Technical note

TARGETED ENRICHMENT OF KINNEX FULL-LENGTH RNA LIBRARIES WITH IDT xGEN HYBRIDIZATION AND WASH KIT V3

Overview

For RNA sequencing, targeted enrichment is considered a favorable alternative to whole transcriptome sequencing because it can capture more isoform diversity of targeted genes, requiring less sequencing depth and enabling higher sample multiplexing. The PacBio® Iso-Seq® method for full-length RNA sequencing has been used to capture single genes[1], highly similar paralogs or pseudogenes[2], and low-abundance long non-coding RNAs for annotation purposes[3]. Targeted enrichment may be the right choice when the goal of sequencing is to:

- Identify and quantify transcripts from a known set of genes
- Characterize alternative 5' starts and 3' ends
- Detect lowly expressed transcripts
- Maximize sample multiplexing capacity
- Reduce bioinformatics analysis time

This technical note describes the procedure for targeted enrichment using IDT xGen Hyb and Wash kit v3 for Kinnex™ full-length RNA libraries.

Required materials and equipment

- For a comprehensive list of materials and equipment, please refer to the PacBio **Kinnex full-length RNA protocol**[4] and the **IDT xGen protocol**[5].
- For cDNA generation, use the Iso-Seq express 2.0 kit, which is compatible with the IDT targeted enrichment and Kinnex full-length RNA library construction. The input to Iso-Seq express 2.0 is 300 ng of total RNA, ideally with RIN ≥ 7. Lower-quality RNA may result in shorter cDNA, while total RNA lower than 100 ng may result in the incomplete capture of the full diversity of the targeted genes.

Library preparation

Library preparation will follow the **Kinnex full-length RNA protocol** with modifications outlined here.

1. Generate full-length cDNA using the Iso-Seq express 2.0 kit.
2. Enrich for targeted genes using the **IDT xGen Hyb Wash v3 protocol.**
3. Continue through the Kinnex PCR and pooling step (step 4) in the **Kinnex full-length RNA protocol**.
4. The targeted Kinnex library is compatible for sequencing on Sequel® II/IIe, Revio®, and Vega™ systems.

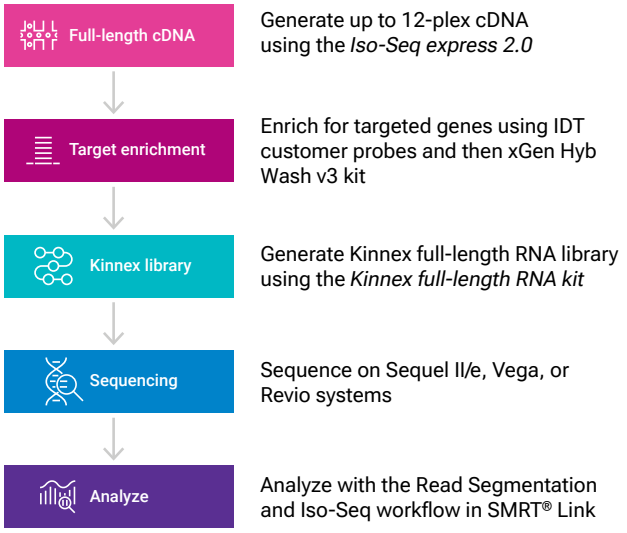


Figure 1. Library workflow for generating targeted Kinnex full-length RNA libraries using the IDT xGen Hyb Wash v3 kit.

SMRT® Link informatics and analysis recommendations

Targeted Kinnex full-length RNA data can be analyzed the same way as whole transcriptome Kinnex datasets using the **SMRT Link Read Segmentation and Iso-Seq** workflow or **through the command line**. Note, however, that these workflows do not have specific analyses for targeted gene lists and will output gene and isoform information for all detected transcripts.

