC isolation

CTCs contribute to the initiation of metastasis by detaching from the primary tumor and circulated to distant organs via the bloodstream. Once at a new location, they invade tissues and divide, forming secondary colonization sites. Detecting and analyzing CTCs is crucial for understanding disease progression and tailoring appropriate treatment strategies. One of the groundbreaking technologies facilitating this process is magnetic bead technology.

Invitrogen™ Dynabeads™ magnetic bead technology employs superparamagnetic particles, that allow beads to be magnetic only in a magnetic field but have no residual magnetism when removed from the magnetic field. The superparamagnetic particles are coated with specific antibodies that bind to certain targets on cells, allowing for their isolation when a magnetic field is applied. Dynabeads magnetic beads provide an automation-friendly tool for isolation of circulating biomarkers. Generally, large beads are optimal for working on open platforms, while smaller beads are optimal for microfluidics. Positive isolation can be utilized to separate CTCs expressing cancer-specific markers, whereas depletion can be utilized to deplete leukocytes from blood samples for marker-independent CTC enrichment, leaving the target cells untouched.

Positive isolation techniques use Dynabeads magnetic beads coupled with antibodies that target surface markers on CTCs (Figure 2). This method results in a high yield of pure CTCs from blood samples. Dynabeads products used for positive CTC isolation include Invitrogen™ Dynabeads™ Epithelial Enrich (EpCAM) and Invitrogen™ CELLection™ Dynabeads Epithelial Enrich (EpCAM). These products target the epithelial cell adhesion molecule EpCAM, a prominent epithelial marker on CTCs. Alternatively, researchers can apply their own specified target antibody by either using biotinylated antibodies with Invitrogen™ Dynabeads™ Streptavidin™ beads or by covalently coupling their antibodies using the Invitrogen™ Dynabeads™ Antibody Coupling Kit.

In contrast, depletion techniques deplete leukocytes from blood samples, enriching the CTCs in the process (Figure 3). This is achieved using Dynabeads magnetic beads coupled to anti-CD45 antibodies, which bind to leukocytes, allowing for their removal and hence enriching CTCs in the

sample. Both isolation techniques are efficient and yield high purity CTCs, making them suitable for downstream analyses.

Positive isolation and depletion workflows can be automated using Thermo Scientific™ KingFisher™ sample purification instruments. Kingfisher instruments help provide high throughput, improved reproducibility, and reduced manual handling errors.

In conclusion, magnetic bead technology has significantly simplified the process of isolating CTCs. By providing a high yield of pure CTCs, magnetic-bead-based isolation enables researchers to gain valuable insights into cancer progression and metastasis.

Advancements in cancer biomarker isolation: Harnessing magnetic bead technology for exosome isolation

EVs are categorized into different subtypes that include apoptotic bodies, oncosomes, and nanovesicles due to differences in biogenesis, release pathway, size and function.

Traditionally, the isolation of exosomes uses methods such as different forms of ultracentrifugation that can be labor-intensive, involving differential, cushion, density gradient ultracentrifugation, ultrafiltration, and size exclusion chromatography (SEC). These methods can be time-consuming and may require a large amount of starting material but low exosome yield. However, the rapid kinetics and ion exchange properties of Dynabeads magnetic beads may offer a more efficient and less labor-intensive alternative (Figure 5). A key feature of Dynabeads magnetic beads for any isolation protocol is the rapid binding kinetics of the beads.

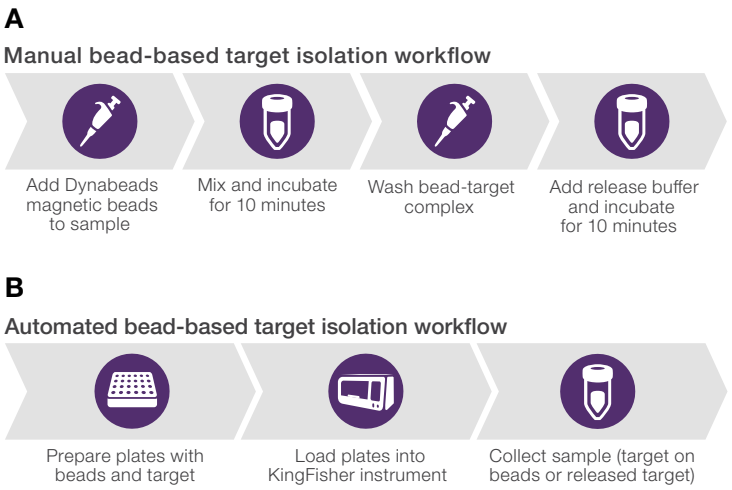


Figure 4. The proximity of the beads to the targets in the solution enables short incubation times and therefore fast protocols for both manual (A) or automated (B) exosome isolation workflows.

