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Encapsulating individual living yeast cells for screening and recovery with Xdrop® Sort

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Summary

- Living yeast cells can be encapsulated in Xdrop DE20 (1.6 pl) or DE50 (100 pl) droplets.
- The yeast cells can proliferate in DE50 droplets.
- Based on their fluorescence, DE20 droplets containing yeast cells can be sorted using Xdrop Sort in a workflow that is sensitive, accurate, and high-throughput.

Introduction

Encapsulating living microbial cells in highly stable, double-emulsion (DE) droplets enables sensitive, accurate, high-throughput screening with the possibility to retrieve the cells of interest. This single-cell resolution can be applied to the study of cellular events with extracellular secretion of proteins, such as enzymes[1], antibodies[2], or metabolites[3]. Cellular proliferation studies for drug discovery and clinical assessment are also possible[4–6].

Here, we show the use of Xdrop Sort for the encapsulation in DE droplets and subsequent sorting of living yeast cells (*Saccharomyces cerevisiae*) expressing green fluorescent protein (GFP). We discuss the effects of emulsion size on cell viability and proliferation, and show that living yeast cells can be recovered from DE droplets after sorting.

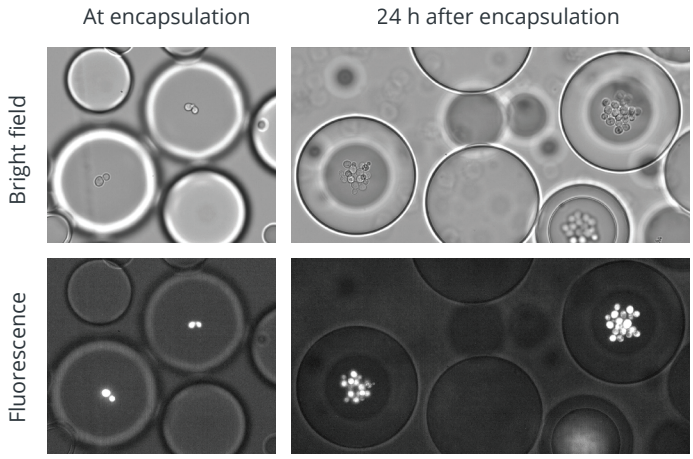

Figure 1. Bright field and fluorescence microscopy images of representative fields showing yeast cells in Xdrop DE50 droplets immediately after encapsulation and after 24 h incubation of the yeast cells in droplets at 30°C. The medium is YPD.

Encapsulation in double emulsion (DE) droplets

Yeast cells expressing GFP or mKate2 (red fluorescent protein) were cultured from a single colony and grown overnight in liquid yeast extract–peptone–dextrose medium (YPD).

The cells were then encapsulated using Xdrop DE20 and Xdrop DE50 Cartridges, which produce double-emulsion droplets with two different inner volumes: 1.6 pl and 100 pl, respectively. For the Xdrop DE20 Cartridge, 40 µl of cells (10^4 cells/µl) were pipetted into each sample well of the cartridge. For the Xdrop DE50 Cartridge, 100 µl of cells (500 cells/µl) were pipetted into each sample well. See the instrument manuals for more information on cartridge setup and encapsulation runs.

The droplets containing the cells were transferred to static microtiter plates and incubated at 30°C for 24 h.

Yeast cell viability after encapsulation and incubation

In both the DE20 and DE50 droplets, yeast cells appeared viable after encapsulation, with proliferation observed in the occupied DE50 droplets (Figure 1). After 24 h, the number of cells in the occupied DE50 droplets had risen from 1 or 2 to more than 10.

Sorting of GFP-expressing yeast cells in DE20 droplets

Yeast strains expressing either GFP or mKate2 were encapsulated as described above. The droplets were mixed 1:1000 to create a ~0.1% frequency of GFP to mKate2 droplets. Two 200 µl aliquots of droplets were prepared (~5 million DE20 droplets each) and loaded into an Xdrop DE20 Sort Cartridge for sorting.

Xdrop Sort detected 225 GFP-positive droplets from aliquot 1 and 234 from aliquot 2, and sorted 207 and 221 GFP-positive droplets, respectively, equating to a mean recovery of 93%. Figure 2 shows the Droplet Viewer screen from Xdrop Sort detailing the detection and sorting of the droplets for aliquot 1. The data for the Droplet Viewer report is automatically generated by Xdrop Sort to allow the user to investigate the success of the sorting process.

