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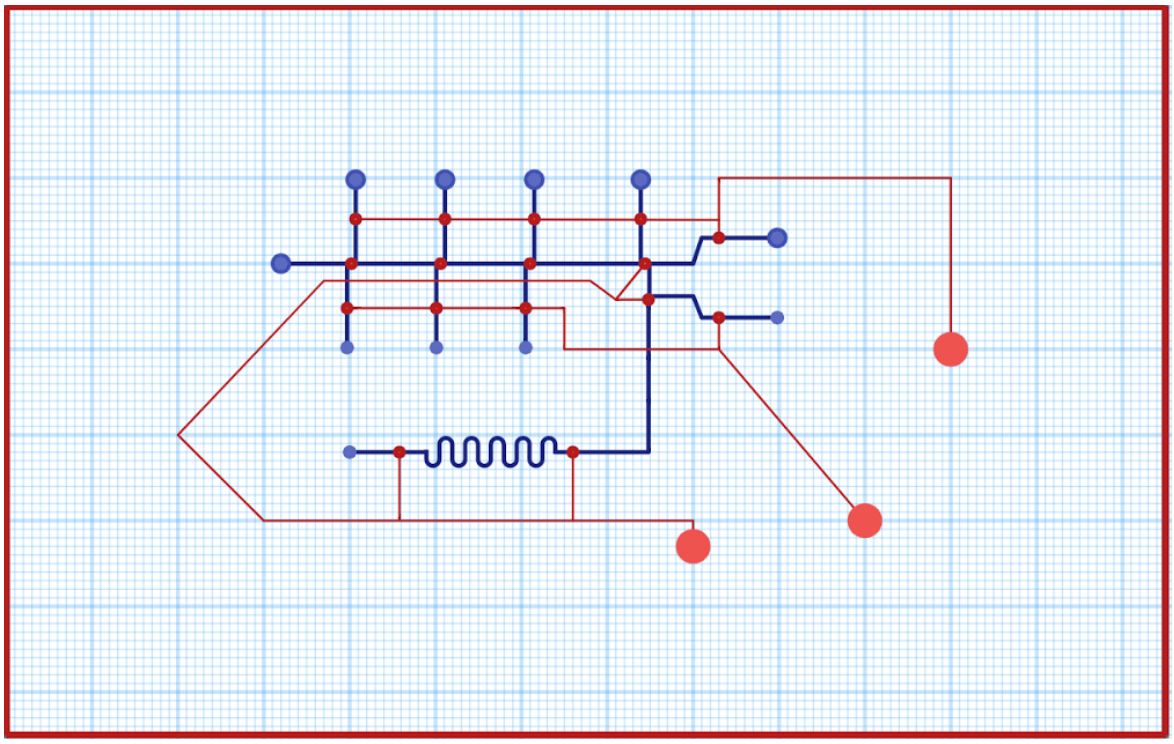
# Overview

