

UNDERSTANDING CANCER WITH PRECISION GENOMICS

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INCORPORATING GENOMICS IN PERSONALIZED CANCER MEDICINE

Adenocarcinomas are cancers that develop in the glands lining different organs and include breast, stomach, prostate, lung, pancreatic, and colorectal cancers.¹ Cancer epidemiology suggests that adenocarcinomas have familial origins. For example, family history serves as a high-risk factor for prostate, breast, and colon cancers, where heritable factors account for 42 percent of prostate cancer, 27 percent of breast cancer, and 35 percent of colon cancer cases. These estimates include highly heritable Mendelian disorders such as *BRCA1*- and *BRCA2*-associated breast cancers and *FAP*-associated colon cancer, as well as several genetically complex cancers.^{1,2}

Cancer Biomarkers

Many genetic factors serve as cancer biomarkers, including oncogenes and tumor suppressor genes. Oncogenes control cell growth and proliferation, whereas tumor suppressor genes regulate terminal differentiation and apoptosis. In addition, there are tumor-specific biomarkers that are upregulated or downregulated in specific tumor subtypes. Mutations in any of these genes lead to tumorigenesis. Several molecular diagnostic techniques help researchers identify and discover new cancer biomarkers that facilitate early disease diagnosis. Additionally, biomarker identification leads to improved treatment efficacy and allows researchers to classify and stage complex tumors.²

Biomarker Discovery Techniques

Microarrays, quantitative PCR (qPCR), digital PCR (dPCR), and capillary elec-

trophoresis (CE) are common methods for cancer diagnosis and biomarker discovery, determining gene expression patterns that vary between normal and cancerous cells. DNA microarrays allow researchers to examine the expression of thousands of genes at once. Based on differential gene expression patterns, researchers identify causative or disease-associated genetic variants and classify cancer subtypes.³ This information is crucial for physicians when determining the appropriate treatment and predicting its success in cancer patients. Additionally, microarrays are cost-effective tools in research for developing personalized patient care. For example, increased estrogen receptor gene expression could improve the therapeutic response of breast cancer treatments that target those receptors. Microarray profiling of the estrogen receptor gene informs doctors whether to choose chemotherapy over surgery for breast cancer patients.⁴

Like microarrays, amplification-based methods such as qPCR and dPCR also analyze multiple genes simultaneously in a single reaction. These techniques are important in clinics because scientists can analyze several gene targets at once from biopsy tissues, lowering reagent cost and preserving precious tissue samples for follow-up microscopic assessments.⁵

CE is another advantageous cancer diagnostic tool as it collects nucleic acids from small sample volumes and separates them with polyacrylamide gel electrophoresis.⁶ Coupled with other detection techniques, researchers use CE to characterize DNA and RNA molecules from tumors. For example, hereditary cancers often possess splicing abnormalities in susceptibil-

ity genes, such as the splicing errors common in *BRCA2*-associated breast cancer.^{6,7} Using CE, researchers detect small variations in nucleotide sequences and identify rare cancer-causing isoforms.

