MERIT Internship Report

School of Science, Department of Chemistry
Biomolecular Chemistry Laboratory Dr. 3rd year
MERIT 8th
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Internship Period

2022/1~2022/3

Host Company

On-chips Biotechnologies Co., Ltd; User Support Department。

Research Topic

Development of the screening model for the protein-based Ca^{2+} sensor.

Research Background

Calcium ion (Ca^{2+}) plays a vital role in cellular signals and physiological processes such as muscle contraction and neurotransmitter release. Abnormalities in Ca^{2+} signaling can have severe pathological consequences, including cardiovascular disease, Alzheimer's disease, etc. Therefore, investigation of the fundamental roles of Ca^{2+} in cellular signals is necessary for the fields of biology and medicine. Besides, a successful approach to monitor Ca^{2+} dynamics in cells has been the use of fluorescent Ca^{2+} probes. To date, the development of fluorescent Ca^{2+} sensors has proceeded for more than two decades. Generally, two types of Ca^{2+}indicators have been widely used: chemical Ca^{2+} indicators and protein-based Ca^{2+} indicators. Compared to chemical Ca^{2+} indicators, protein-based Ca^{2+} indicators are genetically encoded, which makes them easily optimized by simply mutating their genetic information and then screening the variants. However, one of the most challenging problems for optimizing protein-based Ca^{2+} indicators is that the screening process is way too time-consuming.

To develop a novel optimization process that is faster and more efficient, we applied our droplet technology and cell-sorting techniques for sensor development. Herein, we prepared water-in-oil droplets containing one cell averagely by On-chip Droplet Generator and sorted the droplet with the highest fluorescence intensity. According to our developed process, thousands of variants can be screened at one time.

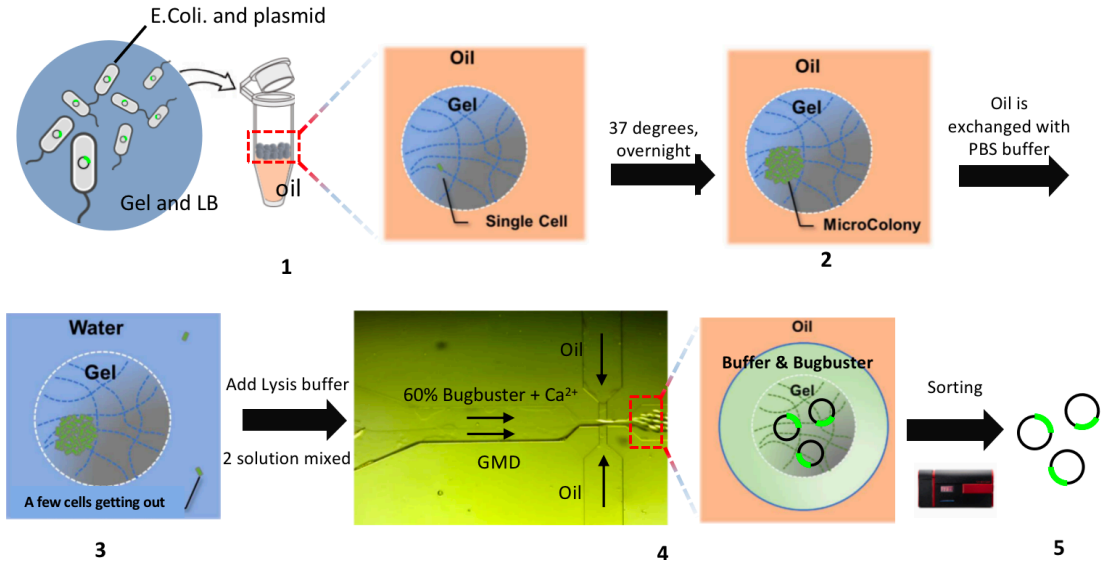

Figure 1

As demonstrated in **Figure 1**, we first prepared gel balls containing a single colony in oil by droplet generator. Then, we got the microcolony after 37 degrees incubation and performed the buffer exchange. Afterwards, to lysis the microcolony and extract the proteins, we used the droplet generator again to produce the droplets that mix gel balls and lysis solution. After 1 hour of incubation at 37 degrees, the gel balls were filled with fluorescence protein and we screened the droplet to collect those gel balls with the highest fluorescence intensity.

