Application Note

Efficient Discovery of B Antigen-Cleaving Enzymes Using Ultrahigh-Throughput Droplet Microfluidics

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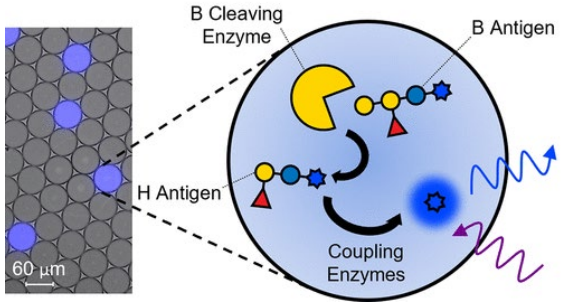
Ultrahigh-Throughput Single Emulsion Droplet Screening for the Discovery of New B Antigen Cleaving Enzymes

ACS Catal. 2024, 14, 12884−12894

https://pubs.acs.org/doi/10.1021/acscatal.4c02165



Graphical Abstract: Enzyme Screening Strategy

Introduction

ABO blood type mismatch is a critical challenge in blood transfusion. B-type red blood cells (RBCs) carry B antigens, and transfusions from a B-type donor to an incompatible recipient can lead to immune reactions, potentially resulting in death. The goal is to convert B-type RBCs to universal O-type RBCs by removing the terminal sugar (galactose) from the B antigen. Previous enzyme-based methods for this conversion have either been inefficient or required impractical amounts of enzyme. This study presents a **droplet-based microfluidic screening system** to discover new enzymes from metagenomic libraries capable of cleaving the B antigen efficiently.

Methods

A high-throughput single emulsion droplet microfluidic system was developed to encapsulate single cells from metagenomic libraries along with a fluorogenic B antigen substrate within water-in-oil droplets (Fig. 1). Fluorescent droplets, containing cells capable of cleaving the B antigen, are then detected using fluorescence-activated droplet sorting via the On-chip Sort, a microfluidic-based cell sorter. The positive droplets then undergo validation, cloning, and characterization of the discovered enzymes.

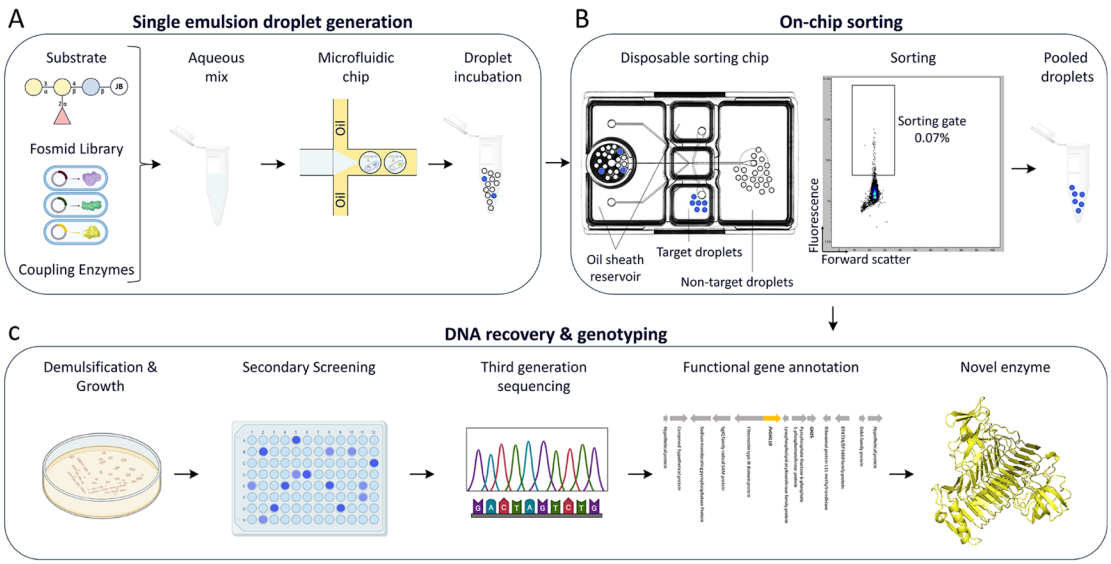


Figure 1. Workflow of the screening process: (A) Droplet Generation: Single cells from a metagenomic library are encapsulated in pL-sized droplets along with the B antigen substrate and coupling enzymes. (B) Sorting: Fluorescent droplets are sorted using On-chip Sort (On-chip Biotechnologies) (C) Cloning and Validation: Positive hits are cloned, sequenced, and characterized to confirm their ability to cleave the B antigen.