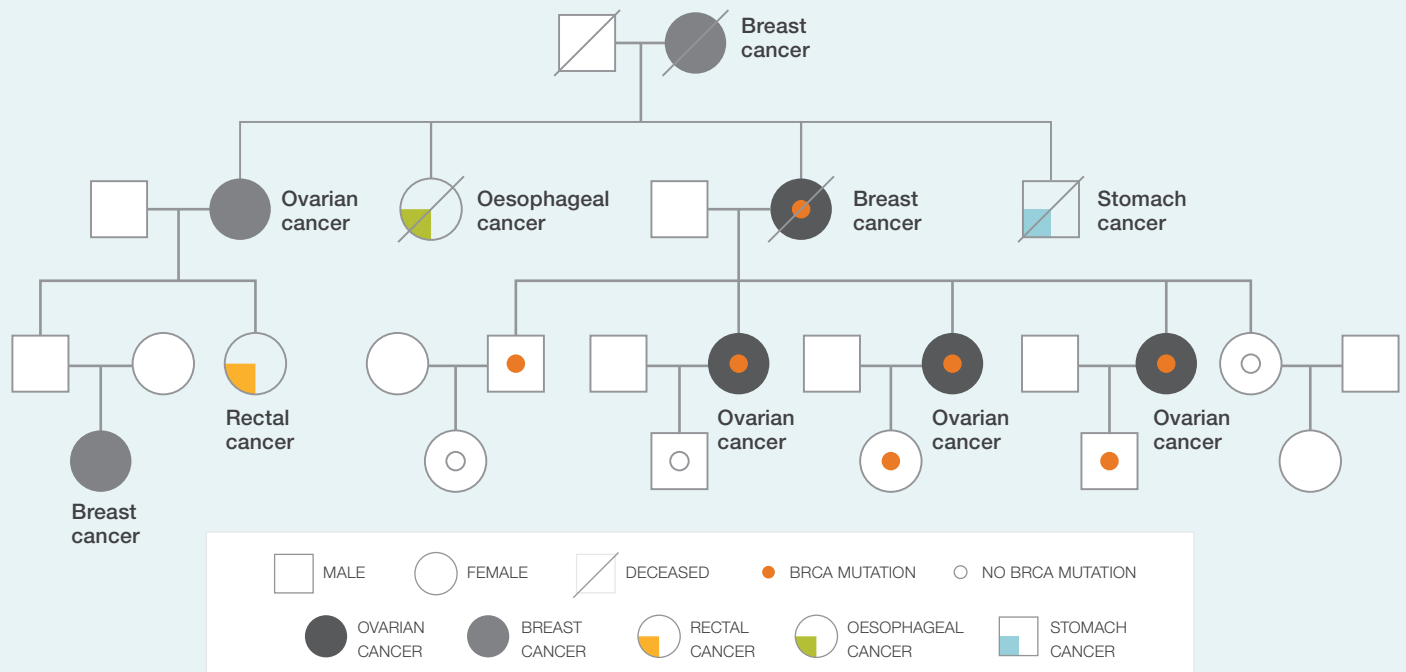
Traditionally, cancer researchers use these methodologies to tease apart tissue biopsy samples and understand the tumor landscape. However, tumors have molecular and cellular heterogeneity, and tissue biopsies, which are usually difficult to obtain, do not capture cancer's complexity. Therefore, researchers recently began to perform genomic analyses to profile circulating DNA in the blood of patients with cancer. Combining NGS with dPCR, liquid biopsy extends cancer research towards identifying cancer at early stages and predicting successful treatment plans.

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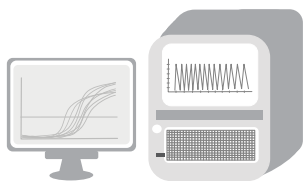
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Defeating Cancer with Molecular Approaches

Precision genomics is a powerful tool in cancer clinics. Many adenocarcinomas have a hereditary component, allowing researchers to determine causative gene variants and assess risk in family members. For instance, the pedigree below shows a family history of adenocarcinomas. With the following genetic screening methods, researchers identified a highly pathogenic *BRCA1* variant in this family causing breast, stomach, and lung cancers.¹



Adapted from Li et al.¹



Quantitative PCR (qPCR)

qPCR quantifies target gene expression, including low-expression mRNA species, allowing researchers to characterize individual tumors.



Microarray

Microarray panels measure thousands of transcripts from a sample, offering a genome-wide view of gene expression patterns in healthy versus cancer cells.



Digital PCR (dPCR)

Digital PCR accelerates routine cancer diagnostics by detecting and quantifying trace amounts of cell-free DNA from blood with precision.



Capillary Electrophoresis (CE)

CE detects nucleotide changes, enabling cancer-causing variant characterization.

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DECODING CANCER SIGNATURES FROM EXTRACELLULAR VESICLES

Rodney Ouellette, MD, PhD

Scientific Director, Atlantic Cancer Research Institute

Associate Professor, Université de Moncton



Cancer cells produce vesicles that include chemical signatures such as DNA, RNA, microRNA, proteins, and metabolites. Researchers develop methodologies to isolate these vesicles circulating in the bloodstream and characterize them with genomic methodologies. Cancer researcher Rodney Ouellette interrogates the communication between cancer and immune cells by decoding messages present in such vesicles.

Why do you study extracellular vesicles?

