Takara Bio’s NGS toolbox:

sneak peek into low-input, long-read sequencing with ONT technology

that’s
GOOD
science!®

NGS oncology research portfolio

Industry gold standard for low-input and single-cell NGS library preparation

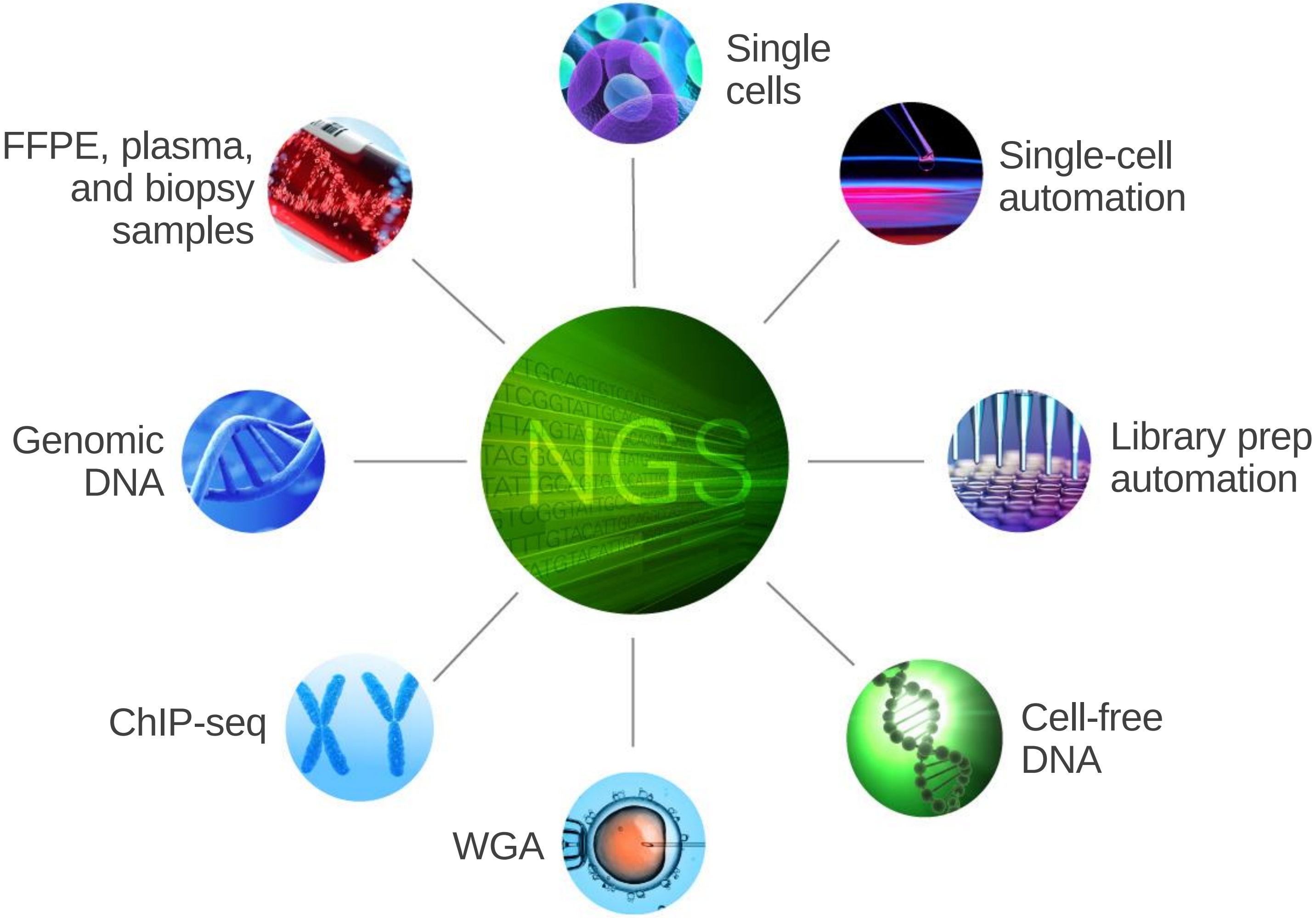
① DNA-seq solutions

Leverages ThruPLEX® and PicoPLEX® technologies

② SMART-Seq® RNA-seq solutions

Leverages SMART® technology

Technologies for DNA-seq



Single cells

Single-cell automation

Library prep automation

Cell-free DNA

WGA

ChIP-seq

Genomic DNA

FFPE, plasma, and biopsy samples

DNA-seq

- Leverages PicoPLEX and ThruPLEX technologies
- High sensitivity
- Robust and highly reproducible
- Simplified workflows and single-tube protocols

Technologies for RNA-seq

Single cells

Single-cell automation

FFPE, plasma, and biopsy samples

Low-input eukaryotic samples

Library prep automation

Immune profiling

Prokaryotic samples

SMART-Seq RNA-seq

- Leverages SMART technology
- Confidence in full-length sequence information
- Highest sensitivity in terms of transcripts identified
- Uniform gene-body coverage

