Interrogating Cancer-Predisposing Germline Variants on the MiniSeq™ System

Identify predisposition genomic variants linked to cancer using the most accessible sequencing platform from Illumina.

Highlights

- **Streamlined Sample-to-Data Germline Testing Solutions**
  Simple workflows with minimal hands-on time
- **Comprehensive Variant Detection with Expert-Selected and Custom Panels**
  Complete assays to detect predisposition genes, variants, and hotspots 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

Introduction

Next-generation sequencing (NGS) technology has led to recent breakthroughs in cancer research, including associations between genomic variants and cancer predisposition.[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 analytical sensitivity.[5, 6] PCR-based genotyping also offers a quick and simple workflow and high analytical 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 analytical sensitivity (down to 1% allele frequency limit of detection), higher discovery power with the ability to profile hundreds of genes simultaneously, and increased resolution.[8, 9] Labs worldwide are taking advantage of NGS to examine multiple cancer-predisposing alterations with a lower cost, faster turnaround time, and lower tissue requirements compared to Sanger/CE sequencing and PCR-based genotyping.

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

Using targeted sequencing methods, cancer researchers can focus on a select set of genes, gene regions, or hotspots with known or suspected predispositions toward cancer (Figure 1). Focused panels for targeted cancer sequencing featuring expert-selected content

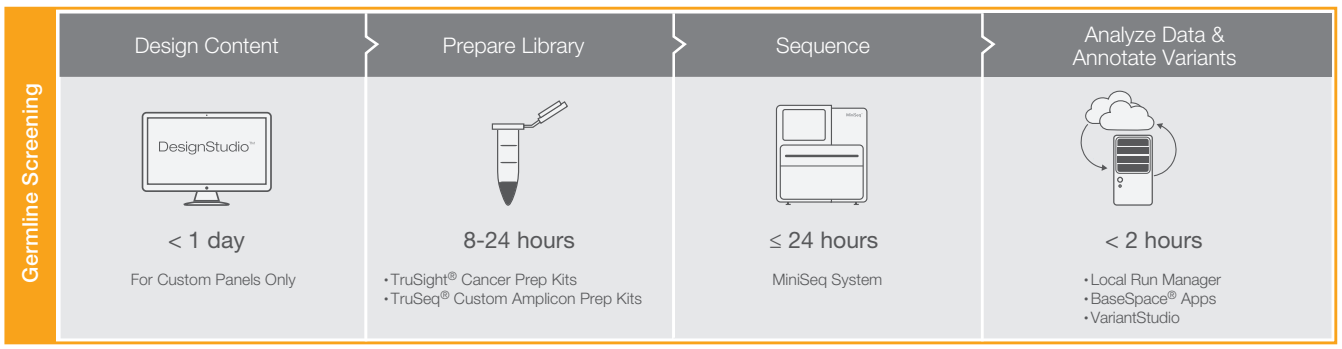

Figure 2: MiniSeq System Germline Screening Workflow—The integrated workflow enables streamlined library preparation, sequencing, and data analysis, allowing for cost-effective studies for a broad range of samples.

significantly narrow the scope of a sequencing project, reducing cost and data analysis burdens. Because it assesses a limited set of genes, targeted cancer sequencing allows for deeper coverage of these regions of interest.

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 around the world, 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). These were developed by Illumina scientists and optimized for the MiniSeq System following industry guidelines and expert recommendations. The BaseSpace Environment enables labs to analyze, archive, and share sequencing data securely, and delivers expert-selected tools in an intuitive user interface that simplifies informatics analysis. The Illumina service and support team is 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 it 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.

Simple, Streamlined Workflows

MiniSeq System workflows simplify germline variant detection and enable researchers to maximize productivity (Figure 2). Researchers can use a fixed panel of 94 genes and 284 single nucleotide polymorphisms (SNPs) known to be associated with a predisposition toward cancer with the TruSight Cancer Sequencing Panel. Also, researchers can perform customized germline profiling with user-defined panels of up to 1536 amplicons generated with the TruSeq Custom Amplicon Low Input Library Prep Kit.

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 profiling of genetic variants, including the TruSight Cancer panel.

The amplicon generation method employed by TruSeq Custom Amplicon Low Input uses a hybridization-extension-ligation approach. This creates a single strand template from a double-stranded DNA population that is later amplified via PCR.

TruSight Cancer Library Prep

The TruSight Cancer Sequencing Panel is compatible withTruSight Rapid Capture kits, providing a single, integrated library preparation and enrichment workflow. This rapid prep requires only 50 ng of input DNA, takes 1.5 days, and eliminates the need for mechanical shearing.