Extracellular vesicles (EVs) are extremely heterogeneous. If you sequence a single EV, you would only get a fraction of what the host cell contains. However, if a cancer cell produces thousands of them, there is a high likelihood of the entire cell represented in all of its EVs. We study EVs that contain information about the parental cells in lung cancer, triple-negative breast cancer, and glioblastomas. If one is looking for a cancer-specific gene mutation or a cancer-associated microRNA or protein, there is a very good chance of finding it in the EV. Moreover, vesicles from cancer cells interact with healthy cells, allowing us to investigate how they interact with immune cells.

We first capture these vesicles from a patient's liquid biopsy, which is a blood or urine test providing a full picture of tumor heterogeneity. We are interested in how liquid biopsy can help predict treatment response, particularly for patients receiving immuno-oncology drugs, where we see clear differences between patients that respond to the drug versus non-responders. While successful predictions

help us avoid side effects in the drug-responder patient cohort, we work on developing new therapies for non-responders.

Do normal cells produce extracellular vesicles?

All cells have the ability to produce EVs. You can isolate EVs from a healthy person's blood indicating they have a normal physiological function. In a healthy person, the EV amount is low. When we look at cancer patients' plasma or blood, we see a dramatic increase in EV numbers, and the proportion of EVs that come

but not in host cells. It seems that the cell is efficiently pumping out certain factors it does not want. We also observed that an EV's constituents have effects on the immune system either through molecules like PDL-1, immunoglobulins, or T cell receptors. Therefore, we believe they are potentially interacting and disrupting the immune system.

Which methods help you screen and characterize EVs?

When you work with EVs, it is almost like working with single cells. We do not

“When we look at cancer patients' plasma or blood, we see a dramatic increase in EV numbers, and the proportion of EVs that come from cancer compared to the healthy cells is also much higher.”

—Rodney Ouellette, Atlantic Cancer Research Institute

from cancer compared to the healthy cells is also much higher. Hence, cancers have turned the EV mechanism into overdrive. Some research suggests they are sending messages to the immune system. We believe that EVs are making the immune system less responsive and unable to mount a defense against cancer.

Do EVs have an active role in cancer progression?

It is a complex question and there are many theories around this. We know that sometimes a cell wants to get rid of material that may be preventing it from proliferating rapidly or to avoid apoptosis. We often observe that microRNAs and other regulatory molecules are present in EVs

know how much gene levels vary between EVs and among EVs from different cells. Therefore, we employ sequencing. Over the past few years, we developed methodologies that allowed us to compare patient samples. We mostly obtain extremely low amounts of RNA and DNA as a starting material, therefore, we developed PCR amplification methods to increase the yield. We also employ next-generation sequencing for transcriptomic analysis. We obtain 5-7 nanograms of starting material which is too low for sequencing, but our new protocols help us improve the detection limit. We often use digital PCR to validate sequencing results.

This interview has been edited and condensed for clarity.

CIRCULATING TUMOR DNA PREDICTS PATIENT SURVIVAL IN LUNG CANCER

Atocha Romero, PhD

Director, Liquid Biopsy Unit

Department of Medical Oncology

Hospital Universitario Puerta de Hierro-Majadahonda



Cancer cells either release nucleic acids freely or in encased membrane vesicles in the blood. This circulating tumor DNA is a gold mine for researchers hunting for tumor biomarkers. Liquid biopsy expert Atocha Romero uses molecular profiling techniques to characterize circulating tumor DNA to predict lung cancer treatment success and patient survival.

Why do you incorporate liquid biopsy in your research?

The incidence and mortality of lung cancer is very high. We need to test many biomarkers because most lung cancer therapies are targeted against specific molecules. It is critical to analyze as many biomarkers as possible. Without known biomarkers, the patient might not receive the best treatment for their tumor. Lung cancer tumors are usually difficult to access due to the anatomy of the lung. Most of these tumors are often diagnosed in patients in their 60s, making it difficult to obtain biopsy material

for biomarker analysis because invasive procedures might not be an option in elderly people. Therefore, we focus on liquid biopsies.

I run the liquid biopsy lab, and the main focus of our research is to evaluate how liquid biopsy can improve the diagnosis and management of lung cancer patients. There is a lot of evidence that the amount of circulating tumor DNA correlates well with tumor bulk sequencing data, allowing us to use it as a surrogate for disease monitoring. By analyzing circulating tumor DNA, we can anticipate tumor progression diagnosed on CT scans.

