Finding High Producing Clones with Monoclonality Assurance in One Day.

Background

In the discovery and development of novel biopharmaceuticals (antibodies) for therapeutic use Cell Line Development is an integral process in the biomanufacturing of therapeutic proteins. Mammalian cells make ideal factories for protein production as they have similar molecular mechanisms to human cells for protein modifications such as glycosylation[1].

During the development of a mammalian cell line there are some desirable attributes as well as criteria specified by regulatory bodies (such as ICH, FDA, EMA) which state that cell lines for therapeutic use must be cloned from a single cell progenitor to ensure genetic and phenotypic consistency which require proof of monoclonality[1, 2].

Five key characteristics desired from a host cell line are:

1. Productivity - must generate high product yields
2. Scalability - grow robustly
3. Stability - be able to grow in serum-free, chemically defined conditions
4. Quality – desired product quality
5. Monoclonality - well-defined clonal cell lines derived from a single cell

Being able to provide each of these characteristics from a cell line creates a substantial bottleneck when screening for and isolating rare cells. Traditionally, selection of single clones was achieved by the resource-intensive method of limiting dilution, and more recently aided by semi-automated technologies such as fluorescent activated cell sorting (FACS), colony picking and single-cell printers. Once isolated, clones are imaged by instruments such as cell-in-well imagers and then assessed for their productivity before being progressed into small scale bioreactors for batch production.

With the market demand for biotherapeutics increasing at a dramatic rate there is a pressing need to substantially shorten the development workflow. Cyto-Mine® utilises established picodroplet technology to fast-track the generation of cell banks comprised of high-producing clones derived from single cells with monoclonality assurance.

The present study illustrates the process by which the Cyto-Mine® IgG secretion assay measures the productivity of hundreds of thousands of single cells encapsulated in highly consistent picoliter droplets or ‘test tubes’ while simultaneously providing verification of clonality.

Aims & Objectives

This application note will demonstrate how Cyto-Mine®

- Identifies high producing clones from a mixed population.
- Accurately identifies clonality.
- Significantly shortens the Cell Line Development workflow by using a single fully integrated instrument.

Box 1. The Cyto-Mine® Single Cell Analysis and Monoclonality Assurance System overcomes the limitations of current technologies by screening hundreds of thousands of individual cells for secreted target protein, and then isolating and dispensing the highest producers with high viability to microplate wells (Figure 1).

Figure 1. Cyto-Mine® proprietary technology finds and isolates high-producer clones from complex cell populations.

Methods

Cyto-Mine® emulates the limiting dilution and productivity screening process but in a much more efficient, higher throughput and fully automated manner (Box 1).

Single Cell Encapsulation

Using Poisson distribution statistics, cells are encapsulated into 300 pL picodroplets of preferred culture medium at a dilution level that optimizes the number of picodroplets containing only a single cell.

Antibody Secretion Assay

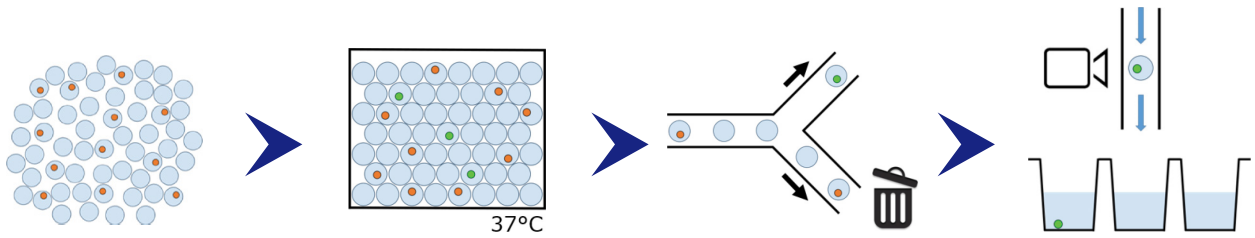
Next, the cells are incubated to enable the secreted target protein to accumulate inside the picodroplet, which is captured and detected by animal origin free (AOF) IgG detection reagent present in the culture medium (Box 2). The miniaturized scale means that secreted IgG can be quantified after just 0.5 to 2 hours.

Sorting

The highest-secreting single cells are then sorted by fluorescence intensity for collection.

