# Single-cell application development with the ICELL8® cx system:  One platform, endless possibilities

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# Single cells: a brief historical perspective for Takara Bio

## 2012

Ramsköld, D. **Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells.** *Nat. Biotechnol.*

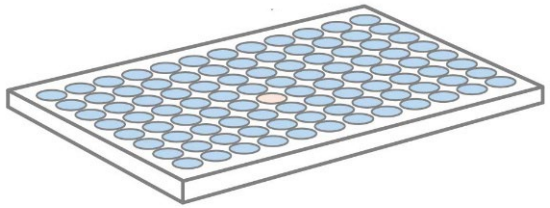
- SMARTer® Ultra® Low RNA Kit for Illumina® Sequencing
- 12 cells
- 3 cultured cell types
- Basic clustering

## 2018

Schaum, N. **Single-cell transcriptomics of 20 mouse organs creates a *Tabula Muris*.** *Nature*

- 10x and Smart-seq2 methods
- 100,000 cells
- *De novo* cell-type identification by tSNE

# Takara Bio solutions for single-cell research



## SMART-Seq® Single Cell Kit

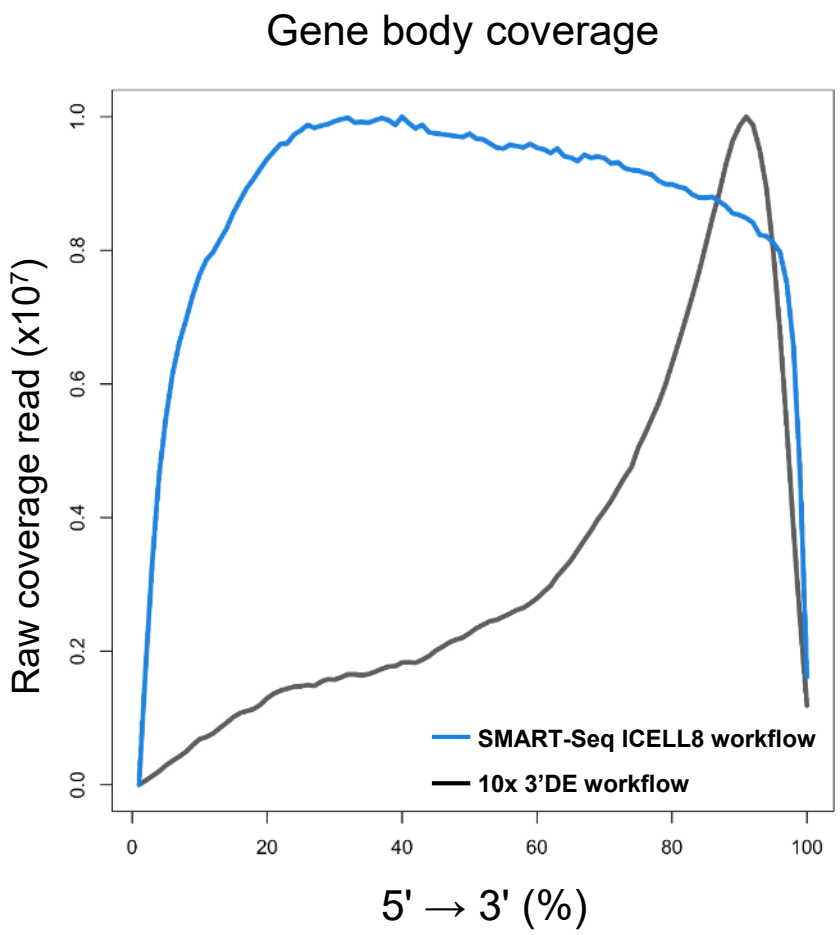
- Chemistry optimized for increased performance on single cells with very, very low RNA content
  - QC performed with 2 pg of Mouse Brain RNA
- Best kit for single-cell or nuclei applications
- Robust full-length chemistry
- Highest sensitivity and reproducibility
- Easily adaptable to automation protocols


