Picodroplets for Biologics Discovery and Cell Line Development: Accelerating the Discovery and Development Process

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1. Abstract

Although antibody therapeutics dominate drug development, prolonged workflows and increasing costs during their discovery and development hinder their success[1-3]. Cyto-Mine® offers significant advantages in the number of cells that can be processed, cost per run, and the speed with which projects can be completed. Sphere Fluidics has developed Cyto-Mine® Chroma - a new instrument - that enables the use of more fluors, multiplexed assays, and a broader range of applications. Early applications of the Cyto-Mine® Chroma platform will enable cell isolation based on cell surface markers, viability, and titer of secreted proteins. Moreover, in combination with re-injection-based workflows, it will facilitate complex assays like combining more than one cell type in a single droplet (crucial for binding assessment, cytotoxic assays, and advancing antibody development).

2. Cyto-Mine®

Figure 1: The Cyto-Mine® platform

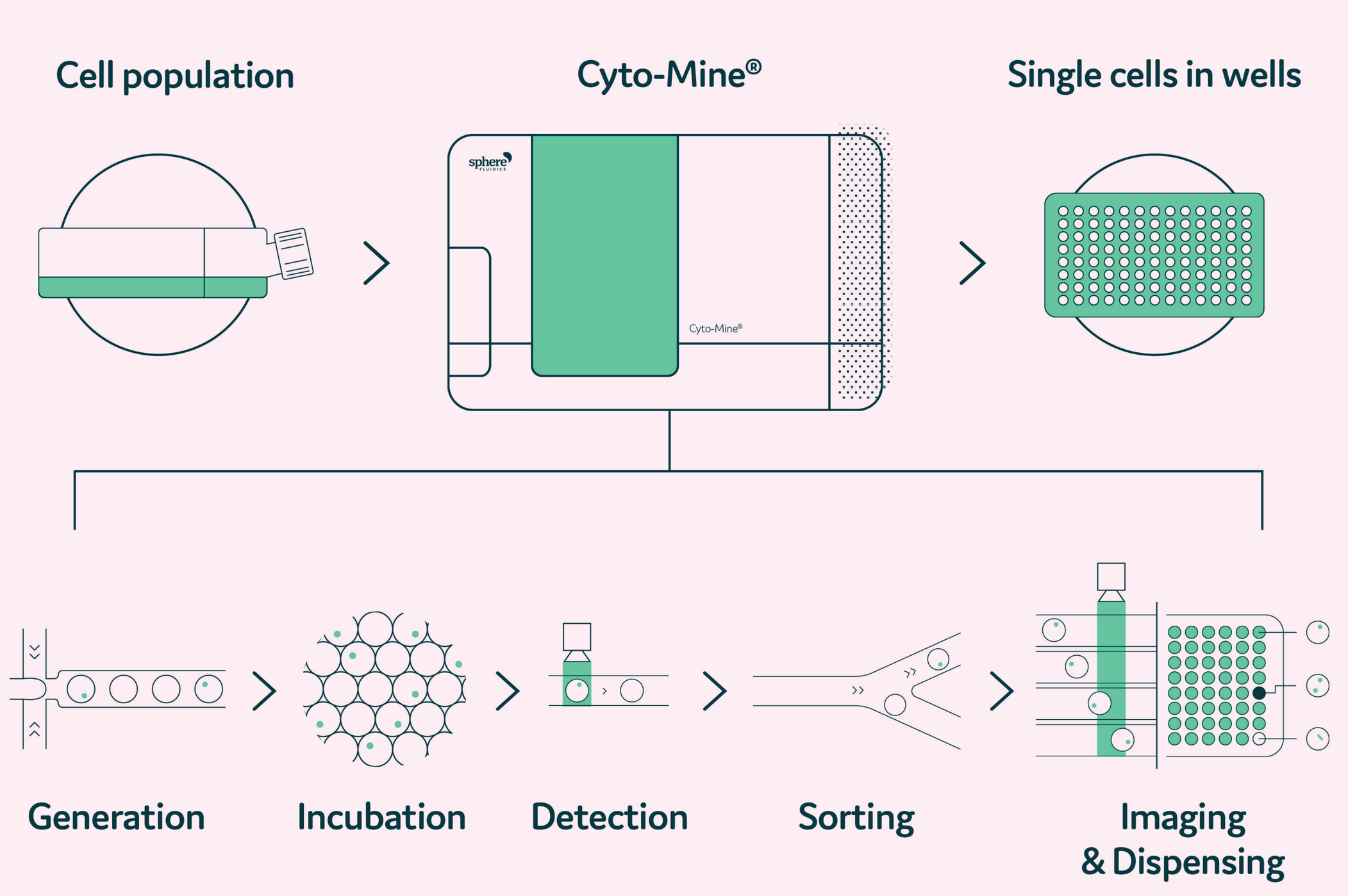


Figure 2: A Cyto-Mine® workflow. Integration of single cell screening, sorting, isolation, and verification using a fully integrated microfluidic process.

	Cyto-Mine®	Cyto-Mine® Chroma
Fully integrated	Y	Y
Automated microfluidic	Y	Y
Software & hardware control system	Y	Y
Encapsulate single cells in picodroplets	Y	Y
Selectively sort & image picodroplets	Y	Y
Ensure monoclonality	Y	Y
Dispense 'hits' into microtiter plate	Y	Y
Identify & isolate rare cells/cells with high productivity	Y	Y
Number of lasers	1	4
Assay multiplexing	N	Y
Reduce CLD/AbD process time	Y	YY

Table 1: Features of Cyto-Mine® and Cyto-Mine® Chroma

Cyto-Mine® harnesses picodroplet technology to find rare and valuable variants within heterogeneous cell samples. The machine integrates single-cell encapsulation (generation), a fluorescence-based optical detection method followed by sorting for isolation of 'hits', verification by imaging of monoclonality assurance, and single-cell dispensing into microtiter plates.

