LONG-READ RNA SEQUENCING
BEST PRACTICES

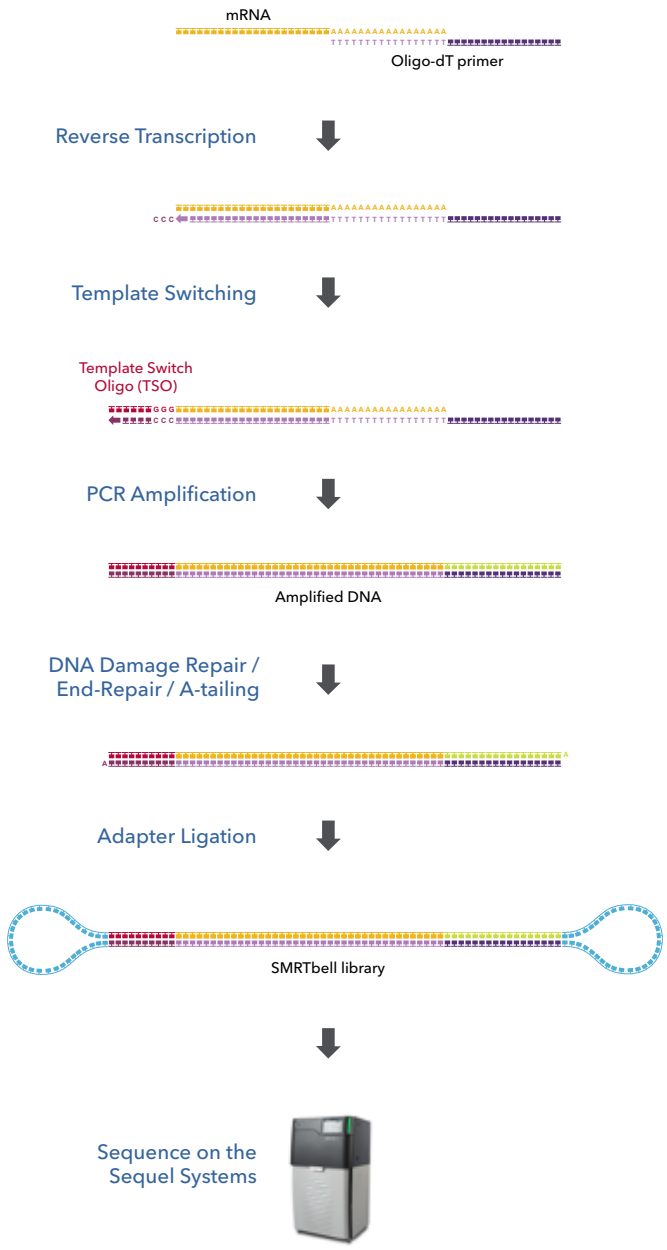
HUMAN BIOMEDICAL RESEARCH

PLANT AND ANIMAL SCIENCES

RNA SEQUENCING

With Single Molecule, Real-Time (SMRT®) Sequencing and the Sequel® Systems, you can easily and affordably sequence complete transcript isoforms in genes of interest or across the entire transcriptome. The Iso-Seq® method allows users to generate full-length cDNA sequences up to 10 kb in length — with no assembly required — to confidently characterize full-length transcript isoforms.

FROM RNA TO FULL-LENGTH TRANSCRIPTS

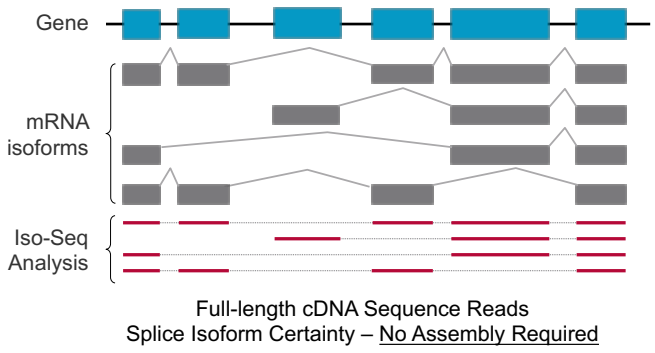


WORKFLOW RECOMMENDATIONS

- Prepare full-length cDNA from 300 ng of total RNA using the NEBNext® Single Cell/Low Input cDNA Synthesis & Amplification Module kit[1]
- Use the SMRTbell® Express Template Prep Kit 2.0 to prepare libraries in one day[2]
- Multiplex up to 12 samples[2]
- Scale throughput on Sequel Systems
  - Use the Sequel II System to generate up to 4 million* full-length, non-concatemer (FLNC) reads per SMRT Cell 8M
  - Or use the Sequel System to generate up to 500,000* FLNC reads per SMRT Cell 1M

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

DETERMINATION OF TRANSCRIPT ISOFORMS



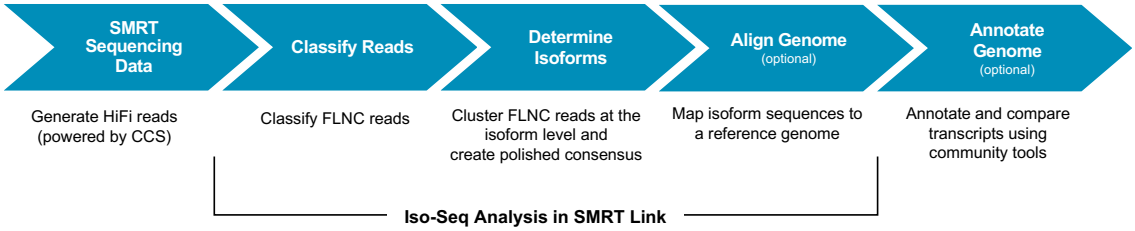
The Iso-Seq method allows you to produce evidence-based genome annotations, discover novel genes and isoforms, and improve RNA-seq quantification and allele-specific isoform expressions.

