iRAGE:

Independent DNA Recovery from an Agarose Gel after Electrophoresis

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Abstract

iRAGE, Independent Recovery from Agarose Gel after Electrophoresis, is a device that autonomously extracts DNA from an agarose gel for further laboratory use. The process is controlled by a microcontroller that monitors the extraction process with three sensors: the heat sensor, the transmittance sensor and current meter. The heat sensor ensures that the chemical system does not exceed a threshold of 65°C, the melting point of agarose. The current meter monitors whether current is running through the gel, forcing the DNA out. The transmittance sensor, emits light through the buffer in which the DNA is extracted, is composed of a photodiode that transduces the transmitted light into a voltage. By detecting the change in transmittance the microcontroller can determine when elution is over and shut the system off, outputting any information in graphical form to a LCD screen.

Introduction

The objective of this project was to create a new device that electrically extracts DNA from agarose gels. iRAGE is meant to work with little effort from the user. Once the user turns our device on they can walk away without focusing any further attention on the instrument. This involves sensors to monitor whether a connection is made across the gel (current meter), keep the system from overheating (heat sensor) and verify when all DNA has been eluted (concentration sensor). At the end of the process, iRAGE consistently extracts ≥20% the DNA put in.

iRAGE has sensors that have been calibrated to interact with a chemical system. The heat sensor checks whether the system is too hot causing the gel to melt. The device lowers the voltage applied across the gel accordingly. The current meter checks if the gel and buffer are not connecting the electrical current. Its feedback alerts the user by sound. The concentration sensor determines when DNA has stopped eluting into the buffer. Its feedback shuts the system down. Overall this device will free up more laboratory time for the user.

It is important to note that this device is meant to elute very small fragments on the order of $0 < X \leq 500$ base pairs. Larger fragments run very slowly and need a higher voltage supply for elution.

The project was conceptualized and is funded by our principal investigator, Professor Nader Pourmand.
Block Diagram

Figure 1
This is a functional block diagram of the electrical components and how they interact with the mechanical device. The arrows point out the flow of data between the microcontroller and other components. The mechanical diagram illustrates how each element will react to the two buffer and gel system.

Figure 1 shows a block diagram for the iRAGE device. Each sensor interacts with the mechanical apparatus and the chemical system inside. Feedback from the sensors tells the microcontroller whether it is necessary to drop the voltage in case of overheating, alert the user if the current is not connected, or turn the system off if there is no change in transmittance due to completed extraction.
Components

The iRAGE device will consist of multiple electrical components and a specific physical design. There are 5 mechanical and 6 electrical components.

- **Mechanical**
  - Gel Holder
  - Catch Basin
  - Base
  - Boom
  - Cap
- **Electrical**
  - Controlled Voltage Source
  - Heat Sensor
  - Concentration Sensor
  - Control Unit
  - Ammeter
  - External Power Source

The mechanical apparatus holds the buffers and gel band in a certain orientation. The electrical components are external to the device and are placed according to the components of the mechanical apparatus with which they must interface. These components are described in detail in sections 2-7.

1. *Preliminary Analysis of Mechanical Design*

1.1. **Overview of Chemical, Mechanical and Thermodynamic tests to understand the properties of Electroelution**

First we experimented with the elution process in order to qualitatively understand what factors hindered DNA mobility or caused DNA contamination. We tested different mechanical designs, vertical and horizontal, to observe any advantageous aspects of either one. We calculated and measured heat accumulation in the biochemical system during elution to understand what variables (cross-sectional area, length of apparatus, etc.) play a role in our designs. DNA samples were tested and purified to monitor the loss of product due to reactive materials and dilution. By taking into account biomolecular mobility, organic chemical reactivity and mechanical thermodynamics we were able to eliminate and maximize aspects of our apparatus to help efficiently interface electrical hardware and biomolecular environments. These are described in detail in the following subsections.

1.2. **Preliminary tests with Team-Fabricated Mechanical Designs**

All of the tests were done with several different apparatus designs that our team fabricated. We came up with three basic schemes to each design that are shown Figure 2.
These are the three basic designs of all the apparatuses that we fabricated. The top left illustration is a vertical design that separates the two buffers by two caps glued together. We drilled a hole through the caps so the buffer in the bottom well can connect to the agarose gel in the top. The agarose gel sits on top of the cap, separating the buffer in the bottom well and the buffer just above the gel. The design to the left of it is the same except there is a mesh in between the lower well and the agarose gel. This mesh is a micro-pore film. Below the two vertical designs is a horizontal design that consists of two tubes with two more caps glued together. Each tube for each prototype varies in size.