3. Increasing efficiency of Cell Line Development processes with Cyto-Mine®

Cyto-Mine® increases success rate in gene integration, clone selection and monoclonality assurance

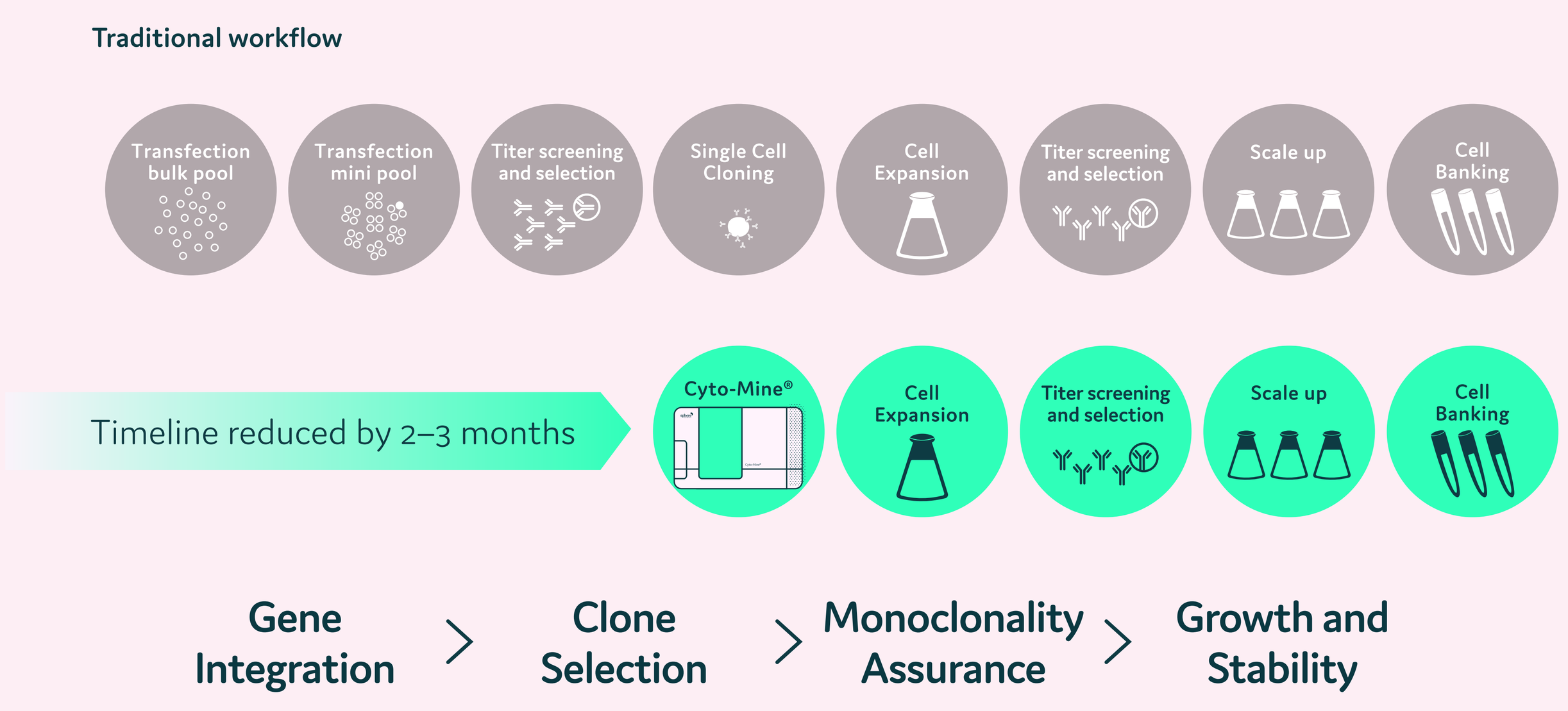


Figure 3: Traditional CLD processes are time-consuming. Cyto-Mine® reduces time and increases success rates. At a single cell level, the clones are selected based on their productivity. Selected single cells are dispensed into microplates with image assurance on their monoclonality. Cells dispensed into microplates can be monitored for growth over time.

