

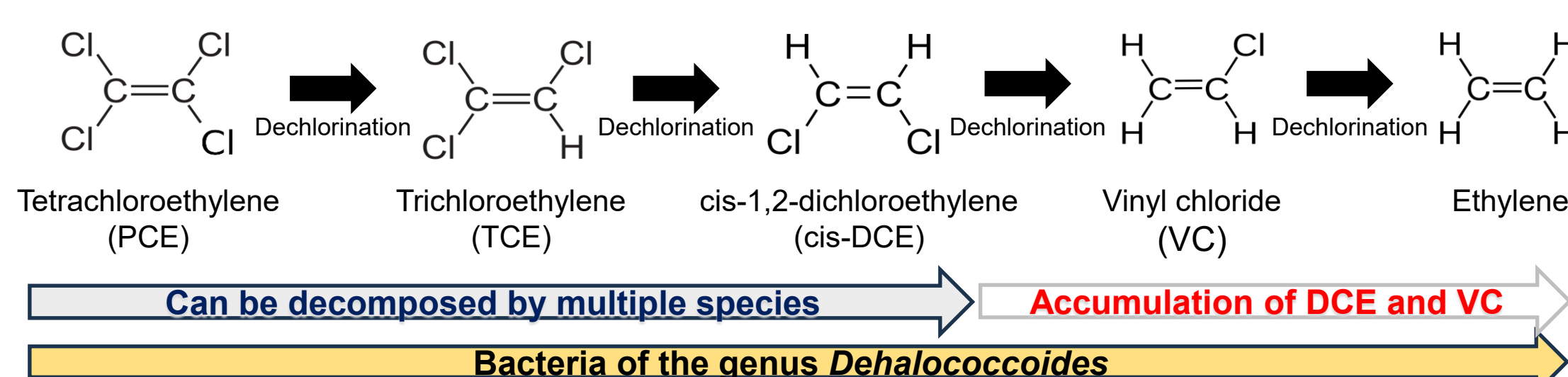
Isolation of Bacteria of the Genus *Dehalococcoides* Using a Microfluidic Chip-based Cell Sorter

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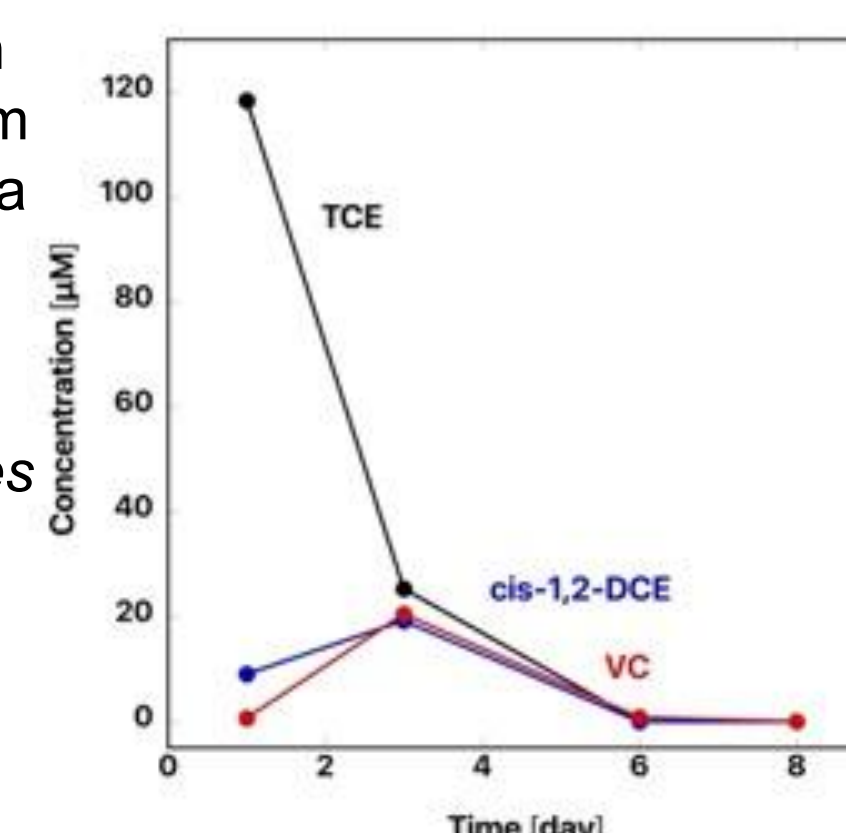
Introduction

