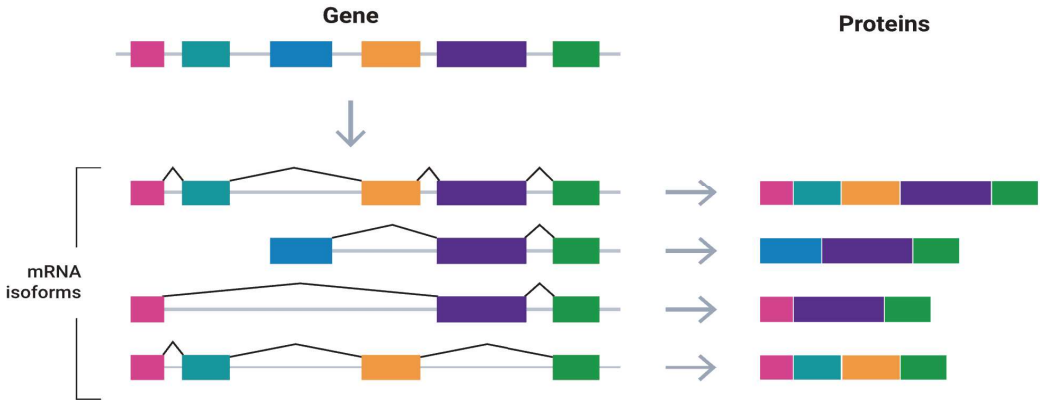
PacBio

Introducing

KINNEX™ kits

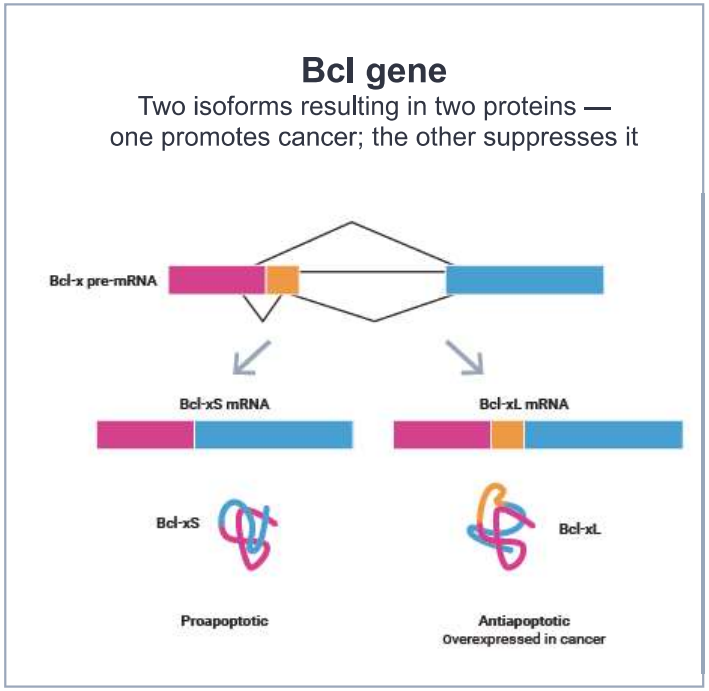
for scalable, cost-effective RNA sequencing at isoform level resolution