Gene integration - increasing efficiency using picodroplets

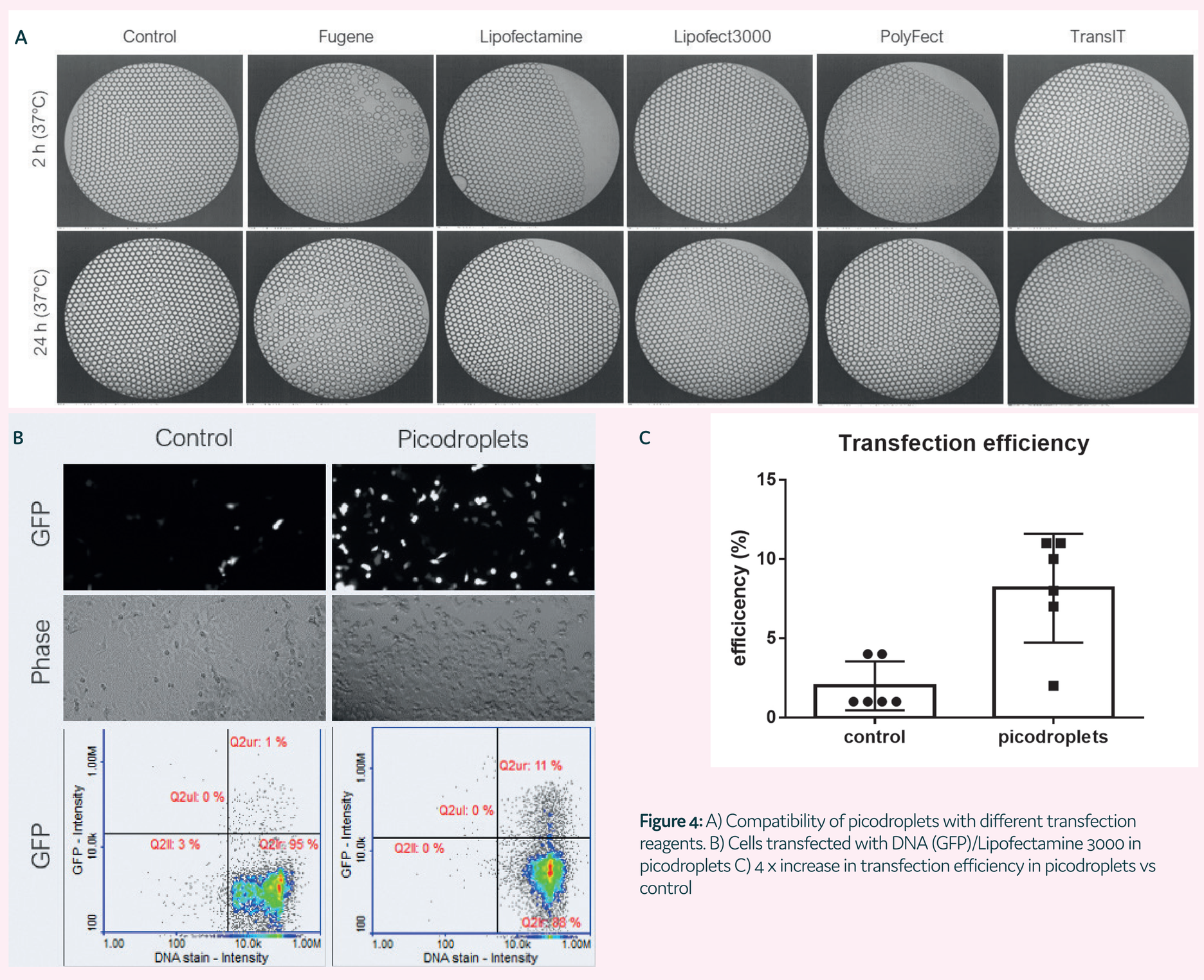


Figure 4: A) Compatibility of picodroplets with different transfection reagents. B) Cells transfected with DNA (GFP)/Lipofectamine 3000 in picodroplets C) 4 x increase in transfection efficiency in picodroplets vs control

Clone selection using FRET Assays

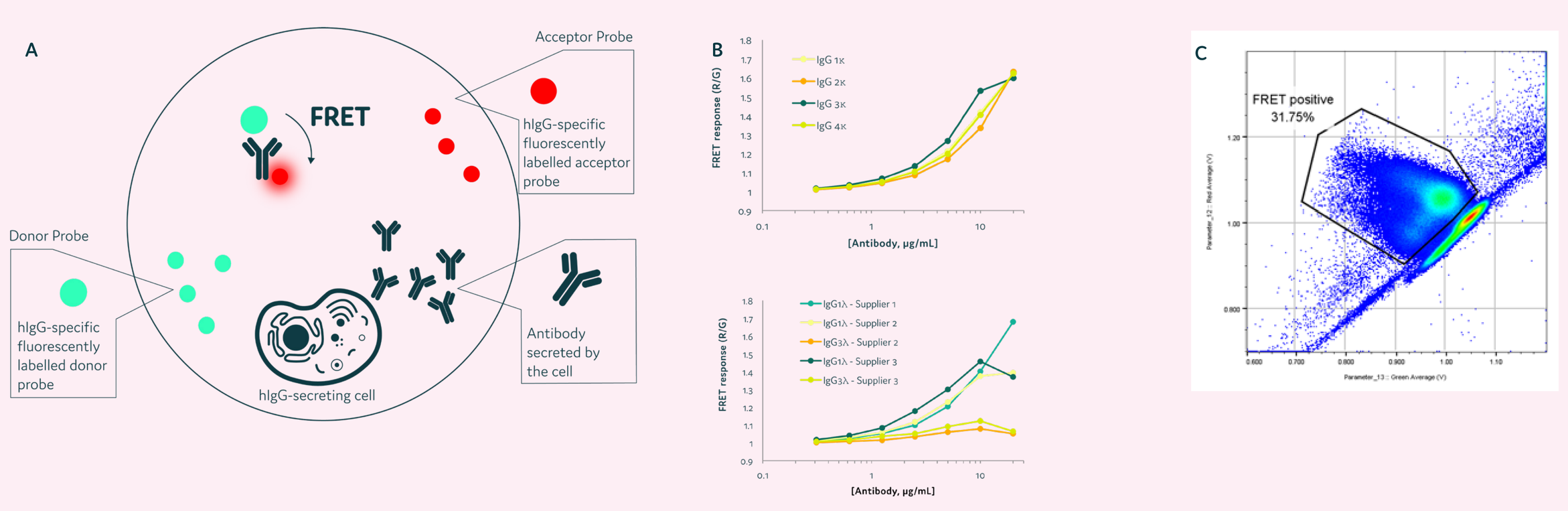


Figure 5: Clone Selection using CytoCellect®PLUS. A&B) The kit detects secreted IgG antibodies regardless of their light chain. C) The kit can be used with Cyto-Mine® for selection of high producing clones, even if IgG light chain (kappa or lambda) is unknown.

