

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

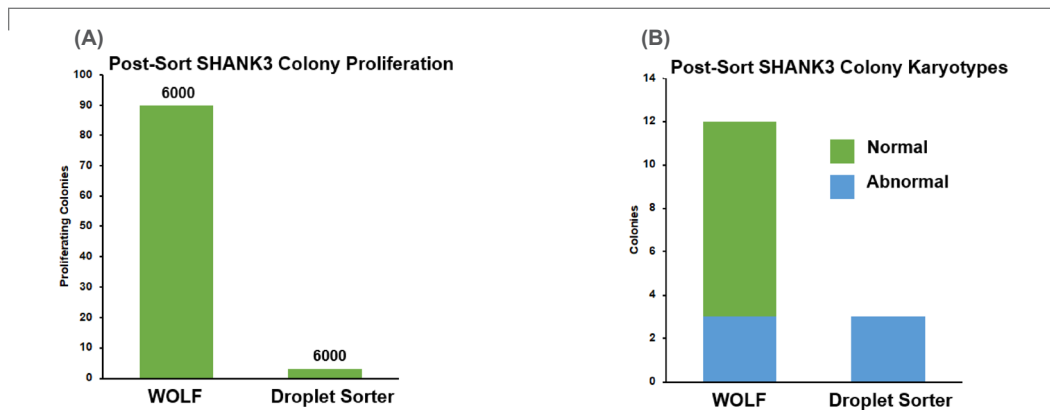
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Background

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, NanoClect) 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.

Autism Spectrum Disorder hiPSCs



Alzheimer's Disease hiPSCs

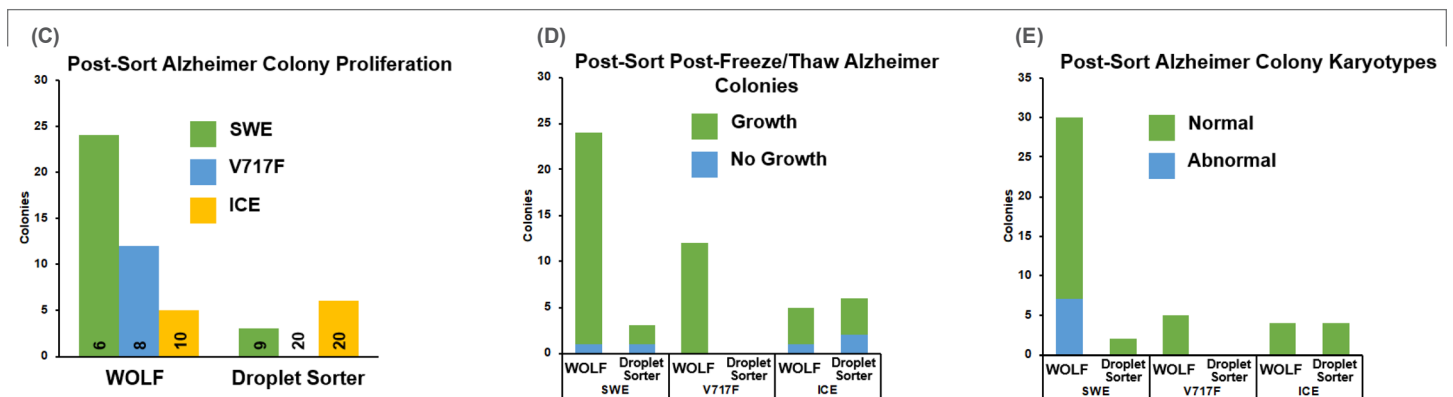


Figure 1. Viability and genomic integrity after sort: (A) Number of colonies that proliferated after sorting and plating for the SHANK3-knockdown CRISPR-modified cells. Numbers above bars indicate number of cells sorted and plated. (B) SHANK3 karyotyped colonies that displayed genomic abnormalities. (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.



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.

For more information, visit nanocellect.com or email info@nanocellect.com