Isoforms — not genes — are the drivers of biology and disease



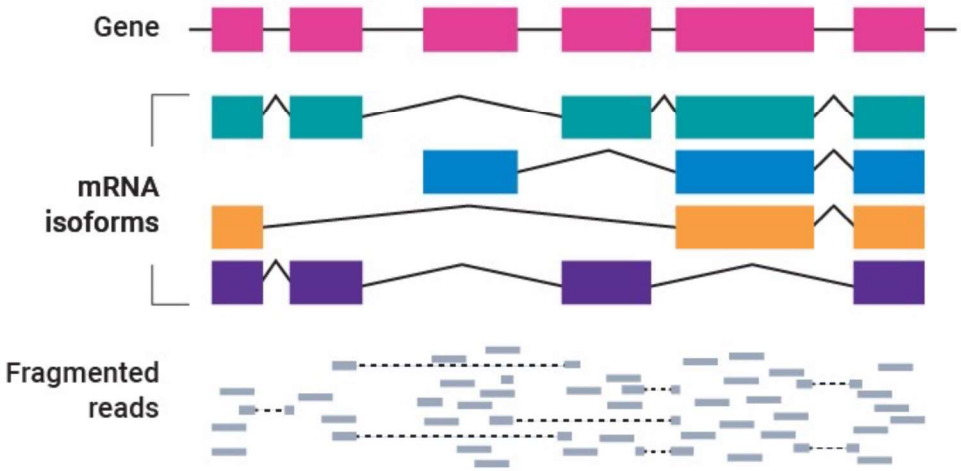
Alternative splicing (AS) in genes makes many different isoforms by choosing different exon combinations

Different isoforms → different proteins → different functions (or dysfunctions!)



Long-read sequencing helps provide a more complete view of the transcriptome

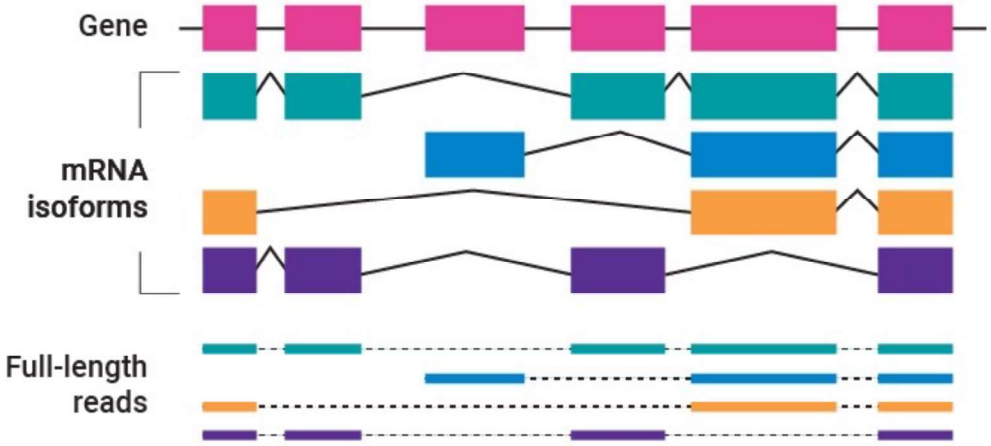
Short-read sequencing



Short-read sequencing can only assemble ~20 to 40% of human transcriptomes

PARTIAL view of transcriptomes

Long-read sequencing



PacBio's long-read sequencing offers superior isoform discovery power

COMPLETE view of transcriptomes

PacBio

Kinnex kits: solutions for RNA applications with scalability at affordable cost

Kinnex single-cell RNA kit

Upgrade to *MAS-Seq for 10x Single Cell 3'* kit for 10x 5' support

Cell type-specific isoform discovery with the highest accuracy

Exceptional data providing answers short reads cannot

Kinnex 16S rRNA kit

Full-length 16S rRNA for species identification

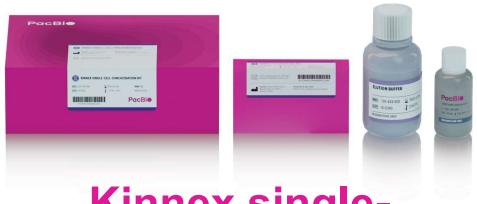
Exceptional data at the same price as short reads

Kinnex full-length RNA kit

Full-length isoform quantification with flexible sample multiplexing.