## Ligation

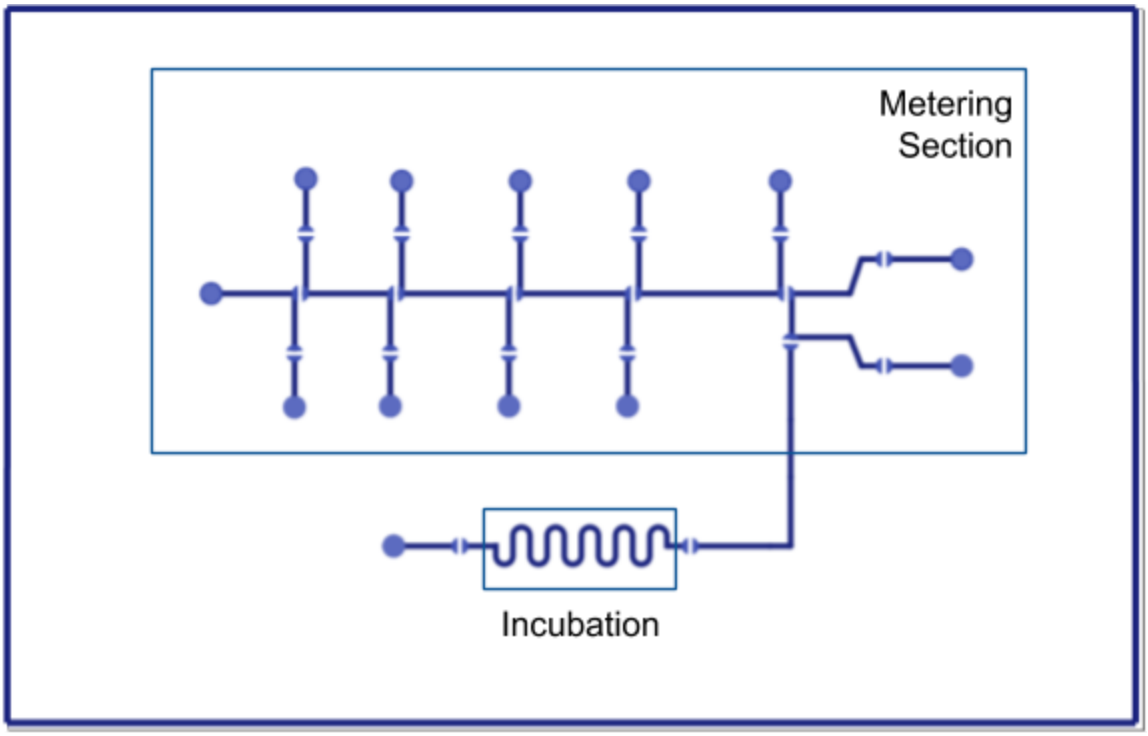
Designed by Sarah Nemsick
Date Completed: 10/10/2017

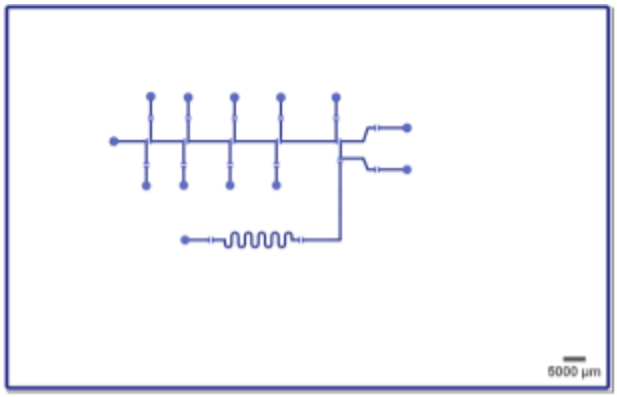


Ligation is a commonly used protocol in synthetic biology. In molecular cloning ligation is the process by which external DNA is inserted into a vector DNA, often a plasmid, using the enzyme DNA ligase. The newly formed DNA, or recombinant DNA, can then be analyzed or transformed.

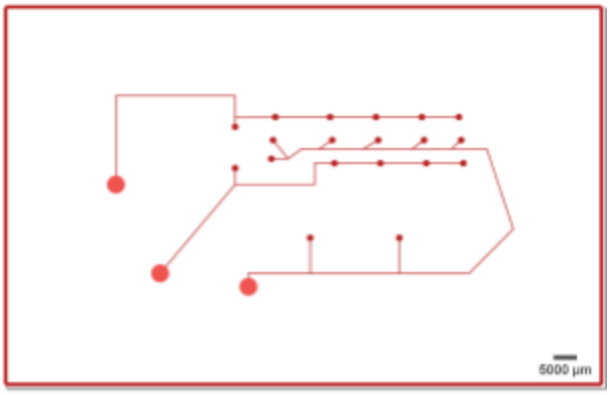
This microfluidic chip is designed to perform ligation. T4 DNA Ligase Buffer, vector DNA, insert DNA, water, and T4 DNA Ligase are metered on the chip and are then mixed together. The solution then undergoes incubation, after which the recombinant DNA solution can pipetted out from the chip and used in further molecular cloning procedures.

