Technical Note

Single-Cell isoform Sequencing with ArgenTag and PacBio® Kinnex™ kits

ArgenTag PacBio

Overview

This technical note outlines the experimental conditions for preparing PacBio Kinnex libraries using cDNA generated with the ArgenTag Single-Cell RNA kit for Long-Read Sequencing.

ArgenTag cDNA is compatible with both the Kinnex full-length RNA kit (8-fold concatenation) and the Kinnex 16S rRNA kit (12-fold concatenation). The choice of kit depends on the experimental goal: the 8-fold kit maximizes read length, while the 12-fold kit maximizes sequencing throughput.

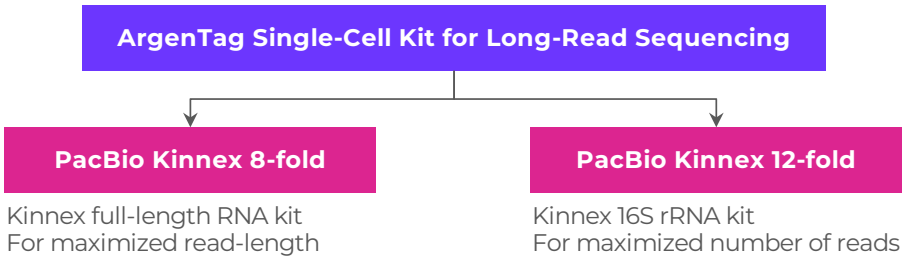

Figure 1: PacBio Kinnex kit options compatible with the ArgenTag Single-Cell RNA Kit for Long-Read Sequencing.

Required materials and equipment

- ArgenTag Single-Cell kit (cat #AT001-SD2) for Long-Read Sequencing and third party materials described in section 3 of the protocol [1].
- For the cDNA cleanup (step 8.C), we recommend AMPure® PB beads (PacBio cat #100-265-900).
- Reagents and equipment from the selected Kinnex protocol [2, 3], starting from the Kinnex PCR step.
- Note that the TSO artifact removal step is not required. ArgenTag cDNA is inherently TSO artifact-free, streamlining library preparation to ~5 hours.

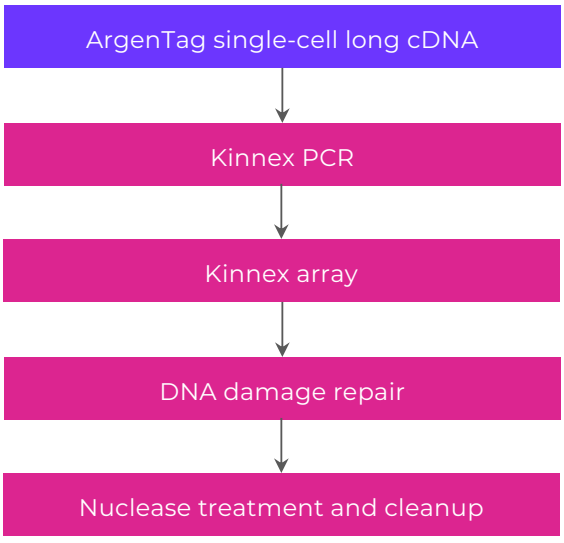

Figure 2: Library preparation workflow for generating Kinnex libraries from ArgenTag single-cell barcoded long cDNA. Steps include Kinnex PCR, array formation, DNA damage repair, and nuclease treatment and cleanup.

Kinnex library preparation

Following cDNA QC per the ArgenTag user manual (step 8.D) — ideal cDNA should have an average peak above 1,200 bp with fragments in the 1,000–5,000 bp range exceeding 30% of total molecules — proceed directly to the Kinnex PCR step using 55 ng input for 8-fold concatenation (Kinnex full-length RNA kit, step 4.1 [2]) or 35 ng for 12-fold concatenation (Kinnex 16S rRNA kit, step 3.1 [3]) (Figure 2). Library preparation then continues according to the respective PacBio Kinnex protocol, and sequencing is set up in SMRT® Link Sample Setup using the same parameters as standard Kinnex libraries.