Exceptional data with more reads in less time

Kinnex kits for full-length RNA, single-cell RNA, and 16S rRNA sequencing

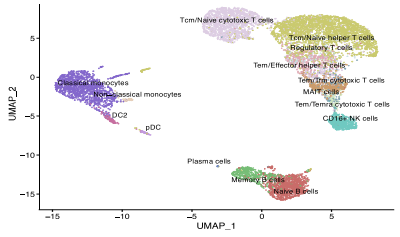


Kinnex single-cell RNA kit

Upgrade to *MAS-Seq for 10x Single Cell 3'* kit

Support 10x 3' and 5'; up to 4-plex

40M reads (Sequel II and IIe systems)

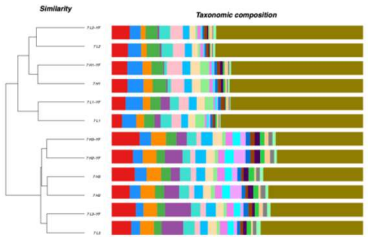


Kinnex 16S rRNA kit

Full-length 16S rRNA for species identification

Up to 1,536-plex

25M reads (Sequel II and IIe systems)

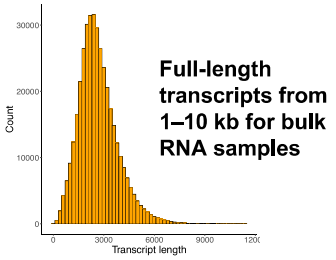


Kinnex full-length RNA kit

Full-length RNA sequencing

Up to 48-plex

15M reads (Sequel II and IIe systems)



Kinnex RNA sequencing Kits

15-20 kb

High-throughput RNA isoform sequencing using programmed cDNA concatenation

Aziz M. Al'Khafaji, Jonathan T. Smith, Kiran V. Garimella, Mehrtash Babadi, Victoria Popic, Moshe Sade-Feldman, Michael Gatzen, Siranush Sarkizova, Marc A. Schwartz, Emily M. Blaum, Allyson Day, Maura Costello, Tera Bowers, Stacey Gabriel, Eric Banks, Anthony A. Philippakis, Genevieve M. Boland, Paul C. Blainey & Nir Hacohen

Kinnex full-length RNA kit

Kinnex 16S rRNA kit

Kinnex single-cell RNA kit

Kinnex™ full-length RNA kit

Scalable, cost-effective full-length RNA sequencing with exceptional quality

Kinnex full-length RNA kit
(103-072-000)
12 rxn

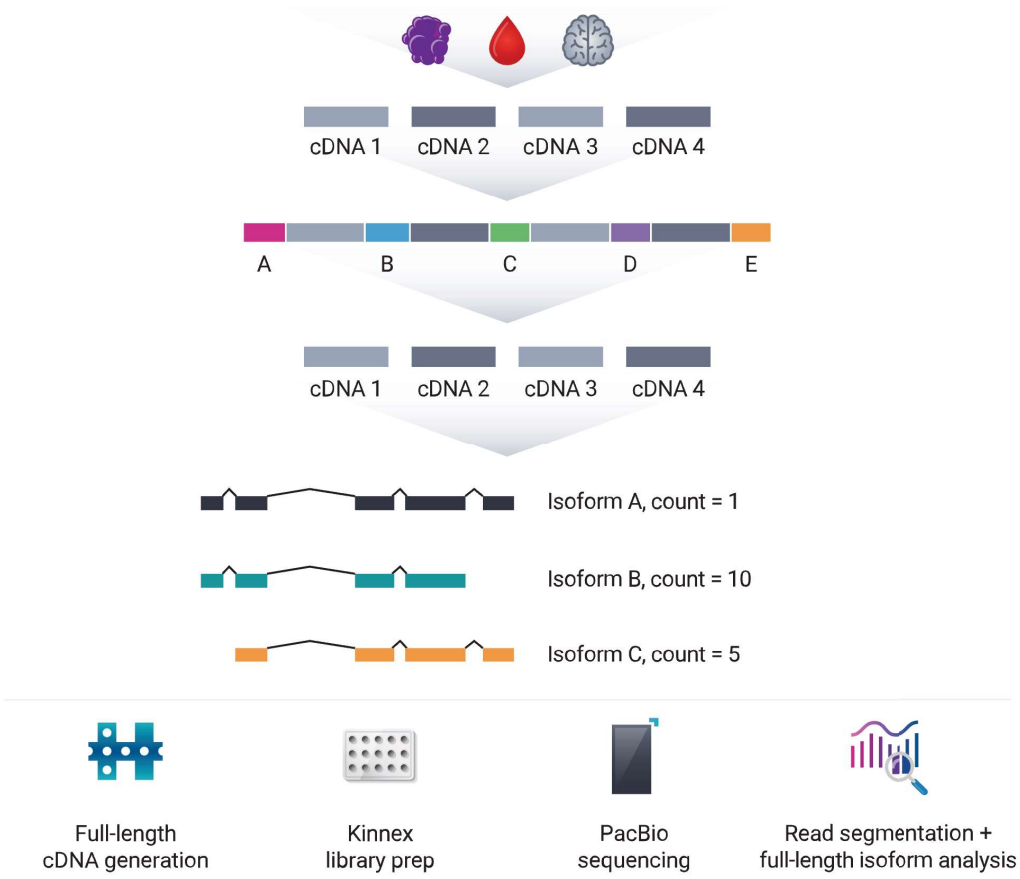
Iso-Seq *express 2.0* kit
(103-071-500)
24 rxn