Evolution of Takara Bio’s single-cell SMART-Seq® technology

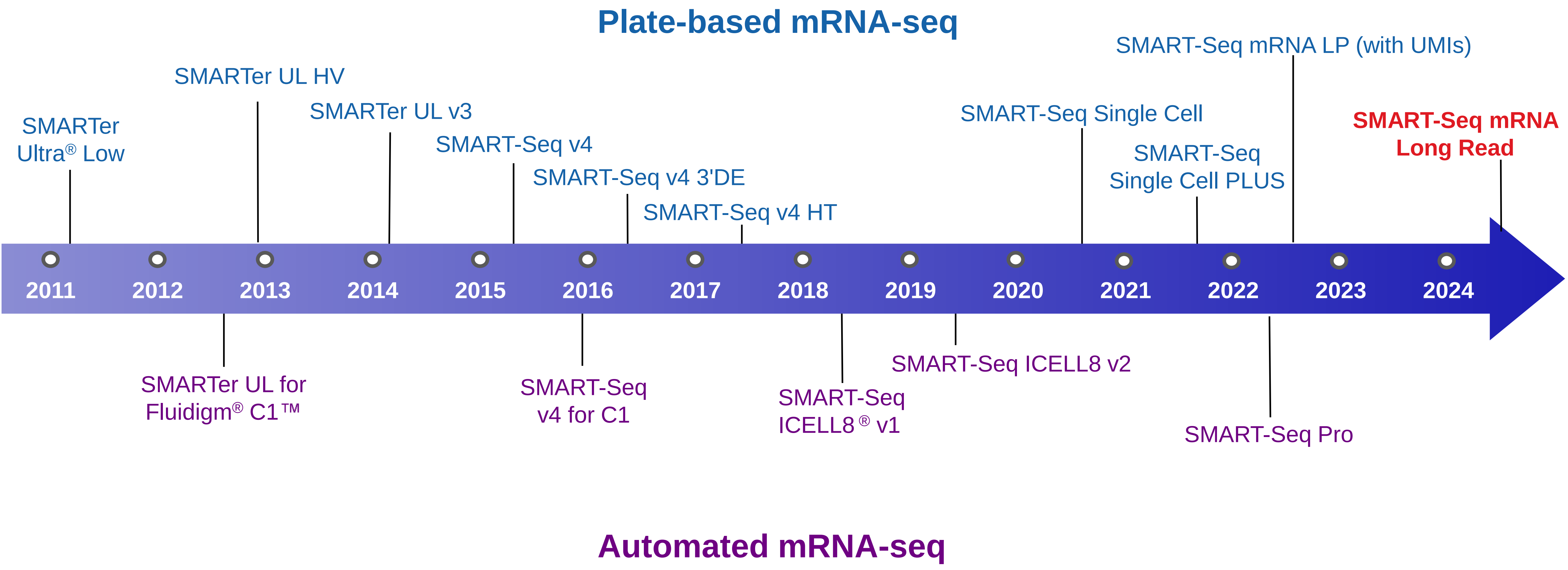


Plate-based mRNA-seq

SMARTer Ultra® Low

SMARTer UL HV

SMARTer UL v3

SMART-Seq v4

SMART-Seq v4 3'DE

SMART-Seq v4 HT

SMART-Seq Single Cell

SMART-Seq Single Cell PLUS

SMART-Seq mRNA LP (with UMIs)

SMART-Seq mRNA Long Read

2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024

SMARTer UL for Fluidigm® C1™

SMART-Seq v4 for C1

SMART-Seq ICELL8® v1

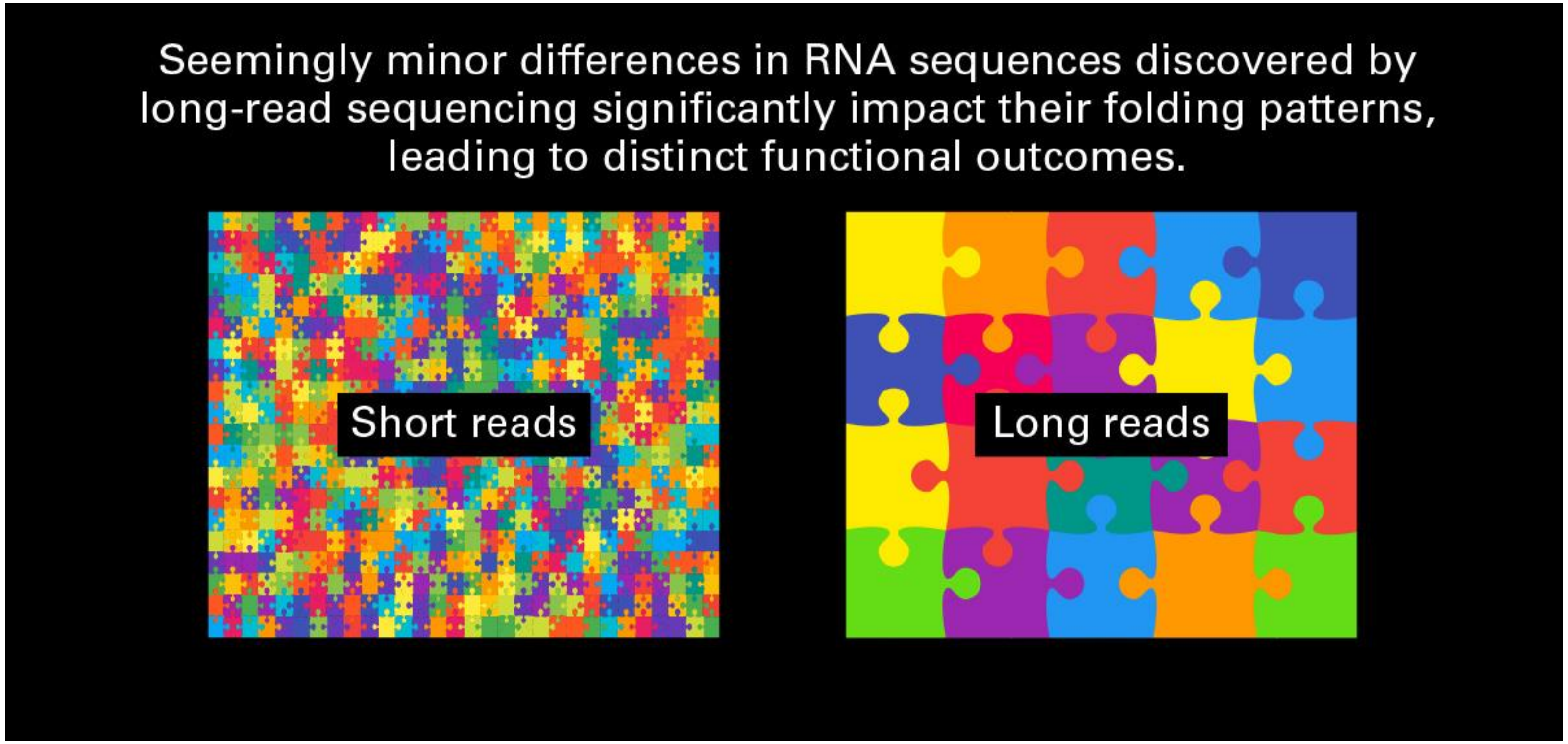
SMART-Seq ICELL8 v2

SMART-Seq Pro

Automated mRNA-seq

Why long-read RNA-seq?

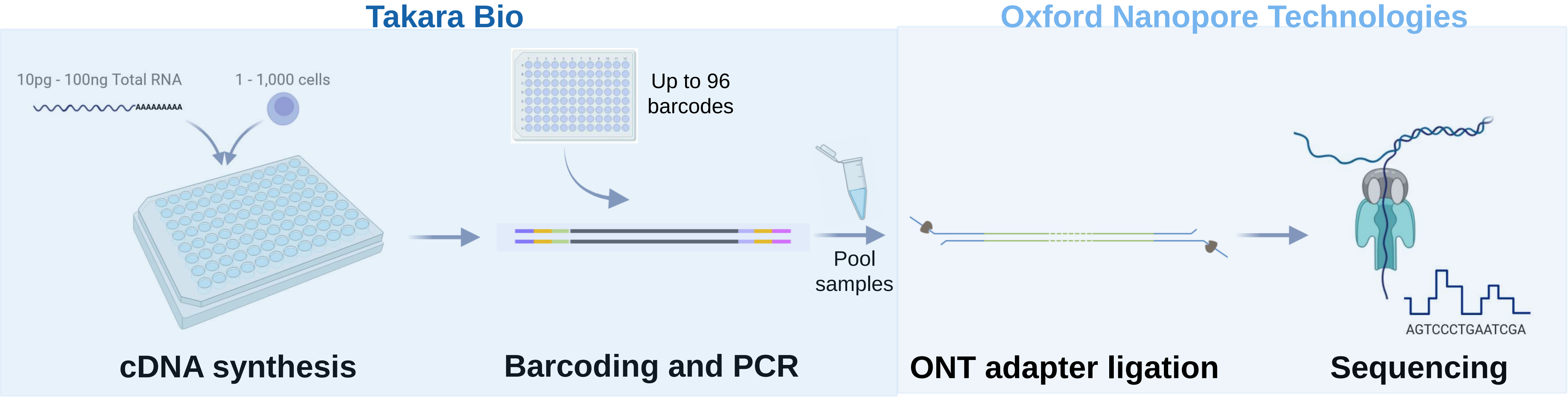
Long-read approaches effectively detect and characterize isoforms and gene fusions



Seemingly minor differences in RNA sequences discovered by long-read sequencing significantly impact their folding patterns, leading to distinct functional outcomes.