IDT INTEGRATED DNA TECHNOLOGIES PacBio

Dataset: Targeted enrichment in human brain and UHRR samples

We applied the targeted Kinnex full-length RNA workflow to **human brain and UHRR samples**. Twenty-nine genes implicated in neurodegenerative, neuromuscular, and autoimmune disorders were selected for probe synthesis by IDT (Table 1). Commercially available human brain and UHRR total RNA were split into 6 technical replicates each, then three replicates from each sample were subject to IDT targeted enrichment. The 12 barcoded cDNA samples were then pooled and made into a single Kinnex full-length RNA library and sequenced on one Revio SMRT® Cell and one Vega SMRT Cell each (Figure 2). The runs yielded 7.6M and 4.6M HiFi reads, and, after read segmentation, 59.7M and 36.2M S-reads, respectively.

We calculated the on-target rate by aligning the S-reads to GRCh38 and determining the fraction of reads that overlapped with the targeted gene BED file. Between 99.1–99.9% of the S-reads mapped to GRCh38, with an on-target rate of 85–86% for the IDT capture samples, compared to only 1.2–1.3% for whole transcriptome data (Figure 3). We observed no differences in on-target rate or captured transcript lengths between Revio and Vega datasets, though the targeted dataset has a narrower range of transcripts (1100–1670 bp for 25–75% percentile) compared to the whole transcriptome dataset (1100–2000 bp for 25–75% percentile).

ABCA7	*CD2AP*	*HTT*
APH1A	*CD33*	*INPP5D*
APOE	*CELF1*	*MAPT*
APP	*CLU*	*MEF2C-AS1*
BACE1	*CR1*	*MS4A6A*
BIN1	*EPHA1*	*NCSTN*
BSG	*FERMT2*	*NME8*
C9orf72	*FMR1*	*PICALM*
CASS4	*GRN*	*SPDEF*
	HLA-DRB1	
	HLA-DRB5	

Table 1. List of 29 genes used in the IDT targeted Kinnex full-length RNA dataset.

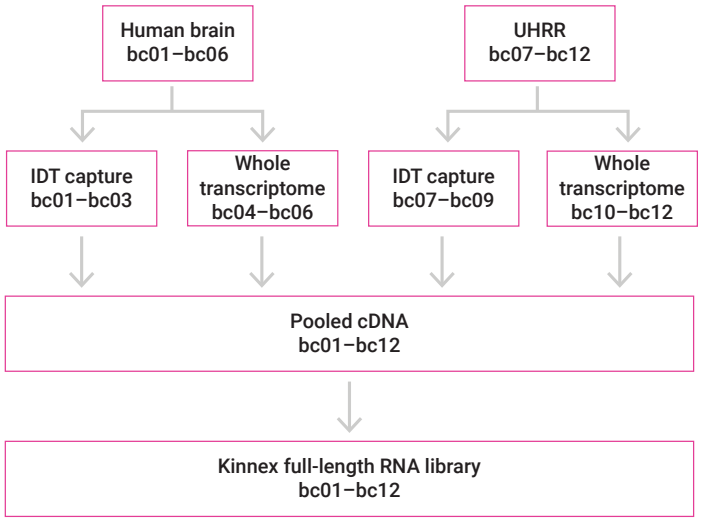
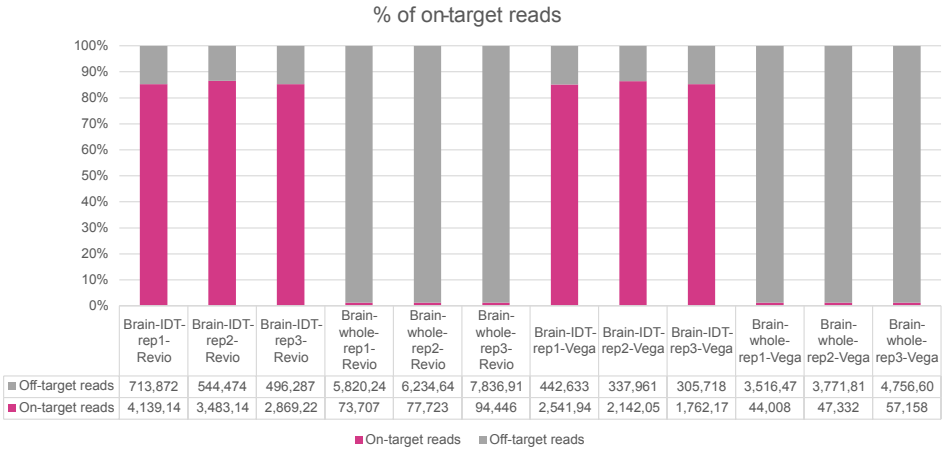


Figure 2. Experimental design for targeted Kinnex full-length RNA sequencing.



