Gentle enrichment of plant protoplasts and nuclei using a microfluidic cell sorter

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Abstract

Cell isolation via gentle cell sorting is needed for numerous biological workflows in plant biology including gene characterization and function, proteomics and transcriptomics, single-cell genomics, improved cell line development through gene engineering/CRISPR-Cas9 editing, and breeding. Sorting protoplasts or nuclei is challenging with droplet cell sorters due to the high pressures, shear stress and osmotic changes which damage protoplasts and nuclei making them unusable for downstream cell culture or genomic applications. Traditional cell sorters limit the use of custom sheath buffers, which quickly degrade the fluidics components in the instruments, yet are needed to support cell viability during and post sort. Lastly, traditional cell sorters are restricted to sorting smaller cell sizes (mostly < 30 µm), due to the inability to hold a stable drop delay with larger nozzles (200 µm or larger) and low sheath and sample pressures (< 10 psi). Protoplasts and nuclei share sensitivity and stress induction when enriched via traditional cell sorting. The WOLF™ microfluidics cell sorter offers a solution that efficiently enriches for populations of interest with low shear stress and supports cell viability by enabling scientists to use culture medium as sheath. Here we demonstrate isolation and successful separation of tomato leaf protoplast classes, and nuclei from the leaves of Roma tomato and green bell pepper plants.

Microfluidics Cell Sorting Technology

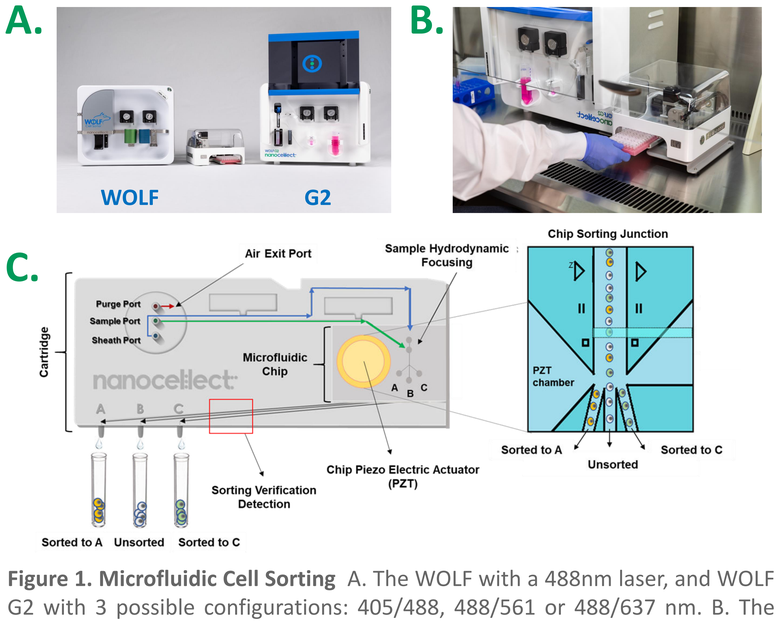
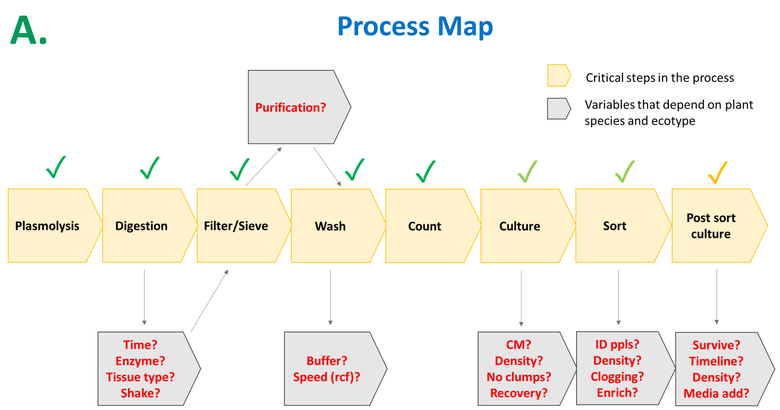
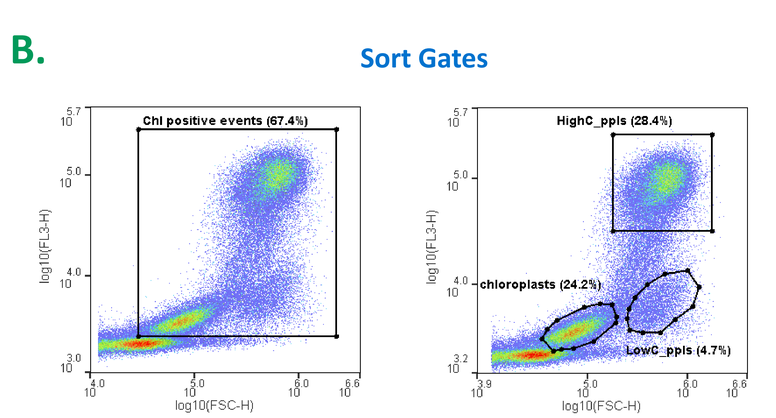


Figure 1. Microfluidic Cell Sorting A. The WOLF with a 488nm laser, and WOLF G2 with 3 possible configurations: 405/488, 488/561 or 488/637 nm. B. The WOLF and WOLF G2 are light-weight and compact enough to fit into a BSC. C. Disposable microfluidic cartridge allows for flexibility with custom sheath fluid and low sorting pressures of <2 psi. As events pass through the sorting junction, the PZT pulses pushing the event to the left or right channels. Undesirable events will pass through the middle channel.