SMART-Seq mRNA Long Read (LR) Kit

Leveraging Takara Bio's highly sensitive, full-length SMART-Seq technology for sequencing with Oxford Nanopore Technologies (ONT).



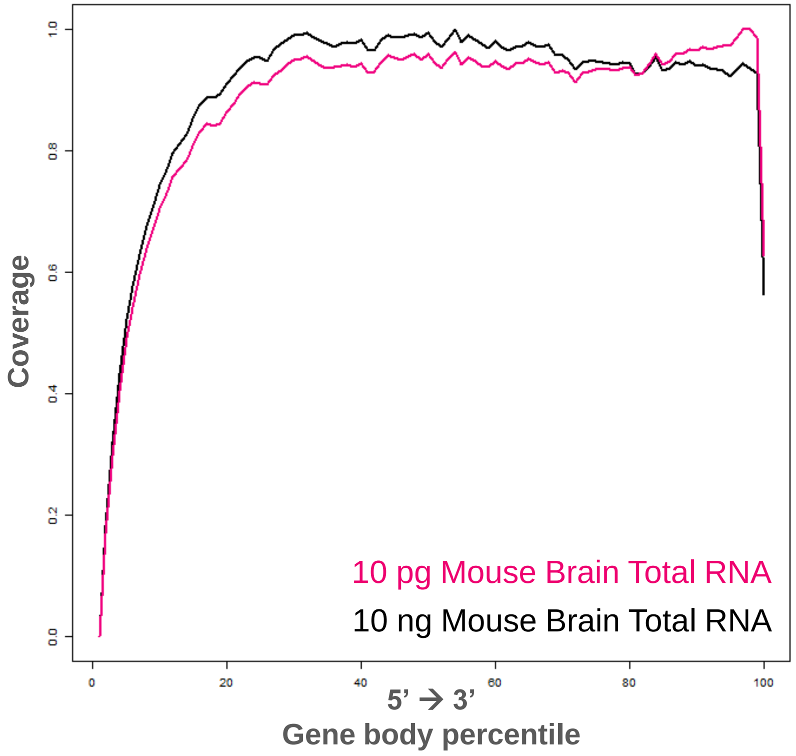
- Full-length cDNA synthesis from 10 pg–100 ng total RNA or 1–1,000 cells
- Barcoded cDNA allows for single-reaction library prep
- Multiplexing up to 96 samples

- Single-day library prep and same-day sequencing
- Full-length sequencing and real-time data analysis

Created using BioRender.com

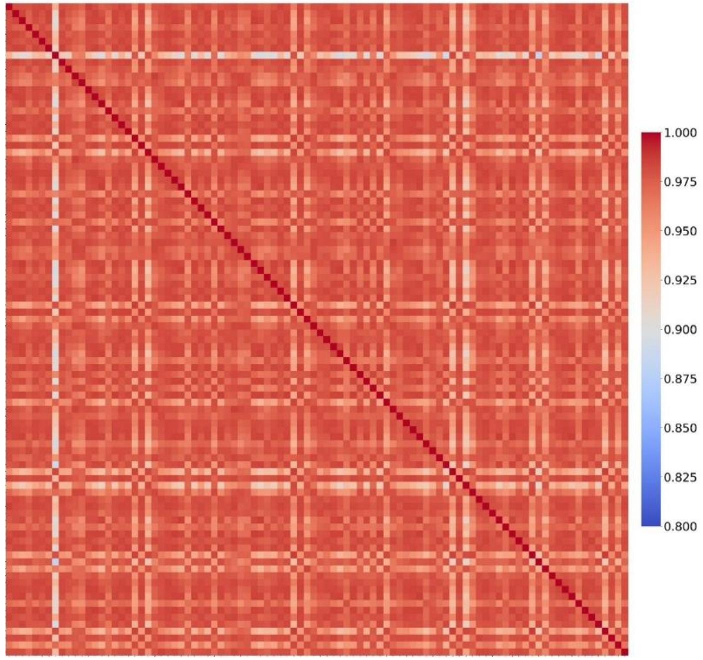
SMART-Seq mRNA LR generates highly reproducible full-length cDNA

Even coverage across the genome and a high demux rate



Gene body coverage

10 ng Total Mouse Brain RNA



Pearson correlation coefficient

- **Average: 0.968**
- **Range: 0.876–0.983**

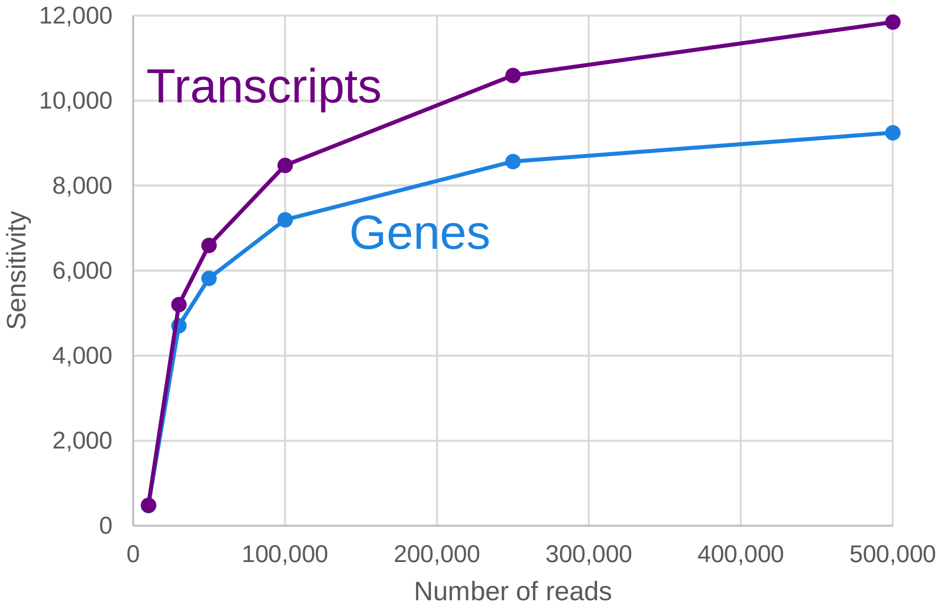
Demux: 97.8%
Fold change across barcodes: 2.4

