TruSeq® Amplicon - Cancer Panel

Rapidly detect hundreds of critical cancer mutations with the fastest and easiest multiplexed amplicon assay optimized for the MiSeq® system.

Highlights

- **Most relevant cancer loci in a single comprehensive panel**
  Sequence hundreds of cancer hotspots from selected genes such as *BRAF*, *KRAS*, and *EGFR*
- **Shortest time to project completion**
  Go from genomic DNA to fully analyzed data in two days
- **Unprecedented amplicon and sample multiplexing**
  Generate the highest quality and most accurate data with the ability to reliably detect mutations below 5% frequency

Introduction

The TruSeq Amplicon - Cancer Panel (TSACP) is a highly multiplexed targeted resequencing assay for detecting somatic mutations across hundreds of mutational hotspots in cancer genomes. TSACP provides a streamlined workflow, including a quality control assay for DNA from formalin-fixed, paraffin-embedded (FFPE) samples, simple bead-based sample normalization, automated cluster generation nd paired-end sequencing, and on-instrument data analysis (Figure 1). Leveraging the long paired-end read capability, speed, and high data quality of the MiSeq system, entire projects can now be accomplished in days instead of months. TSACP enables highly sensitive mutation detection within important genes, including *BRAF, KRAS*, and *EGFR*. Mutations in these genes are linked to many cancers, including melanoma, colorectal, ovarian, and lung cancer. The unique ability of this assay to screen precious FFPE samples for these important variants will unlock a wealth of genomic information from many tumor types.

Comprehensive Screening for FFPE Samples

The TruSeq Amplicon - Cancer Panel provides pre-designed, optimized oligonucleotide probes for sequencing mutational hotspots in > 35 kilobases (kb) of target genomic sequence. Within in a highly multiplexed, single-tube reaction, 48 genes are targeted with 212 amplicons. Table 1 contains a complete list of the oncogenes included in the panel.

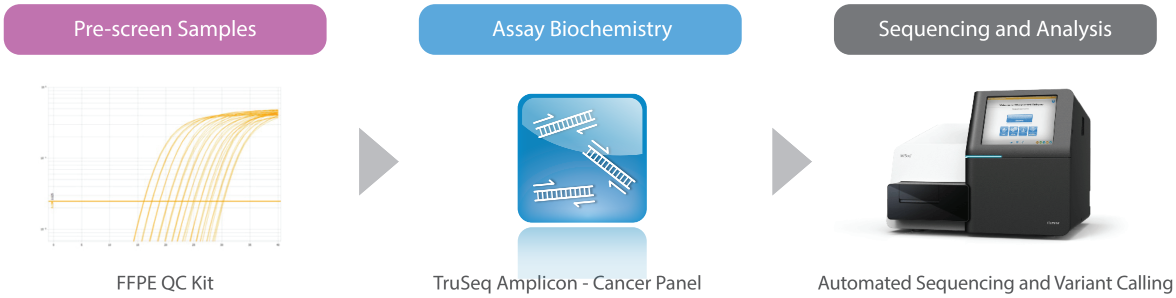
The TSACP is uniquely suited for detection of somatic mutations in FFPE samples. Prior to amplicon preparation, a simple qPCR-based

Table 1: TSACP Cancer-Related Genes

ABL1	*EGFR*	*GNAS*	*MLH1*	*RET*
AKT1	*ERBB2*	*HNF1A*	*MPL*	*SMAD4*
ALK	*ERBB4*	*HRAS*	*NOTCH1*	*SMARCB1*
APC	*FBXW7*	*IDH1*	*NPM1*	*SMO*
ATM	*FGFR1*	*JAK2*	*NRAS*	*SRC*
BRAF	*FGFR2*	*JAK3*	*PDGFRA*	*STK11*
CDH1	*FGFR3*	*KDR*	*PIK3CA*	*TP53*
CDKN2A	*FLT3*	*KIT*	*PTEN*	*VHL*
CSF1R	*GNA11*	*KRAS*	*PTPN11*	
CTNNB1	*GNAQ*	*MET*	*RB1*	

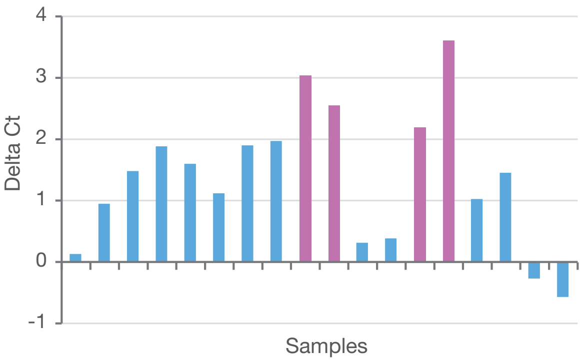
Cancer-related genes represented in the TSACP. For a full list of target regions, see the manifest file[1] (MyIllumina login required).

