Cancer research

Advancing cancer research with genetic analysis tools

applied biosystems

Introduction

Cancer is a genetic disease. Whether inherited or acquired, all of the myriad changes in protein expression, immune signaling, and cell growth and death that define cancer are ultimately caused by genetic dysfunction. Probe any deeper than this into the biology of cancer, and the details become less clear. Only a fraction of cancer-associated genetic variants have been identified, and with them only partial understanding of the events they provoke. New advances in gold-standard genetic technologies such as quantitative PCR (qPCR), capillary electrophoresis (CE) sequencing, and microarrays continue to drive fundamental and applied discoveries in oncology. More recent technologies such as next-generation sequencing (NGS), microfluidic digital PCR (dPCR; see sidebar), and Applied Biosystems™ castPCR™ (competitive allele-specific TaqMan™ PCR) technology are enabling researchers to pursue new territory with greater sensitivity, precision, and speed. This eBook explores how genetic technologies have enabled some of the most recent and exciting discoveries in several areas of cancer research.

Contents

Digital PCR

Digital PCR (dPCR) is a PCR approach that directly quantifies nucleic acid amounts from isolated Applied Biosystems™ Absolute Q™ Assays on individual nucleic acid molecules. dPCR enables fast and highly precise quantitation of genetic mutations. It can be especially valuable for detecting variants that occur at low frequency relative to wild type background DNA, such as in liquid biopsy or precious tumor samples. dPCR has quickly become a standard approach for nucleic acid quantitation in oncology, cell and gene therapy development, and other research applications. For more information, click here.

castPCR technology

Applied Biosystems™ castPCR™ (competitive allele-specific TaqMan™ PCR) technology is a fast, highly specific, and sensitive approach to detecting mutant alleles in the presence of large amounts of wild type alleles. castPCR technology uses a mutant allele–specific TaqMan™ primer that competes with a blocker oligonucleotide on the wild type allele. The blocker prevents binding of the mutant allele–specific primer to the wild type allele to suppress wild type allele amplification and reduce background signal. Only the mutant allele is amplified, which means higher specificity. castPCR is used in cancer research to detect and measure low-frequency somatic mutations in cancer-associated genes. For more information, click here.

Section 1: Molecular mechanisms of cancer

Exploring molecular mechanisms of malignancy

The promise of precision medicine and personalized cancer treatments is deeply rooted in building a deeper understanding of the molecular mechanisms that differentiate cancer onset, progression, and metastasis across cancer types and individual patients. Genetic mutations and epigenetic modifications are inextricably linked. Gene mutations can disrupt epigenetic mechanisms, and epigenetic modifications can drive genome instability and mutagenesis [1]. Here, we discuss the cellular mechanisms involved in cancer development and the contributions of genetic analysis technologies in furthering our understanding.

Genetic mechanisms

Sanger sequencing and Applied Biosystems™ TaqMan™ qPCR assays are gold-standard approaches to detecting, identifying, and quantifying expression of mutated genes to reveal possible mechanisms of cancer progression. One mechanism is oxygen deprivation, which stimulates vascularization of solid tumors and promotes cell mobility. Islam et al. used Sanger sequencing and TaqMan Assays (qPCR) together to investigate possible impacts of genetic mutations on oxygen availability in the tumor microenvironment in adrenal gland cancers, and subsequent implications for metastasis. Using Sanger sequencing by capillary electrophoresis, they identified multiple copy number mutations in an endothelial signaling protein gene, *EPAS1*, which is induced by hypoxia and is implicated in many cancers. The copy number changes were then correlated with *EPAS1* mRNA overexpression by qPCR to measure gene expression. The amplification and expression mutations were associated with tumor location, suggesting a role in carcinogenesis and metastasis as well as potential for predicting disease progression [2].

Genotyping with TaqMan Assays is a powerful approach to associate genetic mutations with alterations in cancer-related pathways and cancer cell behavior. For example, *TP53* missense mutations are the most frequent gene mutations across all human cancers. They result in protein gain of function (GOF), which is implicated in cancer progression and malignancy. In addition, loss of wild type *TP53* through loss of heterozygosity (LOH) is found in more than 93% of human cancers. GOF mutations are often found together with LOH mutations in metastatic cancers. In colorectal cancer (CRC), for example, LOH in wild type *TP53* and mutant p53 GOF are both required for metastasis. However, little is known about how they cooperate to promote malignant progression. To investigate this mechanism, Nakayama et al. genotyped organoid cell lines by SNP genotyping using TaqMan Assays to explore GOF–LOH relationships in different CRC driver gene mutations. This study revealed that the combination of both types of mutations activated inflammation and growth factor pathways, which may contribute to malignancy. The authors suggested that inhibition of mutant p53 GOF or suppression of *TP53* LOH might present opportunities for preventing CRC metastasis [3].

Epigenetic mechanisms

Approximately 10–15% of colorectal cancers (CRCs) have a high frequency of mutation in repetitive DNA sequences, referred to as microsatellite instability (MSI), which is thought to result primarily from mismatch repair (MMR) defects. Hypermethylation of promoter regions can be one cause of MMR defects. DNA methylation, microRNA overexpression, and histone modification are all epigenetic modifications that can cause gene expression changes that have been implicated in carcinogenesis and cancer progression [4-6]. Cells that are MMR-deficient usually have many mutations, which may also lead to cancer. However, in sporadic cancers, mutations in repair genes themselves are rarely the cause of MMR deficiency. Inhibition of DNA repair genes by epigenetic events is a more likely cause. Sequencing and expression analysis are fundamental to revealing genes that are impacted by epigenetic mechanisms and their roles in cancer progression. Studies using Sanger sequencing have identified mutations that indicated that most sporadic CRCs with high MSI originated from somatic MMR mutations [4,7]. Therapies that modify epigenetic patterns are leading to new research approaches for some cancers [1].

MicroRNA (miRNA) dysregulation is another epigenetic cause of MMR deficiency that is associated with carcinogenesis, malignant transformation, and metastasis in multiple types of cancers. When overexpressed, miRNA bound to mRNA can cause the mRNA to degrade or be poorly translated [8]. Although the canonical targets of miRNAs are noncoding promoter regions, recent evidence suggests that miRNAs can bind to sites within coding sequences as well. Binding of miRNA within coding sequences may be implicated in some cancers. A recent study revealed a missense mutation within the coding sequence of an adult-type granulosa cell tumor (AGCT) suppressor gene. The gene mutation provided a target site for a miRNA mutation. Subsequent binding of the miRNA resulted in degradation of the tumor suppressor mRNA and haploinsufficiency of the tumor suppressor itself. miRNA expression analysis using TaqMan Assays revealed that abundance of the miRNA mutation and the tumor suppressor variant were highly correlated with malignancy. The authors suggested that this missense mutation may provide a promising therapeutic target and prognostic parameter for patients with AGCT [9].

Microarrays have been a cornerstone technology for nucleic acid expression analysis for over 20 years. They remain a valuable tool for investigating the implications of genetic regulatory mechanisms in cancer. For example, miRNAs are known to regulate the transition of epithelial cells to mesenchymal cells (epithelial-to-mesenchymal transition, EMT). EMT is an essential process in enabling dissipation of cancer cells [10]. Colonization of cancer cells into a neoplastic mass is also essential for metastasis. The mechanism of mesenchymal-to-epithelial transition (MET) has been proposed as a contributor to colonization. However, less is known about the molecular mechanisms of MET compared to EMT. Xu et al. explored the role of miRNA in MET in liver-metastasized CRC. Liver metastasis is the most common cause of death in patients with CRC. In this study, Xu et al. profiled miRNA expression levels in CRC cells using RT-qPCR with Applied Biosystems™ TaqMan™ human miRNA microarrays that contained probes for all known mature miRNAs. This study revealed a decrease in the rate of invasion of CRC cells in liver that corresponded to upregulation of a miRNA that was derived from hepatocyte exosomes, suggesting an important role for miRNA in liver metastasis in CRC [10].

Conclusions and outlook

Every new genetic mutation that is discovered to influence malignancy is a potential target for improvements in cancer therapy. Thorough characterization of the impact a mutation has on malignancy is the most powerful determinant in revealing how it may contribute to improved cancer medicine. Genetic technologies such as qPCR, capillary electrophoresis, and microarrays are advancing rapidly to enable researchers to dig deeper into various mechanisms of malignancy and drive novel discoveries toward cancer characterization and pathogenesis.

