



Explorations in Bacteriology Using Microfluidics

My research this summer consisted two main projects, both of which involved the characterization and amplification of microbial genomic DNA.

Counting Cyanobacteria Genome Copy Number

Background

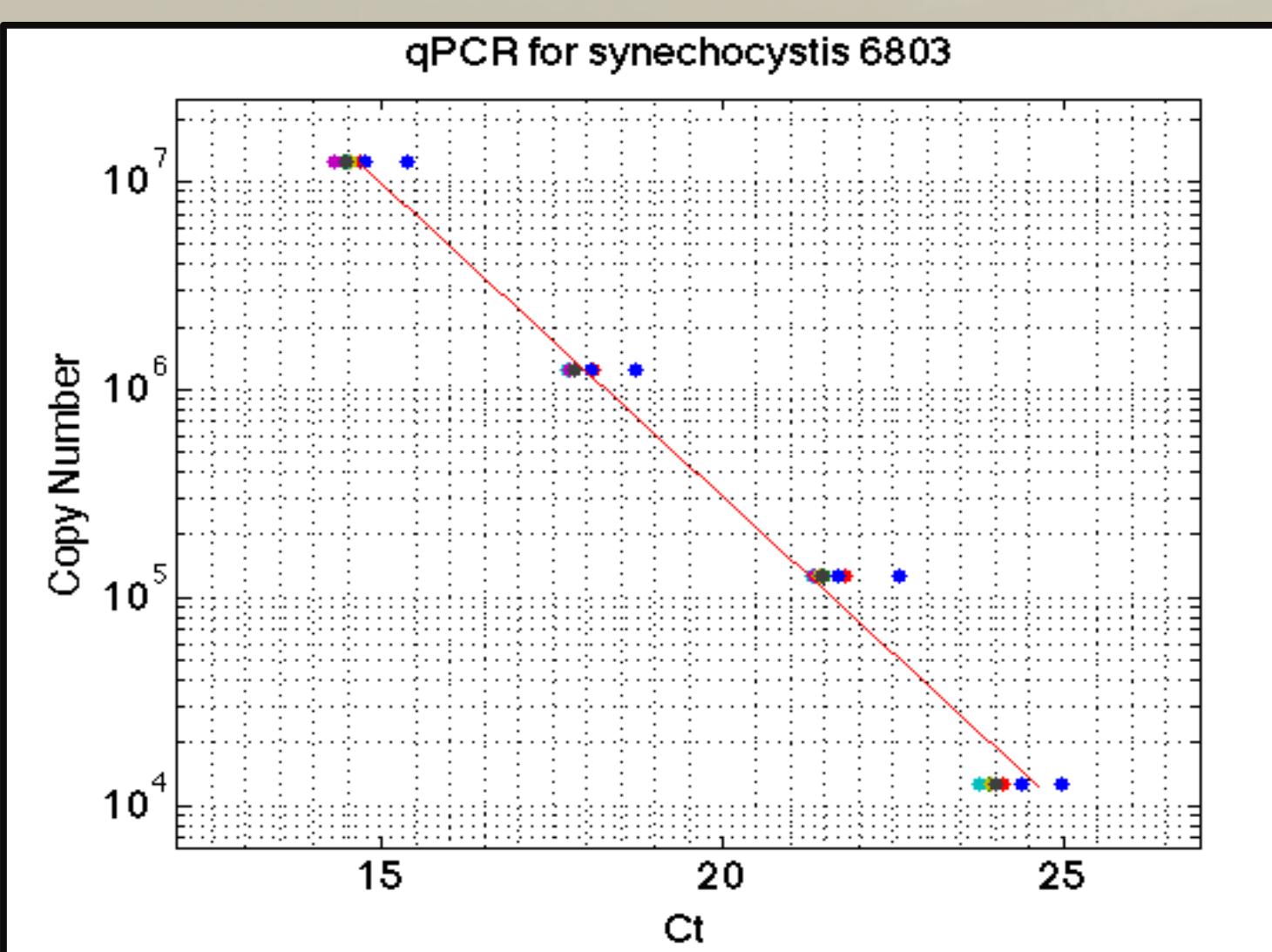
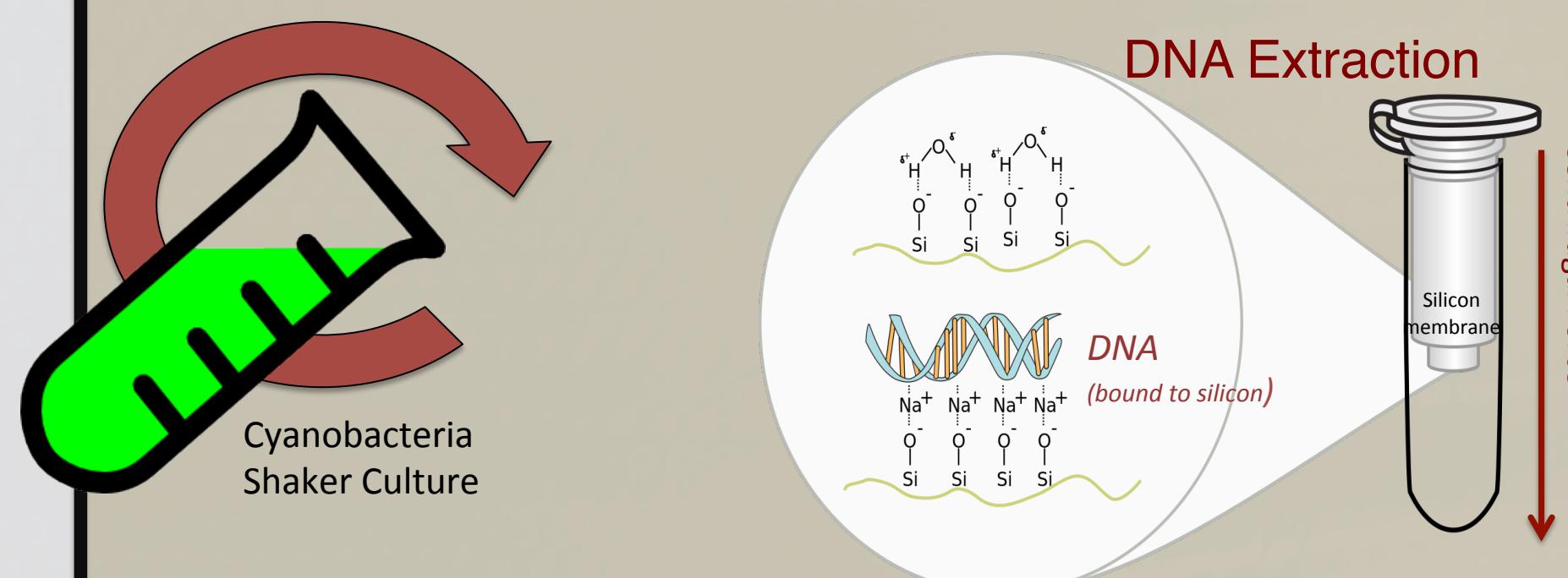
Bacteria frequently harbor multiple copies of their genome. This is called Polyploidy. Griese *et al.*¹ used fluorescence imaging to find that a strain of cyanobacteria called **Synechocystis PCC 6803** (Syn. 6803) is “highly polyploid”, with over 200 copies of its genome. I set out to more accurately count the genome copy number of Syn. 6803 using digital PCR.

