



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.<sup>10</sup> 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),<sup>11</sup> the National Comprehensive Cancer Network (NCCN),<sup>12</sup> and the World Health Organization.<sup>13</sup> 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.<sup>14</sup> 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.<sup>15</sup>

#### 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.<sup>16, 17</sup> 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)<sup>18</sup>
- 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

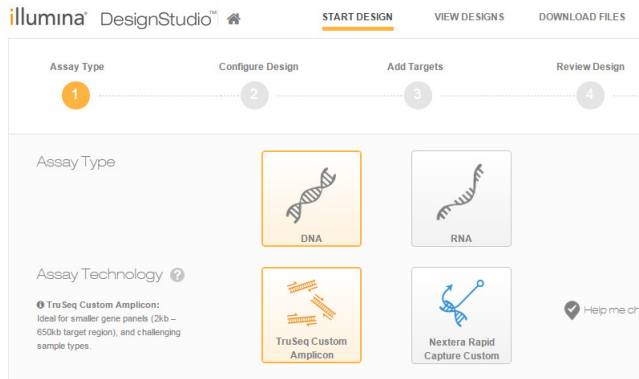
Illumina methods for library preparation include capture-based target enrichment and amplicon generation (Figure 3). With target enrichment, specific regions of interest are captured by hybridization to biotinylated probes, then isolated by magnetic pull-down. This highly multiplexed approach enables 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.<sup>19</sup>

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.

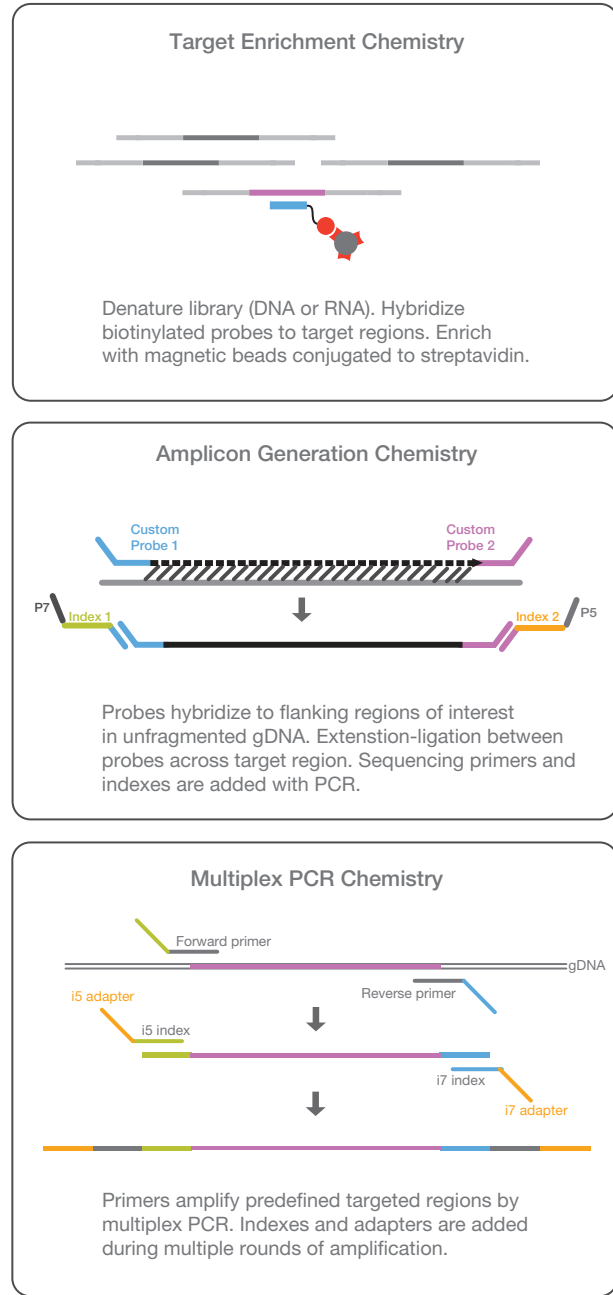
### Custom Panel Design

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.<sup>†</sup>



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

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



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

### 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).

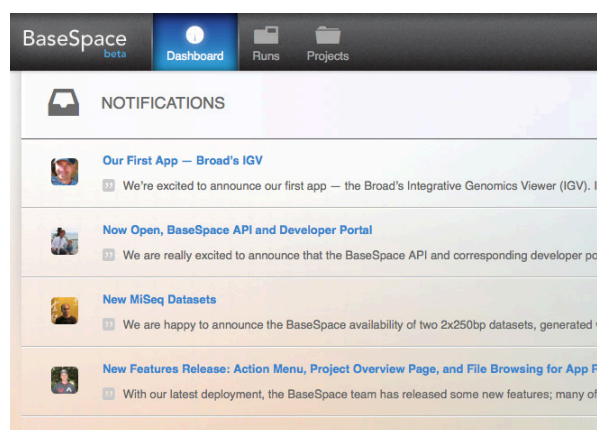


Figure 6: BaseSpace Dashboard—The BaseSpace Environment features an intuitive, click-and-go user interface to empower any researcher to perform their own informatics.