What information do you get from liquid biopsies?

We sequence circulating tumor DNA and we developed an internal data analysis pipeline to filter out germline and clonal hematopoiesis-derived mutations. By calculating the sum of all the mutant allele frequencies from the detected mutations, we determine the amount of ctDNA in blood.

How does circulating tumor DNA correlate with tumor growth?

I work with Mariano Provencio, the president of the Spanish Lung Cancer Group, who led a clinical trial that yielded excellent results in terms of survival in locally advanced cancer patients with operable tumors. This NADIM trial¹ evaluated the efficacy of a combination of immunotherapy and chemotherapy in the neoadjuvant setting.

Our team evaluated the utility of circulating tumor DNA in this NADIM trial. Imaging techniques are limited in their ability to evaluate tumor response to immunotherapy treatment because the presence of inflammation confounds the results. Even if tumor growth is not present, inflammation surrounding the tumor might give a false impression. Therefore, it is difficult to assess the clinical response based on imaging in immunotherapy-based treatments. In the context of NADIM trial, we found that non-detectable levels of circulating tumor DNA after neoadjuvant treatment were significantly associated with improved survival. Indeed, ctDNA levels more accurately predicted long-term survival than radiologic assessments in the NADIM study. Based on this analysis, we conclude that circulating tumor DNA offers an accurate measure of tumor response to treatment, being an early surrogate of patients' survival that could be used as a trial endpoint in the neoadjuvant setting.

This interview has been edited and condensed for clarity.

Reference

- 1) M. Provencio et al., "Overall survival and biomarker analysis of neoadjuvant nivolumab plus chemotherapy in operable stage IIIA non-small-cell lung cancer (NADIM phase II trial)," *J Clin Oncol*, 40(25):2924-33, 2022.



Atocha Romero from Hospital Universitario Puerta de Hierro-Majadahonda using QuantStudio Absolute Q Digital PCR System in liquid biopsy for potential cancer management and detection.

GENETIC ANALYSIS TECHNOLOGIES TO SUPPORT CANCER RESEARCH

Cancer is a disease mediated by genetic alterations, and as such, tools that can analyze the genome are instrumental in understanding the mechanisms that lead to cancer, detecting cancerous cells, and providing avenues for research on cancer treatments. The Human Genome Project resulted in the development of many tools that generated sequence information and used that sequence information to understand human health and disease, including cancer. Thermo Fisher Scientific has been at the forefront of the development of these tools and offers a broad portfolio of solutions in cancer research.

In this section, we give a brief overview of the technologies in our continuum and how they could be used for cancer research. The flexibility of these solutions is vast, and can be easily adapted to investigate the research questions being asked.

Microarray methods and tools

When a normal cell goes down a path that ultimately ends in a cancerous cell, it acquires many mutations that may range from single-nucleotide changes and small indels to copy number changes and large chromosomal rearrangements. One way to characterize these genomic changes is to use high-density DNA microarrays. For these analyses, hundreds of thousands of probes tiled across the genome are arrayed onto a single chip. Sample hybridization to these arrays can determine which sequences are present and in how many copies (Figure 1). The advantage of microarrays is that hundreds of thousands to millions of sequences can be interrogated in a single experiment. If the appropriate probes that detect specific mutations are present on the chip, they can also detect common SNP