Thermo Fisher Scientific is committed to providing cancer researchers with powerful genetics solutions that help 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, NGS, or CE
- Extract precise data from limited and challenging samples such as formalin-fixed, paraffin-embedded (FFPE) tissue, precious solid-tumor biopsy samples, or liquid biopsies, by dPCR or qPCR
- Obtain key insights from multiple perspectives provided by different technologies

References

1. Baylin SB, Jones PA. Epigenetic Determinants of Cancer. Cold Spring Harb Perspect Biol. 2016;8(9):a019505. Published 2016 Sep 1. doi:10.1101/cshperspect.a019505
2. Islam F, Pillai S, Gopalan V, et al. Identification of Novel Mutations and Expressions of EPAS1 in Phaeochromocytomas and Paragangliomas. Genes (Basel). 2020;11(11):1254. Published 2020 Oct 24. doi:10.3390/genes11111254
3. Nakayama M, Hong CP, Oshima H, et al. Loss of wild-type p53 promotes mutant p53-driven metastasis through acquisition of survival and tumor-initiating properties. Nat Commun. 2020;11(1):2333. Published 2020 May 11. doi:10.1038/s41467-020-16245-1
4. Chang SC, Li AF, Lin PC, et al. Clinicopathological and molecular profiles of sporadic microsatellite unstable colorectal cancer with or without the CpG island methylator Phenotype (CIMP). Cancers (Basel). 2020;12(11):3487. Published 2020 Nov 23. doi:10.3390/cancers12113487
5. Fawzy MS, Ibrahiem AT, AlSel BTA, et al. Analysis of microRNA-34a expression profile and rs2666433 variant in colorectal cancer: a pilot study. Sci Rep. 2020;10(1):16940. Published 2020 Oct 9. doi:10.1038/s41598-020-73951-y
6. Senga SS, Grose RP. Hallmarks of cancer-the new testament. Open Biol. 2021;11(1):200358. doi:10.1098/rsob.200358
7. Cheng LY, Haydu LE, Song P, et al. High sensitivity sanger sequencing detection of BRAF mutations in metastatic melanoma FFPE tissue specimens. Sci Rep. 2021;11(1):9043. Published 2021 Apr 27. doi:10.1038/s41598-021-88391-5
8. Iorio MV, Croce CM. Causes and consequences of microRNA dysregulation [published correction appears in Cancer J. 2020 Nov/Dec;26(6):561]. Cancer J. 2012;18(3):215-222. doi:10.1097/PPO.0b013e318250c001
9. Shin E, Jin H, Suh DS, et al. An alternative miRISC targets a cancer-associated coding sequence mutation in FOXL2 [published correction appears in EMBO J. 2021 Aug 16;40(16):e108163]. EMBO J. 2020;39(24):e104719. doi:10.15252/embj.2020104719
10. Xu H, Lan Q, Huang Y, et al. The mechanisms of colorectal cancer cell mesenchymal-epithelial transition induced by hepatocyte exosome-derived miR-203a-3p. BMC Cancer. 2021;21(1):718. Published 2021 Jun 19. doi:10.1186/s12885-021-08419-x

Section 2: New approaches to cancer detection

Introduction

Using genetic technologies to detect cancer early in carcinogenesis is one of the paramount intentions of precision medicine. Oncogenes are in a constant and delicate balance with cancer suppression genes. Excessive growth signals from either initiator or suppressor genes can send cell proliferation and apoptosis out of control, enabling cancer to take hold. Early identification of cancer gene expression signatures may be essential for defining individualized treatment strategies to inhibit disease progression [1]. Here, we discuss how genetic analysis technologies such as next-generation sequencing (NGS), qPCR, dPCR, microarrays, and capillary electrophoresis are continuing to lead the cutting edge of cancer detection in basic and applied research areas.

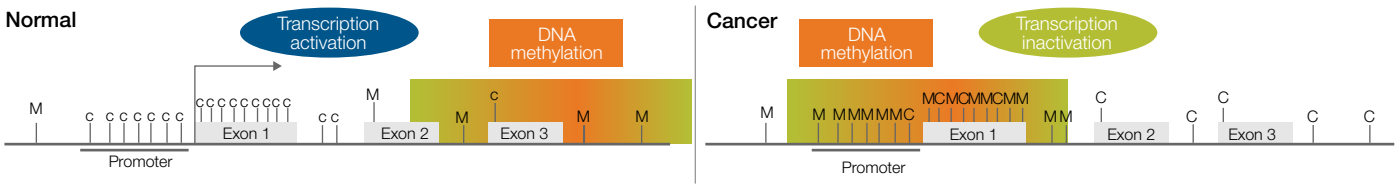
Solid-tumor biopsy

About 90% of cancers in adults and 40% of cancers in children are solid tumors. Microscopic examination of tissue biopsies has long been the gold standard for distinguishing benign from malignant solid tissue masses. Molecular profiling of solid tumors can take several weeks. Tumor heterogeneity can also complicate diagnosis [2]. Genetic technologies are enabling new approaches to solid-tumor analysis that may help detect and differentiate solid tumors in a quicker manner.

New developments in ion semiconductor–based NGS, such as Ion Torrent™ NGS technology, have made major contributions in characterizing somatic mutations in cancer genomes. Targeted NGS panels allow simultaneous analysis of multiple targets, which can eliminate the need for reflex testing and reduce the amount of sample required for mutation analysis. To assess the feasibility of ion semiconductor sequencing for solid-tumor detection, Mehrotra et al. analyzed the ability of several different ion semiconductor sequencing systems, including the Ion S5™ XL System, to detect variants in a variety of solid-tumor cancers in archived FFPE samples. Using comprehensive Ion AmpliSeq™ cancer panels covering cancer hotspots and entire coding regions of cancer-related genes, the authors found good concordance for mutation detection sensitivity and specificity across all systems. The systems demonstrated strong variant detection performance, and their panel capacity, ease of use, and sequencing speed showed strong potential for future clinical use [3].

Detection of pancreatic ductal adenocarcinoma (PDAC) is complicated by the fact that tissue masses from nonmalignant chronic pancreatitis (CP) can cause the same signs and symptoms as malignant neoplasms. Resistance to available therapies, late clinical presentation, and difficult early diagnosis all contribute to poor outcomes. PDAC also has greater genetic heterogeneity than any other tumor, suggesting that no single molecular biomarker and no single molecular targeted therapy will work for diagnosing and treating all PDAC patients. Gress et al. explored the potential of multiplexing mRNA and miRNA markers to differentiate and diagnose PDAC from CP. They developed a platform that incorporates both mRNA and miRNA markers on a single Applied Biosystems™ TaqMan™ array microfluidic card. RT-qPCR was used for simultaneous analysis of miRNA and mRNA expression patterns using minimal amounts of biopsy sample. Their studies using this multi-omics platform yielded 100% diagnostic accuracy of PDAC vs. CP and yielded superior classification compared to classifiers that were based on only miRNA markers. The authors also concluded that the TaqMan array cards offer the additional advantages of a high degree of standardization and limited hands-on time [4].

In cases of challenging tumor detection, some molecular detection technologies can be limited by cost, accuracy, availability of enough tissue, and time to conduct the assay. Accurate and timely tumor characterization from very small sample amounts can be particularly vital for aggressive, dispersed, or inoperable tumors such as gliomas, the most common tumor of the brain. Due to the difficulty of extracting tissue from the brain, glioma samples are precious, and biopsies generally yield very little sample. Saxena et al. used Applied Biosystems™ TaqMan™ Assays with high-density nanofluidic dPCR to detect epidermal growth factor receptor (*EGFR*) mutations from minute quantities of sample from solid glioblastoma tumors. They detected the EGFR variant III (EGFRvIII), which is associated with treatment resistance and poor prognosis, within 24 hours of surgery. They were also able to characterize the heterogeneous distribution of mutations in complex glioma tumors. From this study, the authors conclude that dPCR is a faster and more sensitive diagnostic assay than whole-genome sequencing for detection of the EGFRvIII mutation [2].



Identification of genetic mutations in very little tumor sample is leading to some exciting revelations in cancer detection. Chalela et al. used multiple genetic technologies to demonstrate that driver mutations can appear in histologically noncancerous cells, even without producing clonal expansion and without coincident mutation in the primary tumor cells. They used Sanger sequencing to identify specific exons within the *EGFR* or *KRAS* oncogenes. castPCR technology and, independently, qPCR confirmed mutation status, and dPCR confirmed the presence of the *EGFR* or *KRAS* mutations. Using these technologies, DNA extracted from normal lung tissue and lung adenocarcinoma (ADC) revealed *EGFR* or *KRAS* mutations in nontumoral lung cells even in patients with early-stage lung adenocarcinoma who had tested negative for those mutations [5].

Liquid biopsy

Solid-tissue biopsy is the most widely used method for detecting and categorizing tumors. However, tissue biopsy requires invasive tissue extraction, which is not always feasible, and the information it yields is limited, both spatially and temporally. Solid-tumor biopsies may not capture complex tumor heterogeneity or changes in malignancy over time. Noninvasive access to evidence of cancer mutations via circulating tumor DNA (ctDNA) in bodily fluids such as blood, lymph, urine, and semen can be an important tool to detect cancer at early stages. Liquid biopsy can also be a valuable approach to track efficacy of therapy over the course of treatment, monitor patients for relapse or metastasis, or assess malignancy in inaccessible tissues. Detecting ctDNA is often challenging because the DNA molecules bearing the cancer mutations are only a small fraction of the total circulating cell-free DNA (cfDNA) collected in the liquid sample.

The high sensitivity of Absolute Q dPCR assays with microfluidic dPCR is exceptionally well suited to detecting mutations in cfDNA in liquid biopsies. In a recent study, Siggillino et al. assessed ongoing response of non-small cell lung cancer (NSCLC) to targeted treatment by measuring plasma levels of *EGFR* mutations. They conducted quantitative analyses comparing dPCR with TaqMan Assays, amplification-refractory mutation system PCR, and peptide nucleic acid (PNA)-mediated PCR clamping. In their studies quantifying *EGFR* mutation detection, dPCR with TaqMan Assays yielded the highest sensitivity and best correlation of liquid biopsy with tissue biopsy. The authors concluded that dPCR is a robust method for absolute quantitation and monitoring of the evolution of mutations over time [6].

