



PLANT AND ANIMAL
SCIENCES

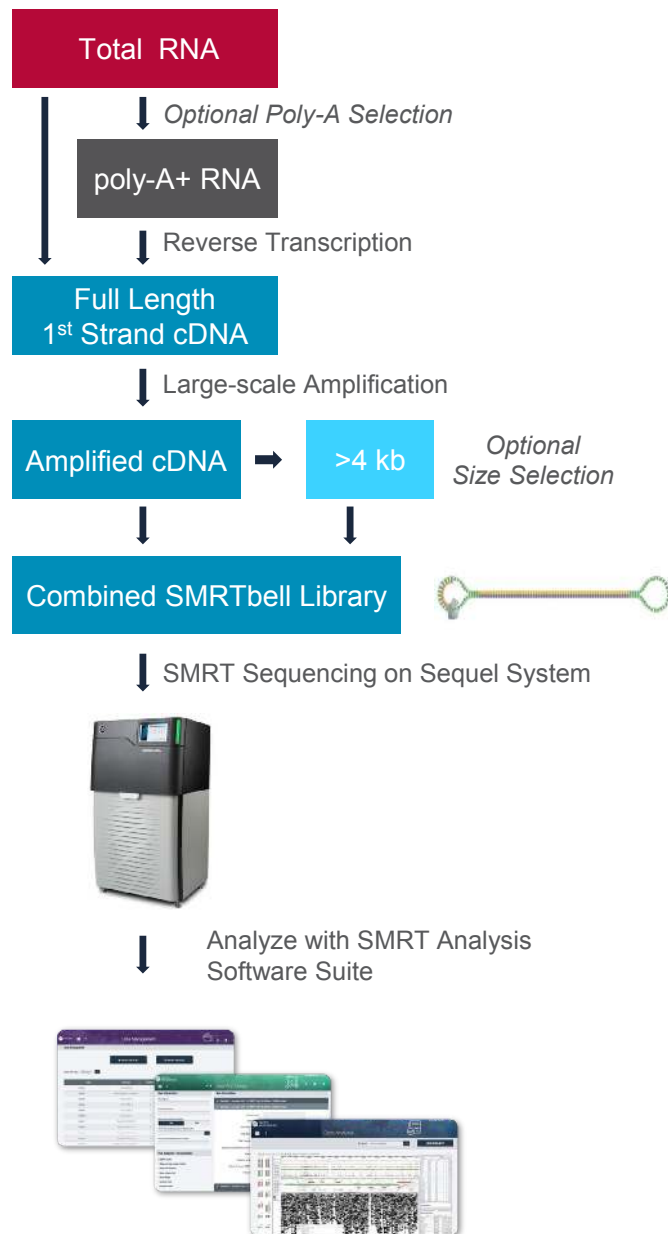


HUMAN BIOMEDICAL
RESEARCH

LONG-READ RNA SEQUENCING BEST PRACTICES

With Single Molecule, Real-Time (SMRT®) Sequencing and the Sequel® System, you can easily and affordably sequence transcript isoforms of up to 10 kb in their entirety. The Iso-Seq® method allows users to generate full-length cDNA sequences – with no assembly required – in order to confidently characterize the full complement of transcript isoforms within targeted genes, or across an entire transcriptome.