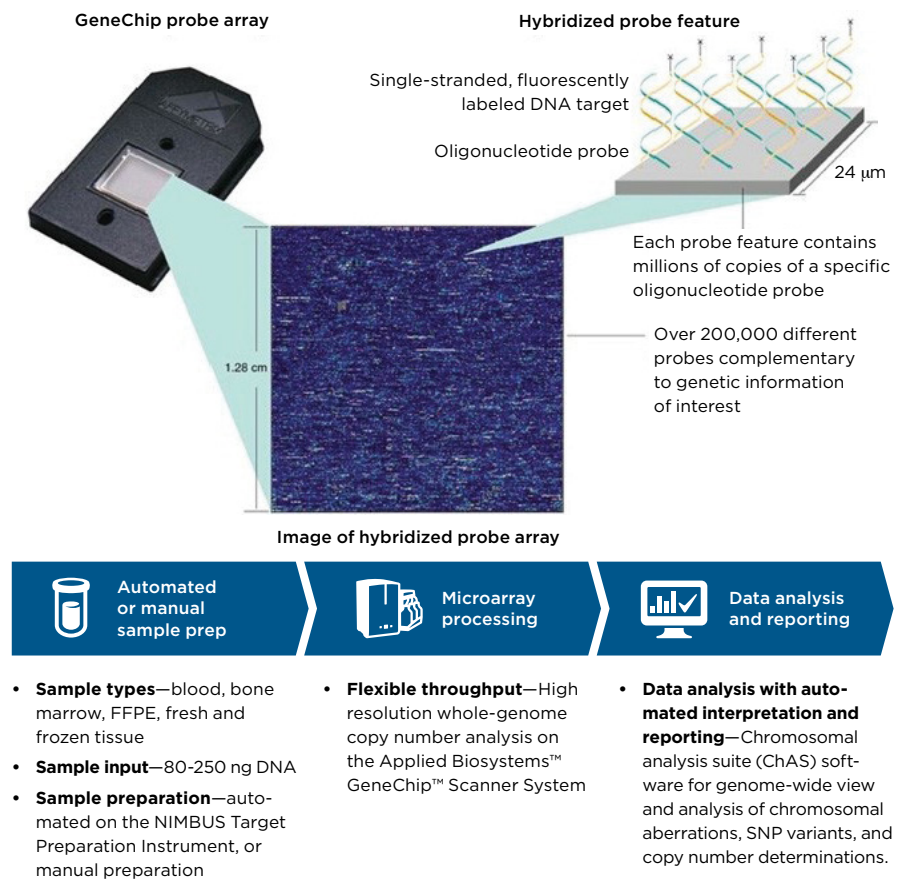


Figure 1. Basics of a microarray assay workflow.

variants. Both copy number aberrations (CNAs) and somatic mutations are important drivers of hematological malignancies. Cytogenetic investigation of these malignancies has become an integral part of disease evaluation, prognosis, and prediction of response to therapy. Current analysis of hematological malignancies involves multiple sequential tests and laborious workflows. However, implementation of high-resolution copy number microarrays in research laboratories has created an unprecedented opportunity to profile multiple relevant driver events

in hematological malignancy samples. Whole-genome microarrays that cover both polymorphic (e.g., single-nucleotide polymorphisms, or SNPs) and non-polymorphic regions of the genome are called Hybrid-SNP arrays and can be used to assess DNA copy number alterations at much higher resolution than with conventional cytogenetic analyses.

The Applied Biosystems™ CytoScan™ HD Suite—comprising includes hybrid-SNP arrays, automated and manual target preparation options, fully kitted reagents, instrument for array processing, and genetic analysis software

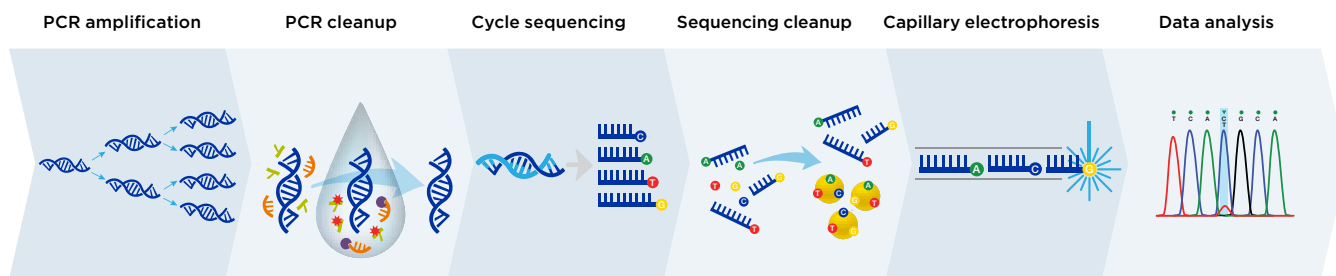


Figure 2. Basics of a Sanger sequencing workflow. The target region that will be sequenced is amplified by PCR. The primers and PCR reagents are removed before a second linear amplification is performed. This step generates the fragments that are chain-terminated with a fluorescent dideoxynucleotide. The cycle sequencing reaction is purified, and the resulting fragments are separated by capillary electrophoresis and detected with a laser. The sequence can be read from the lengths of the fragments and the colors of the dideoxynucleotide terminators.

is a comprehensive, high-resolution, whole-genome solution designed to assist in the understanding and characterization of biomarkers in hematological malignancies. The CytoScan™ HD assay interrogates all relevant CNAs associated with lymphoid and myeloid disorders using a single microarray-based assay. The assay covers all the major lymphoid disorders associated with acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL), as well as myeloid disorders associated with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), and multiple myeloma (MM). In addition to superior performance, the CytoScan HD Suite does not require additional cell culture or cell arrest prior to karyotyping.

