CellSelect™ Software

HUMAN-MOUSE MIXED CELL STUDY

Confidence in your single-cell sequencing

CONTROL - CellSelect Software identifies single-cells, you choose which cells to process

CONFIDENCE - Single-cell capture rates over 97%

SPEED - Confirmed isolation of live single cells in minutes without intervention

It is critical that your single-cell isolation technology provides robust and confident isolation of individual cells.

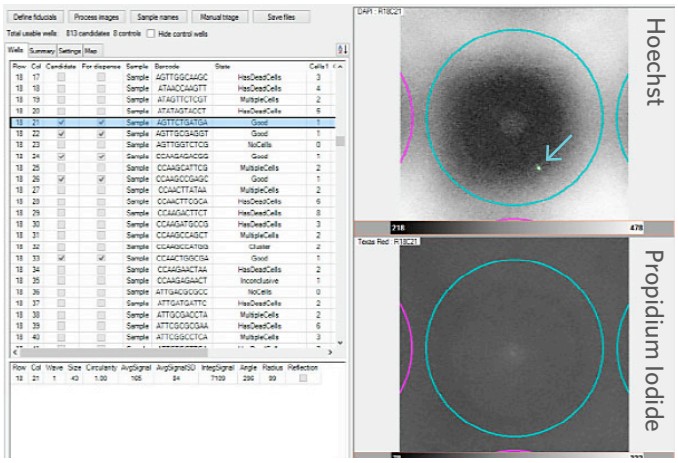
The ICELL8™ Single-Cell System employs an imaging station and proprietary CellSelect software to ensure only single-cells of your choice are analyzed. The Imaging Station is an automated optical system for rapid image capture, scanning every well across multiple parameters in minutes. CellSelect Software interrogates these images of each well, and evaluates Hoechst dye staining for single-cell identification and Propidium Iodide for cell viability (Figure 1). A confidence score is determined for each well, and only those single cells meeting a sufficient confidence score will be selected for processing. Advanced settings in the software allow for fine-tuning of your detection parameters for each sample type, and allows establishing custom criteria for cell selection.

In the following data we show an evaluation of mixed populations of human and mouse cells. This experiment demonstrates the power of the system to discretely isolate and identify individual cells with high fidelity.[1] See *Methods* for details.

Results and Discussion

This mixed species experiment was conducted to demonstrate the single-cell isolation and confirmation capabilities of the ICELL8 system.

A Selected Well



B Rejected Well

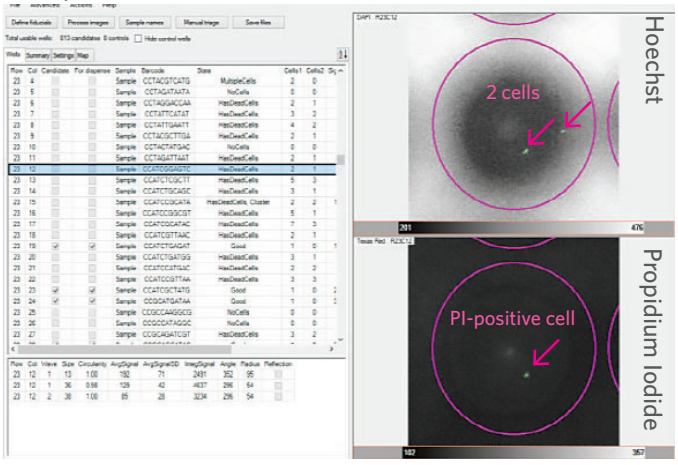


FIGURE 1. **The CellSelect™ software provides automated identification of cells in multiple channels:** CellSelect Software identifies: **A)** Single cell containing well with no dead cells present. **B)** Wells containing multiple cells or any PI positive cells and automatically de-selects them for further processing.

The CellSelect software was used to automatically identify 1,268 viable single-cell containing wells. After a manual review of these wells, 652 single-cells were selected for processing and sequencing.[2] On average, over 4,000 unique genes and 20,000 unique molecular identifiers per cell were sequenced.. An analysis of this data identified 5 wells containing doublet cells expressing genes consistent with both the mouse and human genomes. These results suggest that the CellSelect software in conjunction with manual review can increase the single-cell capture rate to over 97% (figure 2). This double occupancy rate provides a high confidence of single-cell isolation and high confidence to proceed with sample sequencing.

