WOLF Cell Sorter for Single-Cell Cloning and Cell Line Development

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Abstract

Generation of stable monoclonal cell lines is required for a wide variety of applications, including the development of biologics, such as therapeutic antibodies, or to create CRISPR-engineered cells for disease-in-a-dish models. While the demand for engineered cell lines has increased, production of monoclonal lines is still limited by the current technology for single-cell sorting and dispensing.

Here we contrast the limitations of traditional technologies with newer technologies, such as microfluidic sorters and dispensers (WOLF® Cell Sorter, NanoCellect®, Inc.) that can analyze, sort and dispense single cells into 96- or 384-well plates with high viability and outgrowth. We highlight that both robust immortalized cell lines (e.g. CHO) and more sensitive cells can be used to generate monoclonal cell lines in a sterile format with little cellular stress. The results reveal that the WOLF cell sorting platform can efficiently isolate targeted single cells while maintaining high viability and can be used for the isolation of single cell clones for applications such as CRISPR modified stem cells and antibody production CHO cells.

Microfluidic Cell Sorting Technology

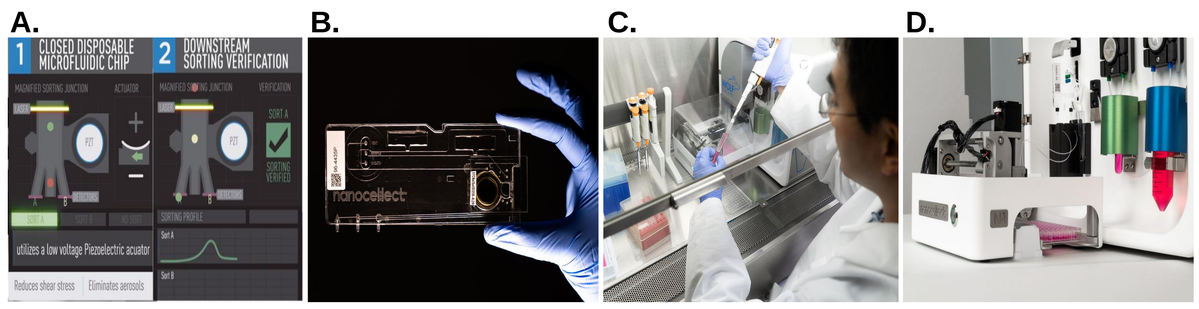

Figure 1.Microfluidic Cell Sorting A. The microfluidic sorting mechanism of the WOLF Cell Sorter uses a gentle piezo actuator to gently sort cells at <2 psi of pressure. **B.** The microfluidic-based single-use cartridge for the WOLF sorter. **C.** The WOLF Cell Sorter and N1 Single-Cell dispenser, in a TC hood, is an easy-to-use, aerosol-free, sterile and disposable system for selection and sorting of cells in bulk, or directly into 96- or 384-well plates. **D.** The WOLF Cell Sorter and the N1 Single-Cell module.

Workflow

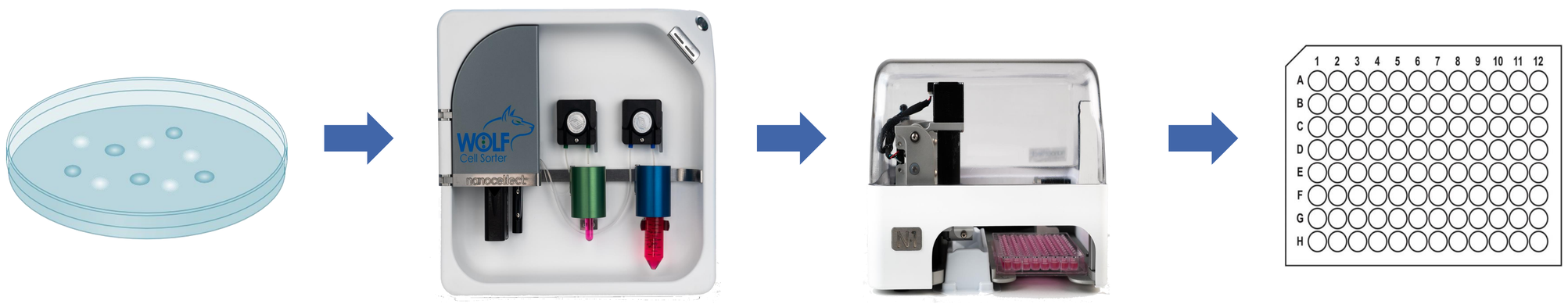

Figure 2. Single-cell cloning workflow. The mixture of cells is collected in a microfuge or FACS tube in buffer of choice and loaded onto the WOLF Cell Sorter with ~50mL of the sheath fluid of choice. Target cells are selected (based on up to 5 optical flow cytometry parameters) and then dispensed into 96-well plates, pre-filled with media. Cells are incubated in culture and imaged or assayed to confirm genetic and monoclonal status.