Result

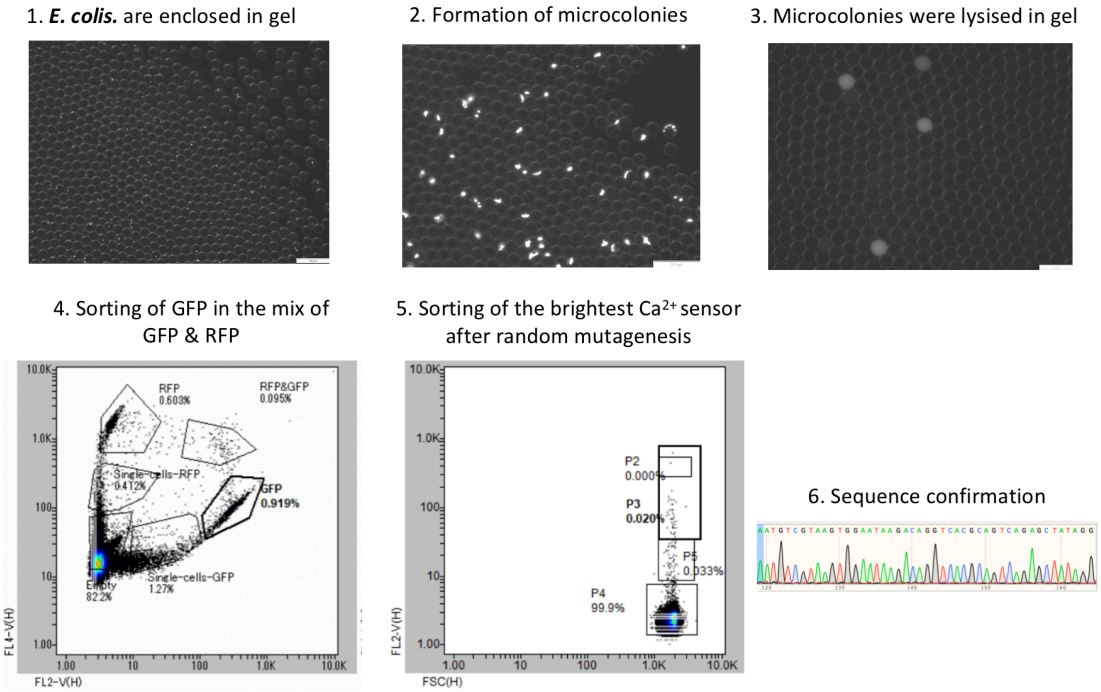

Figure 2

The results are shown in the **Figure 2**. 1. The single colonies were successfully enclosed in the gel by controlling the LB concentration with colonies. 2. After incubation at 37 degrees overnight, we obtained the microcolonies in the gel. 3. Then, we mixed the Bugbuester solution with gel balls by involving gel balls into droplets to colonies. 4. Successful sorting of GFP & RFP two types of proteins. 5. Mixed mutated library of Ca^{2+} sensor (GCaMP6) after random mutagenesis was successfully sorted by on-chip sorter. 6. DNA was extracted from the sorted droplets and the sequence was confirmed.

Conclusions & Perspective

We developed a standard workflow for analyzing or sorting fluorescence protein-based protein complexes according to their fluorescence intensity and wavelength. We hope this technique would be a powerful tool to accelerate protein engineering.

Acknowledgement

I would like to express our sincere thanks to Mr. Honma and Dr. Ishige for accepting and supervising this internship. I deeply appreciate Professor Campbell and Associate Professor Takuya Terai of our laboratory for supporting this internship. I am also grateful to the MERIT program for providing this valuable opportunity.