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TruSight® Tumor 15

Evidence-based gene content and an efficient workflow solution for evaluation of common somatic variants in FFPE solid tumor samples.

Highlights

- **Increased Lab Efficiency**
  Streamlined, 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 leader insights, 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 15 of the 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 inprocess 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. These DNA libraries are compatible with the MiniSeq™, MiSeq®, or MiSeqDx® System (run in research mode). Data are analyzed using on-instrument reporting software or the TruSight Tumor 15 BaseSpace® App.

User-Friendly Analysis and Reporting

Results from on-instrument software or the TruSight Tumor 15 BaseSpace App are presented in a predefined report denoting the presence or absence of biologically significant 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. For a list of the specific variants covered in the predefined report, see the Report Definitions file on the TruSight Tumor 15 Support Page.[2]

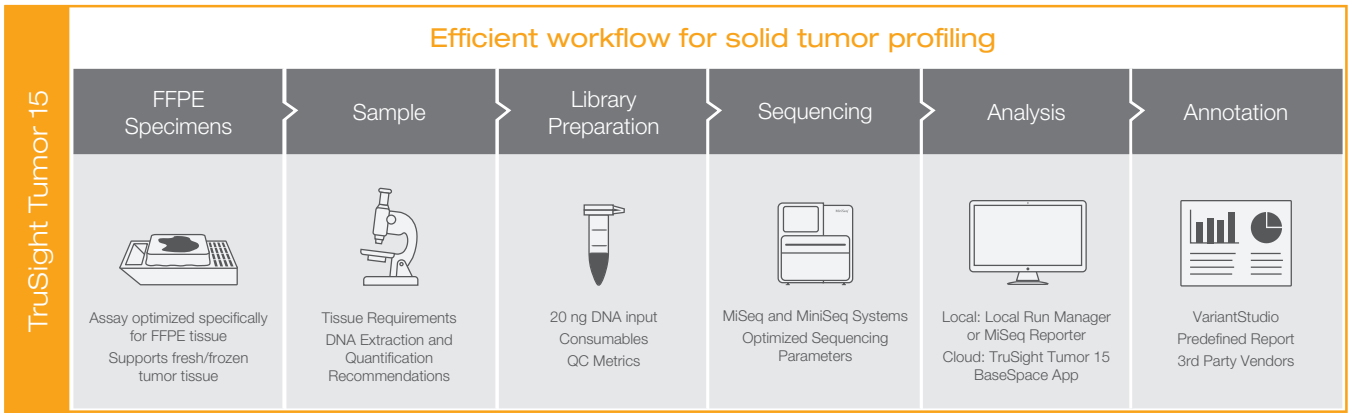

Figure 1: TruSight Tumor 15 Workflow—TruSight Tumor 15 is optimized for seamless integration into current lab workflows, going from extracted DNA to data in approximately 36 hours. The assay can be run on the MiniSeq or MiSeq Series of sequencing systems.

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)[3] and the European Society for Medical Oncology (ESMO).[4] Independent consortia publications and late-stage pharmaceutical research also influenced the design of TruSight Tumor 15.[5-7] 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 the oncology community, 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.

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	24 hours (MiniSeq) or 27 hours (MiSeq)
Sequence Run	2 × 151 bp
Sample Throughput	8 samples per run using MiniSeq High Throughput Kit
	8 samples per run using MiSeq v3 chemistry
Variant Frequency	5%
Amplicon Coverage	93.5% of bases covered at ≥ 500×

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,[7,8] TruSight Tumor 15 provides uniform coverage of target regions, identifying somatic mutations as low as 5% variant allele frequency with ≥ 500× minimum coverage (Table 2).

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, inprocess quality recommendations are provided during library preparation process, supporting sample success (Figure 3). These quality recommendations include final library concentration and expected library size.

