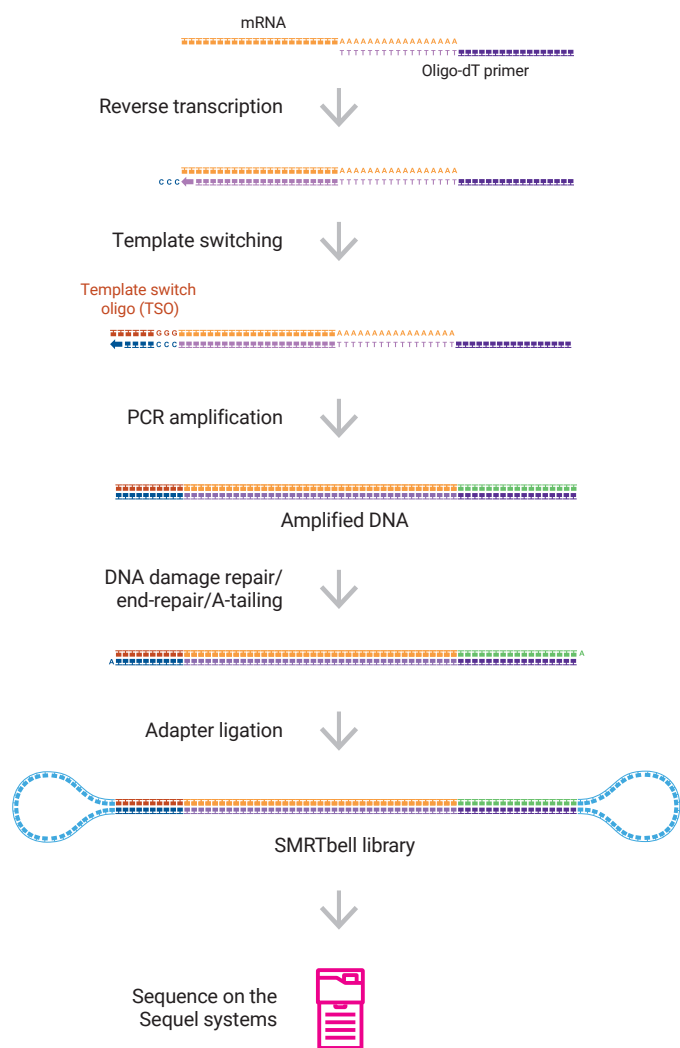# LONG-READ RNA SEQUENCING — BEST PRACTICES

With Single Molecule, Real-Time (SMRT®) sequencing and the Sequel® II or Sequel IIe system, 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

mRNA
Oligo-dT primer

Reverse transcription

Template switching

Template switch oligo (TSO)

PCR amplification

Amplified DNA

DNA damage repair/ end-repair/A-tailing

Adapter ligation

SMRTbell library
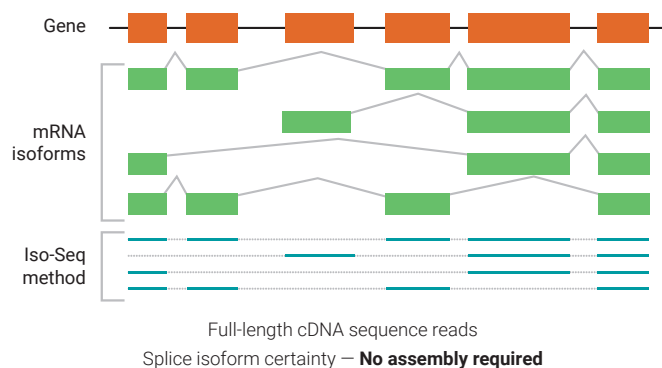
Sequence on the Sequel systems

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® prep kit 3.0 to prepare libraries in one day[2]

- Multiplex up to 12 samples[2]

- Use the Sequel II or Sequel IIe system to generate up to 4 million* full-length, non-concatemer (FLNC) reads per SMRT Cell 8M

