

Molecular Understandings of Microbial Ecology in a Complex Environment

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Question 1:

Can you determine diatom and bacteria abundance in a complex environment using Q-PCR with broad primer sets?

Background:

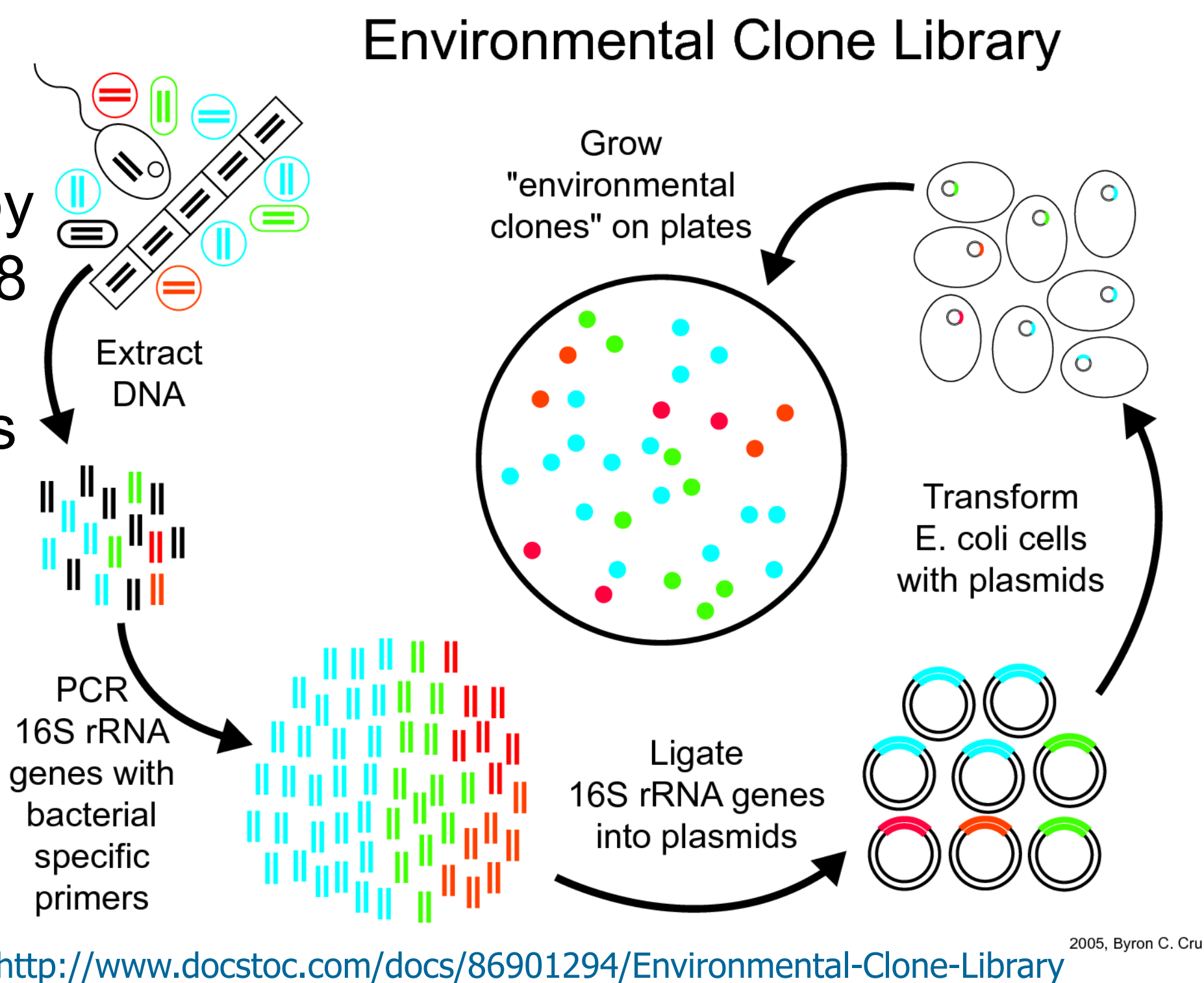
Treatment wetlands to serve as natural barriers for chemical contaminants that can affect potable water supplies and sensitive aquatic habitats. This project will begin to provide an understanding of the microbial communities present within a constructed wetland periphyton biofilm cell (below) using QPCR to quantify the amount of bacteria and diatoms in the biofilm



