# Streamlining monoclonal CHO cell line generation using droplet microfluidics

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## Introduction

**Overall Aim:**
To utilise droplet microfluidics to increase the efficiency and speed of selection of high productivity monoclonal stable CHO cell lines using the Cyto-Mine®.

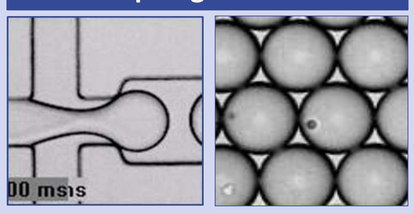
Droplet microfluidics exploits the aqueous droplets in oil to facilitate the screening of millions of single cells in a day. In principle, any assay which is performed in the well of a microtiter plate at the 10-100ul scale can be converted to a pL assay within a droplet. In partnership with Sphere Fluidics, we are developing a fully integrated droplet microfluidic device (Cyto-Mine®) and relevant droplet-based assays to support cell line generation workflows.

## Cyto-Mine® workflow overview

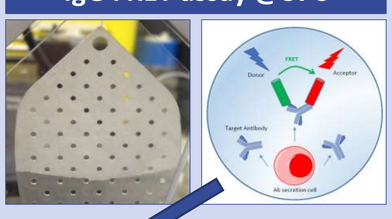
**Cyto-Mine®**

**Sample loading**

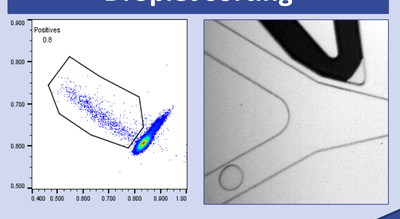
**Droplet generation**



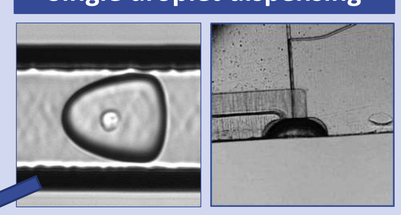

**IgG FRET assay @37C**



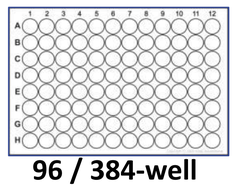

**Droplet sorting**




**Single droplet dispensing**



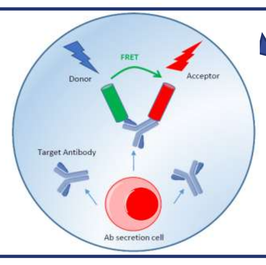
**Output**



**96 / 384-well plates**

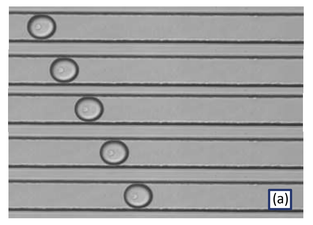
### FRET assay

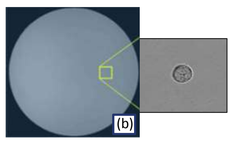
A FRET Assay was designed to be performed in a 300pL droplet. FRET pairs bind to two sites on the antibody secreted from the CHO cell during the incubation period. The higher the concentration of expressed antibody in the droplet, the greater the fluorescence from the red acceptor fluorophore, and a lower fluorescence from the green donor fluorophore.




### Monoclonality confirmation

*'For recombinant products, the cell substrate is the transfected cell containing the desired sequences, which has been cloned from a single cell progenitor...'* ICH Harmonised Tripartite Guidelines.




Single cell deposition confirmed by (a) images of droplet recorded pre-dispensing and (b) single cell image within well post dispensing.

## Monoclonal CHO cell line generation

### Proof of concept: separation of high and low productivity CHO Cells

**High** and **low** productivity cell populations were differentially fluorescently stained (CellTracker Deep Red or CellTrace Violet respectively) and mixed at a **1**:**49** ratio. The cell mixture was then loaded on to the Cyto-Mine®, incubated and sorted based on FRET read-out. The scatter plot in **Figure 1** shows three populations are clearly visible, (a) high productivity cells, (b) low productivity cells and (c) are droplets containing no cells. After sorting for the high productivity cells (a), and depositing those droplets into 96-well plates, the wells were imaged in bright-field and fluorescent channels on a NyOne imaging device (Synentec). Analysis of these images showed a 49 fold enrichment of High productivity cells (**Figure 2**). This demonstrates the Cyto-Mine® is capable of separating two cell populations based on productivity.

