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Application note

Kinnex single-cell RNA kit for 10x Visium HD 3’ Spatial Gene Expression

Spatial transcriptomics offers a comprehensive view of RNA expression within the context of a tissue’s three-dimensional structure, enabling understanding of cell function based on location, its neighbors, and the local microenvironment. This is particularly crucial for studying development, cancer, neurobiology, and pathology (Williams et al. 2022). The 10x Visium HD 3' Spatial Gene Expression assay offers subcellular (2 µm), single-cell resolution that allows precise mapping of RNA expression across complex tissues with high-density, contiguous coverage. Sequencing readout is required for the Visium HD 3' Spatial Gene Expression assay, however, with short-read sequencing, only gene-level information can be captured. In contrast, PacBio® HiFi long-read sequencing can cover the entire RNA transcript along with the spatial barcodes and UMIs.

The *Kinnex™ single-cell RNA kit* is compatible with cDNA generated using the Visium HD 3' Spatial Gene Expression assay for creating a sequencing-ready library that can run on PacBio sequencing systems. A custom bioinformatics pipeline supports processing HiFi reads into gene- and isoform-level count matrices that can be analyzed with standard spatial transcriptome tools.

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Kinnex for high-definition, spatially resolved isoform sequencing

The *Kinnex single-cell RNA kit* utilizes the MAS-Seq method for throughput increase. MAS-Seq (Al'Khafaji et al., 2024) is a concatenation method for increasing throughput by joining cDNA molecules into longer concatenated fragments. HiFi reads generated from sequencing the concatenated molecules can then be bioinformatically broken up to retrieve the original cDNA sequences. The result is higher throughput and reduced sequencing needs for cost-effective spatial isoform sequencing.

The PacBio Visium HD support workflow processes the full-length cDNA sequences to classify them against a reference annotation (e.g., GENCODE) to identify novel genes and isoforms. The output consists of gene- and isoform-level spatial count matrices that are compatible with tertiary analysis software (Figure 1).

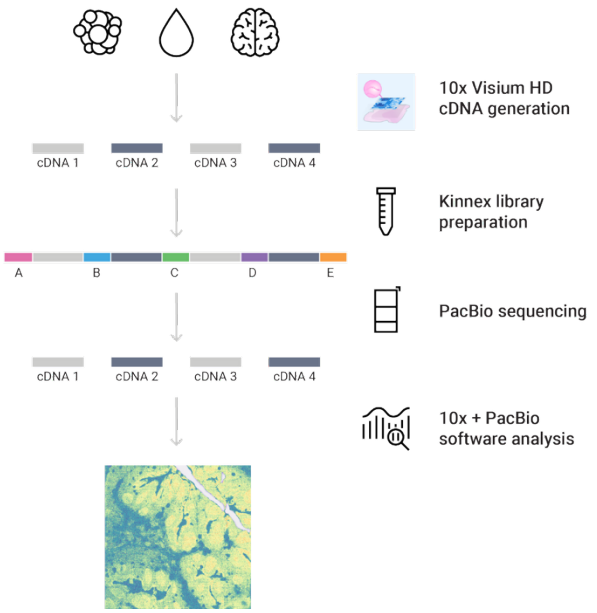

Figure 1. Kinnex for spatial isoform sequencing. Spatially-barcoded cDNA molecules are concatenated into larger insert libraries and sequenced, then analyzed using SMRT® Link and other supporting software tools.

Kinnex library workflow

The Kinnex library workflow begins with 15–75 ng of 10x Visium HD cDNA and produces a Kinnex library that is ready for sequencing.

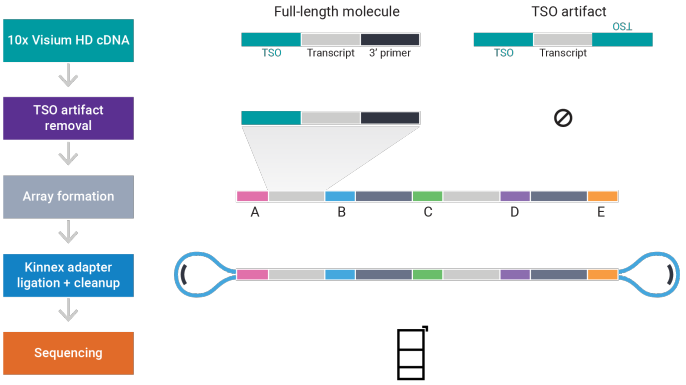

Figure 2. Kinnex library workflow for 10x Visium HD 3' Spatial Gene Expression cDNA.

cDNA molecules are first removed of TSO (template-switching oligos) artifacts, then concatenated to form ordered arrays. Kinnex adapter ligation ensures enrichment of full arrays, which are then sequenced on PacBio long-read sequencers (Figure 2).