In recent years, soil and groundwater contamination caused by chloroethylenes has become a serious issue. Due to their harmful effects on human health such as carcinogenicity and neurological disorders, decontamination is necessary. Among various decontamination methods, bioremediation—using microbes to decompose contaminants—has gained significant attention. Chloroethylenes are decomposed through reductive dechlorination reactions by various anaerobic bacteria. Among anaerobic bacteria, only *Dehalococcoides* spp. are capable of completely dechlorinating dichloroethylene and vinyl chloride into harmless ethylene. Therefore, *Dehalococcoides* spp. are expected to be applied in bioremediation.



However, *Dehalococcoides* spp. are known to be difficult to isolate due to their extremely low decomposition activity and slow growth rates, as well as their reliance on symbiotic bacteria for these activities. Isolation is important to clarify various biological properties of symbiotic bacteria.

We have constructed a novel microbial consortium with high trichloroethylene (TCE) decomposition activity from a contaminated site containing chloroethylenes in Chiba Prefecture, Japan (right figure). We have also isolated three bacterial strains from a known microbial consortium with TCE decomposition activity that appeared to be symbiotic microbes for *Dehalococcoides* spp. (table below). In the present study, we conducted experiments to obtain novel bacterial strains of the genus *Dehalococcoides* from the microbial consortium using droplets and the symbiotic microbes.



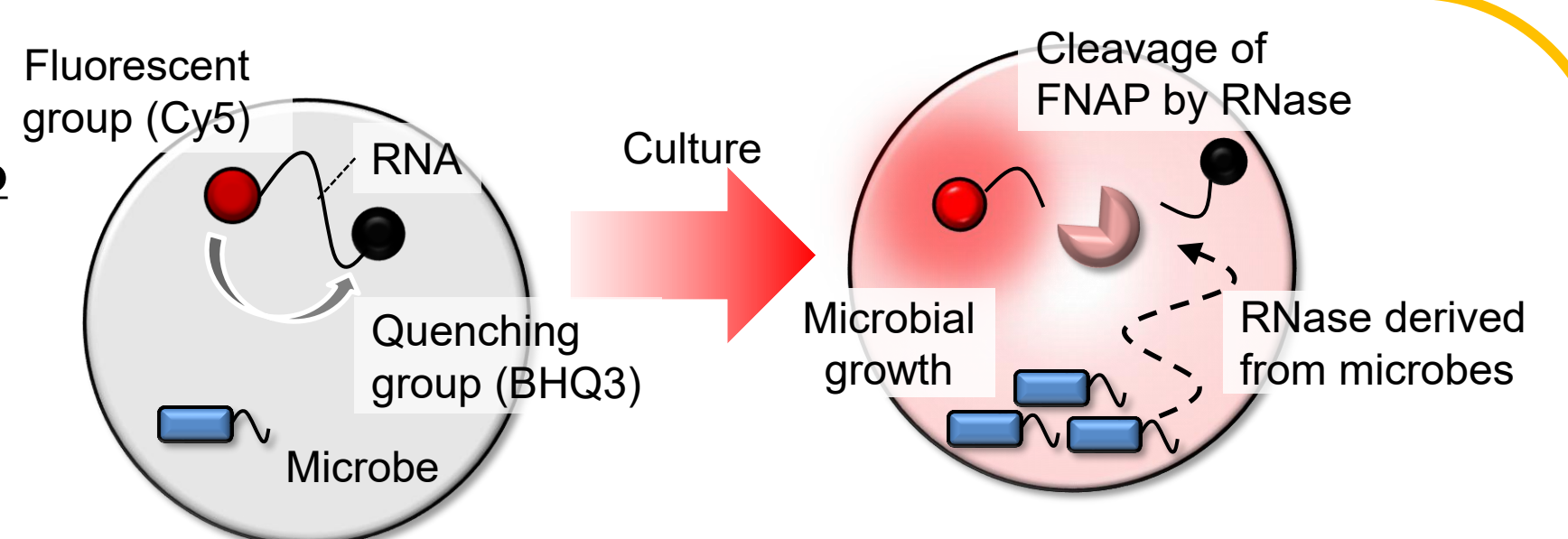
TCE-decomposing activity of the novel microbial consortium.

