PacBio

Whitepaper

Full-length bulk and single-cell RNA sequencing for neuroscience research

The ability to characterize bulk and single-cell transcriptomes at the isoform level is critical for neuroscience research. Many genes associated with neurological diseases or brain development have complex alternative splicing (Licatalosi & Darnell, 2006), and there is emerging potential in utilizing alternative splicing defects for RNA-based therapeutics (Nikom & Zheng, 2023).

The Iso-Seq® method, full-length RNA sequencing using PacBio® HiFi sequencing, can sequence full-length isoforms without the need for computational inferencing, reducing errors in isoform identification (Figure 1).

The Kinnex™ RNA kits, based on the MAS-Seq method for concatenating amplicons such as cDNA, further increases throughput on the PacBio long-read sequencers. Here, we highlight publications using Iso-Seq in neuroscience research to show how bulk and single-cell Iso-Seq data help identify novel isoforms, determine disease-specific isoform expressions, delineate splicing patterns during brain development, and help identify new drug candidates.

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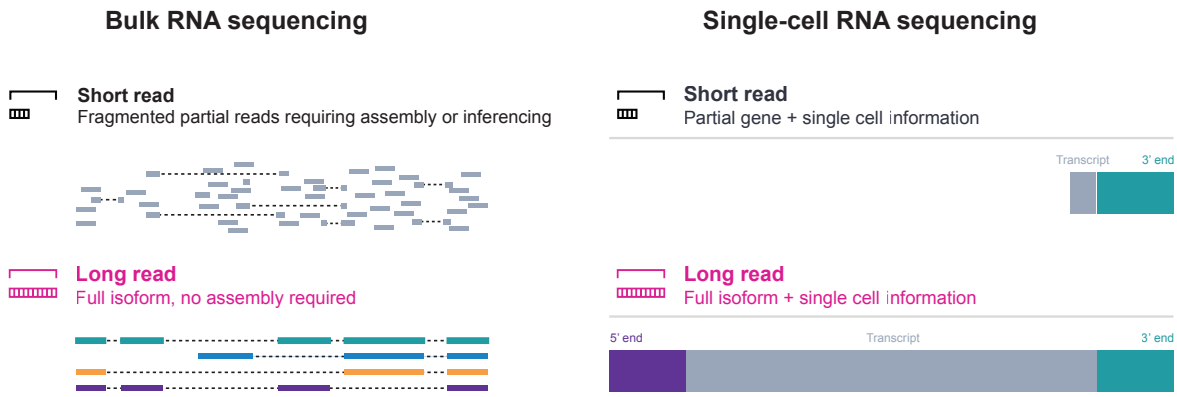

Figure 1. Comparison of long- vs short-read RNA sequencing. In bulk transcriptome sequencing, short reads required computational assembly or inference to identify transcripts, whereas long reads resolve the full-length cDNA molecule. In single-cell transcriptome sequencing, short reads are limited to the ends of a cDNA molecule and can only offer gene-level information. In contrast, long reads can provide isoform information at the single-cell level.

Spotlight: Kinnex concatenation kits

The Kinnex RNA kits include the Kinnex full-length RNA kit, the Kinnex single-cell RNA kit, and the Kinnex 16S rRNA kit. Kinnex RNA kits are all based on the MAS-Seq method, which joins amplicons into longer concatenated fragments. HiFi reads generated from sequencing the concatenated molecules can then be bioinformatically broken up to retrieve the original amplicon sequences.

The main difference between the three Kinnex kits are the number of Kinnex primers that determine the concatenation factor (and therefore the throughput increase). The concatenation factors were determined to best suit the target amplicon sizes to maximize HiFi sequencing yield.

Learn more about Kinnex at https://pacb.com/kinnex

Novel splicing and neuropsychiatric disorders

Alternative splicing occurs at high frequency in the brain compared to the rest of organs and tissues (Mazin et al. 2021) and is thought to contribute to the functional complexity of the nervous system. Treutlein et al. (2014) sequenced the neurexin genes in murine prefrontal cortex (PFC) samples and found an evolutionarily-conserved, novel alternatively spliced exon in *Nrxn1α* and *Nrxn3α* genes, as well as 274 unique splice isoforms for Nrxn1α. This work demonstrates how alternative splicing affects critical processes for synaptic transmission.

Flaherty et al. (2019) applied a similar targeted approach to characterize 123 *Nrxn1α* isoforms in human PFC and hiPSC-derived neurons for studying the effect of heterozygous intragenic deletions in neuropsychiatric disorders. The hiPSC-neurons showed splicing dysregulation of *Nrxn1α*, where wild-type isoform expression was reduced, but mutant isoform expression increased. Importantly, certain mutant isoforms were shown to significantly perturb neuronal activity that cannot be mitigated by overexpression of wild-type isoforms. These data suggest that overexpression of even a single mutant isoform could be sufficient to perturb neuronal activity.