Figure 2
1.3. Horizontal design and separating buffers by an agarose gel

We designed our first apparatus horizontally to test whether agarose gel is able to separate the buffers and electrically connect a current. This would determine whether the agarose gel was conductive enough for elution. We observed that the agarose gel was able to completely separate both buffers and connect the current between the electrodes. Since we are able to connect through an agarose gel, our team fabricated more apparatuses with the intention of separating both buffers completely.

![Figure 3](image)

This horizontal design is made of two centrifuge tubes that are roughly 200µL in volume. In the picture, the left compartment is filled with clear TAE buffer. The right compartment is filled with a blue TAE buffer. The agarose gel is in the orange cap. Both buffers are completely separated, but a current is running through.

1.4. DNA and intercalated agents are a dense mixture, resisting electrophoretic mobility

We tested vertical and horizontal designs to observe how we could optimize the movement of DNA fragments through agarose gels. From experimental trials we concluded that biomolecule movement through a vertical design was much faster than a horizontal one. We concluded that the density of the DNA molecule was causing a significant effect on mobility.

The DNA samples that we use in our experiments are intercalated with dyes such as SYBR-Green. We did this to mimic the use of dyes that are used in gel electrophoresis, and to see the DNA move through the gel under ultraviolet light. The added weight of an intercalating agent causes a higher density to the already dense DNA mix. Figure 4, illustrates how intercalation works.

![Figure 4](image)

The diagram shows two DNA strands. The left strand has no intercalating agents, but the right does. What happens is a molecule of a specific structure inserts in between a base pair of nucleic acids. The red is an intercalating agent inbetween the base pairs on the DNA strand.
We first observed this dense effect when pipetting a DNA/SYBR Green mix into liquid agarose. **Figure 5** illustrates our observations.

![Figure 5](image)

After the agarose gel with the DNA mix is allowed to dry in the tube, the cap is taken off. The diagram shows the DNA mixture at the bottom of the agarose gel which is in the middle of the tube and cap. The DNA mixture sunk to the bottom of the gel before it had hardened demonstrating the high density of a normal stained DNA sample.

We suspected that the density of the stained DNA sample and gravity was causing a separate force of movement. We diagrammed how this movement through a horizontal design might look. **Figure 6** illustrates what forces are affecting DNA movement—the positive electrode by charge-to-mass ratio and the downward by gravitational pull. The gravitational pull is based on the proportion of DNA density to buffer density.

![Figure 6](image)

In this diagram we have portrayed the electrodes as point charges that have a basic electric field in between them. These field lines which are shown to travel from the positive electrode on the right, to the negative one on the left are analogous to the direction of the current flow. The circle in the middle is what we have stipulated as our DNA
sample. Inside the apparatus the DNA is moving according to what we have denoted as four main force vectors. The first is the force that is determined by the magnitude of the charge on the DNA. The second is the frictional force which is directly proportional to the mass of the DNA. The third is the gravitational force that is determined by the density ratio between the DNA sample and the phase that the sample is in: gel or buffer. The fourth is the normal force reacting against the gravitational pull downward. As the DNA moves downward it collides with water, Tris-acetate and other molecules in the buffer, causing a normal force resisting its gravitational fall.

When our DNA sample is moving through the gel and buffer, there is a constant gravitational force that is driving the DNA sample downward. We thought that the sample would either homogenize in the buffer or form DNA clumps on the positive electrode. However we mostly saw DNA falling to the bottom of the horizontal tube. This did not cause a problem for us, but it led us to believe that the gravitational force was significant in the movement of DNA in our apparatus. Figure 7 depicts what we observed.

![Figure 7](image)

The diagram shows DNA at the bottom of the tube. In reality the tube does not open up at the bottom, but we showed it this way to illustrate how some DNA is not reacting to the current. This is apparent as the force vectors that react to the positive charge and the frictional force are absent.

Since we know our DNA sample is significantly affected by gravity, our team decided to utilize this force and use a vertical design concept to hasten the elution process.

1.5. Resistance through a vertical design with and without a mesh

We fabricated several vertical apparatuses to test overall resistance between a vertical design with a mesh and without a mesh. Having a significantly larger resistance based on a mesh could cause various problems. First, a higher resistance could produce more heat in the chemical system thus melting the agarose gel. Second, due to Ohm’s Law, \( V = IR \), if the resistance of the system is larger, the current will decrease since we are applying a constant voltage. Current is the measure of how much charge is moving per time, and decreasing it would mean a slower DNA elution from the gel. Figure 4 shows some of the apparatuses that we used to experimentally distinguish resistance with a mesh versus not.
These are a few of the vertical apparatuses that we fabricated. Picture A shows an apparatus that is held together by a cap. B, shows a design in which we implemented a mesh. Below the mesh is a opening which opens to the catching buffer. C, shows another design in which both tubes are held together by a perforated cap.

