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TruSight® Tumor 15

Evidence-based gene content and a comprehensive workflow solution for research of common somatic variants in FFPE solid tumor samples.

Highlights

- **Increased Lab Efficiency**
  Simple, comprehensive workflow with detailed QC steps and a simple, predefined variant report
- **Enhanced Productivity**
  Rapid turnaround with only 3.5 hours of hands-on time, going from DNA to data in approximately 36 hours
- **Relevant Gene Content for Solid Tumors**
  Somatic variants selected from relevant industry guidelines, key opinion leaders, and pharmaceutical research
- **Sensitive Variant Detection from Low DNA Input**
  Accurate somatic variant detection of 5% allele frequency using 20 ng DNA from FFPE tissue samples

Introduction

Next-generation sequencing (NGS) technology has led to recent breakthroughs in cancer research, including associations between several genomic variants and tumorigenesis.[1] Labs worldwide are using NGS to examine multiple cancer-associated alterations in parallel, resulting in a lower cost and faster turnaround time compared to iterative single-gene assays. TruSight Tumor 15 uses NGS to assess the 15 most commonly mutated genes in solid tumors. The panel is part of a streamlined workflow solution that accelerates tumor profiling, going from DNA sample to results in approximately 36 hours. TruSight Tumor 15 enables labs to apply NGS for accurate, affordable tumor analysis.

High Operational Efficiency

TruSight Tumor 15 features a comprehensive workflow that simplifies NGS, enabling seamless integration into existing lab practices. The panel evaluates 15 significant genes in a single assay, offering a more efficient approach to tumor profiling than single-gene analysis. Samples can be multiplexed so that up to 8 samples can be run in parallel.

Simple, Comprehensive Workflow

The TruSight Tumor 15 workflow extends beyond library preparation to include tissue requirements, DNA extraction and quantification recommendations, and in-process qualification steps. This comprehensive workflow can be easily integrated into laboratory workflows, going from DNA to data in approximately 36 hours (Figure 1). Library preparation is completed in 7 hours, with only 3.5 hours of hands-on time. DNA libraries are loaded directly onto a reagent cartridge for sequencing on the MiSeq® System or MiSeqDx® System (run in research mode) using MiSeq v3 chemistry, without additional processing. Data are analyzed using on-instrument MiSeq Reporter software.

User-Friendly Analysis and Reporting

Results from MiSeq Reporter software are presented in a predefined report denoting the presence or absence of somatic variants. Further options for streamlined variant filtering, classification, and annotation are available through VariantStudio software. The simplified analysis tools included with the panel allow any researcher to explore and understand sequencing data, with minimal bioinformatics resources.