Pearson correlation coefficient

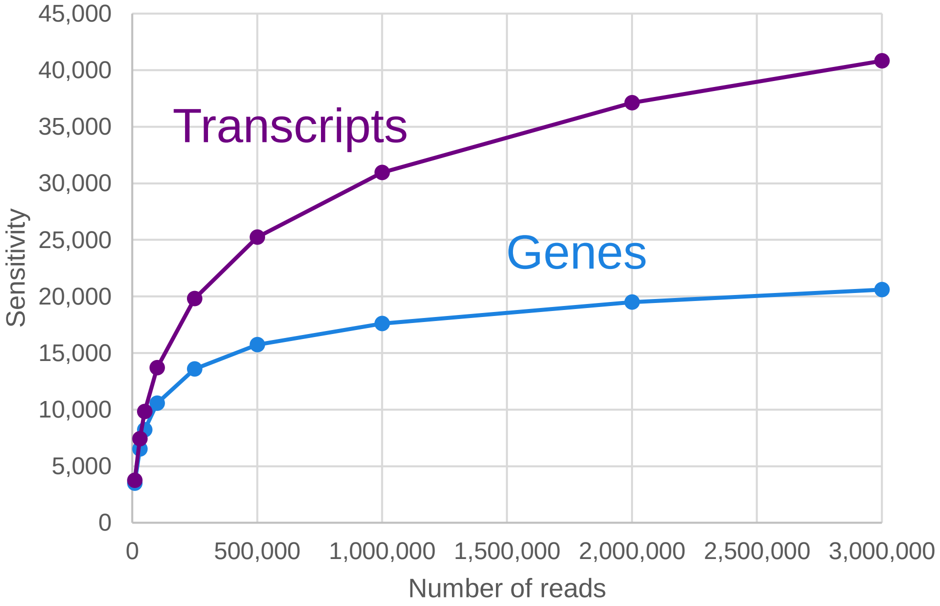
SMART-Seq mRNA LR provides unparalleled sensitivity

Downsampling of reads reveals high sensitivity across a broad input range

10 pg Mouse Brain Total RNA

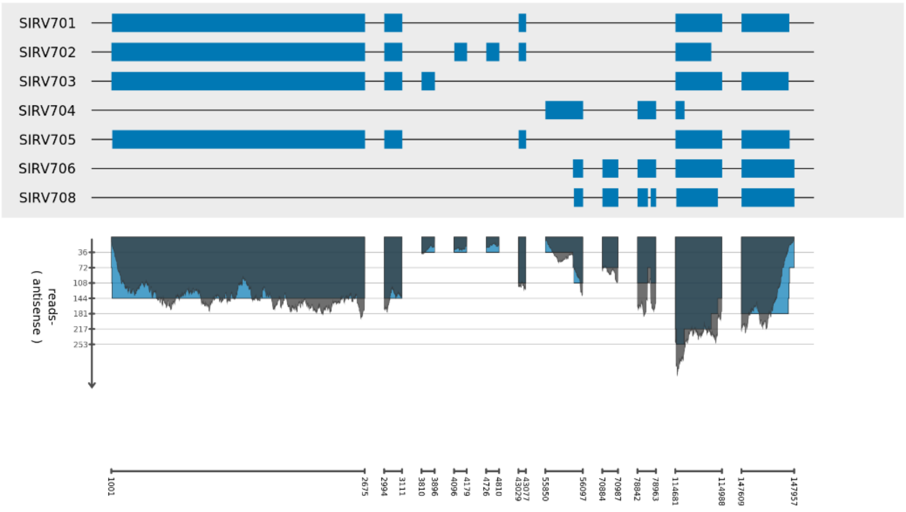


10 ng Mouse Brain Total RNA



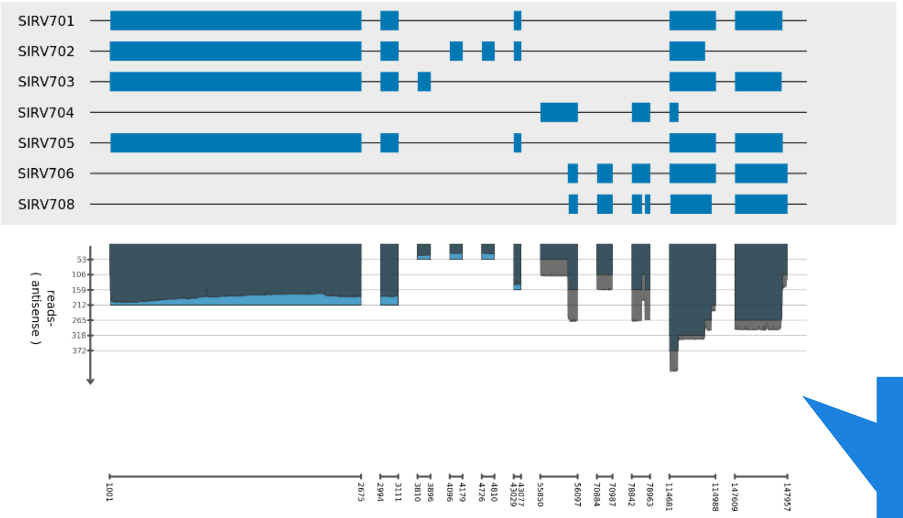
Detection of full-length isoforms using SIRV spike-ins

SMART-Seq mRNA LR generates higher % of full-length isoforms



SMART-Seq mRNA (Illumina®)

Isoform length: 919 bp – 2492 bp



SMART-Seq mRNA LR (ONT)

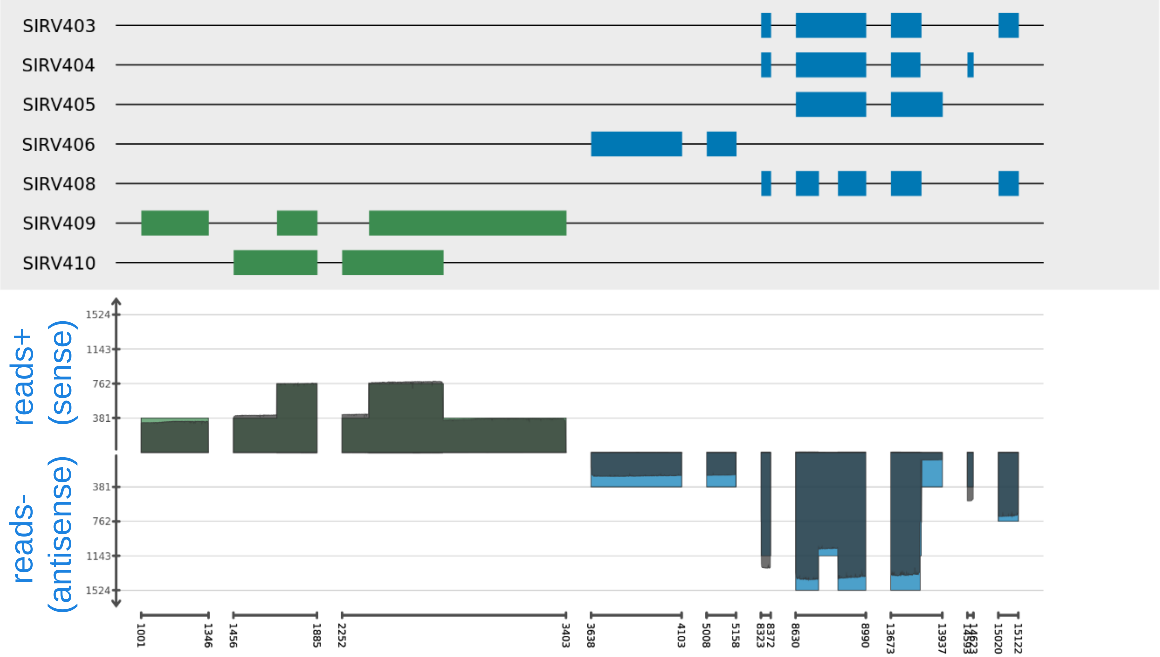
89% full-length isoforms detected.