Plant Protoplasts





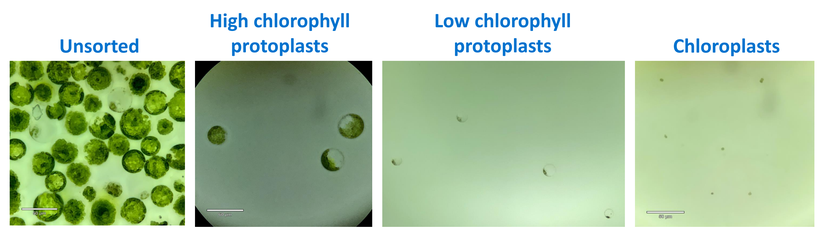
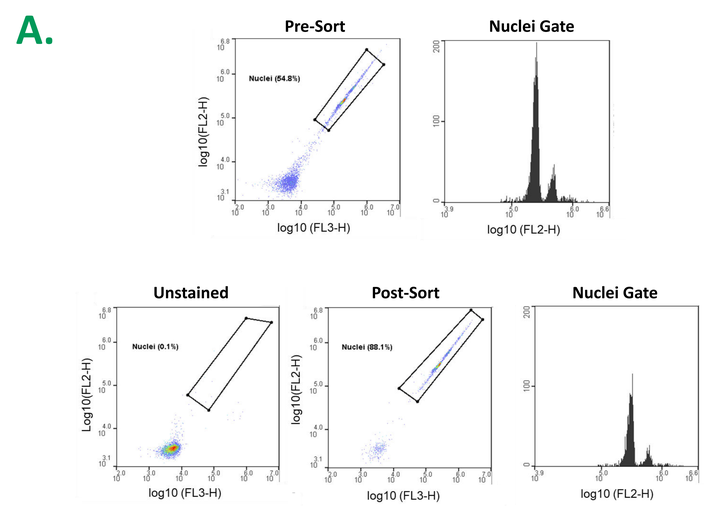


Figure 2. Plant protoplast isolation and cell sorting. (A) Process map – yellow boxes indicate steps, grey boxes are variables that change with plant ecotype and tissue source, and checkmarks indicate status of the process steps (green = pass, yellow = needs to be improved, red = fail). (B) Sorting gates for tomato protoplasts – plots measured by FL3 fluorescence (red = chlorophyll) on the Y-axis and FSC (estimated size) on the X-axis. Left plot shows large gate encompassing chlorophyll positive events and the right plot breaks down the chlorophyll containing events into three distinct populations. (C) Images of unsorted protoplasts (left) and the three populations of sorted tomato protoplasts: high chlorophyll, low chlorophyll, and free chloroplasts.

Plant Nuclei



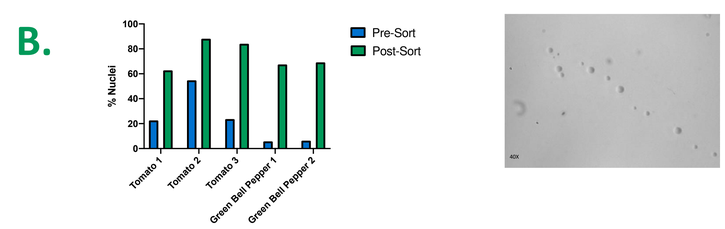


Figure 3. Gentle nuclei sorting and enrichment of Roma tomato nuclei via WOLF microfluidics cell sorter. (A) Unstained (pre-sort) and stained preparations of nuclei (post-sort). Nuclei were identified as PI-FL2+/PI-FL3+. Multiple populations were identified suggesting different cell cycle stages and/or ploidy of the plants. (B) Enrichment of plant nuclei via sorting (left) and microscope image of enriched nuclei (right).

Conclusions & Future Applications

CONCLUSIONS:

- WOLF successfully identified and enriched for intact protoplasts and nuclei.
- Protoplast enrichment success is dependent on suitability of the cell culture system for sorting.
- Methods of protoplast isolation vary from crop to crop and between plant ecotypes.
- WOLF successfully isolated and enriched intact plant nuclei.

FUTURE APPLICATIONS:

- Rare cell population enrichment for downstream omics applications.
- Enriching for CRISPR-Cas9 gene editing events.
- Regeneration of whole plants from sorted single cells.