Positively charged Invitrogen™ Dynabeads™ Intact Virus Enrichment beads bind to negatively charged exosomes, viruses, or proteins within 10 minutes (Figure 4). Following capture, the exosomes (or other negatively charged vesicles) can be released from the beads in 10 minutes by adding an anion with a stronger relative affinity than the bound vesicle. This short and easy enrichment approach can be simplified even further by using the KingFisher purification system. The automated isolation method allows larger numbers of samples to be processed in only 10–20 minutes with high reproducibility, reduced hands-on time, and minimal error rates.[1,2]

Dynabeads magnetic beads enable researchers to directly isolate specific exosome subpopulations through immuno-affinity capture. The process uses Dynabeads magnetic beads along with primary antibodies to common exosomal surface markers (CD9, CD63, CD81, and EpCAM). In addition, Invitrogen™ DynaGreen™ CaptureSelect™ Anti-IgG-Fc (Multi-Species) Magnetic Beads offer a sustainable, microplastic-free smaller bead option for capturing specific exosome subpopulations from liquid biopsy samples in only 40 minutes. This process can also be automated for high throughput needs.

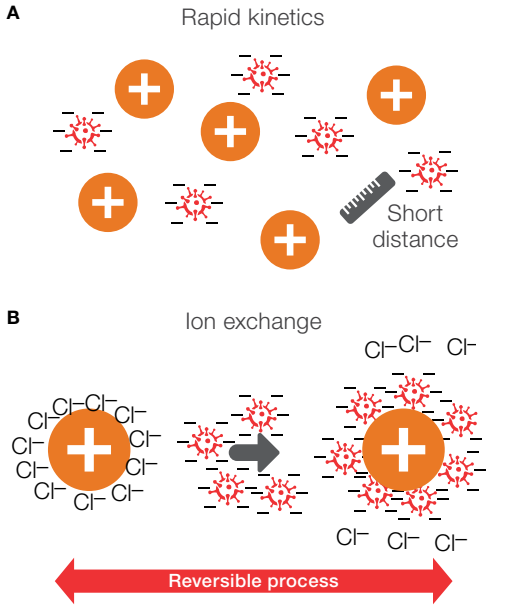


Figure 5. Isolation principle. (A) The positively charged Dynabeads Intact Virus Enrichment beads are near the negatively charged exosomes, enabling rapid binding kinetics and a fast isolation protocol. (B) For isolation of negatively charged exosomes or viruses, positively charged Dynabeads Intact Virus Enrichment beads protected with Cl^- ions are used. Exosomes added to the Dynabeads Intact Virus Enrichment beads will replace the Cl^- ions and bind to the bead surface. An anion with higher relative affinity can subsequently be added to replace the exosomes and thus release them into the sample.

Learn more

Exosome isolation and monitoring from cell culture and urine

References

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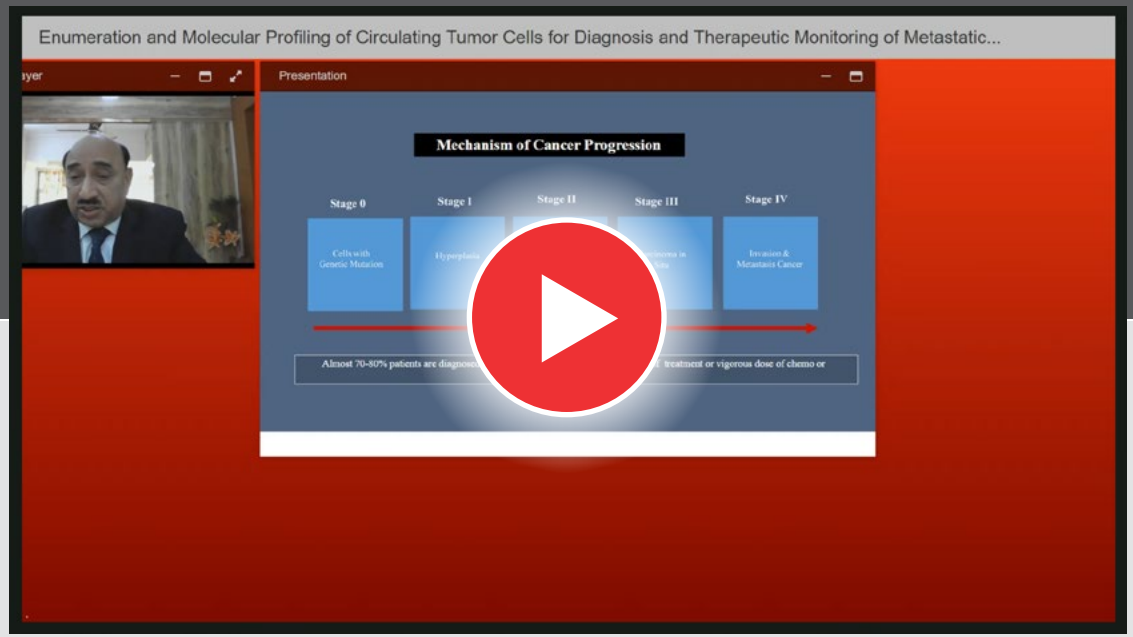
Clinical research

Early exploration in cancer research

New approaches to investigating cancer in liquid biopsy and three-dimensional tissue cell models are providing opportunities for researchers to gain early insights into cancer mechanisms. Advancements in enumeration of CTCs may reveal new insights into the impact of CTC burden on early detection, monitoring, and metastasis. Organoids can model cancer tissues to investigate growth, cellular structure, and microenvironment. Spheroids may be developed to mimic cell behavior for drug screening and resistance.