* 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

Gene

mRNA isoforms

Iso-Seq method

Full-length cDNA sequence reads
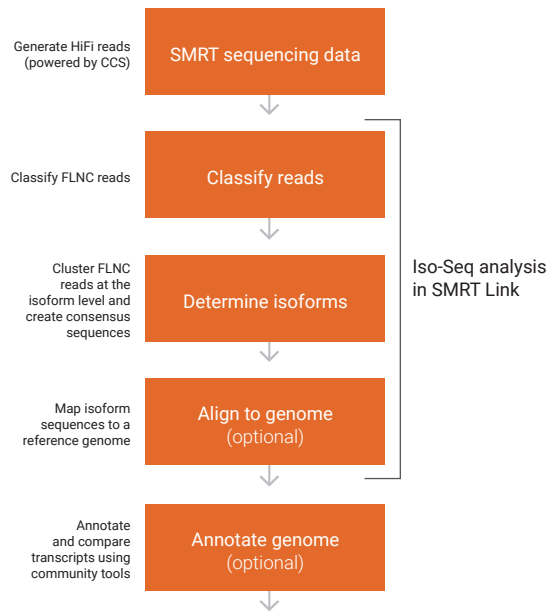Splice isoform certainty — **No assembly required**

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 expression.

With a single SMRT® Cell 8M you can:

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

PacBio

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 TAMA[5], SQANTI3[6], and tappAS[7]

Generate HiFi reads (powered by CCS) → **SMRT sequencing data**

Classify FLNC reads → **Classify reads**

Cluster FLNC reads at the isoform level and create consensus sequences → **Determine isoforms**

Map isoform sequences to a reference genome → **Align to genome** (optional)

Iso-Seq analysis in SMRT Link

Annotate and compare transcripts using community tools → **Annotate genome** (optional)
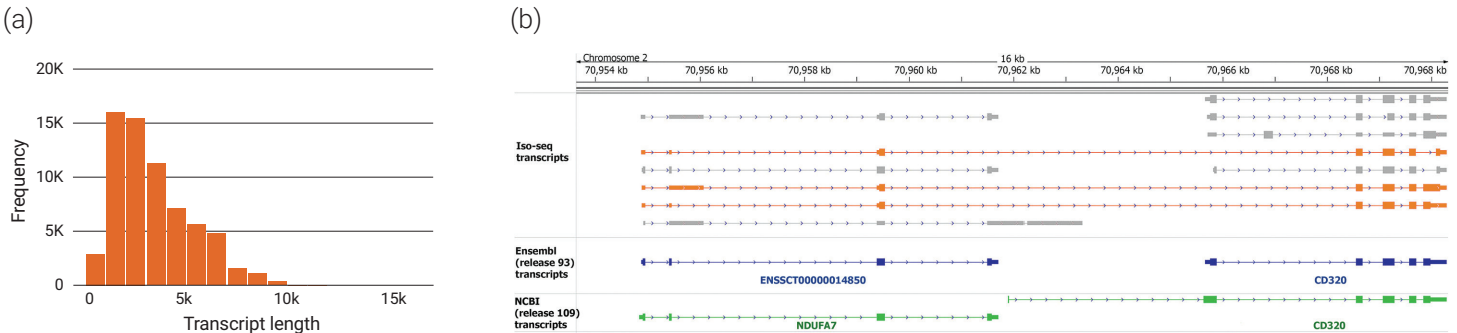
Accurately detect alternative splicing events in specific genes
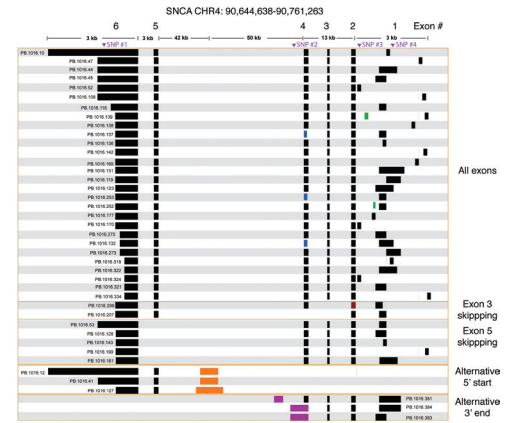
The Iso-Seq method enables detection of complex alternative splicing of the synuclein alpha (SNCA) gene using targeted enrichment.[8]

Improve genome annotation across the transcriptome

(a)

(b)

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: **Preparing Iso-Seq™ libraries using SMRTbell® prep kit 3.0.** 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. Iso-Seq bioinformatics documentation**: https://isoseq.how/**
5. Community tool TAMA: **https://github.com/GenomeRIK/tama**
6. Community tool SQANTI3: **https://github.com/ConesaLab/SQANTI3**
7. Community tool tappAS: **https://github.com/ConesaLab/tappAS**
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.

Learn about long-read RNA sequencing: **pacb.com/isoseq**

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