Profiling Cancer-Associated Genetic Variants on the MiniSeq™ System

Identify driver mutations in tumorigenesis and progression using an accessible sequencing platform from Illumina.

Highlights

- **Streamlined Sample-to-Data Tumor Profiling Solutions**
  Simple workflows with minimal hands-on time
- **Comprehensive Variant Detection with Expert-Selected and Custom Panels**
  Complete assays for somatic variant detection in a broad spectrum of cancers
- **Easy Data Analysis**
  Walk-away, intuitive data analysis performed locally or in the cloud
- **End-to-End Illumina Support**
  Expert Illumina specialists available globally to provide installation, training, and support

Figure 1: Targeted Sequencing Enables Variant Detection—Targeted sequencing enables researchers to detect somatic variants associated with a broad spectrum of cancers.

Introduction

Next-generation sequencing (NGS) technology has led to recent breakthroughs in cancer research, including associations between genomic variants and tumorigenesis.[1-4] With the ability to sequence multiple genes and samples simultaneously, high-throughput NGS offers distinct advantages over traditional capillary electrophoresis (CE)/Sanger sequencing methods and PCR-based genotyping.

Sanger sequencing is the current gold standard method and offers a quick and simple workflow. Yet it has low scalability due to increasing sample input requirements, low discovery power, and low sensitivity (down to 20% allele frequency limit of detection).[5, 6] PCR-based genotyping also offers a quick and simple workflow and high sensitivity. However, it can only interrogate a limited set of mutations, has virtually no discovery power, and is not scalable when processing multiple samples or the input requirement is high.[7] NGS offers higher sensitivity (down to 5% allele frequency limit of detection), higher discovery power with the ability to screen hundreds of genes simultaneously, and increased resolution.[8, 9] Labs worldwide are taking advantage of NGS to examine multiple cancer-associated alterations with a lower cost, faster turnaround time, and lower tissue requirements compared to Sanger/CE sequencing and PCR-based genotyping.

Using targeted sequencing methods, cancer researchers can focus on a select set of genes, gene regions, or hotspots with known or suspected associations to cancer (Figure 1). Focused panels for targeted cancer sequencing featuring expert-selected content significantly narrow the scope of a sequencing project, reducing cost and data analysis burdens. Because it assesses a predefined set of genes, targeted cancer sequencing allows for deeper coverage of these regions of interest and higher sensitivity to call variants.

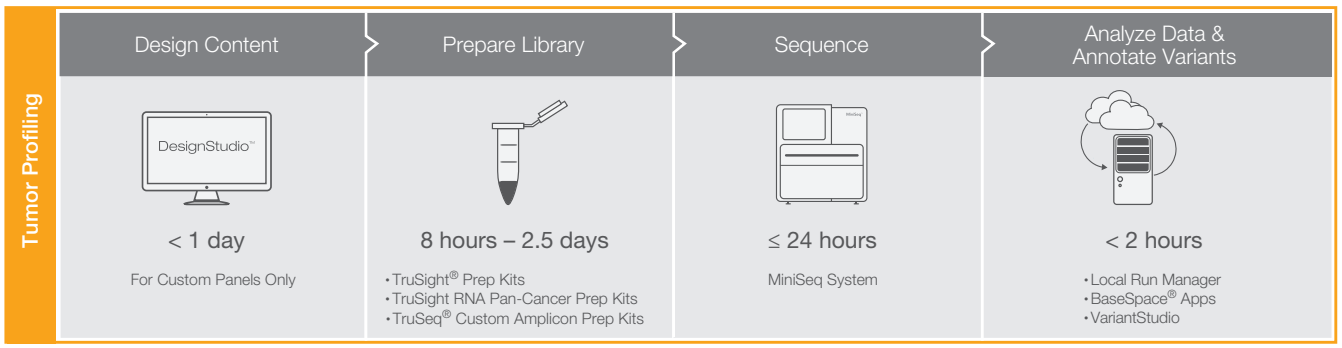

Figure 2: MiniSeq System Tumor Profiling Workflow—The integrated workflow enables streamlined library preparation, sequencing, and data analysis, allowing for cost-effective studies for a broad range of samples.