Genetic tests

Sanger sequencing is widely used to interrogate genes for small fragment mutations. It can be a fast, accurate, and sensitive approach to detecting cancer mutations. Bai et al. used human glioma cell lines to develop a Sanger sequencing assay accompanied by RT-qPCR with a TaqMan Assay to test for promoter mutations in a telomerase reverse transcriptase promoter gene (*TERTp*) that is associated with the diagnosis and poor prognosis in gliomas. They used the assay to analyze the frequency of *TERTp* mutations among different types of gliomas, tissue preparations, patient ages, tumor locations, and pathologic stages. The authors suggested that a fast, straightforward, and accurate method such as Sanger sequencing may aid in earlier diagnosis and prognostic assessment of gliomas [7].

Acute myeloid leukemia (AML) exemplifies the need for rapid and sensitive cancer mutation detection. Approximately 50% of AML patients present with normal karyotypes; however, multiple cellular regulatory genes carry mutations that impact prognosis and survival. Olarte et al. used castPCR technology to detect mutations in the isocitrate dehydrogenase (*IDH*) genes from AML patient samples. castPCR technology revealed a higher frequency of *IDH1* mutations than *IDH2* mutations; *IDH1* mutations are associated with unfavorable prognosis, while *IDH2* mutations are not associated with any change in survival. Some *IDH* mutations present opportunities as therapeutic targets [8]. Quicker detection of important mutations would enable patients to benefit from targeted therapies sooner.

As understanding the genetic factors that impact disease among different geographic and ethnic populations becomes more important, so does understanding population structures to develop new approaches for individualized cancer detection. Mighri et al. conducted haplotype analysis using NGS and Sanger sequencing to investigate the genetics and origin of the *BRCA1*-c.211dupA mutation, which has been found only in Tunisian families with breast cancer. The mutation has a severe phenotype, including onset at a young age, underscoring the need for early detection. Breast cancer patients with the *BRCA1*-c.211dupA mutation are already using their diagnosis to guide therapeutic decisions [9].

New approaches in data analysis may also offer improvements in genetic tests for cancer detection. In some cases, imaging methodologies used to diagnose solid tumors can result in false positives due to benign tissue masses. De Rienzo et al. sought to determine whether molecular testing could reduce false positives. They developed a test based on gene expression ratio using Applied Biosystems™ SYBR Green™ dye–based RT-qPCR to determine expression levels of several genes associated with lung cancer in normal and cancerous lung tissues. They determined the test to be a very sensitive and specific approach to reliably differentiate normal lung from cancerous tissue [10].

Conclusions and outlook

Early cancer detection remains as one of the most pursued and challenging goals in cancer medicine. Liquid biopsy is gaining traction as new discoveries demonstrate its advantages in detecting circulating tumor DNA in a noninvasive or minimally invasive manner when compared to tissue biopsy. Detection of genetic mutations that are associated with cancer development, particularly in cases with familial predisposition, is becoming mainstream for early disease management and in some cases, prevention. As researchers uncover novel mutations and predictive biomarkers involved in cancer pathogenesis, sensitive genetic analysis (PCR) and sequencing (NGS and Sanger sequencing) technologies continue to power such discoveries.

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- Obtain key insights from multiple perspectives provided by different technologies

References

1. Yang Z, Sweedler JV. Application of capillary electrophoresis for the early diagnosis of cancer. Anal Bioanal Chem. 2014;406(17):4013-4031. doi:10.1007/s00216-014-7722-y
2. Saxena D, Sheikh S, Kao G, et al. Rapid and ultrasensitive digital PCR (dPCR) profiling of EGFRvIII in tumor cells and tissues. Neurooncol Adv. 2019;1(1):vdz030. doi:10.1093/noajnl/vdz030
3. Mehrotra M, Duose DY, Singh RR, et al. Versatile ion S5XL sequencer for targeted next generation sequencing of solid tumors in a clinical laboratory. PLoS One. 2017;12(8):e0181968. Published 2017 Aug 2. doi:10.1371/journal.pone.0181968
4. Gress TM, Lausser L, Schirra LR, et al. Combined microRNA and mRNA microfluidic TaqMan array cards for the diagnosis of malignancy of multiple types of pancreatico-biliary tumors in fine-needle aspiration material. Oncotarget. 2017;8(64):108223-108237. Published 2017 Nov 21. doi:10.18632/oncotarget.22601
5. Chalela R, González-García JG, Khilzi K, et al. EGFR and KRAS Mutations in Lung Parenchyma of Subjects With EGFR/KRAS Wild-Type Lung Adenocarcinoma. Pathol Oncol Res. 2021;27:598292. Published 2021 Mar 24. doi:10.3389/pore.2021.598292
6. Siggillino A, Ulivi P, Pasini L, et al. Detection of EGFR Mutations in Plasma Cell-Free Tumor DNA of TKI-Treated Advanced-NSCLC Patients by Three Methodologies: Scorpion-ARMS, PNAClamp, and Digital PCR. Diagnostics (Basel). 2020;10(12):1062. Published 2020 Dec 7. doi:10.3390/diagnostics10121062
7. Bai H, Bai S, Li X, et al. Establishment and Validation of the Detection of TERT Promoter Mutations by Human Gliomas U251 Cell Lines. Biomed Res Int. 2021;2021:3271395. Published 2021 Jun 1. doi:10.1155/2021/3271395
8. Olarte I, García A, Ramos C, et al. Detection Of mutations in the isocitrate dehydrogenase genes (IDH1/IDH2) using castPCR™ in patients with AML and their clinical impact in Mexico City. Onco Targets Ther. 2019;12:8023-8031. Published 2019 Oct 1. doi:10.2147/OTT.S219703
9. Mighri N, Hamdi Y, Boujemaa M, et al. Identification of novel BRCA1 and RAD50 mutations associated with breast cancer predisposition in Tunisian patients. Front Genet. 2020;11:552971. Published 2020 Nov 6. doi:10.3389/fgene.2020.552971
10. De Rienzo A, Yeap BY, Cibas ES, et al. Gene expression ratio test distinguishes normal lung from lung tumors in solid tissue and FNA biopsies. J Mol Diagn. 2014;16(2):267-272. doi:10.1016/j.jmoldx.2013.11.008

Section 3: Cancer biomarkers, vaccines, and therapeutics

Introduction

Few cancers arise from single mutations. In most cancers, multiple genetic events may be involved including mutations, chromosomal aberrations, molecular deficiencies, and epigenetic modifications. The heterogeneity of potential mechanisms, types and subtypes of malignancies, and individual responses is vast. Despite such diversity, current treatment strategies for some cancers rely on a single approach for all patients who display similar pathological manifestations. Ongoing advancements in genetic technologies are revealing genetic and epigenetic biomarkers associated with specific subtypes, phenotypes, and genotypes. Identifying genetic biomarker signatures that can differentiate diagnosis, risk levels, and treatment strategies for individual patients is a significant focus of precision medicine research. Here, we discuss the recent discoveries and advancements made in the field of cancer biomarker, and vaccine and therapeutics research that have been powered by several genetic analysis technologies.

Discovering biomarkers for disease development

Circulating tumor DNA (ctDNA) has drawn attention as a potential source of biomarkers from liquid biopsies. However, in most circumstances the amount of ctDNA is too low to detect using conventional genetic approaches. Hagi et al. developed a method using the Ion Torrent™ NGS system that incorporates molecular barcodes into DNA fragments for sequencing. Their approach eliminates background errors to detect rare mutations in plasma from patients with esophageal cancer at about a 40-fold lower variant allele frequency compared to the traditional molecular barcode approach [1].

Genetic technologies can be highly valuable for differentiating genetic variants implicated at different stages of cancer progression. For example, microRNAs (miRNAs) have been determined to be involved in many mechanisms of malignancy. Oncogenic miRNAs (oncomiRNAs) have been identified that control cell growth and proliferation, cell death, cancer invasion, metastasis, and immune signaling. With so many areas of influence, miRNAs may be a valuable source for biomarker discovery.