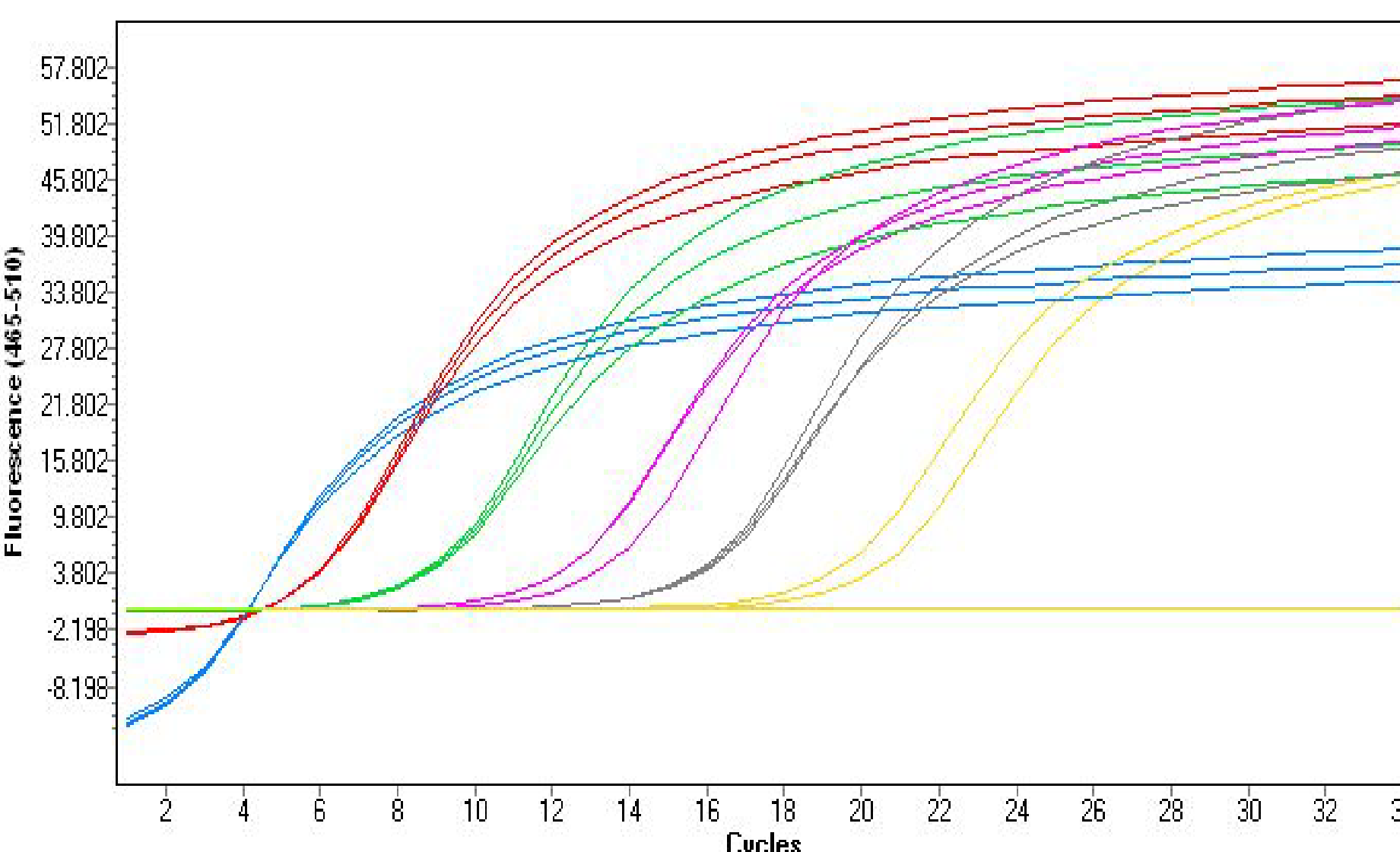
Methods:

Clone Library:

Collection of bacterial 16s DNA generated by EUB338 and EUB 518 primers. Each individual sequence is ligated and transformed into a single cell which is then grown up on plates resulting in amplification and division of each DNA fragment.