Clone selection - using FC Fusion to determine productivity

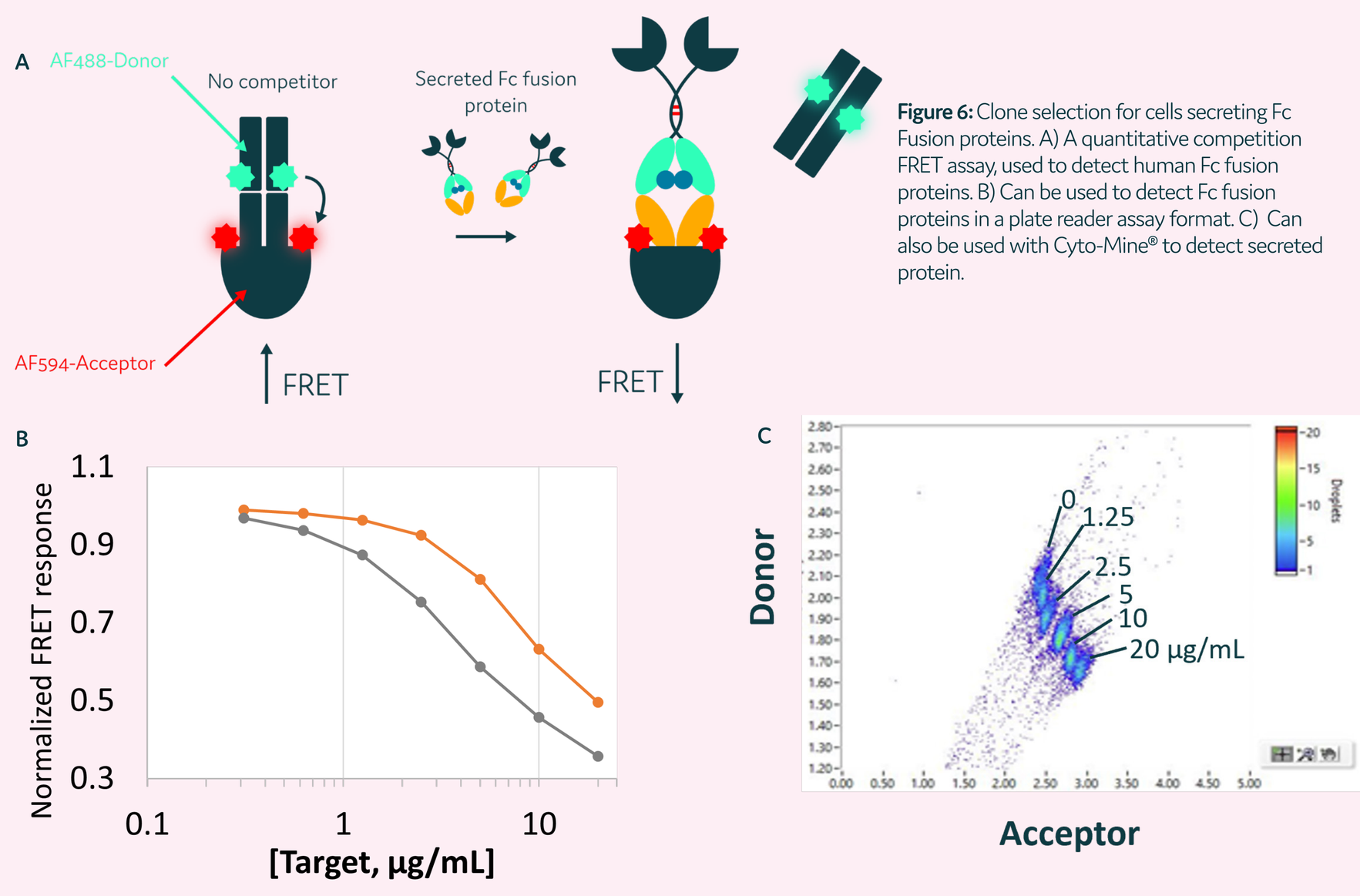


Figure 6: Clone selection for cells secreting Fc Fusion proteins. A) A quantitative competition FRET assay, used to detect human Fc fusion proteins. B) Can be used to detect Fc fusion proteins in a plate reader assay format. C) Can also be used with Cyto-Mine® to detect secreted protein.

Monoclonality assurance

- Cyto-Mine® has been specifically designed to ensure monoclonality via accurate and reliable single cell dispensing.
- Cyto-Mine® dispenses and reports clonality of cells with an accuracy of 98.9%.

