

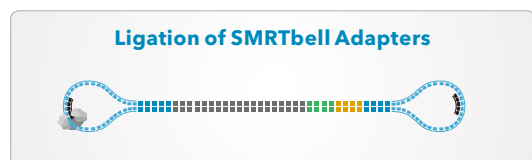
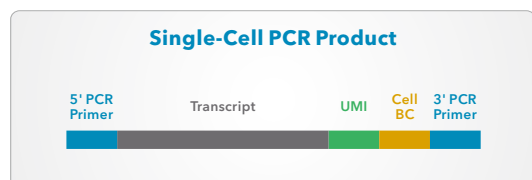
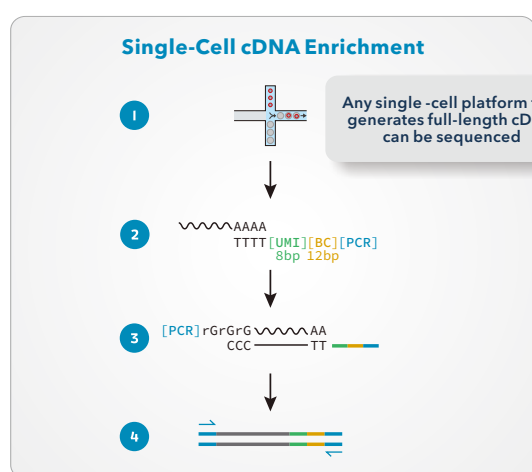
# SINGLE-CELL RNA SEQUENCING WITH HIFI READS

## BEST PRACTICES



With PacBio® single-cell RNA sequencing using the [Iso-Seq® method](#), you can now distinguish between alternative transcript isoforms at the single-cell level. The highly accurate long reads (HiFi reads) can span the entire 5' to 3' end of a transcript, allowing a high-resolution view of isoform diversity and revealing cell-to-cell heterogeneity without the need for assembly.

### FROM RNA TO FULL-LENGTH TRANSCRIPTS AT A SINGLE-CELL LEVEL



### WORKFLOW RECOMMENDATIONS

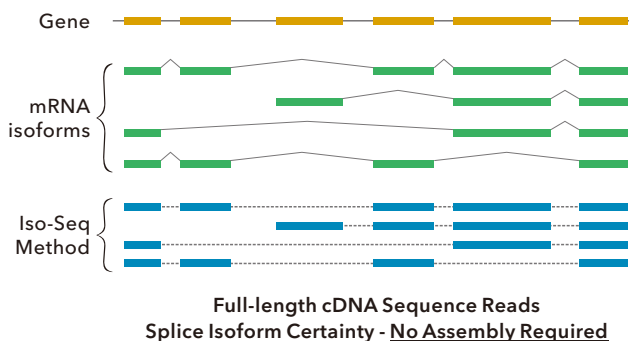
- Enrich for single-cell cDNA using a single-cell sorting platform that generates full-length cDNA\*
  - Template switch oligo (TSO)-based cDNA synthesis methods are recommended
  - The final single-cell cDNA product consists of 5' primer, transcript, poly-A tail, unique molecular index (UMI), cell barcode and 3' primer
  - To generate matching short-read data, save 5% of the material
  - Additional PCR cycles can be added if necessary
- Start library preparation with at least 160 ng of input cDNA (post-single-cell platform PCR reaction) for 1-2 SMRT® Cell 8M<sup>1</sup>
  - More starting material will be required for sequencing multiple SMRT Cells 8M
- Prepare libraries with the SMRTbell® Express Template Prep Kit 2.0 in one day<sup>2</sup>
- Use HiFi reads on the Sequel® II or IIe Systems to generate 3 million full-length reads from one SMRT Cell 8M to obtain ~1,000 unique molecules for 3,000 single cells\*\*
- Use 24 hr movies with 2 hrs pre-extension time<sup>1</sup>
- For human samples, run up to 240 SMRT Cell 8M/year at a cost of ~\$1,300/SMRT Cell 8M, excluding single-cell enrichment cost<sup>†</sup>

\* Number of usable reads, containing the UMI and cell barcode, vary by single-cell platform. Any platform that generates full-length cDNA is compatible with the single-cell RNA sequencing workflow.

\*\* Read lengths, reads/data per SMRT Cell type and other sequencing performance results vary based on single-cell platform, sample quality/type and insert size.

† Prices, listed in USD, are approximate and may vary by region. Pricing includes library and sequencing reagents run on a Sequel II or IIe System and does not include instrument amortization or other reagents.

### DETERMINATION OF TRANSCRIPT ISOFORMS

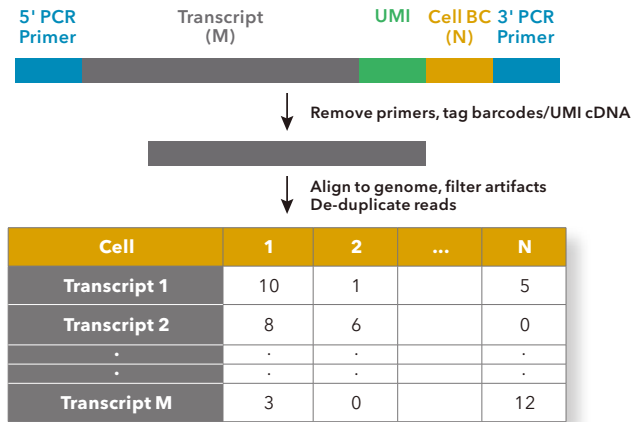


Single-cell RNA sequencing using the Iso-Seq method allows you to discriminate alternative transcripts in the context of full-length isoform, all at a single cell level<sup>3,4,5,6,7</sup>



