

# Full-Length RNA-seq of Alzheimer Brain on the

# PacBio Sequel II System

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RNA SEQUENCING

## Abstract

The PacBio Iso-Seq method produces highquality, full-length transcripts and can characterize a whole transcriptome with a single SMRT Cell 8M.

We sequenced an Alzheimer whole brain sample on a single SMRT Cell 8M on the Sequel II System. Using the Iso-Seq bioinformatics pipeline followed by SQANTI2 analysis, we detected 162,290 transcripts for 17,670 genes up to 14 kb in length. More than 60% of the transcripts are novel isoforms, the vast majority of which have supporting cage peak data and polyadenylation signals, demonstrating the utility of long-read sequencing for human disease research.

Full-Length Isoforms using Iso-Seq

	Known	Novel	Total
Genes	17,051	619	17,670
Isoforms	51,660	110,630	162,290

Table 1. Number of genes and isoforms detected in the Alzheimer brain sample after Iso-Seq analysis and SQANTI2 filtering. All post-filtered isoforms have all junctions supported by public (Intropolis) RNA-seq data.

# **Complex Splicing in Alzheimer Brain**

#### PLD3 Isoforms with Alternative Starts/Ends

	chr19:40,348,500-40,382,886 34,387 bp. enter position, gene symbol, HGVS or search terms go									
chr19	(q13.2) 19p13.3	19p13.2	p13.11 19p1	2 19q12 q1	3.11 13.	.12 19 <b>c</b> 1	13.2	13.32 q13.3	3 13.42	13.43
Alzheimer Brain Iso-Seq 2019 (post-Filter)										
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PB.17533.11	<b>▶→→→→→→→→→→</b> →	· <del>›››››››››››››</del>	<del>&gt;</del>	<del>}}}}}</del>	$\rightarrow$	<del>&gt;&gt;&gt;&gt;&gt;</del>	$\rightarrow \rightarrow \rightarrow$	******	$\rightarrow\rightarrow\rightarrow\rightarrow\rightarrow$	
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PB.17533.13		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	·····	·····			$\rightarrow$	· · · · · · · · · · · · · · · · · · ·	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
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The data is publicly available at: https://downloads.pacbcloud.com/public/dat aset/Alzheimer2019 IsoSeq/

Full-Length RNA-Seq on Sequel II System

# **Iso-Seq Express kit**<sup>[1]</sup>

- 300 ng total RNA
- Full-length cDNA



Min: 80 bp





#### PSEN2 Isoforms with Fusion to COQ8A



#### Saturation of Known Transcripts



Most Isoforms Near CAGE peak + polyA Signal

Category	Count	CAGE peak within 50 bp	polyA Motif Detected			
FSM	32,649	70%	72%			
ISM	19,011	37%	62%			
NIC	84,610	36%	55%			
NNC	25,323	57%	72%			
Antisense	321	24%	43%			
Intergenic	376	24%	38%			

#### Alzheimer Brain Shows High Splicing Complexity

Number of Isoforms per Gene



### Conclusions

- The Iso-Seq method produces high-quality, full-length transcripts up to 15 kb
- 1-day prep, 1-day sequencing, 1-day bioinformatics analysis
- Iso-Seq method reveals complex alternative splicing in Alzheimer brain sample

### **Bioinformatics**

Iso-Seq3 in SMRT Analysis

- Reads to ORF in 1 day
- Downstream community tools<sub>[2]</sub>





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