Spotlight: Targeted approaches for isoform sequencing with the Iso-Seq method

Many Iso-Seq studies chose targeted sequencing to increase sequencing coverage on disease genes of interest. We describe the benefits and disadvantages of different targeted approaches here.

Amplicon sequencing: Primers are designed based on conserved regions of a gene (e.g., first and last exon). This approach is the most cost-effective for sequencing one or two genes, but can only capture transcripts that have the target starts and end, which could lead to missing transcripts with alternative start or end sites.

Hybrid capture: Hybridization probes are designed across gene bodies to capture transcripts with variable start and end sites. This approach is the most versatile as it can be used for as few as one gene and as many as thousands of genes. It is more costly than amplicon sequencing, but requires less coverage than whole transcriptome sequencing.

Cataloging alternative splicing in neurodegenerative diseases

Alzheimer's Disease (AD), Dementia with Lewy bodies (DLB), and Parkinsons Disease (PD) share overlapping neuropathologies and clinical manifestations, where many of the same genes are implicated in disease association. Efforts to catalogue novel isoforms therefore often include samples from multiple disease types. Tseng et al. (2019) sequenced the *SNCA* gene in PD, DLB, and control samples using the Iso-Seq method and showed complex usage of alternative start sites and variable 3' UTR lengths. Evans et al. (2024) used a similar approach to sequence the *SNCA* gene in human dopaminergic neurons and showed that annotated *SNCA* transcripts accounted for only 5% of expression, whereas the majority of transcripts use alternative 5' and 3' UTRs (Figure 2). Together, these studies show that HiFi sequencing reveals novel transcripts for genes implicated in neurodegenerative disease.

Humprey et al. (2025) focused on creating a comprehensive isoform atlas for microglia, generating Iso-Seq data for 30 post-mortem brain samples and detected 35,879 novel isoforms and 2,238 novel genes not found in GENCODE. The expanded transcriptome added new noncoding regulatory isoforms including intron retention, antisense, and readthrough fusions. The authors then applied different QTL analyses using the augmented transcriptome and identified 456 novel eQTL genes and 5,658 tuQTL novel isoforms. By significantly expanding the microglial transcriptome with HiFi sequencing, this study links splicing with potential disease risk.

Course et al. (2023) used a targeted approach to characterize variation in *PSEN1* and *PSEN2* isoforms in control, familial, and sporadic Alzheimer's disease brains. The *PSEN1* isoforms were found largely to be similarly expressed among the three sample cohorts, while a *PSEN2* exon 9B splice variant was found to be elevated in the sporadic Alzheimer's disease cases. This work illustrates how isoform-resolved analysis can reveal subtle, disease-associated splicing differences that may be invisible at the gene-expression level.

Extending the targeted approach from bulk to single-cell transcriptome, Liu et al. (2024) characterized 50 disease-related genes in AD, DLB, PD, and control single-nuclei libraries. Not only did the authors identify novel isoforms shared across all sample groups, certain genes, for example, *MAPT* and *CLU,* exhibited major isoform switching events in excitatory neurons in AD and DLB samples. This study shows how single-cell isoform profiling with HiFi sequencing can establish disease-associated isoform diversity.

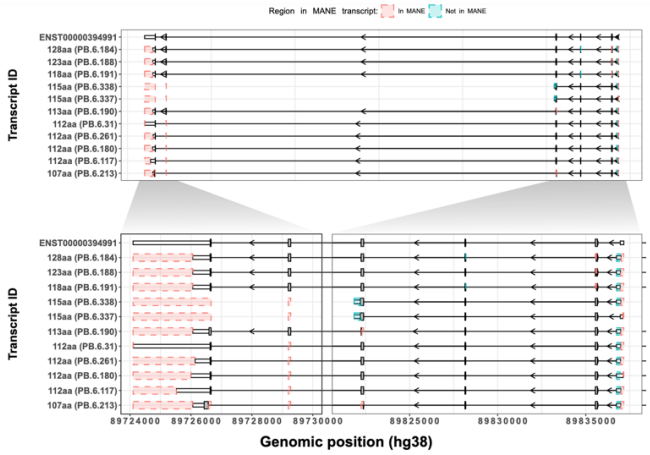

Figure 2. Novel coding SNCA isoforms discovered in Evans et al. (2024) as compared to the MANE select reference.