- Normalized spike-ins from Lexogen (SIRV-Set 4) were used to assess isoform detection.
- SIRVs were spiked into 10 ng mouse brain control RNA at known concentration and coverage was assessed.

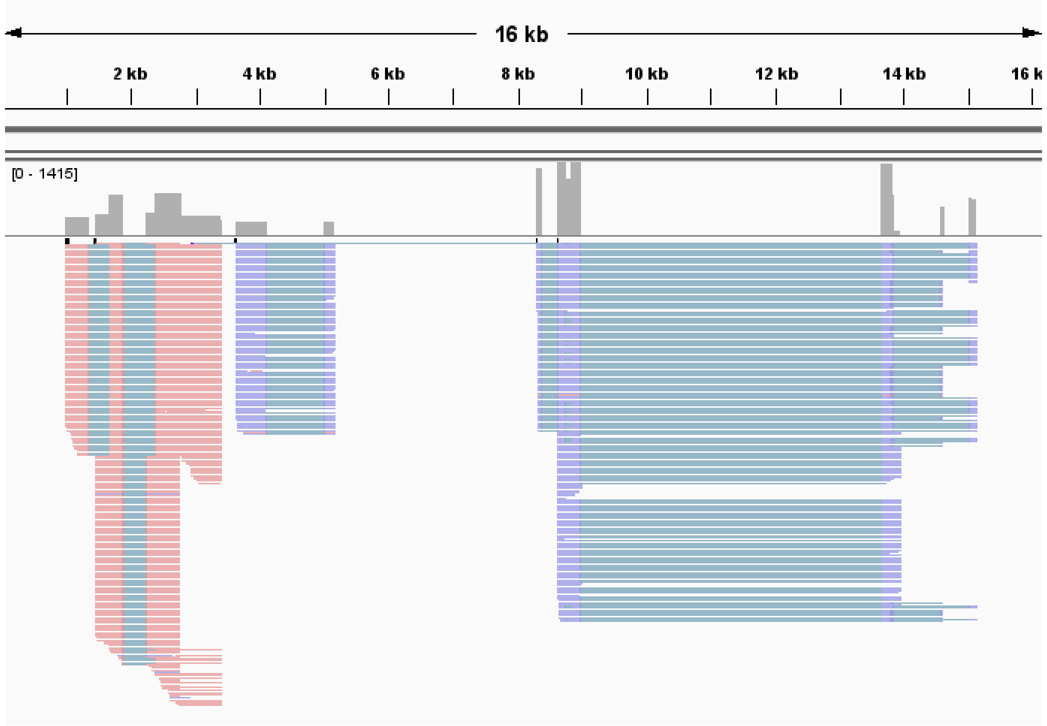
Detection of full-length isoforms using SIRV spike-ins

