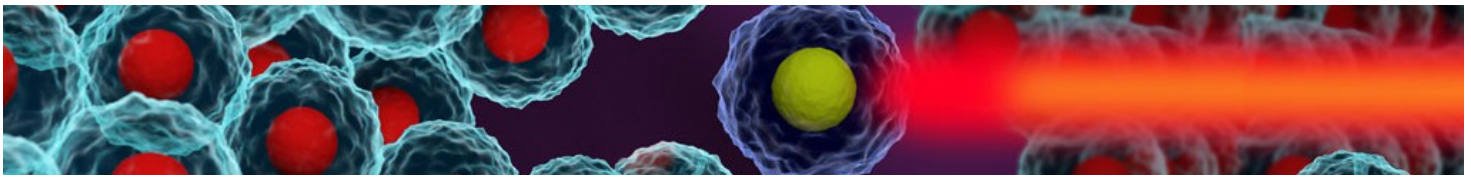
Single-cell analysis of Alzheimer's disease



In recent years, sequencing approaches have shed light on the genomic, epigenomic, and transcriptional dysregulation of Alzheimer's disease and other age-linked disorders. Genome-wide association studies have identified numerous risk loci (Kamboh et al. 2012; Jansen et al. 2019). RNA-seq reveals widespread transcriptomic dysregulation and altered splicing (Raj et al. 2018). Chromatin accessibility profiling demonstrates epigenomic changes in elderly and Alzheimer's-afflicted brains (Gjoneska et al. 2015; Klein et al. 2019).

However, brain tissue is extremely heterogeneous, and this represents an enormous research challenge due to the cellular basis of Alzheimer's disease: microglia and astrocytes promote inflammation, oligodendrocytes retract their protective myelin sheaths, neurons die and sever synaptic connections, and each of these pathologies is the result of unique disruptions in individual cell types. While a large proportion of sequencing work has been done using bulk tissue rather than single cells, breakthroughs in single-cell sequencing technologies have allowed researchers to begin examining the genomic, epigenomic, and transcriptional landscapes of individual cells. This has allowed the identification of Alzheimer's disease-associated cell types and revealed age- and Alzheimer's-linked genomic mosaicism (Bushman et al. 2015; Keren-Shaul et al. 2017; López-Sánchez et al. 2017).

By understanding the cellular basis of Alzheimer's disease, investigators will be more capable of monitoring and tailoring prophylactic and therapeutic interventions. Researchers have only begun to scratch the surface of what single-cell sequencing methods can tell us about this debilitating illness, but we know these approaches will require sensitive, reproducible, and high-throughput methods.

Fortunately, we're up to the task.

Highlighted products

Ultra-sensitive single-cell sequencing

Our new SMART-Seq Single Cell Kit (SSsc) incorporates our proprietary SMART (**S**witching **M**echanism **a**t 5' end of **R**NA **T**emplate) technology to offer unprecedented sensitivity from intact single cells or nuclei. It offers greater sensitivity and reproducibility than Smart-Seq2 with a reduced percentage of dropouts (Figure 1). Indeed, its optimized chemistry outperforms all currently available full-length sequencing methods for single-cell applications, including our gold-standard SMART-Seq v4 technology (Figure 2), which currently powers the transcriptomic arm of the Allen Cell Types Database. These features make SSsc the ideal chemistry specifically for single-cell applications and an optimal choice for characterizing the transcriptional diversity of the CNS. Additional benefits include its compatibility with automation platforms and a user-friendly, plate-based workflow. Finally, for high-throughput workflows, we offer both our automation-friendly SMART-Seq HT Kit and our ICELL8 cx Single-Cell System—an advanced automation platform for single-cell sequencing workflows (more in the next section, below).