- **Generate up to 12 different barcoded cDNA** using Iso-Seq *express 2.0* kit
- **Additional multiplexing possible** with four unique barcoded Kinnex adapters
- **From total RNA to sequencing-ready library in two days**
- **Throughput:** 15 million reads (Sequel II/IIe system) and 40 million reads (Revio system) — no short reads needed!
- **SMRT Link generates isoform classification** with abundance information — go directly into tertiary analysis!

PacBio

* Study design, sample type, and level of multiplexing may affect the number of SMRT Cells required.

Kinnex full-length RNA kit for high-accuracy, full-length isoform sequencing



- Input 300 ng total RNA, RIN ≥7
- Generate up to 12-plex barcoded cDNA using *Iso-Seq express 2.0 kit*
- 2-day library preparation
- Kinnex full-length RNA *Run Design* with auto-analysis
- Isoform-classification software to identify novel genes and isoforms with abundance information

Kinnex™ single-cell RNA kit

Isoform-resolution single-cell RNA sequencing with the highest accuracy

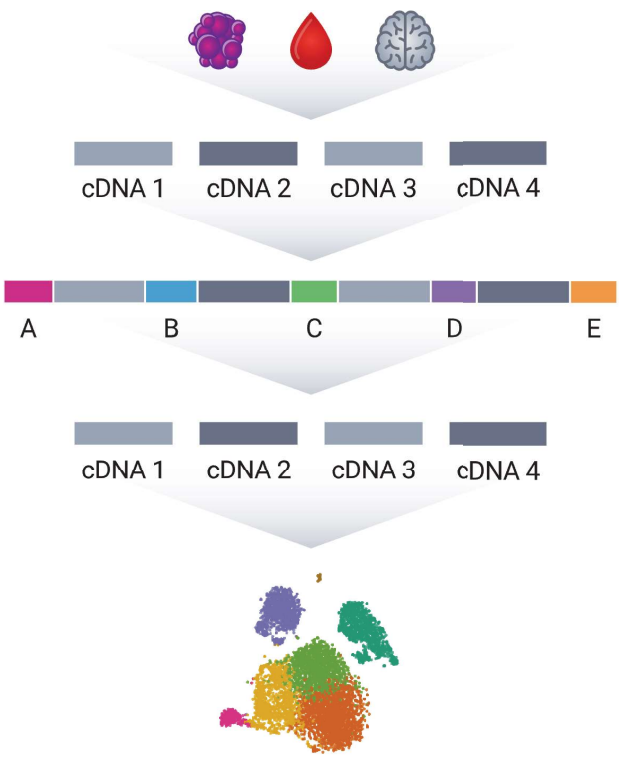
Kinnex single-cell RNA kit
(103-072-200)
12 rxn