# Chip Design



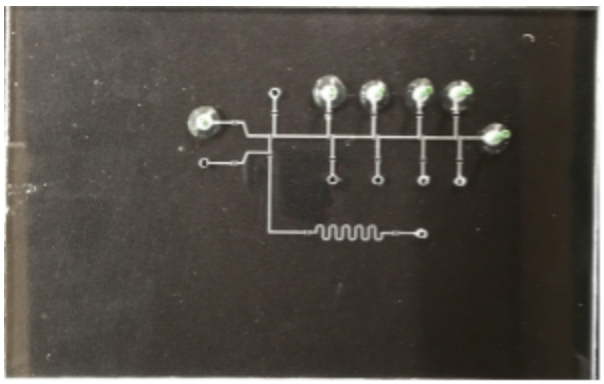




Flow Layer

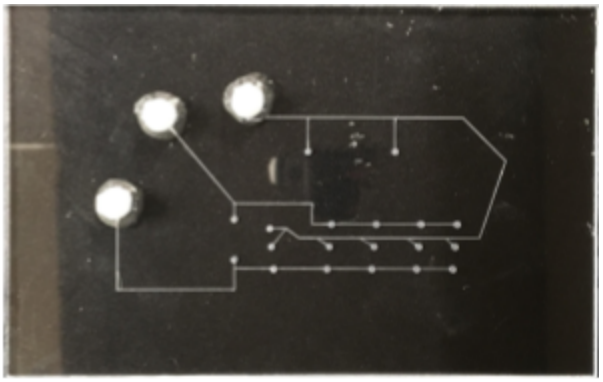



Control Layer

# Milling Guidelines



Flow Layer



Control Layer

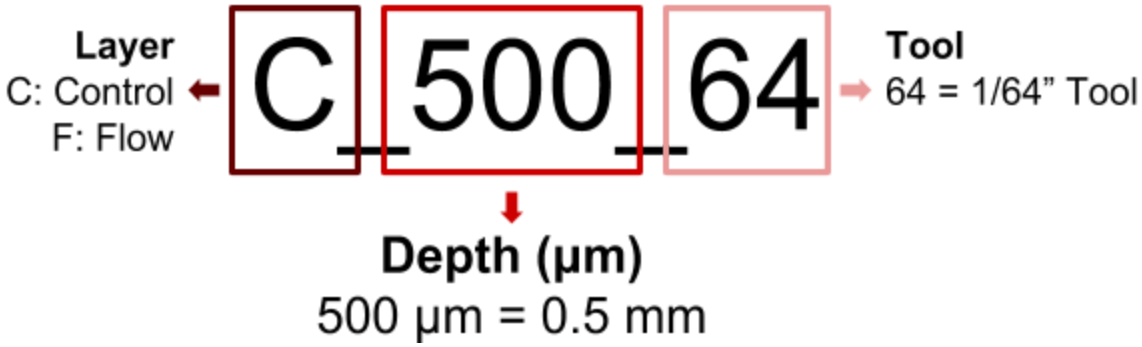
## Milling Guidelines

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

### Notes

1. This chip should be milled on thick polycarbonate (5.00mm < $Z_{Polycarbonate}$).
2. This chip requires thin PDMS (0.24mm < $Z_{PDMS}$ < 0.26mm)