The Applied Biosystems™ OncoScan™ CNV Plus Assay is a microarray-based whole-genome copy number assay designed to query genomic DNA that has been degraded, such as DNA extracted from FFPE samples. It enables the detection of relevant copy number variants (CNVs) such as copy number gain and loss, LOH, cnLOH, ploidy, allele-specific changes, mosaicism, clonal heterogeneity, and chromothripsis. It also includes probes that can query a panel of driver somatic SNPs commonly found in solid tumors. Similarly, the Applied Biosystems™ OncoScan™ CNV Assay has the same copy number coverage as the OncoScan CNV Plus Assay, but does not include somatic SNP mutation probes.

Sanger sequencing methods and tools

Sanger sequencing is the trusted standard for obtaining DNA sequence information. It powered the Human Genome Project, and investigators continue to rely on this method to generate highly accurate reliable sequencing results. Sanger sequencing is a specialized form of fragment analysis—it relies on chain-terminating fluorescent nucleotides to generate a series of fragments that differ by one nucleotide (Figure 2). Thermo Fisher offers fast and straightforward Sanger sequencing workflows that provide a high degree of accuracy, long-read capabilities, and simple data analysis. Applied Biosystems™ BigDye™ Terminator v1.1 and Terminator v3.1 cycle-sequencing chemistries are the gold standard for Sanger sequencing by CE. After cycle sequencing, there are various options for cleanup before electrophoresis, including Applied Biosystems™ Centri-Sep™ purification columns and plates, ExoSAP-IT™ enzyme mix, and BigDye™ XTerminator™ kits. An entire sequencing workflow can be completed in a few hours with minimal hands-on time from sample to answer, providing the flexibility to support a diverse range of applications in many research areas.

Discovery-based genomic research, such as NGS, often uncovers novel or unexpected variants or other sequence anomalies. Investigators look for ways to verify these new discoveries using orthogonal methods. Sanger sequencing is the method of choice for confirming NGS

results because of its workflow simplicity and unambiguous results. For these confirmatory studies, short amplicons, usually covering only the region to be confirmed, need to be sequenced. Moreover, minor allelic variants, present in a heterogeneous sample, can be identified and confirmed by Sanger sequencing. Applied Biosystems™ Minor Variant Finder Software is easy-to-use desktop software designed for the accurate detection and reporting of minor variants in Sanger sequencing traces with a detection level of minor alleles as low as 5%. On a test set of 632,452 base positions, it exhibited a 5% limit of detection with 95.3% sensitivity and 99.83% specificity.¹ Minor Variant Finder Software can also readily align sequences with the human reference genome and VCF files from NGS experiments, providing a smooth workflow for NGS confirmation with annotations in the dbSNP database.

Digital PCR methods and tools

Digital PCR (dPCR) is a method that quantifies sequences present in a sample by counting the number of copies of the target sequence. The basis of dPCR is that a nucleic acid sample is physically compartmentalized into thousands of parallel PCR reactions, such that each reaction well contains one target molecule on average (Figure 3). In this scenario, some reactions may not contain the target molecule at all, while others will contain one or more copies. The collection of these compartmentalized reactions is subjected to endpoint PCR, and the number of wells with a positive signal and no