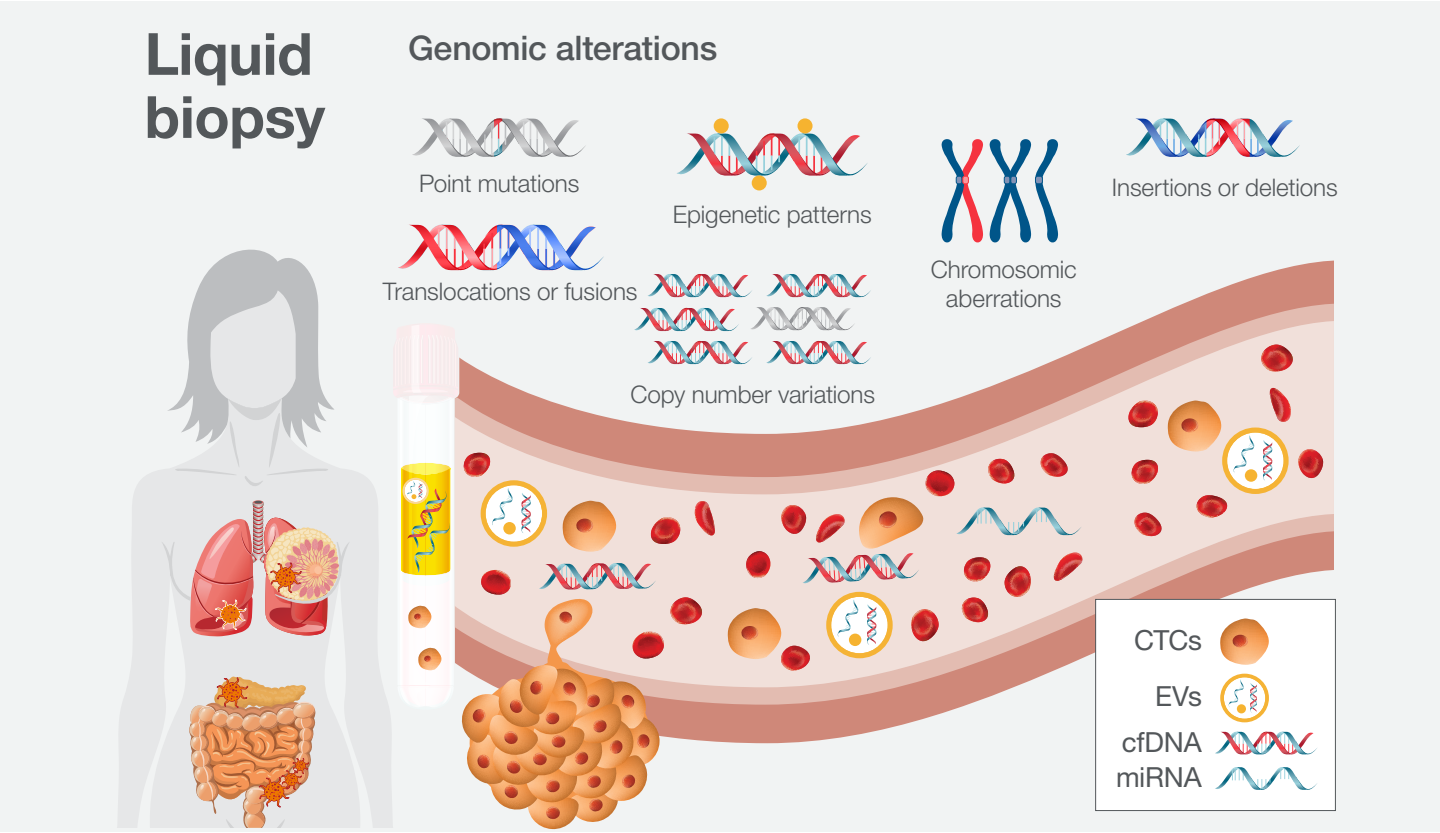
Combatting metastasis is a prevailing challenge for cancer researchers, oncologists, and patients. It is the primary indicator of fatal disease in many cancers. Aberrant expression of miRNA is associated with metastasis in a wide range of cancers. De Miguel-Perez et al. used Applied Biosystems™ TaqMan™ microRNA assays and RT-qPCR to track miRNA expression from extracellular vesicles in serum following surgical

resection of CRC tumors. Tracking revealed expression levels for multiple miRNAs that correlated with progression of CRC. The researchers developed a selectively designed panel of extracellular vesicle–derived miRNAs that might provide reliable and specific biomarkers for early prediction of metastatic CRC from noninvasive liquid biopsies [2]. Studies using TaqMan Assays have also revealed upregulated miRNAs associated with CRC in solid tissue specimens [3].

Metastasis is also the primary indicator of fatal breast cancer. Dwedar et al. used TaqMan microRNA assays and qPCR to reveal higher levels of cell-free circulating miR-10b in serum from breast cancer patients compared to healthy individuals. The miR-10b variant was also upregulated as tumors progressed to metastasis. The authors speculate that circulating miR-10b could be used as a potential biomarker for noninvasive diagnosis and prognosis of breast cancer [4].

In prostate cancer, the inability to differentiate slow-growing tumors from aggressive tumors has led to a rise in biopsies of insignificant tumors and overtreatment. Ruiz-Plazas et al. turned to an uncommon biopsy fluid, semen, as a possible source of genetic insights to stratify patient risk levels and potentially guide appropriate treatment. Analysis of oncomiRNA expression using RT-qPCR with SYBR Green dye revealed a panel of oncomiRNAs that differentiated aggressive from nonaggressive forms of prostate cancer. An assay that reveals aggressive prostate cancer subtypes based only on information obtained from semen may be a valuable approach to guiding more effective treatment options [5].

Almost all cases of cervical cancer are caused by human papilloma virus (HPV). Long noncoding RNAs (lncRNAs) have been associated with cervical cancer, but how HPV infection leads to aberrant lncRNA expression and pathogenesis remains a mystery. In one recent study, sequencing and RT-qPCR revealed 194 lncRNA isoforms that were differentially regulated



coincidentally with progression of cervical lesions resulting from HPV infection. Primer-walking qPCR revealed that alternative promoters, RNA splicing, and polyadenylation generated at least 14 of those isoforms. Interestingly, high expression levels of one lncRNA was found to be a biomarker for better survival rate in the study. These studies also revealed a lncRNA that is coregulated with an important DNA repair factor that is associated with high incidence of high-risk HPV-induced cancers. The authors suggested that these findings might lead to biomarkers for early diagnosis and treatment strategies for HPV-induced cervical cancer [6].

Discovering biomarkers to gauge response to treatment

Identifying biomarkers that aid in predicting how a patient might respond to treatment is another important intention for personalized medicine research. For example, immune checkpoint inhibitors (ICI) have become promising immuno-therapies for treating a variety of cancers. However, not all patients benefit from ICI immunotherapy and currently there is no reliable biomarker for predicting potential outcomes of ICI treatment [7,8].

Using Ion Torrent™ RNA sequencing, Hwang et al. identified two multigene signatures and two individual genes that independently predict durable benefits for treatment of non-small cell lung cancer (NSCLC) with anti-programmed cell death protein (PD)-1 antibody [7]. Wang et al. used RT-qPCR with TaqMan Assays to query gene expression levels in a panel of ICI-related proteins that are associated with clinical outcomes in clear renal cell cancer (ccRCC). They correlated expression levels and calculated risk scores for risk of advanced disease, recurrence, or death in early-stage, untreated ccRCC patients. The study revealed a panel of biomarkers for soluble ICI-related proteins that might assist in risk assessment for survival prognosis in ccRCC [8].

Expanding insights into cancer vaccine research

Because cancer cells closely resemble normal, healthy cells and individual tumors all present their own distinguishing antigens, developing effective cancer vaccines requires more sophisticated approaches than those used in conventional pathogen vaccines. Tumor cells, immune cells, peptides, viral vectors, and nucleic acids have all been investigated as possible platforms for cancer vaccines.

Genetic solutions for research into preventive oncovirus vaccines

As of 2019, human tumor viruses account for an estimated 12–20% of cancers worldwide [9]. Vaccines that target oncoviruses have already been proven to prevent certain cancers. Hepatitis B virus (HBV) can cause liver cancer and HPV causes almost all cases of cervical cancer. Four vaccines have been approved by the U.S. Food and Drug Administration (FDA) to prevent HBV or HPV infection. Although the incidence and death rates from cervical cancer have plummeted since

the first HPV vaccine was approved by the FDA in 2006, cervical cancer still remains a leading cause of cancer deaths in women [10]. Epstein-Barr virus, human immunodeficiency virus, human herpes virus 8, Merkel cell polyomavirus, and human T-lymphotropic virus 1 (HTLV-1) are all known to cause cancer. The work to develop truly preventive vaccines for viral cancers is not complete.

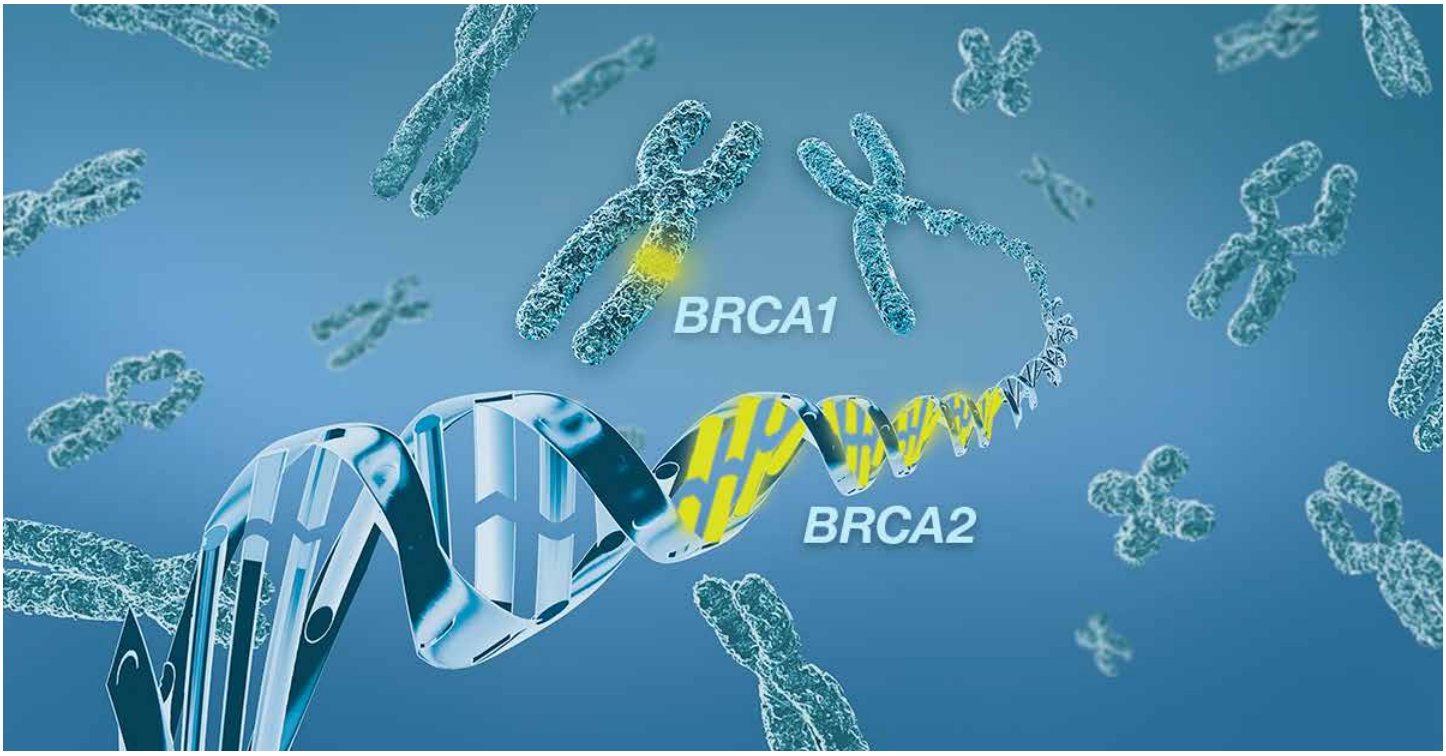
For example, most cases of genital high-risk HPV infection resolve spontaneously; however, persistent infections can progress to cancer. To understand how differences in innate immunity control papillomaviruses, Scagnolari et al. investigated the role of key components in the rapid immune control of papillomavirus infection. They used TaqMan Assays with RT-qPCR to explore alternative splicing and viral replication in various strains of genetically deficient mice. These studies revealed a signaling pathway that controls viral replication and clearance of virus-transformed abnormal cells. Understanding mechanisms of early viral clearance may provide vital insights into how disruption of innate immunity could convert a transient infection into a persistent infection, revealing potential approaches to preventing HPV infection [11].