	Brain-IDT-rep1-Revio	Brain-IDT-rep2-Revio	Brain-IDT-rep3-Revio	Brain-whole-rep1-Revio	Brain-whole-rep2-Revio	Brain-whole-rep3-Revio	Brain-IDT-rep1-Vega	Brain-IDT-rep2-Vega	Brain-IDT-rep3-Vega	Brain-whole-rep1-Vega	Brain-whole-rep2-Vega	Brain-whole-rep3-Vega
Off-target reads	713,872	544,474	496,287	5,820,24	6,234,64	7,836,91	442,633	337,961	305,718	3,516,47	3,771,81	4,756,60
On-target reads	4,139,14	3,483,14	2,869,22	73,707	77,723	94,446	2,541,94	2,142,05	1,762,17	44,008	47,332	57,158

Figure 3. Percentage of on-target reads for IDT targeted enrichment vs whole transcriptome Kinnex full-length RNA data.

Targeted enrichment enabled detection of more known and novel isoforms in the targeted genes (Figure 4). In some cases, the targeted genes were expressed at lower levels and could only be detected with enrichment (e.g. *CR1, NME8).*

High replicate reproducibility was observed for the same Kinnex library sequenced on Revio and Vega systems (Pearson correlation: 0.99 for transcript counts), with same-sample different-replicate correlation ranging between 0.91 and 0.99 (Figure 5a). There is good correlation in the transcript abundances of target genes between the whole transcriptome and IDT capture data, indicating that transcript abundances are largely preserved through enrichment (Figure 5b).

Conclusion

Targeted enrichment is a powerful tool for detecting rare and lowly expressed isoforms in a defined set of genes. The Kinnex full-length RNA kit on PacBio long-read systems enables quantitative detection of full-length transcript isoforms with multiplexing capacity.

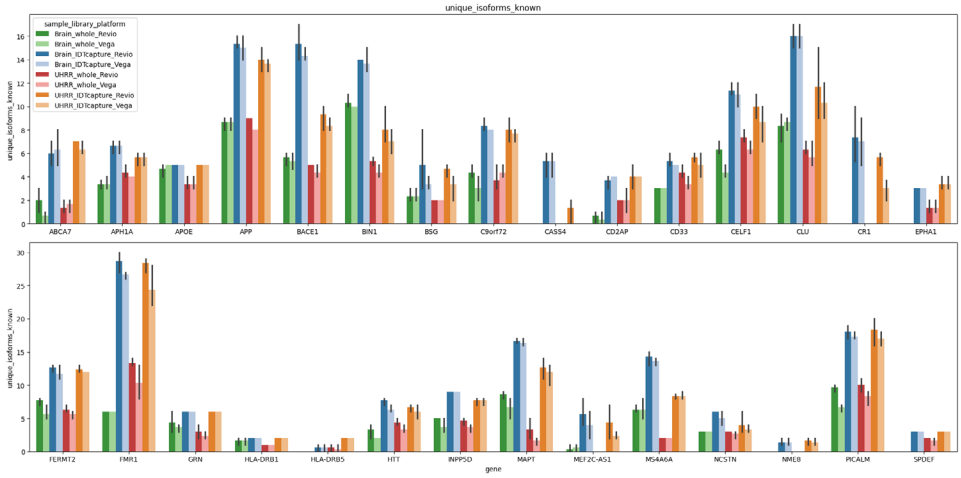


Figure 4. Number of unique isoforms detected for the 29 targeted genes. Targeted enrichment enabled detection of more isoforms compared to whole transcriptome. In some cases, some genes were not captured due to low expression levels in the whole transcriptome data but were captured in the IDT-Kinnex dataset.

