BROAD CLINICAL LABS

PRODUCT DATASHEET

Transcriptome Capture

Product Overview

Transcriptome Capture (TCap) is an alternative to traditional transcript enrichment methods, including poly(A) selection and ribosomal depletion, and is optimal for low-input and degraded samples, including formalin-fixed paraffin-embedded (FFPE) tissues.

The assay begins with preparation of a stranded, UMI-enabled cDNA library from isolated RNA, then hybridization of the library to DNA oligonucleotide probes to enrich for mRNA transcript fragments. The probe set used is the TWIST Bioscience Alliance Clinical Research Panel, which covers the complete mitochondrial genome and ACMG59 genes, as well as RefSeq and Online Mendelian Inheritance in Man (OMIM) putative gene sequences, Catalog of Somatic Mutations in Cancer (COSMIC) variants, key promoters, and other motifs that have been identified as potential cancer hot spots. TCap enables expression analysis and correlation of expression with DNA variants.

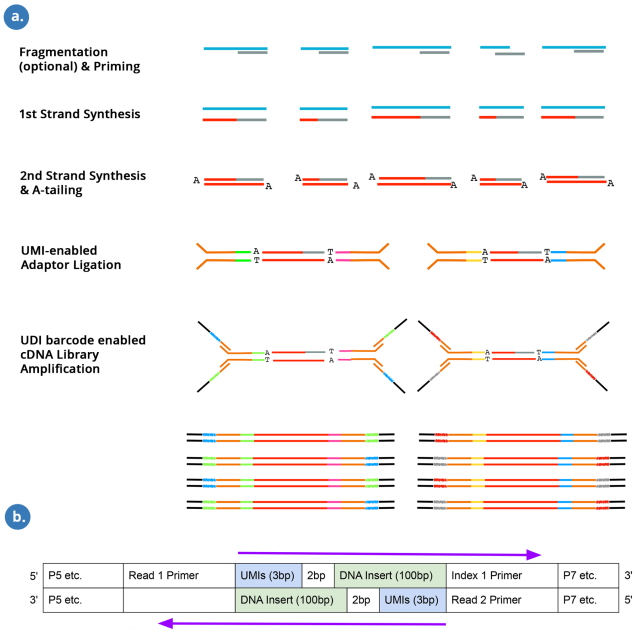

Figure 1a. Schematic of the pre-hybridization library creation workflow, which generates a UMI-enabled, sequenceable library.

Figure 1b. Stubby-Y UMI's are sequenced in line with cDNA inserts, resulting in a read structure of 3M2S146T8B8B3M2S146T, where 3M is the UMI, 2S is the space, 146T is the Template, and 8B is the index barcode.

Sample	Median CV Cov.	% Correct Strand Reads	% mRNA	% PF Reads Aligned	% Dup.
S1	1.09	98.5	84.1	99.3	38.3
S2	1.16	98.0	82.4	94.6	50.8
S3	1.02	98.4	86.0	97.4	39.1
S4	0.959	97.6	85.7	98.6	59.9
S5	1.11	97.6	88.0	98.4	58.1
S6	1.29	96.1	87.6	98.8	54.8

Table 1. Representative analytical (hg19) and quality metrics generated using FFPE derived RNA

What's Included

- Stranded cDNA Synthesis
- UMI enabled cDNA library construction
- Targeted Capture
- Illumina Sequencing (2 x 151 bp) aligned in pairs
- Data analysis including alignment
- Data delivery
- Sample Fidelity QC may be performed upon request; only when matched DNA has been fingerprinted

Input Requirements

- Typically used for FFPE tissue or low quality RNA samples; other specimens can also be processed (blood, stool, cell pellets)
- ≥550 ng (≥12 ng/µL concentration) of purified total RNA; ≥1µg preferred
- DV200 scores >30%

Data Deliverables

- 50 Million reads aligned in pairs
- STAR genome aligned BAM (hg19 or hg38)
- STAR transcriptome aligned BAM (hg19 or hg38)
- Alignment, RNA, insert size and duplication metrics
- Data will be delivered to a Cloud workspace.