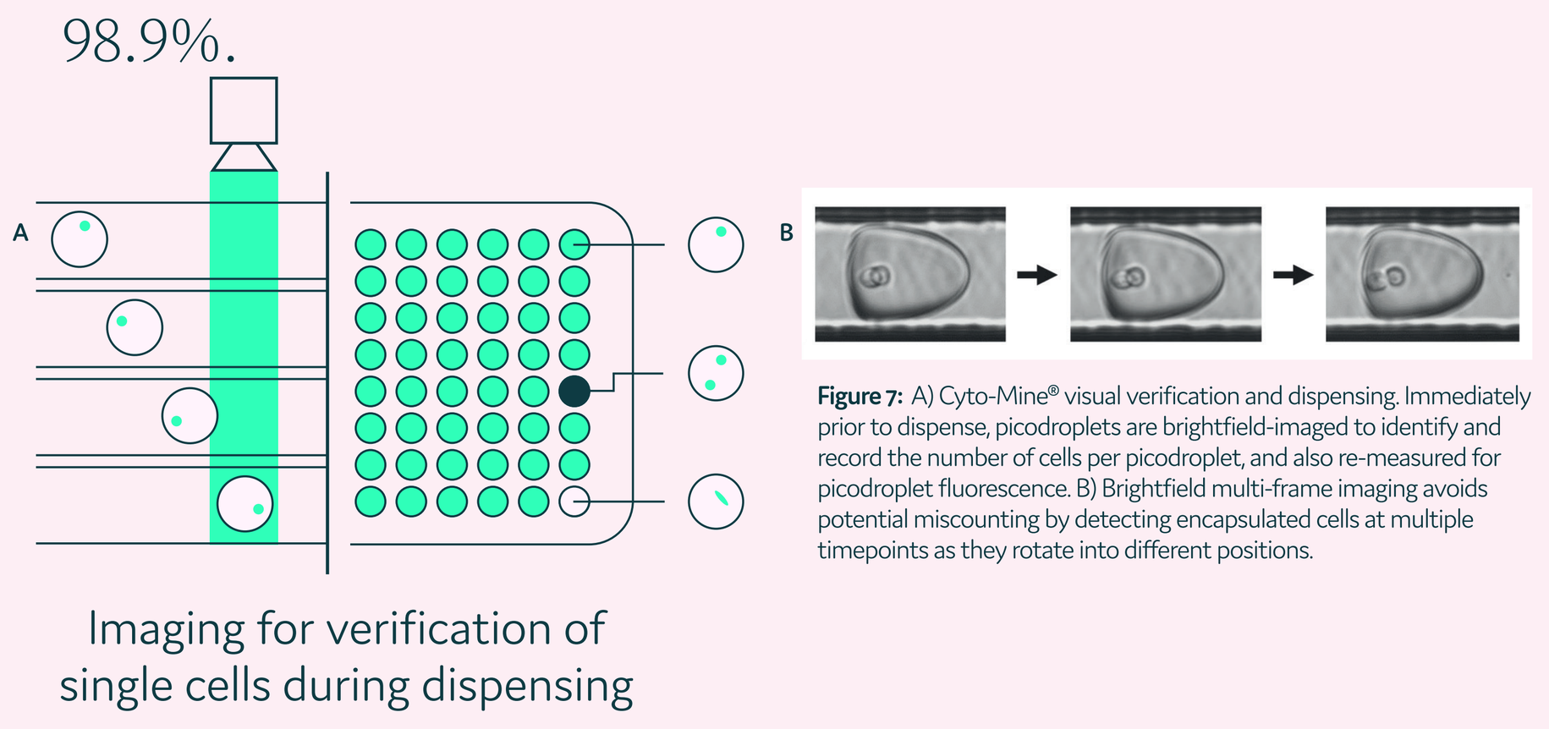


Figure 7: A) Cyto-Mine® visual verification and dispensing. Immediately prior to dispense, picodroplets are brightfield-imaged to identify and record the number of cells per picodroplet, and also re-measured for picodroplet fluorescence. B) Brightfield multi-frame imaging avoids potential miscounting by detecting encapsulated cells at multiple timepoints as they rotate into different positions.

Growth and stability - maintaining outgrowth

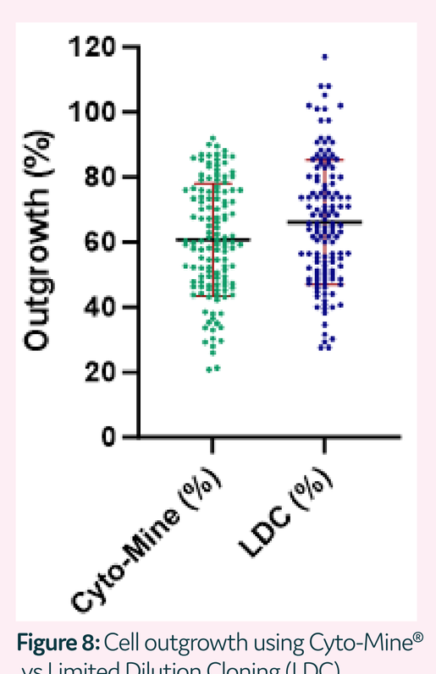


Figure 8: Cell outgrowth using Cyto-Mine® vs Limited Dilution Cloning (LDC)

- Single cells isolated using Cyto-Mine® have high viability and show comparable growth compared to Limited Dilution Cloning