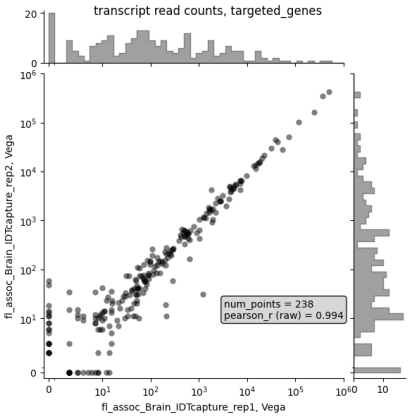


Figure 5a. High replicate reproducibility across technical replicates. Transcript abundances based on full-length read counts were compared between rep1 and rep2 of brain-IDT capture samples run on the Vega system.

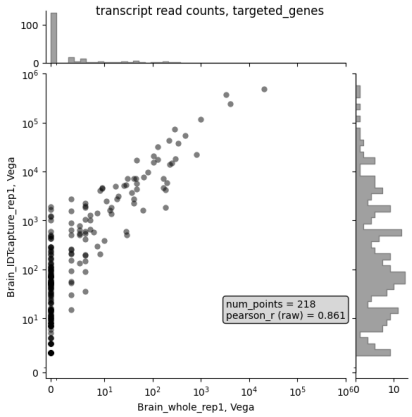


Figure 5b. Transcript abundance correlation between targeted enrichment and whole transcriptome show a moderate correlation, while the IDT capture data detects considerably more isoforms due to targeted enrichment.

Further, by comparing IDT-enriched vs whole transcriptome Kinnex data on the same samples on both the Revio and Vega systems, we demonstrated:

- Compatibility of IDT Hyb Wash kit v3 with the Kinnex full-length RNA kit
- High on-target rate (~85%) using IDT probe-based capture
- High sequencing reproducibility within and across PacBio long-read systems
- Preservation of transcript abundances with the targeted enrichment process

Taken together, these results demonstrate PacBio RNA sequencing with IDT target enrichment as a robust workflow for isoform characterization in targeted genes.

REFERENCES

1. Evans, J. R., et al. (2024). The diversity of SNCA transcripts in neurons, and its impact on antisense oligonucleotide therapeutics. *bioRxiv*, 2024-05. https://doi.org/10.1101/2024.05.30.596437
2. Gustavsson, E. K., et al. (2024). The annotation of GBA1 has been concealed by its protein-coding pseudogene GBAP1. *Science Advances*, 10(26), eadk1296. https://doi.org/10.1126/sciadv.adk1296
3. Kaur, G., et al. (2024). GENCODE: massively expanding the lncRNA catalog through capture long-read RNA sequencing. *bioRxiv*, 2024-10. https://doi.org/10.1101/2024.10.29.620654
4. PacBio procedure & checklist – Preparing Kinnex libraries using the Kinnex full-length RNA kit
5. IDT protocol – xGen Hybridization and Wash Kit v3 for PacBio Targeted Kinnex
6. PacBio troubleshooting guide – SMRT Link Kinnex full-length RNA
7. Dataset – IDT targeted enrichment vs whole transcriptome Kinnex (brain and UHRR samples): https://downloads.pacbcloud.com/public/dataset/Kinnex-full-length-RNA/DATA-Revio-IDT-BrainUHRR/

Questions? Visit **pacb.com/contact** or contact your local customer service representative

Research use only. Not for use in diagnostic procedures. © 2025 Pacific Biosciences of California, Inc. ("PacBio"). All rights reserved. Information in this document is subject to change without notice. PacBio assumes no responsibility for any errors or omissions in this document. Certain notices, terms, conditions and/or use restrictions may pertain to your use of PacBio products and/or third-party products. Refer to the applicable PacBio terms and conditions of sale and to the applicable license terms at pacb.com/license. Pacific Biosciences, the PacBio logo, PacBio, Circulomics, Omniome, SMRT, SMRTbell, Iso-Seq, Sequel, Nanobind, SBB, Revio, Onso, Apton, Kinnex, PureTarget, SPRQ, and Vega are trademarks of PacBio.