Experiment Design

- Single Syn. 6803 cells, sorted with FACS, were lysed with lysozyme at 37°C
- Lysis product was pre-amplified to a concentration appropriate for dPCR
- Pre-amplification product was then loaded into the dPCR microfluidic chip and ran
- Copy number was calculated using dPCR quantification, accounting for pre-amplification

1. Cell Culture & DNA Extraction

Cyanobacteria Syn. 6803 was inoculated into test tubes and grown at 37°C for >7 days before cell harvest and extraction of genomic DNA using lysozyme and silicon membrane centrifugation.



3. Primer Characterization

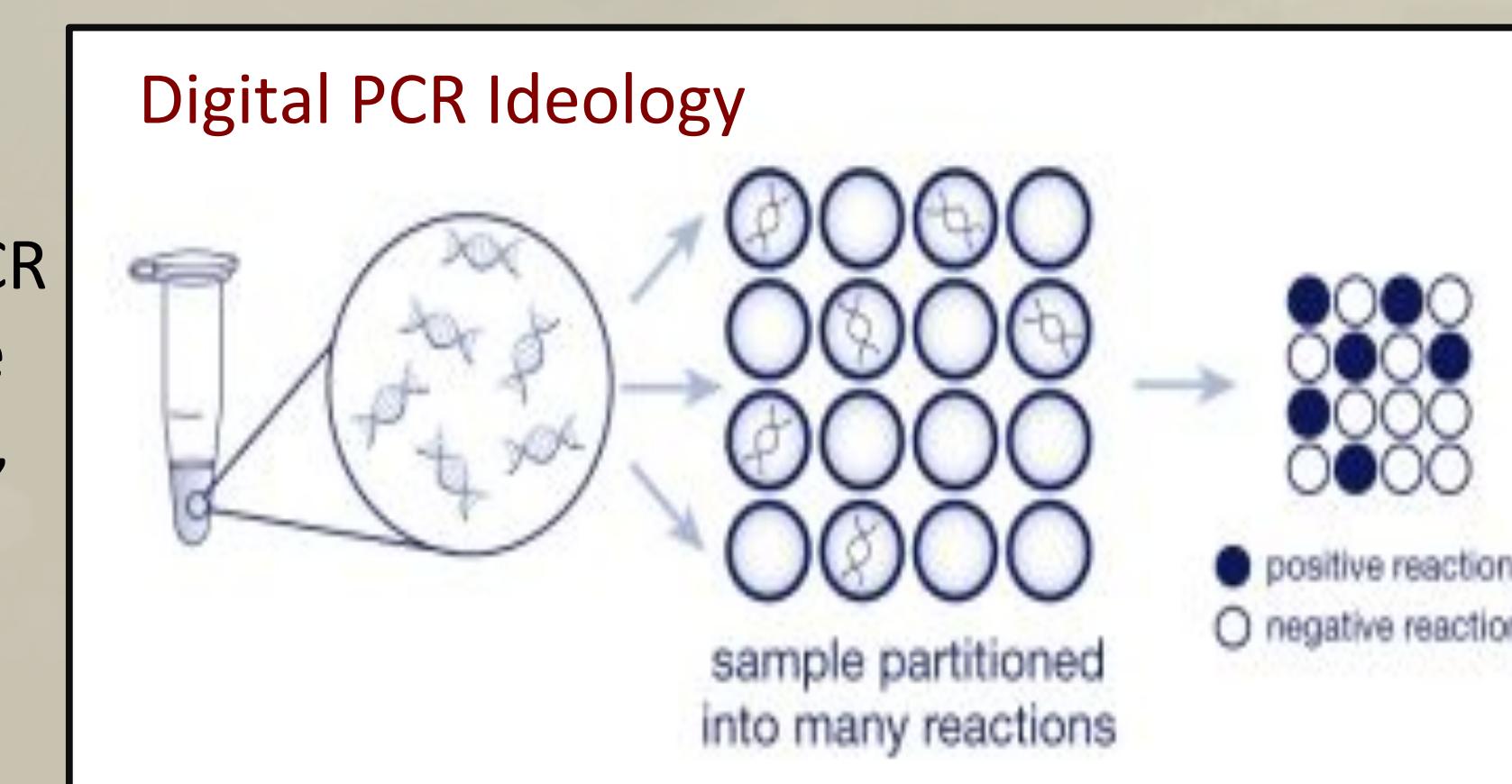
Primer efficiency was characterized using qPCR and linear regression. When 100% efficient, DNA quantity (fluorescence) will double after each PCR temperature cycle. My primers were characterized to be approximately 100% efficient.

$$F = F_i (2 - \epsilon)^{\text{Cycle}}$$

F = Fluorescence, $(2 - \epsilon)$ = Efficiency

4. Digital PCR (dPCR)

Digital PCR accurately quantifies DNA by splitting the PCR reaction into many (770) chambers such that only some chambers contain DNA template. During thermocycling, only the chambers which contain DNA template will undergo PCR, increasing their fluorescence. DNA is quantified by counting these chambers.



Results and Conclusions:

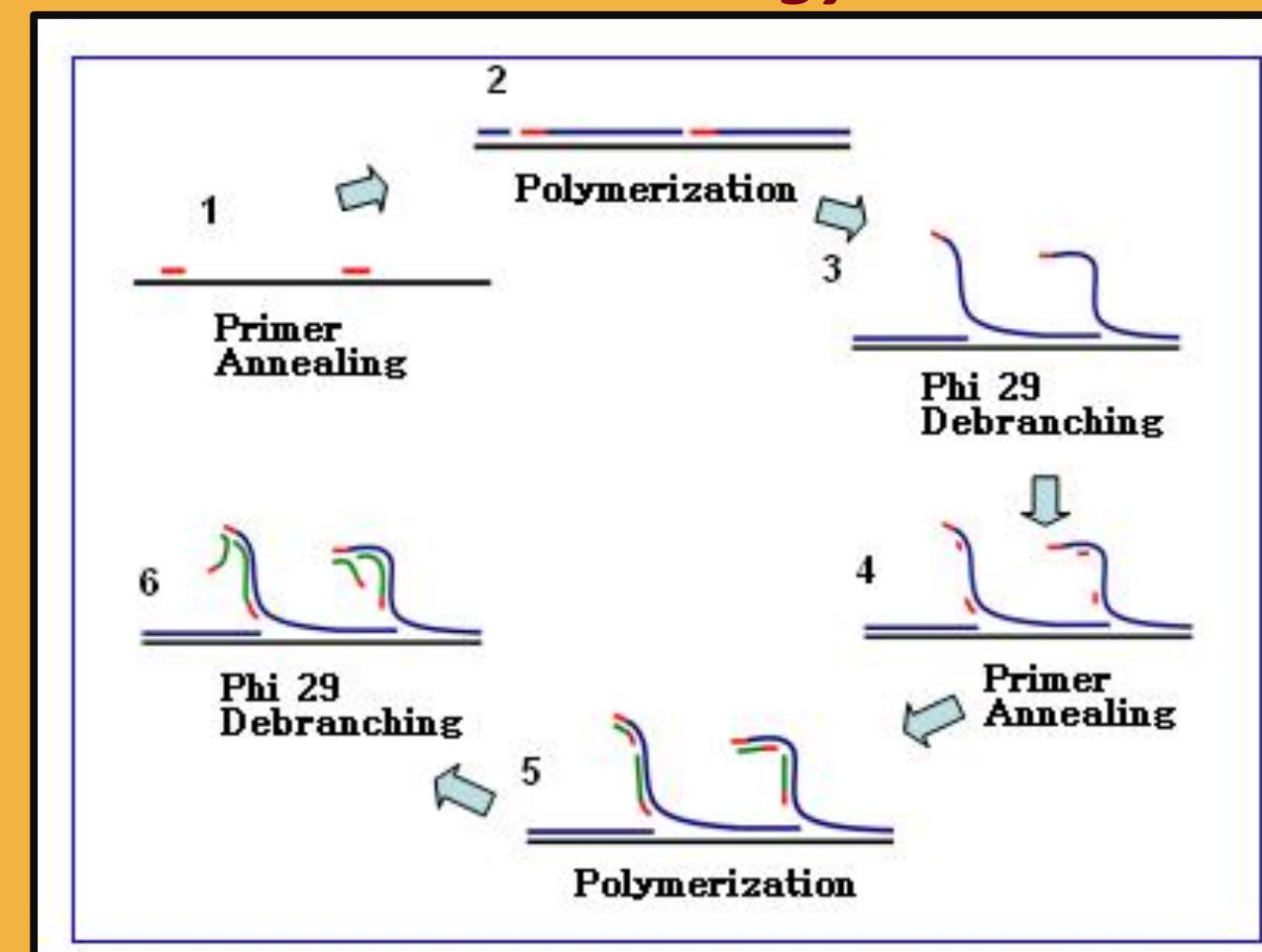
Initial experiments performed using all 8 primers in pre-amp and dPCR run yielded overabundant DNA concentrations, attributed to amplification of non-genomic DNA. The experiment was repeated using only single primers, which yielded low amounts of DNA in all cases, which was attributed to cells not lysing. Lysis duration was extended to 18 hours but failed to yield results. Results from dPCR using extracted gDNA support theory of failed lysis.

Multiple Displacement Amplification (MDA) Reactions on Microfluidic Chips

Background

Multiple Displacement Amplification (MDA) is an isothermal (30°C) DNA replication method, frequently used as a first step in DNA sequencing.

MDA Reaction Ideology



Advantages

- May amplify genomic DNA of unknown sequence
- Does not require thermocycling
- High yield and genome coverage
- Low error frequency

Disadvantages

- Non-specificity may lead to amplification of DNA contaminants
- Amplification bias is generated as the probability of amplicon amplification increases with amplicon abundance.
- Allelic dropout, the non-amplification of one of a pair of heterozygous alleles, is common