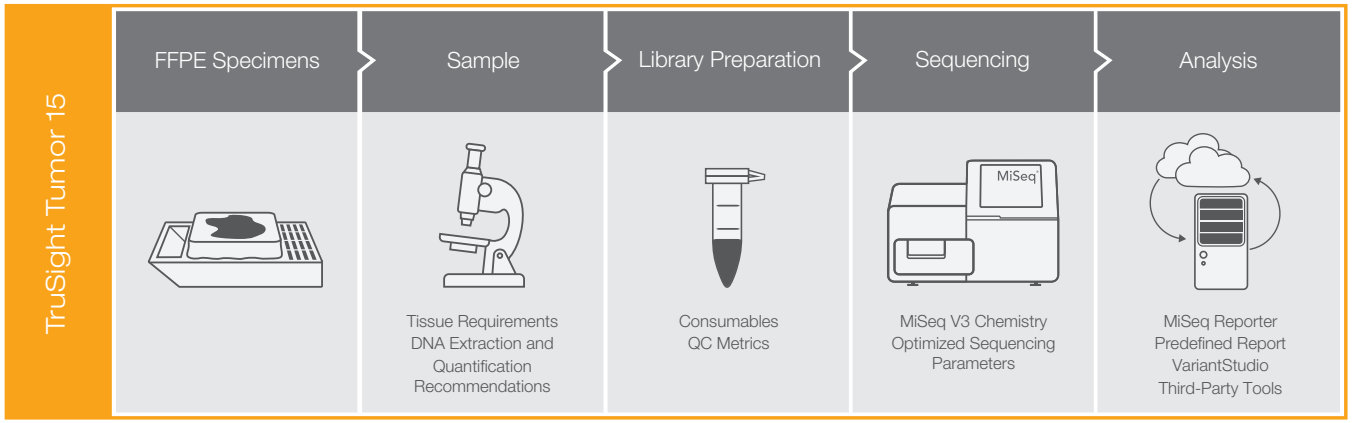

Figure 1: TruSight Tumor 15 Workflow—TruSight Tumor 15 is optimized for seamless integration into current lab workflows, going from extracted DNA to data in approximately 36 hours.

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Relevant Gene Content

The content for TruSight Tumor 15 focuses on relevant regions in 15 cancer-associated genes (Table 1). The gene content was carefully selected to include content cited by industry organizations such as the National Comprehensive Cancer Network (NCCN)[2] and the European Society for Medical Oncology (ESMO).[3] Independent consortia publications and late-stage pharmaceutical research also influenced the design of TruSight Tumor 15.[4,5] These genes and gene regions include single nucleotide variants (SNV) and insertions and deletions (indels) that have demonstrated involvement in solid tumors. By harnessing the expertise of recognized authorities in cancer research, TruSight Tumor 15 enables researchers to focus resources on relevant genes that are most likely to play a role in tumorigenesis.

Table 1: Gene Content on TruSight Tumor 15

AKT1	*GNA11*	*NRAS*
BRAF	*GNAQ*	*PDGFRA*
EGFR	*KIT*	*PIK3CA*
ERBB2	*KRAS*	*RET*
FOXL2	*MET*	*TP53*

Optimized for Low DNA Input and FFPE Tissue

Archival FFPE tumor samples often contain degraded DNA that introduces data inaccuracies, and the extraction process yields small amounts of usable DNA for NGS. The library preparation and sequencing methods for TruSight Tumor 15 are designed to address these challenges. Sample quantification guidelines are provided to ensure reliable, high-quality sequencing data from FFPE tissue. By maximizing sample success rates across multiple tumor types, the panel enables conservation of limited samples and resources.

Sensitive, High-Confidence Variant Detection

Deep sequencing using NGS provides the high sensitivity to reveal somatic variation in tumor subpopulations. Illumina sequencing by synthesis (SBS) chemistry is the most widely adopted NGS technology, generating > 90% of global sequencing data.* When paired with high-quality sequencing on the MiSeq Systems,[6,7] TruSight Tumor 15 provides uniform coverage of target regions, identifying somatic mutations at 5% variant allele frequency with ≥ 500× average coverage (Table 2). Such high sensitivity provides the accuracy needed to identify low-frequency variation from FFPE tumor samples with confidence (Table 3 and Table 4).

The TruSight Tumor 15 library preparation assay follows a tiling method that uses 2 oligo pools for multiplex PCR (Figure 2). This strategy enables coverage of larger DNA regions, produces higher coverage uniformity, and reduces the presence of primer dimers and FFPE-induced artifacts. This results in high accuracy and sensitivity. Instead of requiring a separate, preliminary qPCR reaction, in-process quality recommendations are provided during library preparation process, supporting sample success (Figure 3). These quality recommendations include final library concentration and expected library size.

Table 2: Specifications

Parameter	Details
Panel Size	44 kb
Content	250 amplicons
Amplicon Size	Average ~150–175 bp
DNA Input Requirement	20 ng total (10 ng × 2 reactions)
Library Preparation Time	7 hours total time, 3.5 hours hands-on time
Sequence Run Time	27 hours on the MiSeq System
Sequence Run	2 × 151 bp
Sample Throughput	8 samples per run using MiSeq v3 chemistry
Variant Frequency	5%
Amplicon Coverage	93.5% of bases covered at ≥ 500×

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

Table 3: TruSight Tumor 15 Performance With Characterized FFPE Cell Lines

Gene	Mutation	Reported Frequency	Detected Frequency	Coverage
BRAF	V600E	10.5%	9.5%	26,128×
EGFR	G719S	24.5%	24.0%	24,617×
KRAS	G13D	15.0%	18.3%	4191×
KRAS	G12D	6.0%	11.5%	4194×
NRAS	Q61K	12.5%	16.0%	10,037×
PIK3CA	E545K	9.0%	11.5%	22,944×
PIK3CA	H1047R	17.5%	19.0%	14,867×
cKIT	D816V	10.0%	14.8%	4040×

DNA from the HD-C751 FFPE cell line (Horizon Diagnostics) containing known variants was evaluated using the TruSight Tumor 15 assay and sequenced on the MiSeq System.