With proper full array formation and adequate sequencing, one SMRT® Cell on the Vega™ and Revio® systems are expected to achieve 50–60 million and 100–120 million cDNA sequences, respectively (Table 1).

Table 1. Target Kinnex 10x Visium HD library performance

Metric	Performance
Sample preparation time	2 days
Expected library size	11,000–16,000 bp
Target P1 loading	60–80%
Expected HiFi yield per SMRT Cell	2.5–3.5 million HiFi reads (Vega system) 6–7.5 million HiFi reads (Revio system with SPRQ™ chemistry)
Expected full array %	85–92%
Expected read yield per SMRT Cell	50–60 million reads (Vega) 100–120 million reads (Revio + SPRQ)

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Kinnex Visium HD bioinformatics workflow

The SMRT Link *Read Segmentation* workflow bioinformatically deconcatenates the HiFi reads into segmented reads (S-reads) that represent the original individual 10x Visium HD cDNA molecules. The S-reads can be processed through a combined 10x and PacBio software pipeline to produce gene- and isoform-level count matrices that are compatible with tertiary spatial analysis tools such as Space Ranger and Seurat (Figure 3).

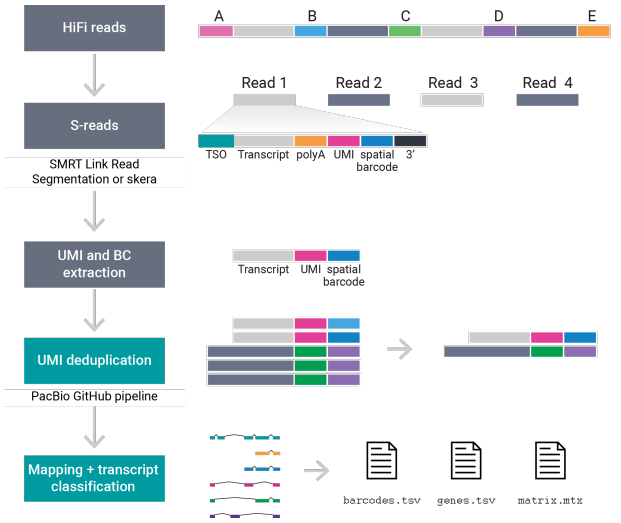

Figure 3. Workflow for analyzing Kinnex 10x Visium HD spatial data using 10x and PacBio supported pipeline.

Read segmentation

HiFi reads are segmented into individual segmented reads (*S-reads*) that represent the original 10x Visium HD cDNA sequences.

UMI/BC extraction & UMI deduplication

Primers and polyA tails are removed, but also used to orient the read into 5'–3' orientation. Spatial barcode and UMI information are extracted using the 10x Genomics Space Ranger pipeline. A PacBio pipeline reimports the spatial barcodes and UMIs into a BAM format for processing. Reads are then deduplicated based on spatial barcodes and UMIs.

Mapping and transcript classification

Deduplicated reads are mapped to the reference genome and classified against a transcript annotation (e.g., GENCODE). Finally, a gene- and isoform-level spatial matrix is output for tertiary analysis.

Kinnex 10x Visium HD spatial datasets

Commercially available human samples were purchased from BioIVT and mouse samples were purchased from Charles Rivers Laboratories. Spatially barcoded cDNA was generated using the Visium HD 3' Spatial Gene Expression kit and made into Kinnex libraries. Each Kinnex library was sequenced on four Revio SMRT Cells using SPRQ chemistry. The data was analyzed using the *Read Segmentation* workflow in SMRT Link v13.1 followed by the 10x and PacBio Visium HD software pipeline (Table 2) and visualized using Space Ranger and Seurat (Figure 4). The Kinnex data showed a high barcode identification rate (83—93%), which was in concordance with matching short-read data.

