APPLICATION NOTE

Samplix

# Improving the throughput and efficiency of screening for rare, active enzyme variants in very large libraries

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**Summary**

- During bulk cultivation, bacterial cells producing inactive enzyme variants can outgrow cells producing active but rare variants.
- Encapsulating cells into droplets prevents rare cells to be out-competed, making screening ineffective.
- Xdrop greatly increases the throughput for fluorescence-based screening, improving enzyme engineering efficiency.

## Introduction

Library sizes of >$10^{12}$ enzyme variants can be generated during enzyme engineering. However, conventional microtiter plate- or agar plate-based screening systems only allow a few thousand variants to be screened, leaving most of the generated variants unexplored. Therefore, ultrahigh-throughput screening (uHTS) technologies are critical for exploring the diversity of enzymes in a library.

In uHTS, a link must be made between each signal and the activity of the enzyme variant; and between the genotype and the generated signal (phenotype). Samplix's two sizes of double-emulsion droplets, designated DE20 (volume 1.6 pl) and DE50 (100 pl), form a closed compartment for cell growth and assays. This provides the link between genotype and phenotype.

Moreover, cells producing rare active enzyme variants are often outgrown in bulk cultivation, greatly complicating the identification of potentially improved variants. Encapsulated cells grow in isolation, so rare active variant-producing cells are not out-competed.

Samplix has developed a workflow using Xdrop and the Xdrop DE20 Cartridge to encapsulate living bacterial cells producing an enzyme library with rare active variants in DE20 droplets. The double-emulsion droplets support:

- Fluorescence-based analysis in a unique single-cell format and sorting using a cell sorter or Xdrop Sort.
- Recovery of the cells producing the desired variants for further growth and analysis.

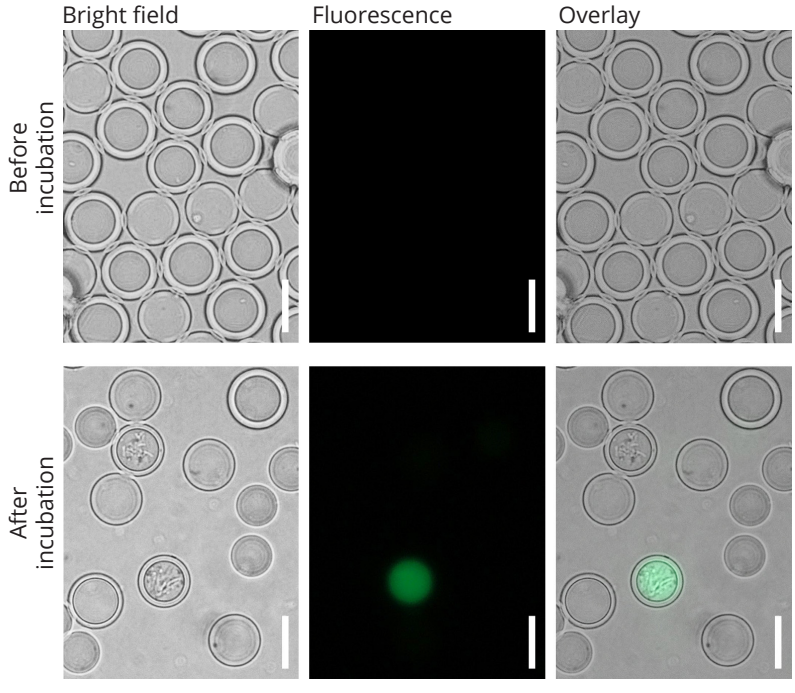


**Figure 1**. Bright field (first column), fluorescence microscopy (second column) and overlay (third column) images of Escherichia coli cells in 1.6-pl double-emulsion droplets (DE20 droplets). The top panels show the encapsulated cells before incubation. The bottom panels show them after 16 h incubation at 30°C.

Cells that produce an active endoglucanase variant convert the co-encapsulated fluorogenic substrate into fluorescein molecules (bottom center and bottom right). The scale bar is 20 µm.