Reinterpreting genetic risks associated with *de novo* variants

Cataloging new isoforms, and even new genes, in previously understudied brain tissues can help reprioritize variants associated with neurological conditions. Patowary et al. (2024) used bulk and single-cell long-read RNA sequencing on mid-gestation human neocortex and identified more than 140,000 novel isoforms and added ~27 Mb transcribed portions of the human genome not previously found in Gencode. The authors augmented Gencode with the newly discovered isoforms using the Ensembl Variant Effect Predictor (VEP) tool and uncovered more severe consequences for 1.24% of all variants associated with autism spectrum disorder (ASD) and intellectual disability (ID)/developmental disorders (DD). Additionally, retraining SpliceAI with the new isoforms predicted a larger proportion of cryptic splice sites, which are known be a mechanism underlying ASD/ID/DD risks, contributing expansive single-cell data to a better understanding of genetic risk.

Detecting cell-type specific splicing patterns in developing brains

The developing brain presents a unique opportunity to study splicing differences across timepoints and different cell types. Joglekar et al. (2024) created a single-cell atlas for the mouse hippocampus and visual cortex across four postnatal time points and contrasted it against adult mouse brains using long-read single-cell RNA sequencing. The authors found that isoform variability was highest across cell subtypes and less so across different brain regions or time points. Certain cell types, particularly thalamic and cerebellar astrocytes, exhibited strong variability in transcription start sites (TSS), polyadenalytion (PolyA) sites, and exon regulation, implying that cell types can display specialized splicing patterns depending on their region of origin. Splicing volatility peaked across all cell types in mouse adolescence, with some cell-type specific exons transiently changing inclusion status and losing their cell-type specificity. Further, it was found that cell type identities may be partially defined by splicing, rather than gene expression alone (Figure 3).

In a similar study, Patowary et al. (2024) re-clustered cells from mid-gestation brains at the isoform level, and found that certain cell types – progenitors transitioning into neurons and early-born excitatory neurons – were split into additional clusters, providing higher-resolution cell maturation stages than those observed by gene-level clustering. This work adds to the conclusion that isoform-level data provides a better understanding of brain development than gene expression alone.

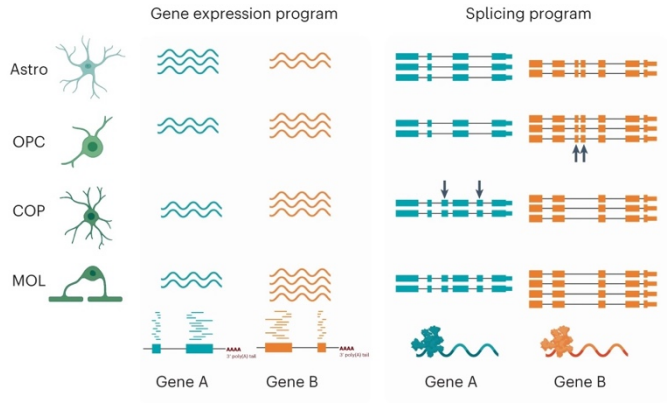

Figure 3. Findings from Joglekar et al. (2024) suggest that while subtypes in the oligodendrocyte lineage have similar gene expression patterns, the OPC splicing pattern more closely resembles that of astrocytic splicing.

Towards developing RNA-based therapeutics

Identification of specific isoforms that are differentially expressed in diseases have the potential to become targets for therapeutics. Evans et al. (2024) designed anti-sense oligonucleotides (ASOs) targeting the diverse 3' UTRs in the *SNCA* gene and showed that one of the candidates was able to reverse Parkinson's Disease (PD) cellular pathology in midbrain dopaminergic neurons. Importantly, targeted Iso-Seq data showed many previously unannotated 3' UTRs for the *SNCA* gene, indicating that ASO sequences designed against the canonical 3' UTR would only target a small portion of the total *SNCA* expression. Thus, generating a comprehensive transcriptome map in the target tissue or cell types is paramount for effective RNA-based therapeutics design.

In a different study, Gustavsson et al. (2025) used targeted Iso-Seq to identify novel amyloid precursor protein (APP) transcripts shown to encode for a new 100 amino acid ORF, different from the canonical APP-C99, that is generated independently of β-secretase cleavage. Further, these novel APP transcripts are detected in full-length APP KO neurons, suggesting alternative pathways of amyloid-β (Aβ) generation and aggregation. The authors note that this finding might imply that current therapeutic approaches targeting canonical Aβ production might fail to fully reduce amyloid levels, and that a combination approach targeting multiple steps in the amyloidogenic pathway is likely needed.

Conclusion

Taken together, these studies demonstrate how long-read RNA sequencing with PacBio offer advantages for studying neurological development, conditions, and diseases due to its ability to characterize full-length isoforms both at the bulk and single-cell level with high accuracy. Neuroscience research using PacBio sequencing can resolve the full complexity of RNA splicing in the brain, and has already revealed novel coding isoforms, refined cell subtypes during brain development, identified disease-associated splice patterns, and showed promise for expediting therapeutic development.