<table>
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<th>Length (mm)</th>
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<th>Resistance (Ω)</th>
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<tr>
<td>70</td>
<td>201.06</td>
<td>Yes</td>
<td>13450</td>
</tr>
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</table>

The graph shows that the use of a mesh causes more resistance through the chemical system. 1 on the x-axis means “no mesh” and 2 is “mesh.”
1.6. Large dilution of DNA renders sample undetectable by spectrophotometer

When our team eluted DNA into different amounts of buffer, we realized that it was difficult or impossible to see the DNA under UV-light in a buffer after it left the gel. Our spectrophotometer readings showed no absorption at the 260 nm wavelength. We concluded that we were diluting the sample so much that it was impossible to detect absorption.

Most of the beginning apparatuses that we tested had very large compartments. They all ranged from 500 µl to 1000 µl. When eluting DNA samples of only a few hundred nano-grams, the DNA becomes almost undetectable by spectroscopy if the concentration is under 1 ng/µl.

We designed an experiment in which we diluted small samples of DNA and checked their concentration. A fragmented sample with a concentration of 45 ng/µl was used as the basis for this project. We pipetted 5 µl of DNA into a centrifuge tube and added 10 µl of water for each optical density scan. Before doing the experiment we calculated that it would take a dilution of over 225 µl to show no absorption signal. This was about right. It wasn’t until 200 µl when the absorption signal at 260 nm was almost zero.

In our final design we wanted a catch volume small enough so that any amount of DNA could be detected. After discussing with Professor Nader Pourmand we came to the conclusion that our catch must be 50-60 µl in size. Every apparatus thereafter was designed to elute DNA into compartments 100 µl or smaller. Our final design was fabricated to hold roughly 60 µl.

1.7. Heat accumulation in the apparatus as a function of time

Our team used different apparatuses to test how temperature would change as a function of time, cross-sectional area and length between electrodes. We tested these variables knowing the following mathematical relationships:

\[ I = \text{Current in amps} \]
\[ R = \text{Resistance in ohms} \]
\[ t = \text{Time in seconds} \]
\[ H = \text{Heat in joules} \]
\[ m = \text{Mass in grams} \]
\[ S = \text{Specific Heat in J/m}^0\text{C} \]
\[ T = \text{Temperature in Celsius} \]
\[ l = \text{length between} \]
\[ p = \text{electrical resistivity} \]
\[ A = \text{Cross-sectional Area} \]

\[ I^2Rt = H \]
\[ mST = H \]
\[ I^2Rt = mST \]
\[ \frac{I^2Rt}{mS} = T \]
\[ R = \frac{\rho * l}{A} \]

We started this experiment when we observed that the agarose gel was melting when it was in a small-cross section of a tube or when we ran the elution too long. Either all the gel would melt or only parts of the gel would melt. We graphically analyzed one of our trials to illustrate how sensitive temperature is to cross-section and electrode distance. This is shown in Figure 10 and 11.
The following graph shows how temperature changes as a function of cross-section. The data for this function was taken from one of our trials with the horizontal apparatus. It illustrates the exponential relationship between cross-section area and temperature. The curve is represented by the function:

\[ R = \frac{\rho \cdot l}{A} \]

\[ \frac{IR^2t}{mS} = T \]

\[ \frac{\rho^2 l^2 It}{A^2 mS} = T \]

\[ \frac{(560.99\Omega m)^2 (0.07m)^2 (0.010A)(1s)}{A^2(1g)(4.186\text{J/g}^\text{C})} = T \]

\[ \frac{3.68389}{A^2} = T \]

\( \rho = \) electrical resistivity, \( l = \) length of the conductor (the length of the apparatus), \( A = \) cross section of tube

\( R = \) resistance, \( m = \) mass of the conductor, \( t = \) time, \( \rho = \) electrical resistivity, \( l = \) length of the conductor, \( A = \) cross section of tube

The electrical resistivity was calculated by knowing the length, average cross section of the tube and the resistance. All were experimentally derived.

