**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 picodroplet 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:

<table>
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<tr>
<th>Instrument</th>
<th>Standard</th>
<th>Higher Stringency</th>
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<tr>
<td>FACS Aria 3</td>
<td>‘Cell’ gate on FSC/SCC plot</td>
<td>Live cell gate (7-AAD negative)</td>
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<tr>
<td>cellenONE X1</td>
<td>Sample mapping (automatic)</td>
<td>User defined circularity, diameter, and elongation parameters</td>
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   Figure 1. Automated mapping of cell samples on cellenONE X1 to set isolation parameters: circularity, diameter and elongation.

2. **RECOVERY**

   Polyclonal mouse hybridomas were sorted into collection tubes using the FACS Aria 3 to obtain 1,000 cells per tube. Concentrations were diluted to bias towards high recovery: 50 cells/well for the cellenONE® X1 and 4 events/sec for the FACS Aria 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.

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

**RESULTS**

1. **VIABILITY**

   The inclusion of dead cell stain (7-AAD) improved the outgrowth of the FACS Aria 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 FACS Aria 3.
   - Plate processing was 40 min for the FACS Aria 3 and 60 min for the cellenONE® X1.
   - The cellenONE® X1 processed plates resulted in significantly better outgrowth.

**CONCLUSION**

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

- **VIABILITY** - 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.

**FUTURE WORK**

- At the time of experiments, optimal 76-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.