Genetic solutions for research into therapeutic cancer vaccines

Strategies that enable the immune system to identify and destroy cancer cells at a very early stage of oncogenesis are being aggressively pursued to develop vaccines for cancers with a noninfectious etiology. Genetic technologies that enable development of delivery vector constructs, assessment of vaccine delivery, and measurement of expression levels of target molecules are essential to building new insights and opportunities for therapeutic cancer vaccines.

Adenoviruses are highly effective vectors to deliver vaccine epitopes and drive tumor cell lysis to treat some cancers. They can be grown to high titers, have genomes that are easily manipulated, and induce robust transgene expression. However, the immune system presents a significant barrier to widespread use of adenoviruses. First, much of the general population has been exposed to adenoviruses so preexisting immunity is high. Second, administering an adenovirus-based vaccine as therapy to a cancer patient who has not been exposed to adenovirus may elicit a dangerous immune response. Understanding how the immune system circumvents adenovirus-mediated epitope expression and cytolysis may provide valuable insight towards developing more effective adenovirus-based cancer vaccines.

A recent study identified a cytosolic antibody receptor (*TRIM21*) that inhibits both adenovirus-mediated epitope expression and cytolysis. Sequencing and RT-qPCR with TaqMan Assays were used to conduct transcriptome-wide analysis of expression levels of intact genes, knockout variants, and point mutations in viral and immune system genes following adenovirus administration. These studies revealed that *TRIM21* both neutralizes transgene expression and inhibits antigen-specific cytotoxic T cells, preventing the adenovirus vaccine from inducing antitumor immunity. The mechanisms of these pathways hold potential to increase or decrease *TRIM21* activity as needed for specific cancer therapeutic applications [12].



With the success of mRNA vaccines for COVID-19, interest is growing for mRNA as a vector for antigens that will initiate a cancer-killing immune response. mRNA cancer vaccines offer several advantages over other vaccine platforms including high potency, safe administration, rapid development potential, and cost-effective manufacturing. However, mRNA vaccine applications can also be limited by instability, innate immunogenicity, and inefficient *in vivo* delivery [13]. Positive results from research studies are fueling continued investigation. For example, Li et al. identified a CpG oligodeoxynucleotide that works in combination with an mRNA-based vaccine to boost immune response against melanoma tumors. The investigators used RT-qPCR to measure induction of the immune response based on increased expression of multiple cytokine genes. The combined approach provided stronger antitumor effect than either component alone [14].

Building genetic insights for cancer therapeutics

Heterogeneous cancers are characterized by diverse molecular alterations and multiple clinical profiles. Genetic tools that can detect and investigate cancer mutations may yield insights into therapeutic success or resistance, contribute to identification of new therapy targets, and ultimately support development of more personalized treatment strategies.

Targeted cancer therapy research

Mutations in genetic targets for cancer therapy usually result in either loss of function (LOF) and accumulation of dysfunctional proteins in tumors, or oncogenic gain of function (GOF) effects such as tumor proliferation, metastasis, and drug resistance. Precise molecular and genetic profiling to identify relevant mutations in tumor cells is gaining interest as a source of potential opportunities for targeted treatment options [15]. Development of targeted cancer therapies faces two primary genetic challenges: 1) identifying mutations that can influence the efficacy of targeted therapies; and 2) identifying variants in individual genomes to determine their likelihood to respond to specific targeted therapies.

Monoclonal antibody (mAb)-based inhibitors of programmed death ligands (PD-L) are one class of targeted therapy that has shown remarkable antitumor activity in a variety of cancers. However, some patients, even with high levels of PD-L1 antigen, do not respond to currently available mAb therapies. Park et al. demonstrated that natural killer (NK) cell variants can elicit different immune responses in cells with high PD-L1. They used PCR and Sanger sequencing to genotype NK cells from a variety of human cancer and healthy cell lines, revealing a specific genotype that induced an antibody-dependent cytotoxicity response. The authors conclude that combining NK cell therapy with anti-PD-L1 mAbs offers a valuable immunotherapeutic strategy for certain cancer profiles [16].

Chimeric antigen receptor T (CAR T) cell therapy is another strategy that has shown promising clinical results for some cancers, but it also faces challenges. Using animal models for CAR T cell therapy in humans can cause cross-species reactivity, so animal models are unlikely to be reliable indicators of response. In particular, on-target off-tumor behavior of CAR T cells can result in severe adverse events in patients who express the target antigen on both tumor and healthy tissue. Because CARs are highly specific for human antigens, on-target off-tumor toxicity is difficult to predict in current animal models [17,18]. Castellarin et al. developed a mouse model that uses high-throughput TaqMan Assays with qPCR to enable tuning of human *HER2* expression levels and can be used to test on-target off-tumor toxicity of CAR T cells. The authors concluded that this model is an effective preclinical tool to improve prediction of safety and efficacy of CAR T cell therapies [18].

Nontargeted cancer therapy research

Resistance to chemotherapy can be one of the most troubling circumstances for cancer patients, physicians, and oncology researchers. Tremendous heterogeneity across types of cancers and patients confounds identification of cancer therapeutics for broad use. Elucidating genetic mechanisms of chemoresistance may reveal valuable insight towards identifying new therapeutic targets and developing more effective therapeutic agents.

In some cancers, chemotherapeutic resistance is thought to arise from variable expression of Notch receptors within tumors. The genetic mechanisms of Notch expression variability that result in chemoresistance remain elusive. Kumar et al. identified a specific Notch ligand that drives metastasis and chemoresistance in breast cancer. Using RT-qPCR to correlate expression levels of the Notch ligand mRNA and the ligand, they determined that combination therapy that targets both shows almost complete response to doxorubicin in Notch ligand–positive tumors [19].

Cytotoxic chemistry is currently the best line of defense against triple-negative breast cancers (TNBC), which do not express the estrogen receptor, progesterone receptor, or *HER2* markers that are targets for other breast cancer therapies. Even with cytotoxic chemotherapy, TNBC patients have poor prognosis. Broad et al. turned to microarray-based transcriptomics and TaqMan miRNA assays to reveal an interferon-signaling pathway that is associated with chemoresistance in TNBC cells. The authors suggested that inhibition of this pathway could present a new research approach to improve future outcomes in TNBC [20].

Conclusions and outlook

To prevent and treat cancer is a key driving motivation for many cancer researchers. Discovery of biomarkers that can predict cancer development and progression, as well as treatment to response, is providing researchers tools to develop improved therapeutics. Rapid progress on novel vaccine platform development as well as personalized cancer therapy is providing those affected by or predisposed to developing cancer, a new hope to a cure or prevention. Genetic technologies such as PCR, NGS, capillary electrophoresis, and microarrays are enabling researchers to develop model systems to study the disease, evaluate efficacy of vaccines and therapeutic agents, and determine genetic markers that influence cancer onset or response to therapy. The findings from such studies are advancing the cancer field toward individualized evaluation and treatment, given the strong influence of genetics on the disease.