Identification of all isoforms with reads in the correct stranded orientation

Isoform length: 600 bp – 1597 bp



SIRVsuite visualization

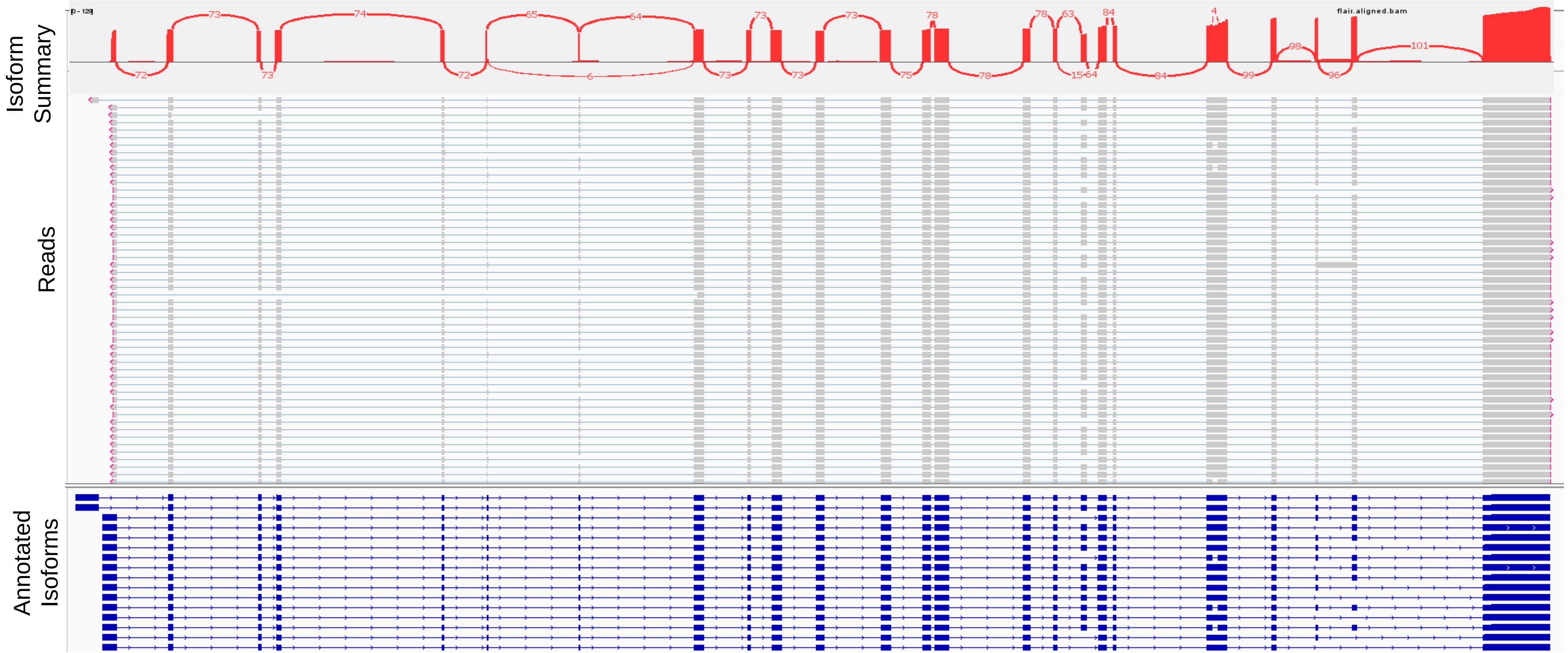


Genome browser view

95% full-length isoforms detected

Isoform and full-length transcript detection in Mouse Brain Total RNA – *Nbr1*

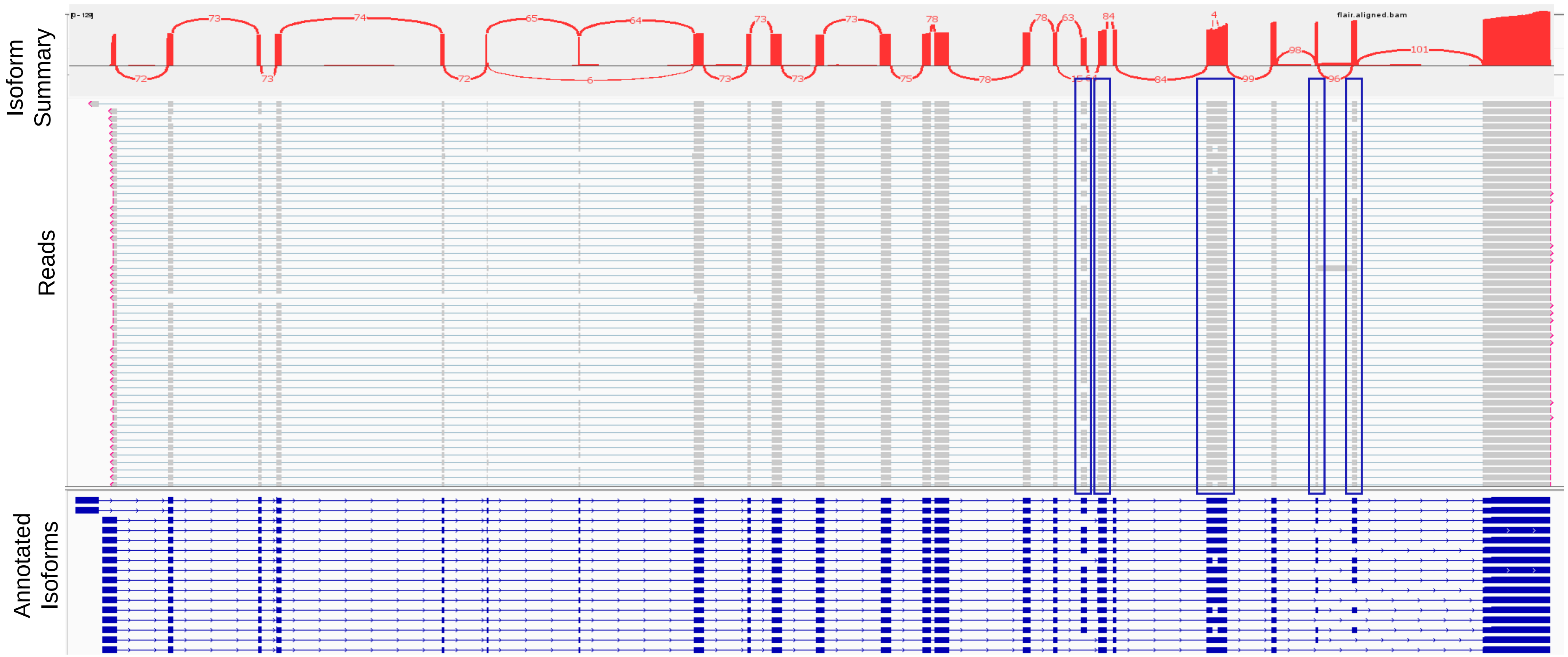
Detect full-length transcripts and different isoforms with 10 pg Mouse Brain Total RNA



Nbr1 transcript is ~4.5 kb

Isoform and full-length transcript detection in Mouse Brain Total RNA – *Nbr1*

Detect full-length transcripts and different isoforms with 10 pg Mouse Brain Total RNA

