The WOLF "cuts" time from CRISPR workflows

Introduction: The CRISPR/Cas9 system is ideal for genome-wide knockout and gain-of-function screening experiments due to the ease, efficiency, and irreversibility of genetic modifications. Here we highlight how NanoCellect's WOLF Cell Sorter saves time and resources in progressing from hypothesis to fully edited and tested cell lines for challenging academic pursuits or next generation cell therapies. Specifically, the WOLF Cell Sorter achieves analysis and selection of highly sensitive and fragile cells that would be difficult or impossible to select with traditional cell sorting technologies.

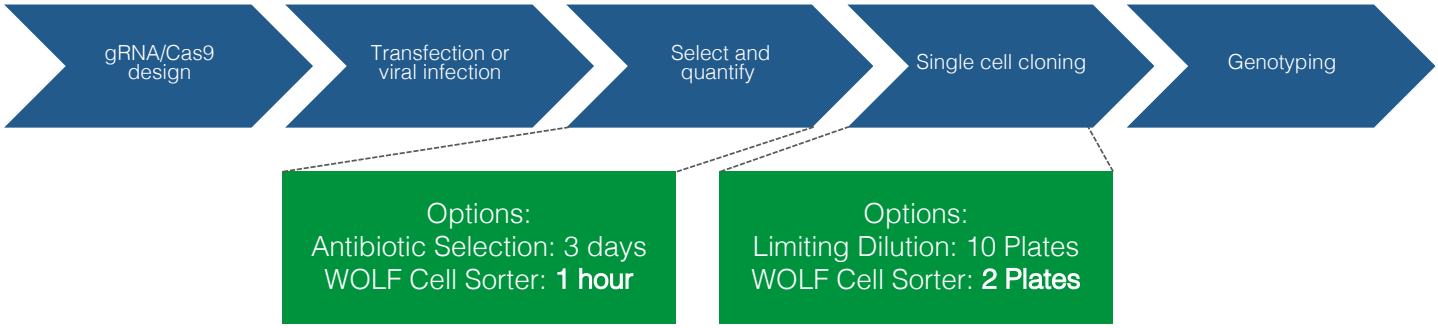

Figure 1. Workflow schematic for CRISPR gene editing.

Enriching and calculating efficiency: There are various methods to deliver gRNA/Cas9 into target cells depending on the cell type. These include transfection, electroporation, or lentivirus infection. Having a fluorescent label or antibiotic resistance designed into the constructs allows for the efficiency of your edits to be determined and cells to be enriched. Using fluorescent labels (GFP being the most popular) the WOLF Cell Sorter can measure the efficiency of transfection, while enriching target cells. Without a cell sorter, antibiotic selection requires **3 days** to eliminate un-transfected cells. Without selection, one can still use the WOLF Cell Sorter to efficiently dispense single-cell into 96- or 384-weill plates for genotyping.

Single-cell cloning: Single-cell cloning is critical to select desired cells from a homogenous population. Limiting dilution is a very common technique, but is highly inefficient. The WOLF Cell Sorter directly sorts into 96- or 384-well plates with very high single-cell precision (92%) and viability for high colony outgrowth (86%). Traditional fluorescent activated cell sorters use droplets to sort and this reduces viability (Fig. 2A). With pressures up to 70 PSI and high shear stress cells can die, have loss of RNA integrity, or select for cells with abnormal karyotypes. In contrast, the WOLF Cell Sorter uses a gentle sorting mechanism with only 2PSI of pressure—greatly increasing the viability of a wide variety of cell types, including stem cells.

Phenotyping by Flow Cytometry: CRSPR rescue screens can be used to discover the genetic mechanisms of drugs or other interventions. The WOLF Cell Sorter can be used to identify and sort specific cells that are "hits' from screens by virtue of genetic markers or in the change of proteins, such as receptors, that can be labeled with fluorescent antibodies.

Summary: The WOLF is a versatile (analyze, sort, dispense), compact, and easy-to-use cell sorter that can be used in gene editing labs of any size. Cutting out days and complexity from workflows is valuable and the WOLF ensures a higher rate of viable single cells in a well. Whether the experiment requires analysis, sorting or plating, the WOLF's gentle, sterile microfluidic technology is designed to help you move forward with greater confidence and efficiency.

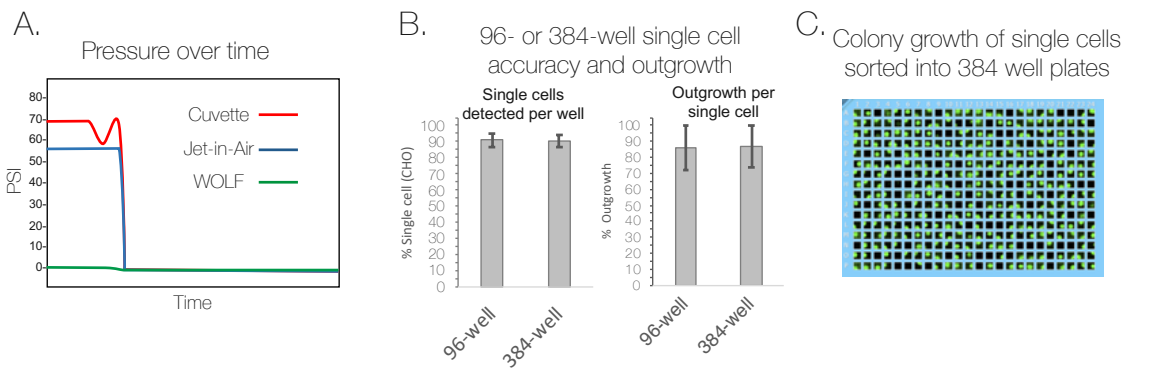

Figure 2. Gentle cell sorting improves cloning success: *A. Illustration of pressures experienced by cells while being sorted on traditional cell sorters: Cuvette (red), Jet-in-Air (blue) or the WOLF (green). B. Deposition rate of single cells directly sorted into 96 or 384-well plates and (C) an example of high colony outgrowth from 384 single sorted cells using the WOLF Cell Sorter.*