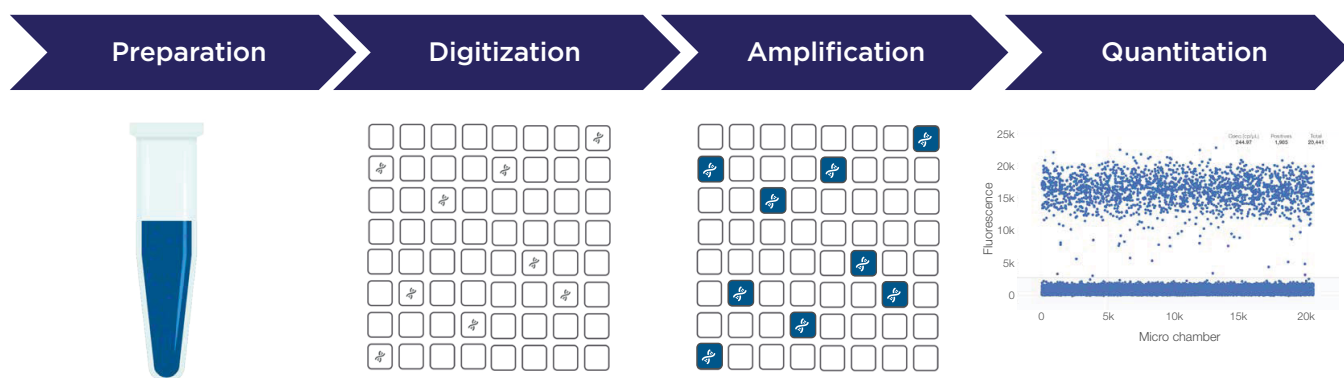


Figure 3. Basics of a digital PCR assay. dPCR reactions on the QuantStudio Absolute Q dPCR system make use of Absolute Q dPCR assays (TaqMan chemistry) to detect a target sequence. In dPCR assays, the sample is diluted and loaded into a matrix containing thousands of individual reaction chambers. If the dilution is correct, some of the chambers will have a target and some will not. Subjecting the chambers to PCR will cause a positive signal in the chambers where there is a target, but no signal where there is no target. The wells that are positive and negative are counted, and the starting concentration can be calculated based on dilution and other factors. Because the sample is loaded such that there is one molecule per microchamber, an overabundance of one sequence will not necessarily overwhelm detection in wells that have a rare sequence.

signal are tallied. The number of target copies is calculated from the fraction of negative reactions, based on the assumption that the segregation follows a Poisson distribution (thus accounting for the possibility that multiple target molecules occupy the same reaction). The number of individual reactions influences the sensitivity of the assay—the more reactions there are, the lower the limit of detection and the higher the accuracy.

dPCR is often used to detect rare mutant alleles in cancer samples and is used to analyze circulating tumor DNA (ctDNA) in liquid biopsy research. Because the entire sample is compartmentalized into individual wells, the detection of rare alleles is not masked by an overabundance of normal alleles. The sensitivity is dependent on the amount of DNA; to achieve a sensitivity of 0.1% (1 in 1,000 copies), 1,000 copies are needed. For diploid genomic DNA (gDNA), at least 6 ng of input gDNA is required for this sensitivity. Thus, the amount of recoverable DNA limits the sensitivity of the assay.

The Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System consolidates all workflow steps into a single plate, transforming a multi-step, multi-instrument workflow into a one-step qPCR-like workflow. Absolute Q™ Liquid Biopsy dPCR Assays on the QuantStudio Absolute Q dPCR sys-

tem detect and quantify the most common cancer-related mutations (e.g., *EGFR*, *BRAF*, *KRAS*, *PIK3CA*, *JAK2*), as well as therapy-resistant mutations (e.g., *EGFR T790M*). Absolute Q Liquid Biopsy dPCR Assays have been verified to detect allele frequencies as low as 0.1% and are guaranteed to perform on the QuantStudio Absolute Q dPCR system. The Absolute Q Liquid Biopsy dPCR Assays run on the QuantStudio Absolute Q Digital PCR System provides a precise, cost-effective, and rapid method for monitoring response and resistance to treatment by testing for relevant cancer-driver and therapy-resistant mutations. Absolute Q Liquid Biopsy Assays are also complementary to OncoPrint Liquid Biopsy NGS Assays when it is necessary to orthogonally validate mutations.

Conclusions and outlook

The Applied Biosystems brand continues to develop innovative tools that facilitate cancer research. For any of the problems spanning the genetic analysis continuum, from discovery-based whole-genome research, through focused research on distinct collection of sequences, to precise, sensitive detection of specific sequences, the Applied Biosystems™ portfolio has the solution that addresses any research needs. For more information, contact your sales representative.