Figure 1: TSACP Workflow



The TruSeq Amplicon - Cancer Panel is a comprehensive assay for examining the most relevant cancer mutations, even in challenging FFPE samples. With the shortest time to project completion, researchers can go from genomic DNA to fully analyzed data in less than two days with the MiSeq system.

Figure 2: FFPE Screening Improves Assay Performance



DNA extracted from 18 FFPE samples was screened with the Illumina FFPE QC Kit prior to amplicon prep. ΔCt was calculated by the difference between the Ct number of each sample and of the control DNA (not shown). Samples that generated successful amplicon sequencing data are indicated by blue bars; those that did not are shown by purple bars. Using a cutoff value of 2, the assay outcome of this particular library preparation was 100%.

Table 2: Specificity and Uniformity Across Samples

Sample	% Specificity	% Uniformity	Variant Type	% Allele Frequency
Control	93.5	94.0	NA	NA
1	91.2	94.4	Indel	29.2
2	93.9	91.5	SNV	2.95
3	93.5	91.5	SNV	2.90
4	91.6	94.9	Indel	18.9
5	93.1	91.2	SNV	8.70
6	93.9	91.6	SNV	40.8
7	92.3	92.6	SNV	37.1
8	90.3	93.5	SNV	39.5
9	93.5	92.1	SNV	7.80

Nine FFPE samples representing multiple cancer types were screened using the TruSeq Amplicon - Cancer Panel. Insertion/deletion (indels) and single nucleotide variants (SNVs) were called with high specificity and uniformity.

DNA quality control step allows sample pre-screening. This up front sample quality control step accurately predicts assay performance from fragmented DNA, reducing time and reagent costs (Figure 2).

The TruSeq Amplicon assay chemistry begins with hybridization of the pre-mixed, optimized oligonucleotide probes upstream and downstream of the regions of interest. Each probe includes a target capture sequence and an adapter sequence used in a subsequent amplification reaction. A proprietary extension-ligation reaction extends across the region of interest, followed by ligation to unite the two probes. This creates a new template strand and gives the assay excellent specificity. Extension-ligation templates are PCR amplified and two unique sample-specific indices are incorporated. The final reaction product contains amplicons that are ready for sequencing. An integrated bead-based normalization procedure allows simple volumetric library pooling, avoiding laborious qPCR-based quantification methods. Pooled amplicon libraries can be loaded directly onto the MiSeq system without additional processing.

Unprecedented Multiplexing, Excellent Data

With the ability to combine hundreds of amplicons per sample and up to 96 samples per MiSeq run, the TSACP provides an unprecedented level of sample multiplexing, while providing excellent specificity and uniformity. An example TSACP experiment was performed following the workflow described in Figure 1 using 2 × 150 bp MiSeq reads. Representative uniformity data, with percentage of bases at least 0.2× the mean sequencing depth, are shown for nine cancer samples plus one control (Table 2). Specificity values, defined as the percent of sequenced bases passing filter and aligning to the desired target regions, are also shown in Table 2. Excellent coverage uniformity and specificity were obtained, with > 91% of bases covered at > 0.2× of the mean coverage and > 90% of reads on target.

TSACP maintains excellent coverage, even at high read depths. In another experiment (Figure 3A), two runs with twenty pooled samples gave > 90% uniformity and specificity. A single sample sequenced at > 27,000× provided similar results, demonstrating consistently high specificity and uniformity at extremely high read depths. In Figure 3B, one sample chosen from the Run 1 pool of twenty samples showed > 95% of bases are covered at 500× depth, enabling low frequency variant detection. With highly specific amplicon targeting, combined with the most accurate TruSeq sequencing chemistry, TSACP lets researchers detect mutations below 5% frequency with high confidence (Figure 4).

Summary

In a single experiment, researchers can access the most content of any commercially available amplicon cancer panel, including critical cancer-related genes such as *BRAF*, *KRAS*, and *EGFR*. The TruSeq Amplicon - Cancer Panel delivers confidence and simplicity with unparalleled specificity and uniformity.

