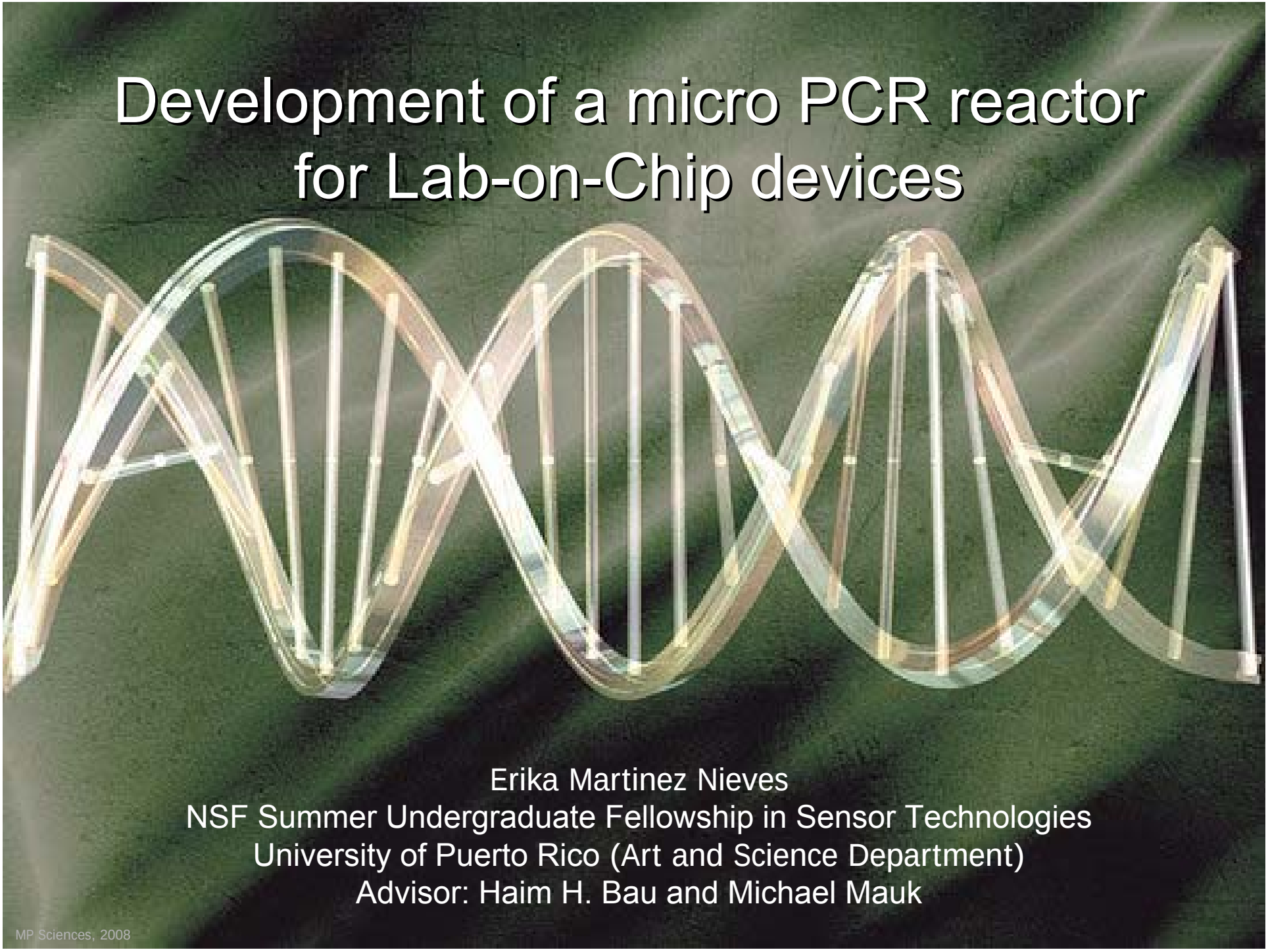
Development of a micro PCR reactor for Lab-on-Chip devices



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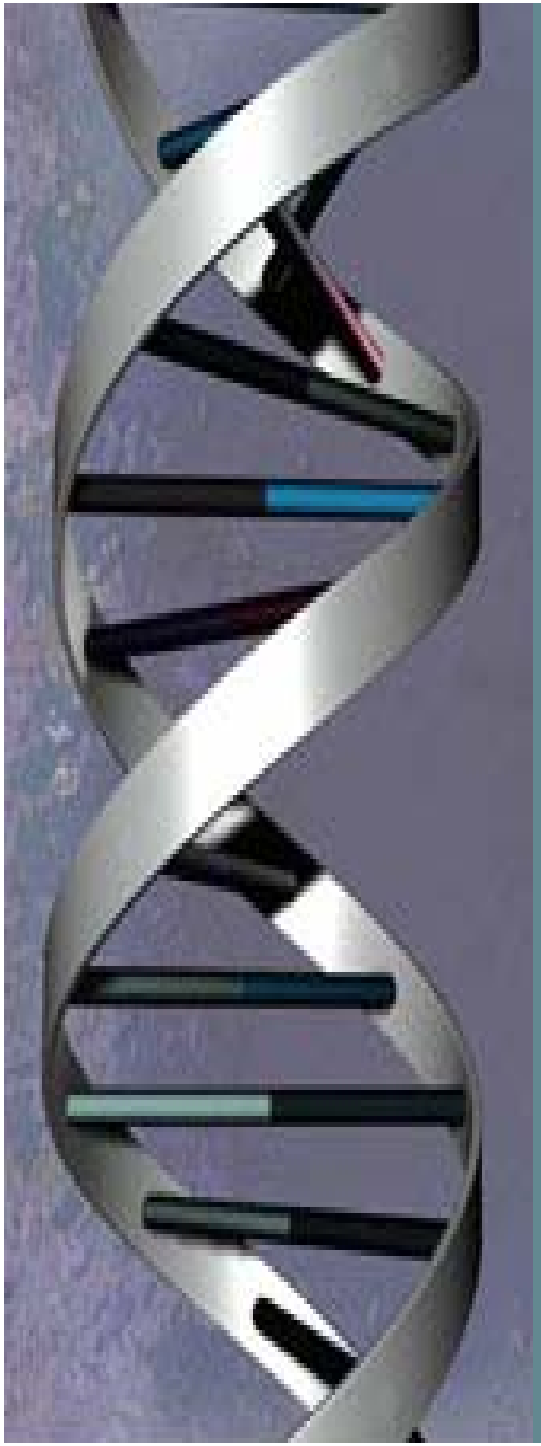
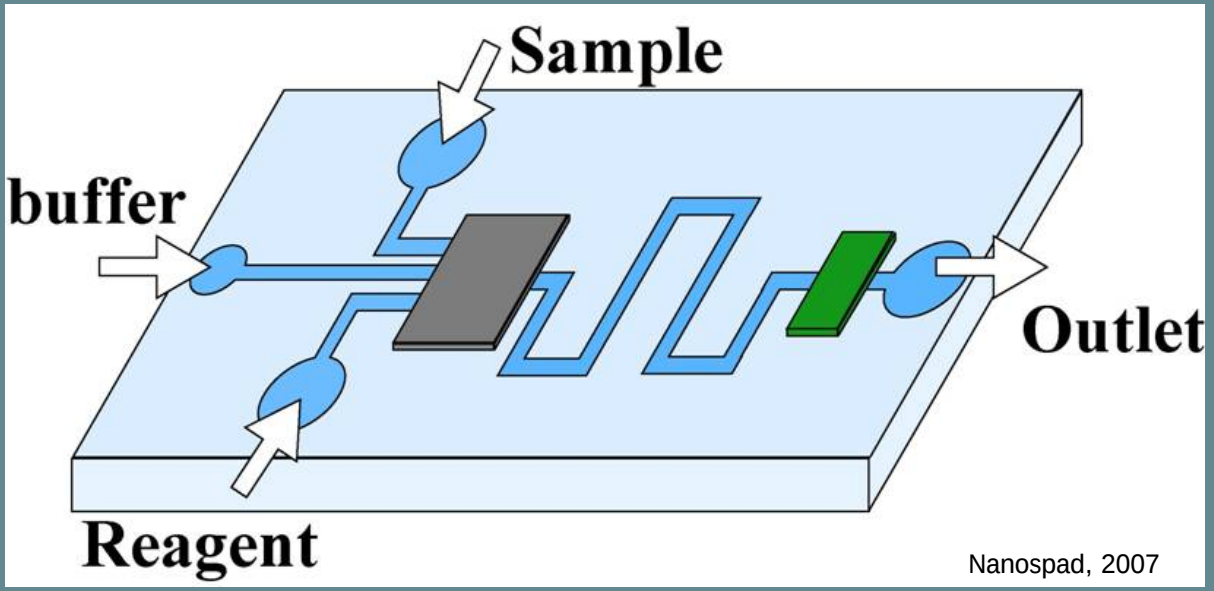
What are LOC Devices?