The MiniSeq System delivers a clear, complete, cost-effective toolset for targeted cancer sequencing. It harnesses industry-leading Illumina NGS technology, used in > 90% of all NGS publications, with over 26,000 peer-reviewed publications in all.*

The MiniSeq System is supported by a suite of Illumina library preparation solutions and simple, streamlined sample-to-data workflows (Figure 2). Illumina scientists developed and optimized these assays for the MiniSeq System following industry guidelines and expert recommendations. The BaseSpace platform - the Illumina genomics computing environment - enables labs to analyze, archive, and share sequencing data securely. It delivers expert-selected tools in a simple, intuitive user interface that simplifies informatics analysis. The Illumina service and support team are available globally throughout the entire workflow, from library preparation to data analysis, to offer training, assistance, and answer questions 24 hours a day, 5 days a week.

The MiniSeq System is the most affordable Illumina sequencing system to acquire, and is cost-efficient to run, even for low numbers of samples. It makes the quality and reliability of Illumina NGS accessible to labs of all sizes. With the MiniSeq System, the move to targeted cancer sequencing is easier than ever.

Tumor Profiling Applications

The MiniSeq System enables deep investigation and rapid profiling of solid tumors and hematological malignancies.

Solid Tumors

The advent of molecular profiling overcame the limitations of traditional solid tumor classification methods that relied on the morphology of tumor cells and the surrounding tissue.[10] Today, molecular profiling is a standard technique used to help classify solid tumors. Molecular profiling is included in established guidelines from the College of American Pathologists (CAP),[11] the National Comprehensive Cancer Network (NCCN),[12] and the World Health Organization.[13] In turn, genomic technology has evolved to meet molecular profiling needs. NGS provides a comprehensive method for assessing genetic mutations associated with solid tumors, including lung, colon, breast, melanoma, gastric, and ovarian cancers. NGS methods such as TruSight Tumor 15 and TruSeq Custom Amplicon Low Input preserve precious sample material by requiring lower DNA input. Moreover, NGS methods save time by assaying multiple targets simultaneously, compared to traditional iterative or reflexive methods.

Solid tumor profiling on the MiniSeq System also supports traditionally challenging formalin-fixed paraffin-embedded (FFPE) samples, such as preserved tumor tissue. The ability of Illumina targeted cancer sequencing solutions to accommodate FFPE DNA grants researchers access to the abundance of information contained in these samples.

Hematological Malignancies

The many stages of hematopoietic differentiation provide multiple opportunities for mutations that lead to distinct cancer subtypes.[14] For this reason, molecular evidence of a clonal process is critical to understanding disease etiology and how cancer subtypes relate to therapeutic options and prognosis. Current methods for assessing myeloid malignancies can be effective, but are time-consuming and can be expensive when looking at multiple variants, and may not determine the underlying genetic cause of the disease.

In contrast to traditional single-gene methods, NGS offers advancements in sensitivity and scale, enabling rapid and accurate profiling of hematological malignancies. NGS methods such as TruSight Myeloid can assess many relevant genes and identify multiple classes of genetic mutations at one time. The TruSight Myeloid panel also delivers high sensitivity for greater visibility into important drivers of hematological cancer.[15]

TruSight RNA Pan-Cancer Panel

The TruSight RNA Pan-Cancer panel enables researchers to assess many variants in multiple cancer types, including both hematological malignancies and solid tumors. By covering a broad range of genes, TruSight RNA Pan-Cancer delivers discovery power, providing insight into the mutational changes driving malignancies. Targeted DNA panels have a limited ability to detect gene fusions, one of the most frequent mutational changes in cancers.[16, 17] Benefits of TruSight RNA Pan-Cancer include:

- Detection of gene fusions (including those with novel fusion gene partners) and fusion transcripts (more easily and reliably identified by RNA analysis)[18]
- Confirmation that DNA variants are truly expressed in the cancer being studied (and therefore are relevant targets of interest)
- Identification of aberrantly expressed genes with no DNA evidence (due to altered epigenetic state)

TruSight RNA Pan-Cancer is an excellent companion to Illumina targeted DNA panels, enabling researchers to assess gene expression profiles, detect somatic variants and gene fusions, and confirm expression of somatic variants in all cancer types. TruSight RNA Pan-Cancer is compatible with the MiniSeq system providing an economical solution for the assessment of 1385 genes in all cancer types from both fresh and FFPE samples.