## ICELL8 cx Single-Cell System

- Open platform with integrated nanodispensing + imaging systems
- Visual identification and unique processing of individual cells
  - Up to ~1,500 cells
  - Three colors (blue, green, red)
- Flexible to function with Takara Bio or user-developed chemistries, including:
  - ATAC-seq, CUT&Tag, and SMART-Seq full-length mRNA sequencing
- Bioinformatic support available for mapping and further classification of your cells of interest

# SMART-SEQ ICELL8

# What does it mean to be full length?

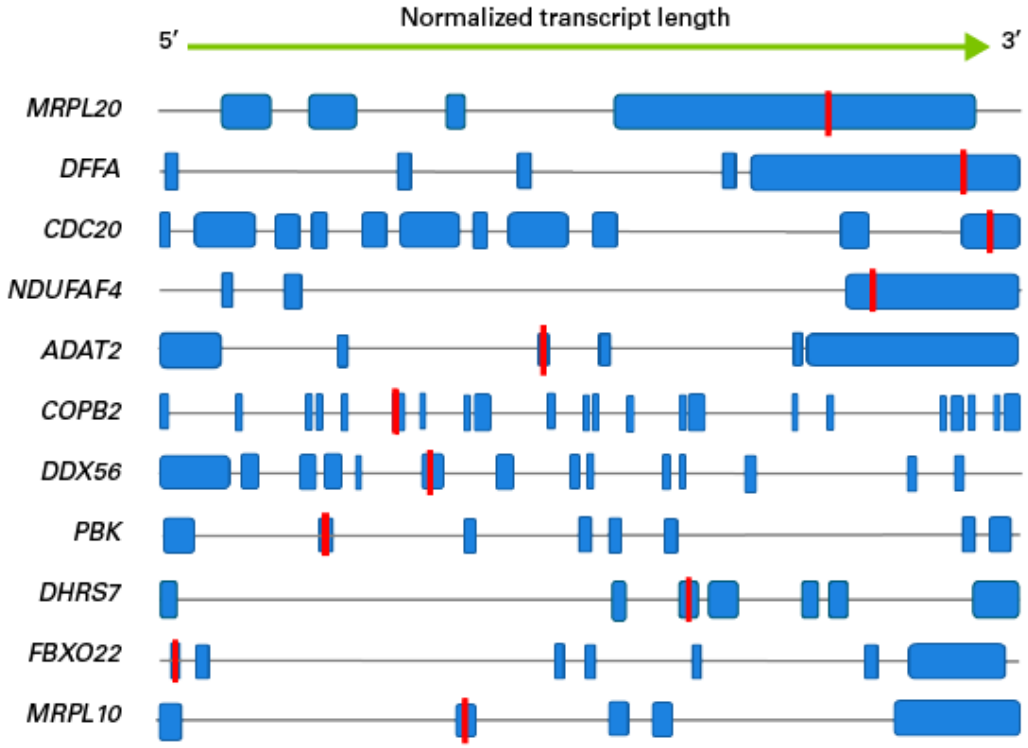



- Full-length protocol has paired-end reads – both reads contain information about the gene (barcodes are identified through index reads)
- This leads to >2X coverage of the genes identified than a single-end technology with the same number of clusters

Data from HEK 293 cells (10x v2)

# Improved SNP detection with full-length information

| | | SMART-Seq ICELL8 workflow | | 10x 3'DE workflow | | |
|---|---|---|---|---|---|---|
| Gene | Wt / variant | Total reads | Variant reads | Total reads | Variant reads | Location |
| *MRPL20* | G / C | 662 | 163 | 127 | 27 | Exon 4 (total 4) |
| *DFFA* | C / T | 136 | 45 | 71 | 21 | Exon 5 (total 5) |
| *CDC20* | G / A | 161 | 84 | 144 | 86 | Exon 11 (total 11) |
| *NDUFAF4* | A / C | 225 | 74 | 98 | 42 | Exon 3 (total 3) |
| *ADAT2* | A / C | 107 | 25 | 17 | 5 | Exon 3 (total 6) |
| *COPB2* | C / A | 209 | 144 | ND | ND | Exon 6 (total 22) |
| *DDX56* | G / A | 108 | 32 | ND | ND | Exon 6 (total 14) |
| *PBK* | C / G | 160 | 55 | ND | ND | Exon 2 (total 8) |
| *DHRS7* | T / C | 175 | 173 | ND | ND | Exon 3 (total 7) |
| *FBXO22* | C / T | 169 | 55 | ND | ND | Exon 1 (total 7) |
| *MRLP10* | G / T | 231 | 137 | ND | ND | Exon 2 (total 5) |