Thermo Fisher Scientific is committed to providing cancer researchers with powerful genetic solutions that enable them to pursue the greatest expectations of precision cancer medicine, ultimately providing more personalized treatment strategies and better patient outcomes in the future.

Thermo Fisher can help you achieve your most exciting cancer research goals with a full range of cancer genomics and transcriptomics solutions.

- Uncover cryptic cancer heterogeneity by qPCR, dPCR, microarray or CE
- Extract precise data from limited and challenging samples such as FFPE tissue, precious solid tumor biopsy samples, or liquid biopsies, by microarray, dPCR or qPCR
- Obtain key insights from multiple perspectives provided by different technologies

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Analysis of Mutant Alleles in Liquid Biopsies: from Discovery to Monitoring

Dr. Stephen Jackson

Associate Director, Product Applications,
Thermo Fisher Scientific

The use of quantitative PCR & Digital PCR for human disease research has increased substantially. Get insights in this on-demand webinar on how these powerful technologies have propelled some key human disease research areas and opened opportunities for new applications in the research landscape.

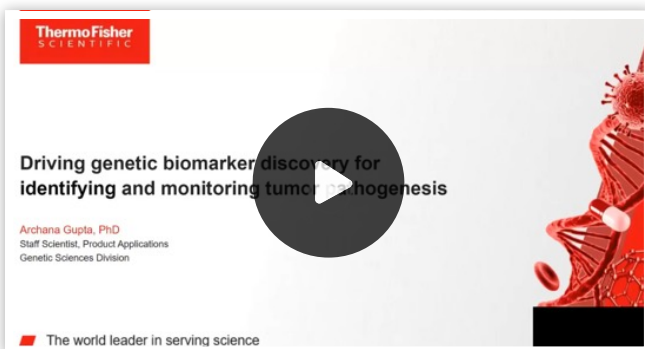


Analysis of Mutant Alleles in Liquid Biopsies: from Discovery to Monitoring

Dr. Atocha Romero

Head of the Liquid Biopsy Laboratory at Hospital
Universitario Puerta Hierro, Majadahonda, Spain

Dr. Atocha Romero from Hospital Universitario Puerta de Hierro-Majadahonda discusses her research into clinical utility of digital PCR in liquid biopsy for potential management and detection of cancer

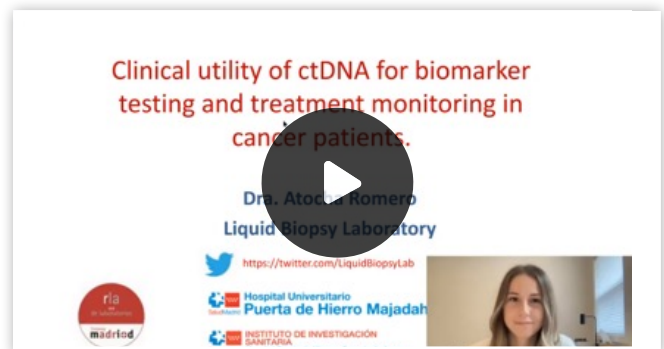


Driving Genetic Biomarker Discovery for Identifying and Monitoring Tumor Pathogenesis

Dr. Archana Gupta

Staff Scientist in the Genetic Sciences Division
- Thermo Fisher Scientific

Learn in this on-demand webinar on how genetic biomarkers have become increasingly important for disease prediction, early disease detection and progression, and options for intervention. Genetic markers such as microRNA, mRNAs and/or DNA either cell-free or packaged in circulating tumor cells (CTCs) and extracellular vesicles sourced from liquid biopsy provide the basis for biomarker research that contributes to precision medicine options for cancer.



Digital PCR (dPCR) in Liquid Biopsy for Management and Detection of Cancer

Dr. Atocha Romero

Head of the Liquid Biopsy Laboratory at Hospital
Universitario Puerta Hierro, Majadahonda, Spain

Learn in this on-demand webinar on the analysis of circulating tumor DNA (ctDNA) by dPCR, which has several applications:

- ctDNA genotyping can be used for non-invasive biomarker testing, which is especially relevant in testing lung cancer samples
- ctDNA levels correlate well with tumor bulk
- ctDNA levels are of prognostic significance, as ctDNA levels have been shown to correlate well with survival outcomes