Simple and Streamlined Workflows

MiniSeq System workflows simplify tumor profiling and enable researchers to maximize productivity (Figure 2). Researchers can choose from a suite of assays, enabling targeted cancer sequencing studies to be tailored for interrogation of genomic alterations in a broad spectrum of cancers. The MiniSeq System supports the following solutions:

- TruSight Tumor 15 – Focused panel to assess 15 genes with relevant solid tumor somatic variants
- TruSight Myeloid – Fixed panel to assess exonic regions of 15 full genes and key hotspots of 39 additional genes in myeloid malignancies
- TruSight RNA Pan-Cancer – Comprehensive assessment of gene expression, variant, and fusion detection in 1385 oncology-related genes
- TruSeq Custom Amplicon Low Input Dual Strand – User-defined panels for customizable tumor profiling of up to 1536 amplicons

* Data calculations on file. Illumina, Inc. 2015.

Library Preparation

Illumina methods for library preparation include capture-based target enrichment and amplicon generation (Figure 3). With target enrichment, specific regions of interest are captured by hybridization to biotinylated probes, then isolated by magnetic pulldown. This highly multiplexed approach enables a wide range of applications for the discovery, validation, or screening of somatic variants. The TruSight RNA Pan-Cancer panel uses this method for library preparation.

Amplicon generation involves 2 submethods depending on the product chosen (Figure 3). TruSeq Custom Amplicon Low Input and TruSight Myeloid employ a hybridization-extension-ligation approach, creating a single strand template from a double-stranded DNA population that is later amplified via PCR. TruSight Tumor 15 utilizes a multiplexed PCR approach, amplifying the predefined targeted regions from genomic DNA. Sequencing of resulting amplicons from either method is useful for the discovery of rare somatic mutations in complex samples such as heterogenous tumors mixed with germline DNA.[19]

The TruSeq Custom Amplicon Low Input Dual Strand approach is an additional feature useful for somatic tumor profiling. This approach creates a mirror amplicon to the original amplicon generated during library preparation. This virtually reduces all idiosyncratic and systemic "noise" resulting from FFPE deamination, storage oxidation, and any other artifacts that arise in the handling, preparation, and resequencing process. The resulting DNA interrogation provides an accurate picture in profiling analysis.

Custom Panel Design

The TruSeq Custom Amplicon Low Input assay empowers researchers to create a custom panel targeting genes and regions of interest using Illumina DesignStudio™ software, a free, easy-to-use, online tool that provides optimized coverage (Figure 4). DesignStudio produces TruSeq Custom Amplicon Low Input probes with an average of > 94% *in silico* coverage across all gene sets. Illumina Concierge services offer support for optimization of probe design, functional evaluation and optimization of custom panels, and increasing target coverage.†

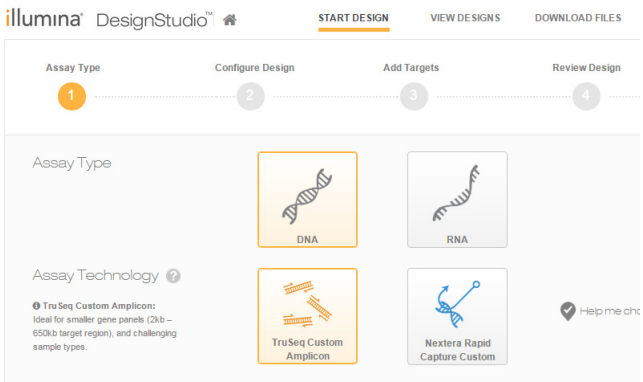

Figure 4: Custom Probe Design—Researchers can use DesignStudio to visualize targeted genomic regions and attempted amplicons to assess design coverage and score.