Applications in cancer research:

Enumeration and molecular profiling of circulating tumor cells for diagnosis and therapeutic monitoring of metastatic cancer



[Learn more about Dr. Pravin D. Potdar's methods] for the enumeration and isolation of CTCs as well as the significance of molecular profiling of CTCs for the study and monitoring of metastatic cancers.

Dr. Pravin Potdar is a founding member and former Vice President of the Molecular Pathology Association of India (MPAI) and has served as Faculty and Professor of Genetics and Stem Cell Biology at Dr. APJ. Abdul Kalam Education and Research Centre, Mumbai. Dr. Potdar is a former Head and Chief of the Department of Molecular Medicine and Biology at Jaslok Hospital and Research Centre, Mumbai, India, where he established a Molecular Diagnostics and Stem Cell Research laboratory and carried out research programs in the fields of cancer, neurological and genetic disorders, infectious diseases, diabetes mellitus, and stem cells. With over 30 years of scientific research experience and more than 93 published papers in cancer and stem cell research, Dr. Potdar's work includes developing several mesenchymal and hematopoietic stem cell lines from various normal and tumor tissues, adipose tissue, human placental membrane, dental pulp cells, and blood cells.

Applications in cancer research:

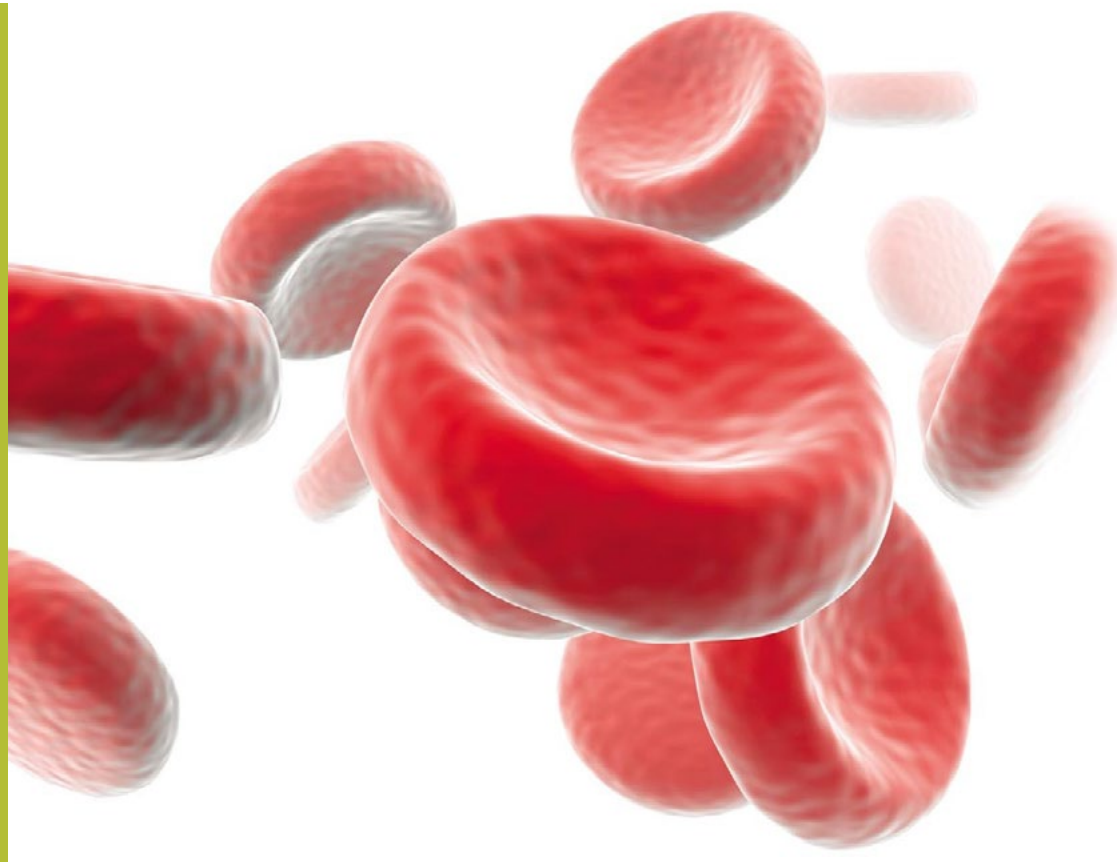
RNA Isolation from organoids and spheroids

Gain insights from scientists who share their expertise on RNA extraction methods and workflows for 3D cell culture, with a special focus on cancer applications.



Organoids and spheroids have become valuable tools in various research areas, such as drug discovery, toxicology, disease modeling, and regenerative medicine, as they provide a more accurate representation of complex biology compared to 2D models. In this webinar, Thermo Fisher scientists Laura Chapman, Anupriya Gupta, and Jay Bhandari share their expertise on RNA extraction methods and workflows for 3D cell culture, with an emphasis on cancer applications.

Innovations in blood cancer research



Types of blood cancer

Cancer arises from tissues, however, when that tissue is bone marrow, abnormalities in the production and function of blood cells and their components can result in hematologic cancers such as leukemia, lymphoma, and myeloma. **Hematologic cancer research** is a dynamic and rapidly evolving field focused on understanding and developing effective treatments for blood cancers. Hematological cancers, including leukemia, lymphoma, and multiple myeloma, arise from abnormalities in the production and function of blood cells and their components.

