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\textsuperscript{®} 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

Gene body coverage

Data from HEK 293 cells (10x v2)
Improved SNP detection with full-length information

- 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

- 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

- 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

- ~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

- 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

- 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
  – Poster 134, 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
that’s GOOD science!