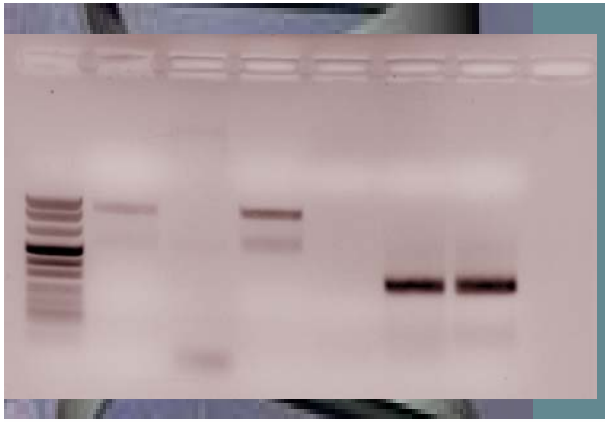
- Lab-on-chip (LOC) devices are composed of multiple microchannels and chambers where different stages of DNA analysis take place.
- They are designed to provide timely and accurate diagnosis for patients without the need for highly skilled personnel or advanced laboratories.
- The main challenge is to design devices to function in an area with limited resources.



What are LOC Devices?

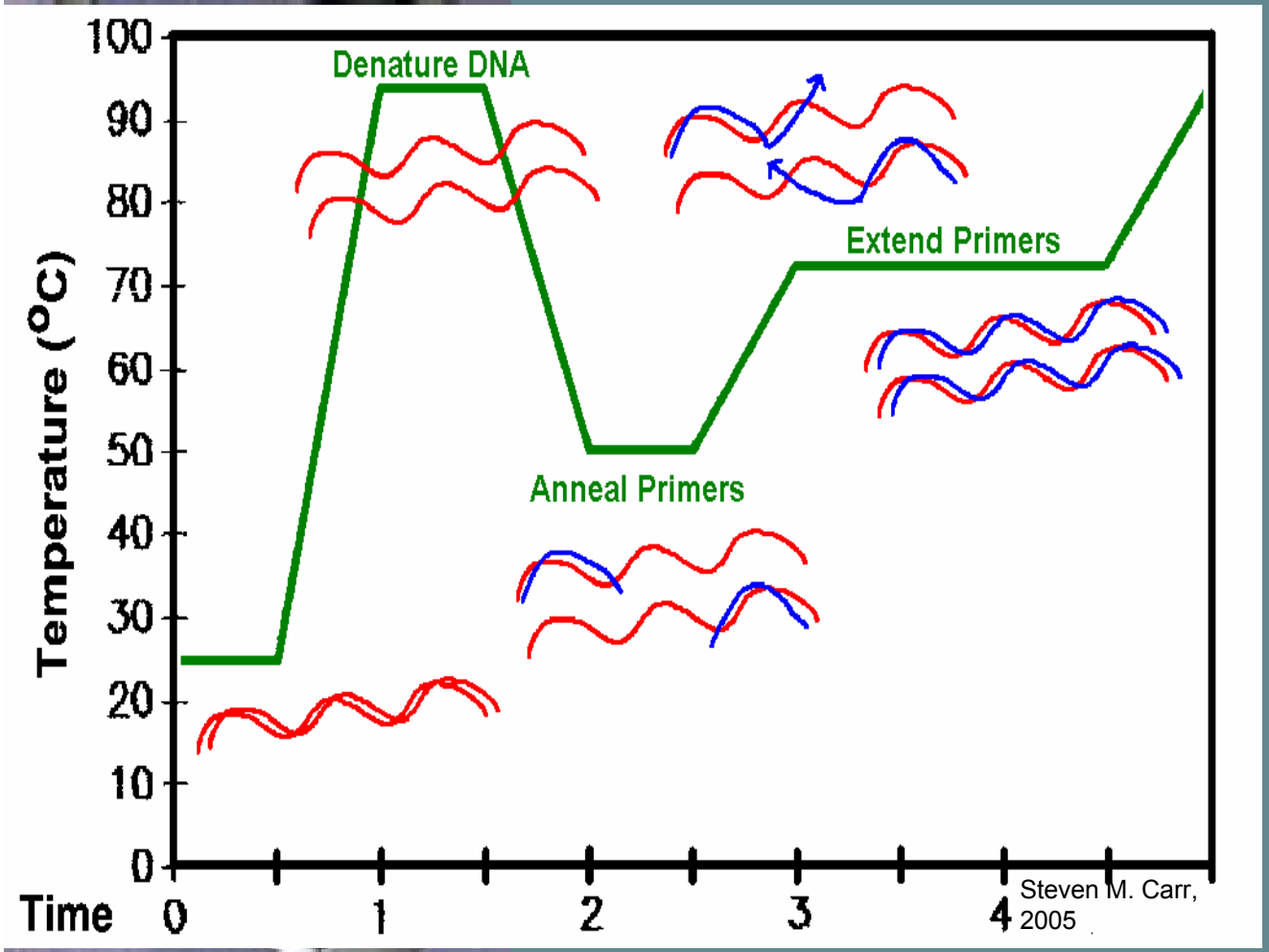


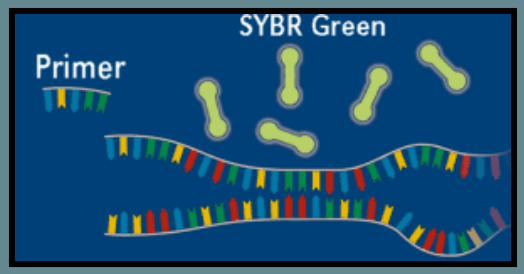
Nanospad, 2007

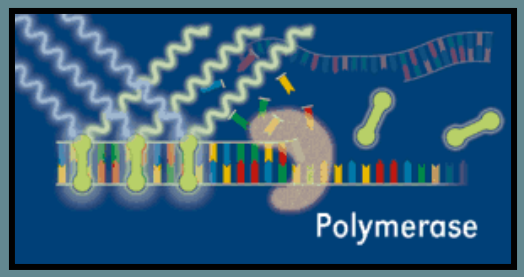


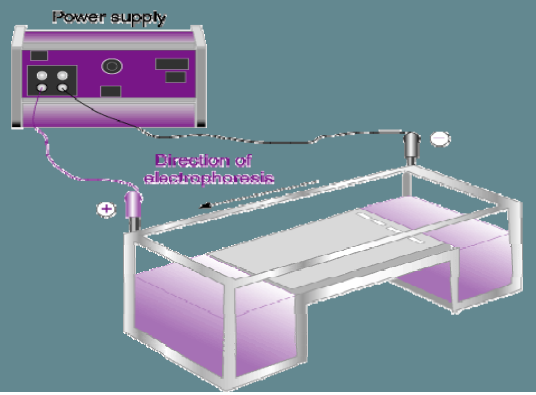
What is a PCR Process?

- **Polymerase Chain Reaction** consists of making multiple copies of a piece of DNA by repeating a reaction a specific number of times.









Problem:

- The process of performing PCR on a LOC device needs to be improved because of the following limitations:

  -unknown status of the starting DNA during PCR

  -excessive time consumption

Possible Solution:

- Use RT-PCR:

  Real Time Polymerase Chain Reaction is a technique where DNA is copied and fluorescence data is gathered throughout the entire amplification process

- Benefits over conventional PCR:

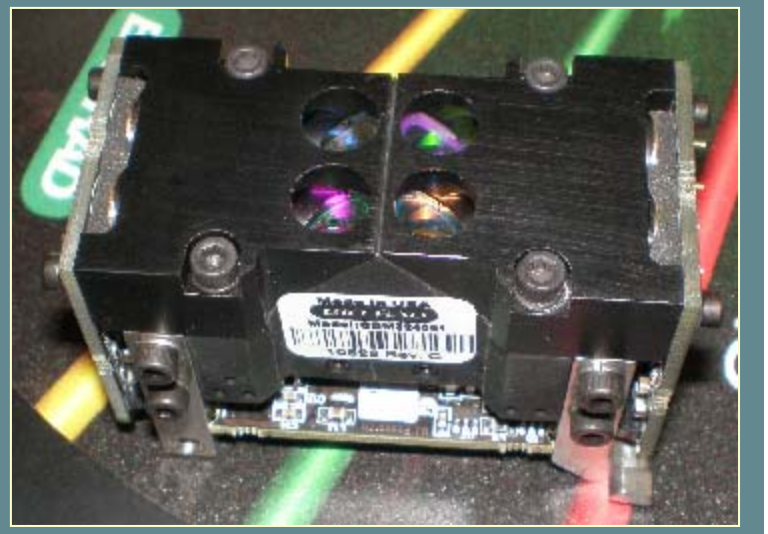
  -No gel is necessary, thus time is reduced

  -The status of the initial DNA is known

  -More accurate results

  -Graphical and numerical analysis are available

RT-PCR

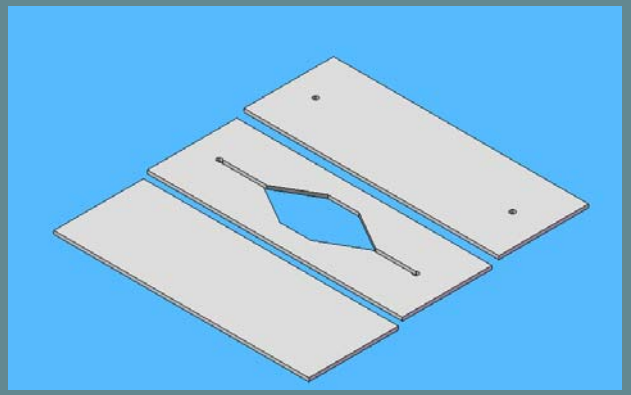


Approach:

- Make a PCR chamber in acrylic to simulate a LOC device and study the PCR process occurring in the chamber using RT-PCR.

PCR Chip

Single chamber, acrylic plastic chip.