Gene	Variant	Chr	Coordinate	Classification	Type	Genotype	Exonic	Filters	Qualit
MSH6	AC>AC/A	2	48030639	...	deletion	het	yes	PASS	
MSH6	A>A/AC	2	48030639	...	insertion	het	yes	PASS	
MSH6	A>A/ACC	2	48030639	...	insertion	het	yes	PASS	
CTN1B1	C>C/A	3	41266101	...	snv	het	yes	PASS	
PDGFRA	A>G/G	4	55141055	...	snv	hom	yes	PASS	
FBXW7	TC>TC/T	4	153241155	...	deletion	het	yes	PASS	
EGFR	G>G/A	7	55241707	...	snv	het	yes	PASS	
GNAQ	GAAA>...	9	80343587	...	deletion	het	yes	R8	
GNAQ	GAAA>G...	9	80343587	...	deletion	het	yes	R8	
GNAQ	GAA>GAA/G	9	80343587	...	deletion	het	yes	R8	
GNAQ	GA>GA/G	9	80343587	...	deletion	het	yes	R8	

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.<sup>20</sup>

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.

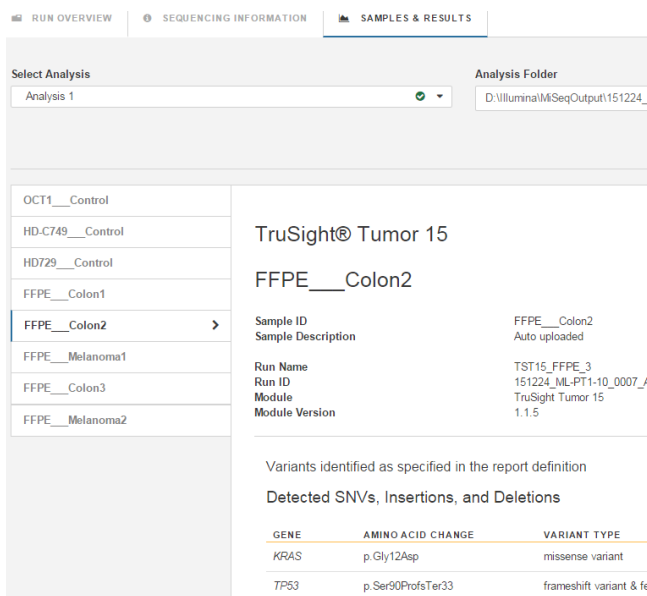
Sample libraries were prepared from 20 ng of total input DNA following this protocol. Sequencing data sets were generated from 3 DNA controls of known variant compositions and 10 FFPE-extracted DNA samples from lung, colon, melanoma, and breast tumors previously characterized using the MiSeq® System.

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

**Table 2: TruSight Tumor 15 Performance with Characterized Horizon Sample**

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

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	<i>KRAS</i> G12S	<i>KRAS</i> G12S	22.3%	21,134x
FFPE_Colon2	<i>KRAS</i> G12D	<i>KRAS</i> G12D	11.5%	4322x
FFPE_Colon3	<i>BRAF</i> V600E	<i>BRAF</i> V600E	25.5%	140,040x
FFPE_Colon4	<i>KRAS</i> G12V	<i>KRAS</i> G12V	33.4%	5256x
FFPE_Colon5	<i>KRAS</i> G13D	<i>KRAS</i> G13D	33.0%	4156x
FFPE_Melanoma1	<i>BRAF</i> V600E	<i>BRAF</i> V600E	65.7%	106,924x
FFPE_Melanoma2	<i>KRAS</i> G12R	<i>KRAS</i> G12R	4.1%	54,622x
FFPE_Melanoma3	<i>BRAF</i> V600E	<i>BRAF</i> V600E	93.5%	61,838x
FFPE_Melanoma4	<i>BRAF</i> V600K	<i>BRAF</i> V600K	22.2%	8075x
FFPE_Breast1	<i>AKT1</i> E17K	<i>AKT1</i> E17K	37.3%	56,438x

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.