Results

A fosmid library was created using *E. coli* with metagenomic DNA sourced from the feces of AB blood type donors. This library was encapsulated in droplets and cultured for 48 hours to detect any α-galactosidase activity that cleaves the terminal sugar from the B antigen. From the screening of 1.4×10^5 droplets, 93 (0.07%) positive droplets with a fluorescence exceeding a set threshold (>3-fold background) were collected. After secondary screening of the recovered clones, *Pv*GH110, a glycoside hydrolase family 110 α-1,3-galactosidase, was successfully identified. This enzyme was derived from *Phocaeicola vulgatus*, a bacterium in the human gut microbiome.

Subsequent cloning, expression, and purification of the *Pv*GH110 in *E. coli* allowed for the evaluation of its enzyme activity. When compared to AgaBb and *Bf*Gal110A, which are known for having the highest specific activities against the B antigen, *Pv*GH110 demonstrated similar conversion efficiency. Furthermore, in the presence of dextran, which enhances enzyme reactions through macromolecular crowding effects, *Pv*GH110 was able to completely convert B-type red blood cells into O-type red blood cells (Fig. 2).

The use of droplet technology in this study enhanced the screening efficiency compared to traditional well-plate-based methods and significantly reduced the quantities of enzyme and substrate needed. For instance, only about 30 µg of substrate was used in this screening, whereas conventional methods would have require approximately 75 mg, marking a 2500-fold reduction. The study presents a robust, efficient, and accessible system for discovering new enzymes capable of converting B-type RBCs to O-type. The identified enzyme, *Pv*GH110, is a promising candidate for clinical applications, and the method could be further utilized to discover other antigen-cleaving enzymes, paving the way for future advancements in blood compatibility and transfusion medicine.

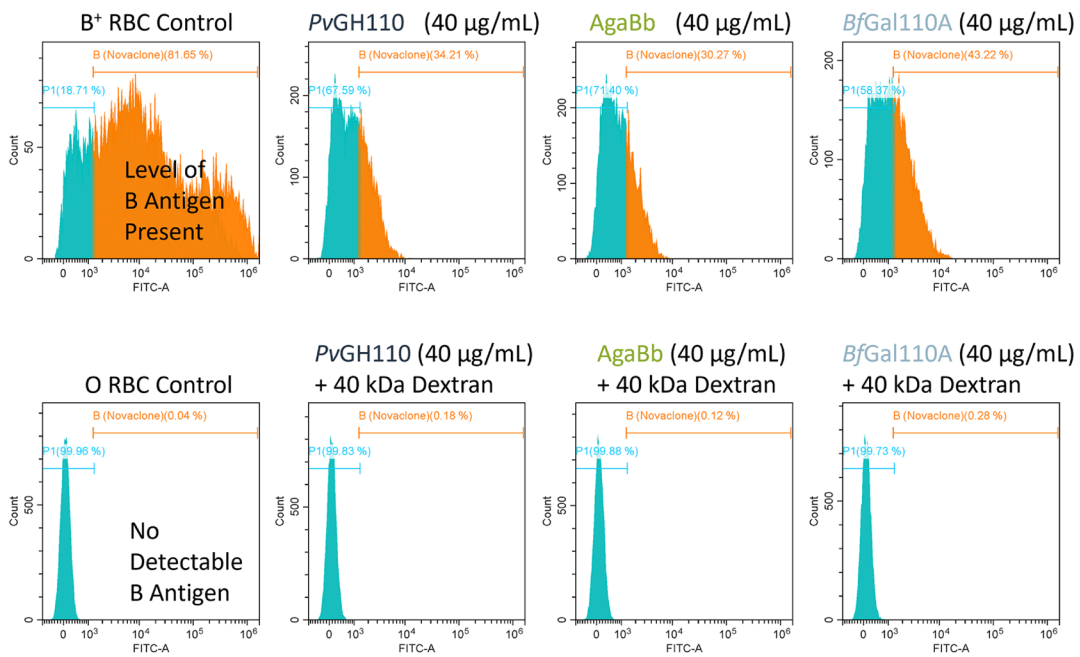


Figure 2. Evaluation of B antigen-cleaving enzyme activity: This figure shows the activity of the newly discovered enzyme, *Pv*GH110, in converting B+ RBCs to O-type RBCs. It compares the conversion efficiency of *Pv*GH110 with known enzymes (AgaBb, *Bf*Gal110A), evaluated in the presence and absence of dextran.

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