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

References

1. Hagi T, Kurokawa Y, Takahashi T, et al. Molecular Barcode Sequencing for Highly Sensitive Detection of Circulating Tumor DNA in Patients with Esophageal Squamous Cell Carcinoma. Oncology. 2020;98(4):222-229. doi:10.1159/000504808
2. de Miguel Pérez D, Rodriguez Martínez A, Ortigosa Palomo A, et al. Extracellular vesicle-miRNAs as liquid biopsy biomarkers for disease identification and prognosis in metastatic colorectal cancer patients. Sci Rep. 2020;10(1):3974. Published 2020 Mar 4. doi:10.1038/s41598-020-60212-1
3. Fawzy MS, Ibrahiem AT, AlSel BTA, Alghamdi SA, Toraih EA. Analysis of microRNA-34a expression profile and rs2666433 variant in colorectal cancer: a pilot study. Sci Rep. 2020;10(1):16940. Published 2020 Oct 9. doi:10.1038/s41598-020-73951-y
4. Dwedar FI, Shams-Eldin RS, Nayer Mohamed S, Mohammed AF, Gomaa SH. Potential value of circulatory microRNA10b gene expression and its target E-cadherin as a prognostic and metastatic prediction marker for breast cancer. J Clin Lab Anal. 2021;35(8):e23887. doi:10.1002/jcla.23887
5. Ruiz-Plazas X, Altuna-Coy A, Alves-Santiago M, et al. Liquid Biopsy-Based Exo-oncomiRNAs Can Predict Prostate Cancer Aggressiveness. Cancers (Basel). 2021;13(2):250. Published 2021 Jan 11. doi:10.3390/cancers13020250
6. Liu H, Xu J, Yang Y, et al. Oncogenic HPV promotes the expression of the long noncoding RNA lnc-FANCI-2 through E7 and YY1. Proc Natl Acad Sci U S A. 2021;118(3):e2014195118. doi:10.1073/pnas.2014195118
7. Hwang S, Kwon AY, Jeong JY, et al. Immune gene signatures for predicting durable clinical benefit of anti-PD-1 immunotherapy in patients with non-small cell lung cancer. Sci Rep. 2020;10(1):643. Published 2020 Jan 20. doi:10.1038/s41598-019-57218-9
8. Wang Q, Zhang J, Tu H, et al. Soluble immune checkpoint-related proteins as predictors of tumor recurrence, survival, and T cell phenotypes in clear cell renal cell carcinoma patients. J Immunother Cancer. 2019;7(1):334. Published 2019 Nov 29. doi:10.1186/s40425-019-0810-y
9. Brubaker J. The 7 Viruses That Cause Human Cancers. American Society for Microbiology. January 25, 2019. Accessed January 19, 2022. https://asm.org/Articles/2019/January/The-Seven-Viruses-that-Cause-Human-Cancers
10. National Cancer Institute. Large Study Confirms that HPV Vaccine Prevents Cervical Cancer. October 14, 2020. Accessed January 19, 2022. https://www.cancer.gov/news-events/cancer-currents-blog/2020/hpv-vaccine-prevents-cervical-cancer-sweden-study
11. Scagnolari C, Cannella F, Pierangeli A, et al. Insights into the Role of Innate Immunity in Cervicovaginal Papillomavirus Infection from Studies Using Gene-Deficient Mice. J Virol. 2020;94(12):e00087-20. Published 2020 Jun 1. doi:10.1128/JVI.00087-20
12. Bottermann M, Foss S, van Tienen LM, et al. TRIM21 mediates antibody inhibition of adenovirus-based gene delivery and vaccination. Proc Natl Acad Sci U S A. 2018;115(41):10440-10445. doi:10.1073/pnas.1806314115
13. Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. Mol Cancer. 2021;20(1):41. Published 2021 Feb 25. doi:10.1186/s12943-021-01335-5
14. Li Q, Ren J, Liu W, Jiang G, Hu R. CpG Oligodeoxynucleotide Developed to Activate Primate Immune Responses Promotes Antitumoral Effects in Combination with a Neoantigen-Based mRNA Cancer Vaccine. Drug Des Devel Ther. 2021;15:3953-3963. Published 2021 Sep 18. doi:10.2147/DDDT.S325790
15. Jin J, Wu X, Yin J, et al. Identification of Genetic Mutations in Cancer: Challenge and Opportunity in the New Era of Targeted Therapy. Front Oncol. 2019;9:263. Published 2019 Apr 16. doi:10.3389/fonc.2019.00263
16. Park JE, Kim SE, Keam B, et al. Anti-tumor effects of NK cells and anti-PD-L1 antibody with antibody-dependent cellular cytotoxicity in PD-L1-positive cancer cell lines. J Immunother Cancer. 2020;8(2):e000873. doi:10.1136/jitc-2020-000873
17. Kato D, Yaguchi T, Iwata T, et al. GPC1 specific CAR-T cells eradicate established solid tumor without adverse effects and synergize with anti-PD-1 Ab. Elife. 2020;9:e49392. Published 2020 Mar 31. doi:10.7554/eLife.49392
18. Castellarin M, Sands C, Da T, et al. A rational mouse model to detect on-target, off-tumor CAR T cell toxicity. JCI Insight. 2020;5(14):e136012. Published 2020 Jul 23. doi:10.1172/jci.insight.136012
19. Kumar S, Nandi A, Singh S, et al. Dll1+ quiescent tumor stem cells drive chemoresistance in breast cancer through NF-κB survival pathway. Nat Commun. 2021;12(1):432. Published 2021 Jan 18. doi:10.1038/s41467-020-20664-5
20. Broad RV, Jones SJ, Teske MC, et al. Inhibition of interferon-signalling halts cancer-associated fibroblast-dependent protection of breast cancer cells from chemotherapy. Br J Cancer. 2021;124(6):1110-1120. doi:10.1038/s41416-020-01226-4

Section 4: Genetic analysis technologies to support cancer research

Introduction

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.

The portfolio of solutions from Thermo Fisher can be thought of as a continuum that gives researchers options at every stage of their research journey, based on the types of questions being asked (Figure 1). For example, some investigators might be interested in **discovery**-based research, using unbiased queries spanning the entire genome to uncover new sequences or relationships between genes. These types of experiments are facilitated by Ion AmpliSeq™ next-generation sequencing (NGS) workflows or by Applied Biosystems™ microarray solutions. Once a set of sequences of interest is decided, investigators might move to a **focus**-based model, where these selected sequences are analyzed in medium-throughput analyses such as Sanger sequencing to validate, confirm, and focus on a defined region across many samples; or Applied Biosystems™ TaqMan™ panels to focus on sets containing many genes. Finally, if a very specific set of mutations or genes needs to be analyzed in a large number of samples with minimal effort, individual TaqMan Assays may be used for **detecting** these sequences.

Importantly, these approaches and technologies are complementary to each other. For example, a researcher might start with a discovery-based approach to catalog the mutations commonly found in a tumor, then focus on those mutations by confirmatory Sanger sequencing in more samples, and finally design a TaqMan probe to the mutation(s) that can be used to stratify patients for a research program. Our solutions continuum is meant to ease the research path from discovery to insight.

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

Figure 1. The genetic analysis continuum. In oncology research, the tools and techniques used depend on the question being asked, the scale of the data that need to be analyzed, and the logistical needs of the investigator. Applied Biosystems™ genetic analysis systems fall into a continuum, from very large-scale **discovery**-based research to medium-scale **focused** research to very targeted **detection** of specific genes and mutations.

sequences are present and in how many copies (Figure 2). 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 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 nonpolymorphic regions of the genome 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 microarrays, reagents, and analysis software—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.

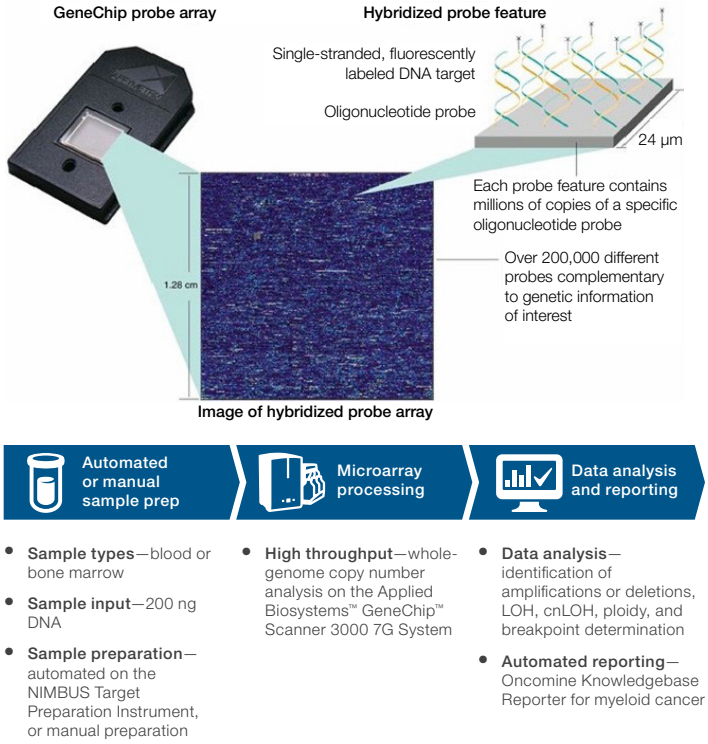


Figure 2. Basics of a microarray assay.

Finally, the Applied Biosystems™ Axiom™ Precision Medicine Research Array includes over 800,000 genotyping probes. The targets on this array were selected to emphasize variants that are commonly seen in clinical research, including common cancer variants, clinically actionable variants, pharmacogenomic variants, and more. The common cancer markers were chosen from the list of published variants associated with cancer phenotypes identified via a genome-wide association study (GWAS), as per the National Human Genome Research Institute and the European Bioinformatics Institute (NHGRI-EBI) GWAS Catalog, as well as some recently published and unpublished cancer-associated SNPs. The clinically actionable variants include relevant variants from ClinVar and enable assessment of actionable genetic risk across a wide range of populations. Finally, the pharmacogenomic variants in the array prioritize variants that are derived from the Clinical Pharmacogenetics Implementation Consortium™ (CPIC™) guidelines and variants contributing to star alleles.