Bioinformatics analysis

After sequencing, HiFi reads are segmented into individual S-reads using Read Segmentation in SMRT Link or using skera via the command line. S-reads retain ArgenTag barcoding sequences and can be demultiplexed and analyzed using one of two approaches [detailed instructions in 4, 5, 6]:

1) AT taggy demux + update_rc.sh + PBIso-Seq
- input: S-reads (.bam)
- steps: AT Taggy Demuxer, + update_rc.sh +, PB Iso-Seq workflow (step 6+), Pigeon.
- output: GTF, gene and isoform classification, gene- and isoform-level count matrix, isoform classification.

2) AT taggy demux + AT pipeline (research-grade tool)
- input: S-reads (.fastq)
- steps: AT Darwin QC, AT Taggy Demuxer, AT Flames counter, AT Ise Seurat, SQANTI3.
- output: GTF, gene count matrix, transcript count matrix, isoform classification.

Metric	Kinnex 8 Revio®	Kinnex 12 Revio SPRQ™
HiFi reads	1,964,147	4,789,198
Segmented reads (S-reads)	14,879,640	55,971,586
Mean S-read length	1,626 bp	1,346 bp
S-reads with valid barcodes	13,930,445 (94%)	52,848,068 (94%)
Deduplicated reads	8,445,767 (57%)	30,420,071 (54%)

Table 1: Sequencing metrics for MDA-MB-453 libraries using Kinnex 8-fold (Revio) and Kinnex 12-fold (Revio SPRQ) concatenation strategies. Data available here.

Kinnex dataset example: MDA Cell line

To demonstrate the high quality of the ArgenTag platform with Kinnex, we generated cDNA following the standard ArgenTag protocol [1] from the human breast cancer cell line MDA-MB-453 followed by either Kinnex 8-fold or Kinnex 12-fold library preparation. The 8-fold library was sequenced on one Revio SMRT® Cell using original chemistry, and the 12-fold library was sequenced on one Revio SMRT Cell using SPRQ chemistry (Table 1).

Analysis was performed using Read Segmentation from SMRT Link to obtain the S-reads that represent the individual cDNA molecules, followed by AT taggy demux script and the remainder of the PacBio Iso-Seq® pipeline as described in Bioinformatics Analysis (approach 1).

As expected, the 8-fold concatenation resulted in slightly longer median transcripts (1,691 bp) than 12-fold concatenation (1,452 bp), though with the higher throughput enabled by the Revio SPRQ chemistry, the 12-fold dataset captured a broader isoform diversity (Figure 3). Overall, the higher throughput of the 12-fold run resulted in more UMIs and genes captured per cell (Table 2), though in terms of isoform proportions and cell clustering, the two datasets were highly concordant (Figure 4, Figure 5) as would be expected.

Metric w QC Seurat w- Mito and Ribo genes	Kinnex 8 Revio	Kinnex 12 Revio SPRQ
Number of cells	6,020	6,012
Number of genes	13,546	15,690
Median UMIs per cell	1,024	3,650
Median genes per cell	756	1,759
wo- Mito and Ribo genes	Revio	Revio SPRQ
Number of cells	6,015	6,011
Number of genes	13,375	15,475
Median UMIs per cell	943	3,214
Median genes per cell	711	1,678

Table 2. Cell and gene-level quality metrics for Kinnex MDA-MB-453 dataset using Seurat, with and without ribo/mito genes. Seurat QC filtering criteria: genes detected in fewer than 3 cells and cells with fewer than 200 detected genes were removed; cells exceeding the 99th percentile for gene or UMI counts, or with >15% mitochondrial reads, were also excluded.

