Isolating Induced Pluripotent Stem Cells using the WOLF G2

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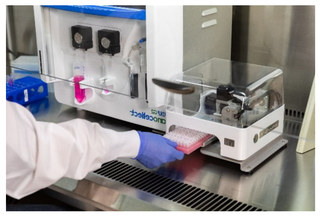
ABSTRACT

The culture and sorting of induced pluripotent stem cells (iPSCs) require special care that can be both time consuming and laborious in order to achieve reliable results. Although maintaining a homogeneous stem cell culture is possible, researchers greatly benefit from other tools to succeed and reduce laborious maintenance. Microfluidic cell sorting is essential to the success of gently sorting homogeneous stem cells and eliminating unwanted cells. Here we demonstrate how the dual laser WOLF G2 Cell Sorter was imperative to identify and sort naïve stem cell populations. The WOLF G2 accurately identified and enriched hiPSCs that were labeled with two cell surface markers SSEA-4 (Stage Specific Embryo Antigen 4) and TRA-1-60-R (Tumor-related Antigen-1-60 [R]), that are widely used to label undifferentiated stem cells. High purity and viability of the stem cells was also affirmed by successful seeding and adherence of the sorted iPSCs into a new 6-well plate. NanoCellect's microfluidic sorting technology enables researchers to generate consistent high-quality results.

MICROFLUIDIC SORTING AND SAMPLE PREPARATION

A.

B.



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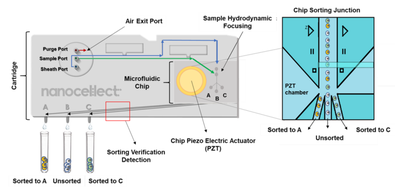
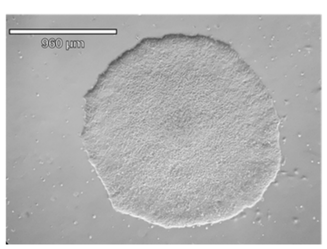


Figure 1. Microfluidic Cell Sorting A. The WOLF (left) and WOLF G2 (right) cell sorters' distinguishing capabilities are based on a microfluidic cartridge sorting technology to gently sort cells accurately. The WOLF uses a single 488 nm laser while the WOLF G2 has 2 lasers available in 3 different configurations that includes the 488 nm laser with either the 405 nm, 561 nm or 637 nm laser **B.** Due to the compact design, the WOLF or WOLF G2, along with N1 Single Cell Dispenser, can easily fit into a biosafety cabinet enabling a sterile sorting environment. **C.** The entire sorting process occurs on a disposable microfluidic cartridge; everything the sample and sheath touches is sterile and free from sample-to-sample contamination.



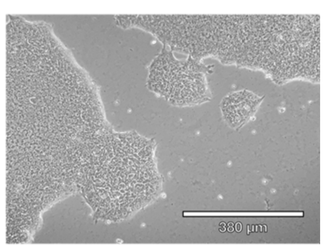


Figure 2. Morphology of human iPSCs cultured on vitronectin in DMEM/F-12 50/50 with HiDef-B8: A. Representative image of a healthy compact colony with < 30% differentiation and < 70% confluency (40x magnification). **B.** Representative image of colonies with distinct borders, well defined edges, and large nucleus to cytoplasm ratio (100x magnification).

WORKFLOW OVERVIEW

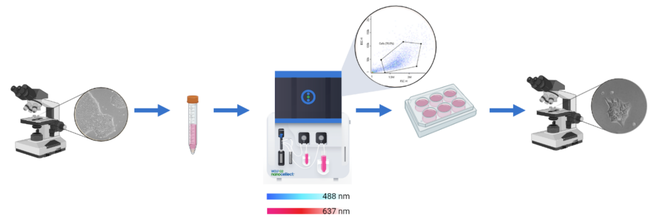
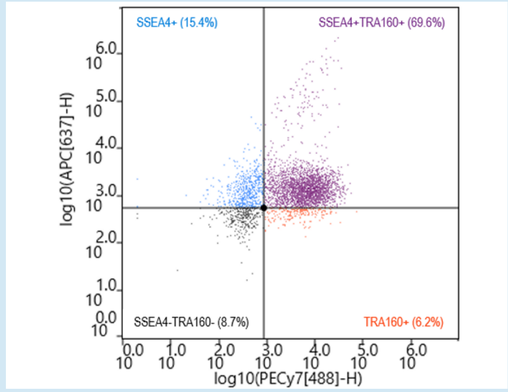


Figure 3. A simplified workflow: The experimental process begins with general iPSC maintenance and a visual confirmation of healthy cells. Once appropriate wells are selected, sample preparation is necessary to create a single cell suspension. Single cell suspension is separated into various stained cell tubes and applied to the WOLF G2. Appropriate lasers are chosen to be compatible with the 488 nm and 637 nm configuration. Double stained single cell suspension is replated into prepared plates and imaged on subsequent days for cell health and growth.

IPSC BULK SORTING

A.