Standards: Are made from a clone and has a known concentration and amplicon length which is used to determine the copy number per microliter. Dilutions are used to make the standard curve (left).

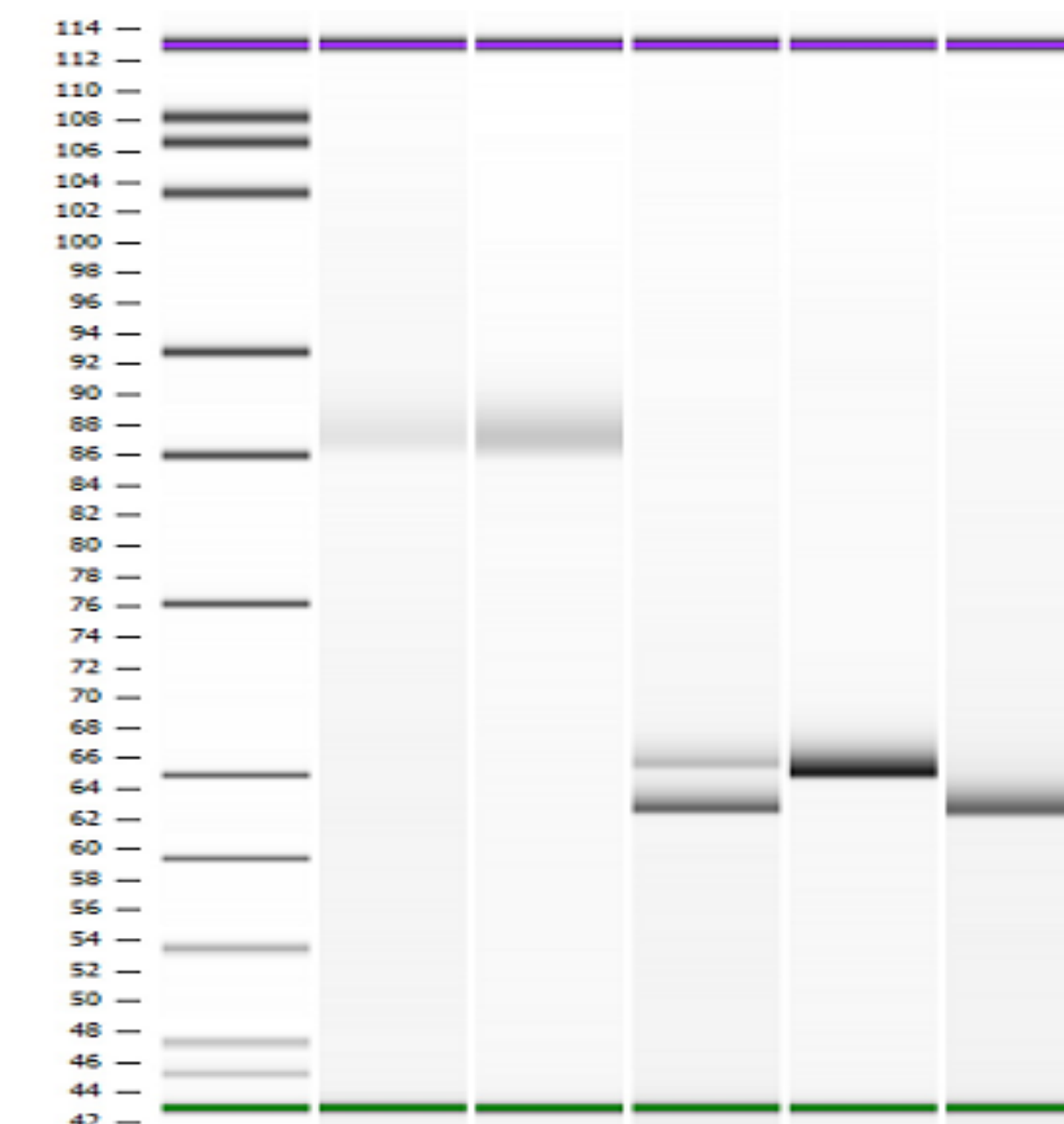


Q-PCR: Quantitative real time polymerase chain reaction. SYBR green, a fluorescent dye, binds all double stranded DNA and detection is monitored by measuring increase in fluorescence as the DNA is amplified