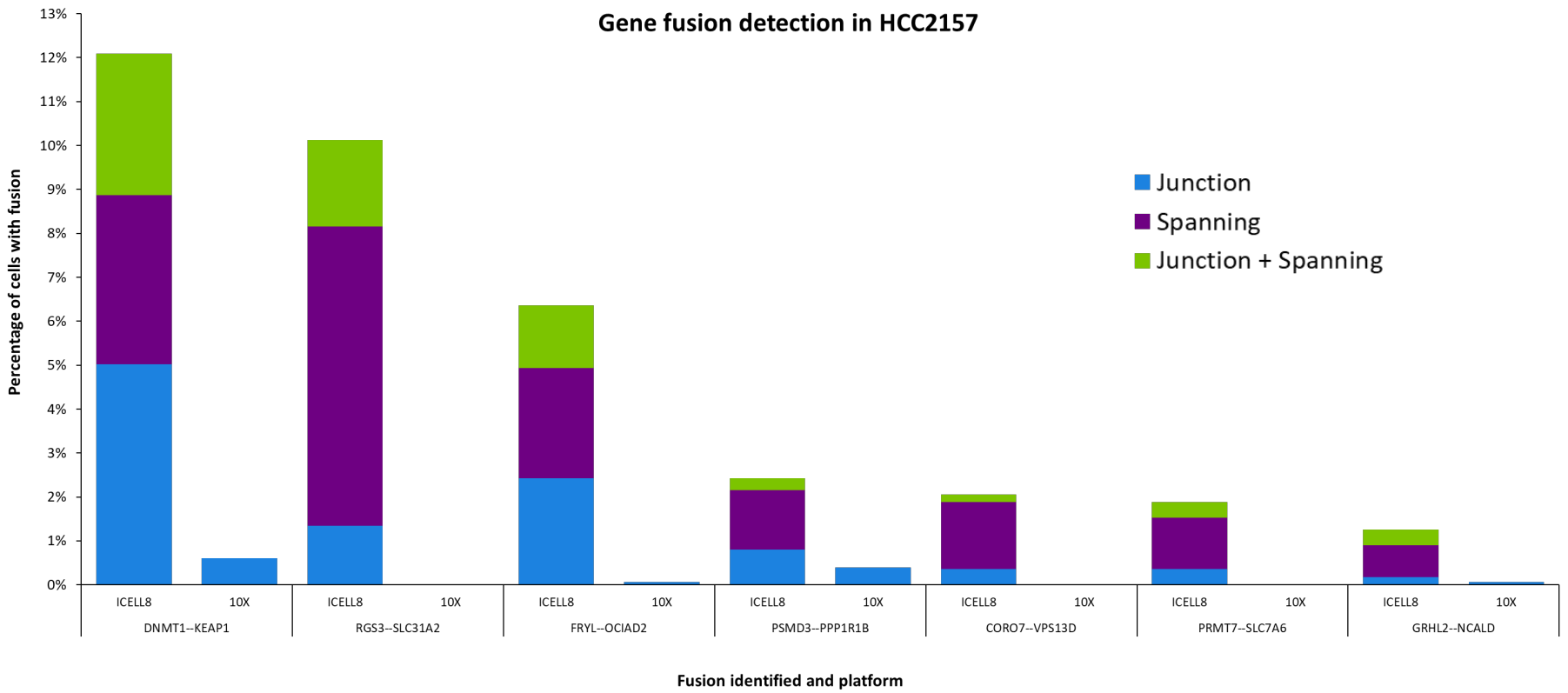

*Exon maps are not drawn to scale.*

- More SNPs across the whole transcript were detected in samples prepared with SMART-Seq chemistry on the ICELL8 cx system
- 10x 3′ DE (v3) chemistry was not able to detect (ND) SNPs at the 5′ end of genes