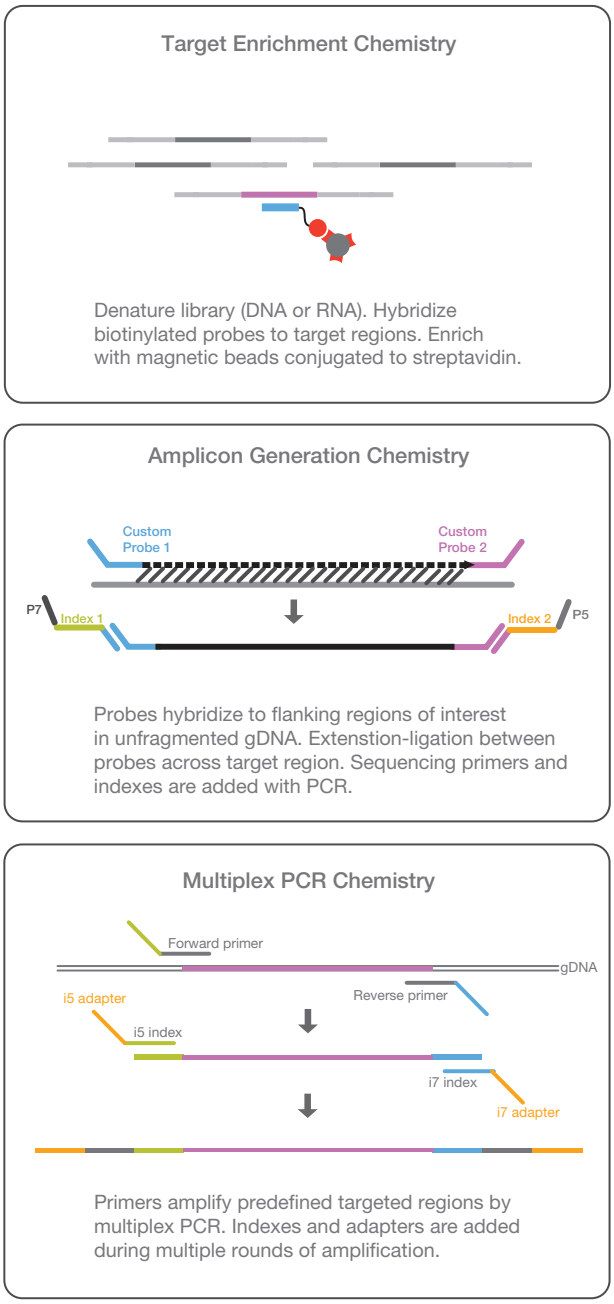

Figure 3: Library Preparation Methods—Illumina methods for sequencing library preparation include targeted enrichment, amplicon generation, and multiplex PCR.

† For more information on Illumina Concierge service, contact an Illumina representative.

Sequencing on the MiniSeq System

Whether using target enrichment or amplicon generation methods for library preparation, after sample libraries are prepared they can be easily sequenced on the MiniSeq System (Figure 5). It integrates clonal amplification and sequencing into a fully automated process on a single instrument. This eliminates the need to purchase and operate expensive, specialized equipment.

The MiniSeq System features load-and-go operation and an intuitive user interface that provides simple, step-by-step guidance through each stage of the sequencing run. It takes less than 5 minutes to load and set up a MiniSeq System. Sequencing runs can be completed in ≤ 24 hours. MiniSeq reagent kits are available in Mid-Output and High-Output formats, allowing optimization of study designs based on read-length, sample number, and output requirements.

Figure 5: MiniSeq System—The MiniSeq System harnesses the latest advances in SBS chemistry and an easy, integrated workflow.

Simplified Data Analysis and Bioinformatics

Data analysis with the MiniSeq System requires no informatics expertise or command-line experience. It features Local Run Manager software, an onboard system for creating a run, monitoring status, and automated sequencing data analysis post-run. Local Run Manager features a modular design that allows users to install and update individual analysis modules as needed, which generate simple reports for various sequencing applications.

In addition, sequencing data generated with the MiniSeq System can be instantly transferred, stored, and analyzed in the BaseSpace Computing Environment (Cloud-based or Onsite). BaseSpace Applications (Apps) provide expert-preferred data analysis tools in an intuitive, click-and-go user interface designed for informatics novices (Figure 6). These Apps support a range of common sequencing data analysis needs such as alignment, variant calling, and more. The BaseSpace ecosystem provides one of the largest collections of commercial and open-source analysis tools currently available. VariantStudio enables rapid filtering, identification, and annotation of disease-associated variants in flexible, structured reports (Figure 7).