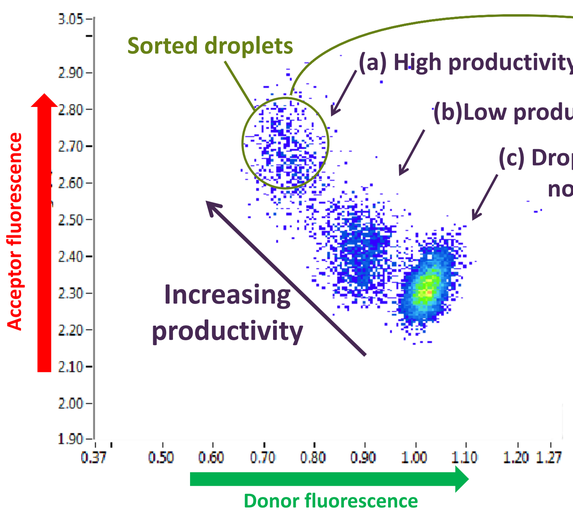



**Figure 1.** FRET assay scatter plot after incubation, showing separated cell populations by antibody productivity.

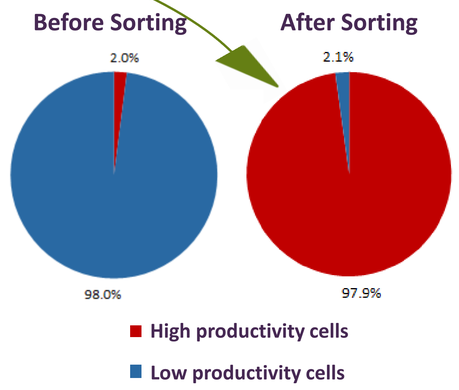
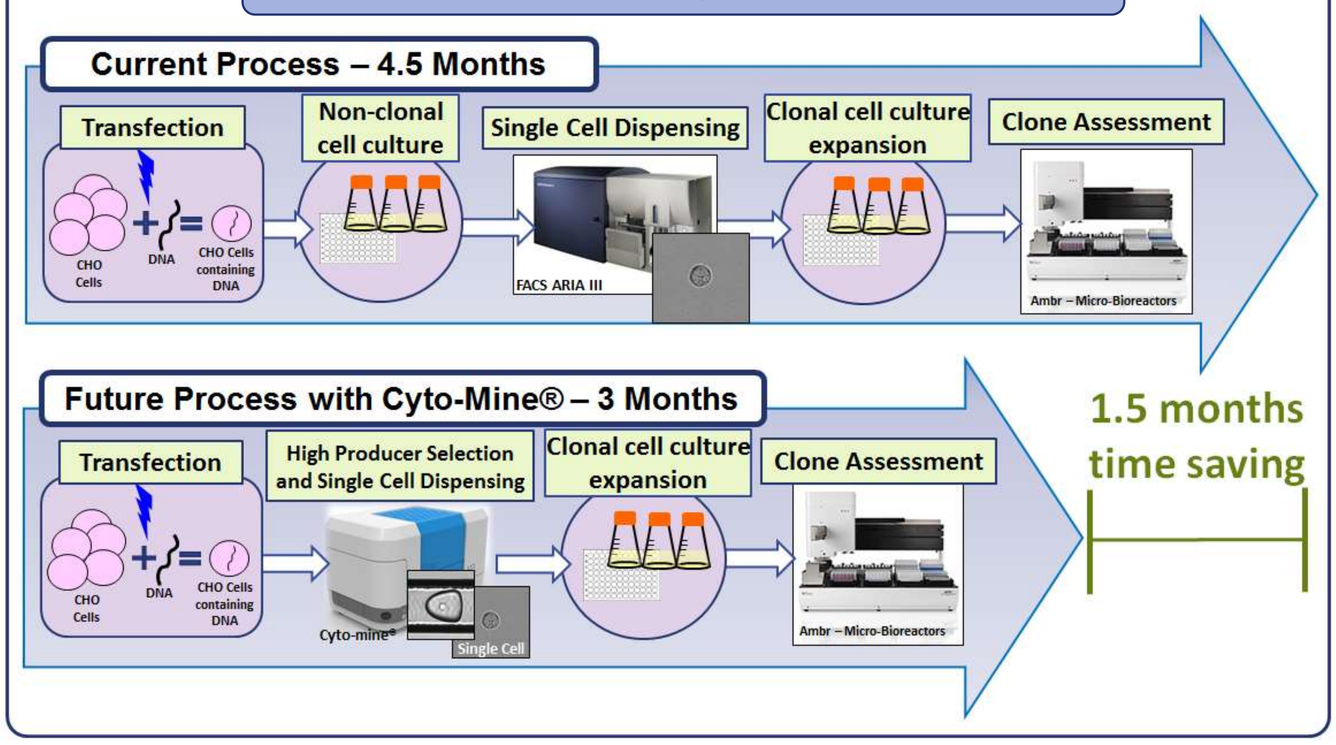



**Figure 2.** High productivity cell and low productivity cell abundance before and after high productivity cell enrichment on the Cyto-Mine®.

### Reduction in cell line generation timelines




### Cloning directly from a transfection pool

Cloning of high productivity cells directly from a recently transfected pool.

A host CHO DG44 cell line was transfected with a plasmid containing the sequence for an IgG4 antibody. The transfected cells were pooled and incubated at 37°C. The viability of the culture was assessed over 14 days (**Figure 3)**. In this experiment the time of increasing cell viability was observed on day 10, and we sorted on day 11. The transfection pool was loaded on to the Cyto-Mine® and incubated for 1h at 37°C. **Figure 4** shows the sorting gate identifying the whole population of droplets showing increased FRET and therefore antibody expression (droplets with no cells not gated). **Figure 5** shows the sorted population gated only for high productivity droplets.