There are three primary classes of hematologic malignancies:[1]

- **Leukemia** is characterized by the abnormal production of white blood cells in the bone marrow.
- **Lymphoma** starts in the lymphatic system.
- **Multiple myeloma** is a cancer of plasma cells.

Each of these blood cancers can further be categorized into various subtypes based on specific characteristics and cell types involved.

The importance of hematologic cancer research

Hematological cancer research aims to advance understanding of the genetic, molecular, and cellular mechanisms underlying these diseases. Through innovative research methods and therapeutic approaches, researchers aim to improve patient outcomes, enhance early detection strategies, and ultimately, find cures for these complex and challenging malignancies. Here, we explore recent advancements that are transforming hematological cancer research, highlighting the importance of early detection and the innovative approaches being employed with the potential for better treatment outcomes in the future. Tailored targeting of the unique characteristics and mechanisms of each type of blood cancer may allow for more effective, precise, and personalized treatment while minimizing harm to healthy cells, ultimately helping lead to better outcomes and reduced side effects.

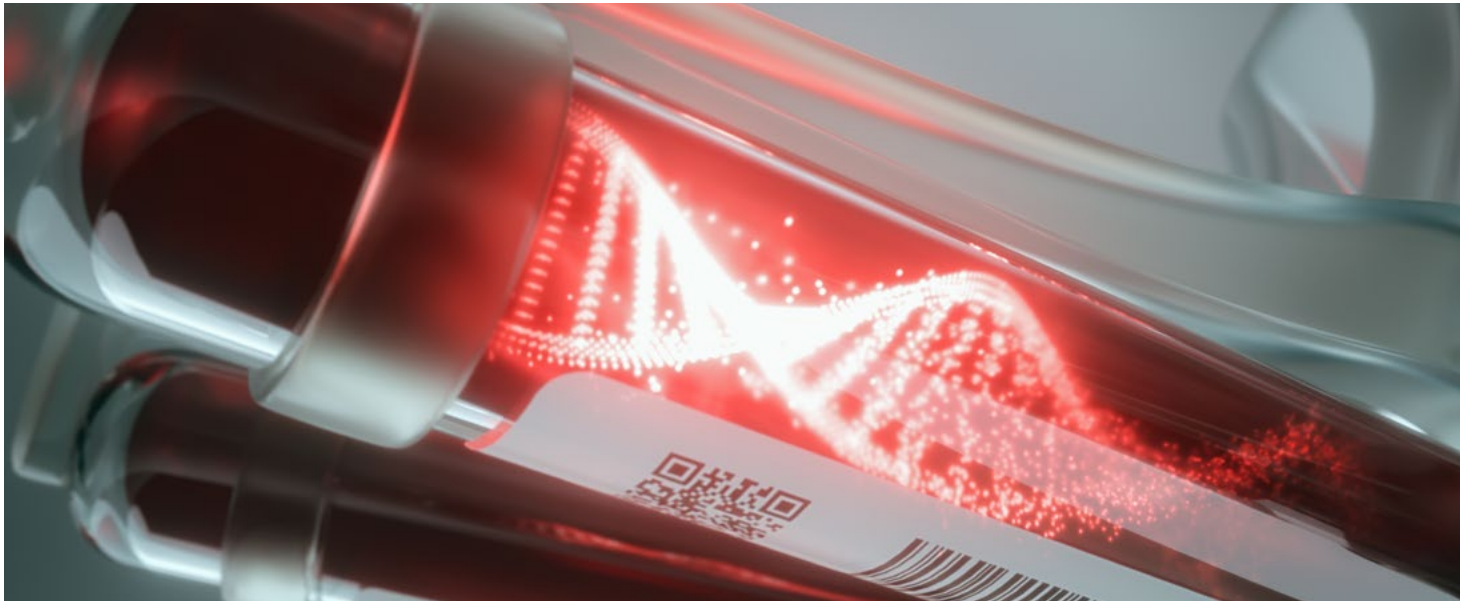
Early detection of cancer using blood cells

Early detection of hematological malignancies can play a pivotal role in helping improve patient outcomes.[2] Identification at an early stage can enable timely intervention, which may lead to more effective treatment strategies and potentially improved outcomes. Research efforts are underway to develop innovative techniques for early detection, including **molecular profiling**, **liquid biopsies**, and advanced analytical technologies.

Molecular profiling and antigen testing

Molecular profiling techniques have dramatically changed hematological cancer research with new approaches to unraveling the genetic complexity of these diseases. Researchers utilize advanced genomic technologies, such as **NGS**, to identify specific genetic alterations and mutations associated with different types of hematological cancers.[3] These genetic biomarker signatures can provide valuable insights into disease classification, prognostication, and potential therapeutic targets. By advancing understanding of the genetic abnormalities driving hematological cancers, researchers may be able to develop targeted therapies and personalized treatment approaches to enable potentially improved patient outcomes.