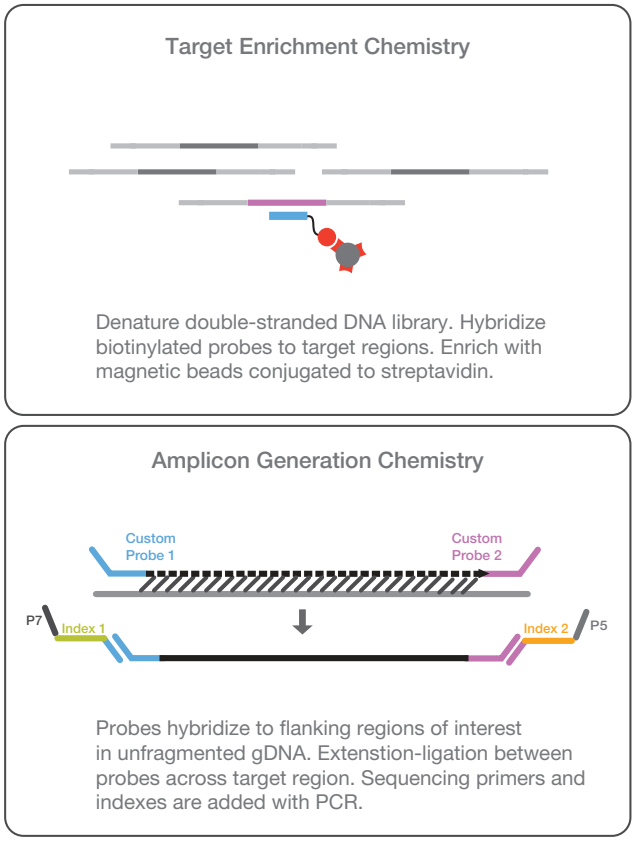

Figure 3: Library Preparation Methods — Illumina methods for sequencing library preparation include targeted enrichment and amplicon generation.

The TruSight Rapid Capture Sample Prep Guide is an easy-to-follow protocol for preparing DNA sequencing libraries. It leads users through each step of library preparation, listing necessary reagents and indicating safe stopping points.

TruSeq Custom Amplicon Low Input Library Prep

The TruSeq Custom Amplicon Low Input assay empowers researchers to create a custom amplicon 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 designs with an average of > 94% *in silico* coverage across all gene sets. Illumina Concierge services offer support for probe design, functional evaluation of custom panels, and increasing target coverage.†

TheTruSeq Custom Amplicon Low Input assay features simple, streamlined library preparation that is automation friendly. It can be completed in 6.5 hours, with only 3 hours of hands-on time. The TruSeq Custom Amplicon Low Input Library Prep Reference Guide contains everything a first-time user needs, including an overview of the protocol, tips and techniques, detailed protocol steps, kit contents, and a list of user-supplied consumables.

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

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

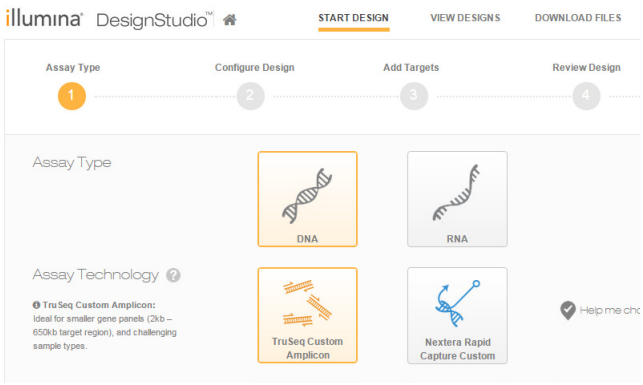

Figure 4: Custom Probe Design — Researchers can use DesignStudio to visualize targeted genomic regions and attempted amplicons to assess design coverage and score.

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 from sequencing data (Figure 7). An easy-to-use application, VariantStudio allows for flexible report generation that summarizes and annotates disease-relevant variants in a structured format.

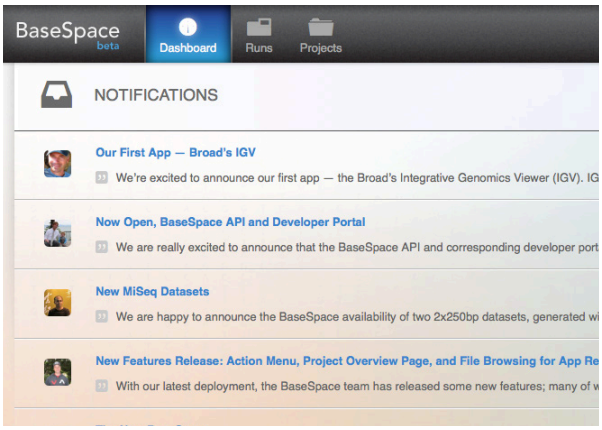

Figure 6: BaseSpace Dashboard—The BaseSpace Environment features an intuitive, click-and-go user interface to empower any researcher to perform their own informatics.

