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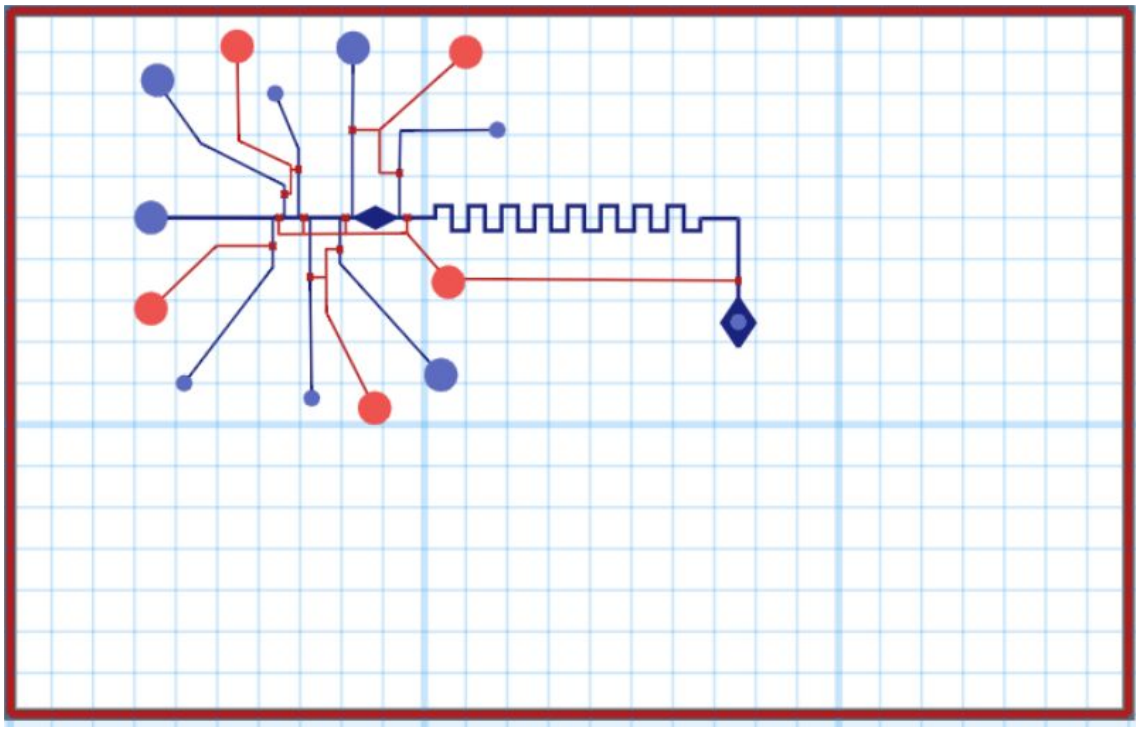
Overview

DNA Digest

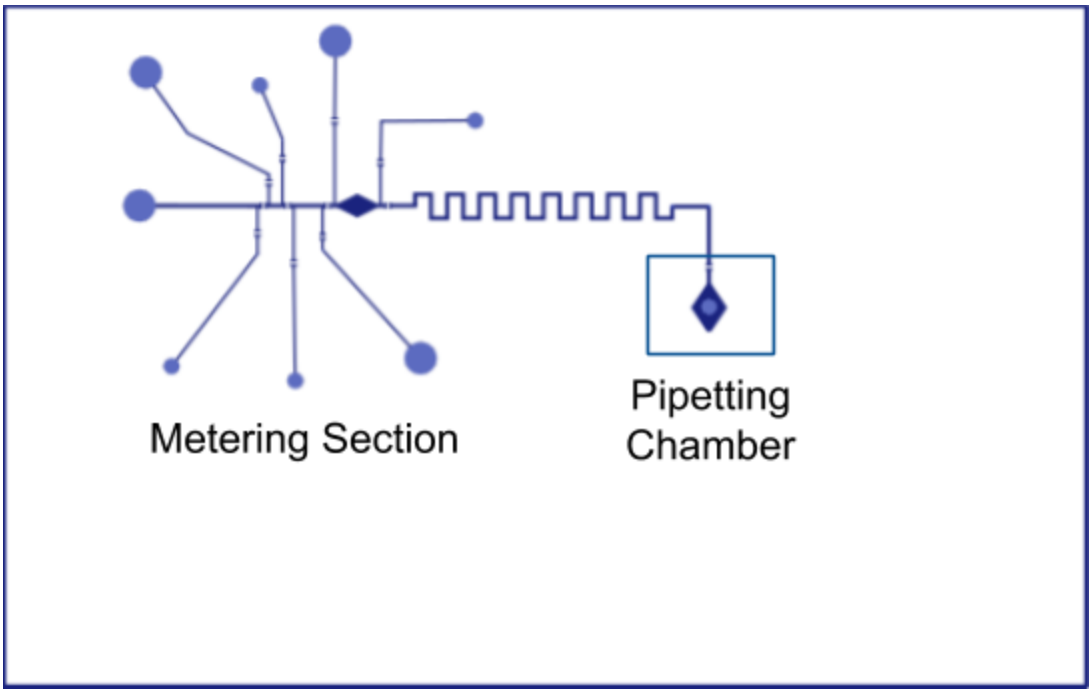
Designed by Dinithi Samarasekera
Date Completed: 09/29/2017