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

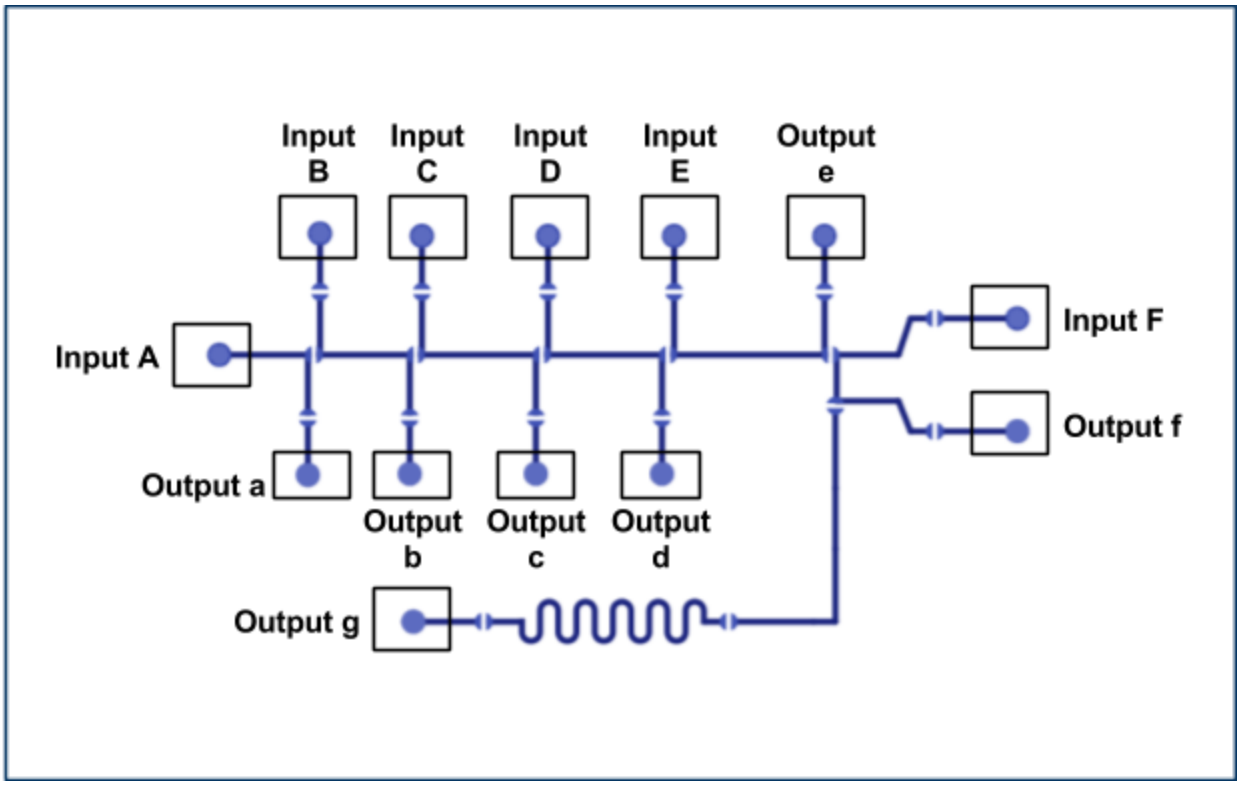
| Order | Layer Name |
|---|---|
| 1. | F_500_64 |
| 2. | F_1000_64 |
| 3. | F_4500_16<br>*If $Z_{polycarbate}$ > 6.00 mm, depth = $Z_{polycarbate}$-1000 um* |
| 4. | F_PORTS_16 |
| 5. | Border |

## Control Layer

| Order | Layer Name |
|---|---|
| 1. | C_150_100 |
| 2. | C_1000_64 |
| 3. | C_PORTS_8 |
| 4. | Border |