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Table 4: TruSight Tumor 15 Performance With FFPE Tumor Samples

Gene	Variant	Reference Sequence	Alternate Sequence	Depth	Frequency
BRAF	V600E	A	T	38,111×	66.3%
AKT1	E17K	C	T	33,141×	39.0%
KRAS	G13D	C	T	7403×	34.5%
NRAS	Q16R	T	C	13,682×	42.7%
PIK3CA	E545K	G	A	76,822×	49.4%

DNA from FFPE tumor samples was extracted and then evaluated using the TruSight Tumor 15 assay and sequenced on the MiSeq System.

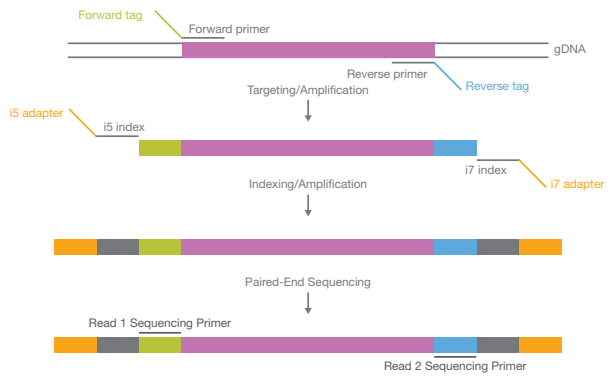

Figure 2: TruSight Tumor 15 Chemistry—The TruSight Tumor 15 assay uses a multiplex PCR approach, resulting in high accuracy and sensitivity.

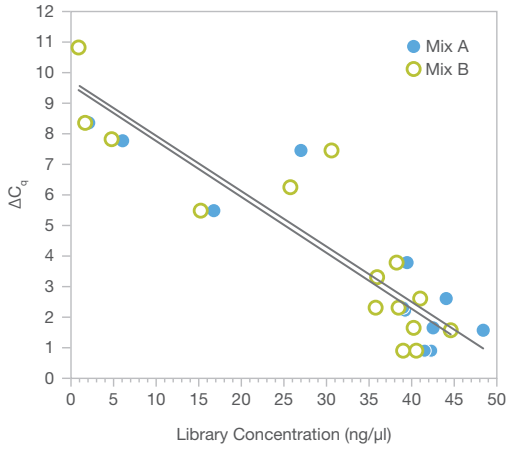

Figure 3: Concordance of Quality Metrics—The quality threshold used for TruSight Tumor 15 (20 ng/µl) is highly correlated with ΔC_q results from DNA quality evaluation using qPCR. Mix A and Mix B denote the 2 different libraries prepared per sample.

Summary

TruSight Tumor 15 offers a comprehensive workflow solution for the detection of the most common somatic variants found in solid tumors. Developed according to evidence-based guidelines, with input from key opinion leaders and late-stage pharmaceutical research, the panel enables labs to use the power of NGS technology to focus on the most relevant genes and analyze low-frequency variants from FFPE DNA with confidence. By assessing 15 genes in a single assay, this panel offers a streamlined, economical workflow solution that can be implemented easily by labs using NGS for the first time.

Ordering Information

Product	Catalog No.
TruSight Tumor 15 Kit Includes library preparation panel, 3 sequencing cartridges, and sequencing reagents sufficient for 24 samples	OP-101-1001
TruSight Tumor 15 Includes library preparation consumables, oligos, and indexes sufficient for 24 samples	OP-101-1002

Learn More

For more information about Illumina technology for oncology applications, visit www.illumina.com/oncology.

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