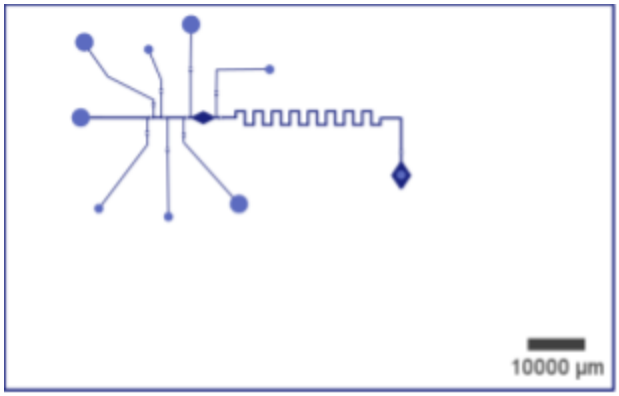


DNA digestion, or DNA fragmentation, is a basic protocol in synthetic biology. This is typically performed prior to analysis of the DNA sequence, or in order to perform further protocols. Restriction enzymes are mixed and then incubated with DNA in a buffer solution. This yields DNA fragments cleaved at specific sites according to the enzymes used.

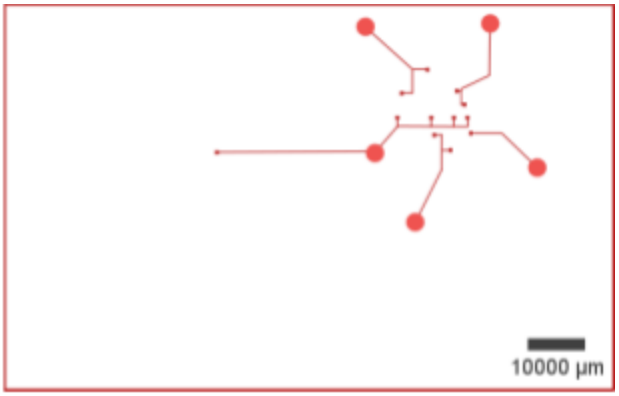
This microfluidic chip design performs DNA digestion. The desired DNA segment, restriction enzymes, water and buffer solution are inputted and mixed. The resulting solution is then sealed in an incubation chamber on top of which a heating element is placed. After the designated incubation time has passed, the liquid can then be transferred from the chip using a pipette.