<i>Youngiibacter</i> sp.
<i>Petrimonas sulfuriphila</i> IBARAKI
<i>Desulfovibrio desulfuricans</i>

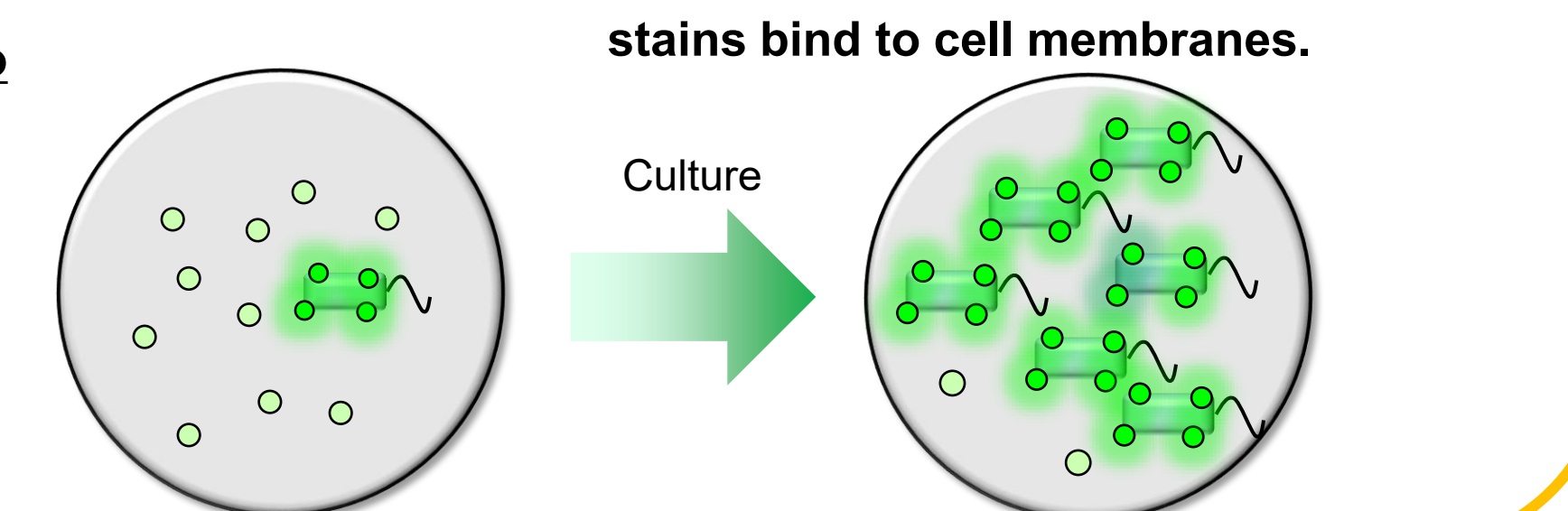
Symbiotic microbes used in this study.