Data from K562 cells

# Fusion identification in a breast cancer cell line

ICELL8 = SMART-seq ICELL8 workflow
10X = 10x 3'DE workflow




- Fusions were identified in 70% of SMART-Seq cells, but only 30% of 10x cells
- SMART-Seq chemistry allows for spanning read identification—supporting data

Data from HCC2157 cultured cells

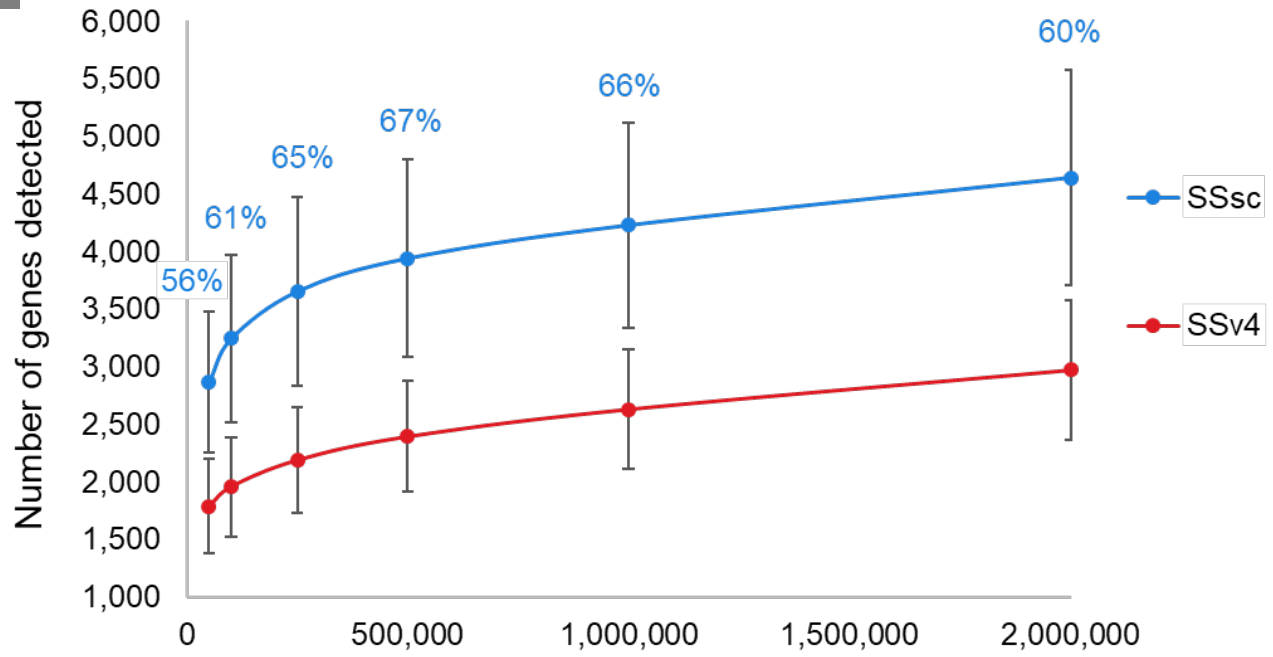
# SMART-SEQ SINGLE CELL KIT (SSsc)

# Studies to illustrate performance of the new SSsc kit

- Comparisons with SMART-Seq v4 kit (SSv4)
  - PBMCs
- Comparison with Smart-seq2 (SS2)
  - Cultured lymphoblast cells
- Comparison with NEBNext Single Cell kit
  - Primary T cells
- Customer data comparison
  - Primary B and T cells