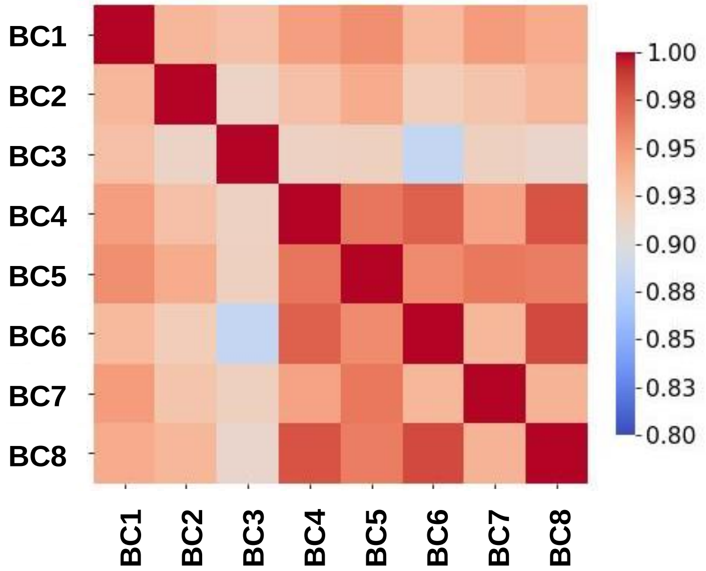


Nbr1 transcript is ~4.5 kb

SMART-Seq mRNA LR is compatible with single cells

Generate highly reproducible data without 3’ or 5’ bias for single cells

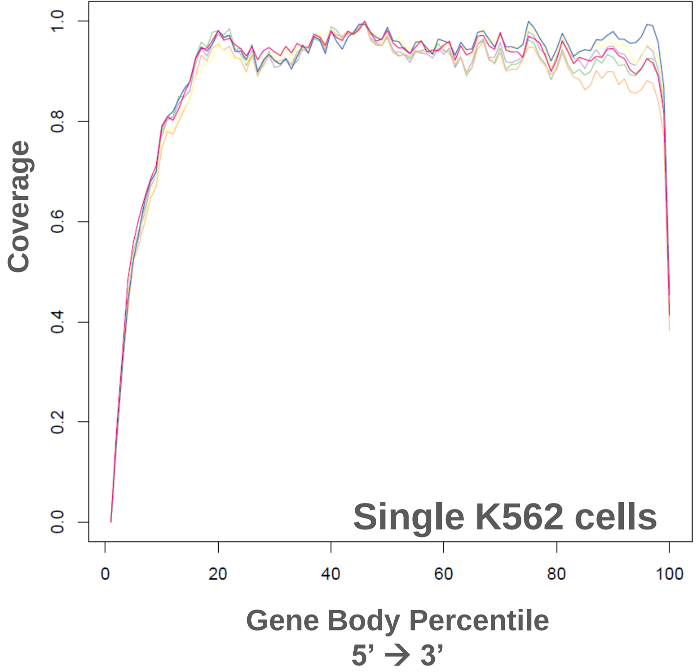
Pearson correlation



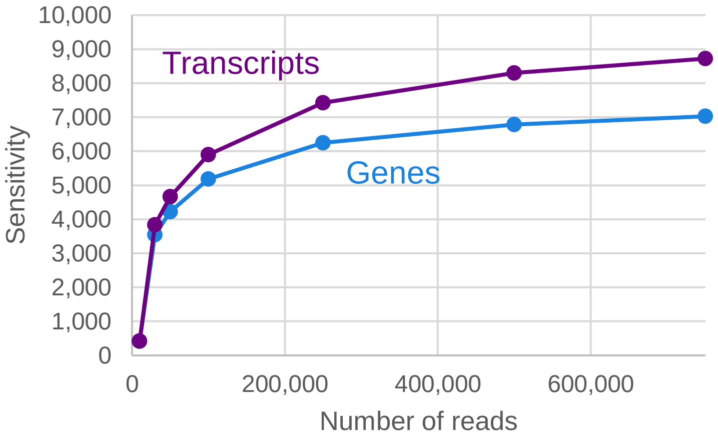
Pearson correlation coefficient

- **Average: 0.94**
- **Range: 0.88–0.98**

Gene body coverage



Sensitivity

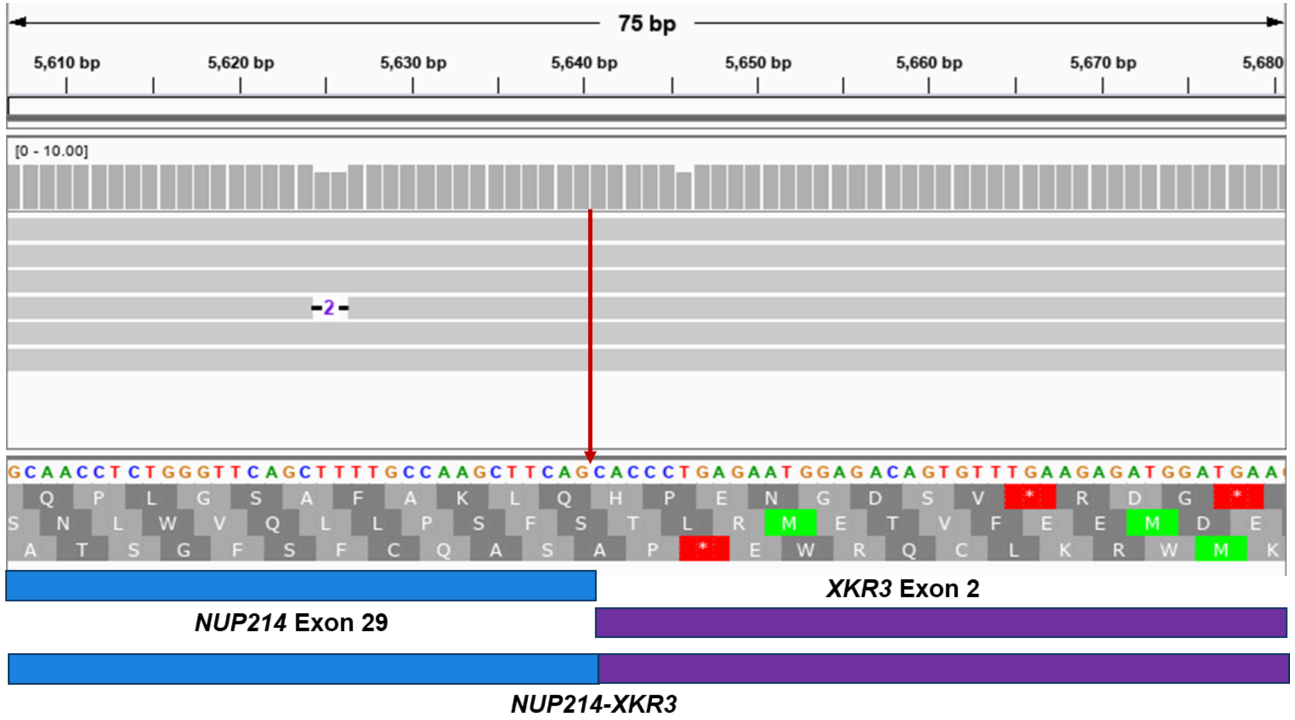


Sample type: FACS-sorted, single K562 cells

Gene fusion detection in single cells

SMART-Seq mRNA LR detects gene fusions

Single-cell *NUP214::XKR3* fusion



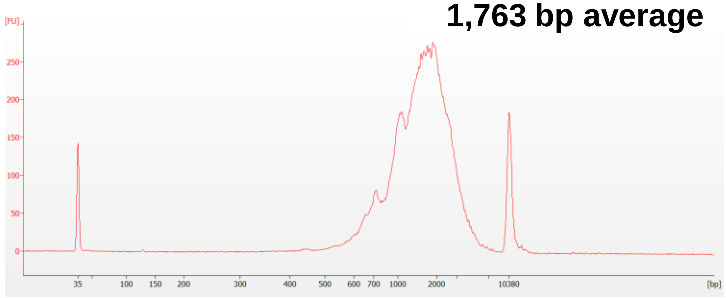
6 reads with *NUP214::XKR3* fusions

1 of 13 cells had 6 fusion reads

SMART-Seq mRNA LR vs the competition

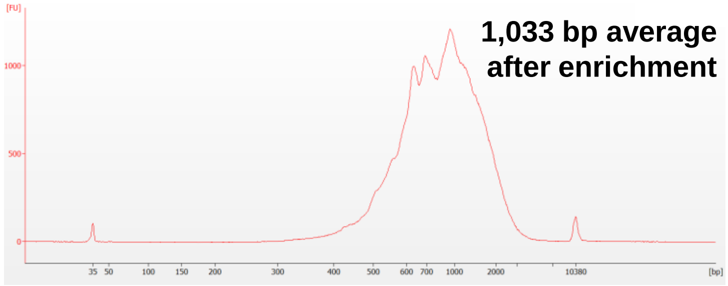
SMART-Seq mRNA LR generates longer cDNA and longer reads

Takara Bio’s barcoded cDNA



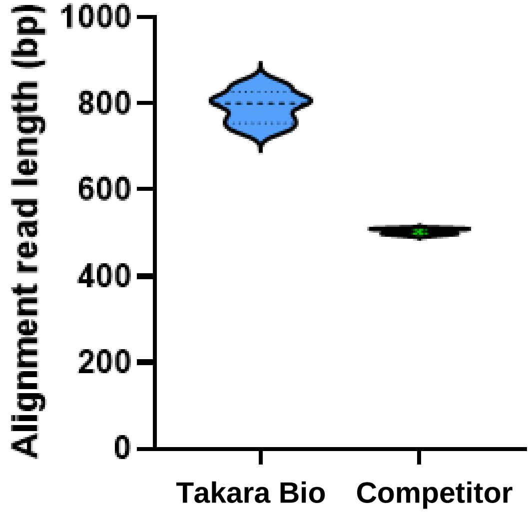
1,763 bp average

Competitor’s barcoded cDNA



1,033 bp average after enrichment

Read alignment comparison

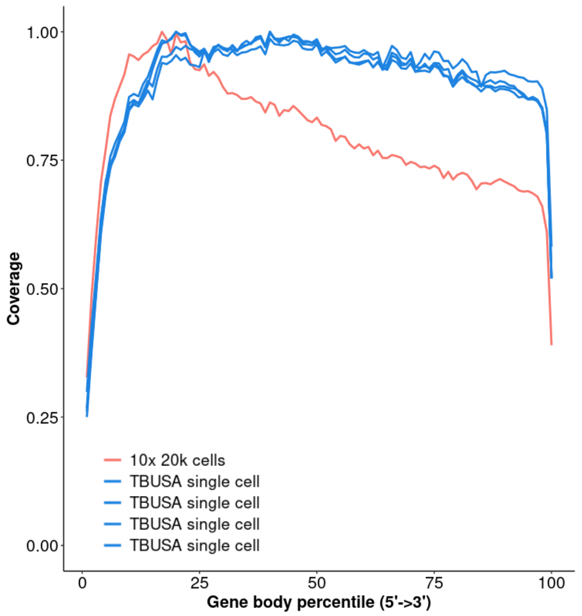


- Single-cell cDNA from K562 cells was prepared with either Takara’s SMART-Seq mRNA LR versus a competitor’s kit
- ONT sequencing adapters were added using the SQK-LSK114 kit according to manufacturer’s instructions

