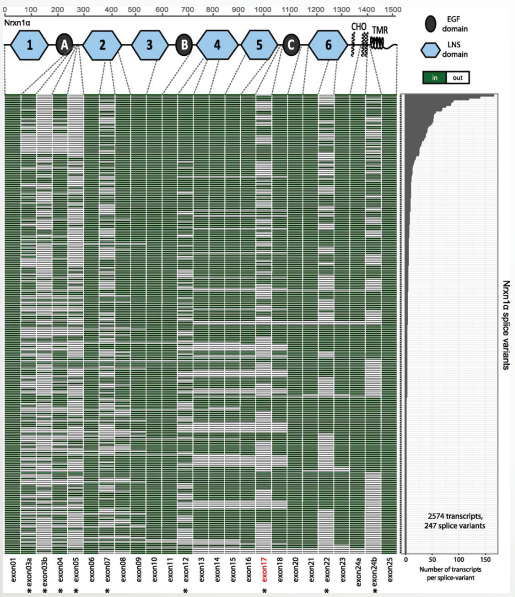
PACIFIC BIOSCIENCES®

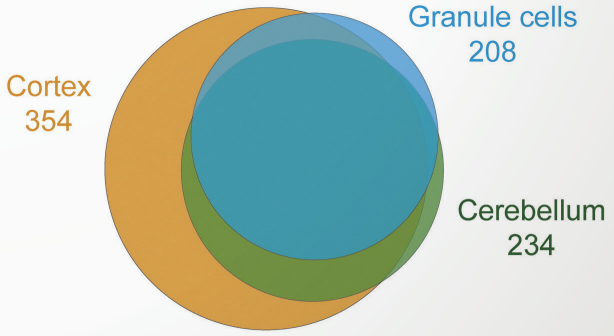
Full-length Transcripts No Assembly Required

The **Iso-Seq™ application** generates full-length cDNA sequences, from the poly-A tail to the 5' end of transcripts - eliminating the need for transcript reconstruction and inference - giving **complete, unambiguous information about alternatively spliced exons, transcriptional start sites**, and poly-adenylation sites. Be confident in the characterization of the full complement of isoforms within targeted genes, or across an entire transcriptome.

- Directly detect full-length mRNA and lncRNA transcripts
- Discover novel genes, gene isoforms, and gene fusion events
- Evidence-based gene annotation
- Improve quantitation accuracy of RNA-seq data with sample-specific gene models
- Detect allele-specific transcript isoform expressions

Complex Isoforms of Neurexin 1α Gene Detected Using Isoform Sequencing with PacBio

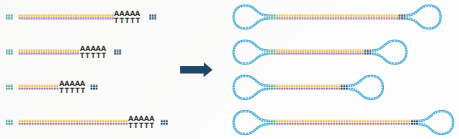




A. Transcript map of 247 unique alternatively spliced isoforms of the Neurexin 1α gene (~4-5 kb) generated with a targeted Iso-Seq method.[1]
B. Neurexin 1α isoform variants distribution detected across three cell types.[2]

From RNA to Evidence-based Gene Models

Iso-Seq Sample Preparation

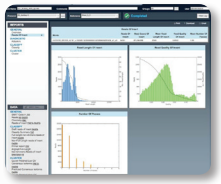


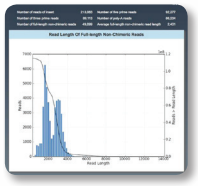
- Prepare full-length transcripts with from total RNA (~50 ng) or mRNA
- Optional size-selection protocol for transcripts > 3 kb
- Support for targeted genes and multiplexing

SMRT® Sequencing on the PacBio® RS II

- Sequence full-length transcripts up to 10 kb
- Scalable throughput:
  - Profile multiplexed targeted transcripts in a single SMRT Cell
  - Increase sequencing depth for more comprehensive transcriptome characterization

Iso-Seq Analysis in SMRT Analysis





- No assembly required; no inference of isoforms
- Output high-quality, full-length transcript sequences
- Fully *de novo*, no genome reference required
- Open-source computational pipeline

References

1. Treutlein, B., et al. (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.
2. Schreiner, D., et al. (2014) Targeted Combinatorial Alternative Splicing Generates Brain Region-Specific Repertoires of Neurexins. *Neuron*, **84** (2), 386-398.
3. Au, K. F., et al. (2013) Characterization of the human ESC transcriptome by hybrid sequencing. *Proceedings of the National Academy of Sciences*, **110** (50), E4821-E4830.
4. Sharon, D., et al. (2013) A single-molecule long-read survey of the human transcriptome. *Nature biotechnology* **31**, 1009-1014.
5. Tilgner, Hagen, et al. (2014) Defining a personal, allele-specific, and single-molecule long-read transcriptome. *Proceedings of the National Academy of Sciences* **111** (27), 9869-9874.
6. Thomas, S., et al. (2014) Long-read sequencing of chicken transcripts and identification of new transcript isoforms. *PloS one* **9** (4), e94650.
7. Zhang, W. (2014) PacBio sequencing of gene families - a case study with wheat gluten genes. *Gene* **533** (2), 541-546.
8. Clark, T (2015, January) Single Molecule, Real-Time Sequencing of Full-length cDNA Transcripts Uncovers Novel Alternatively Spliced Isoforms *Poster presented at Plant and Animal Genome XXIII Conference*. San Diego, CA.
9. Pacific Biosciences, (2014, Oct) PacBio Data Release: Whole Human Transcriptome from Brain, Heart, and Liver. [blog post] Retrieved from http://blog.pacificbiosciences.com/2014/10/data-release-whole-human-transcriptome.html

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