Fragment analysis methods and tools

Fragment analysis is a highly flexible method that can be applied to a wide variety of research fields. The flexibility afforded with the choice of PCR primers (PCR being a necessary first step in any fragment analysis experiment) means that a specifically sized fragment corresponding to a PCR target sequence is straightforward to generate (Figure 3). Coupling this with the ability to label fragments with up to four different fluorophores, researchers have great flexibility in their fragment analysis experimental design. Below, we describe some examples of how fragment analysis can be used in common cancer research problems.

Mutation detection

Oncology researchers frequently need to investigate or verify a limited number of variations in one or more genes of interest. The Applied Biosystems™ SNaPshot™ Multiplex System is a versatile and economical method to perform SNP genotyping. Up to ten SNP markers (on different genes) can be investigated simultaneously by using PCR amplification, followed by dideoxy single-base extension (SBE) using an unlabeled primer, and then capillary electrophoresis of the resulting fragments. After electrophoresis and fluorescence detection, the alleles of a single marker appear as different colored peaks at roughly the same size in the electropherogram plot [1]. This technology has been used for simultaneous screening of four hotspot mutations in *PIK3CA*, a gene involved in the phosphatidylinositol 3-kinase pathway, which plays an important role in cancer pathogenesis [2].

Microsatellite instability assay

Microsatellite instability (MSI) is a hallmark of several cancers and is characterized by changes to the length of microsatellites due to defect in mismatch repair mechanisms (MMR). Microsatellites are genetic motifs consisting of 1–6 base pair repeats. These sequences are susceptible to replication errors that can result in deletions and insertions. Normally, these errors are corrected by DNA MMR; however, when deficiencies in the DNA MMR system are present, microsatellite replication errors accumulate in the genome [3]. In addition to its prognostic value for certain types of cancer, including colorectal [4] and endometrial, MSI can also serve as a predictive biomarker for immune checkpoint therapy response [5].

The Applied Biosystems™ TrueMark™ MSI Assay interrogates 13 mononucleotide MSI markers. Eight of these markers are derived from literature and guidelines from the National Cancer Institute [6], and the other five markers were internally identified for monomorphism and high sensitivity in multiple cancer types. The assay also contains two sample identification markers to determine sample mix-up or contamination [7]. To facilitate analysis of MSI data, we developed TrueMark™ MSI Analysis Software. This is a desktop-based software that takes the fragment analysis files of the TrueMark MSI Assay and provides an easy-to-interpret analysis of the results. A groundbreaking feature of the software is a proprietary algorithm that can analyze samples without the need for running normal sample controls concurrently, saving time and expense.

Loss of heterozygosity (LOH)

MLPA™ (multiplex ligation-dependent probe amplification) technology is a flexible technique that is commonly used to detect aberrations in gene copy number such as LOH. It is based on the ligation and PCR amplification of up to 50 multiplexed pairs of probe oligonucleotides, which hybridize to the loci of interest. Each oligonucleotide pair is designed to give an amplification product of a specific length; by using sequence-tagged ends, all ligated probes can be amplified with a single primer pair in a PCR reaction. The forward PCR primer carries a fluorescent label, allowing for the detection and quantification of size-separated probes on an automated capillary electrophoresis system [8].

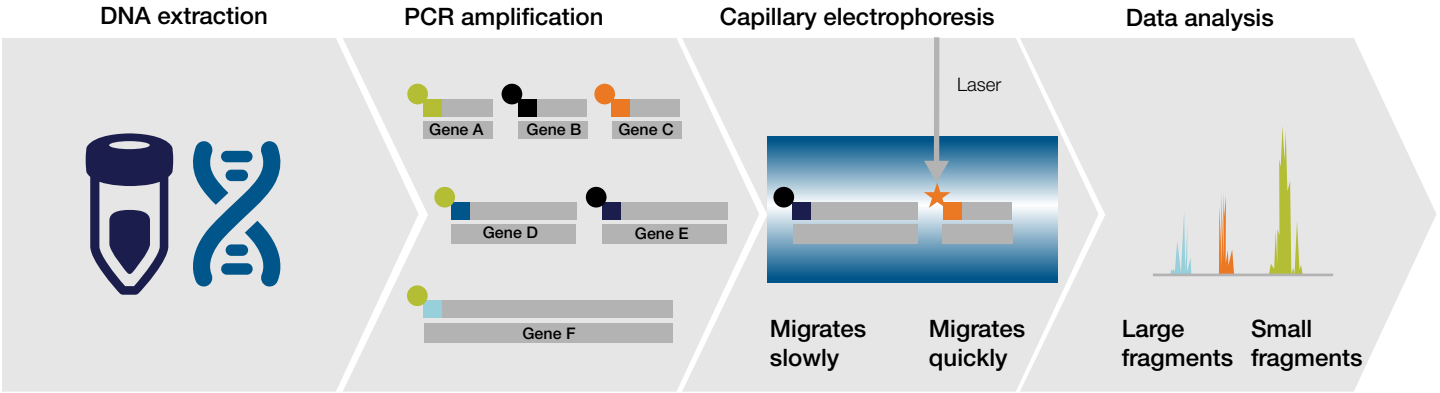


Figure 3. Basics of a fragment analysis assay. PCR primers are designed such that different target sequences are amplified with primers with different fluorophore labels, and that will generate different sized amplicons. Following PCR, the amplicons are electrophoretically separated by size in a capillary. A laser excites the fluorophores as the fragments migrate through the capillary. The size and color of the resulting fragments reflects the abundance of the target sequence in the sample.

Several cancers are characterized by germline point mutations in specific genes, such as the *BRCA1* mutation in breast cancer and *BRCA2* mutation in ovarian cancer. However, even for such well-characterized cancers, mutations involving genomic rearrangements have been found in a subset of cases [9]. The MLPA assay can be used to interrogate these genomic loci in cases where the initial screen is negative, even though there is a high probability of disease inheritance. For instance, the MLPA assay has been used to show deletions and duplications in the *BRCA1* gene in patients with a history of breast cancer but who were negative for *BRCA* point mutations [10].

MLPA technology can also be used to detect epigenetic modifications involving DNA methylation [11] that contribute to cancer formation. The methylation-specific MLPA (MS-MLPA) assay has probes that bind and ligate over a GCGC sequence, which is also a cleavage site for a methylation-sensitive restriction enzyme, HhaI. The enzyme cleaves the probes that are ligated and hybridized to unmethylated DNA, while the probes bound to methylated DNA remain intact and are subsequently amplified by PCR. This assay has been used to determine promoter methylation status of tumor suppressor genes *p15* and *p73* in acute myeloid leukemia cell lines [11].

Epigenetic modification

Changes in DNA methylation patterns are also characteristic of oncogenesis. Genome-wide hypomethylation or hypermethylation, particularly of cytosines 5′ to guanosines (CpG) within the promoter regions of tumor repressor genes, has been shown to be associated with cancer development [12]. The SNaPshot multiplex assay can also be used to investigate DNA methylation patterns following bisulfite treatment of genomic DNA. Sodium bisulfite converts unmethylated cytosines into uracil, which are subsequently replaced as thymine in PCR; in contrast, methylcytosines remain unchanged. This feature can be used to distinguish base differences by PCR amplification, T/C genotyping using SNaPshot single-base extension (SBE) multiplex assay, followed by fragment analysis.

Cell line authentication and human sample matching

Cancer cell lines are heavily relied upon for biomedical research, including cancer-related studies. To ensure reproducibility of scientific research, cell line authentication (CLA) is of paramount importance to confirm the cell's origin, as well as to check for contamination and genomic instability. CLA is performed by generating a profile of highly variable short tandem repeat (STR) markers from microsatellite loci with varying number of repeats for a particular cell line/type, and then comparing it with the allelic profiles present at these loci against known standards. A study aimed at authenticating 278 human tumor cell lines used in China found that nearly 46% of the samples were either cross-contaminated or misidentified [13]. Needless to say, such findings can have massive implications on the conclusion of studies that utilize such cell lines. The Applied Biosystems™ CLA GlobalFiler™ PCR Amplification Kit generates a molecular fingerprint for 24 different STR loci, while the Applied Biosystems™ CLA IdentiFiler™ Plus PCR Amplification Kit analyzes 16 STR loci.

In addition to CLA, STR typing can be used for human sample matching. CAR T cell therapy is a potent cellular immunotherapy that has seen great success in the treatment of hematological malignancies. CAR T cell preparation involves the isolation, activation, engineering, and expansion of patient-derived T cells. Every step of this process can affect the efficacy and consistency of a CAR T cell product, and requires thorough characterization to ensure that the product meets predesigned specifications. STR typing can be used to confirm that the CAR T cells match the starting T cell population, ensuring the identity of the final CAR T product [14].

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 4). 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 [15]. 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.

PCR methods and tools

The portfolio of TaqMan Assays is the most comprehensive set of real-time PCR (qPCR) products available for analyzing gene expression, miRNA levels, protein abundance, copy number variation, SNP genotyping, and rare-allele mutation detection. The typical TaqMan Assay consists of forward and reverse primers to amplify a target by PCR (Figure 5). TaqMan Assays also contain a sequence-specific fluorescent probe, designed with a minor groove binder (MGB) moiety at the 3′ end that increases the melting temperature (T_m) of the probe and stabilizes probe–target hybrids. This means that TaqMan MGB probes can be significantly shorter than traditional probes, providing better sequence discrimination and flexibility to accommodate more targets. In addition, TaqMan probes have a nonfluorescent quencher (NFQ) that binds to the fluorophore, until it is cleaved by the polymerase, and minimizes background. All TaqMan Assays come with a performance guarantee.

