

Microfluidic Handling of DNA for Spatial Genome Research

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Primary CNF Tools Used: ABM Contact Aligner, Class II Photoresist Room, P7 Profilometer

Abstract:

Nuclear envelope invaginations that contain endoplasmic reticulum (ER), mitochondria and other cytosolic organelles have been linked to cancer, and can be used as tumor biomarkers. To gain a better understanding of how this occurs, the organization and composition of chromatin near nuclear invaginations needs to be examined. Assuming that these invaginations are involved in cell signaling and transcription, they may control disease-specific phenotypes through spatial gene regulation.

To explore spatial control of gene regulation, we have developed a method that enables chromatin isolation from small (femtoliter scale) targeted volumes within the nucleus. The method, we call Femto-seq, uses localized nonlinear excitation to photo-biotinylate small nuclear volumes so that chromatin can be obtained using affinity purification, and then sequenced. To improve the throughput and efficacy of Femto-seq, here we demonstrate a microfluidic purification device utilizing a single fluidic channel with a functionalized surface and mixing elements that improve sample yield and the level of purification. When validated and employed, our microfluidic devices will enhance the capacity of Femto-seq to elucidate spatial gene regulation patterns associated with nuclear invaginations.

The device was fabricated using multilayer SU-8 photolithography. Herringbone structures were added along the fluidic channel to encourage “chaos” in flow patterns of our sample through the channel, increasing the capture efficiency and improve our purification yield. We utilized a negative resist and our design was cast into PDMS and bonded to a glass surface. Our device was characterized through fluorescence microscopy and biochemical assays to first, validate that a streptavidin-biotin complex is formed using a glass functionalization protocol, and then to optimize an elution protocol suitable for our device and quantify our yield and purity of biotinylated deoxyribonucleic acid (DNA).

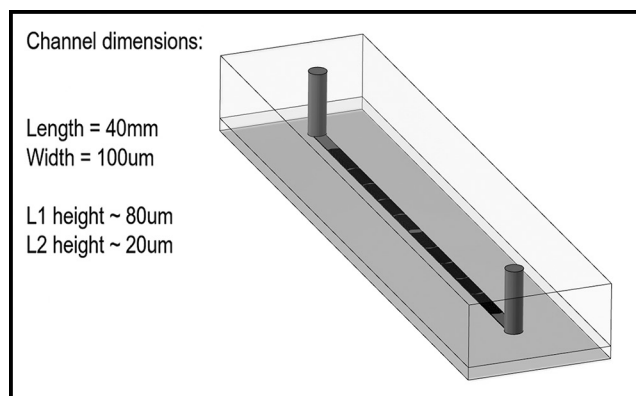


Figure 1: Schematic design of microfluidic device.

Summary of Research:

Our project goal for this summer was to fabricate and optimize a microfluidic device that can immobilize biotinylated DNA. The device design was already determined by mentor Jack Crowley, which was a single fluidic channel that had added mixing elements called herringbones on the top of the channel, both embedded in a layer of polydimethylsiloxane (PDMS) as shown in Figure 1.

The PDMS is then binded onto a glass slide to close the channel. To achieve this goal, the project had three main parts. The first part was the wafer fabrication and the device construction, and our methodology for this part was to use multi-layer SU-8 soft photolithography because the design required the fluidic channel to be treated as one layer during photolithography, and the herringbones to be treated as another; the second part was to functionalize the device surface in order to immobilize streptavidin on to the glass substrate and take advantage of the very strong affinity between streptavidin and biotin; the final part was validation and characterization of the device, and to set up an engineering system to optimally operate the device.

The first portion of the project took the longest because we were in unfamiliar territory working with multi-layer photolithography — so there was a lot of trial and error. Issues arose when we weren't able to achieve proper contact between our silicon wafer and our photomasks, but by talking to Aaron Windsor and Chris Alpha from the CNF staff, we were able to gather some tips and tweak our fabrication process, which yielded better results.

Upon being able to consistently acquire usable channels from this process we were able to move on to the functionalizing portion of the project. This portion involved functionalizing fully constructed devices and validating the protocol worked as intended.

To do this we used fluorescent microscopy and flowed fluorescent biotin through functionalized devices, to image and search for bright spots which correlate to immobilized biotin. We were able to see that we were immobilizing some biotin, so the next step was to set up an experiment to characterize our device. We chose to use DNA gel electrophoresis to do this as we are able to quantify the DNA bands in the agarose gel after the process, so we would be able to get quantitative data.

We set up the experiment by using DNA gel extraction on a DNA ladder to biotinylate a single length of DNA, and then flowing the ladder and the biotinylated DNA through our device and collecting the flow through. The flow through was run through the gel alongside some baseline samples to learn as much as we could about our device.

Conclusions and Final Steps:

Our next steps begin with completing an engineering system to operate the microfluidic chip. This includes setting up a system that adds an oscillatory washing step within the fluidic channel to increase our purification yield, and would also include adding washing and buffer steps. After this system has been set up, a DNA gel electrophoresis experiment can be run on the device flow-through to validate that biotinylated DNA can be captured using our microfluidic device design and the surface functionalization protocol used works as expected.

Once these have been validated the next step would be to quantify the devices performance, and compare the amount of cells needed to provide a sufficient DNA sample via Femto-seq, and determine the reduction of cells needed for this process from the beginning of the summer, which was 30,000 cells, to now. Next, elution steps to break the streptavidin-biotin complex formed in this device should be tested and optimized.

The goal is to find a protocol that does minimal damage to the device itself to encourage longevity of the materials used. Part of this elution step would be to develop a flow-through collection protocol that leads to minimal loss of the DNA we worked so hard to affinity capture in the channel. Furthermore, ideally after the steps to optimize this device's function have been established, the system to control the device itself should become programmable, to make device operation as automatic as possible.

References:

- [1] Ma, S., et al. 2018. *Sci. Adv.* 4(4).
- [2] Stroock, A.D., et al. 2002. *Science* 295, 647-51.