# Higher performance with primary samples

A

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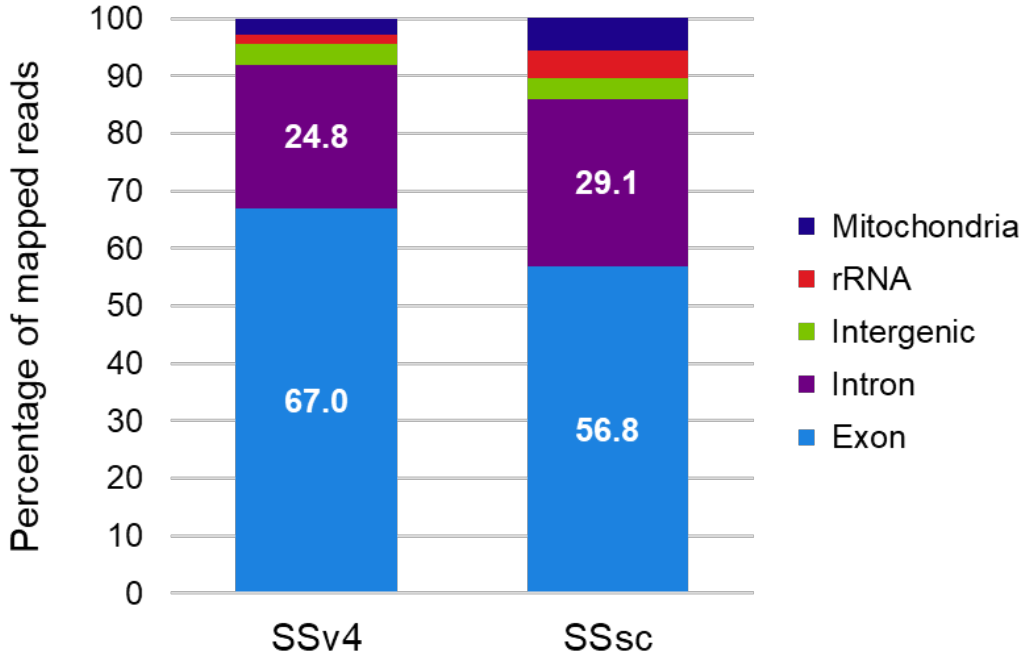


- Consistently, ~60% more genes are detected in the cells processed with SSsc, regardless of the sequencing depth used for the analysis
- Similar read distribution between the two chemistries

Data from PBMCs

# SSsc outperforms SS2

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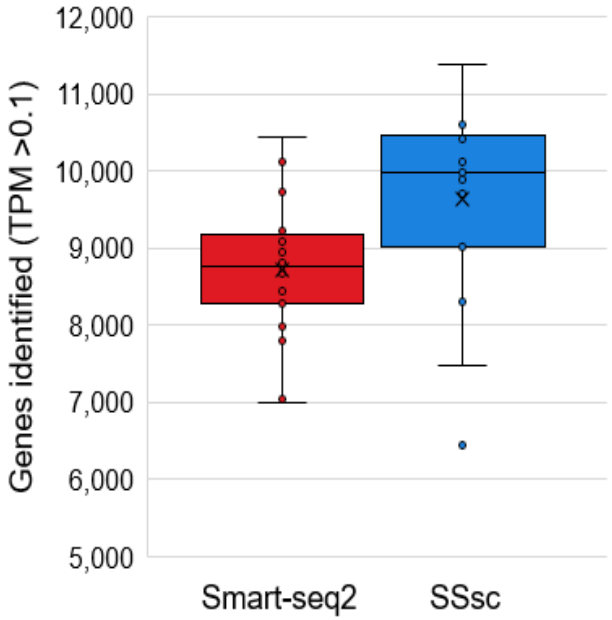