A comprehensive profile at the molecular level can be pivotal in understanding and detecting genetic alterations that may be relevant to these cancer types or transplant needs. Molecular profiling from hematological samples requires high quality DNA and RNA isolated from the



origin sample. Innovative approaches to the way researchers can obtain these nucleic acids are coming to light. Sequential DNA/RNA isolation with the **Applied Biosystems™ MagMAX™ Sequential DNA/RNA Kit** provides the ability to isolate these analytes sequentially with a single sample utilizing automated protocols on KingFisher automated purification systems.

Antigen tests are important tools in the cancer research field as they offer insights into the growth and monitoring of cancer and, potentially, its treatment. Human leukocyte antigen (HLA) typing is a blood antigen test that identifies antigens on the surface of cells and tissues. The HLA genes are located in the major histocompatibility complex, which is a DNA locus that codes for cell surface proteins that are essential in the process of binding antigenic peptides during the immune response.[4] These genes are considered the most polymorphic genetic system in humans, with more than 35 thousand alleles described, and high levels of diversity inside and between human populations. HLA typing has hence become an important test for stem cell and solid organ transplantation, various disease associations, and pharmacogenetics to screen for drug hypersensitivity.

NGS technologies have transformed the HLA typing field by generating high-resolution data and resolving most allelic ambiguities without multiple reflexive tests. High-performance NGS HLA assays demand high-quality genomic DNA from biological samples such as peripheral blood. Automated DNA extraction using the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Apex system can offer the consistency needed to generate high-quality genomic DNA, suitable for downstream HLA typing through downstream sequencing analysis.

The impact of biomarkers in liquid biopsies

The discovery and validation of protein or genetic biomarkers within blood samples have had a significant impact on the advancement of hematological cancer studies. For hematological cancers in particular, liquid biopsies to detect biomarkers in blood and other bodily fluids have provided a powerful, less invasive, and real-time approach for monitoring disease progression, treatment response, and minimal residual disease.[5] Researchers are continuously exploring the potential of liquid biopsies and biomarker studies as research tools for early detection, disease monitoring, and the identification of therapeutic targets for hematologic diseases.

Hematological based disease modeling and preclinical research

Disease modeling and preclinical research are vital components of hematological cancer investigation. Researchers mimic the biological characteristics of hematological cancers using cell lines, animal models, and three-dimensional culture systems.[6] These models enable the investigation of disease mechanisms, drug efficacy, and resistance mechanisms, helping to provide a platform for the development and testing of novel therapeutic strategies. By studying the interactions between cancer cells and the microenvironment, researchers can uncover new insights into disease progression and identify potential targets for intervention.

Paving the way to a cure

Research on hematological cancers is uniting with genetic research in new ways, yielding advancements in understanding the genetic and molecular mechanisms underlying these diseases. Innovations across workflow applications may help contribute to expanding knowledge of the intricacies of these diseases that may offer researchers approaches to develop targeted therapies, personalized treatment approaches, and innovative diagnostic methods. Through ongoing research efforts, hematological cancer research strives to improve patient outcomes, enhance early detection strategies, and ultimately, find cures for these complex malignancies.

Related resources

Application note: Genomic DNA extraction from bone marrow aspirates and peripheral blood mononuclear cells

Blog: Sample types in genomics and oncology research: capability advancements in automation

Application note: Versatile solutions for human leukocyte antigen testing with peripheral blood

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Hematologic cancer

Blood cancer research:

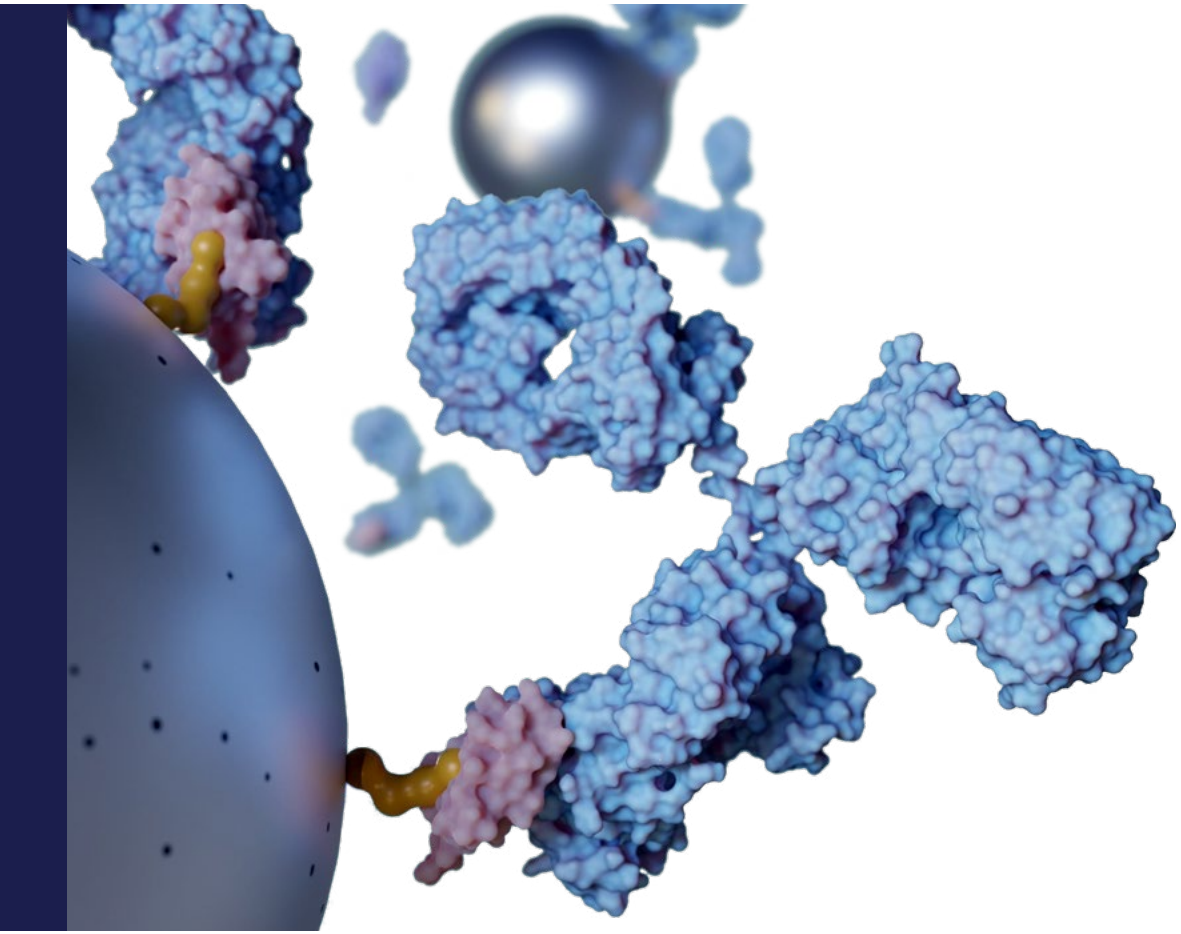
Versatile solutions for human leukocyte antigen testing with peripheral blood