Method

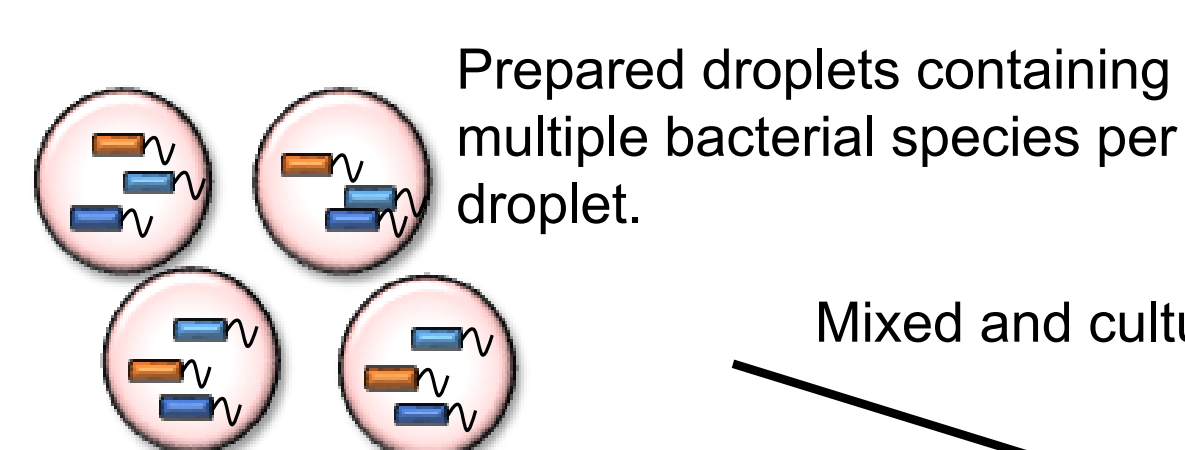
Fluorescence due to On-chip FNAP-sort



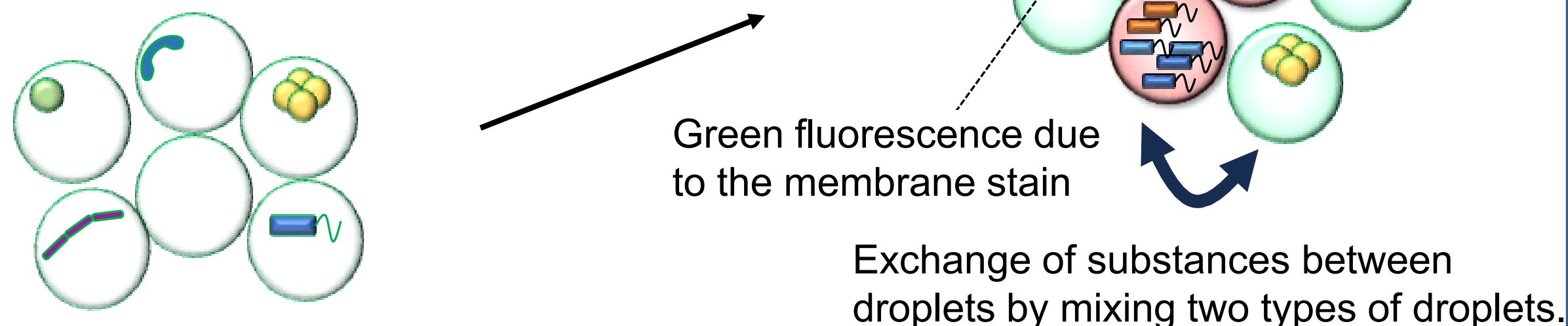
Fluorescence due to membrane stains



✓ Droplets containing three species of symbiotic bacteria

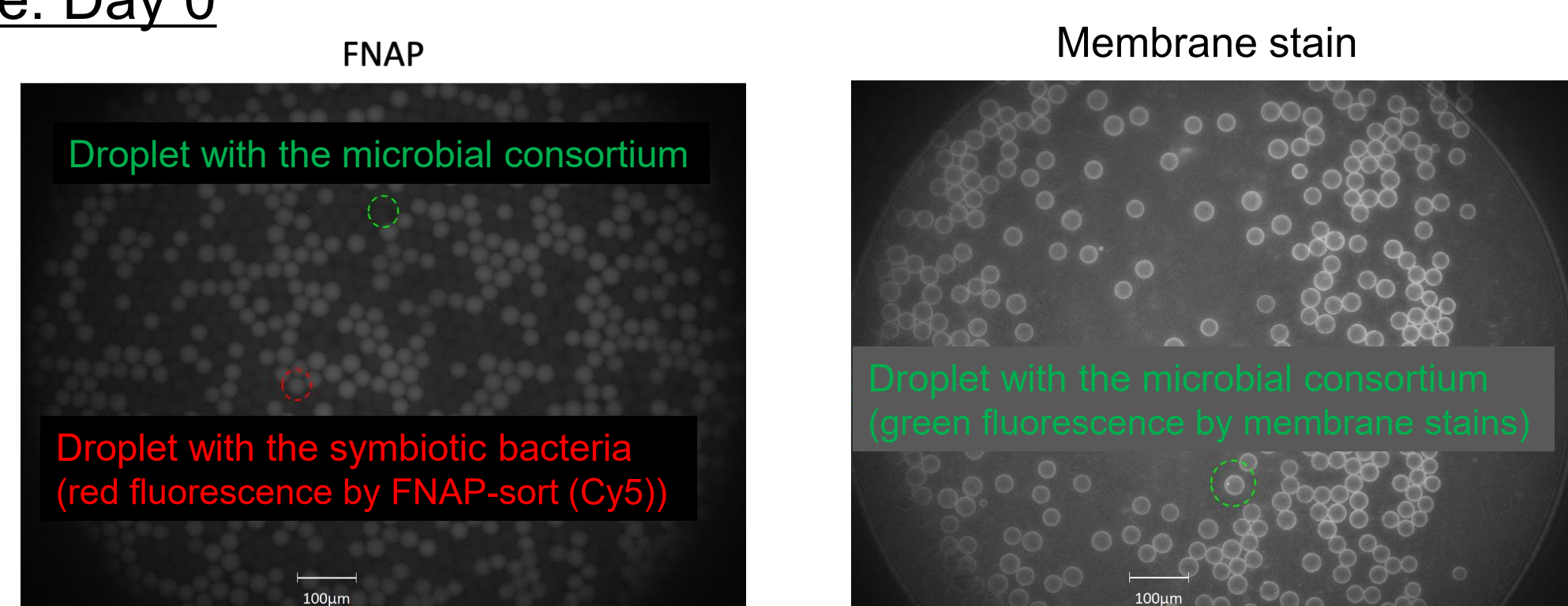


✓ Droplets containing microbial consortia

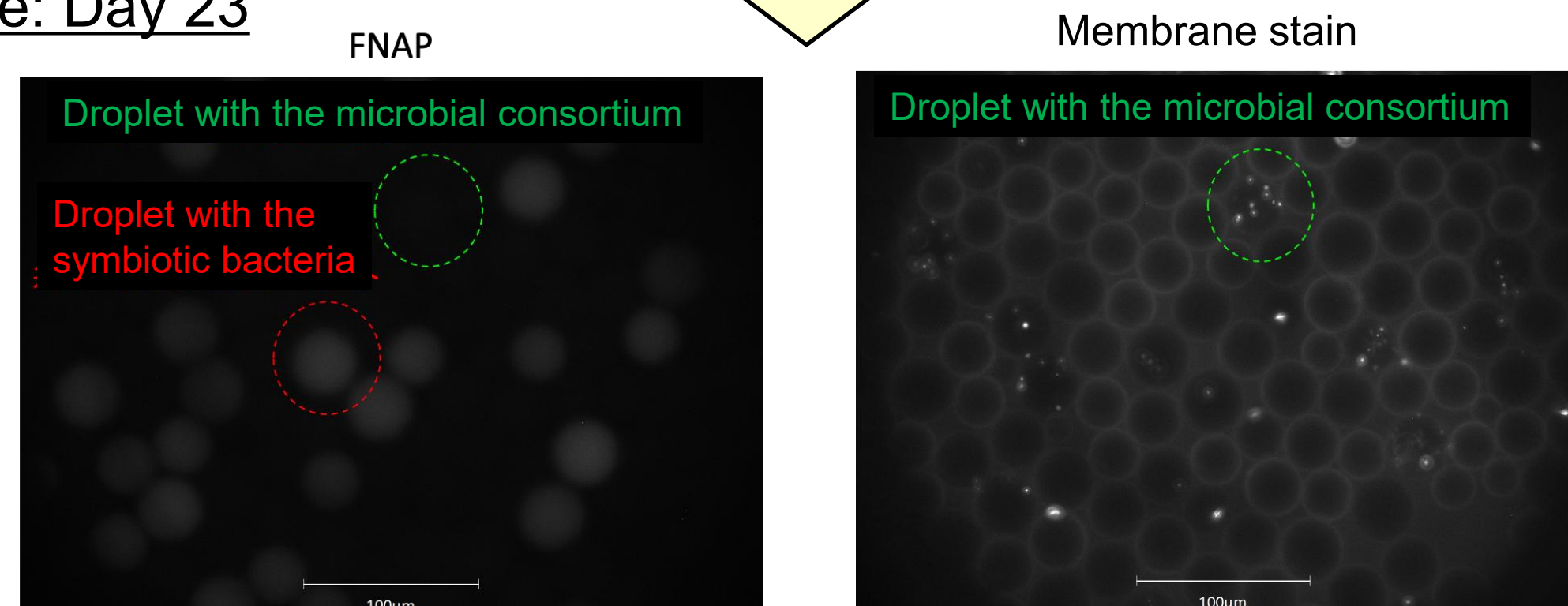


Droplet culture

