

# Full-length cDNA Sequencing of Alternatively Spliced Isoforms Provides Insight into Human Cancer

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## Abstract

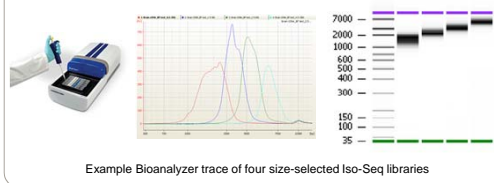
The majority of human genes are alternatively spliced, making it possible for most genes to generate multiple proteins. The process of alternative splicing is highly regulated in a developmental-stage and tissue-specific manner. Perturbations in the regulation of these events can lead to disease in humans. Alternative splicing has been shown to play a role in human cancer, muscular dystrophy, Alzheimer's, and many other diseases. Understanding these diseases requires knowing the full complement of mRNA isoforms. Microarrays and high-throughput cDNA sequencing have become highly successful tools for studying transcriptomes, however these technologies only provide small fragments of transcripts and building complete transcript isoforms has been very challenging.

We have developed a technique, called Iso-Seq sequencing, that is capable of sequencing full-length, single-molecule cDNA sequences. The method employs SMRT Sequencing from PacBio, which can sequence individual molecules with read lengths that average more than 10 kb and can reach as long as 40 kb. As most transcripts are from 1 – 10 kb, we can sequence through entire RNA molecules, requiring no fragmentation or post-sequencing assembly. Jointly with the sequencing method, we developed a computational pipeline that polishes these full-length transcript sequences into high-quality, non-redundant transcript consensus sequences. Iso-Seq sequencing enables unambiguous identification of alternative splicing events, alternative transcriptional start and polyA sites, and transcripts from gene fusion events. Knowledge of the complete set of isoforms from a sample of interest is key for accurate quantification of isoform abundance when using any technology for transcriptome studies.

Here we characterize the full-length transcriptome of paired tumor/normal samples from breast cancer using deep Iso-Seq sequencing. We highlight numerous discoveries of novel alternatively spliced isoforms, gene-fusion events, and previously unannotated genes that will improve our understanding of human cancer.

## Size Fractionation of Iso-Seq Libraries

### Sage Science's BluePippin Size Fractionation

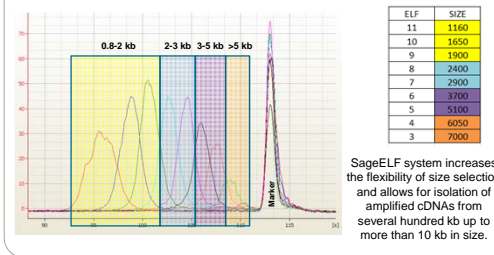


Example Bioanalyzer trace of four size-selected Iso-Seq libraries



Amplified cDNAs after size selection on Sage ELF system.

### Amplified cDNA After Size Fractionation on SageELF System

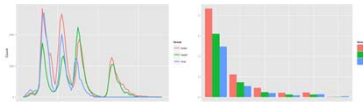


SageELF system increases the flexibility of size selection and allows for isolation of amplified cDNAs from several hundred kb up to more than 10 kb in size.

## Full-Length Human Tissue Transcriptomes

### PacBio Sequencing of Iso-Seq Libraries From 3 Human Tissues

Tissue	Size Fractions Sequenced	Number of Isoforms	Number of Genes	Transcript Lengths
Brain	1.2-2.9 kb	10289	6356	438 – 8823 nt
Heart	1.2-2.9 kb	6896	4351	467 – 8528 nt
Liver	1.2-2.9 kb	6124	3497	439 – 4754 nt



Overview of the dataset showing numbers of transcripts of various sizes and the number of isoforms per gene

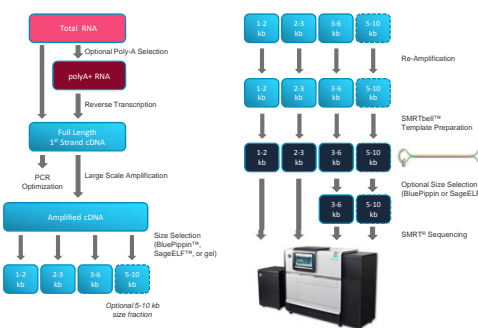
### Full-Length Non-Redundant Transcript Sequences



Two examples of genes with differential alternative splicing across the three tissues

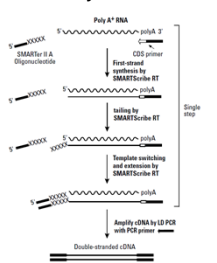
## Sample Preparation Methods

### Iso-Seq Sample Preparation Workflow

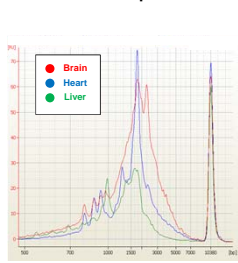


RNA is converted to first strand cDNA using the Clontech SMARTer PCR cDNA Synthesis Kit followed by universal amplification. Amplified cDNA is size fractionated and converted into SMRTbell templates for sequencing on the PacBio RS II.