Figure 3: High Specificity and Uniformity

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Parameter	Run 1	Run 2	Run 3
Number of Samples	20	20	1
MiSeq Output (Gb)	1.8	2.0	1.9
Mean Depth	1,366	1,410	27,019
% Specificity	93.7	94.5	90.7
% Uniformity	96.2	96.2	94.4
Amplicon Dropouts*	0	0	0

*Dropouts are defined as amplicons having no sequencing coverage when the sample achieves a mean sequencing depth of > 1000×.

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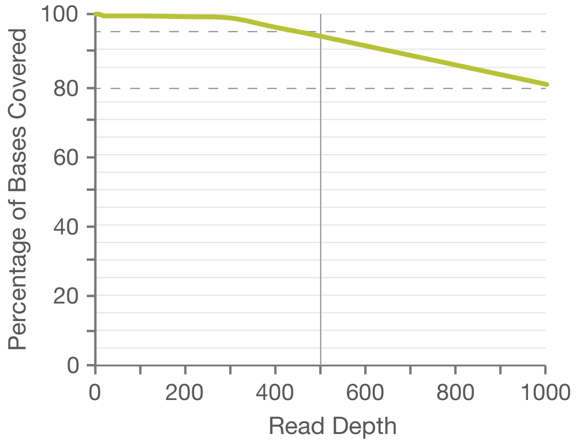
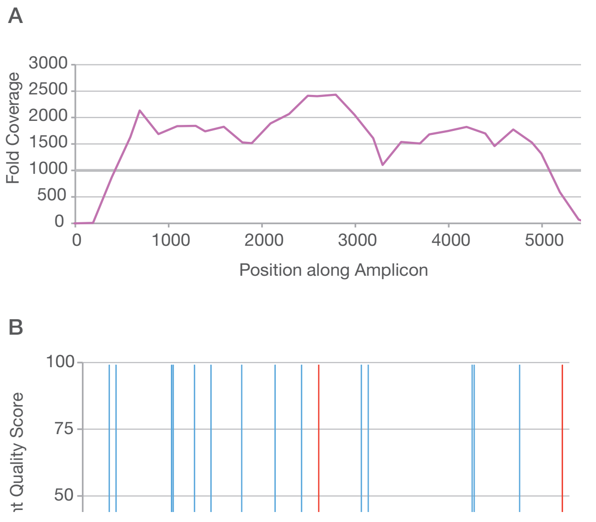


Ⓐ Three TSACP experiments- two with 20 pooled samples and one with a single sample. Excellent assay performance is shown, with > 90% specificity and uniformity, with zero dropouts across the pooled Runs 1 and 2. Similar output, specificity, and uniformity are obtained with the single sample (Run 3), sequenced at an extremely high depth.

Ⓑ Coverage plot from one sample included in Run 1. The percent of bases covered plotted against the read depth shows that > 80% of bases are covered at 1000×, and ~95% are covered at 500× read depth.

Figure 4: Low Frequency Variant Detection



The TSACP detected a 2.95% variant (A to T SNV) in the *BRAF* gene from a colon cancer sample, with a variant score of Q40, or 99.99% confidence (6,048× coverage).

Reference

1. TruSeq Amplicon Cancer Panel Manifest File at MyIllumina.com

TSACP Performance Specifications

Parameter	Details
Panel Size	35 kb
Content	212 amplicons
Amplicon Size	170–190 bp
DNA Input	250 ng
Assay Time	< 7 hrs, 2.5 hrs hands-on
Enrichment Specificity	> 85%
Coverage Uniformity	> 85% at 0.2× mean
Dropouts*	0

*Dropouts are defined as amplicons having no sequencing coverage when the sample achieves a mean sequencing depth of > 1000×.

Ordering Information

Kit	Catalog No.
Illumina FFPE QC Kit	WG-321-1001
TruSeq Amplicon - Cancer Panel (96 samples)	FC-130-1008
TruSeq Custom Amplicon Index Kit (96 indices, 384 Samples)	FC-130-1003
MiSeq Reagent Kit (300-cycles - PE)	MS-102-2002
Optional: TruSeq Index Plate Fixture and Collar Kit (2 Each)	FC-130-1007

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Pub. No. 770-2012-009 Current as of 24 July 2015