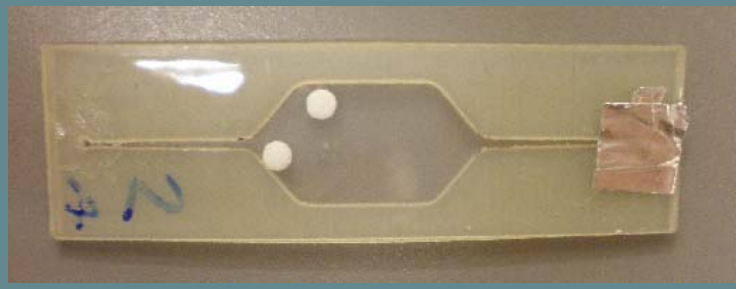
Depth: 0.5 millimeters

Width: 10-15 millimeters

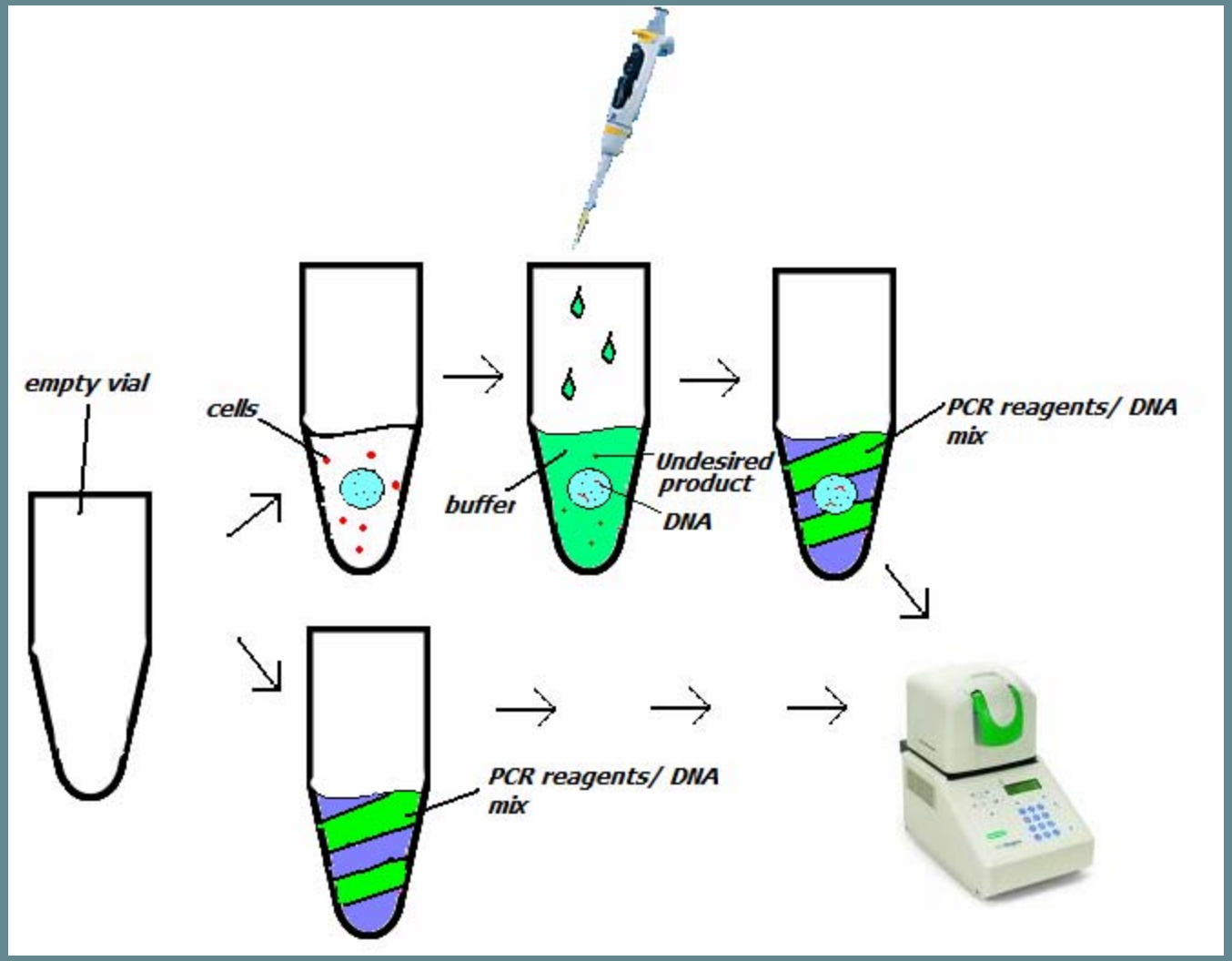
Length: 25-30 millimeters

Bonded with:

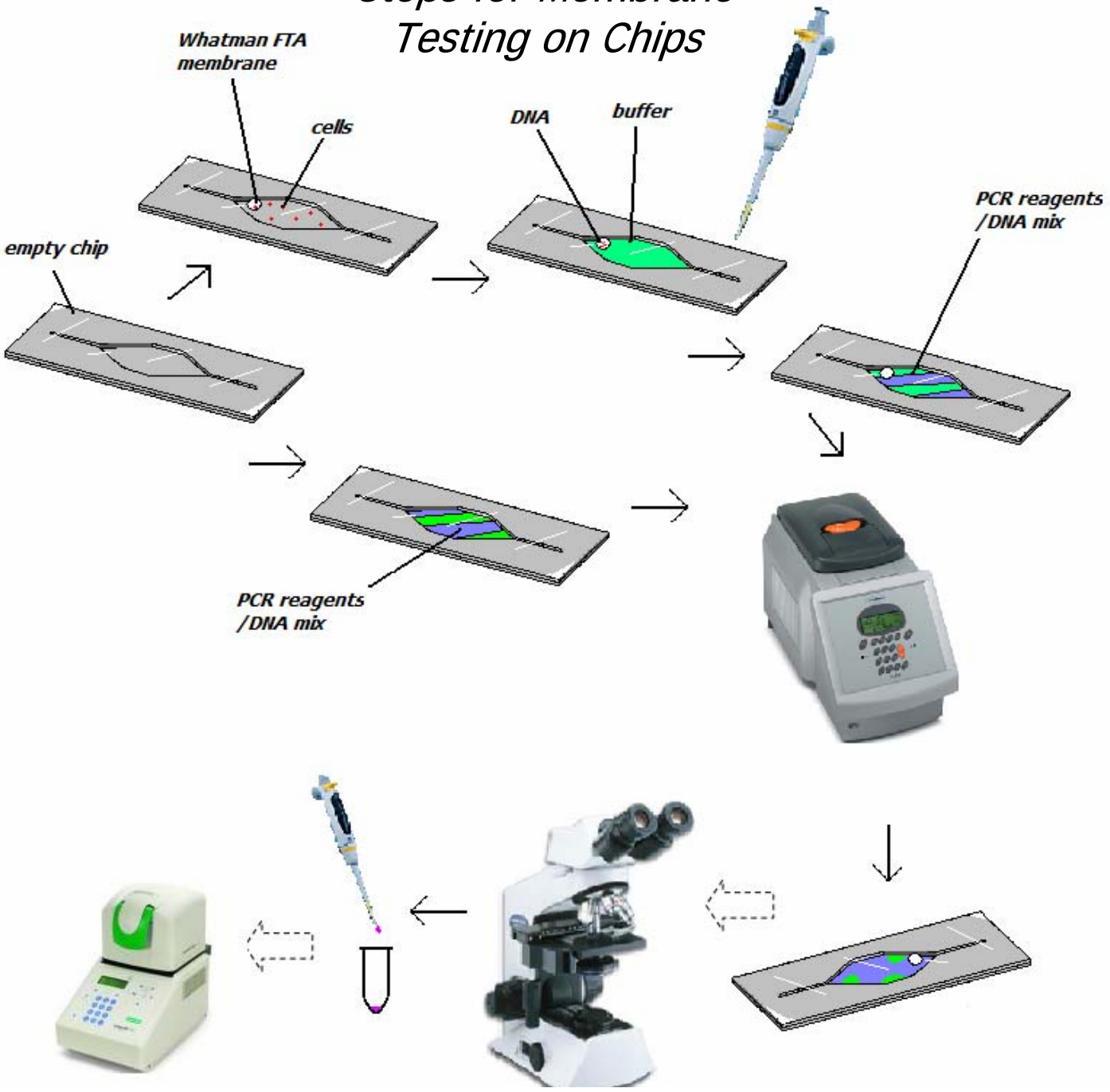
MMA/methanol solution or double-sided tape