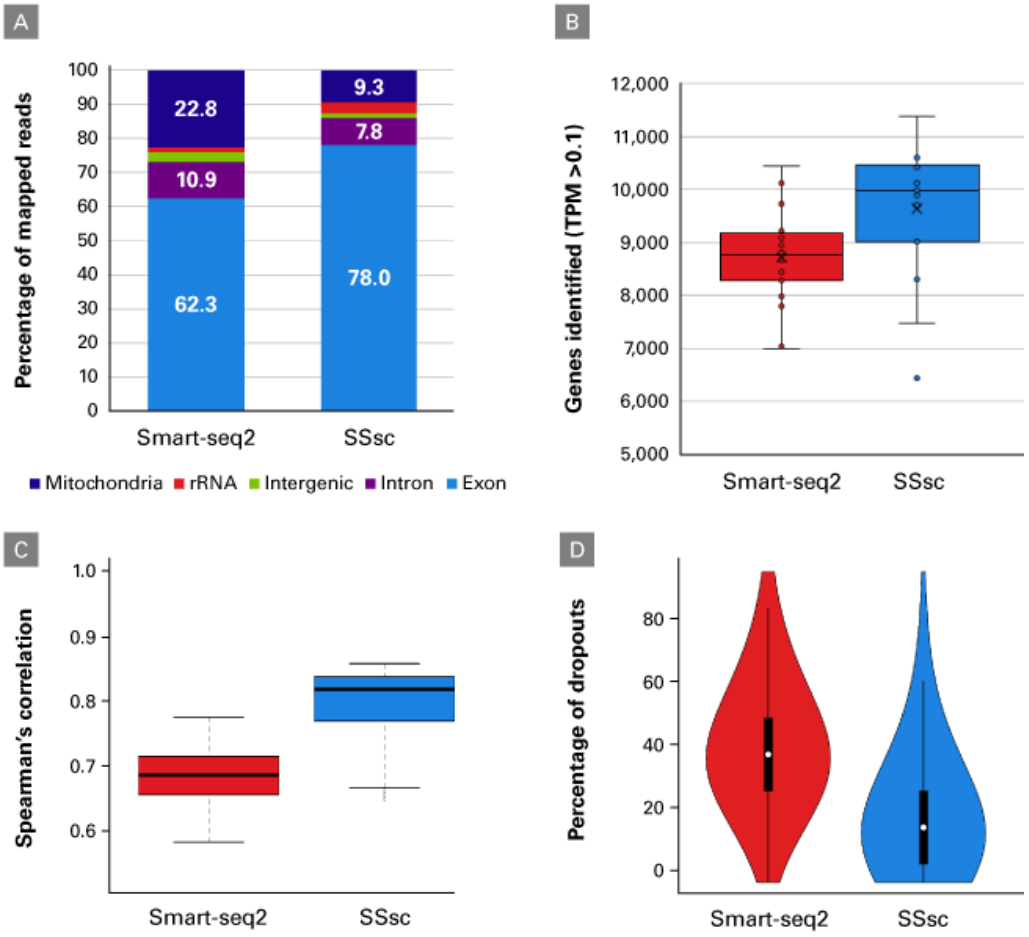


Figure 1. The SMART-Seq Single Cell Kit outperforms the Smart-seq2 protocol. Single cells from the lymphoblastoid cell line GM12878 were processed with SSsc (18 cells) or Smart-seq2 (20 cells) using 19 cycles of PCR. As described in the methods, RNA-seq libraries were generated and sequences analyzed (after normalizing all samples to 1.75 million paired-end reads). **Panel A.** The read distribution varied between the two chemistries, with increased mitochondrial reads using Smart-seq2 and increased exonic reads using SSsc. **Panel B.** More genes were detected in the cells processed with SSsc. **Panel C.** Correlation boxplots showing the intragroup Spearman correlation between all cells processed with either method. The higher Spearman correlation among the cells processed with SSsc indicates a greater reproducibility than the Smart-seq2 method. **Panel D.** The greater reproducibility of SSsc is also demonstrated by the lower dropout rate of the genes detected with a TPM >1.