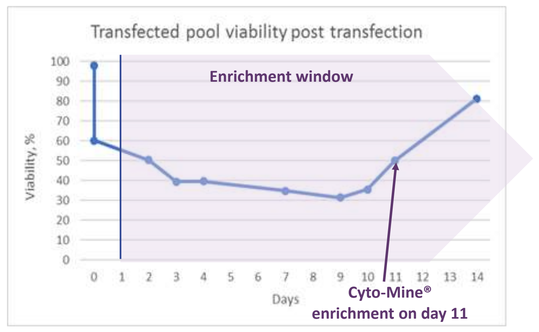



**Figure 3.** Viability of pool post transfection. Potential time for Cyto-Mine® enrichment indicated by the shaded region, estimated from day 1 to day 21 (data not shown).

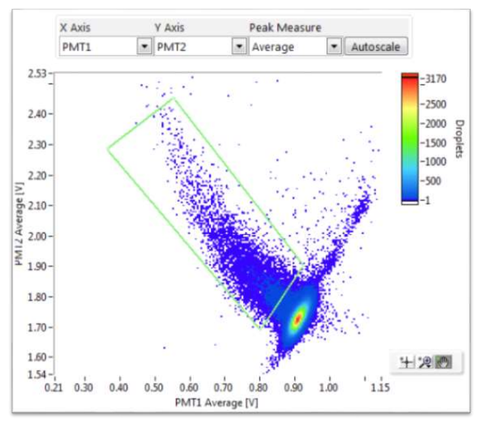



**Figure 4.** Scatter plot showing the spread of productivity of cells in the transfected pool. The green sorting gate identifies the whole population of droplets showing productivity.

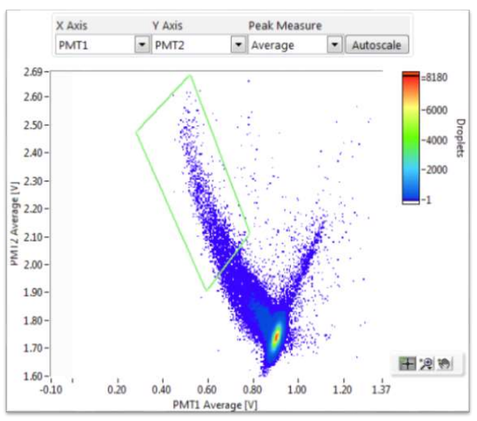



**Figure 5.** Scatter plot showing the spread of productivity of cells in the transfected pool. The droplets containing high productivity population are identified in the green sorting gate.

All recovered clones from 96-well plates were transferred to 24 well plates, where they were assessed for titre after 8 days, **Figure 6**. Clones from the high productivity population gate had statistically higher titres than clones from the gate of the whole population (P value 0.03). The top 40 clones identified by 24-well titre on day 8, were taken through to shake flask (18 high population clones and 22 whole population clones). Batch shake flask overgrow titres are shown in **Figure 7**. The high productivity population clones had a higher mean titre, but this was not statistically significant (P value 0.10). We would expect this as the clones have been triaged down to the highest titre clones only. However, to reach a satisfactory panel of high productivity clones, we had to sort 5 times less clones when using high productivity population enrichment.

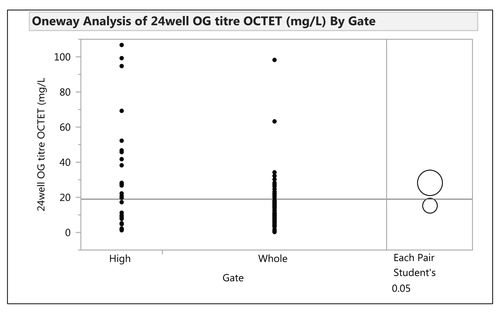



**Figure 6.** Titre of clones in 24-well on day 8, by Octet with PrG sensors. Clones from the high productivity gate had a statistically higher mean titre than clones from whole population gate (P value 0.03).

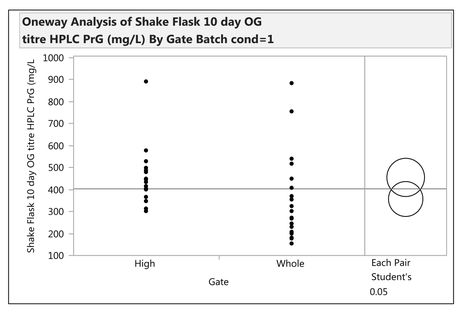



**Figure 7.** Titres of batch shake flask overgrows of top 16 high population clones and top 18 whole population clones by HPLC PrG (top clones based on titre in 24-well plates). P Value 0.10.

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### Summary

Preliminary data of cells sorted 'early' post transfection, indicates suitable high productivity cells can be identified and single cell cloned into microtiter plates with the Cyto-Mine®. Using this method can produce a monoclonal cell line in less than 3 months, with less clones needed to be assessed.

### Next steps

- Further optimisation of the 'early' sorting of post transfection (i.e. Day 1 – Day 21 post transfection).
- Assess clones in fed-batch production processes and stability studies to confirm suitability for manufacturing.