Steps for Membrane Testing in Tubes

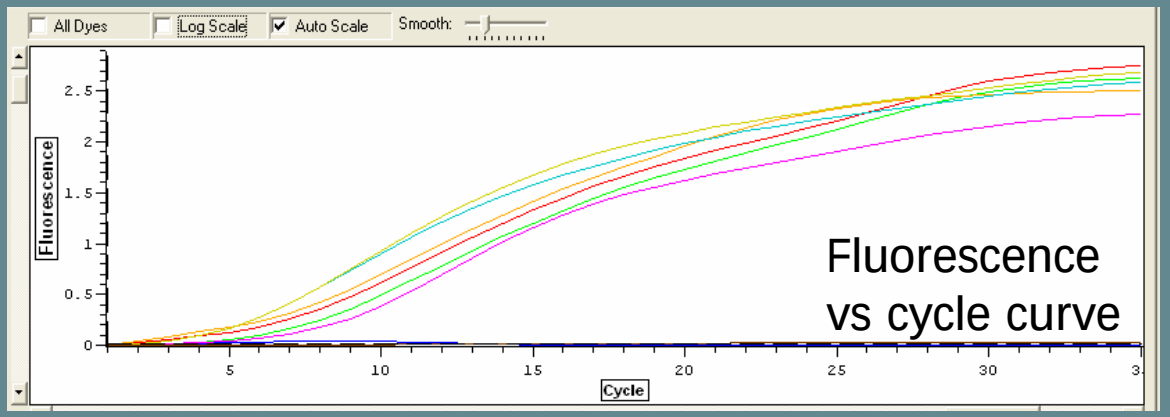


Steps for Membrane Testing on Chips

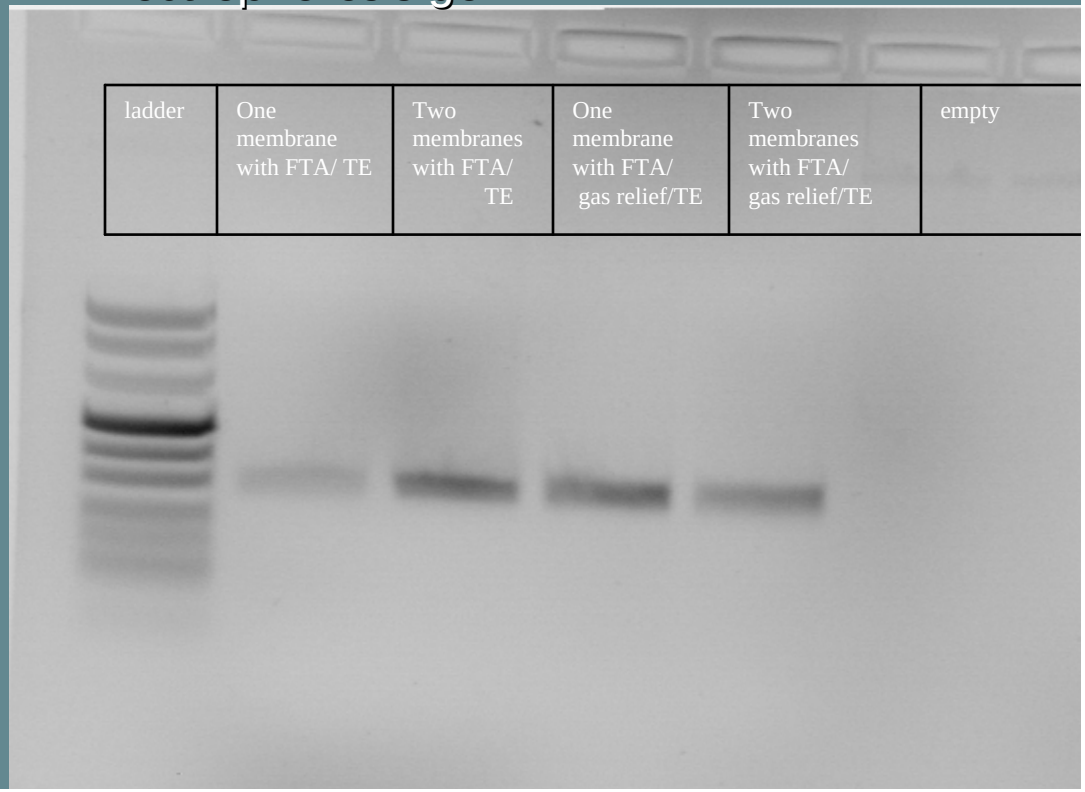


Results:

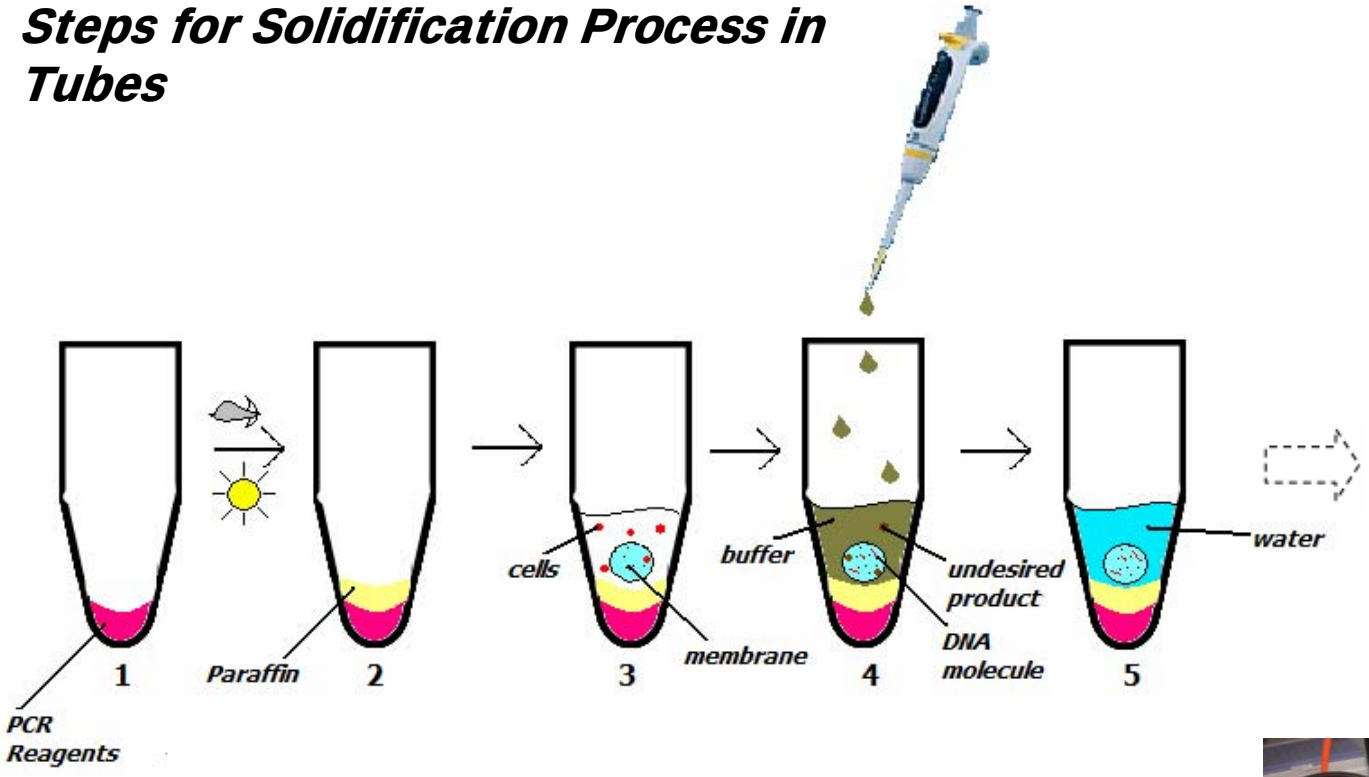
Microscope result:

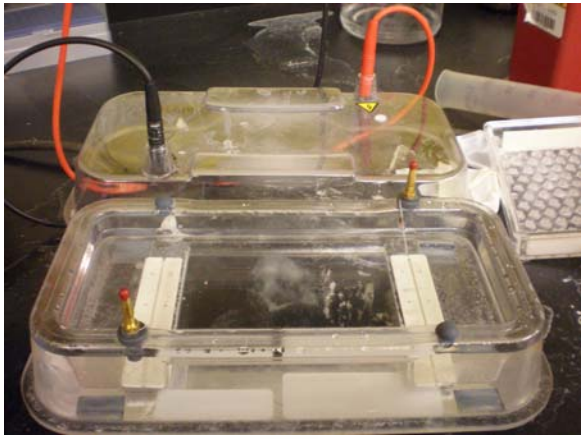


Electrophoresis gel:



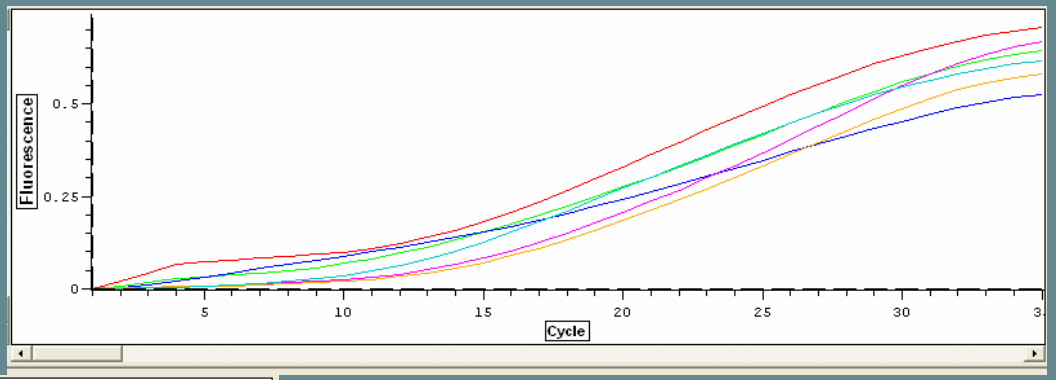
Steps for Solidification Process in Tubes



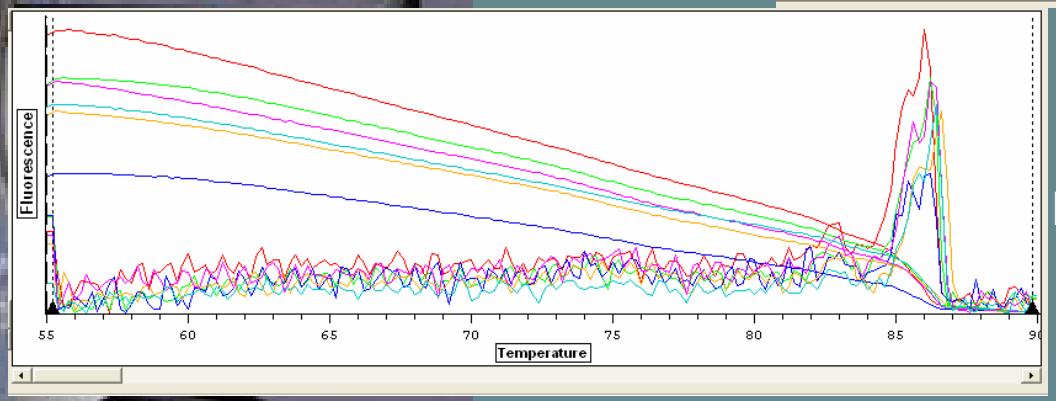


1. *PCR reagents are left to dry overnight.*
2. *In the morning, paraffin is added on top of the PCR reagents.*
3. *After the paraffin is dry, a membrane and cells are added to the vial.*
4. *The remaining cells are removed and the membrane is washed.*
5. *The vial is filled with water and inserted inside the RT-PCR machine.*
6. *Sample is run on gel.*

Tube Results:

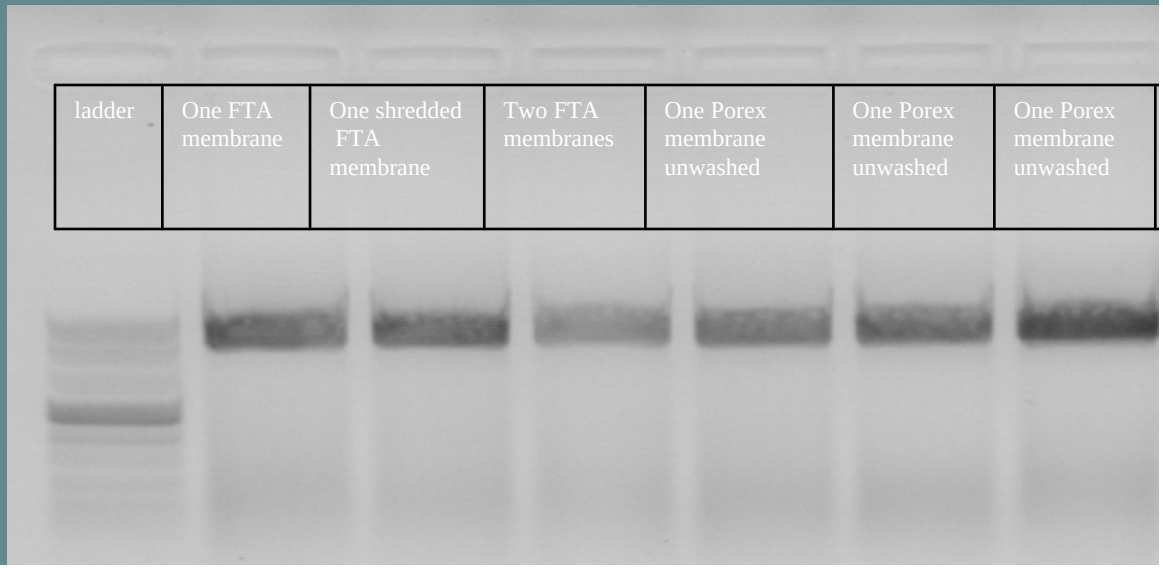


Fluorescence vs Cycle Curve:

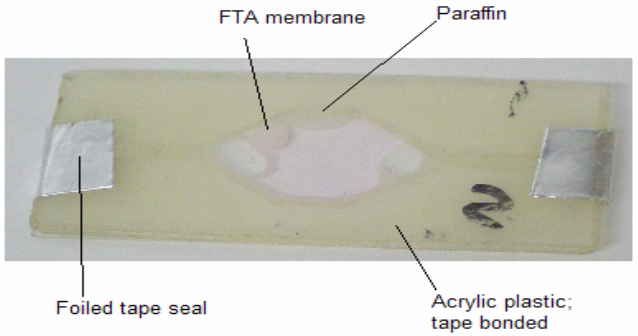


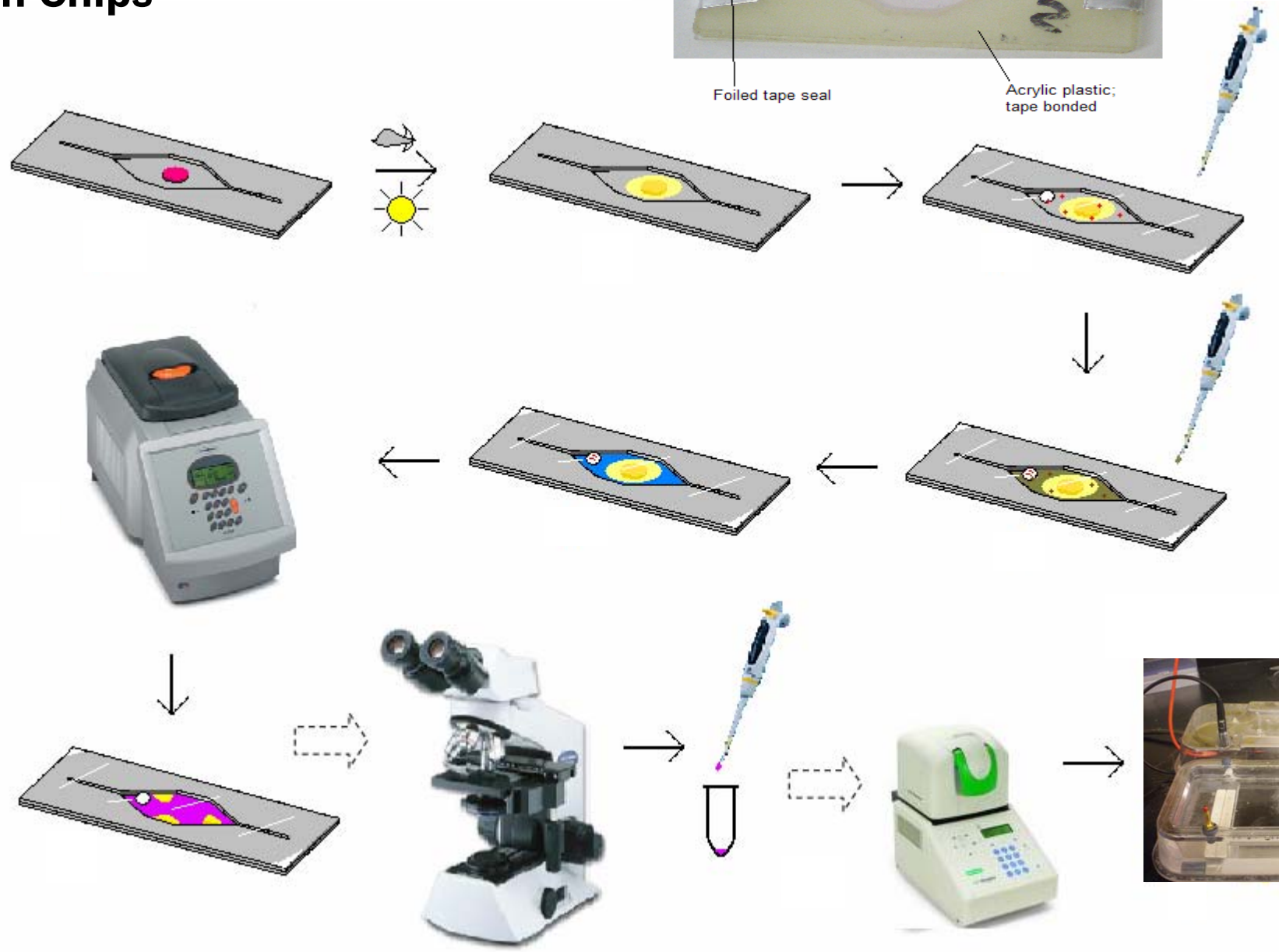
Melting Curve:

Electrophoresis gel:



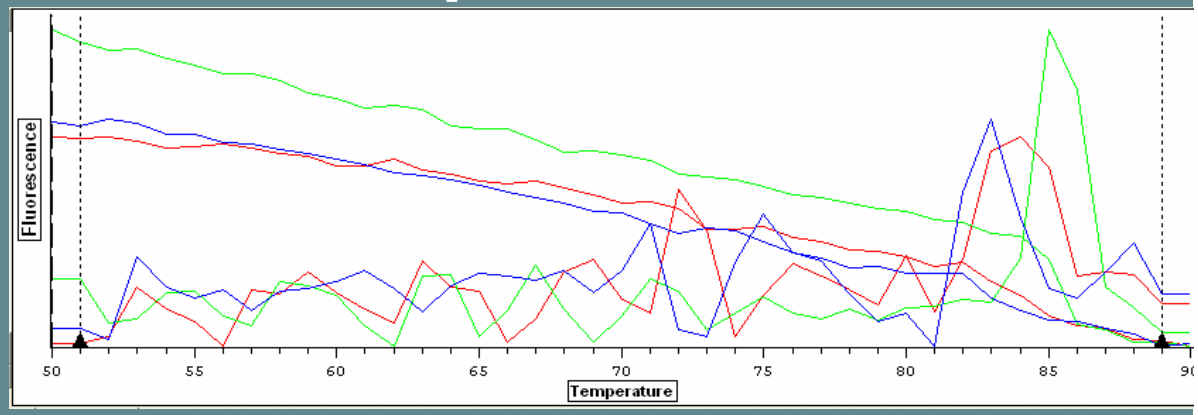
Steps for Solidification Process in Chips



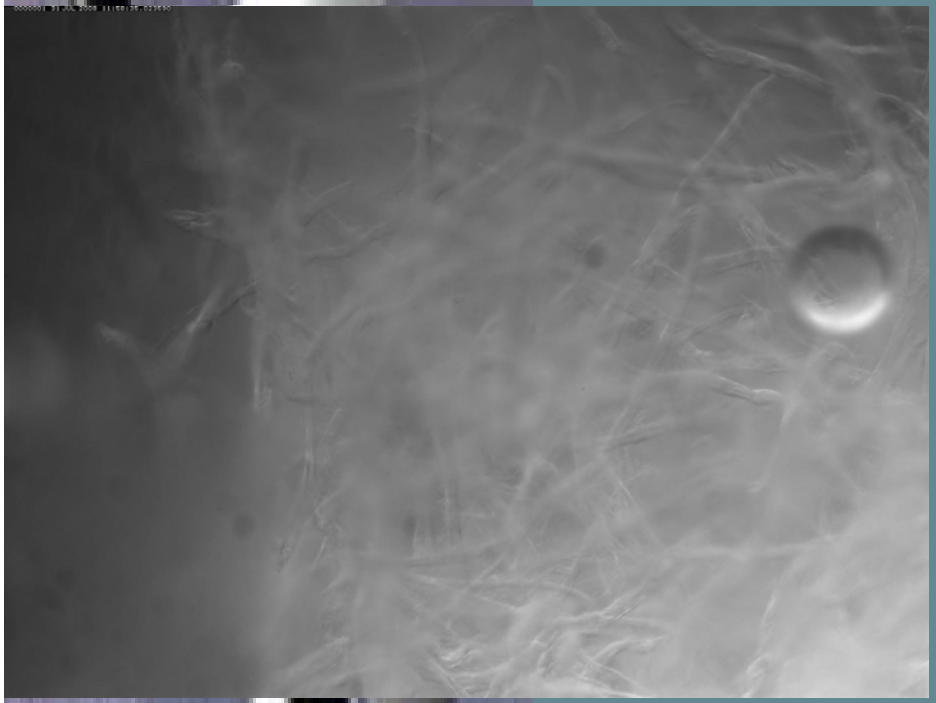


Chip Results:

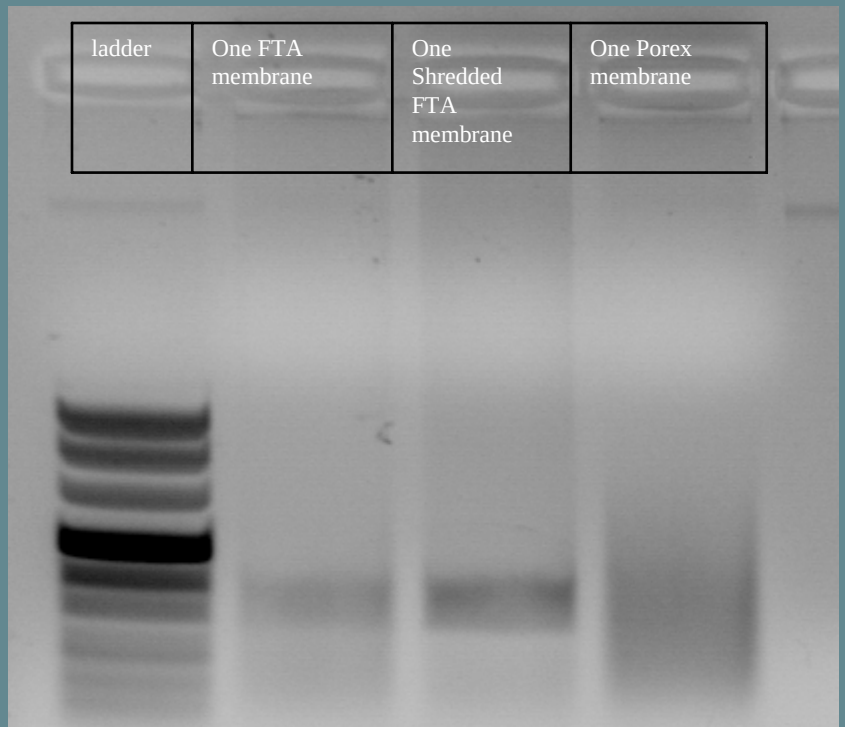
Melting Curve:



Microscope result:



Electrophoresis gel:



Conclusions

- DNA amplification during RT-PCR still occurs in the presence of paraffin or membranes.
- Paraffin is a promising reagent that can be used for the PCR process and as part of the LOC system.
- Membranes are a good possibility to be used in a LOC device with RT-PCR.

Future goals:

- To obtain better melting curve results from the dry storage process in the chip.
- To gather enough information for the integration of the RT-PCR technique into the LOC system.

Penn Engineering

Thank you!!

National Science Foundation
WHERE DISCOVERIES BEGIN