- **Upgrade** from the *MAS-Seq for 10x Single Cell 3'* kit
- **Additional multiplexing possible** with four unique barcoded Kinnex adapters
- **Supports cDNA** generated from 10x *Chromium Next GEM Single Cell 3*' kit (v3.1) and *5'* kit (v2)
- **Supports multiplexing** using barcoded Kinnex adapters (up to 4-plex)
- **Generates sequencing-ready library** from *10x* cDNA in two days
- **80–100 million reads per SMRT Cell on Revio system** — no short reads needed!
- **SMRT Link generates gene- and isoform-count matrix** — go directly into tertiary analysis!
- **Compatible with SMRT Link v13.1 and up**

PacBio

* Study design, sample type, and level of multiplexing may affect the number of SMRT Cells required.

Kinnex *single-cell RNA* kit for single-cell isoform sequencing



Single-cell cDNA generation

Kinnex library preparation

PacBio sequencing

Read segmentation + single-cell isoform analysis

- 10x *Chromium Single Cell 3'* kit (v3.1) and ***5' kit (v2)*** **NEW**
- 15–75 ng cDNA
- 3,000 to 10,000 target cell recovery
- 2-day library preparation
- **Barcoded Kinnex adapters support 4-plex multiplexing** **NEW**
- "Kinnex single-cell RNA" *Run Design* with auto-analysis in SMRT Link v13.1 and up
- Isoform-classification software to identify novel genes and isoforms
- Output compatible with tertiary single-cell analysis tools (e.g., *Seurat*, *Scanpy*, *Kana*)

PacBio

Iso-Seq method reveals thousands of novel isoforms in breast cancer samples

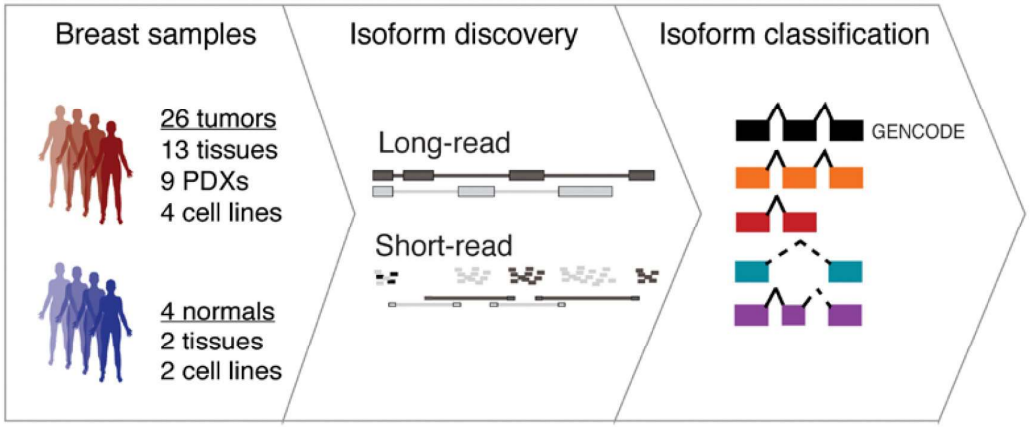
SCIENCE ADVANCES | RESEARCH ARTICLE

CANCER

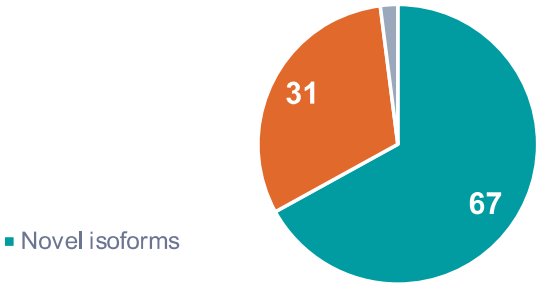
A comprehensive long-read isoform analysis platform and sequencing resource for breast cancer