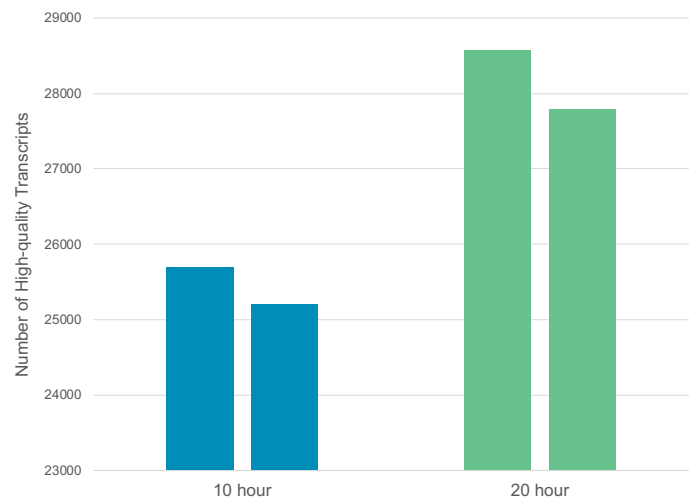
FROM RNA TO ACCURATE GENE MODELS



SAMPLE PREPARATION RECOMMENDATIONS

- Prepare full-length transcripts using the Clontech® SMARTer® PCR cDNA Synthesis Kit with as little as 1 ng of poly-A+ RNA or 2 ng of total RNA¹
- The Sequel Sequencing Kit and protocols eliminate the need for size selection for transcripts <4 kb²
- Optional size-selection protocols to enrich for transcripts >4 kb
- Compatible with standard target enrichment methods, such as NimbleGen SeqCap EZ³ or IDT xGen Lockdown Probes⁴
- Multiplex with sample barcoding⁵
- Scalable throughput
 - Sequence up to 40 Gb, or 400 k-500 k full-length non-chimeric reads, per SMRT Cell 1M*
 - Profile transcripts from multiplexed samples in a single SMRT Cell⁶
 - Survey transcriptomes in 1-2 SMRT Cells on the Sequel System
 - Increase sequencing depth for more comprehensive transcriptome characterization

CHOOSE MOVIE TIMES BASED ON EXPERIMENTAL GOALS – 10 HOURS FOR QUICK SURVEYS AND 20 HOURS TO ENRICH FOR LONG TRANSCRIPTS



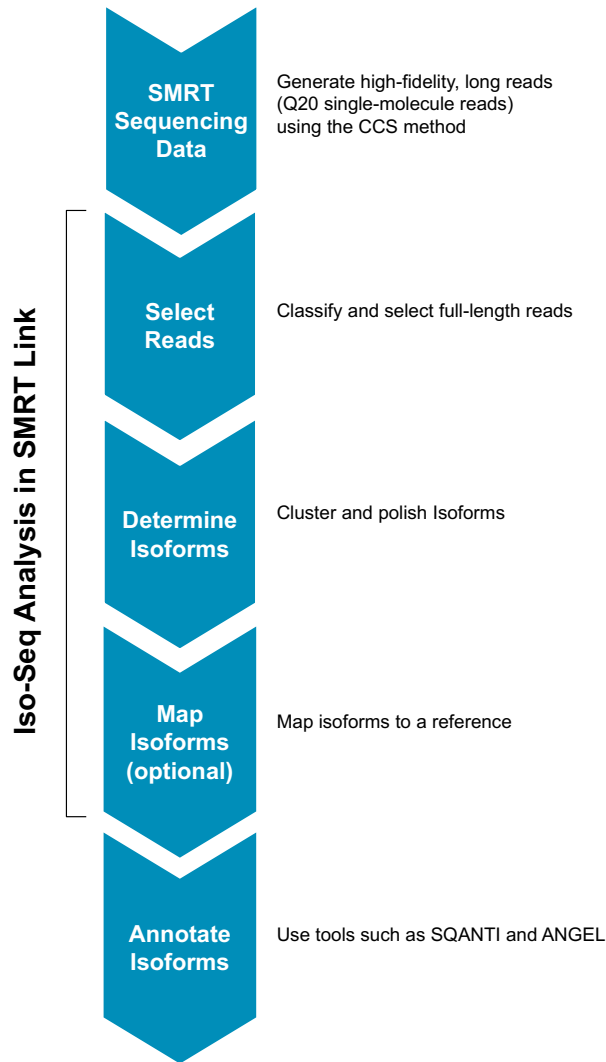
Number of high-quality, full-length transcripts for a diffusion-loaded, non-size selected Iso-Seq library sequenced for 10 hours (left) and 20 hours (right) with Sequel System, Chemistry 3.0, SMRT Link 6.0. The 20-hour run has more full-length sequences than the 10-hour run.

* Read lengths, number of reads, data per SMRT Cell, and other sequencing performance results vary based on sample quality/type and insert size, among other factors.

