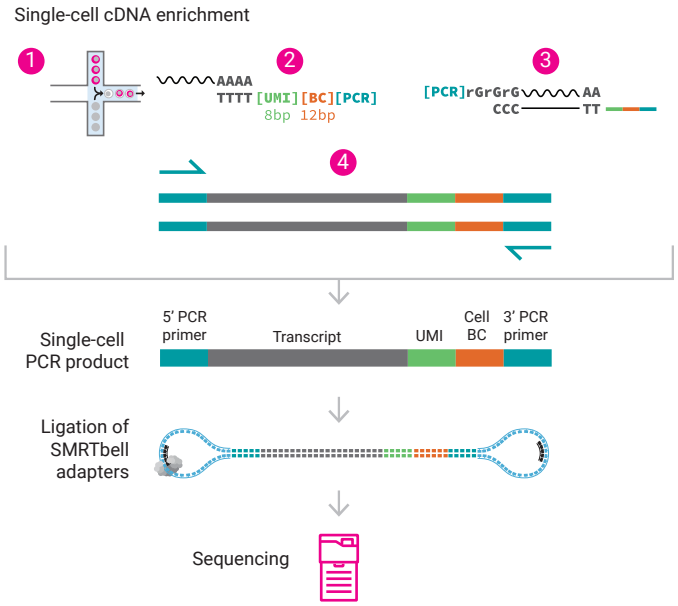
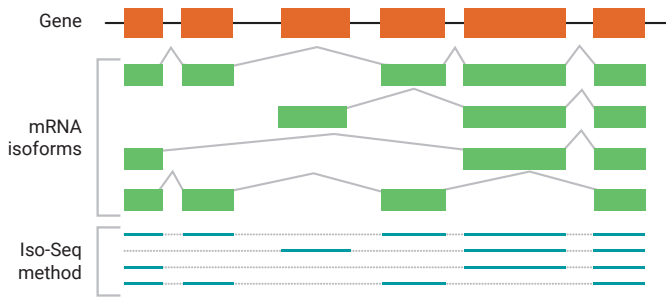
SINGLE-CELL RNA SEQUENCING WITH HIFI READS — BEST PRACTICES

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



Assign alternative isoforms to correct cell type



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[3,4,5,6,7]

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–v2 SMRT® Cells 8M[1]
  - 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[2]
- 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[1]
- For human samples, run up to 240 SMRT Cell 8Ms/year at a cost of ~$1,300/SMRT Cell 8M, excluding single-cell enrichment cost†

* 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.

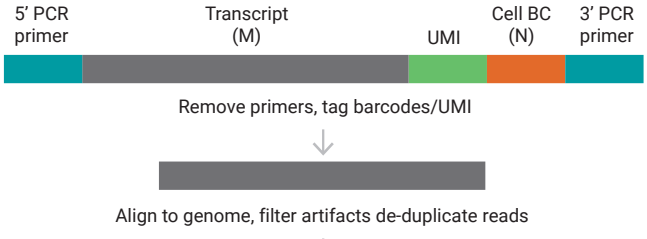
** Read lengths, reads/data per SMRT Cell type, and other sequencing performance results vary based on single-cell platform, sample quality/type, and insert size.

† 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.

PacBio

Data analysis solutions

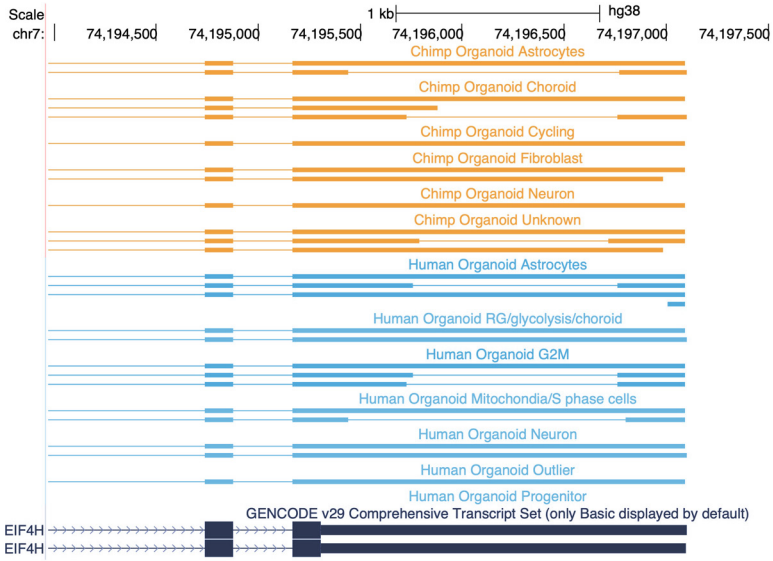
- Analyze HiFi reads which allow accurate single-cell barcode and UMI identification[8]
- Use the single-cell Iso-Seq analysis tools on GitHub[8] to output high-quality, full-length transcript FASTA sequences per UMI, with no assembly required, to characterize transcript variants for each cell



Cell	1	2	...	N
Transcript 1	10	1		5
Transcript 2	8	6		0
.	.	.		.
.	.	.		.
Transcript M	3	0		12

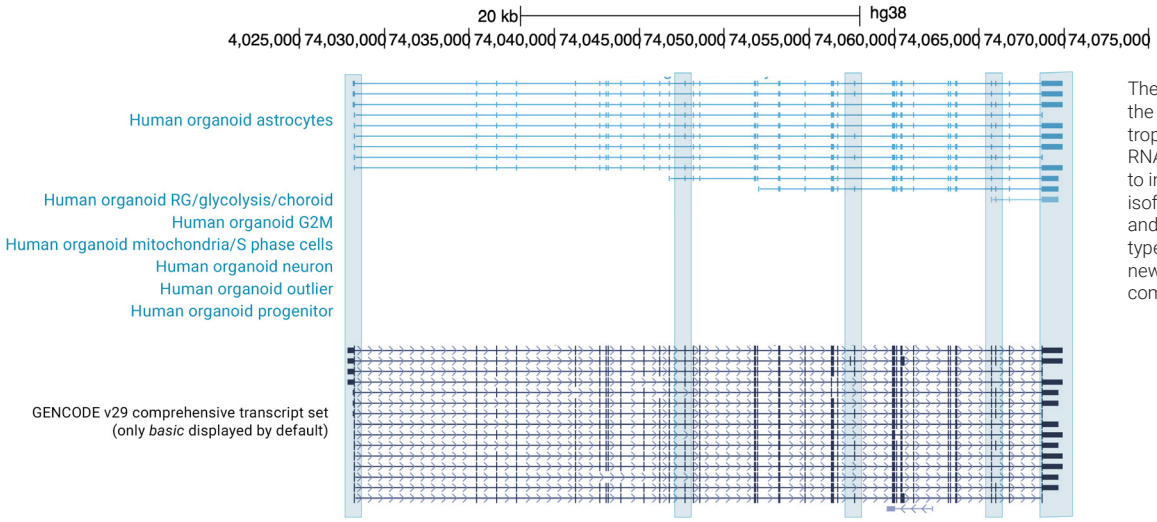
From gene count matrix to isoform count matrix

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**

Learn about single-cell RNA sequencing: **pacb.com/sc-isoseq**

102-193-602 V1 17DEC2021