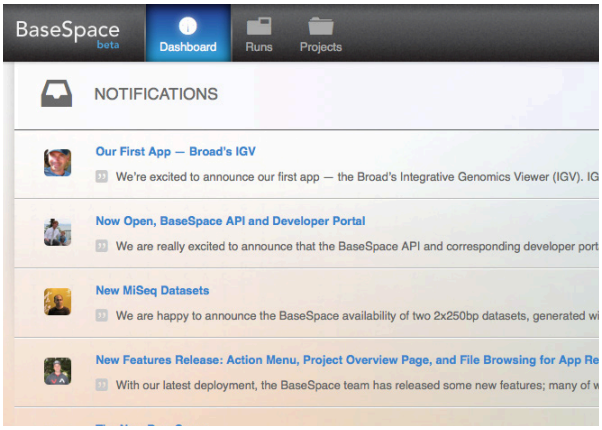

Figure 6: BaseSpace Dashboard—The BaseSpace Environment features an intuitive, click-and-go user interface to empower any researcher to perform their own informatics.

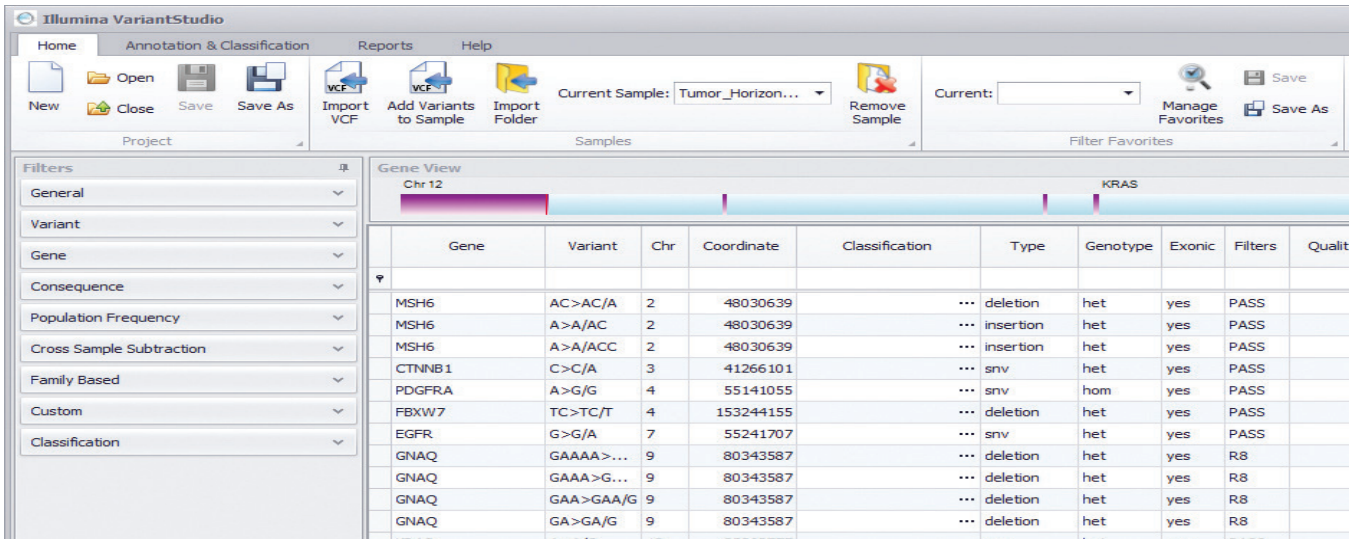

Figure 7: VariantStudio—VariantStudio software features an intuitive user interface that enables easy data analysis and exploration, without requiring informatics expertise. It aggregates information from a broad range of sources into a single database for comprehensive annotation of genomic data. Flexible report generation summarizes and annotates results.

Demonstrated Workflow – TruSight Tumor 15

Library Preparation

The TruSight Tumor 15 library preparation method enables multiplex PCR, which produces higher coverage uniformity and reduces the presence of primer dimers and FFPE-induced artifacts. This results in high accuracy and sensitivity for somatic variant analysis.[20]

The TruSight Tumor 15 Protocol Guide is an easy-to-follow protocol for preparing DNA sequencing libraries, including DNA extraction, quantification, and in-process qualification steps. It leads users through each step of library preparation, listing necessary reagents and indicating safe stopping points.

Sample libraries were prepared from 20 ng of total input DNA following this protocol. Sequencing data sets were generated from 3 DNA controls of known variant compositions and 10 FFPE-extracted DNA samples from lung, colon, melanoma, and breast tumors previously characterized using the MiSeq® System.