It is important to note that this curve only shows us how the temperature rises at a specific voltage across the system. This calculation showed us that temperature in the system can change dramatically if the cross-sectional area of our final mechanical design is not defined properly with the length of the conductor. All other variables can be adjusted, but the cross-section and length are constants after fabrication of the device.
This is a graph of the same trial that we used in Figure 10, but with Temperature as a function of both time and cross-sectional area.

\[
\frac{3.68389t}{A^2} = T
\]

The temperature of the system exponentially rises when the cross-sectional area reaches less than 0.2 meters and beyond as shown in Figure 10. That same exponential climb is shown here. The temperature rises linearly with respect to time.

We experimentally verified these mathematical calculations with different apparatuses and microcentrifuge tubes. Figure 12 illustrates how some of these set-ups looked.
This diagram shows three basic designs that we tested. They varied in cross-sectional area and length. The results are shown in Figure 7. (1) was a horizontal design made of two centrifuge tubes. (2) was an microcentrifuge tube. The thermometer almost filled the entire bath. (3) was a long thin cuvette that we used. The electrode was strung through a pinhole that we made.

We made 5 apparatuses from these 3 types and tested each one to see what final temperature they maxed out at. We assumed that temperature has to come to some steady state value because of heat transfer to the material and outside environment that is in contact with it. The heat capacity can not rise to infinity in reality, so when the temperature stayed steady for a significant amount of time we assumed the system nearly reached equilibrium. Our hypothesis about non-ideal heat accumulation was correct according to experimental results we have in Figure 13.
This is a bar graph of the final temperatures that our different apparatuses reached. There were 5 different apparatuses tested, each were vertical designs. The purple bars of each apparatus are the first trial and the pink bars are the second. There was very little variation between trials. Each trial stayed below the 65°C threshold. We observed that in each trial, the temperature reached a peak where the system did not rise very fast in temperature. When we observed this behavior, we waited for a few minutes and then shut the current off. We concluded that the conductivity of the materials were using caused the system to reach a steady state between heat energy gained and lost. In light of these results, we fabricated another apparatus that was similar to the prototyping designs that we had at the time even though it was not finish.

When we analyzed the results in Figure 13, the rate of change in temperature was decreasing at some point. We took the next step and fabricated an apparatus similar to the dimensions of our main design that we had conceived at that moment. This included the cuvette catch of our final design at 60μl and centrifuge tube to open up the top to the thermometer. We calculated the ideal relationship that could theoretically be shown in our experiments. This is shown in Figure 14.

<table>
<thead>
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<th>Apparatus</th>
<th>Length (mm)</th>
<th>Cross-Section Area (mm²)</th>
</tr>
</thead>
<tbody>
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<td>24</td>
<td>6.1575</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>50.26</td>
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<td>3</td>
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<td>201.6</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>201.06</td>
</tr>
</tbody>
</table>
Figure 14
This graph shows ideally how long it would take for our apparatus to reach 65°C for a given length between the electrodes. The graph is the function:

\[ \frac{2263.2}{X^2} = t \]

Where 2263.2 was determined by the various properties of the apparatus described in Figure 9. The maximum time on the axis is about 15 minutes. The most optimal length between the electrodes would be somewhere less than 2.5 mm.

We kept this in mind and tested the apparatus to see if a length of 15 mm would be too long a length for the design, causing a faster rate in temperature growth. We ran 10 trials with that piece and graphed the final temperature of each trial. We found that these ideal relationships were not as accurate as we thought, and the apparatus we tested did not accumulate enough heat to melt the gel. These observations are graphed in Figure 15.
These results further show that there is some steady state between heat dissipation and heat gained in the apparatus that we used. In this graph, the temperatures reached were near 65°C, but none reached it or went above. The average final temperature was 47°C. We realized this and have taken it into account for our systems integration tests that we are running now.

After analyzing how temperature changes in the many apparatus designs that we fabricated, we concluded that our final design would have a specific length between the electrodes and cross-section area for the gel. We determined this to be 15 mm in length and 6.1575 mm² in cross section.

1.8. Loss of DNA due to reactive materials

To finalize the design, our team researched and tested different plastics to determine which would reduce the reactivity of DNA with our apparatus the most. For the gel holder we decided to use Teflon over the standard plastic polypropylene. We then tested whether material from epoxies would contaminate our DNA samples.

1.8.1. Material for mechanical design

Most of the apparatuses that we built were made of polypropylene a common industrial plastic used to make sample tubes. Figure 16 shows the structure of the molecule.
Polypropylene is a copolymer (there are other forms as well) that is derived from the propylene molecule, a three carbon molecule with a double-bond. The repeating methyl group that hangs off of the main chain causes the helical structure to the plastic much like DNA.