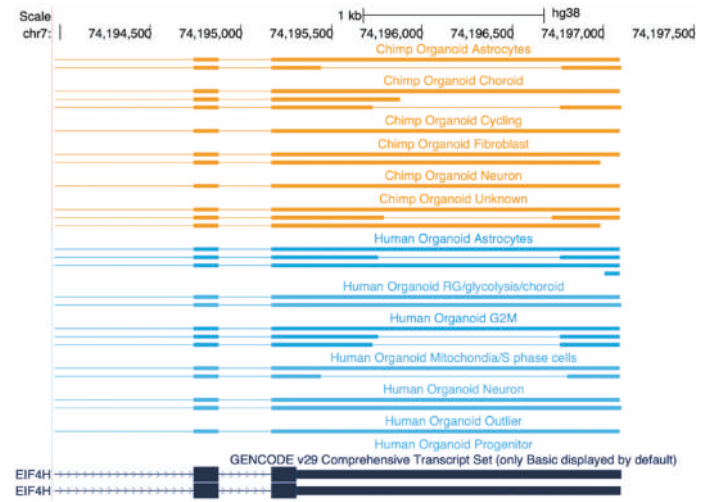
## DATA ANALYSIS SOLUTIONS

- Analyze HiFi reads which allow accurate single-cell barcode and UMI identification<sup>8</sup>
- Use the single-cell Iso-Seq analysis tools on GitHub<sup>8</sup> to output high-quality, full-length transcript FASTA sequences per UMI, with no assembly required, to characterize transcript variants for each cell



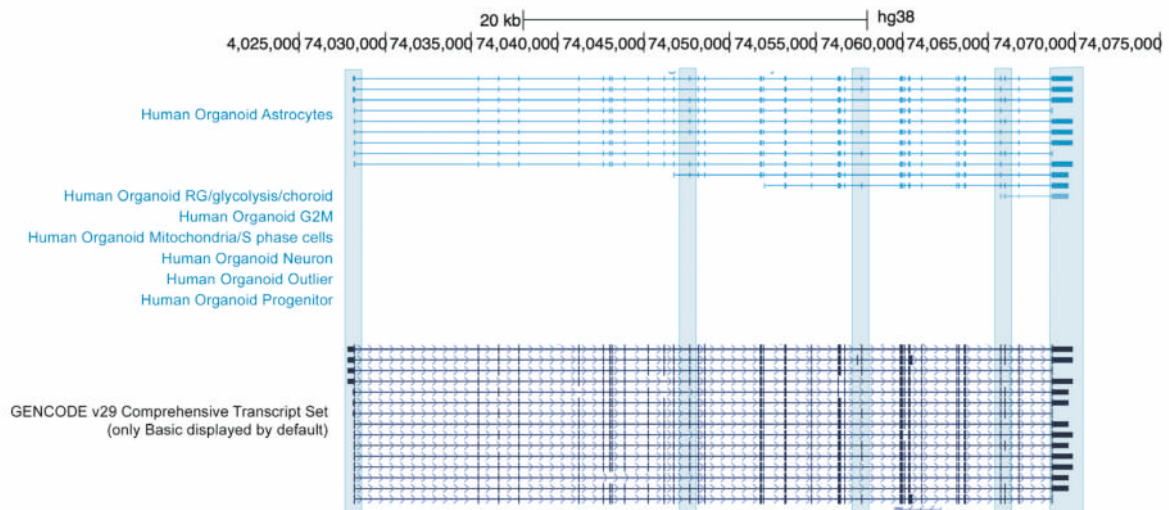
From gene count matrix to isoform count matrix.

## COMPARE ALTERNATIVE GENE-SPLICING EVENTS BETWEEN SPECIES



Assessment of post-transcriptional gene regulation for the EIF4H gene, reveals isoform heterogeneity between cell types.

## ASSIGN ALTERNATIVE ISOFORMS TO CORRECT CELL TYPE



The GENCODE database catalogs the numerous alternatively spliced Tropoelastin isoforms. Single-cell RNA sequencing assigned isoforms to individual cell types with multiple isoforms expressed in astrocytes and absent in other brain-specific cell types. The blue boxes also indicate new alternative splicing events compared to the reference.

## KEY REFERENCES

1. [Overview - Sequel Systems application options and sequencing recommendations](#). PacBio Documentation.
2. Procedure & Checklist - [Preparing single-cell Iso-Seq libraries using SMRTbell express template prep kit 2.0](#). PacBio Documentation.
3. Mincarelli, L. et al. (2020) [Combined single-cell gene and isoform expression analysis in haematopoietic stem and progenitor cells](#). *bioRxiv*.
4. Russell, A. B. et al. (2019) [Single-cell virus sequencing of influenza infections that trigger innate immunity](#). *Journal of Virology* 93: e00500-19
5. Gupta, I. et al. (2018) [Single-cell isoform RNA sequencing characterizes isoforms in thousands of cerebellar cells](#). *Nature Biotechnology* 36: 1197
6. Karlsson K. et al. (2017) [Single-cell mRNA isoform diversity in the mouse brain](#). *BMC Genomics* 18: 126
7. Macaulay et al. (2015) [G&T-seq: parallel sequencing of single-cell genomes and transcriptomes](#). *Nature Methods* 12: 519
8. [Data Analysis Procedure on PacBio GitHub](#)



Single-Cell  
RNA Sequencing