Sequencing on the MiniSeq System

TruSight Tumor 15 sample library pools consisted of 8 samples per High-Output run (16 total; mix of DNA controls and FFPE-extracted tumor samples). Libraries were loaded onto the MiniSeq instrument along with the reagent cartridge and flow cell. Automated cluster generation and paired-end sequencing with a 300-cycle read was set up with Local Run Manager and carried out without any further user intervention, targeting 97% of bases at 500× coverage and taking 24 hours.

Data Analysis

Primary analysis (image analysis, base calling) was performed on the MiniSeq System. Additional analysis (demultiplexing, alignment, and variant calling) was performed with the TruSight Tumor 15 Local Run Manager Module (Figure 8) and VariantStudio.

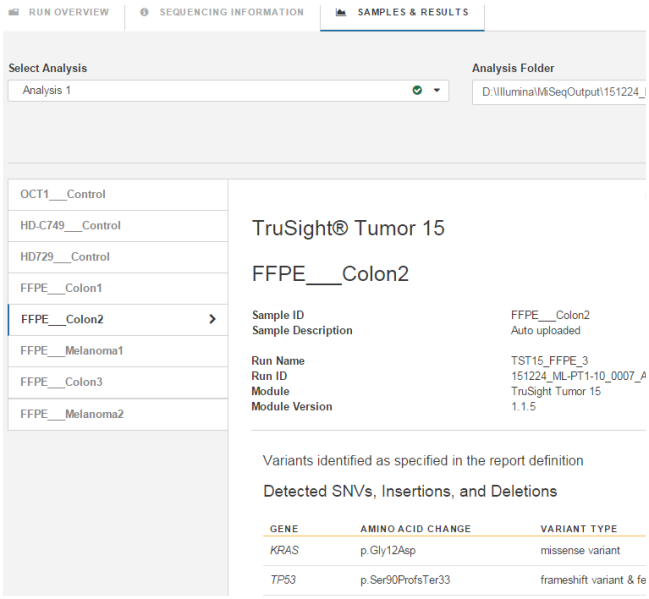

Figure 8: Local Run Manager—Local Run Manager software allows users to create a sequencing run, monitor status, and view results. Onboard data analysis is automatically performed upon run completion.

Results and Discussion

Running the TruSight Tumor 15 sequencing panel on the MiniSeq System achieves at least 95% of bases covered at ≥ 500×, which gives confidence in variant calling (Table 1). It enables detection of variants down to 1% (Table 2). TruSight Tumor 15 run on the MiniSeq System enables variant detection in many different sample types, including low quality FFPE samples (Table 3). Moreover, data generated on the MiniSeq system shows 100% concordance with previously characterized FFPE samples.

Table 1: TruSight Tumor 15 Coverage

Sample ID	Quality	% of Bases ≥ 500×	Amplicon Mean Coverage
FFPE_Colon1	Medium	99.7%	24,219×
FFPE_Colon2	Low	99.9%	20,763×
FFPE_Colon3	Low	99.2%	35,270×
FFPE_Colon4	High	100.0%	18,357×
FFPE_Colon5	High	100.0%	15,769×
FFPE_Melanoma1	Medium	99.7%	32,707×
FFPE_Melanoma2	Low	99.1%	41,640×
FFPE_Melanoma3	High	100.0%	17,285×
FFPE_Melanoma4	Low	95.7%	10,177×
FFPE_Breast1	High	99.1%	15,501×

Table 2: TruSight Tumor 15 Performance with Characterized Horizon Sample

Gene	Mutation	Reported Frequency	Detected Frequency	Coverage
BRAF	V600E	10.5%	12.3%	55,457×
KIT	D816V	10.0%	10.3%	5463×
EGFR	ΔE746-A750	2.0%	2.1%	3553×
EGFR	L858R	3.0%	4.1%	1761×
EGFR	T790M	1.0%	1.2%	18,927×
EGFR	G719S	24.5%	25.6%	41,805×
KRAS	G13D	15.0%	15.3%	6745×
KRAS	G12D	6.0%	7.2%	6742×
NRAS	Q61K	12.5%	11.2%	13,154×
PIK3CA	H1047R	17.5%	18.8%	21,522×
PIK3CA	E545K	9.0%	7.8%	13,250×

DNA from the HD-C749 formalin-fixed cell line (Horizon Diagnostics) containing known variants was evaluated using the TruSight Tumor 15 assay and sequenced on the MiniSeq System. Variants were analyzed using VariantStudio. HD-C749 showed 100% concordance over 7 different runs.