4. Enabling Antibody Discovery using Cyto-Mine®

Cyto-Mine® brings speed and simplicity to antibody discovery processes

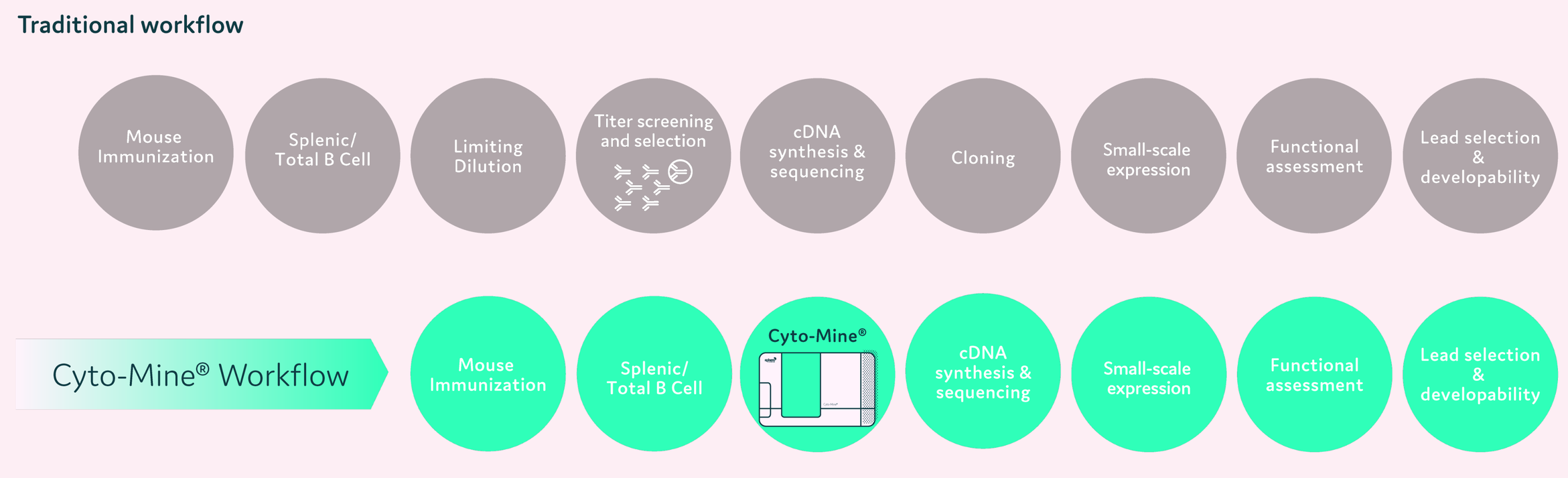


Figure 9: Antibody discovery workflow using Cyto-Mine®

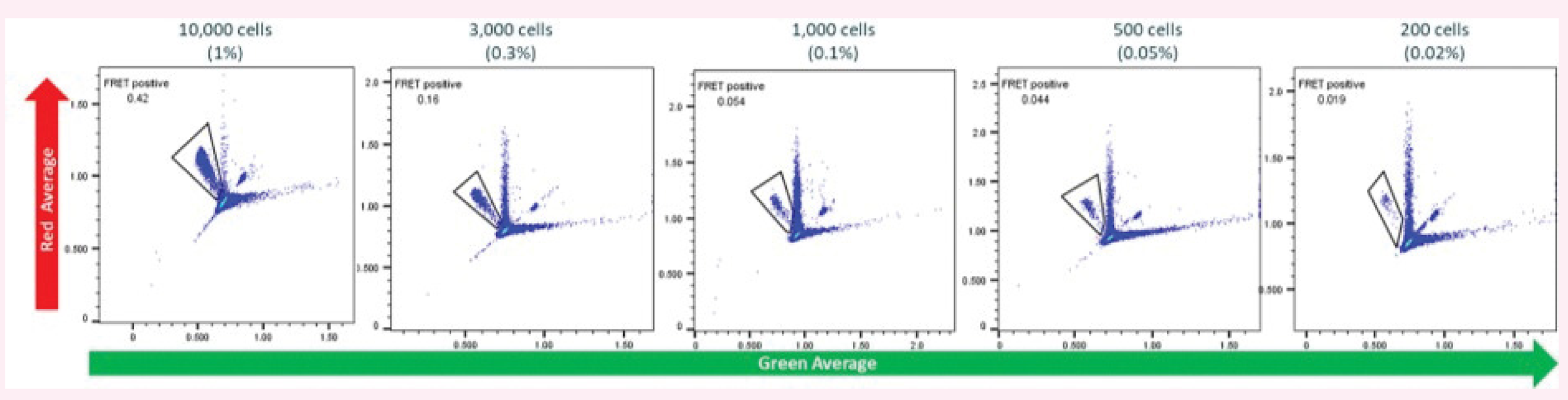


Figure 11: High sensitivity isolation of rare single cells based on their antigen specificity

B cell repertoire screening

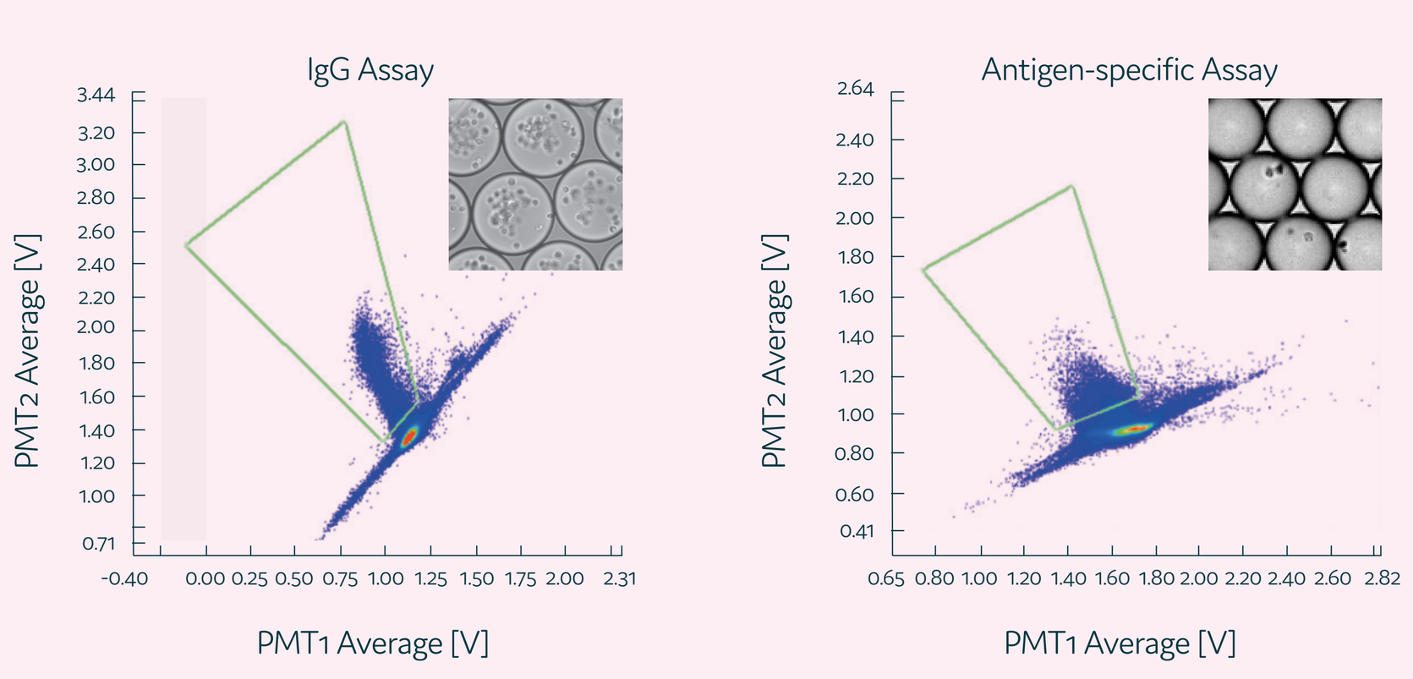
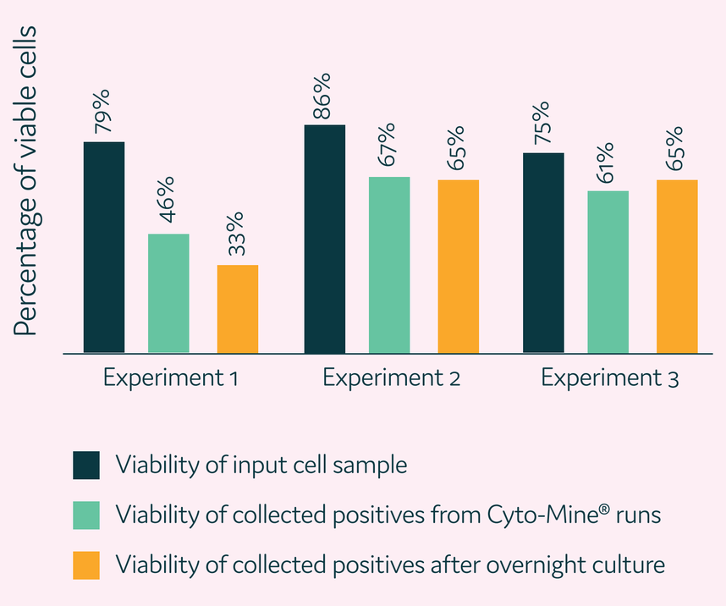


Figure 10: High-throughput single B cell screening using whole B cell repertoire. Selection of B cells based on secretion of IgG and/or antigen specificity.



- Cyto-Mine® used for high-throughput B cell screening and isolation of rare cells based on their productivity.