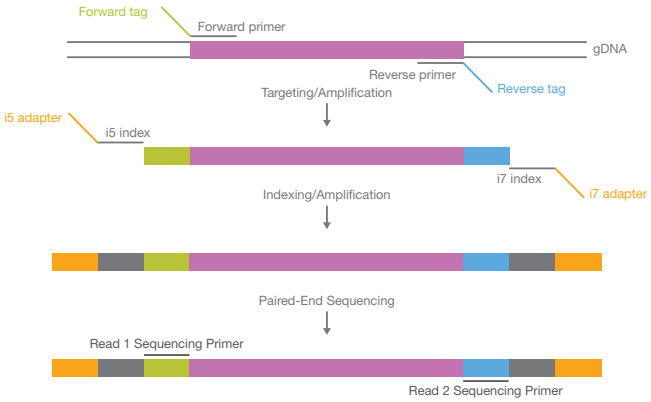

Figure 2: TruSight Tumor 15 Chemistry—The TruSight Tumor 15 assay uses a multiplex PCR approach, resulting in high accuracy and sensitivity.

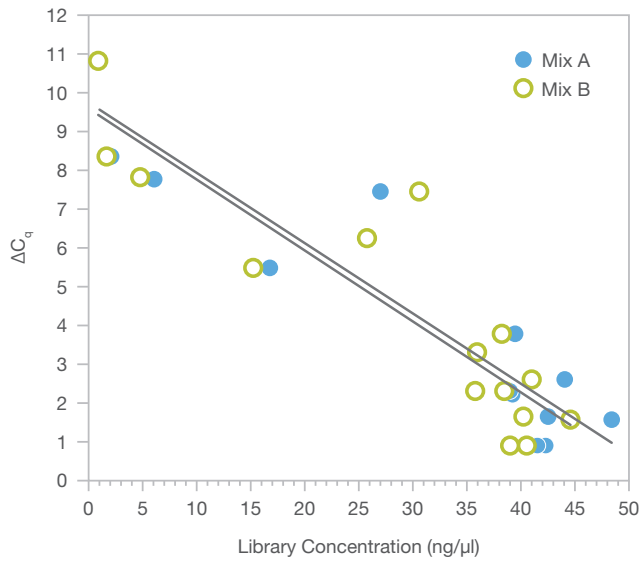

Figure 3: Concordance of Quality Metrics— The quality threshold used for TruSight Tumor 15 (20 ng/µl) is highly correlated with ΔC_q results from DNA quality evaluation using qPCR. Mix A and Mix B denote the 2 different libraries prepared per sample.

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

Table 3: TruSight Tumor 15 Coverage

Sample ID	Sample 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×

DNA was extracted from FFPE tumor samples and then 20 ng of input DNA was evaluated using the TruSight Tumor 15 assay and sequenced on the MiniSeq System. Coverage of ≥ 500× is required for accurate identification of mutations at 5% variant frequency. Sample quality was determined by amplification potential of extracted DNA compared to a non-FFPE control sample in a qPCR assay. High quality is indicated by ΔCq of 0–2. Medium quality is indicated by ΔCq value of 2–4. Low quality is indicated by ΔCq of 4–6.

Table 4: 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 5: 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.

Reliable, Accurate Performance

TruSight Tumor 15 provides the sensitivity and accuracy needed to identify low-frequency variation with confidence in samples of varying quality. High target coverage (at least 93.5% of bases covered at ≥ 500×) provides sensitivity and accuracy required for low-level variant calling (Table 3). TruSight Tumor 15 run on the MiniSeq System enables variant detection in many different sample types, with detection as low as 1% in high-quality DNA (Table 4), and as low as 5% in low quality FFPE samples (Table 5).

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 Includes library preparation consumables, oligos, and indexes sufficient for 24 samples	OP-101-1002
TruSight Tumor 15 MiSeq Kit Includes library preparation panel, 3 MiSeq v3 Kits, sufficient for 24 samples	OP-101-1001
TruSight Tumor 15 MiniSeq Kit Includes library preparation panel, 3 MiniSeq High Output Kits (300 cycles), sufficient for 24 samples	20005610

Learn More

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

References

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