Chip Design



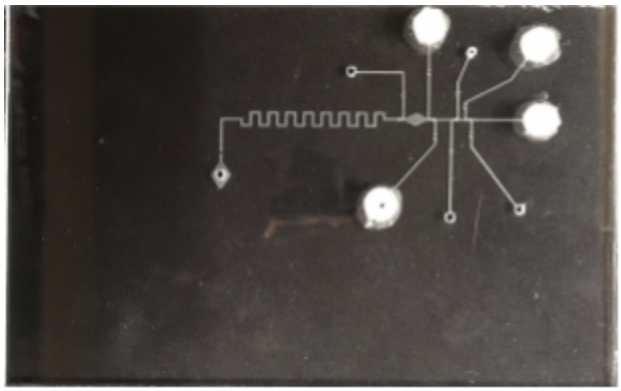


Flow Layer

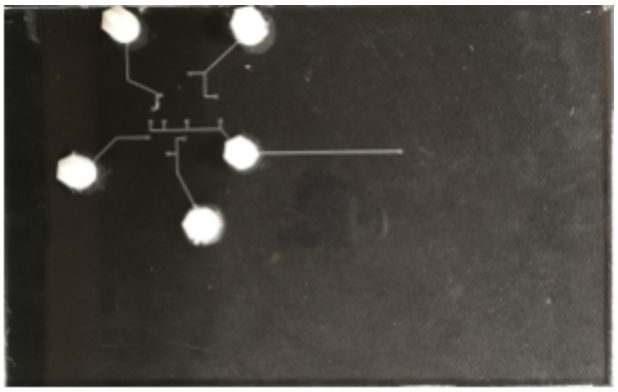


Control Layer

Milling Guidelines



Flow Layer



Control Layer

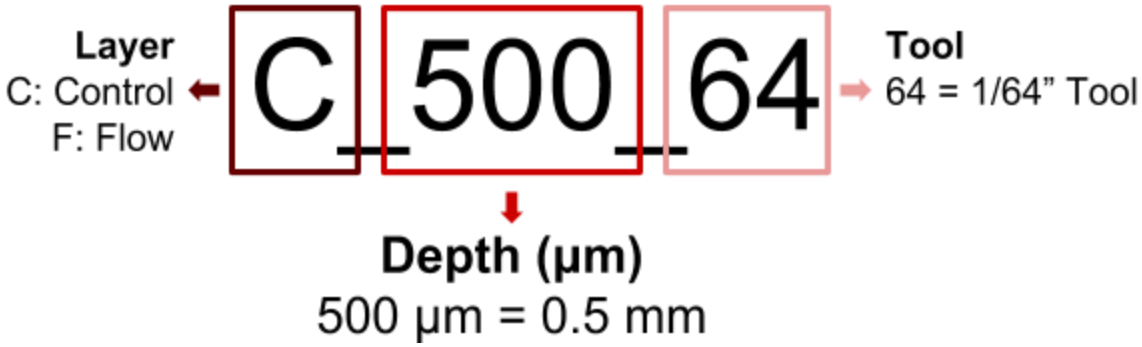
Milling Guidelines

For a comprehensive guide to milling click here. For a list of tool parameters click here.

Notes

1. This chip should be milled on medium or thick polycarbonate (2.75mm < $Z_{Polycarbonate}$).
2. This chip requires thick PDMS (1.0mm < Z_{PDMS} < 1.5mm)

All the required SVGs for milling this chip are provided in the ZIP file. The layer, depth, and tool required for each SVG is listed in the file name. Below is a key describing how to read an SVG file name:



Milling Instructions

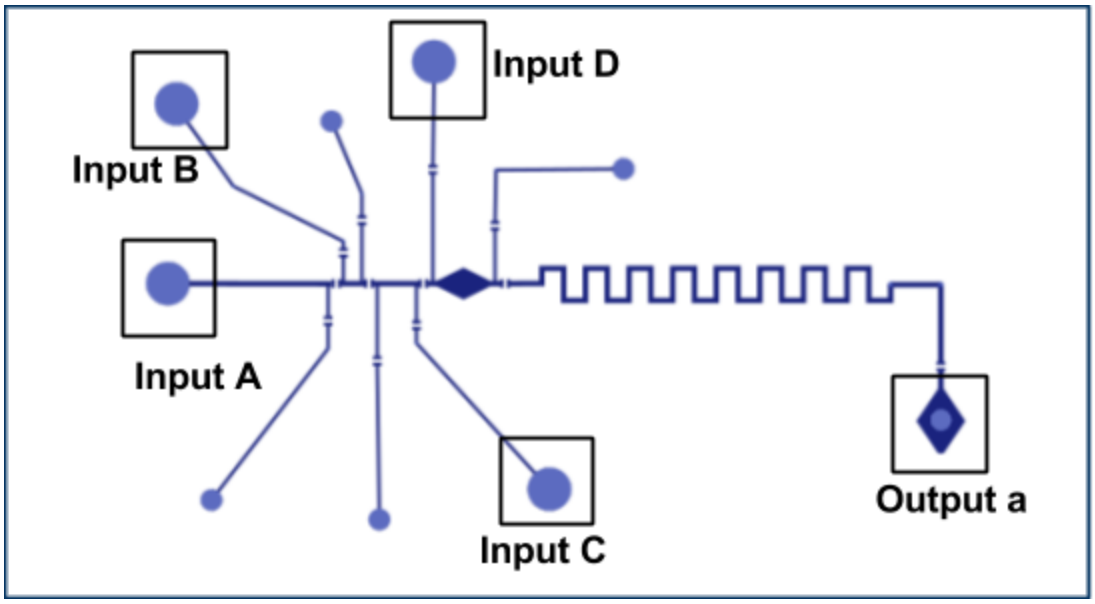
Mill the layers in the order they are listed with the correct depths and using the correct tools.

