cellenONE®: Improved Single Cell Isolation for Cell Line Development

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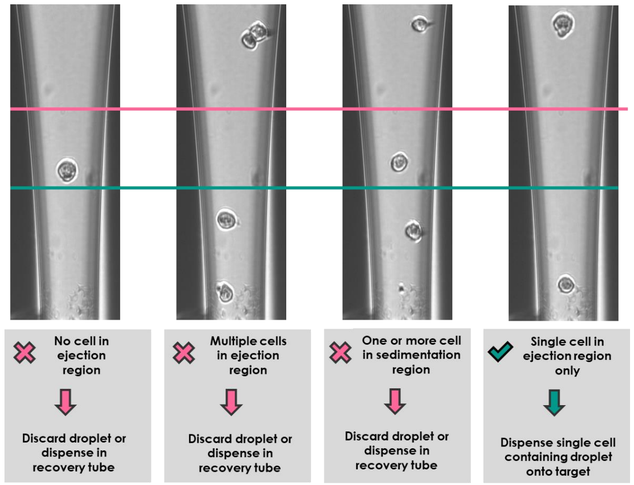
INTRODUCTION

Cost- and time-efficient generation of high-expressing and fast-expanding monoclonal cell lines is critical for the production of biologicals. Common methods for cloning cells are manual dilution and FACS. Manual dilution is a highly inefficient, cost consuming process, as most wells will not contain only a single cell. Flow cytometry enables rapid single-cell isolation but suffers from high shear stress and subsequent poor clonal recovery.

To overcome these challenges, the cellenONE® X1 was developed as a revolutionary platform for automated single cell isolation founded on gentle piezo-acoustic picoliter dispense technology. It enables high-throughput manipulation of cells while providing outstanding isolation precision and viability, enabling high cloning efficiency.

TECHNOLOGY



- Cell suspension is aspirated into an inert glass capillary with piezo ring
- Acoustic-wave generated by electric pulse to formulate highly reproducible droplets (<2% CV) of 50 - 800
- Automated imaging inside the glass capillary determines what will be present in the upcoming drop
- Single cell containing drops are dispensed onto targets, all other conditions are dispensed into a collection tube

METHODS

1. VIABILITY

Single polyclonal mouse hybridoma cells were deposited into 384-well plates under two conditions:

Instrument	Standard	Higher Stringency
FACSAria 3	'Cell' gate on FSC/SSC plot	Live cell gate (7-AAD negative)
cellenONE X1	Sample mapping (automatic)	User defined circularity, diameter, and elongation parameters

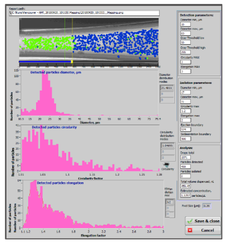


Figure 1. Automated mapping of cell sample on cellenONE® X1 to set isolation parameters: circularity, diameter and elongation.

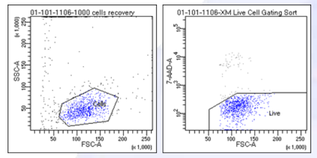


Figure 2. Representative image of gating on FACSAria 3 for general cell population (left) and live cell gating (right).

2. RECOVERY

Polyclonal mouse hybridomas were sorted into collection tubes using the FACSAria 3 to obtain 1,000 cells per tube. Concentrations were diluted to bias towards high recovery: 50 cells/uL for the cellenONE® X1 and 4 events/sec for the FACSAria 3. The aliquots were then loaded into each instrument for single cell isolation into 384-well plates.

For all conditions, the input cell viability was ~ 87%. Plates were stored at 37°C for 1.5 weeks then scanned on an IncuCyte Zoom to measure the number of outgrowing wells and assess recovery.

CONCLUSION

The cellenONE® X1 significantly outperforms the FACSAria 3 in all measured aspects of clonal recovery of hybridoma cells:

VIABILTIY - gentle piezo acoustic dispensing enables greater outgrowth than discriminating live cells as they are still subject to the high shear stresses of FACS after detection.

RECOVERY – cellenONE® X1 can process low cell inputs with minimal losses. Total recovery of cells has the potential to be greater than measured as rejected cells are collected into a recovery tube and may be reprocessed.

RESULTS

1. VIABILITY

Figure 3. Hybridoma outgrowth comparison between FACSAria 3 and cellenONE X1 for both experimental conditions. Wells with <4% confluency were excluded.

- The inclusion of dead cell stain (7-AAD) improved the outgrowth of the FACSAria processed hybridomas by nearly 2 folds.
- Constraining the selection parameters on the cellenONE® X1 did not improve overall outgrowth and was consistent between the conditions.
- The cellenONE® X1 processed plates had overall higher outgrowth (1.2 - 2 folds).

2. RECOVERY

- The cellenONE® X1 recovered 6.4X more cells than the FACSAria 3.
- Plate processing was 40 min for the FACSAria 3 and 60 min for the cellenONE® X1 .
- The cellenONE® X1 processed plates resulted in significantly better outgrowth

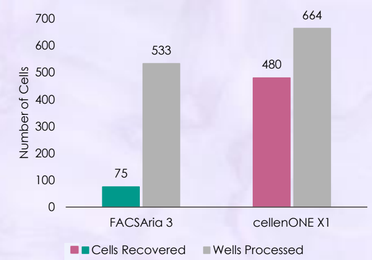


Figure 4. Number of wells processed and containing a single cell after sorting with the an input of 1,000 cells.

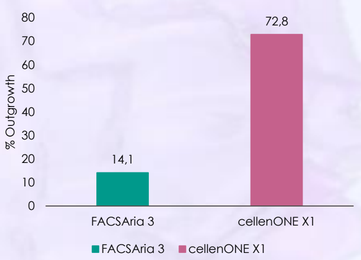


Figure 5. Outgrowth of single cell colonies after sorting of 1,000 cells.

FUTURE WORK

- At the time of experiments, optimal 96-WP processing times were 4 min. Improvements in image processing have since cut this in half.
- Dead-cell stain was utilized to improve the outgrowth obtained by the FACS system but could not be used on the transmission only cellenONE® X1; four channel fluorescence based selection has now been integrated in cellenONE® F1.4 systems.