## Enzyme variant production

We encapsulated a population of cryo stock *Escherichia coli* cells producing an endoglucanase library with an active-to-inactive enzyme variant ratio of 11%. The medium was TB cultivation media containing an antibiotic, IPTG to induce gene expression, and fluorescein di-beta-D-cellobioside (FCB) as a fluorogenic endoglucanase substrate.

The droplets were incubated at 30°C for 16 h on a rotary shaker at 5 rpm. The active enzyme variants convert the co-encapsulated FCB, liberating fluorescein. Inactive variants show no catalytic activity.

Afterwards, droplets were washed and analyzed via fluorescence microscopy. Figure 1 shows the images before (top row) and after (bottom row) incubation.

## Sorting and recovery of viable cells

Sorting was performed using a BD Influx™ Cell Sorter. High fluorescence was detected from 0.8% of the DE20 droplets (Figure 2). Reanalysis of the sorted DE20 droplets via flow cytometry and microscopy showed 57.5-fold enrichment (high fluorescene from 46% of the DE20 droplets). Subsequently, the bacterial cells producing the active endoglucanase variant were released from the DE20 droplets and plated on LB agar plates for further characterization.

## Conclusion

The viability and growth of cultured single cells producing active or inactive enzyme variants is sustainable in Xdrop DE20 droplets. Unlike during bulk cultivation, each cell is encapsulated in isolation, so faster-growing cells cannot outcompete other portions of the population.

This workflow shows that Xdrop could be used in uHTS of large cell libraries with the aim of isolating producers of rare, highly active enzyme variants and/or high-level producers. Here, we have shown that >50-fold enrichment can be readily obtained with the Xdrop workflow. Iterative rounds of enrichment could allow for the identification of so-called super-rare variants, making this a highly advantageous new approach in enzyme engineering.

For more information about Xdrop products and applications, visit samplix.com.



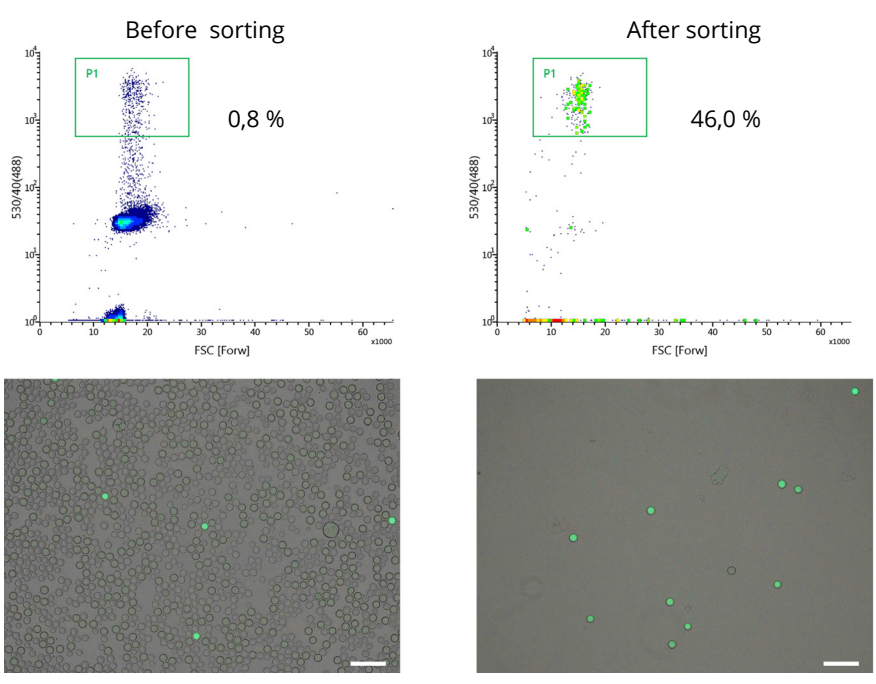


**Figure 2**. Flow cytometry analysis of DE20 droplets containing a bacterial endoglucanase library with rare active variants (top left). Reanalysis of enriched droplets containing active enzyme variants shows a 57.5-fold enrichment (top right). The overlaid brightfield and fluorescence microscopy images show DE20 droplets before sorting (bottom left) and after sorting (bottom right). The scale bar is 100 µm.