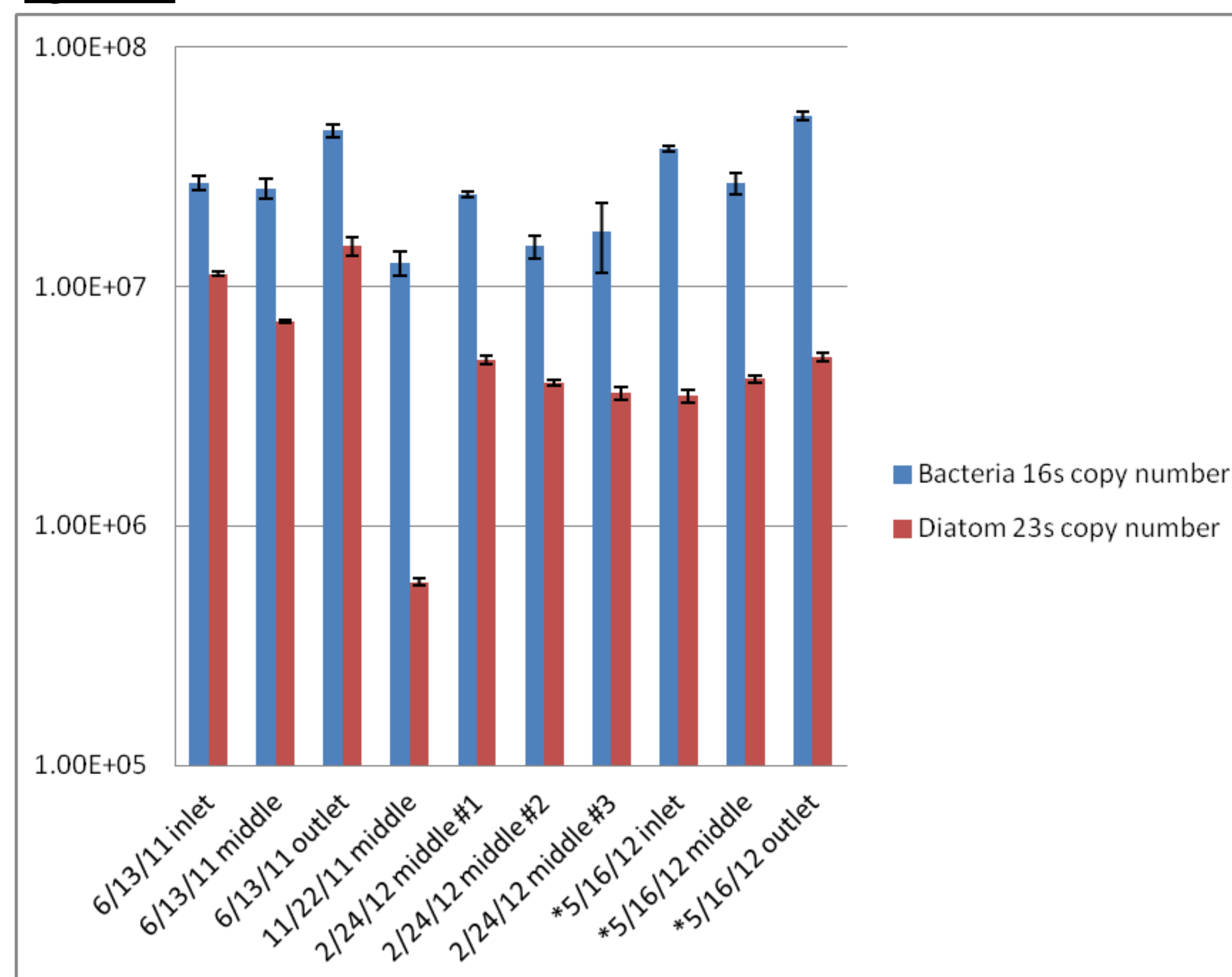
Results:

Clone Library: Bacterial clone library showed that 50% of what the EUB primers were amplifying were 16s chloroplast sequences from the diatoms. To compensated for this the number of "bacterial" copies will be cut in half to better represent the true bacterial count. The diatom clone library showed 100% diatoms sequences and revealed a monoculture of Bacillrophyta present in the biofilm.

Standards: From the clone library, standards were made by re-amplification and gel purification. The concentration and amplicon length were determined by an Agilent Bioanalyzer (right).