Single Cell Dispensing

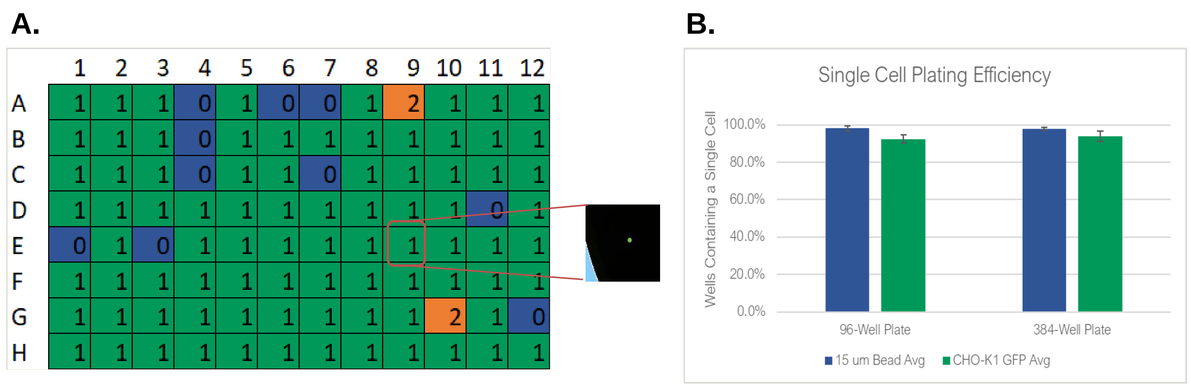

Figure 3. High efficiency single-cell sorting into 96- and 384-well plates with the WOLF Cell Sorter. A. Heatmap view using the Synentec NyOne plate imager a with representative bead image on a 96-well microtiter culture plate. The number in each well indicates the number of control beads or cells counted. **B.** Percent of wells in a plate with single control beads or single CHO-K1 GFP cells dispensed was 98.1 ± 1.2% (beads) and 92.5% ± 2.3% (CHO cells) in 96-well plates. Similar efficiencies were achieved in 384-well plates with 97.8% ± 0.9% (beads) and 93.8% ± 2.8% (CHO).

Antibody Colony Selection

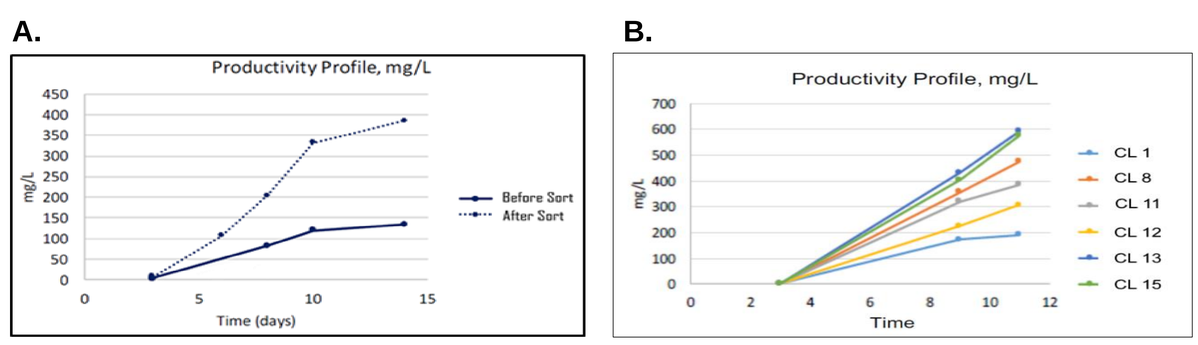

Figure 5. Antibody productivity using the WOLF cell sorter for industrial antibody development. A. Unsorted cells produced 130 mg/L of antibody in a 14-day batch-fed assay. This increased to almost 400mg/L after bulk sorting by selecting high GFP expression. **B.** Single cells were also dispensed into 96-well plates for monoclonal isolation. Six of those colonies were selected to evaluate and produced up to 6x more antibody than unsorted cells. This data was kindly provided by a NanoCellect industrial collaborator.

