

Highlights

- FFPE compatible mRNAseq
- Stranded cDNA capture with targeted probes
- Focused content maximizes discovery power

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

The approach first prepares a stranded cDNA library from isolated RNA, then hybridizes the library to a set of DNA oligonucleotide probes to enrich the library for mRNA transcript fragments.

Transcriptome Capture uses a content bait set containing 38Mb target territory, (including all of our previous exome plus additional coding content), that brings the total coverage of the RefSeq and GENCODE v12 databases to >98%. Typical performance metrics are detailed in Table 1.

Transcriptome Capture Enables:

- Differential Expression Analysis
- Fusion Transcript Detection
- Correlation of expression with DNA variants (Figure 1)

Sample	Insert Size (Bp)	% PF Reads Aligned	% Coding Bases	% Correct Stranded Reads	% rRNA Bases	% mRNA Bases	% UTR Bases	5'-3' Bias
FFPE_TP_1	212	92.69	76.81	96.80	0.62	89.10	12.28	7.05
FFPE_TP_2	241	92.41	74.61	97.33	1.52	85.55	10.94	2.07
FFPE_TP_3	296	92.98	71.77	97.05	4.08	81.19	9.43	3.52
FFPE_TP_4	398	93.61	78.33	96.97	3.52	85.59	7.26	2.58

Table 1. Sequence quality metrics from samples derived from FFPE tissue.

What's Included:

- Sample Receipt & Plating
- DV200 RNA QC
- Stranded cDNA Synthesis
- Target Capture
- Illumina HiSeq 2500 Sequencing (2x 76 Bp Reads)
- Sample Fidelity QC (96 SNP fingerprinting) available with Sample Qualification of matching DNA

Data Deliverable:

- 50 Million reads aligned in pairs
- STAR aligned to human genome assembly (hg19)
- De-Multiplexed, aggregated Picard BAM file with insert size and alignment summary
- Data accessed via secure online digital transfer

Product Inputs:

- 250ng of purified total RNA with DV200 scores >30%*
- FFPE tissue, fresh/frozen tissue, blood, stool, or cell pellets that preferably yield >250ng of RNA (separate charge will be applied for extractions)
- Minimum Sample data including Collaborator Participant ID, Collaborator Sample ID, Gender
- Due to the inherent nature of samples derived from FFPE to contain degraded and crosslinked nucleic acids, all samples submitted to Transcriptome Capture will be accepted "on risk" and subject to billing regardless of data quality

* refer to our application notes for special considerations when working with these material types, www.genomics.broadinstitute.org/products/nucleic-acid-extractions

