SINGLE-CELL RNA SEQUENCING WITH HIFI READS BEST PRACTICES



PACBIO



With PacBio[®] single-cell RNA sequencing using the <u>Iso-Seq[®] method</u>, 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 (postsingle-cell platform PCR reaction) for 1-2 SMRT[®] Cell 8M¹
 - 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 day²
- Use HiFi reads on the Sequel[®] II or Ile 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 time¹
- * 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.

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^{3,4,5,6,7}





DATA ANALYSIS SOLUTIONS

- Analyze HiFi reads which allow accurate single-cell barcode and UMI identification⁸
- Use the single-cell Iso-Seq analysis tools on GitHub⁸ to output high-quality, full-length transcript FASTA sequences per UMI, with no assembly required, to characterize transcript variants for each cell

5' PCR Primer	Transcript (M)			UM	II Cell B (N)	C 3' PCR Primer		
		↓	Remov	ve prin	mers, tag b	arcodes/U	MI cDNA	
		Align to genome, filter artifacts ▼ De-duplicate reads						
Cell	1		2			N		
Transcript 1	1	0	1			5		
Transcript 2	8	3	6			0		
•	· ·		•			•	-	
Transcript N	1 3	3	0			12		

COMPARE ALTERNATIVE GENE-SPLICING EVENTS BETWEEN SPECIES



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

From gene count matrix to isoform count matrix.

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. <u>Overview Sequel Systems application options and sequencing recommendations.</u> 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) <u>G&T-seq: parallel sequencing of single-cell genomes and transcriptomes.</u> Nature Methods 12: 519
- 8. Data Analysis Procedure on PacBio GitHub





Single-Cell RNA Sequencing