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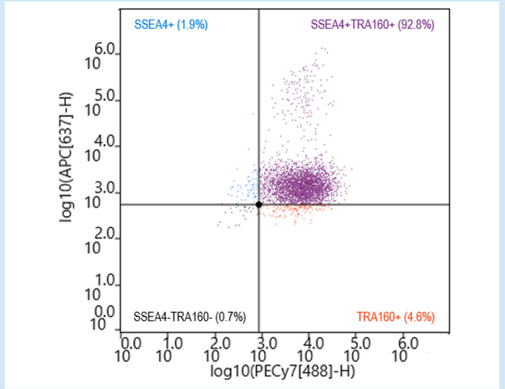
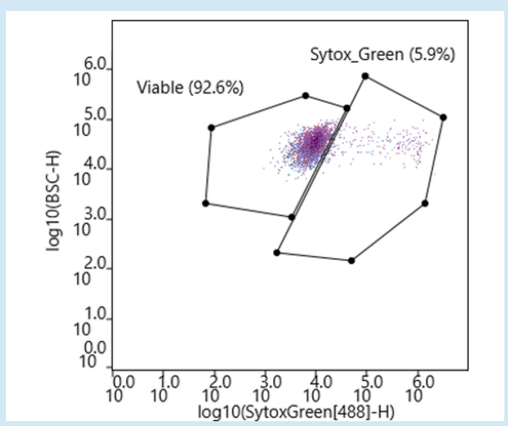


Figure 4. Flow cytometry analysis of SSEA-4 and TRA-1-60-R purity: A. Pre-sort cells were ~69% SSEA-4 and TRA-1-60-R double-positive. **B.** Post-sort cells were ~93% SSEA-4 and TRA-1-60-R double-positive.

A.



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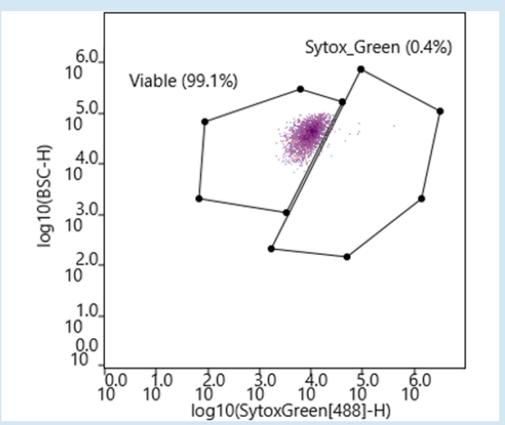
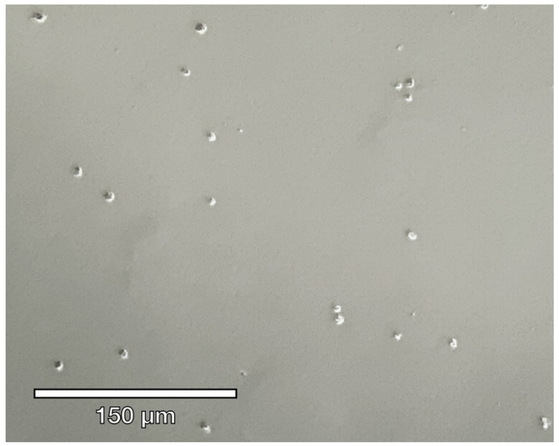


Figure 5. Flow cytometry analysis of SSEA-4 and TRA-1-60-R viability: A. Pre-sort cells stained with SYTOX™ Green Ready Flow™ Reagent displayed roughly 6% dead cells. **B.** Post-sort cells stained with SYTOX™ Green Ready Flow™ Reagent displayed > 99% viable cells.

RESULTS

Prior to sorting, approximately 69% of the cells were positive for SSEA-4 and TRA-1-60-R (Figure 4A) and roughly 6% of the cell sample included dead cells (Figure 5A). After using the gate SSEA4+TRA160+ (top right quadrant) to sort for cells that were positive for both surface markers, the double-positive cells were increased to 93% (Figure 4B). Greater than 99% of the cells were viable post-sort (Figure 5B), reflecting a high viability following microfluidic sorting.

A.



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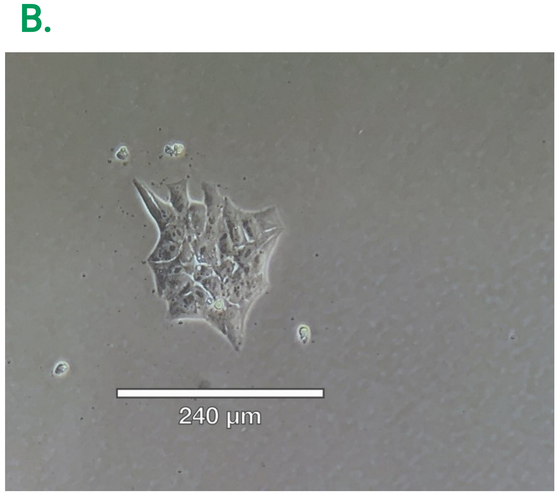

Figure 6. Human iPSCs replated for colony outgrowth: A. Single-cell adherence shown 24 hours after seeding the post-sort sample (100x magnification). **B.** Characteristic iPSC colony emerging, 5 days after seeding (100x magnification).

CONCLUSION

The WOLF G2 accurately identified and enriched hiPSCs that were labeled with two surface markers, SSEA-4 and TRA-1-60-R, widely used to label undifferentiated stem cells. The WOLF G2's ability to identify and sort these cells is imperative for research and development when naïve stem cell populations are needed. Using these surface markers proved successful by starting with a 69.6% double-positive sample and purifying to 92.8%. This data demonstrates that the microfluidic cell sorter was able to purify the initial sample by successfully detecting cells that were actively expressing both surface markers. Given the purification and > 99% viability demonstrates that the WOLF G2 is helpful in stem cell applications. Overall, the gentle sorting and positive identification of such a demanding and delicate cell type has demonstrated the high utility of the WOLF G2.