SMART-Seq mRNA LR vs the competition

SMART-Seq mRNA LR generates more full-length reads

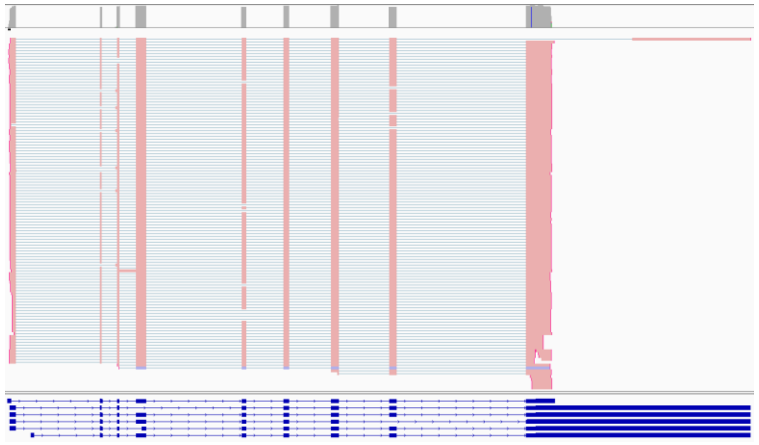
Gene body coverage



Takara's barcoded cDNA

PSMA

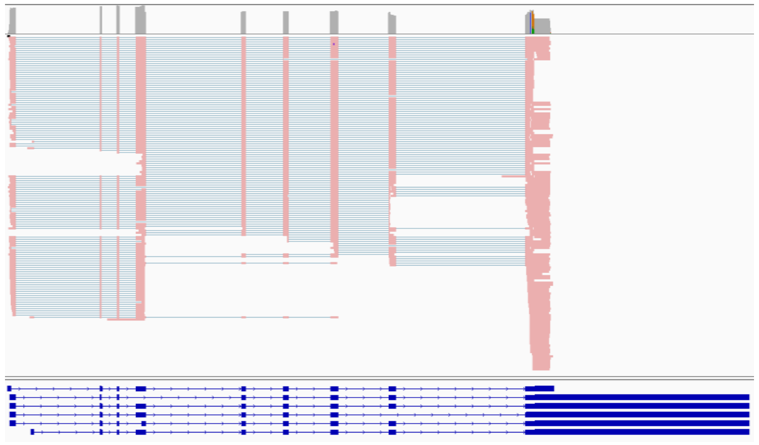
75% FL



Competitor's barcoded cDNA

PSMA

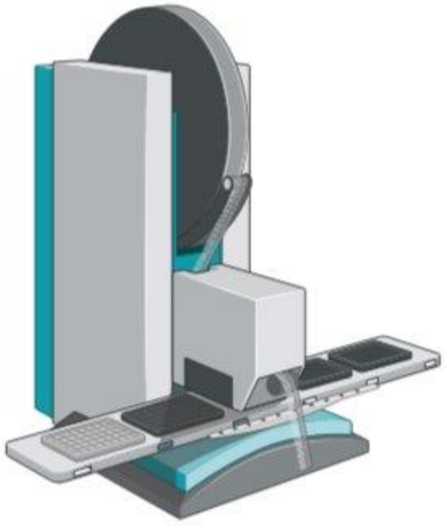
17% FL



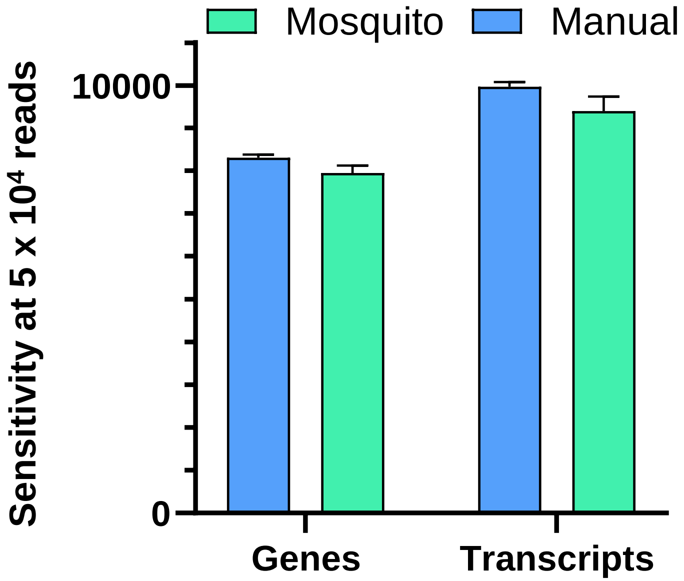
- Single-cell cDNA from K562 cells was prepared with either Takara Bio's SS mRNA LR versus a competitor's kit
- ONT sequencing adapters were added using the SQK-LSK114 kit according to manufacturer's instructions

SMART-Seq mRNA LR is compatible with mosquito HV

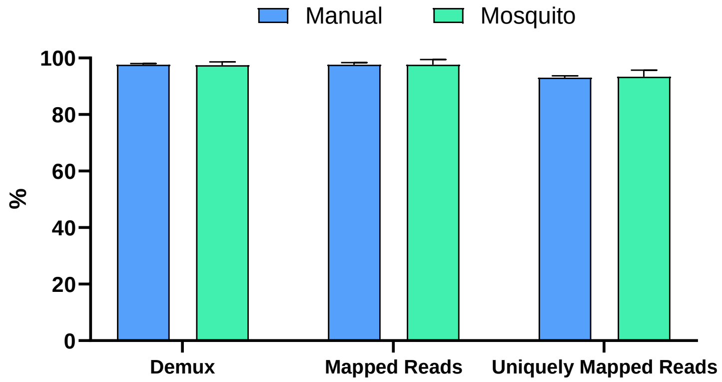
Comparable performance of manual Smart-Seq mRNA LR vs mosquito HV generated data



8X miniaturization



	Mean size of read	Median size of read
Manual	1509.5	1209.6
Mosquito	1480.9	1262.9



- Sorted K562 cells (1 cell/well) in 96 well plate
- Generated cDNA either manually (full-volume) or with mosquito HV (SPT Labtech)

Summary

- Enable full-length, low-input transcriptome analysis
- Identify longer cDNA compared to competitors
- Get high reproducibility
- Detect isoforms and gene fusions
- Automate with miniaturized liquid handling instruments

TaKaRa

that's
GOOD
science!®