# Testing Protocol

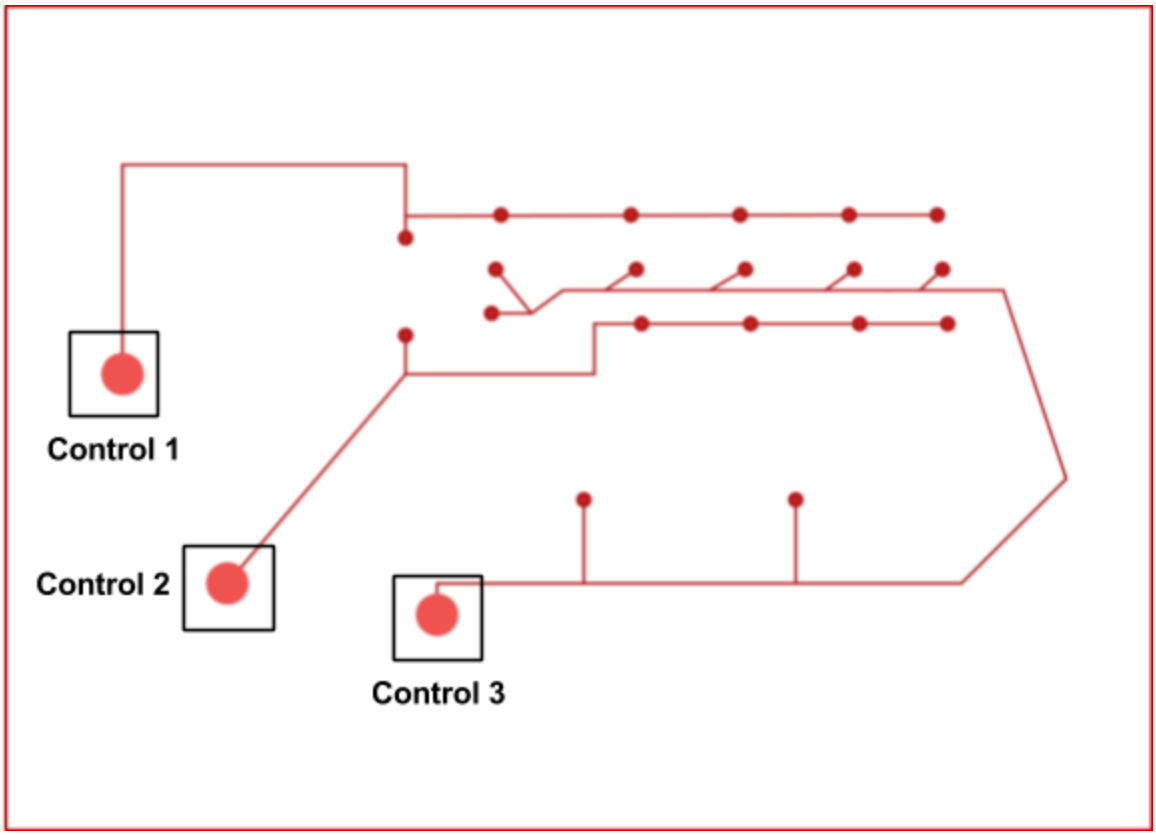
## Flow Layer Setup




| Inputs | | |
|---|---|---|
| **Name** | **Liquid** | **Flow Rate** |
| A | Mineral Oil | 0.5 mL/hour |
| B | T4 DNA Ligase Buffer | 0.5 mL/hour |
| C | Vector DNA | 0.5 mL/hour |
| D | Insert DNA | 0.5 mL/hour |
| E | Nuclease-free water | 0.5 mL/hour |
| F | T4 DNA Ligase | 0.5 mL/hour |

| Outputs | |
|---|---|
| **Name** | **Liquid** |
| a | Excess Mineral Oil |
| b | Excess T4 DNA Ligase Buffer |
| c | Excess Vector DNA |
| d | Excess Insert DNA |
| e | Excess Nuclease-free water |
| f | Excess T4 DNA Ligase |
| g | Final Output |

## Control Layer Setup



# Testing the Chip

**Note: This chip has not been tested in the lab. This protocol is how the chip would be run in theory.**

## Setup

1. Prepare 10 syringes
    a. 5 filled with different colored water
    b. 1 filled with mineral oil
    c. 3 empty 10 mL control syringes
2. Attach your syringe containing mineral oil to Input A
3. Attach the remaining colored water syringes to inputs B-F
4. Attach your waste output tubing to Outputs a-f; this liquid will be excess fluid
5. Attach your output tubing to Output g; this tube should connect to an eppendorf or other small collection receptacle
6. Attach three separate control syringes to Control 1, Control 2, and Control 3
7. If a heating element is being utilized, ensure this element is turned on and at the correct temperature

## Running the chip

8. Open Control 1, then Control 2; you should feel significant resistance while you open these control valves
9. Begin flowing your mineral oil and all colored waters at flow rates of 0.5 mL/hour each
10. Once the mineral oil and colored waters have filled their metering sections and have begun filling the output port, ensure all of their syringe pumps have been turned off
11. Close Control 1, pause, then close Control 2
12. Open Control 3
13. Flow the mineral oil again at 0.5 mL/hour
14. The oil will push and mix the two colored waters
15. When the colored water has moved into the mixer and has begun crossing the final valve, turn off the mineral oil syringe pump
16. Close Control 3
17. Incubate for the required amount of time, depending on the specific protocol
18. Open Control 3
19. Flow the mineral oil again at 0.5 mL/hour until all the colored liquid has been pushed out
20. Collect all of the output colored liquid in your designated receptacle

## Cleaning the Chip

21. Disconnect your output tubing carefully and dispose of all liquid waste in the correct receptacle

22. Disconnect all other syringes
23. Clean the chip following the oil and water protocol listed here
24. Store your chip as detailed in the cleaning protocol