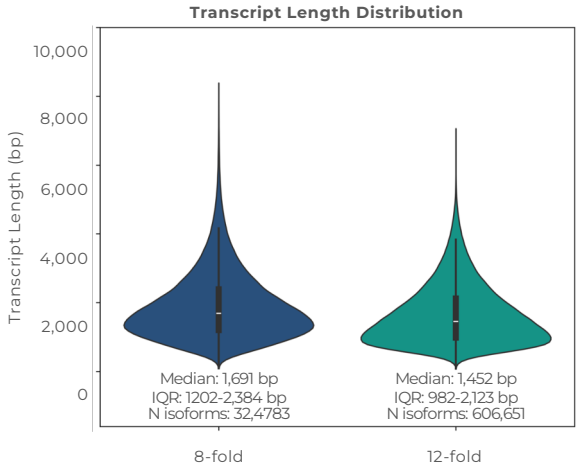

Figure 3: Transcript length distributions for Kinnex MDA-MB-453 dataset.

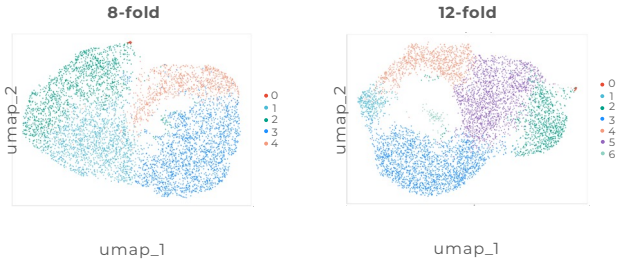

Figure 5: UMAP visualization of MDA-MB-453 cells clustered by gene expression.

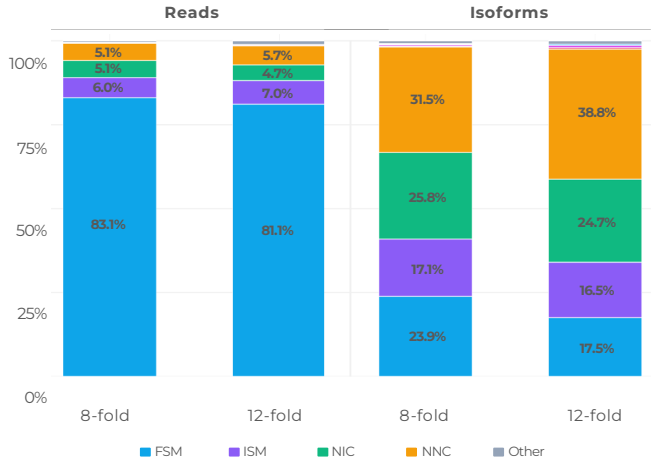

Figure 4: Isoforms detected in Kinnex MDA-MB-453 dataset. Stacked bar charts show the proportion of reads (left) and isoforms (right) assigned to each SQANTI3 structural category. FSM: full-splice match; ISM: incomplete-splice match; NIC: novel in catalog; NNC: novel not in catalog. Negligible values: Antisense, Intergenic, Genic Intronic and Genic Genomic.

ArgenTag single-cell isoform sequencing with PacBio Kinnex: summary

The *ArgenTag Single-Cell RNA Kit for Long-Read Sequencing* delivers end-to-end single-cell isoform sequencing with PacBio Kinnex.

- Fully compatible with Kinnex 8-fold (full-length RNA kit) and 12-fold (16S rRNA kit)
- TSO artifact-free chemistry — no removal step required; ~5h library preparation
- ~6,000 cells recovered per SMRT Cell with >94% valid barcodes
- 83% full-splice match (FSM) reads confirming full-length cDNA integrity
- 8-fold: longer median transcripts (1,691 bp) for deep isoform characterization
- 12-fold: 606,651 isoforms detected for broad transcriptomic profiling
- Compatible with two bioinformatics pipelines for flexible downstream analysis

References

1. Procedure & checklist — ArgenTag Single-Cell kit for Long-Read Sequencing handbook. ArgenTag documentation.
2. Procedure & checklist — Preparing Kinnex libraries using the Kinnex full-length RNA kit. PacBio documentation
3. Procedure & checklist – Preparing Kinnex libraries from 16S rRNA amplicons. PacBio documentation
4. https://github.com/argentagsw/taggy_demux
5. https://www.pacb.com/products-and-services/analytical-software/smrt-analysis/
6. https://isoseq.how/

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Request a consultation Schedule a call with our team to discuss your particular application.

ArgenTag
welcome@argentag.com
argentag.com