Selecting bulk, single cell, or single-nuclei sequencing

Depending on sample availability and study goals, researchers may choose bulk, single-cell, or single-nuclei for their studies.

Bulk transcriptome sequencing: Bulk transcriptome sequencing is more cost-effective compared to single-cell or single-nuclei approaches. The cDNA generated from bulk RNA is also typically longer than those generated from single cell platforms. Bulk transcriptome sequencing is therefore ideal for establishing a reference transcriptome. However, researchers will not be able to distinguish cell type-specific isoforms. ***Recommended kit:*** **Iso-Seq express 2.0 kit with Kinnex full-length RNA kit**

Single-cell RNA sequencing:

Single-cell (and single-nuclei) approaches offer the ability to identify cell type-specific isoform expression, which is a significant advantage over bulk transcriptomes. However, there are additional costs associated with preparing cDNA for compatible single-cell platforms.
Recommended kit: **Kinnex-compatible single-cell cDNA with Kinnex single-cell RNA kit**

Single-nuclei RNA sequencing:

Single-nuclei RNA sequencing is often the preferred approach over single-cell sequencing for brain tissue because of the difficulty with dissociating large and delicate neurons. However, single-nuclei RNA contains many partial or fully unspliced RNA, which hinders data usability for studying spliced isoforms. Some researchers choose exome enrichment (see Hardwick et al. 2022) to enrich for spliced mRNA, while others value unspliced mRNA for detecting intronic SNPs for identifying allele-specific expression (see Simmons et al. 2024).

Recommended kit: **Kinnex-compatible single-nuclei cDNA with Kinnex single-cell RNA kit**

Table 1. Summary of neurological research publications using full-length RNA sequencing.

Study	Samples	Method	Key findings
Treutlein et al. 2014	Adult mice prefrontal cortex	Amplicon-based targeted Iso-Seq	Complex splicing of neurexin genes
Flaherty et al. 2019	hiPSC-NPCs and hiPSC-neurons	Amplicon-based targeted Iso-Seq	Splicing dysregulation of *Nrxn1α*
Tseng et al. 2019	Control, PD, DLB brain	Targeted Iso-Seq	Novel *SNCA* isoforms
Evans et al. 2024	Human dopaminergic neurons	Targeted Iso-Seq	Novel *SNCA* isoforms
Gustavsson et al. 2025	iPSC-derived cortical neurons, astrocytes, microglia, and post-mortem brain	Targeted Iso-Seq	Novel APP isoforms
Humphrey et al. 2025	Microglia	Iso-Seq	Microglia isoform atlas
Course et al. 2023	Control, familial, and sporadic Alzheimer's brain	Targeted Iso-Seq	*PSEN1* and *PSEN2* isoforms
Liu et al. 2024	Control, AD, DLB, PD brain	Single-nuclei with Kinnex*	Novel isoform switching events
Patowary et al. 2024	Mid-gestation brain	Bulk & single-cell Iso-Seq	Novel isoforms, refined cell subtypes based on isoforms
Joglekar et al. 2024	Developing and adult mice brain	Single-cell Iso-Seq	Cell type-specific splicing patterns
Hardwick et al. 2022	Healthy human mid-frontal cortex	Single-nuclei with exome enrichment	Cell type-specific splicing patterns

*Includes use of the original or modified versions of the MAS-ISO-Seq method, the MAS-Seq for 10x Single Cell 3' kit, and all Kinnex RNA kits.

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Nikom, D., & Zheng, S. (2023). Alternative splicing in neurodegenerative disease and the promise of RNA therapies. *Nature Reviews Neuroscience*, *24*(8), 457-473.

Simmons, S. K., et al. (2025). Experimental and Computational Methods for Allelic Imbalance Analysis from Single-Nucleus RNA-seq Data. *bioRxiv*, 2024-08.

Treutlein, B., Gokce, O., Quake, S. R., & Südhof, T. C. (2014). Cartography of neurexin alternative splicing mapped by single-molecule long-read mRNA sequencing. *Proceedings of the National Academy of Sciences*, *111*(13), E1291-E1299.

Tseng, E., et al. (2019). The landscape of *SNCA* transcripts across synucleinopathies: new insights from long reads sequencing analysis. *Frontiers in Genetics*, *10*, 584.

Resources

Application note – Kinnex full-length RNA kit for isoform sequencing

Application note – Kinnex single-cell RNA kit for single-cell isoform sequencing

Webinar – HiFi on the brain: Advancing Parkinson's Disease research using HiFi Sequencing. Speaker: Mina Ryten.

Webinar – Long-read RNA neurology symposium. Speakers: Andy Yang, Jack Humphrey, Michael Gandal, Ana Shahnaee, Emil Gustavsson.

Webinar – Mapping brain isoforms with long-read RNA sequencing. Speaker: Christine Liu.