[Watch our on-demand webinar.](#) Discover how next-generation sequencing (NGS) technologies are transforming HLA typing.

Next-generation sequencing (NGS) technologies transformed HLA typing research by generating high-resolution data and resolving most allelic ambiguities without multiple reflexive tests. High-performance NGS HLA assays demand high-quality genomic DNA from biological samples such as peripheral blood. Watch this on-demand webinar in which Thermo Fisher Scientific scientists and application specialists present the performance of 96 DNA samples extracted from whole blood using the Applied Biosystems™ MagMAX™ magnetic bead-based kit on the Thermo Scientific™ KingFisher™ Flex system for high-resolution HLA typing with the One Lambda™ AllType™ and AllType FASTplex™ NGS kits. This webinar showcases how high-quality DNA positively impacts the HLA typing process and highlights the crucial importance of quality checks on the NGS workflow.

Sample prep

Sample integrity and purity are the keys to confidence in cancer research



Complete sample preparation workflow solutions

As cancer research evolves, tissues, cells, and body fluids are all becoming valuable sources for new insights into the mechanisms of cancer and pathways to potential treatments. Cell-free nucleic acids (cfNAs), CTCs, and exosomes extracted from solid tumor, liquid biopsies, or hematologic samples can hold the keys to helping us understand cancer biology. Analyzing these target biomarkers allows researchers to identify mutations in cancer-associated genes, explore mechanisms of cancer cell function, investigate response to therapies, and identify cancer-associated biomarkers all with the objective of enabling early detection, more informative monitoring, prediction of therapeutic response, and more personalized treatments.

There is a need for streamlined workflows that offer the potential lifespan monitoring of different cancer types, biomarker discovery, and ultimately health status. Versatile and automated sample purification instrumentation, along with optimized kits and reagents can help advance these objectives.

Extracting high-quality nucleic acids from FFPE samples

Although FFPE is excellent for preserving solid tumor tissue morphology, it can be damaging to some biomolecules, diminish nucleic acid integrity, and impede genetic analysis. Nucleic acid extraction from FFPE tissues, which minimizes the impact of degraded samples and preserves the quality of the tissue, can help ensure the success of downstream molecular assays including NGS, RT-qPCR, and gene expression profiling. The AutoLyS M Tube system and **MagMAX FFPE DNA/RNA Ultra Kit** can be combined to help provide a streamlined workflow for high-yield, high-quality nucleic acid isolation from FFPE tissues. The AutoLyS Tube system yields cleared lysates from FFPE tissues without the need for deparaffinization or organic solvents. The MagMAX FFPE DNA/RNA Ultra Kit is designed for simplified, sequential or total isolation of DNA and RNA separately from a single tissue sample. The resulting DNA and RNA both are compatible with a broad range of genetic analysis applications. MagMAX FFPE kits are also scalable for high-throughput or automated needs.

Isolating fragmented DNA

Efficient recovery and isolation of circulating nucleic acid species can be fundamental to preventing invasive sample collection from small tumors or fragile patients. However, isolation can be particularly challenging because peripheral blood also contains circulating DNA derived from normal cells. Thermo Fisher Scientific provides a complete, highly efficient workflow to isolate, purify, and characterize cfDNA even in the presence of normal cell DNA. First, cfDNA is isolated from the relevant biological fluid using the **Applied Biosystems™ MagMAX™ Cell-Free DNA Isolation Kit** either manually with a magnetic stand or automatically on a KingFisher automated purification system. Isolated fragments can ultimately be analyzed through use of **Ion Torrent™ Oncomine™ Cell-free assays** and **Ion Torrent™ Ion GeneStudio™ S5 systems** to enable multiplexed targeted sequencing for highly accurate molecular characterization.