DATA ANALYSIS SOLUTIONS WITH PACBIO SMRT ANALYSIS

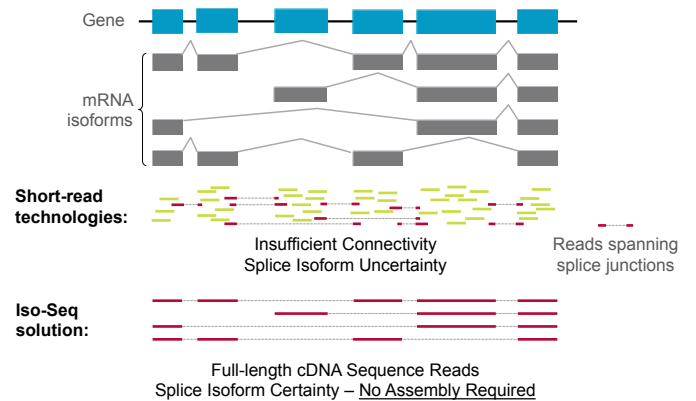
- Use the Iso-Seq protocol in SMRT Analysis to output high-quality, full-length transcript sequences, with no assembly required, to characterize transcripts and splice variants
- Run Iso-Seq analysis with or without mapping to a reference genome
- View tutorial⁷ for running the Iso-Seq protocol in SMRT Analysis

INFORMATICS PIPELINE FOR ISO-SEQ ANALYSIS



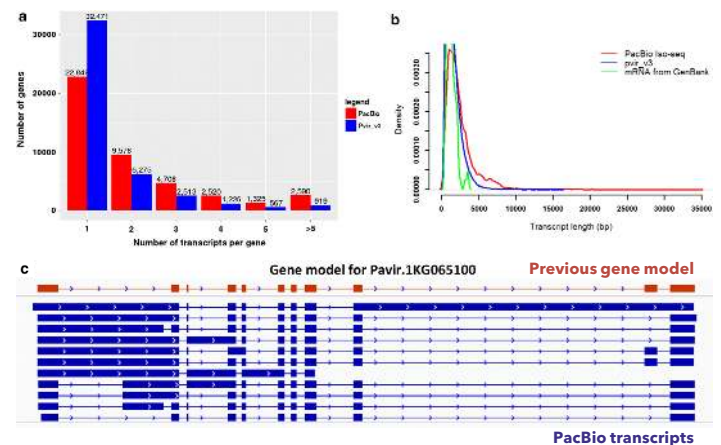
The Iso-Seq protocol, available in SMRT Analysis, generates consensus sequences and determines those transcripts that are full-length by detecting and identifying the 5' primer, Poly-A sequence, and the 3' primer of the reads.

DETERMINATION OF TRANSCRIPT ISOFORMS



The Iso-Seq method allows you to make evidence-based genome annotations, discover novel genes and isoforms, identify promoters and splice sites to understand gene regulation, improve accuracy of RNA-seq quantification for gene expression studies, and distinguish important stress response, developmental, or tissue-specific isoforms.

SIGNIFICANTLY IMPROVE GENOME ANNOTATIONS



Splice isoform analysis in the switchgrass transcriptome using the Iso-Seq method greatly improved genome annotation. A) The Iso-Seq method revealed the diversity of transcripts for each gene in the switchgrass genome. B) The length distribution of the transcripts was significantly higher with PacBio sequencing. C) A comparison of the previous gene model to the PacBio sequences⁸.

KEY REFERENCES

1. Procedure & Checklist – Iso-Seq Template Preparation for Sequel Systems
2. Clark, T. et al. (2017) Full-Length cDNA Sequencing on the PacBio Sequel Platform. *Poster presented at Plant and Animal Genome Conference*. San Diego, CA.
3. Full-length cDNA Target Sequence Capture Using SeqCap® EZ Libraries
4. Full-length cDNA Target Sequence Capture Using IDT xGen® Lockdown® Probes
5. Barcoding Samples for Isoform Sequencing (Iso-Seq Analysis)
6. Best practice for analyzing multiplexed Iso-Seq data
7. Tutorial: Iso-Seq Analysis Application
8. Zuo, C. et al. (2018) Revealing the transcriptomic complexity of switchgrass by PacBio long-read sequencing. *Biotechnology for Biofuels*. 11:170.