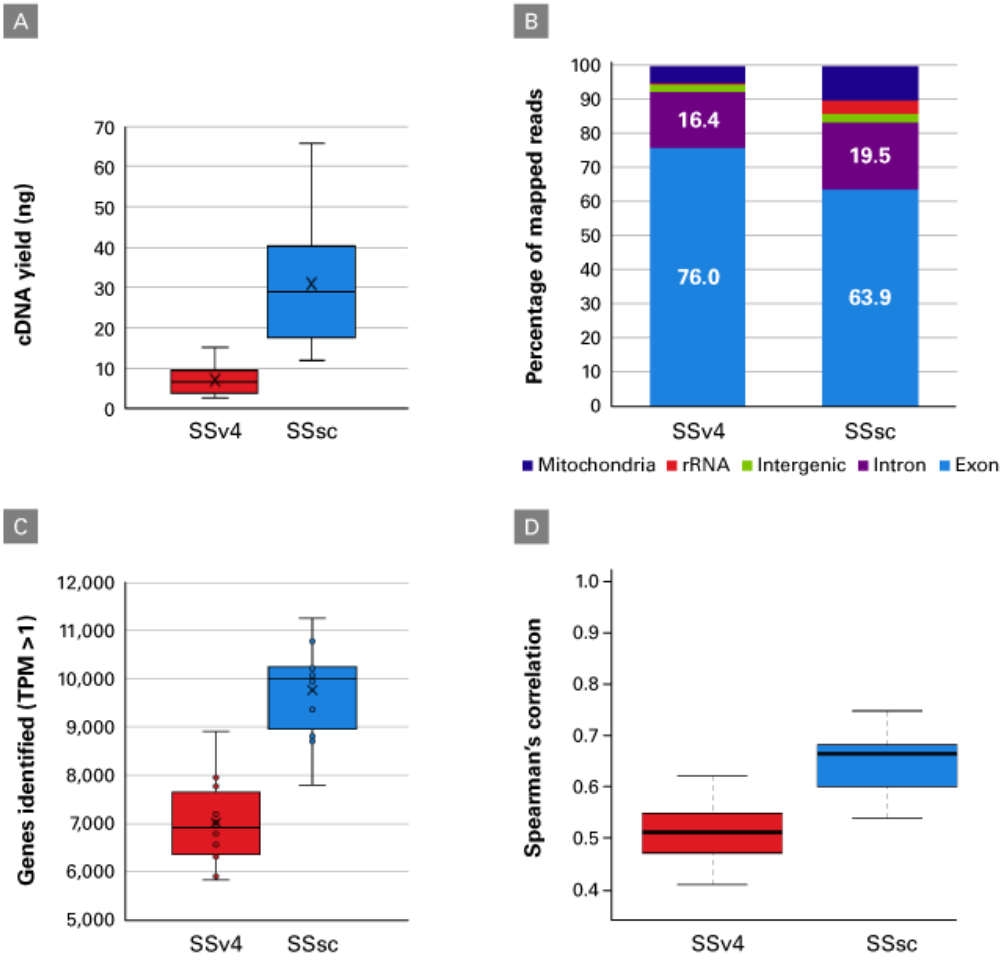


Figure 2. Improved performance for single cells with low RNA content. 12 single cells from lymphoblastoid cell line GM22601 were processed with SMART-Seq v4 (SSv4) or SSsc using 19 cycles of PCR. As described in the methods, RNA-seq libraries were generated and sequences analyzed (after normalizing all samples to 1.25 million paired-end reads). **Panel A.** The cDNA yield generated with SSsc is drastically higher than that generated with SSv4. **Panel B.** The read distribution was fairly similar between the two chemistries. **Panel C.** Over 50% more genes were detected in the cells processed with SSsc. **Panel D.** Correlation boxplots showing intragroup Spearman correlation between all cells processed with either method. The higher Spearman correlation among the cells processed with SSsc indicates a greater reproducibility than SSv4.

In addition to single-cell RNA-seq methods, we also offer best-in-class DNA-seq solutions in the form of our PicoPLEX WGA and PicoPLEX Gold single-cell DNA-seq kits. These kits use our high-performance PicoPLEX technology for single-cell whole genome amplification, employing multiple cycles of quasi-random priming followed by amplification with high-fidelity DNA polymerases to achieve superior performance than MDA-based methodologies. This allows for unbiased and accurate amplification of the genome, enabling the sensitive, reproducible, and accurate genomic coverage needed for identifying single-nucleotide variants (SNVs), copy-number variants (CNVs), insertions, and deletions (Figure 3). Indeed, researchers have cited the use of our PicoPLEX technology to reveal age-linked mosaic CNVs in human brain (Chronister et al. 2019) and neuronal tetraploidy associated with reduced cognition in an animal model of AD (López-Sánchez et al. 2017).