Diogo F. T. Veiga[1]†, Alex Nesta[1,2]†, Yuqi Zhao[1], Anne Deslattes Mays[1], Richie Huynh[1], Robert Rossi[1], Te-Chia Wu[1], Karolina Palucka[1], Olga Anczukow[1,2,3]*, Christine R. Beck[1,2,3]*, Jacques Banchereau[1]*

Veiga *et al.*, *Sci. Adv.* **8**, eabg6711 (2022) 19 January 2022



142,514 splice isoforms detected



Two-third of identified isoforms are **novel** (NNC+NIC)

PacBio

Long reads are one of the only ways to see isoforms in single-cell RNA-Seq

Long-read advantage over short reads

Short-read scRNA-Seq can only reveal gene-level information.
But isoforms — not genes — are often the biological drivers of disease.

Short read
Partial gene + single cell information (100 bp)

In single-cell RNA, short reads are limited to ~50 bp of transcript information — essentially can only give you *gene* information — no matter how much you sequence!

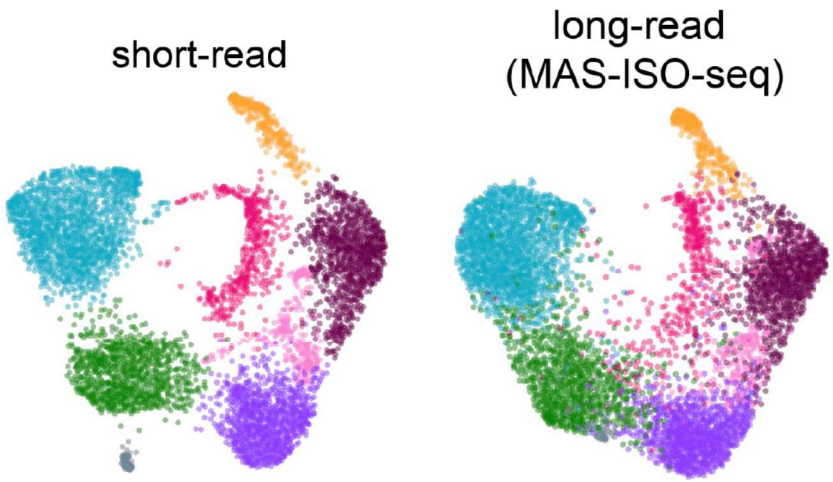
Long read
Full isoform + single cell information (500–2,000 bp)

In single-cell RNA, long reads give you full-length isoform information — the more you sequence, the richer information you get!

