illumina®

TruSight™ Tumor

Deep coverage of 26 genes for low mutant allele detection within regions involved in solid tumors delivered on proven next-generation sequencing technology.

Highlights

- **High accuracy, low mutant allele detection**
  Highly accurate variant analysis at limit of detection below 5% mutant allele frequency across 175 exonic regions with 1000x minimum coverage of each region
- **Optimized for formalin-fixed, paraffin-embedded (FFPE) tissue**
  Exceptional sample success rates with minimal DNA input for accurate base calling even in degraded FFPE samples
- **Best coverage of variants involved in solid tumors**
  Coverage of complete exons for analysis of molecular heterogeneity in highly relevant content selected from CAP and NCCN guidelines, and late stage clinical trials[1–3]

Introduction

Next-generation sequencing (NGS) has helped dramatically increase knowledge of the pathways involved in cancer. Current tumor molecular profiling methods involve multiple single-gene assays, each targeting specific point mutations within hotspot regions. This iterative technique increases costs, requires more DNA and a longer time to generate data. In addition, single-gene genotyping assays reveal only a portion of what is happening within the tumor and can lead to a "one size fits all" view of biology. The same genomic information gained through these tests can be obtained faster and more cost-effectively using NGS technology, while providing additional information on emerging biomarkers implicated in recent clinical studies.

High Accuracy with Low Mutant Allele Detection

Deep sequencing can reveal somatic variation in tumor sub-populations at levels never before possible. TruSight Tumor achieves limits of detection below 5% mutant allele frequency across 175 amplicons (Figure 1A). At this low level of detection, there is a risk that fixation artifacts are either classified as mutations or create an allele bias. This method leverages proprietary biochemistry and bioinformatics techniques to differentiate true variation from artifacts, yielding unprecedented accuracy and specificity (Figure 1B).

Figure 1: TruSight Tumor Performance

A

Sample	HD103	HD111	HD132	HD123	HD118	HD159	HD129	HD129
Gene	*BRAF*	*EGRF*	*EGFR*	*BRAF*	*KRAS*	*KRAS*	*EGFR*	*EGFR*
Mutation	V600E	ΔE746-A750	L861Q	V600K	G12R	G12C	L858R	T790M
Reported Frequency	3.5%	4.0%	4.5%	5.0%	5.0%	6.0%	6.0%	20.0%
Frequency	3.3%	4.6%	4.7%	4.3%	5.2%	6.2%	6.6%	18.8%
Coverage	16470	7469	6132	9731	8747	9381	5347	6403

B

In-House Verification and Pilot	Data
FFPE Samples Evaluated	247
Analytical Sensitivity	> 99.9%
Analytical Specificity	> 99.9%
Total Calls Made	9.2 M bases
Total Calls Verified	1.7 M bases
Library Success Rate post QC	> 99%

A. TruSight Tumor accurately detected mutant allele frequency (MAF) below 5% on Horizon Discovery FFPE cell lines with titrated MAF variants for use in evaluating assay sensitivity.

B. Over 200 libraries were generated from DNA extracted from various FFPE tissue types and laboratory sources. Sequencing of these libraries produced analytical sensitivity and specificity of > 99.9%, and a library success rate of > 99%.

Optimized for Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

Formalin-fixation, paraffin-embedding techniques have long been the standard to protect tissues for downstream analysis and ease of archiving. This process introduces challenges for molecular approaches as it often degrades DNA into small fragments and has the potential to damage the DNA base pairs themselves. This library preparation and sequencing method was designed specifically to unlock the depth of molecular heterogeneity found in precious tumor samples (Figure 2). Illumina's optimized extraction and sample pre-qualification method (QC) provides maximum gDNA yield with archival FFPE tissues. This QC assay recommends a dilution factor based on sample quality, minimizing the DNA input into the library preparation assay resulting in highly uniform libraries (Figure 3).

The Broadest Coverage of Content Implicated in Solid Tumors

The genes involved with solid tumors were carefully selected for this panel to include 26 genes and 175 amplicons of relevant content from CAP and NCCN guidelines, relevant publications, and late-phase pharmaceutical clinical trials. The TruSight Tumor content set provides coverage of entire exons in regions where variation has been cataloged in the COSMIC database[4] in oncogenes, and coverage of all exons in tumor suppressor genes (Figure 4). This provides a more comprehensive view of somatic variation in solid tumors including lung, colon, melanoma, gastric and ovarian—enabling clinical researchers to look beyond point mutations within hot spots in single genes.

Novel bioinformatics tools provide high levels of specificity at exceptionally low levels of mutant allele detection across the 26 genes and 175 amplicons provided (Table 2). The variant calling algorithms and filtering and annotation tools enable a scientist to easily analyze and understand the variation present in a sample.