QPCR:

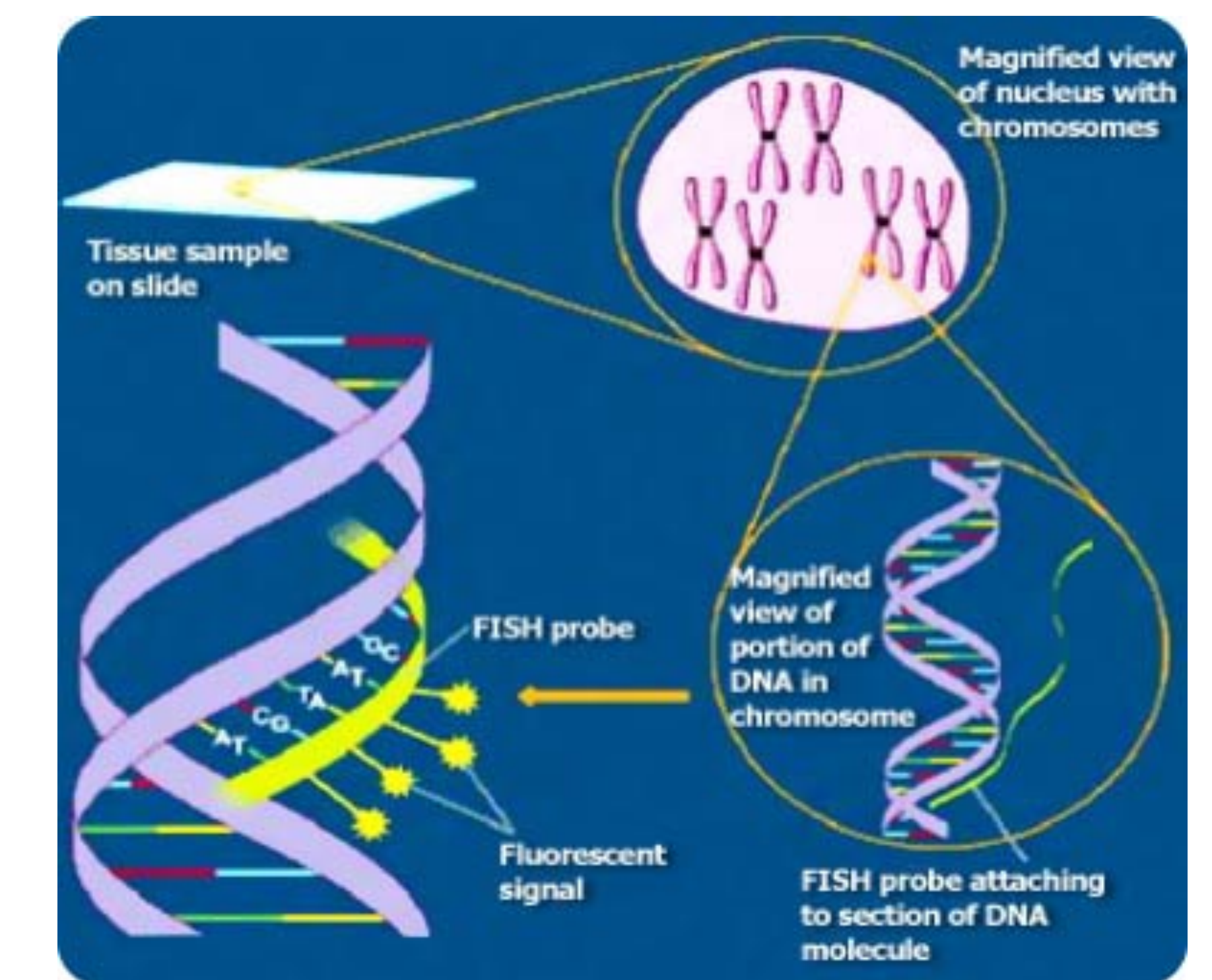


QPCR results show that the periphyton biofilm is relatively homogenous from inlet to outlet. There are also 2-3 times more bacterial 16s copies than 23s copies. Seasonal comparison also shows significantly more bacteria and diatoms in the summer than winter.

Question 2:

What is the binding specificity of a fluorescent in situ hybridization (FISH) probe designed to hybridize with the delta proteobacter geobacter sulfurreducens?