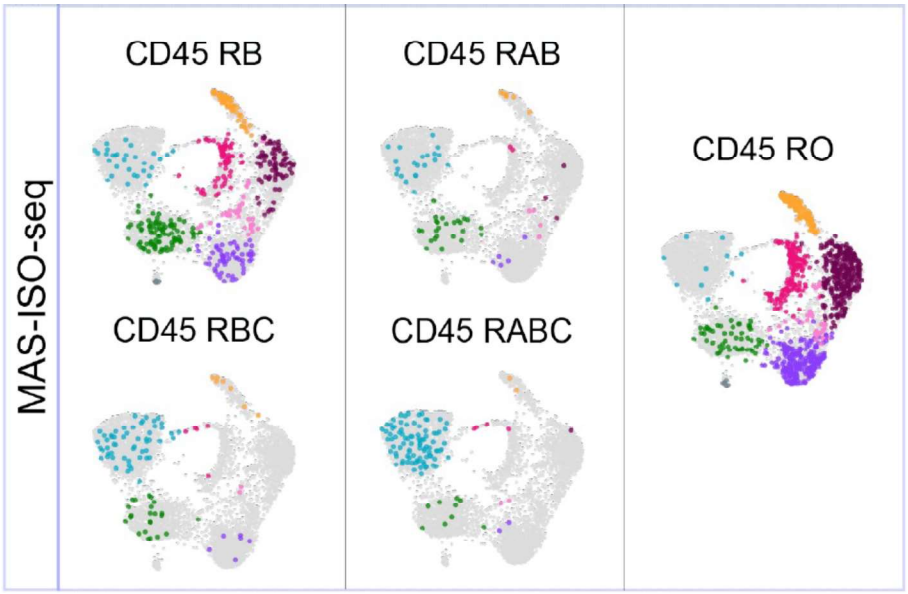
PacBio

New single-cell Iso-Seq method combines gene expression with isoform information

Gene expression and cell clustering equivalent to short reads



…PLUS, isoform information



PacBio

Al'Khalifi, et al. High-throughput RNA isoform sequencing using programmed cDNA concatenation. Nature Biotechnology, 2023.

Single-cell MAS-Seq study

Detection of isoforms and genomic alterations by high-throughput full-length single-cell RNA sequencing for personalized oncology

Arthur Dondi, Ulrike Lischetti, Francis Jacob, Franziska Singer, Nico Borgsmüller, Tumor Profiler Consortium, Viola Heinzelmann-Schwarz, Christian Beisel, Niko Beerenwinkel

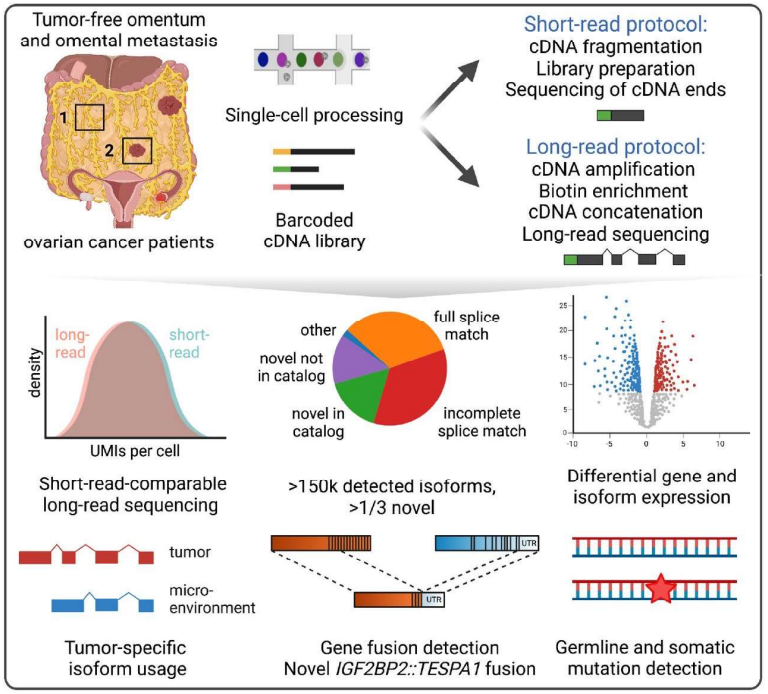
- **Short reads are no longer needed for cell type clustering**

"**cancer cells** expressed **at least twice as many unique isoforms** than other cell types"

- Identified a novel *IGF2BP2::TESPA1* fusion which was misclassified in matched short-read data as high *TESPA1* expression

"**short-read scRNA-seq data** fails to distinguish between gene and fusion expression, potentially **leading to wrong biological conclusions**."

- Germline and **somatic mutation detection**



*"**long-read sequencing provides a more complete picture** of cancer-specific changes."*

PacBio

https://www.biorxiv.org/content/10.1101/2022.12.12.520051v2.full

Many new publications with Kinnex

Long-read single-cell RNA sequencing enables the study of cancer subclone-specific genotype and phenotype in chronic lymphocytic leukemia

Gage S. Black, Xiaomeng Huang, Yi Qiao, Philip Moos, Deepa Sampath, Deborah M. Stephens, Jennifer A. Woyach, Gabor T. Marth

Long-read RNA-seq demarcates *cis*- and *trans*-directed alternative RNA splicing

