Increased Viability and Genomic Integrity of CRISPR-modified hiPS cells selected with WOLF® Cell Sorter Microfluidic Technology

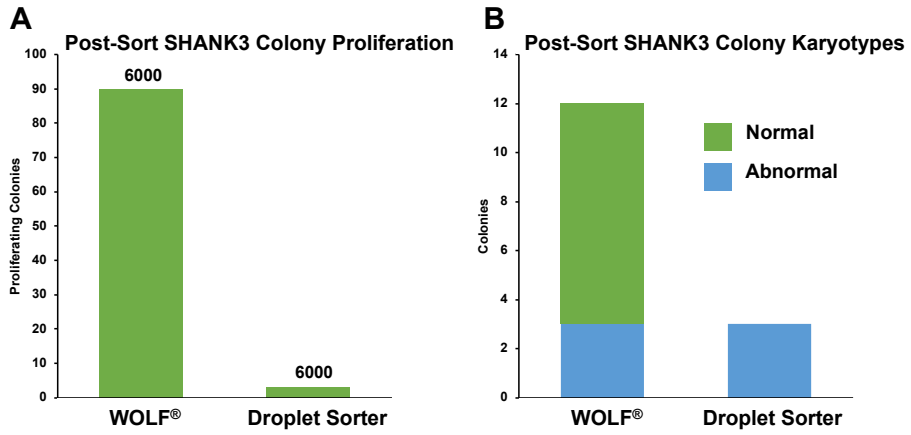
Elizabeth Roberts[1], Allison Songstad[1], Elena Rubio de la Torre[2], Emily Bozek[2], Archana Shankar[1], Gabriela Goldberg[1], Florian Krach[1], Stefan Aigner[1], Gene Yeo[1], Lawrence Goldstein[1] 1. Sanford Consortium for Regenerative Medicine, UCSD. 2. NanoCellect Biomedical, Inc., San Diego, CA

Background: Human iPS cells (hiPSC) can be modified utilizing CRISPR/Cas9 gene editing technology to generate *in-vitro* models of human diseases. Critical shortcomings in the generation of CRISPR-modified clonal lines are: 1) low viability/proliferation, and 2) genomic abnormalities following selection via fluorescent activated cell sorting. To address these issues, we compared microfluidic cell sorting (WOLF® Cell Sorter, NanoCellect) to a traditional electrostatic droplet based cell sorter in Alzheimer's Disease (AD) and Autism Spectrum Disorder (ASD) model cell lines. We evaluated colony formation and karyotypes after sorting.

Methods: Human fibroblasts were reprogrammed into hiPSC using retroviruses containing the four Yamanaka Factors. Resulting hiPSCs were then CRISPR-modified to knock-down AD- or ASD-related genes. GFP was used as reporter for selection. Cells were sorted in parallel with the WOLF® and a traditional droplet-based cell sorter. After sorting, cells were plated on MEFs and grown for 1-2 weeks, picked, expanded, and frozen. Colonies that recovered after cryogenic storage were submitted for microarray karyotype analysis.

Results: ASD-related SHANK3 and AD-related SWE, V717F, and ICE cell lines displayed improvements in viability and genomic integrity following microfluidic sorting on the WOLF®, compared to a traditional droplet cell sorter. The number of proliferating colonies, normal karyotypes, as well as overall freeze-thaw viability were all increased on the WOLF® microfluidic platform compared to a droplet sorter.

Autism Spectrum Disorder hiPCs



Alzheimer's Disease hiPCs

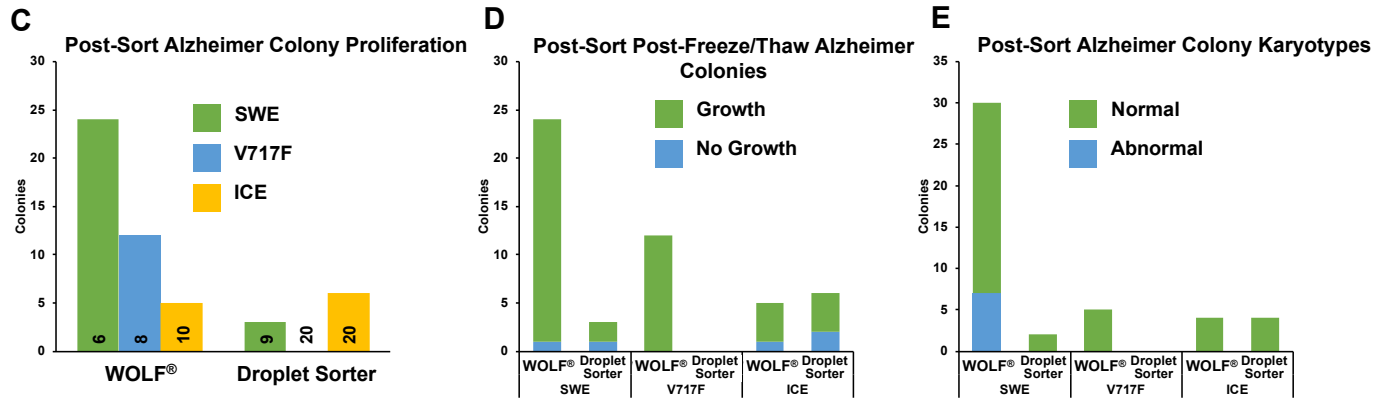


Figure 1: Viability and genomic integrity after sort: **A** Number of colonies that proliferated after sorting and plating for the SHANK3-knockdown CRISPR-modified cells. Numbers above bars indicate number of cells sorted and plated. **B** SHANK3 karyotyped colonies that displayed genomic abnormalities. **C** Number of colonies that proliferated after sorting for the SWE, V717F, and ICE -positive CRISPR modified cells. Numbers inside bars indicate number of cells sorted and plated (x1000). **D** Number of colonies that recovered after cryogenic freeze-thaw measured via trypan blue staining. **E** Number of SWE, V717F, and ICE karyotyped colonies that displayed genomic abnormalities.