Isolating CTCs

Circulating tumor cells (CTCs) are gaining importance as prognostic markers and for research into the monitoring of treatment response. Because of the low number of CTCs in circulation, highly sensitive methods are necessary to capture and detect down to single cells. Dynabeads magnetic beads provide an automation-friendly tool for isolation of circulating biomarkers. Positive isolation can be utilized to separate CTCs expressing cancer-specific markers, whereas depletion can be utilized to deplete leukocytes from blood samples for marker-independent CTC enrichment, leaving the target cells untouched.

In cancer research, the depletion of CD45-positive cells is commonly employed to enrich for CTCs from liquid biopsy samples such as PBMC and whole blood. This allows for the acquisition of untouched and viable CTCs, facilitating subsequent detection and quantification studies. **Dynabeads MyOne CD45 Leukocyte Depletion Beads** are uniform, superparamagnetic beads with a primary monoclonal mouse IgG2a antibody specific for all known isoforms of CD45, a membrane glycoprotein found on all human leukocytes. The product can be used to deplete CD45-positive cells easily and efficiently from viscous samples such as whole blood, in approximately 30 minutes.

For positive cell isolation, Dynabeads magnetic beads coupled to monoclonal antibodies targeting EpCAM can be used. **Dynabeads Epithelial Enrich Beads** allow for high priority isolation of viable cells. **CELLection Epithelial Enrich Beads** provide a positive cell isolation option that releases cells after cell capture to yield pure and viable cells that can be used in any downstream application. Alternatively, there are options to take a more customizable approach and couple other cancer-specific biotinylated antibodies to **Dynabeads Streptavidin magnetic beads for protein and cell capture.**

Enrichment of circulating CTCs is a time-consuming process if working with many samples in parallel and can lead to increased manual handling errors. By utilizing the **KingFisher Sample Purification Systems** with 96 deep-well plates, you can achieve high-throughput recovery of untouched CTCs in approximately 50 minutes after loading the sample and reagents into the plates. The resulting CTCs are viable and suitable for culture, downstream molecular analysis, or proteomic analysis.

Isolation of extracellular vesicles and exosomes

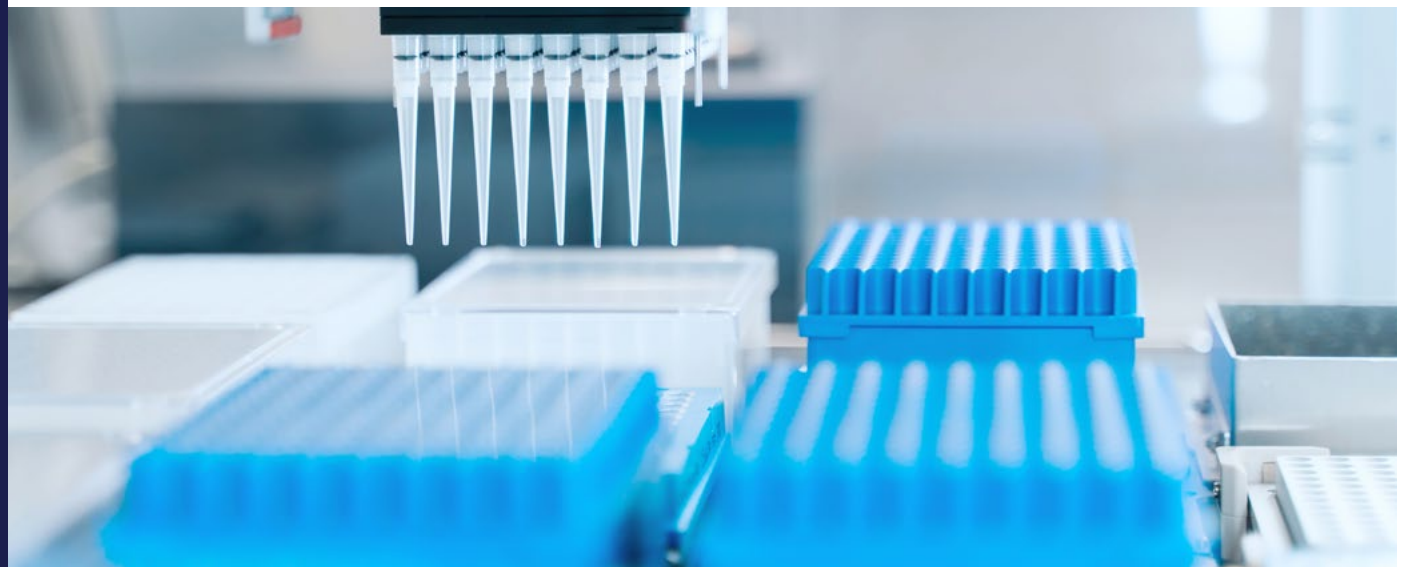
Ultracentrifugation is a conventional method of **exosome isolation**. However, this approach can be time-consuming and damaging to the exosomes. A faster approach to exosome separation from cells and heavy artifacts is to use polymer precipitation to sequester water molecules. This process reduces exosomes solubility, causing their precipitation. The exosomes are then harvested using low-speed centrifugation. Invitrogen™ Total Exosome Isolation reagents and kits leverage this method to offer a simplistic and fast isolation solution that is tailored to specific liquid biopsy sample types.