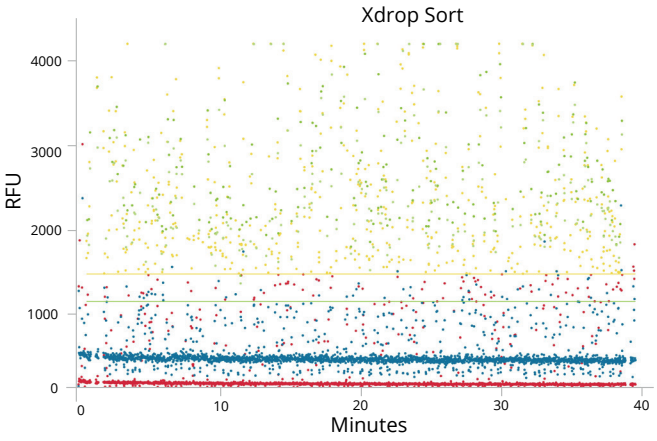

Figure 2. The Xdrop Sort Droplet Viewer analysis of droplet sorting for aliquot 1 shows that during the 40 minutes of sorting, 225 GFP-positive droplets were detected and 207 of these were sorted. Each pair of yellow dots represents a droplet with a fluorescence above the detection threshold (set by the user and indicated by the yellow line) and each pair of green dots represents a droplet that is successfully sorted (with a fluorescence above the sorting threshold, also set by the user and represented by the green line). Red and blue dots respectively represent droplets with fluorescence below the detection and sorting thresholds.

Recovery of living yeast cells from DE20 droplets

Living yeast cells can be recovered from DE droplets after sorting on Xdrop Sort. We plated sorted droplets in a volume of 50 µl directly onto a YPD agar plate and left it to dry for 5 minutes. The plate was incubated at 30°C for 3 days and then photographed with an iBright FL1000 fluorescence imaging system. The count of 90 green and 3 red colonies indicates efficient sorting (Figure 3).

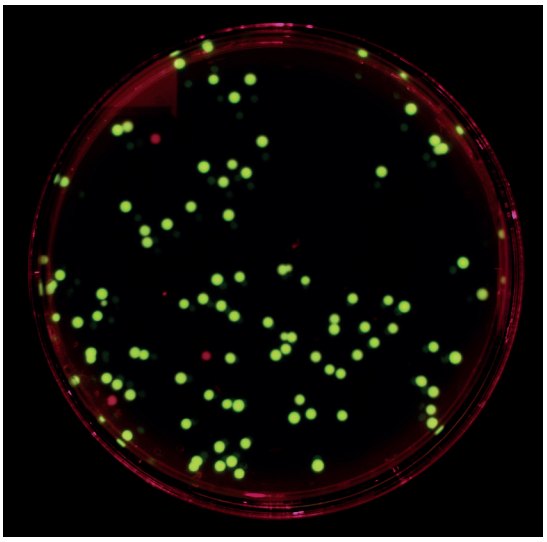

Figure 3. Composite image of yeast colonies after the plating of sorted DE20 droplets containing individual yeast cells directly onto an agar plate. This simple and efficient way of recovering living yeast cells yielded 90 GFP-expressing colonies (green) and just 3 mKate2-expressing colonies (red), indicating a high purity of sorting.

Conclusion

Xdrop Sort offers a robust and efficient method for screening and isolating the desired yeast cells. Here, we successfully used Xdrop Sort to sort GFP-expressing yeast cells from mKate2-expressing yeast cells with high purity and viability. We also showed that the yeast cells stay viable within Xdrop DE droplets and can even proliferate when cultured in the larger DE50 droplet type. Our results highlight the potential of Xdrop Sort for applications in the screening of microbial cells.

How Xdrop Sort works

Xdrop Sort encapsulates single living cells in highly stable double-emulsion (DE) droplets, supporting the analysis of individual cells with specific functionalities. Living cells can be recovered from the droplets. Xdrop Sort can sort Xdrop DE20 droplets with high fluorescence from droplets with low fluorescence to isolate cells with desired functionality. The sorting can be performed on 8 samples in parallel, allowing for the screening of >60 million droplets in just 45 minutes.

Learn more about Xdrop and Xdrop Sort at samplix.com.

References

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