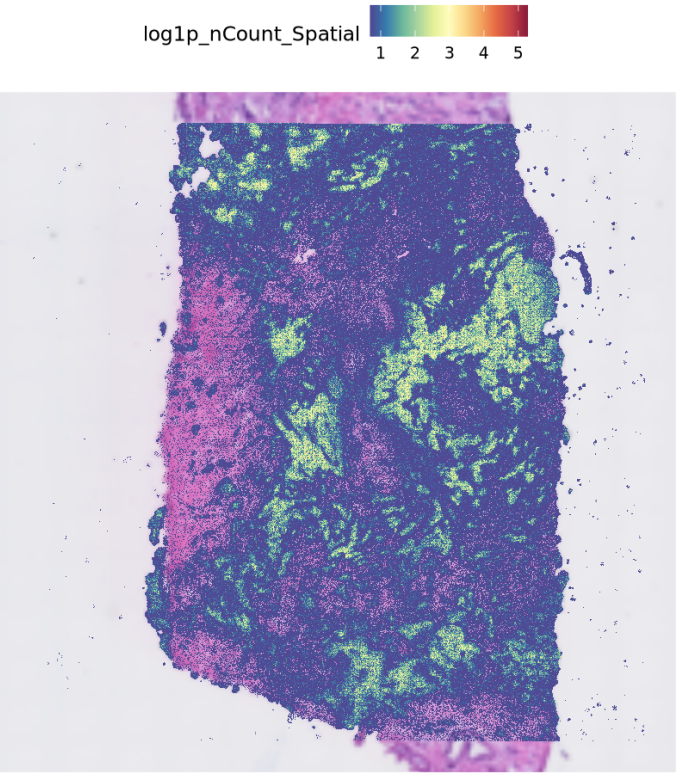

Figure 4. Spatial image of transcript density (log-scale) generated from Kinnex 10x Visium HD 3' Spatial Gene Expression data from breast cancer sample.

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Table 2. Summary statistics of Kinnex 10x Visium HD 3' Spatial Gene Expression data. Each Kinnex 10x Visium HD library was sequenced on four Revio SMRT Cells using SPRQ chemistry. Data was processed using SMRT Link Read Segmentation and the GitHub Visium HD pipeline. Stats marked with + are obtained from running 10x Space Ranger software using the 10x pipeline to convert S-reads into FASTQ files and reflect gene-level information without isoform analysis. Data can be downloaded here.

Metric	Human Breast Cancer	Human Colon Cancer	Human Tonsil	Mouse Brain
8 µm binned squares under tissue+	427,917	674,046	682,432	389,223
HiFi reads	9,139,769	17,433,673	26,176,752	26,504,867
Segmented reads (S-reads)+	142,818,534	275,561,411	413,542,793	418,769,317
Mean S-read length (bp)	592	530	692	694
S-reads with valid barcodes (%)+	92.4%	85.3%	83.6%	92.6%
Deduplicated reads	45,368,854	199,244,211	95,482,635	154,992,501
Sequencing saturation+	41.4%	40.2%	31.3%	59.4%
Mean reads per 8 µm bin+	333.8	408.8	566.0	1075.9
Mean UMIs per 8 µm bin+	96.6	128.4	250.5	318.0
Total genes detected in 8 µm bins+	**23,823**	**23,508**	**25,888**	**22,275**

Kinnex for 10x Visium HD 3' Spatial Gene Expression sequencing: summary

The *Kinnex single-cell RNA kit* supports full-length isoform sequencing for high-definition spatial transcript data from the 10x Visium HD 3' Spatial Gene Expression kit.

- 15–75 ng input cDNA
- 16-fold throughput increase compared to non-Kinnex methods
- No orthogonal sequencing data required
- Fully supported bioinformatics workflow to produce gene- and isoform- spatial count matrices

For more information, visit https://pacb.com/kinnex

References

Al'Khafaji, A. M., Smith, J. T., Garimella, K. V., Babadi, M., Popic, V., et al. (2024). High-throughput RNA isoform sequencing using programmed cDNA concatenation. *Nature Biotechnology*, 42(4), 582-586. https://doi.org/10.1038/s41587-023-01815-7

Williams, C. G., Lee, H. J., Asatsuma, T., Vento-Tormo, R., & Haque, A. (2022). An introduction to spatial transcriptomics for biomedical research. Genome Medicine, 14(1), 68. https://doi.org/10.1186/s13073-022-01075-1

Resources

PacBio Kinnex webpage

PacBio 10x Visium HD GitHub support workflow

10x datasets

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