Project Goal

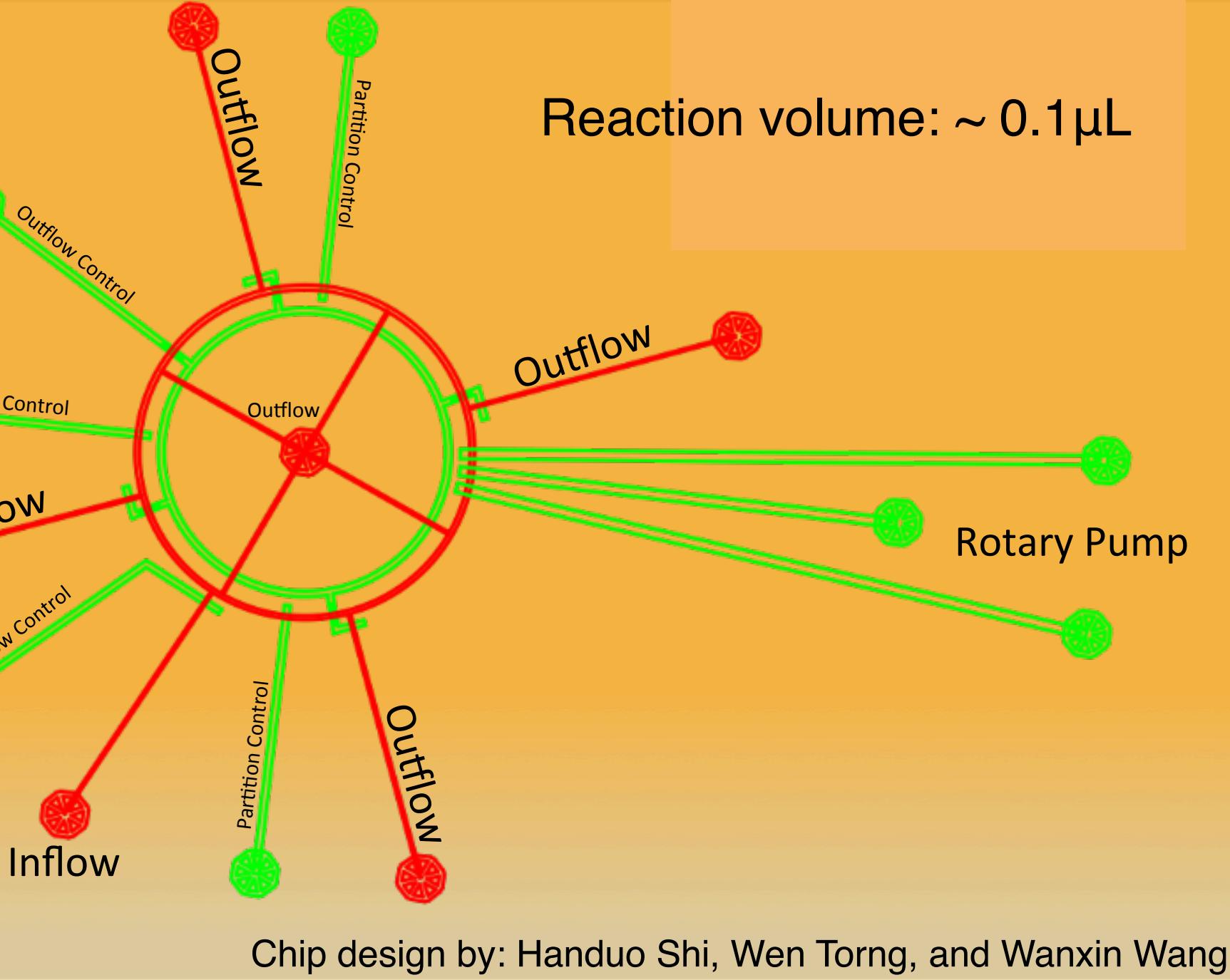
- To reduce and better understand the origin of amplification bias

Approach

- Perform MDA reaction in a microfluidic chamber to allow for the mixing of reactions with different biases to “even out” bias.

Chip Design

The MDA reaction mix flows into the **circular reaction chamber** where the MDA reaction takes place. The reaction chamber is mixed intermittently using the **rotary pump**. After ~3 hours at 30°C the reaction product may be retrieved from the **outflow** ports by pumping TE buffer through the middle outflow port.



Results and discussion

My work consisted of fabrication, testing, and running MDA reactions on this chip. Previously, issues were encountered with leaky valves, which was solved by the dead-end filling of control lines with water. The issue of “sticky” valves (non-opening) was addressed by reducing control layer thickness and increasing post bonding bake time. While this reduced “stickiness”, it allowed for the control membrane to tear more easily, allowing fluid from the control layer to leak into the flow layer. Despite these issues, three MDA reaction runs were performed on the chips, one of which yielded a far greater quantity of DNA product than the others, when quantified using NanoDrop®. The quality of these products remains to be further determined using species specific PCR primers. However, it is likely that contamination of some kind led to the seemingly large quantity of MDA product in one of the reactions.

Citations

1. Griese, M., Lange, C. and Soppa, J. (2011). Ploidy in cyanobacteria. *FEMS Microbiology Letters*, 323: 124–131. doi: 10.1111/j.1574-6968.2011.02368.x

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