Table 3: TruSight Tumor 15 Performance with FFPE Tumor Samples

Sample	Reported Mutation	Detected Mutation	Detected Frequency	Coverage
FFPE_Colon1	*KRAS* G12S	*KRAS* G12S	22.3%	21,134×
FFPE_Colon2	*KRAS* G12D	*KRAS* G12D	11.5%	4322×
FFPE_Colon3	*BRAF* V600E	*BRAF* V600E	25.5%	140,040×
FFPE_Colon4	*KRAS* G12V	*KRAS* G12V	33.4%	5256×
FFPE_Colon5	*KRAS* G13D	*KRAS* G13D	33.0%	4156×
FFPE_Melanoma1	*BRAF* V600E	*BRAF* V600E	65.7%	106,924×
FFPE_Melanoma2	*KRAS* G12R	*KRAS* G12R	4.1%	54,622×
FFPE_Melanoma3	*BRAF* V600E	*BRAF* V600E	93.5%	61,838×
FFPE_Melanoma4	*BRAF* V600K	*BRAF* V600K	22.2%	8075×
FFPE_Breast1	*AKT1* E17K	*AKT1* E17K	37.3%	56,438×

DNA from FFPE tumor samples was extracted and then evaluated using the TruSight Tumor 15 assay and sequenced on the MiniSeq System. Variants were analyzed using VariantStudio. All 10 FFPE samples had 100% variant concordance.

Summary

The MiniSeq System delivers a clear, complete, cost-effective toolset for targeted cancer sequencing. It provides deep investigation and rapid profiling of solid and hematological tumors. Researchers can choose from a suite of proven Illumina assays developed with expert-selected content and following industry guidelines. Or they can work with Illumina scientists to develop their own custom panels. With the MiniSeq System, labs of any size can examine multiple cancer-associated alterations with lower cost, faster turnaround time, and lower tissue requirements compared to iterative Sanger/CE sequencing or PCR-based genotyping.

Learn More

To learn more about targeted cancer sequencing, visit:
www.illumina.com/tumorprofiling

Ordering Information

System Name	Catalog No.
MiniSeq System	SY-420-1001
MiniSeq High Output Kit (75 Cycles)	FC-420-1001
MiniSeq High Output Kit (150 Cycles)	FC-420-1002
MiniSeq High Output Kit (300 Cycles)	FC-420-1003
MiniSeq Mid Output Kit (300 Cycles)	FC-420-1004
TruSight Tumor 15	
TruSight Tumor 15 Includes library preparation consumables, oligos, and indexes sufficient for 24 samples	OP-101-1002
TruSight Tumor 15 MiniSeq Kit Includes library preparation panel, 3 MiniSeq High Output Kits (300 Cycles), sufficient for 24 samples	20005610
TruSight RNA Pan-Cancer	
TruSight RNA Pan-Cancer Panel Set A Includes library preparation consumables and oligos for 48 samples with 12 indexes	RS-303-1002
TruSight RNA Pan-Cancer Panel Set B Includes library preparation consumables and oligos for 48 samples with 12 indexes	RS-303-1003
TruSight RNA Pan-Cancer Set A MiniSeq Kit Includes library preparation consumables and oligos for 48 samples with 12 indexes, plus 6 MiniSeq High Output Kits (150 Cycles)	20005611
TruSight Myeloid	
TruSight Myeloid Sequencing Panel (96 samples)	FC-130-1010
TruSeq Custom Amplicon Index Kit	FC-130-1003
TruSeq Index Plate Fixture and Collar Kit	FC-130-1007
TruSeq Custom Amplicon Filter Plate	FC-130-1006
TruSeq Custom Amplicon Low Input	
TruSeq Custom Amplicon Low Input Kit (96 samples)	FC-134-2001
TruSeq Custom Amplicon Low Input Kit (16 samples)	FC-134-2002
TruSeq FFPE DNA Library Prep QC Kit	FC-121-9999
TruSeq Custom Amplicon Index Kit	FC-130-1003

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Maximize Performance and Productivity with Illumina Services, Training, and Consulting

Whether immediate help is needed during an instrument run, or in-depth consultations are required for sophisticated workflows, Illumina can help. Illumina service and support teams provide a full suite of expedient, customized solutions from initial trainings, to instrument support, and ongoing NGS consultations.

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