Single Cell Outgrowth

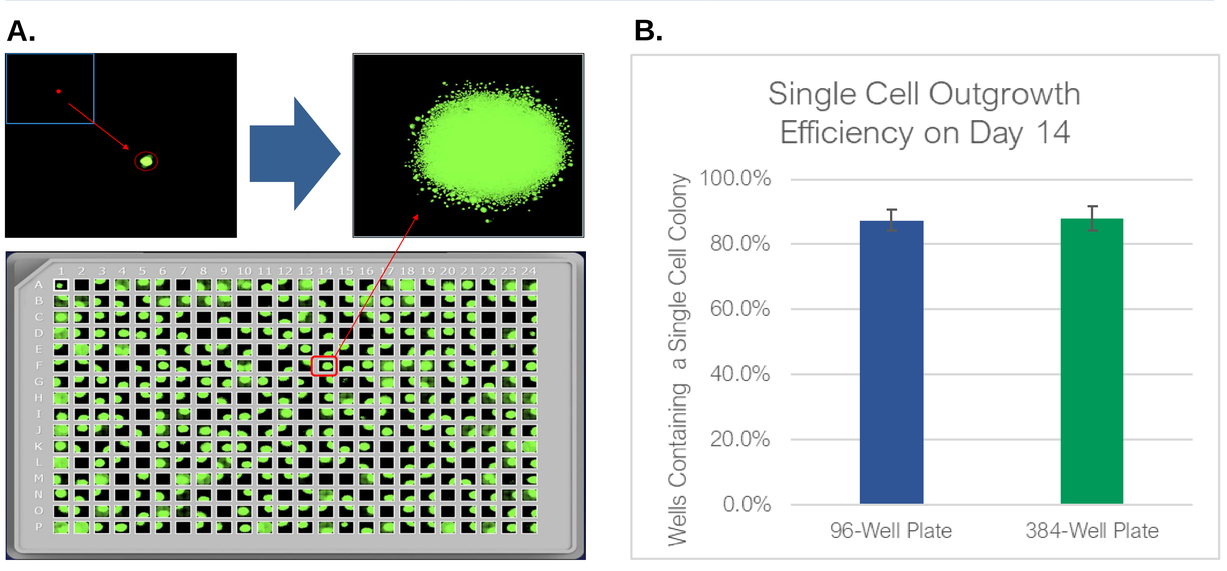

Figure 4. Monoclonal CHO cell outgrowth after analysis, sorting and plating. A. Plates were imaged for fluorescence on day 14 and counted for colony presence. Counts were compared to Day 0 heatmap counts to determine monoclonality (derived from a single plated cell). **B.** After 14 days, the average number of wells on a 96-well plate containing a single cell colony was 87.4 ± 3.3% (N=9) and on a 384-well plate was 88.1 ± 3.7% (N=3).

Sorter Comparison

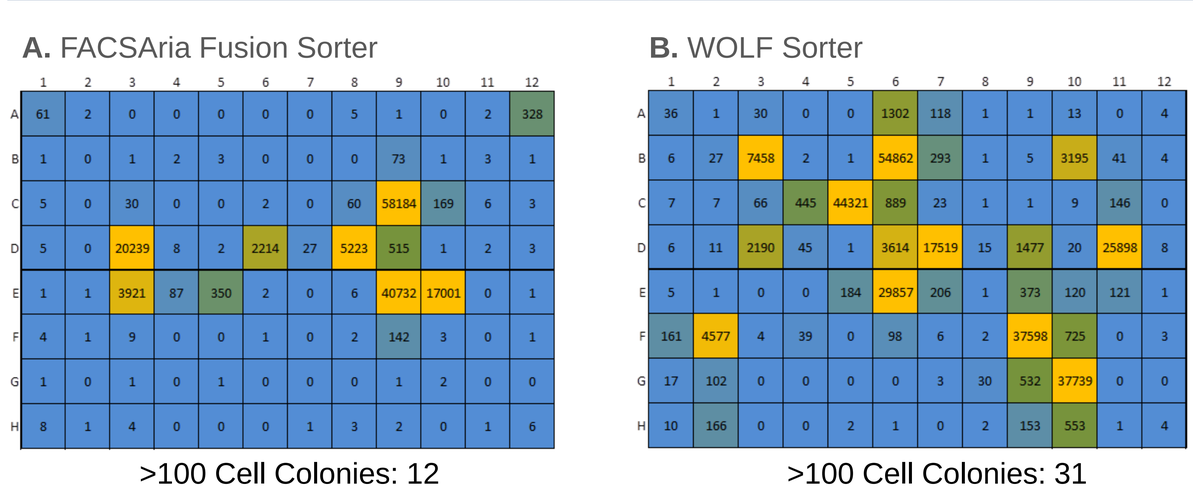

Figure 6. WOLF improved monoclonal outgrowth by >250% over the FACSAria Fusion. Proprietary engineered Jurkat cells were analyzed, sorted and single-cell plated with the FACSAria Fusion (**A**) or the WOLF Cell Sorter (**B**). Wells were analyzed via flow cytometry after incubating for 2.5 weeks incubation. This data was kindly provided by a NanoCellect top 10 biopharma collaborator.