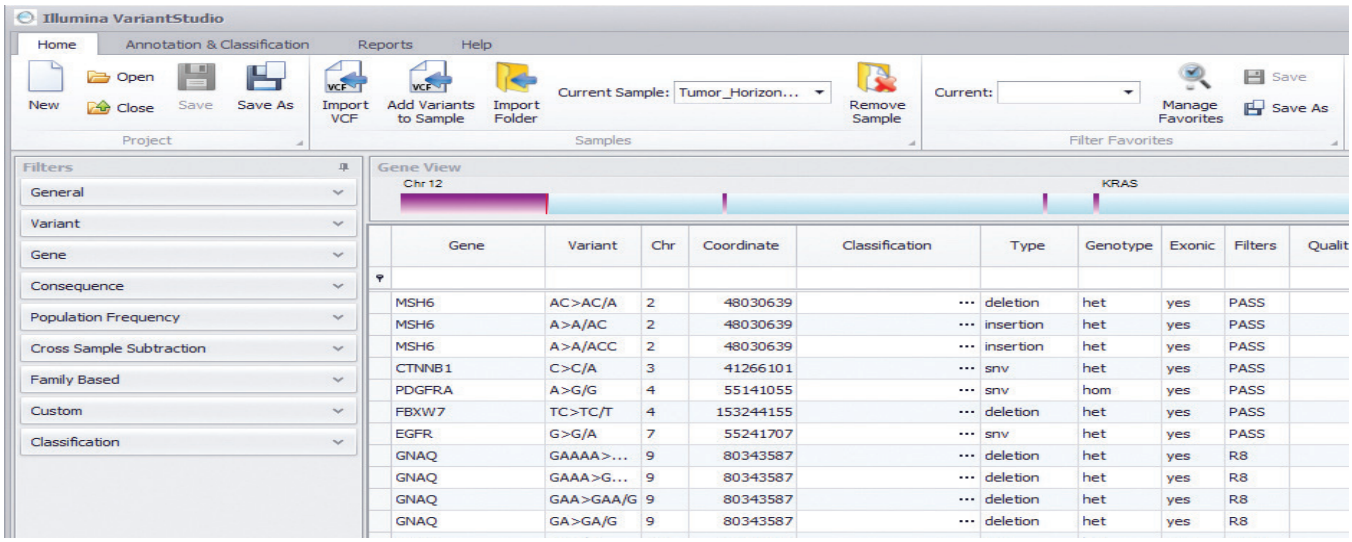

Figure 7: VariantStudio—VariantStudio software features an intuitive user interface that enables easy data analysis and exploration, without require informatics expertise. It aggregates information from a broad range of sources into a single database for comprehensive annotation of genomic data. Flexible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

Demonstrated Workflow - TruSight Cancer

Library Preparation

The TruSight Cancer Oligo Box and the TruSight Rapid Capture Library Prep Kit were used to prepare libraries. DNA samples were extracted from blood, and 50 ng of input DNA were used for each sample in the assay. TruSight Cancer libraries were prepared following the protocol included in the TruSight Rapid Capture Sample Prep Guide. Libraries were quantified and normalized before sequencing on the MiniSeq System.

Sequencing on the MiniSeq System

Sample libraries were loaded onto the MiniSeq instrument along with the reagent cartridge and flow cell. Automated clonal amplification and paired-end sequencing with a 2 × 100 bp-cycle read was carried out without any further user intervention, taking 16 hours.

Data Analysis - Alignment and Variant Calling

Sequence data was analyzed by the BWA Enrichment App in BaseSpace (Figure 8). After demultiplexing and FASTQ file generation, the software uses the Burrows-Wheeler Aligner (BWA) to align the reads against the hg19 homo sapiens reference genome to create BAM files. The Genome Analysis Toolkit (GATK) was then used to perform variant analysis for the target regions specified in the manifest file. The output of GATK are VCF files, which are text files that contain SNPs, indels, and other structural variants. Summary tables were generated to report on enrichment, variant calling, coverage, insert fragment length, and duplicates.