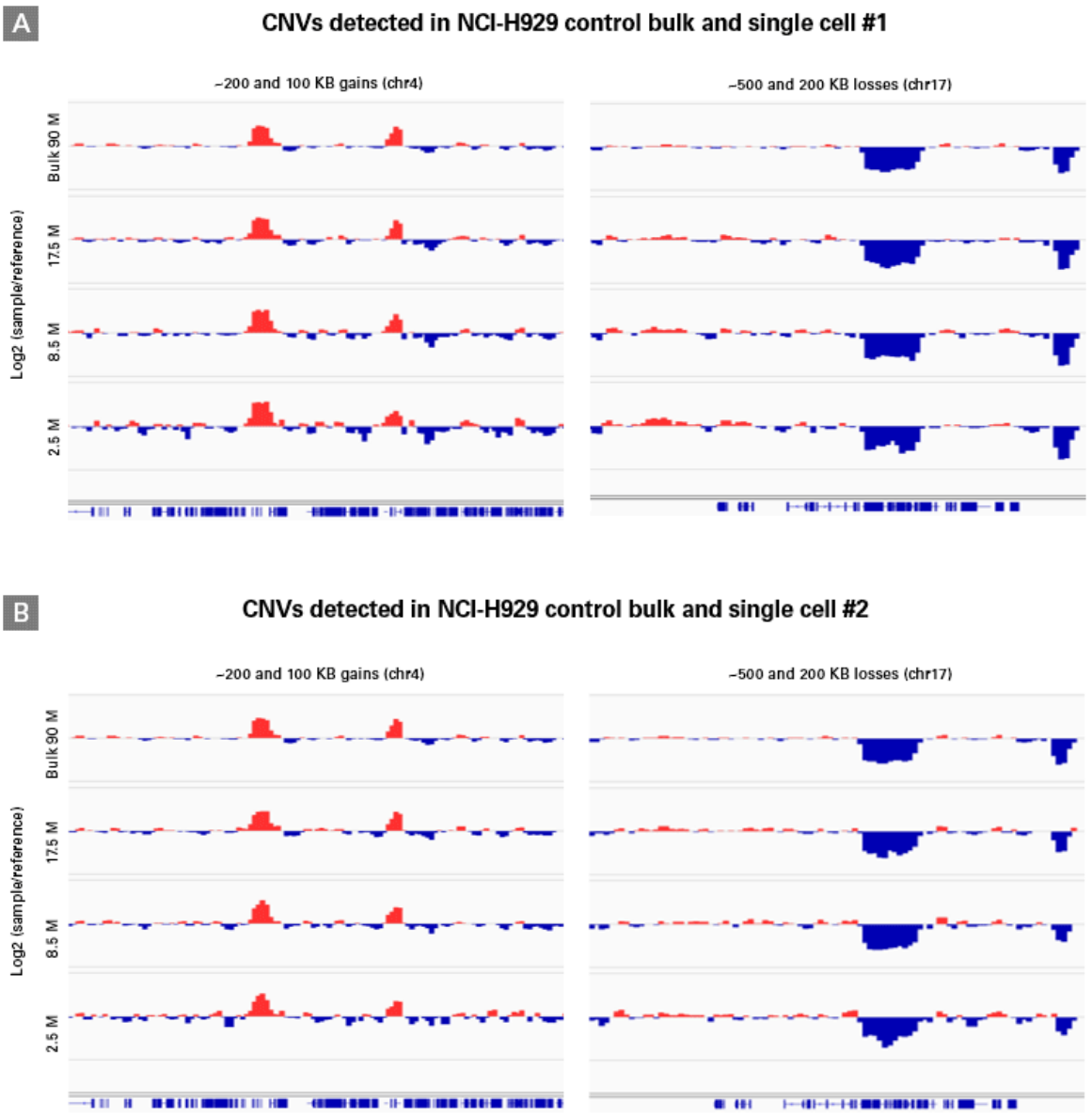


Figure 3. CNVs detected in two individual cells using the PicoPLEX Gold Single Cell DNA-seq Kit. Log_2 ratio of the total number of reads in 50-kb bins from single NCI-H929 cells, shown as one cell in **Panel A** and a second cell in **Panel B**. Red bars represent copy-number gains while blue bars represent losses. The top row of the graphs in each panel depicts the control bulk sample sequenced to a depth of 90 million read pairs. The highly reproducible coverage of the PicoPLEX Gold kit enables the accurate detection of structural variants as small as 100 kb, even at shallow sequencing depths (2.5–8.5 million read pairs).