5. Developing Cyto-Mine® Chroma

Going beyond gene integration

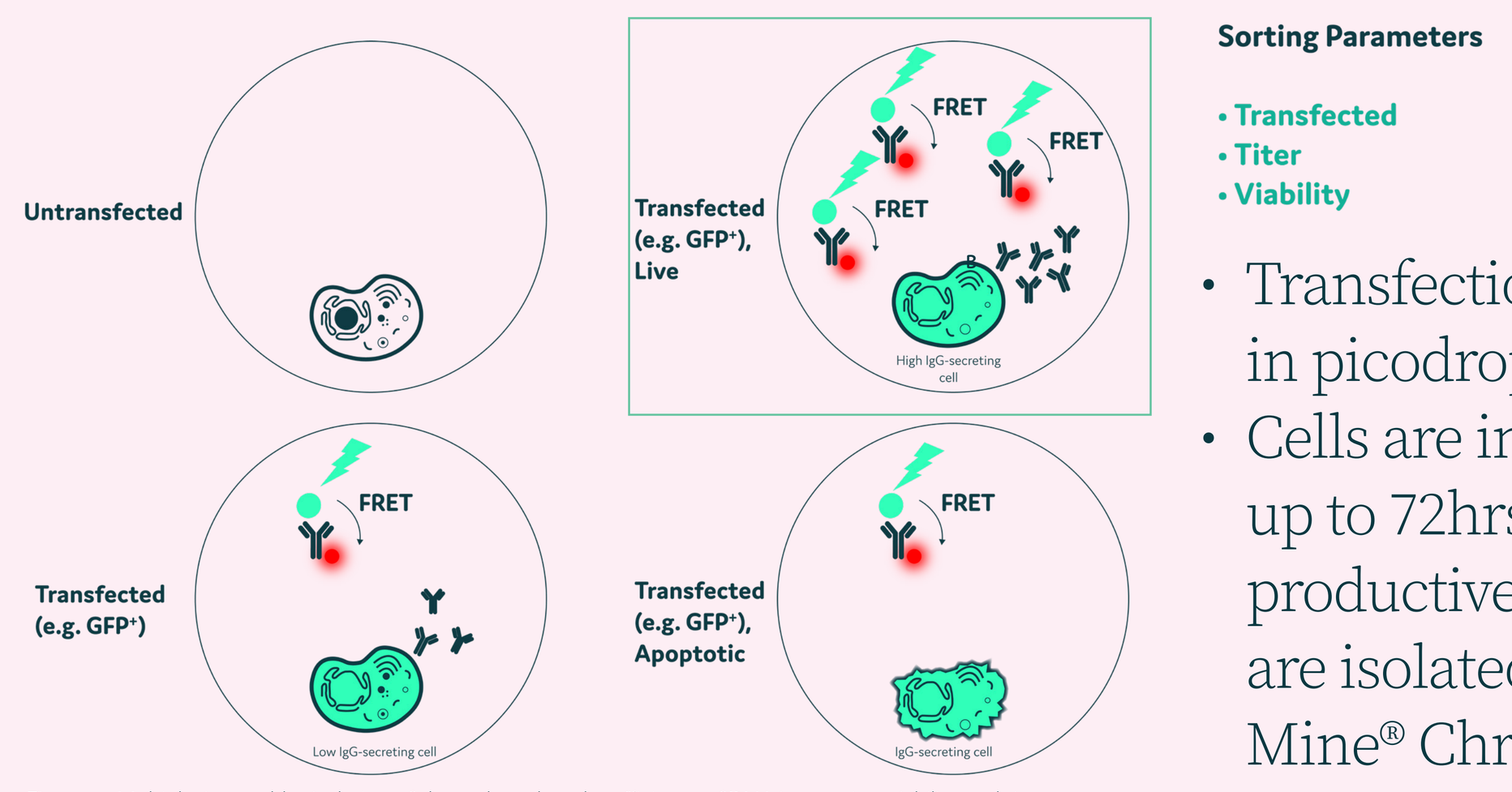


Figure 12: Multiplexing enables isolation of clones based on the efficiency of DNA integration, viability, and titer.

- Transfection takes place in picodroplets
- Cells are incubated for up to 72hrs and the most productive, viable cells are isolated using Cyto-Mine® Chroma

A more efficient selection process

Figure 13: Example selection process. Hybridoma A: Labelled with CellTracker Blue; produces irrelevant IgG. Hybridoma B: Labelled with CellTracker Deep Red; produces an anti-TNF-alpha antibody.