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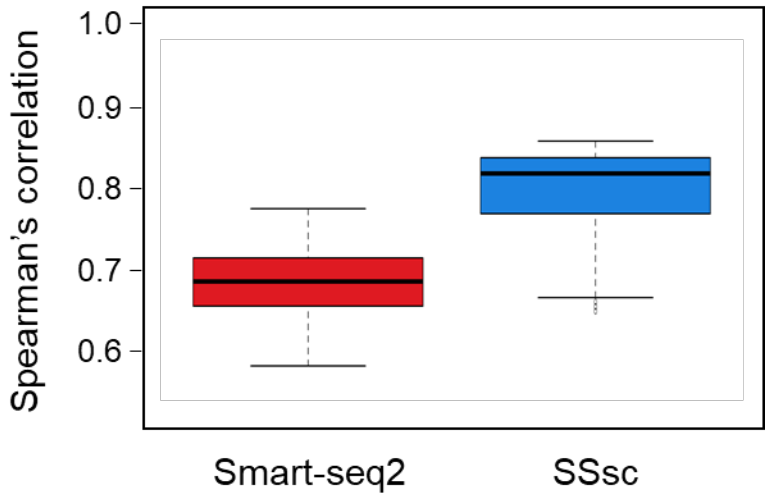
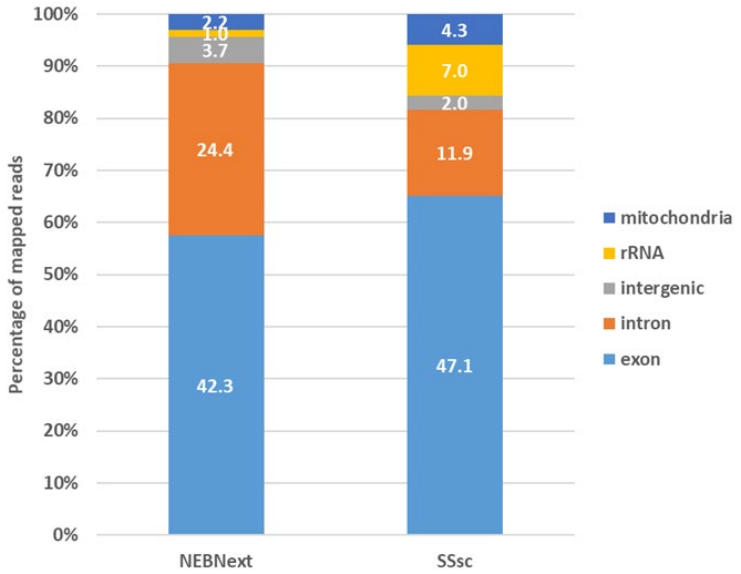



- ~20% more genes identified with SSsc in this study
- SSsc shows higher Spearman's correlation (0.85), indicating higher reproducibility

Data from lymphoblastoid cells (GM12878)

# SSsc is more sensitive than NEBNext Single Cell kit

A

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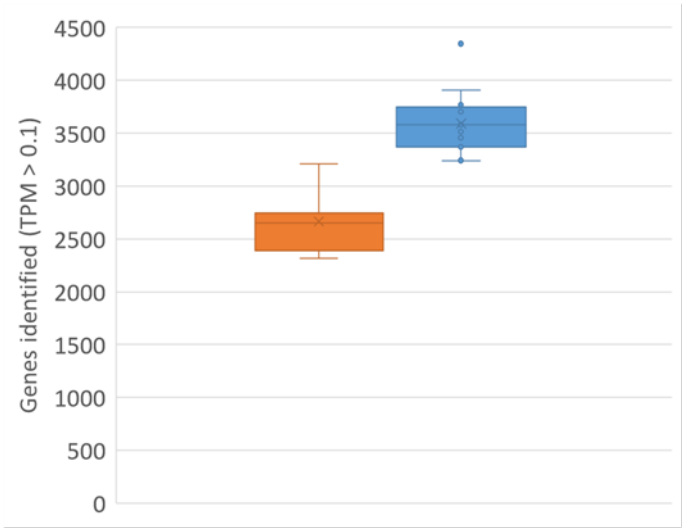


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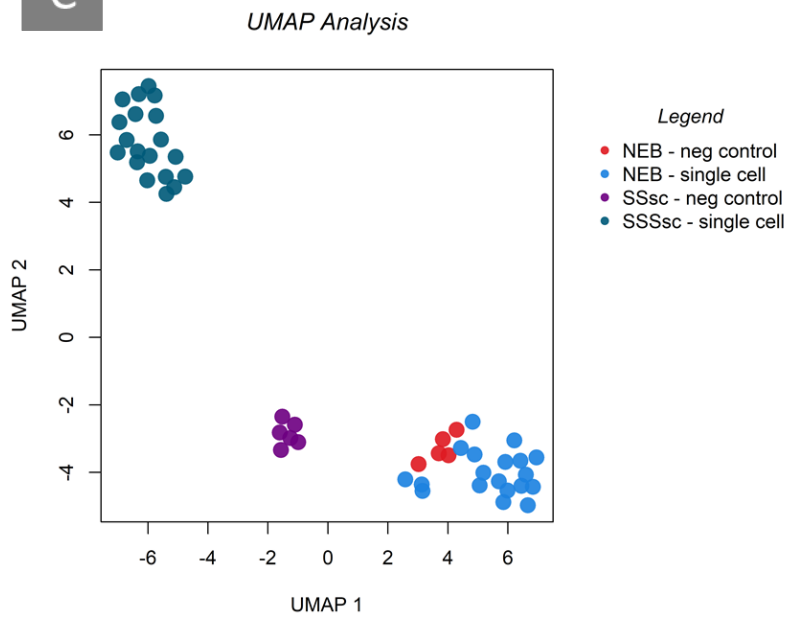