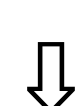
Culture: Day 0



Culture: Day 23

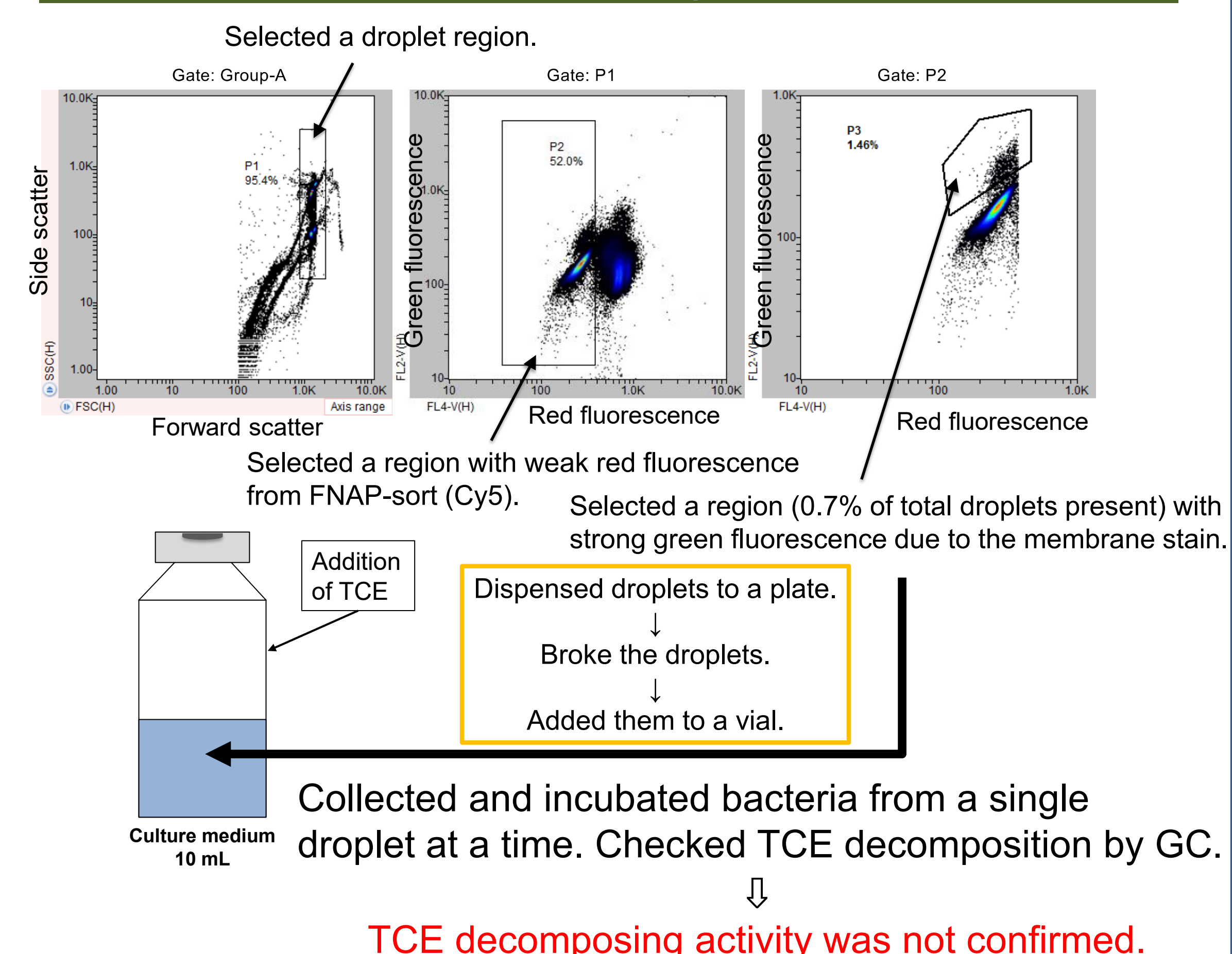


Bacterial growth in the droplets containing microbial consortia was successfully confirmed during the co-culture.



Interaction with droplets containing symbiotic bacteria.

Sorting



TCE decomposing activity was not confirmed.

Conclusions

- ✓ Bacterial growth in the droplets containing the microbial consortium was successfully confirmed during the co-culture with droplets containing the symbiotic bacteria.
- ✓ TCE-decomposing activity in vials was not confirmed.
- ✓ The culture environment needs to be re-evaluated to identify potential issues, such as an inappropriate culture medium (components produced by the symbiotic bacteria may be needed, etc.).