An alternative and highly specific approach to exosome isolation is immunomagnetic separation using Dynabeads magnetic beads. Immunomagnetic separation is well suited for purifying exosome subpopulations from liquid biopsy samples. Exosome-specific antibodies are bound to the surface of these beads for a more efficient purification of exosomes. Dynabeads come pre-coupled with anti-CD9, CD63, CD81, or EpCAM for subpopulation isolation. Invitrogen™ Exosome-Streptavidin Isolation/Detection Reagent is an alternative option that can be coupled with a biotinylated primary antibody for more flexibility. DynaGreen CaptureSelect Anti-IgG-Fc (Multi-Species) Magnetic Beads provide the flexibility to couple exosome specific antibodies while enabling high throughput, rapid, hands-off isolation through KingFisher automated purification protocols. For a more generic exosome isolation approach using magnetic bead separation, Dynabeads Intact Virus Enrichment beads also offer a generic, yet rapid, isolation solution.

This is possible due to the size and charge of exosomes, which is very similar to that of viruses. This short and simple exosome enrichment method can be further simplified using KingFisher instruments as well.

Versatile, automated isolation

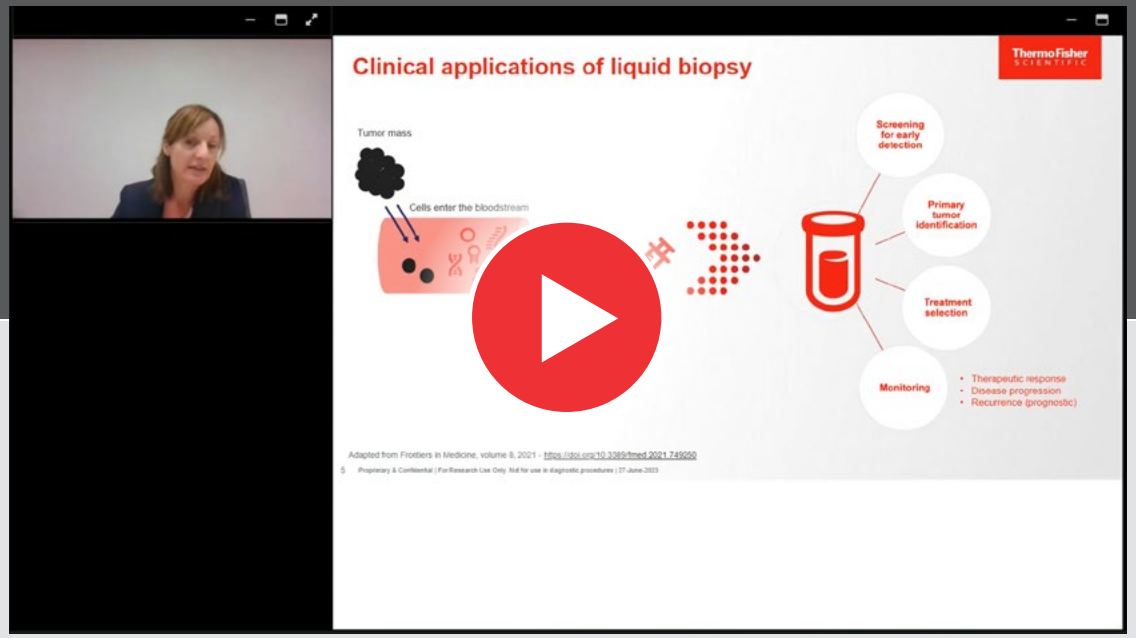
Regardless of your target analyte, **KingFisher automated purification systems** coupled with **MagMAX isolation kits** and **Dynabeads magnetic beads** can support. Through versatile functionality in automating the isolation of DNA, RNA, proteins, exosomes, and cells, these tools provide an efficient sample purification method that helps maximize productivity in labs. High yield and quality purified analytes can be obtained allowing for the reproducibility needed for cancer research workflows and downstream translational research applications.



Liquid biopsy

Cancer research:

Advancing cancer research tools for liquid biopsy



Enhance your understanding of liquid biopsy workflows and assays essential in the sample preparation process.

Learn more about critical steps in the liquid biopsy workflow as Dr. Laure Jobert discusses the essential methods for biomarker enrichment in the sample preparation process. Thermo Fisher Scientific is dedicated to advancing liquid biopsy-based assays, including the use of Dynabeads magnetic beads, to enrich and analyze biomarkers such as circulating tumor cells (CTCs), extracellular vesicles, and circulating tumor DNA (ctDNA).

Ready to gear up for your cancer research?

Take our **sample purification solutions quiz** to uncover the recommended sample prep solutions that fit your needs.

Your cancer research begins here. Discover **end-to-end cancer research workflows**.

Learn more about liquid biopsy ultra-low mutation detection solutions from prep to analysis: **Accelerating the future of liquid biopsy ultra-low mutation detection solutions from sample prep to data analysis**.

Learn more at **thermofisher.com/cancerworkflows**