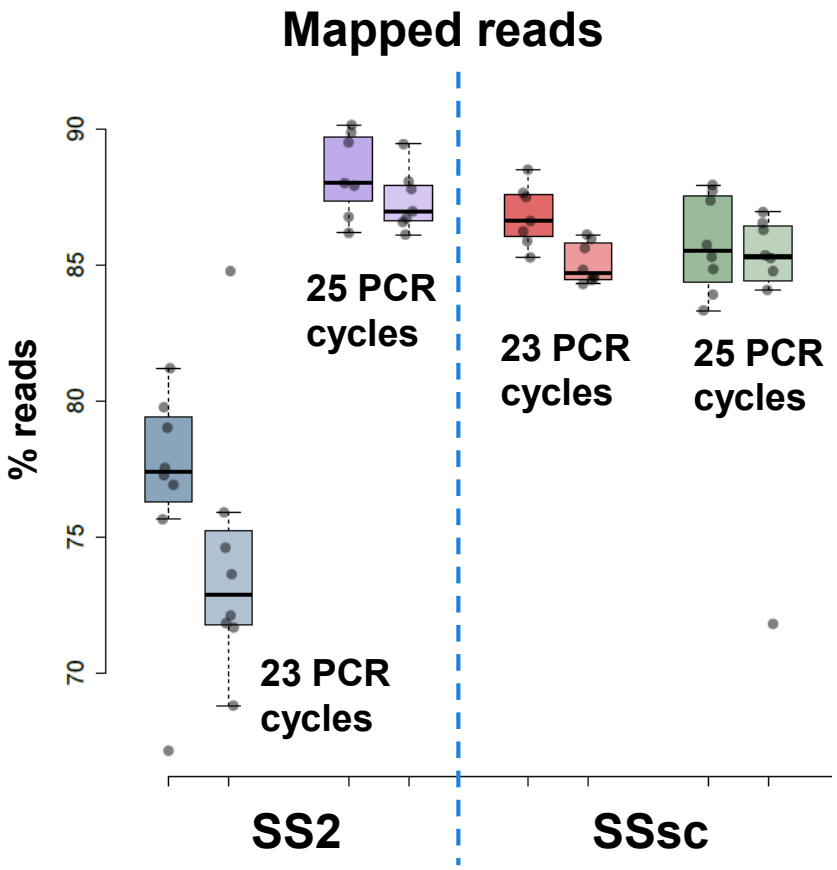

- Greater number of reads mapping to introns with NEBNext Single Cell kit
- More genes (~40%) are detected in the cells processed with SSsc
- Increased cycling was required for the NEBNext Single Cell kit—therefore the negative control performs similarly to the single cells

