



## DESIGN AND MICROFABRICATION OF A LIGASE DETECTION REACTION (LDR) DEVICE

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### ABSTRACT

The objective of this project is to manufacture a microscale Ligase Detection Reaction (LDR) device for detection of rare gene mutations to be incorporated with modular lab-on-a-chip technology. The first step is to determine if the baseline time for the LDR reaction can be reduced. Experiments are being carried out to determine the minimum acceptable time for the reaction. A device for thermally cycling the reagent mix is being modeled by FEA and prototype devices being laid out.

### BACKGROUND

Early detection of cancers requires the ability to detect rare mutations in DNA. The LDR was developed and patented by Francis Barany [1] and his associates at Cornell University. Used in conjunction with the Polymerase Chain Reaction (PCR) in DNA analysis, it is particularly useful for the detection of such rare cancer-associated mutations. The reaction uses PCR products and four primers that are mixed together with distilled water and heated to 95°C. The ligase is added and mixed after 90 seconds of heating. The resulting mixture goes through twenty cycles of 95°C and 65°C for 30 seconds and 4 minutes, respectively, in the macroscale reaction. The total reaction currently takes over two hours. After cycling, the reaction is stopped by cooling to 0°C. The products are then evaluated using a zip code gene array. Figure 1 shows the typical result for the macroscale LDR reaction using a zip code gene array.

There has been significant research in Polymerase Chain Reaction (PCR) miniaturization with reaction times as fast as 20 seconds or less [2]. In order for the LDR to be compatible, it is of the utmost importance to scale down the reaction time so that it is comparable to the PCR. Mitchell in [2] has shown that microfabrication using the LIGA process [3] with polycarbonate can produce reproducible microchips that can withstand the desired temperatures, while minimizing the time and sample volume for chemical and biological analyses, and reduce the cost of fabrication so that the instruments can be used clinically.

### METHODS

Experiments are being conducted to quantify how much the reaction time can be reduced. A test matrix was developed to systematically test what factors affect the reaction in order to reduce the time per cycle. A sample of the matrix is shown in Table 1. Under this system the product obtained after each run is analyzed using a genetic scanner that is able to give the composition of the analyte. This result is compared to the original composition of the successful macroscale reaction and adjustments made throughout the matrix.

The reactants will be driven through the chips using an electric field. Under this field a plug-like movement of the analyte will be realized with a flatter profile than for pressure driven flow [4]. The movement of the mixture is easier to control under this system. In both cases, however, there are still issues of diffusion that have to be resolved.

The desired heating can be achieved by either oscillating the LDR mixture between two isothermal zones heated by resistive heaters, or using a thermoelectric module to cycle the stationary LDR mixture between the two temperatures. FEA models were developed to predict the steady-state temperature profiles for resistive heating. If resistive heating were used some other cooling apparatus will still have to be utilized to stop the reaction. The thermoelectric module cannot only be used for heating but can also be used for cooling which is a very viable option. The dynamic model for the thermoelectric module is currently being developed.

## RESULTS

Table 1. Sample of systematic test matrix.

Run 1		Run 2		Run 3	
0.1 $\mu$ M G12	1 $\mu$ l	0.1 $\mu$ M G12	1 $\mu$ l	0.1 $\mu$ M G12	1 $\mu$ l
1 $\mu$ M czip 11	2 $\mu$ l	1 $\mu$ M czip 11	2 $\mu$ l	1 $\mu$ M czip 11	2 $\mu$ l
1 $\mu$ M com-2	2 $\mu$ l	1 $\mu$ M com-2	2 $\mu$ l	1 $\mu$ M com-2	2 $\mu$ l
200 mM DTT	1 $\mu$ l	200 mM DTT	1 $\mu$ l	200 mM DTT	1 $\mu$ l
10 mM NAD	1 $\mu$ l	10 mM NAD	1 $\mu$ l	10 mM NAD	1 $\mu$ l
2 x buffer	12 $\mu$ l	2 x buffer	12 $\mu$ l	2 x buffer	12 $\mu$ l
Ligase	1 $\mu$ l	Ligase	1 $\mu$ l	Ligase	1 $\mu$ l
94 C 30 s 65 C 4min X 20 cycles		94 C 30 s 65 C 2min X 20 cycles		94 C 20 s 65 C 1min X 20 cycles	
Results		Results		Results	

Chemical analysis will continue using the adjusted matrix until maximum time reduction is achieved.



Figure 1. Result of a macroscale LDR Reaction showing a mutation sticking to the Zip11 code

The temperature profile obtained using resistive heaters modeled in ANSYS (v5.7, ANSYS, Inc., Houston, PA) is shown in Figures 2 & 3.

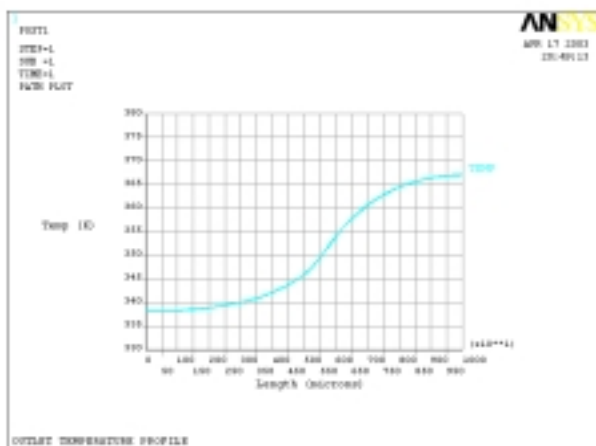


Figure 2. Simulated temperature profile obtained along a length of channel of 1cm using resistive heaters.

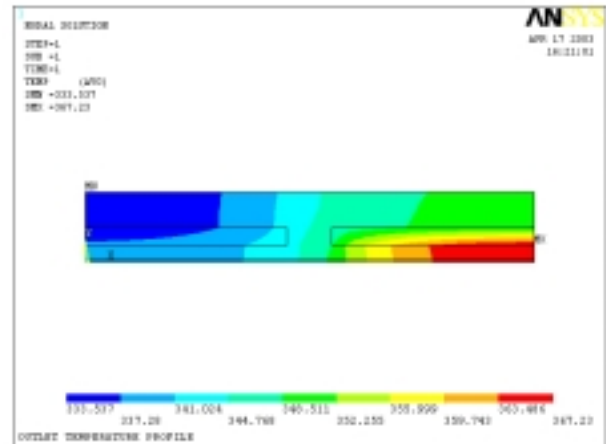


Figure 3. Simulated contour plots obtained across the microchip using resistive heaters.

## CONCLUSIONS

The LDR was only recently developed and provides a means for detecting low abundant mutations. The micro-device will greatly enhance the speed of the reaction making it a viable option in a wider range of analyses.

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## REFERENCES

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