Clonality Assurance

Prior to dispensing, as the picodroplet travels through the microfluidic channels, the encapsulated cells are imaged multiple times to provide verification of clonality. The integrated stages of the Cyto-Mine® process are summarized in **Figure 2.**



Cells encapsulated into picodroplets

Incubation and secretion assay

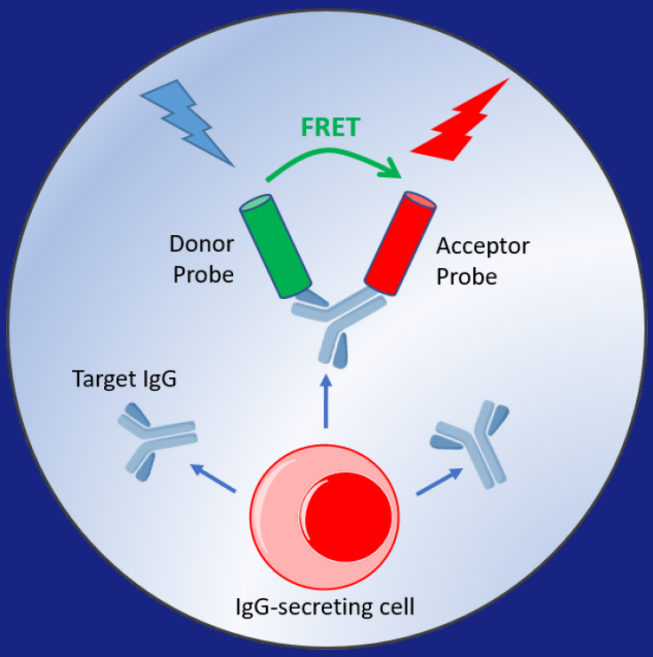
Sorting Positive cells

Single cell verification during dispensing

Figure 2. The Cyto-Mine® workflow integrates the screening, sorting, isolation and verification of high-secreting clones into a fully automated process.

Box 2.

Antibody Secretion Assay



A key enabling component of Cyto-Mine® is its ability to measure the specific IgG production rate of every single cell. The starting cell population requires no prior modification and is simply mixed with the appropriate animal origin free (AOF) detection reagent which includes fluorescently labelled isotype-specific donor and acceptor probes, prior to loading on to Cyto-Mine®. During the in-situ incubation step, IgG secreted by the cell accumulates within the picodroplet. The detection probes bind to the secreted IgG inducing a FRET-mediated shift in fluorescence (**Figure 3**). Cyto-Mine® then measures the fluorescent signal generated and converts it to a quantitative output.

Figure 3: The Cyto-Mine® picodroplet-based IgG secretion assay. A customized pair of IgG-specific AOF fluorescent probes are trapped within each picodroplet. IgG secreted from the encapsulated cell is recognised by the detection probe pair forming a 3-body FRET complex that induces a fluorescent signal.

Results

Antibody Secretion Assay

To validate the Cyto-Mine® IgG secretion assay, 5 separate populations of picodroplets were generated from culture medium spiked with human IgG over a range of concentrations from 0 to 20 mg/L. The 5 picodroplet populations were then pooled together and analysed using Cyto-Mine®. **Figure 4** shows how the different titers resolved into discrete populations. IgG concentrations up to 20 mg/L represent the typical Cyto-Mine® working range, equivalent to a specific productivity (Qp) of up to 144 pg/cell/day assuming a 1-hour incubation. A standard titration curve generated from Cyto-Mine® Scatter Plot data is shown in **Figure 5**.

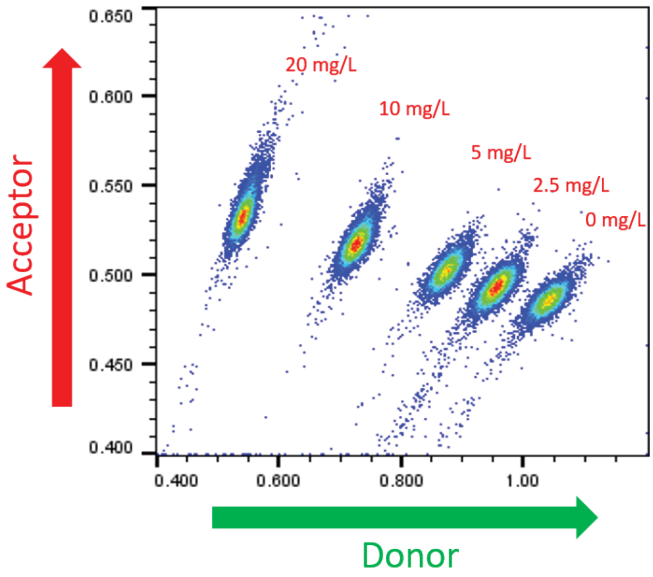


Figure 4: Cyto-Mine® Scatter Plot. Large numbers of individual picodroplets were loaded with the indicated concentrations of human IgG and then resolved using Cyto-Mine® AOF IgG secretion assay and analysis.