Basic FISH procedure

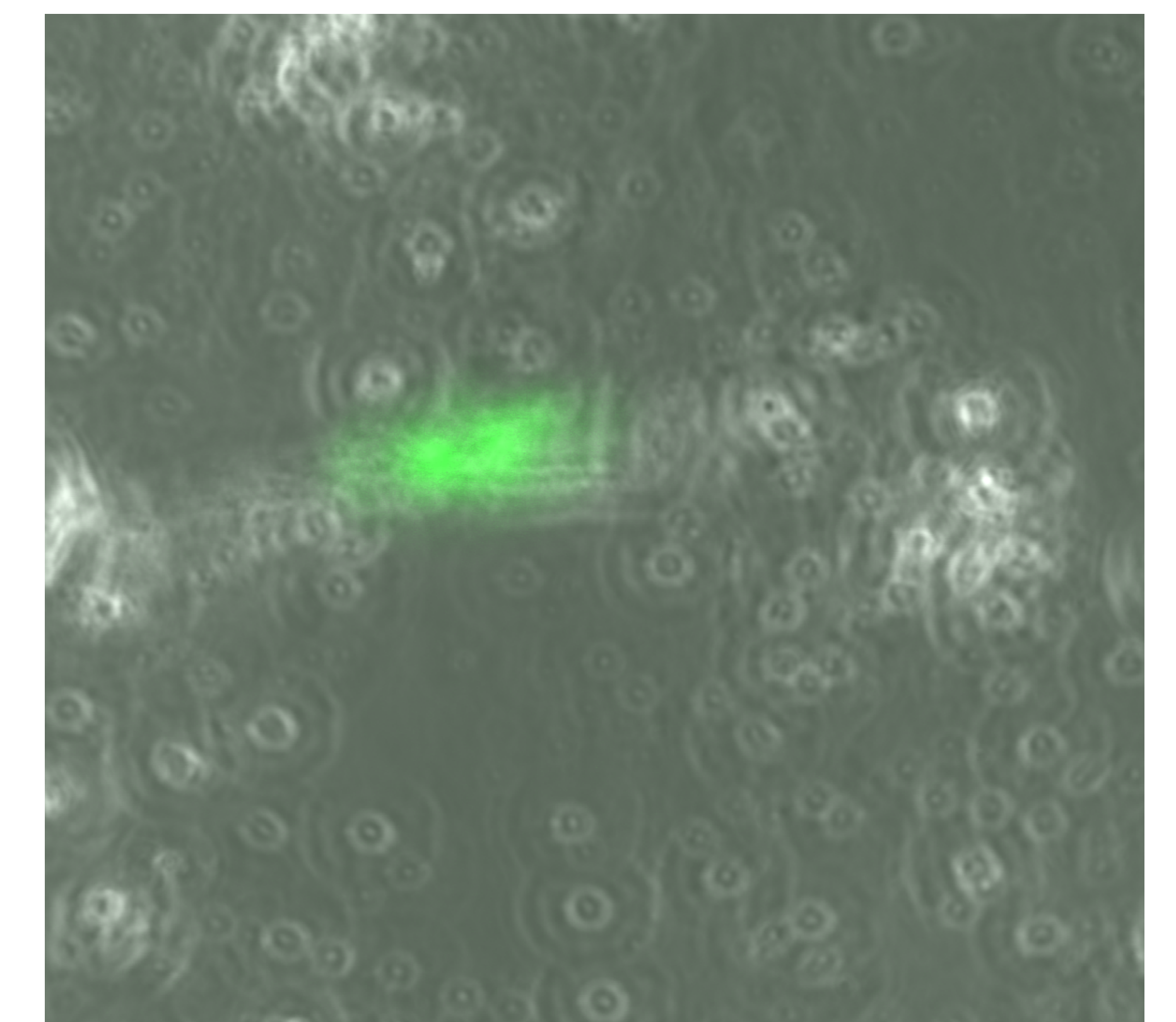


Background

The binding specificity of a FISH probe can be adjusted through adjusting the concentration of formamide in the hybridization buffer.

Optimizing the binding specificity is necessary to be able to confidently identify target microorganisms in complex samples.

Plan: Compose hybridization buffers with varying concentrations of formamide to determine the maximum concentration while still producing proper binding.



Geobacter sulfurreducens stained with the FISH probe delta 495a