In a similar experiment using only automated calling by CellSelect software, a 1.9% occurrence of mixed-species doublets was achieved when a single cell was identified indicating a single-cell isolation rate greater than 96%. The ability to rapidly and accurately identify individual cells through a routine and automated solution substantially reduces subjective bias.

[2]Manual review of wells is a simple process of looking at individual well images and then selecting a box to either include or exclude that well from the down-stream process. Incorporating this additional review is not required for well selection. However, it is a valuable option when precious samples are being evaluated or it is critical that doublet contamination is minimized in the dataset.

Methods

K562 cells, U-87 MG cells that express red fluorescent protein (U87-MG-RFP), NIH 3T3 mouse cells and a mix of U-87 MG-RFP and NIH 3T3 cells were dispensed using the Multi-Sample Nanodispenser (MSND) into 5,184-nanowell ICELL8 chips containing preprinted 5,184 barcoded-oligodT primers. Positive controls (K562 total RNA) and negative controls (1x PBS) were added in 4 wells each. Cells were washed in 1x PBS, counted and stained with Hoechst 33342 and propidium iodide and imaged. After image acquisition and analysis, 90 U87MG human, 90 mouse, and 90 K562 (human) internal single-cell containing wells for each of the single species controls and 382 single-cell containing wells of the two-species mix were down-selected from 1,268 viable single-cell containing wells (candidates) identified by CellSelect™ software. Reaction mix containing MMLV reverse transcriptase was added to the selected individual cell-containing wells based on a CellSelect software generated dispense map. The synthesized cDNAs were pooled and PCR-amplified. Next-generation sequencing (NGS) libraries were prepared using a transposase based method (enriched for 3' ends of mRNAs, Soumillion et.al., http://dx.doi.org/10.1101/003236 2014) and sequenced using a HiSeq 2500 in rapid run mode.

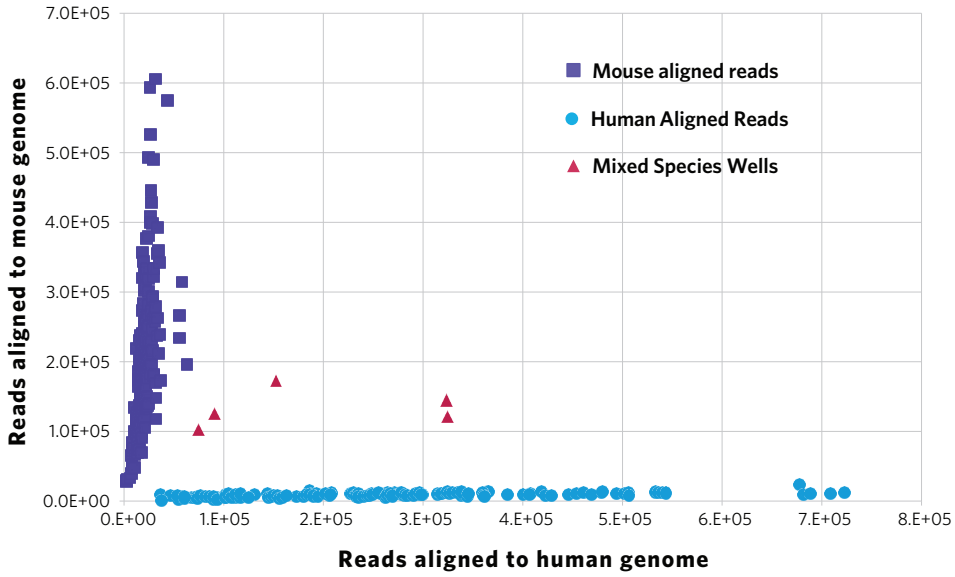


FIGURE 2. **Fidelity of CellSelect™ Calls:** Out of 382 wells processed from a mixture of human and mouse cells, 1.3% of barcodes (5 wells) show sequences consistent with mixed species wells.

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