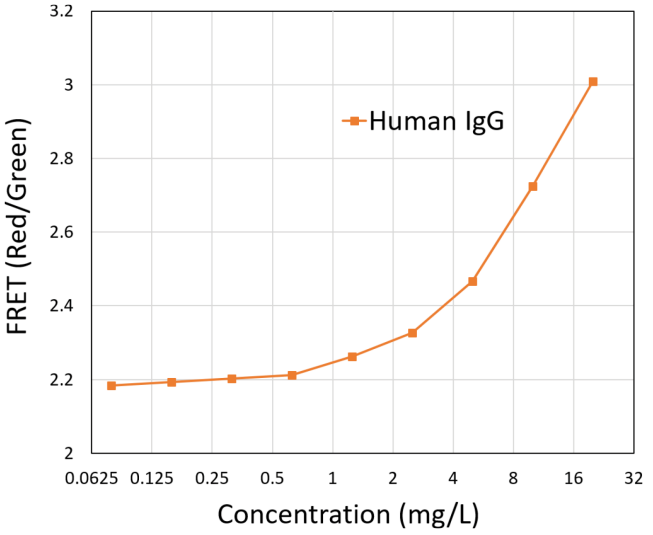


Figure 5: Standard human IgG titration curve derived from Cyto-Mine® AOF IgG secretion assay and analysis.

Screening a CHO cell population for high-producers

A heterogeneous pool of CHO cells stably transfected to express human IgG was mixed with Cyto-Mine® human IgG-specific AOF detection reagent (see Box 2). The cells were encapsulated into picodroplets, incubated for 2 hours, and then analysed using the Cyto-Mine® IgG secretion assay. The results, presented in **Figure 6**, show a population of cells with high acceptor to donor fluorescence ratio, and gated for collection. The bright oval-shaped cluster to the lower-right represents the bulk of data points, which are comprised of picodroplets containing low- or non-producing cells as well as empty picodroplets. The high-producing cells (highlighted high secretors) are clustered in the top left. The Cyto-Mine® software features a gating polygon which is user-customizable for added flexibility so that the highest-value cells can be targeted for collection to microplates.

Cyto-Mine® allows you to screen for single cell productivity from a transfection pool.

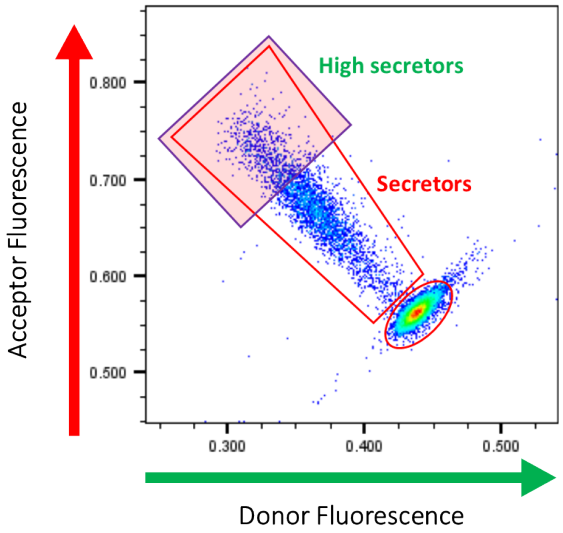


Figure 6: Cyto-Mine® Scatter Plot of FRET signal generated from picodroplet-encapsulated CHO cells incubated with Cyto-Mine® human IgG-specific AOF detection reagent.

Conclusions

These data show the power of Cyto-Mine® for screening large cell populations to find, sort and isolate high-value clones. The assay is quantitative in the range of immunoglobulin normally produced by standard biopharmaceutical production cell types.

With its picodroplet-based IgG secretion assay, Cyto-Mine® offers the following major benefits:

Simplicity:
Seamless, one-step, automated process eliminating the need for multiple instruments

Quality:
GLP-compliant platform with unique ability to mine and isolate rare, single, viable, high-producing cells

Sterility:
End-to-end sterile, disposable and AOF process

Speed and Efficiency:
Allows multiple projects to be run in parallel and frees up user time for other activities

Miniaturisation:
Enables pL-level high-throughput screening with minimal use of assay reagents

Traceability:
Storable images proving single cell status at the point of dispensing provides the necessary documented evidence of monoclonality

Key Point

Cyto-Mine® can select clones based on productivity, straight a transfection pool, and provide monoclonality assurance for each cell. Cyto-Mine® significantly reduces the time from host cell transfection to a cell bank by several weeks, streamlining workflows from multiple resource intensive instruments into one easy-to-use high-throughput system.

References
1. Browne, S.M. & Al-Rubeai, M. Selection methods for high producing mammalian cell lines. Trends Biotechnol. 25, 425–432 (2007).
2. ICH Derivation and characterisation of cell substrates used for production of biotechnological/biological products (Q5D). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals For Human Use, Geneva, Switzerland (Online) (1997).'
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