With a single SMRT Cell 8M you can:

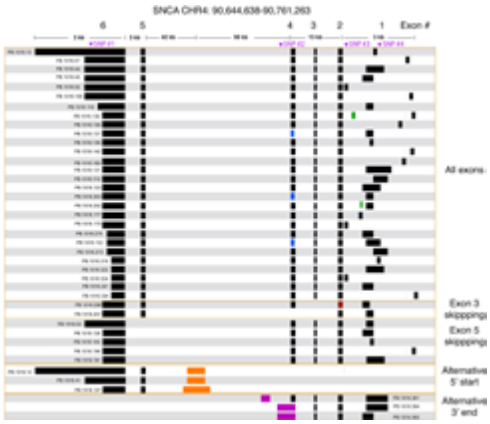
- Characterize a whole transcriptome
- Multiplex multiple tissues for genome annotation

DATA ANALYSIS SOLUTIONS WITH THE PACBIO ANALYTICAL PORTFOLIO

- Generate highly accurate long reads (HiFi reads), with single-molecule resolution using circular consensus sequencing (CCS) mode
- Use the Iso-Seq analysis in SMRT Link to output high-quality, full-length transcript FASTA sequences, with no assembly required, to characterize transcripts and splice variants[3,4]
- Run Iso-Seq analysis with or without a reference genome, and annotate the genome using community tools such as SQANTI[5], TAMA[6], and LoReAn[7]

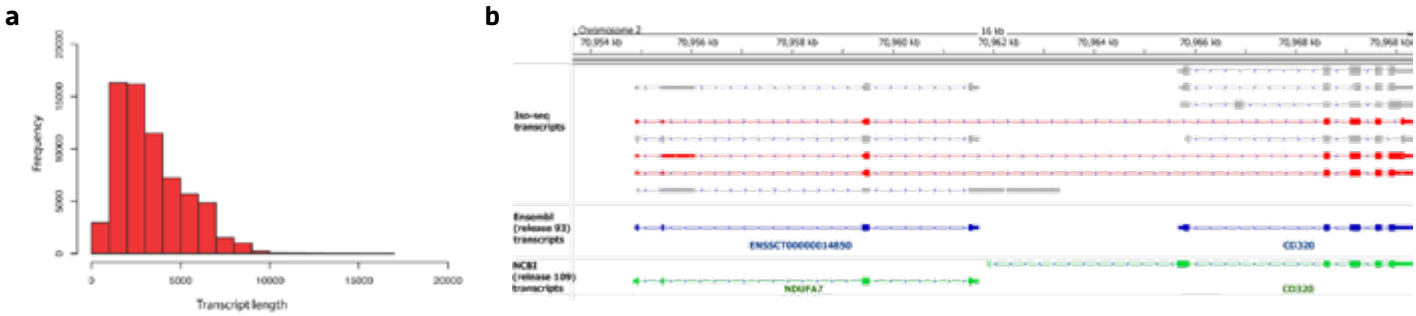


ACCURATELY DETECT ALTERNATIVE SPLICING EVENTS IN SPECIFIC GENES



The Iso-Seq method enables detection of complex alternative splicing of the synuclein alpha (SNCA) gene using targeted enrichment[8].

IMPROVE GENOME ANNOTATION ACROSS THE TRANSCRIPTOME



Full-length isoform analysis of the whole transcriptome of a single white cross-bred pig yielded 67,746 unique transcripts and detected 10,465 novel genes. Shown above (a) length distribution of transcripts and (b) example data showing transcripts covering multiple known genes and predicted protein-coding region in each transcript[9].

KEY REFERENCES

1. NEBNext® Single Cell/Low Input cDNA Synthesis & Amplification Module, Cat#: E6421S
2. Procedure & Checklist - Iso-Seq Express Template Preparation for Sequel and Sequel II Systems. PacBio Documentation
3. Tseng, E. (2019) PAG Conference: Iso-Seq analysis for plant & animal genomes – annotation evaluation & phasing. *Plant and Animal Genome XXVII Conference.* San Diego, CA.
4. Tutorial: Iso-Seq analysis application. *PacBio Tutorial.* https://www.pacb.com/support/training/
5. Community Tool SQANTI2: https://github.com/Magdoll/SQANTI2/
6. Community Tool TAMA: https://github.com/GenomeRIK/tama
7. Community Tool LoReAn: https://github.com/lfaino/LoReAn
8. Tseng, E., et al. (2019) The landscape of SNCA transcripts across synucleinopathies: New insights from long reads sequencing analysis. *BioRxiv, Preprint.*
9. Beiki, H., et al. (2019) Improved annotation of the domestic pig genome through integration of Iso-Seq and RNA-seq data. *BMC genomics*, 20(1), 344.

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