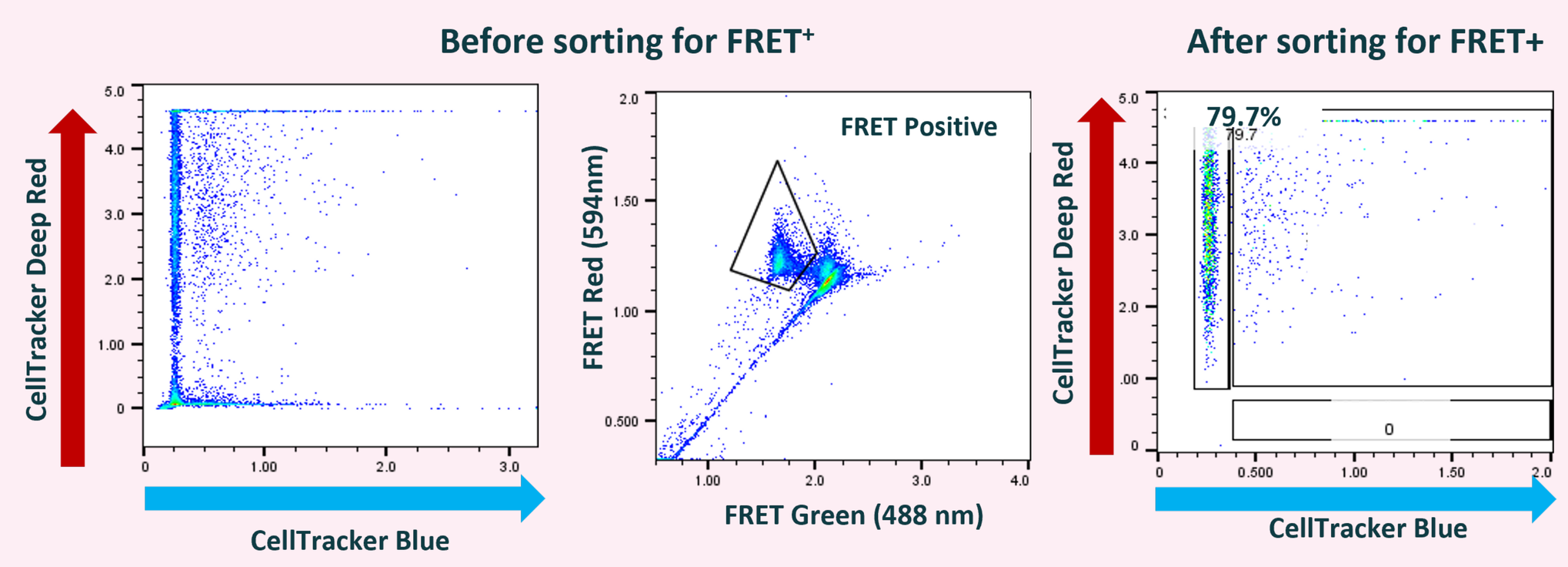


Figure 14: Single cells producing anti-TNF-alpha were detected and isolated using Cyto-Mine® Chroma (recombinant TNF-alpha was used for antigen-specific FRET assay).

- Multiplexing assays in a single droplet improves selection process of high producing clones
- Cells can be imaged for viability, productivity and antigen specificity in a single step
- Additional applications: Mode of action assays, using multiple cells per droplet. Function of a labelled cell type in the presence of unlabelled cell to be evaluated in picodroplets.

6. Expanding Cyto-Mine® Applications: Re-injection

1. Off-line generated picodroplets are injected into Cyto-Mine®
2. Droplets are sorted and dispensed using Cyto-Mine®

Relevant applications include:

- Applications requiring long incubation of cells in droplets
- Applications requiring extensive manipulation during droplet generation

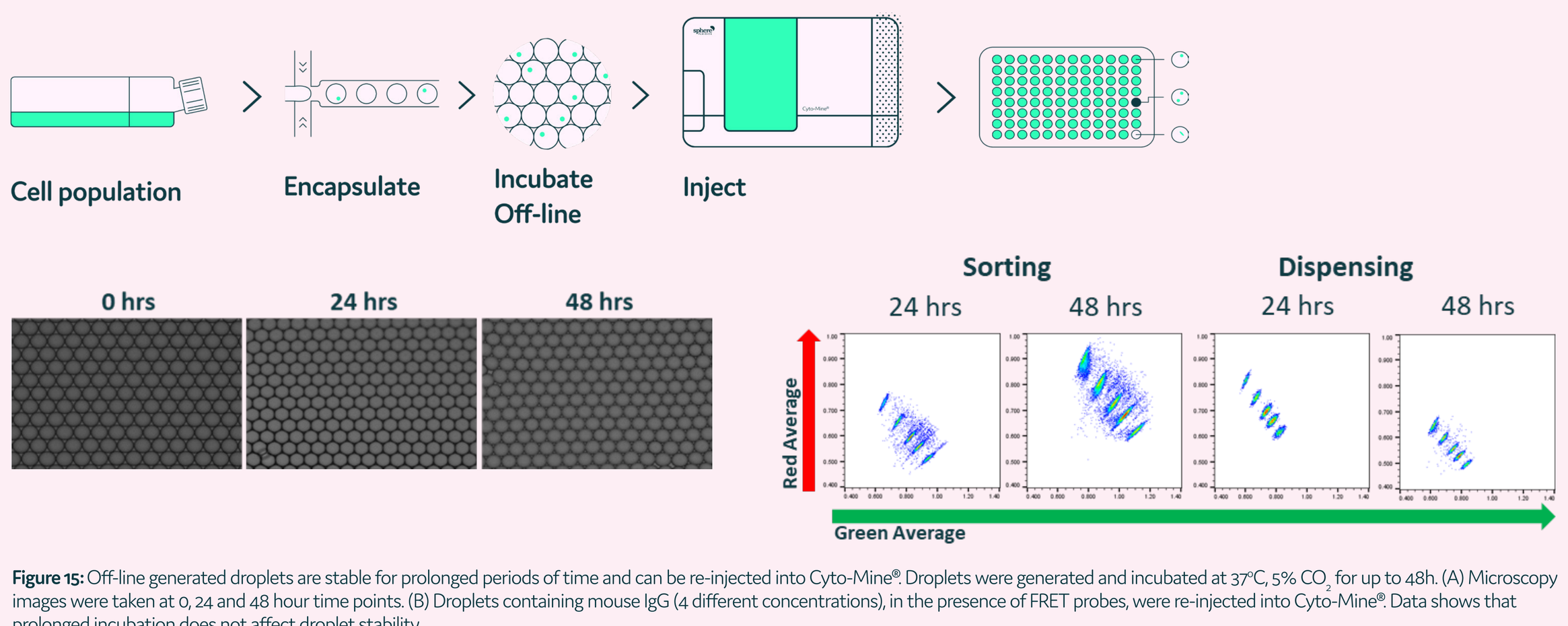


Figure 15: Off-line generated droplets are stable for prolonged periods of time and can be re-injected into Cyto-Mine®. Droplets were generated and incubated at 37°C, 5% CO_2 for up to 48h. (A) Microscopy images were taken at 0, 24 and 48 hour time points. (B) Droplets containing mouse IgG (4 different concentrations), in the presence of FRET probes, were re-injected into Cyto-Mine®. Data shows that prolonged incubation does not affect droplet stability.

References: 1. Kaplon *et. al.,* Antibodies to watch in 2022, Mabs, 2022. 2. Bauer *et. al.,* How can we discover developable antibody-based biotherapeutics?, Front Mol Biosci, 2023. 3. Farid *et. al.,* Benchmarking biopharmaceutical process development and manufacturing cost contributions to R&D, MAbs, 2020.