Combined with Illumina's proven NGS technology, the TruSight Tumor content set delivers unmatched SNP, insertion, and deletion detection across the broadest regions of variation to enable a better understanding of underlying biology leading to improved clinical studies (Table 1 and 2).

Manufactured for the Specific Needs of the Clinical Research Laboratory

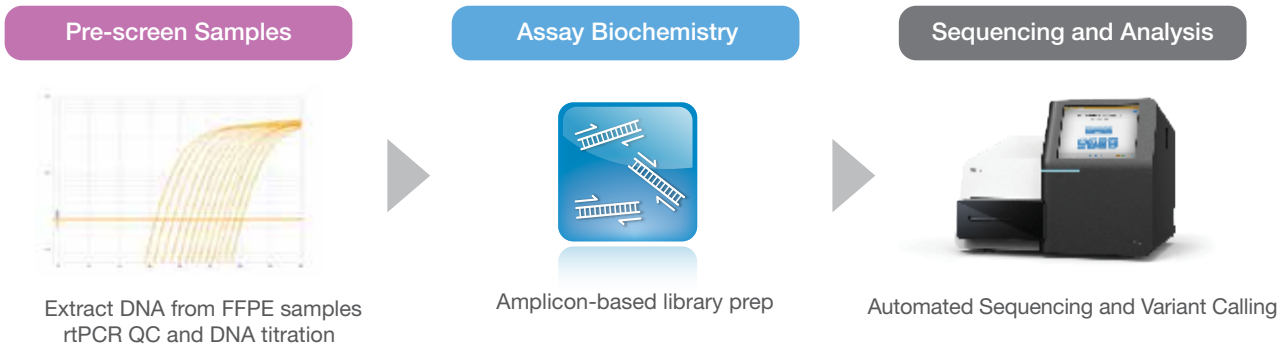
TruSight content sets provides the option of higher quality standards, with a binding supply agreement to enable better laboratory management and minimize laboratory validations.

Table 1: Confirmed Variants

Gene	Variant(s) Confirmed	Reference Sequence	Alternate Sequence	Variant type	Frequency
BRAF	V600K	AC	TT	Dinucleotide	76.1%
BRAF	V600E	A	T	SNP	2.9%
BRAF	V600M	C	T	SNP	3.25%
EGFR	E746_A750del	AGGAATTAAGAGAAGC	A	Deletion	4.6%
EGFR	L747_S752del_insV	GAATTAAGAGAAGCAACATC	GT	In/Del	18.0%
EGFR	delE746_S752insV	GAATTAAGAGAAGCAACATC	GT	In/Del	60.4%
EGFR	delL747_T751insP	ATTAAGAGAAGCAA	AC	In/Del	37.5%
EGFR	L858R	T	G	SNP	2.2%
KIT	S501insAY	C	CTGCCTA	Insertion	32.5%
KIT	V559del	GGTT	G	Deletion	27.5%
KRAS	G12D	C	T	SNP	4.9%
KRAS	G13D	C	T	SNP	10.3%
KRAS	D173D	A	G	SNP	16.5%
PDGFRA	D842_D845del	AGACATCATGCAT	A	Deletion	36.0%
PIK3CA	E545K	G	A	SNP	4.2%

Variants of note are confirmed in previously characterized FFPE samples with TruSight Tumor. This library prep method and bioinformatics solution enables detection of relevant complex variation including dinucleotide changes, insertions, and deletions.

Figure 2: TruSight Tumor Workflow



The TruSight Tumor content set is a comprehensive assay for examining the most relevant cancer genes involved in solid tumors, even in the most challenging FFPE samples. Researchers can go from extracted genomic DNA to data in two days with the MiSeq® system.

Table 2: TruSight Tumor Genes

AKT1	*EGFR*	*GNAS*	*NRAS*	*STK11*
ALK	*ERBB2*	*KIT*	*PDGFRA*	*TP53*
APC	*FBXW7*	*KRAS*	*PIK3CA*	
BRAF	*FGFR2*	*MAP2K1*	*PTEN*	
CDH1	*FOXL2*	*MET*	*SMAD4*	
CTNNB1	*GNAQ*	*MSH6*	*SRC*	

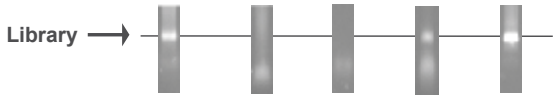
Genes selected from NCCN[1] and CAP[2] guidelines, late-stage clinical trials[3], and relevant publications for lung, colon, melanoma, gastric and ovarian[4].