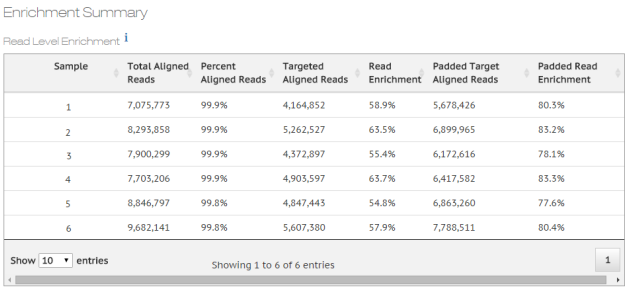

Figure 8: TruSight Cancer Data Analysis in BaseSpace—Summary table generated by the BWA Enrichment App in BaseSpace. Run metrics (aligned reads, aligned bases, and enrichment) are shown.

Results and Discussion

The TruSight Cancer Sequencing Panel provides ≥ 99% of bases covered at 20×, which provides for high confidence in variant calling (Table 1). It also enables ≥ 99% aligned reads and ≥ 77% padded read enrichment for specific and accurate sequencing (Table 1).

The Single Nucleotide Polymorphism database (dbSNP) is a public-domain archive for a broad collection of simple genetic polymorphisms.[10] As most SNVs (single nucleotide variants) and indels (insertions or deletions) are found in the dbSNP database, TruSight Cancer sequencing data produces highly relevant research data and increases the discovery power for identifying new predisposition genetic variants (Figure 9).

Table 1: Coverage Summary for the TruSight Cancer Sequencing Kit

Sample	Mean Region Coverage Depth	Uniformity of Coverage (% > 0.2× mean)	Target Coverage (1×)	Target Coverage (10×)	Target Coverage (20×)	Target Coverage (50×)
1	1068×	94.5%	99.9%	99.4%	99.1%	98.6%
2	1465×	95.2%	100.0%	99.6%	99.5%	99.1%
3	1355×	91.7%	99.7%	99.2%	99.0%	98.5%
4	1248×	97.4%	100.0%	99.8%	99.7%	99.4%
5	1129×	97.4%	100.0%	99.9%	99.6%	99.4%
6	1262×	91.5%	99.8%	99.3%	99.0%	98.4%

Aggregate summary (BWA enrichment) of a representative run on the MiniSeq System with 6 samples using the TruSight Cancer Sequencing Kit.

Variant Summary

SNVs i

Sample	SNVs	SNV Het/Hom Ratio	SNV Ts/Tv Ratio	SNVs (Percent Found in dbSNP)
1	347	6.2	2.8	95.1%
2	291	1.6	2.6	100.0%
3	357	8.2	2.6	95.0%
4	291	1.6	2.6	100.0%
5	355	7.7	2.6	94.9%
6	346	7	2.7	94.5%

Show 10 entries Showing 1 to 6 of 6 entries 1

Indels i

Sample	Indels	Indel Het/Hom Ratio	Indels (Percent Found in dbSNP)
1	6	5	66.7%
2	2	1	50.0%
3	2	1	100.0%
4	1	0	100.0%
5	3	2	100.0%
6	3	2	100.0%

Show 10 entries Showing 1 to 6 of 6 entries 1

Figure 9: TruSight Cancer Variant Summary—The TruSight Cancer Sequencing Panel enables accurate and specific sequencing, provides high confidence in variant calling, and increases discovery power.

Summary

The MiniSeq System delivers a clear, complete, cost-effective toolset for germline screening of predisposition genomic variants linked to cancer. Researchers can choose 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-predisposing alterations with lower cost, faster turnaround time, and lower tissue requirements compared to CE/Sanger sequencing and PCR-based genotyping methods.

Learn More

To learn more about targeted cancer sequencing, visit: www.illumina.com/applications/cancer/research/cancer-dna-sequencing/targeted-cancer-seq.html.

Ordering Information

Product	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 Cancer	**Catalog No.**
TruSight Cancer Sequencing Panel Includes oligos sufficient for 4 enrichments and up to 48 samples	FC-121-0202
TruSight Cancer MiniSeq Bundle Includes oligos, library prep panel, and 2 MiniSeq High Output Kits (300 Cycles), sufficient for 48 samples	20005612
TruSight Rapid Capture (1 index, 8 samples)	FC-140-1101
TruSight Rapid Capture (2 indexes, 8 samples)	FC-140-1102
TruSight Rapid Capture (4 indexes, 16 samples)	FC-140-1103
TruSight Rapid Capture (24 indexes, 48 samples)	FC-140-1104
TruSight Rapid Capture (24 indexes, 96 samples)	FC-140-1105
TruSight Rapid Capture (96 indexes, 288 samples)	FC-140-1106
TruSeq Custom Amplicon Low Input	**Catalog No.**
TruSeq Custom Amplicon Low Input Kit (96 samples)	FC-134-2001
TruSeq Custom Amplicon Low Input Kit (16 samples)	FC-134-2002
TruSeq Custom Amplicon Index Kit	FC-130-1003

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