Automated, high-throughput single-cell sequencing solutions

Our ICELL8 cx Single-Cell System is an open, high-throughput platform that provides an end-to-end solution for isolating, imaging, and generating libraries from single cells. Cells are stained, dispensed into a 5,184-nanowell chip at an average of one cell per well, and imaged. The images are analyzed by the integrated cell selection software, which automatically identifies and processes only nanowells containing single cells to eliminate noise from empty and multiplet-containing nanowells (Figure 4). Targeted nanowells are then processed on-chip, with minimal hands-on time, to generate cDNA or sequencing libraries according to your desired workflow. Many publications have cited the use of our ICELL8 technology to power their high-throughput scRNA-seq analyses, including its usage to transcriptomically profile single nuclei from human cortical and retinal tissue (Hochgerner et al. 2017; Liang et al. 2018).

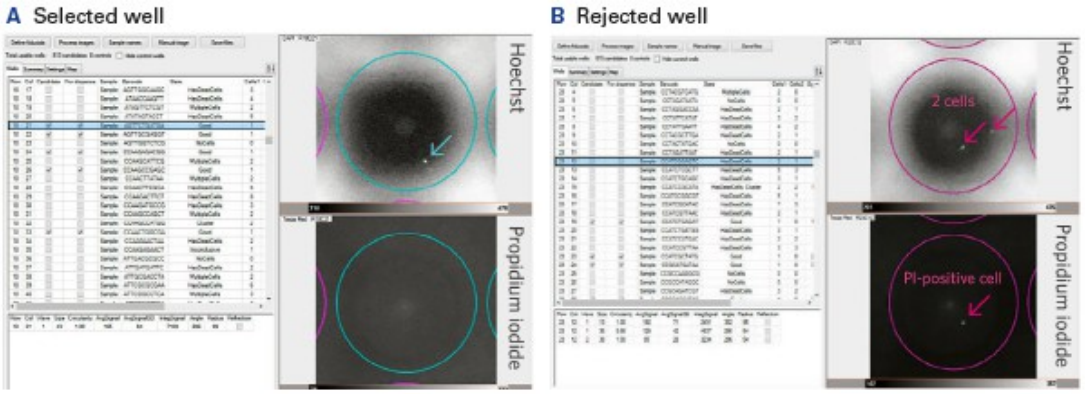


Figure 4. The ICELL8 cx Single-Cell System's integrated CellSelect Software automatically selects only single, live cells for processing. Following imaging of the ICELL8 chip, all 5,184 nanowells are automatically screened for downstream processing. Empty wells and wells containing multiple cells are rejected (**Panel B**) while only cells containing single cells (**Panel A**) are selected and processed, minimizing background noise and ensuring true single cell libraries.

We offer preprinted chips and reagents for several prevalidated NGS applications, including differential expression by 3'-end counting; full-length scRNA-seq for improved detection of SNPs, fusions, and alternative splice variants; and TCR profiling/5'-end differential expression. The greatest strength of our ICELL8 system, however, is its flexibility: researchers have taken advantage of this open platform to develop high-throughput, single-cell workflows for scRNA-seq of intact adult cardiomyocytes, single-cell ATAC-seq, CUT&Tag, and pheno-seq (Yekelchyk et al. 2019; Mezger et al. 2018; Tirier et al. 2018; Kaya-Okur et al. 2019).

We invite you to learn more about the solutions we offer for improving your sequencing workflows. Please reach out to us with any questions or requests and to schedule a trial of this technology via the "contact us" form on the left. If you are on a mobile device, click on the hamburger icon (☰) on the top left of your screen, then scroll down to access the registration form.

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