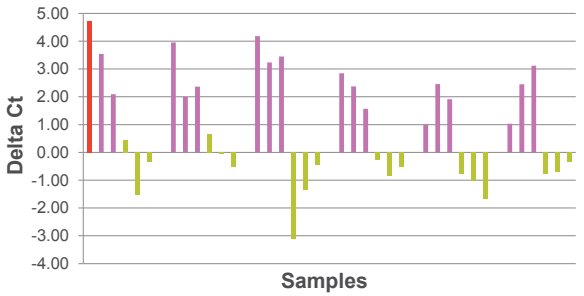
Table 3: Specifications

Parameter	Details
Panel size	21 kb
Content	175 amplicons
Amplicon size	165–195 bp
DNA input requirement	Based on sample QC (typical range: 30–300 ng)
Assay time	< 7 hours, 2.5 hours hands-on
Sequence run time	22 hours
Sequence run	2 × 121 bp
Limit of detection	≤ 5% MAF, reporting limit = 3%
Coverage of each amplicon	Miniumum of 1000× coverage, with 7,000× mean
Amplicon dropouts	0

Figure 3: QC Testing Results in Uniform Libraries

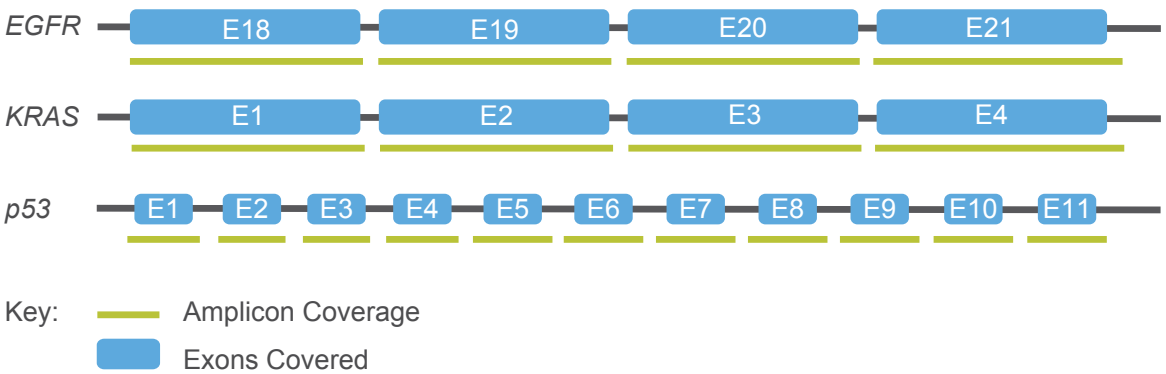
Metric	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Sample area (cm²)	1.5	1.5	1.5	0.2	0.4
DNA mass (ng/ul)	76.8	60.5	3.2	6.6	42.2
DNA QC (dCt)	1.09	6.19	5.36	4.60	1.43





Neither tissue area nor DNA yield were adequate predictors of library performance. This DNA QC method suggests a DNA dilution factor and fixed volume of DNA for library preparation that yields highly uniform libraries. Here we compared tumor, normal and metastasis samples in matched FFPE (purple) and fresh frozen (green) pairs. All but one sample (red) generated a successful library. Using a ΔCT cutoff value of 4, the sample preparation success rate was 100%. The DNA input amount ranged between 30–300 ng, based on the quality of the DNA.

Figure 4: Whole-Exon Coverage of the Genes Involved in Solid Tumors



Look beyond hot spots with 175 amplicons tiled over 82 exons in 26 genes involved in solid tumors progression, prognosis and targeted therapy for a more comprehensive view of tumor heterogeneity.

Ordering Information

Product	Catalog No.	TG Catalog No.*
TruSight Tumor content set Includes library preparation consumables, oligos, DNA QC, and indexes sufficient for 48 samples	FC-130-2001	TG-130-2001

* TG-labeled consumables include features intended to help customers reduce the frequency of revalidation. They are available only under supply agreement and require customers to provide a binding forecast. Please contact your account manager for more information.

References

1. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp#site
2. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, et al. 2013 Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors. Archives of Pathology & Laboratory Medicine Vol 137 Issue 4
3. www.clinicaltrials.gov
4. Forbes SA, Bindal N, Bamford S, Cole C, Kok CY, et al. 2011 COSMIC: Mining COMplete Cancer Genomes in the Catalogue of Somatic Mutations in Cancer. *Nucl Acids Res* 39: D945–50.

Learn More

To learn more about TruSight Tumor and Illumina next-generation sequencing technology, visit www.illumina.com/trusight-tumor

Note regarding biomarker patents and other patents unique to specific uses of products.

Some genomic variants, including some nucleic acid sequences, and their use in specific applications may be protected by patents. Customers are advised to determine whether they are required to obtain licenses from the party that owns or controls such patents in order to use the product in customer's specific application.

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Pub. No. 670-2013-008 Current as of 12 April 2013

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