Giovanni Quinones-Valdez, Kofi Amoah, Xinshu Xiao

Comparison of Single-cell Long-read and Short-read Transcriptome Sequencing of Patient-derived Organoid Cells of ccRCC: Quality Evaluation of the MAS-ISO-seq Approach

Natalia Zajac, Qin Zhang, Anna Bratus-Neuschwander, Weihong Qi, Hella Anna Bolck, Tülay Karakulak, Tamara Carrasco Oltra, Holger Moch, Abdullah Kahraman, Hubert Rehrauer

doi: https://doi.org/10.1101/2024.03.14.584953

The diversity of *SNCA* transcripts in neurons, and its impact on antisense oligonucleotide therapeutics

James R. Evans, Emil K. Gustavsson, Ivan Doykov, David Murphy, Gurvir S. Virdi, Joanne Lachica, Alexander Röntgen, Mhd Hussein Murtada, Chun Wei Pang, Hannah Macpherson, Anna I. Wernick, Christina E. Toomey, Dilan Athauda, Minee L. Choi, John Hardy, Nicholas W. Wood, Michele Vendruscolo, Kevin Mills, Wendy Heywood, Mina Ryten, Sonia Gandhi

Full-length isoform concatenation sequencing to resolve cancer transcriptome complexity

Saranga Wijeratne, Maria E. Hernandez Gonzalez, Kelli Roach, Katherine E. Miller, Kathleen M. Schieffer, James R. Fitch, Jeffrey Leonard, Peter White, Benjamin J. Kelly, Catherine E. Cottrell, Elaine R. Mardis, Richard K. Wilson & Anthony R. Miller

Diversity of ribosomes at the level of rRNA variation associated with human health and disease

Daphna Rothschild, Teodorus Theo Susanto, Xin Sui, Jeffrey P. Spence, Ramya Rangan, Naomi R. Genuth, Nasa Sinnott-Armstrong, Xiao Wang, Jonathan K. Pritchard, Maria Barna

CTAT-LR-fusion: accurate fusion transcript identification from long and short read isoform sequencing at bulk or single cell resolution

Qian Qin, Victoria Popic, Houlin Yu, Emily White, Akanksha Khorgade, Asa Shin, Kirsty Wienand, Arthur Dondi, Niko Beerenwinkel, Francisca Vazquez, Aziz M. Al'Khafaji, Brian J. Haas

Long-read solutions address transcriptomics use cases

Use case	Example using the Iso-Seq method	Citation	Recommended Kinnex solution
Identify alternative splicing patterns	Up to 95% of multi-exon genes are alternatively spliced; characterize AS in disease, cell types	• Reese F, et al. bioRxiv [Preprint]. 2023 • Stergachis A, et al. bioRxiv [Preprint]. 2023	Full-length Kinnex
Identify differentially expressed genes and transcripts (DGE, DTE)	Case-control studies: healthy vs disease, treated vs untreated, time points	• Patowary A, et al. bioRxiv [Preprint]. 2023 • Leung SK, et al. Cell Rep. 2021 • Lienhard M, et al. Bioinformatics. 2023	Full-length Kinnex
Transcript discovery	Profile disease-specific transcript diversity that may serve as therapeutic targets	• Gustavsson E, et al. bioRxiv [Preprint] 2022 • Huang KK, et al. Genome Biol. 2021	Full-length Kinnex
Detect fusion genes	Detect chimeric transcripts resulting from fusion genes to understand tumorigenesis	• Miller AR, et al. J Mol Diagn. 2022	Full-length Kinnex
Characterize differential transcript usage (DTU)	Understand the contribution of specific transcripts in DGE signals	• Leung SK, et al. Cell Rep. 2021 • Xia Y, et al. Nat Commun. 2023	Full-length Kinnex
Characterize cellular heterogeneity	Cell atlas initiatives to characterize cell types in certain tissues; transcriptomic profiling of cellular subpopulations	• Leung SK, et al. Cell Rep. 2021 • Lareau CA, et al. Nat Genet. 2023	Single-cell Kinnex