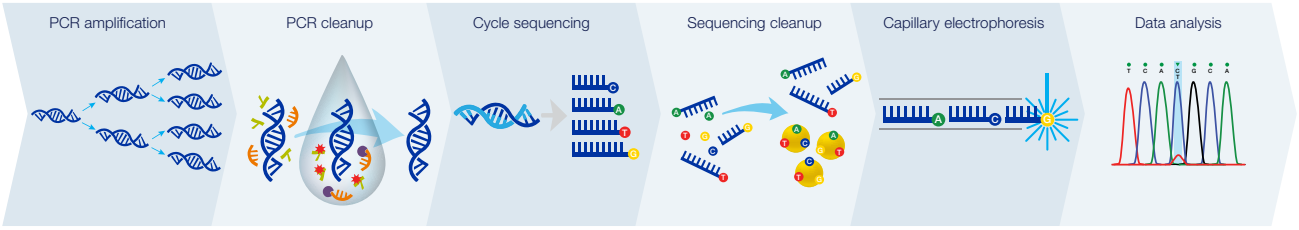


Figure 4. Basics of a Sanger sequencing workflow. The target region that will be sequenced is amplified by PCR. The primers and PCR reagents are removed before a second linear amplification is performed. This step generates the fragments that are chain-terminated with a fluorescent dideoxynucleotide. 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.

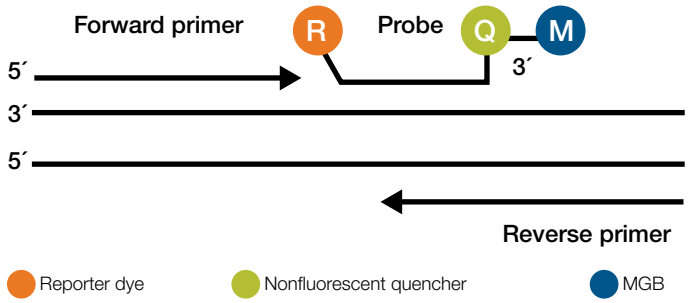


Figure 5. Basics of a TaqMan Assay. Unlabeled forward and reverse oligonucleotide primers define the region that will be queried. A third oligonucleotide binds to the region between the primers. The probe has an end-label fluorophore reporter, a nonfluorescent quencher, and a minor groove binding (MGB) moiety that stabilizes binding of the short probe sequence. When the probe is hybridized to its target sequence, the quencher greatly reduces the fluorescence of the reporter. When the sequence is copied during the PCR reaction, the nuclease activity of the polymerase cleaves the reporter from the rest of the probe, releasing the reporter be detected by a laser in the instrument. The amount of fluorescence is therefore directly related to the amount of target amplified.

One strategy that ensures high-quality PCR data is that investigators follow standardized practices for performing and reporting qPCR results. The MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) recommendations are set of guidelines for qPCR experimental designs and data reporting practices, as well as standards for sharing experimental information with colleagues [16]. The standards are designed to ensure that published qPCR data is meaningful, accurate, and provides researchers with the information necessary to faithfully reproduce results. Thermo Fisher supports these guidelines and provides all information necessary to ensure MIQE compliance when publishing the results of experiments with TaqMan assays. Information requested under the qPCR target, qPCR oligonucleotide, and qPCR protocol sections of the guidelines are readily available in the reagent protocol or from our website.

The TaqMan Assay portfolio is made up of over 20 million predesigned assays. These assays can be ordered as individual assays in tubes. Alternatively, TaqMan arrays contain TaqMan Assays dried down in three array formats: 384-well TaqMan array microfluidic cards (TACs), 96-well and 384-well TaqMan array plates, and Applied Biosystems™ OpenArray™ formats.

To help with choosing the right TaqMan Assays for an experiment, we developed an assay search wizard **(link)**. Querying the gene name, keyword, pathway, or disease can return a list of all the assays that meet the search criteria. The results page highlights the "best coverage" assay for each gene, publications that used the assay highlighted, and provides an opportunity to select and design a combination of assays for an array format. Furthermore, predesigned arrays are available that have been prespotted with gene expression assays targeting common pathways, diseases, and gene families, including several that are important in cancer research.

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 6). 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 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 system detect and

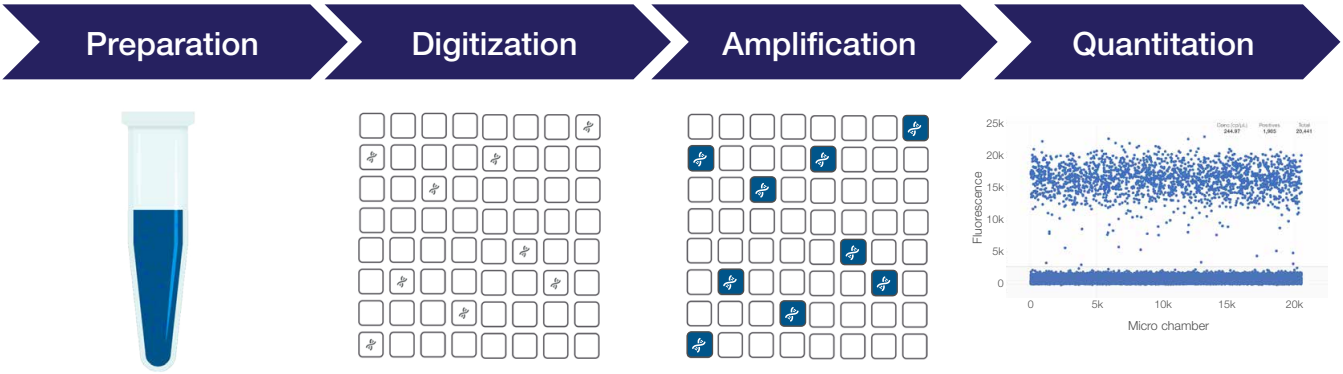


Figure 6. Basics of a digital PCR assay. dPCR reactions on the QuantStudio Absolute Q dPCR system make use of Absolute Q dPCR assays (TaqMan chemistry) to detect a target sequence. In dPCR assays, the sample is diluted and loaded into a matrix containing thousands of individual reaction chambers. If 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.

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 Assay 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.

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

References

1. https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2Fmanuals%2Fcms_042107.pdf
2. Hurst CD, Zuiverloon TC, Hafner C, Zwarthoff EC, Knowles MA. A SNaPshot assay for the rapid and simple detection of four common hotspot codon mutations in the PIK3CA gene. BMC Res Notes. 2009;2:66. Published 2009 Apr 29. doi:10.1186/1756-0500-2-66
3. Reyes GX, Schmidt TT, Kolodner RD, Hombauer H. New insights into the mechanism of DNA mismatch repair. Chromosoma. 2015;124(4):443-462. doi:10.1007/s00412-015-0514-0
4. Kang S, Na Y, Joung SY, Lee SI, Oh SC, Min BW. The significance of microsatellite instability in colorectal cancer after controlling for clinicopathological factors. Medicine (Baltimore). 2018;97(9):e0019. doi:10.1097/MD.0000000000010019
5. Marabelle A, Le DT, Ascierto PA, et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair-deficient cancer: Results From the Phase II KEYNOTE-158 Study. J Clin Oncol. 2020;38(1):1-10. doi:10.1200/JCO.19.02105
6. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res. 1998;58(22):5248-5257.
7. https://www.thermofisher.com/us/en/home/clinical/clinical-genomics/molecular-oncology-solutions/microsatellite-instability-assay.html
8. https://assets.thermofisher.com/TFS-Assets/GSD/Application-Notes/mlpa-assays-seqstudio-genetic-analyzer-app-note.pdf
9. Mazoyer S. Genomic rearrangements in the BRCA1 and BRCA2 genes. Hum Mutat. 2005;25(5):415-422. doi:10.1002/humu.20169
10. Hogervorst FB, Nederlof PM, Gille JJ, et al. Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. Cancer Res. 2003;63(7):1449-1453.
11. Nygren AO, Ameziane N, Duarte HM, et al. Methylation-specific MLPA (MS-MLPA): simultaneous detection of CpG methylation and copy number changes of up to 40 sequences. Nucleic Acids Res. 2005;33(14):e128. Published 2005 Aug 16. doi:10.1093/nar/gni127
12. Skvortsova K, Stirzaker C, Taberlay P. The DNA methylation landscape in cancer. Essays Biochem. 2019;63(6):797-811. doi:10.1042/EBC20190037
13. Huang Y, Liu Y, Zheng C, Shen C. Investigation of cross-contamination and misidentification of 278 widely used tumor cell lines. PLoS One. 2017;12(1):e0170384. Published 2017 Jan 20. doi:10.1371/journal.pone.0170384
14. Mastri M, Ramakrishnan S, Shah SD, et al. Patient derived models of bladder cancer enrich the signal of the tumor cell transcriptome facilitating the analysis of the tumor cell compartment. Am J Clin Exp Urol. 2021;9(6):416-434. Published 2021 Dec 15.
15. https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FLSG%2FApplication-Notes%2Fvariant-detection-tumor-ffpe-samples-sanger-sequencing.pdf
16. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611-622. doi:10.1373/clinchem.2008.112797

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