### Clontech SMARTer PCR cDNA Synthesis Kit

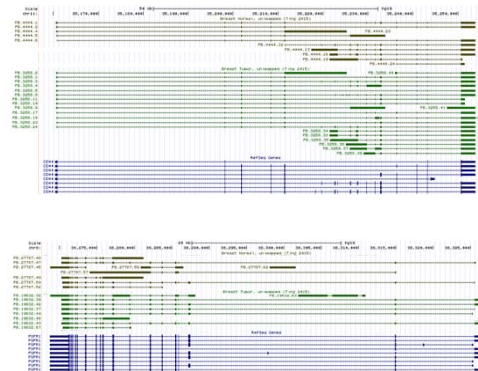


### Size Distribution of Amplified cDNA from Multiple Tissues



## Alternative Splicing Events in Breast Cancer

### Examples of Full-Length Transcripts from Adjacent Breast Tumor and Normal Samples



## Detection of Fusion Genes in Cancer

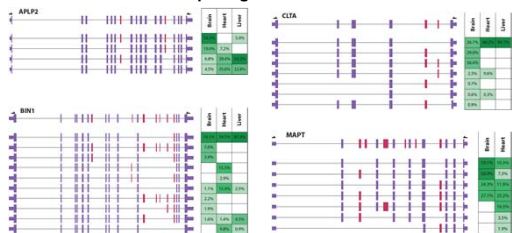
### 93 Gene Fusion Candidates Found in the MCF-7 Cancer Cell Line Iso-Seq Datasets (16 of them are previously known or predicted)

Gene 1	Chrom	Gene 2	Chrom	Literature Support
ARFGEF2	chr20	SULF2	chr20	experimental
BCAS4	chr20	BCAS3	chr17	experimental
ESR1	chr6	CCDC170	chr6	experimental
FOXJ1	chr14	TTC8	chr14	computational
MYH9	chr22	EIF3D	chr22	computational
MYO6	chr6	SENP6	chr6	experimental
PAPPLA	chr14	JCF1	chr14	computational
PQR1	chr8	MATN2	chr8	experimental
RPS8B1	chr17	VMP1	chr17	experimental
RPS8B1	chr17	DIAPH3	chr13	experimental
RBBN1	chr1	APB1	chr1	computational
SLC25A4	chr1	NBP1	chr1	experimental
SYTL2	chr11	PCALM	chr11	experimental
TBL1XR1	chr3	RGS17	chr6	experimental
TLMNG	chrX	SYAP1	chrX	experimental
ZNF217	chr20	SULF2	chr20	computational

Table of known or predicted gene fusions that were detected in the MCF-7 dataset

## Targeted Full-Length cDNA Sequencing

### Sequencing of Full-Length RT-PCR Products Shows Differential Alternative Splicing Across Three Tissues



PacBio sequencing of full-length RT-PCR products simplifies identification of alternatively spliced isoforms and allows for relative quantification of isoform abundance.

## Summary and Resources

### Summary:

- The Iso-Seq method provides full-length cDNA sequences without the need for assembly.
- Improved sample prep and size-selection methods allows for sequencing of transcripts up to 10 kb.
- Alternatively spliced transcripts can be easily identified from either whole transcriptome or targeted sequencing.

PacBio human three tissue dataset available here: <http://blog.pacificbiosciences.com/2014/10/data-release-whole-human-transcriptome.html>

PacBio MCF-7 transcriptome dataset available here: <http://blog.pacificbiosciences.com/2013/12/data-release-human-mcf-7-transcriptome.html>

Additional information and Iso-Seq protocols: <http://www.pacbio.com/applications/iso-seq/index.html>

Details on data analysis of Iso-Seq data can be found here: [https://github.com/PacificBiosciences/cDNA\\_primer/wiki](https://github.com/PacificBiosciences/cDNA_primer/wiki)