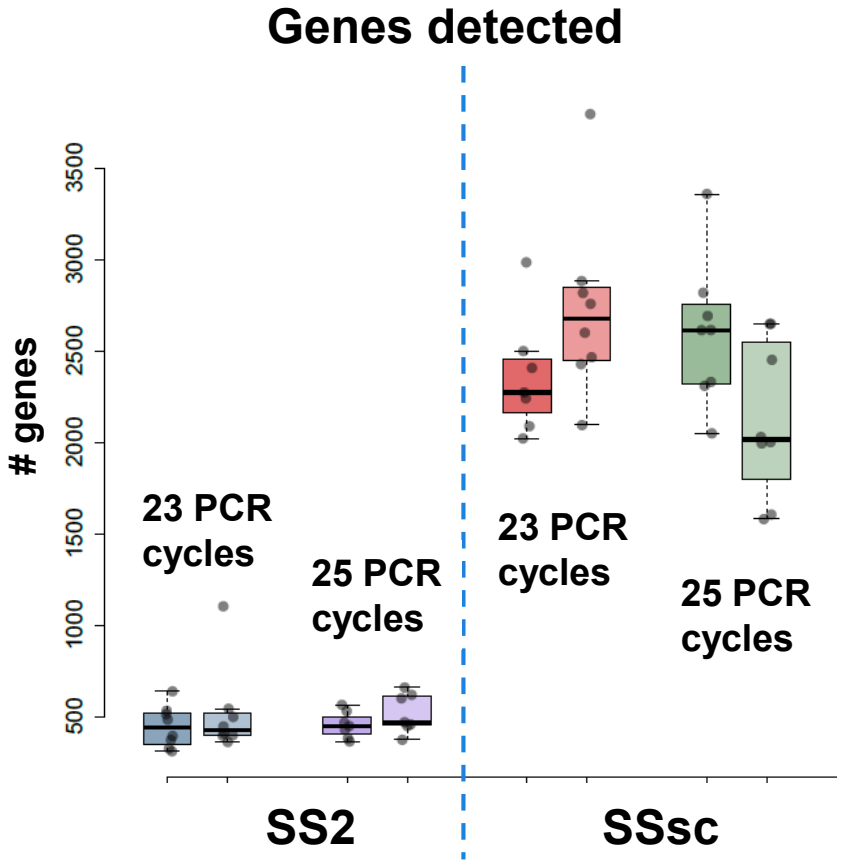
Data from primary T cells

# Datasets from customer using SSsc

- Single Cell Genomics Team Leader at CNAG in Barcelona (Dr. Holger Heyn)
  - Single Cell Genomics Team focuses on the implementation of single-cell sequencing technologies and their application in research and translational contexts
  - Currently a high-volume Smart-seq2 user
  - Did a comparison study using B and T cells with SS2 and SSsc; performed miniaturized reactions for each chemistry

# CNAG:
# SSsc outperforms SS2 for single cells with low RNA content




- Liquid handler: Mantis
- Miniaturized workflows for both chemistries

- Similar number of reads per sample (~500,000)
- SSsc had consistently high performance for the percentage of mapped reads
- SSsc detected at least five times as many genes as SS2

Data from B and T cells

# Conclusions

- The new SMART-Seq Single Cell Kit features a user-friendly, plate-based workflow that starts directly from single cells isolated by FACS or other methods
- Offers unparalleled sensitivity and reproducibility for single-cell, full-length RNA-seq, particularly for cells with very low RNA content (e.g., immune cells)
- Outperforms the Smart-seq2 method in convenience, sensitivity, gene identification, and reproducibility—as seen in both internal and customer-generated data
- Compatible with automation platforms
- Offers the highest confidence for interlaboratory comparisons due to manufacturing with strict quality standards (ISO 13485:2016 certification)

# Takara Bio activities at ABRF

- Automation
  - Poster 147, 11:30: Robust and sensitive detection of gene fusions using high-throughput SMART-Seq chemistry on the ICELL8 cx system
  - Poster 133, 11:30:Utilizing the Rheonix NGS OnePrep™ Solution to automate the Takara Bio ThruPLEX® Tag-Seq HV library preparation kit
  - Poster 132, 12:30: Miniaturization of Ribosomal RNA Depletion and Total RNA Library Preparation in Single Cells
- Immune Profiling
  - Poster134, 12:30: Efficient high-throughput sequencing for quantitative immune profiling using unique molecular identifiers
- DNA-Seq
  - Poster 146, 12:30: ThruPLEX® HV: A Simplified System for Preparation of Molecular-Tagged NGS Libraries from FFPE and cell-free DNA
- RNA-Seq
  - Poster 131, 11:30: Pushing the limits of single-cell RNA-seq with SMART-Seq single cell technology

- Visit us at Booth #104

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