Flow Layer	
Order	**Layer Name**
1.	F_500_64
2.	F_300_100
3.	F_PORTS_8_32 *For this Milling, both the 1/8 and 1/32 endmills are required*
4.	Border

Control Layer	
Order	**Layer Name**
1.	C_300_100
2.	C_PORTS_8
3.	Border

Testing Protocol

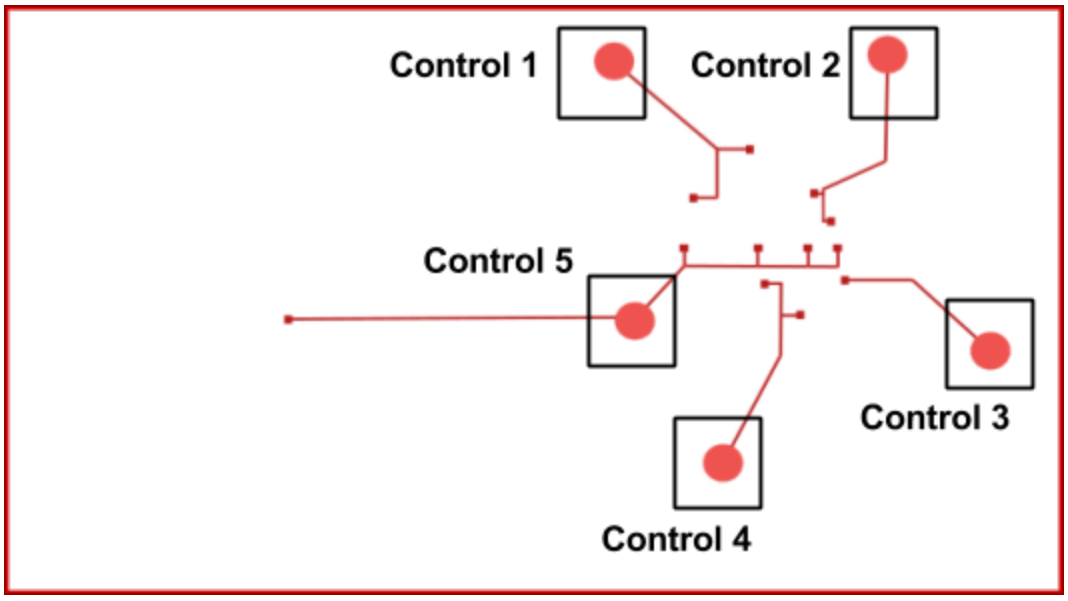
Flow Layer Setup



Inputs		
Name	**Liquid**	**Flow Rate**
A	Oil	0.5 mL/hour
B	DNA Suspension	0.5 mL/hour
C	Buffer and Enzyme Solution	0.5 mL/hour
D	Water	0.5 mL/hour

Outputs	
Name	**Liquid**
a	DNA Fragments in Suspension *Note: The incubation chamber must be sealed prior to use with*

Control Layer Setup



Testing the Chip

Setup

1. Prepare 8 syringes
    a. 3 containing coloured water
    b. 1 filled with mineral oil
    c. 4 control syringes
2. Attach the syringes containing colored water to inputs B,C and D
3. Attach your output tubing to Output a; this tube should connect to an eppendorf or other small collection receptacle
4. Attach two separate control syringes to Control 1 and Control 2

Running the chip

1. Open Control 1, Control 2, Control 3 and Control 4
2. Begin flowing inputs A, B, C and D at 0.5 mL/hour
3. Halt each flow when its respective metered channel on the chip is filled, then close its corresponding Control line
4. Open Control 5 and begin flowing input A at 0.5 mL/hour again
5. When the oil is 1 cm away from crossing the final valve, halt the flow of input A
6. After 20 seconds, close Control 5
7. Leave the mixture to incubate for 1 hour

8. Use a pipette tip to break the seal over the incubation chamber and transfer the DNA digest

Cleaning the Chip

1. Disconnect your output tubing carefully, then disconnect all other syringes
2. Clean the chip following the oil & water protocol listed here
3. Store your chip as detailed in the cleaning protocol