Polypropylene has worked well for our team, but we wanted to optimize our final design in order to reduce as much DNA loss as possible.

Our professor suggested that we use Teflon, polytetrafluoroethylene (PTFE), for our final design. Teflon’s structure contains many fluorines, a highly electronegative element as shown in Figure 17.

High electronegativity of fluorine decreases the Van der Waals forces that would act on other molecules, giving Teflon its inert ability. Fluorine becomes electron rich, giving it a slight negative polarity which acts like a negative charge. Since DNA is negatively charged and Teflon is slightly negative in polarity, the two molecules do not react [10].
In this diagram, a DNA base pair that is connected to a continuous strand (denoted by $R$) shows negative polarity, stipulated by $\delta^-$. The electronegativity of fluorine causes a large electron density on the fluorine atoms themselves, skewing the covalent bond to show that fluorine is slightly more electron-rich. This gives Teflon a slight negative polarity, one that DNA does not stick to.

This led our team to choose Teflon for our final design rather than the normal plastic polymers we had been using.

1.8.2. Use of epoxy in the prototypes and contamination of DNA

We tested the chemical effects of epoxy on our DNA sample since we had used the adhesive to seal electrodes in place and prevent leaking. Epoxy consists of two chemical elements, a resin and a hardener.

The hardener is a polyamine that can form complex structures called ligands in a process called chelation. These epoxy adhesives are filled with specific polyamines that all act as ligands.

Ligands can react with DNA by covalently bonding, intercalating or electrostatically binding to the DNA itself (intercalation is illustrated in Figure 19 and 4). It is this property that can cause epoxy hardeners and other copolymers with reactive functional groups to bind to DNA.
Figure 19
This diagram illustrates the reaction of oligonucleotides with the ligand neotropsin. This is an example of how ligand intercalation affects DNA conformation in hairpin structures [13].

The polymers will only react with the DNA if compounds leach into the buffer. We tested whether epoxy may leach into DNA mixture by coating a centrifuge tube with epoxy and running a current through it. Figure 20 illustrates this experiment.

Figure 20
First a tube is lined with epoxy and allowed to dry. Afterwards, a sample of DNA with a known concentration is pipetted into the tube. The mixture is shaken briefly to help homogenize the solution. The mixture is then run through a spectrophotometer to quantify whether there was DNA loss or not. This same experiment is done with a current run through the tube.

We observed that the epoxy started to leach into the buffer when a current was applied over a period of 10-20 minutes. It was apparent that the epoxy was clouding the buffer. We then tried this experiment by applying epoxy to a pinhole that holds an electrode. There was no apparent leaching of epoxy polymers into the bath. We concluded that the
use of epoxy to seal our positive electrode in our final design would not add to DNA contamination.

### 1.8.3. Oxidation of Electrodes

When running these trials we realized that the lead wires we were using were corroding. Lead was stripping of the positive electrode wire and leaching into the solution. This was a potential contaminant as well. After researching different materials, we formulated an experiment to test what wires were corrosive in buffer solutions. We tested two:

- Silver Chloride
- Platinum

The silver chloride wires were very thin, with a gauge of 28 SWG. This is roughly 0.41 mm diameter. We began testing its corrosive properties and observed that the silver chloride coating strips off the positive electrode wire when a current is run through it. We did not expect this result since silver chloride wires came highly recommended to us. Researching further into this, we learned that silver chloride reacts with Tris buffers to form precipitates in liquid [9].

In light of this problem we decided to test platinum electrodes, the standard electrode used in gel electrophoresis. Since platinum is very expensive, we took an old broken gel kit and stripped the wires from it, and conserved what we used. After testing platinum it was clear that they do not corrode. We concluded that our final design would use platinum wires for the positive electrode.

### 1.9. Biological analysis overview for final design

After experimenting with electroelution we were able to hone our final design to these specific concepts:

- Use of a vertical apparatus to utilize the gravitational effect caused by density of the DNA solution
- Use of a mesh to separate gel and negative electrode buffer from positive electrode
- Use a cross-sectional area of 6.1575 mm$^2$ and 15 mm length between electrodes to keep the system safely within heat constraints
- Elute DNA into a 60 µl compartment to ensure high concentration for significant detection
- Use of Teflon and specific use of epoxy to minimize DNA contamination
- Use of platinum wire for positive electrode

Taking into account these different variables we were able to develop a design (Section 2) that met both our electrical and biological standards.
2. Mechanical - Apparatus Design

After taking into account the biophysical, chemical and thermodynamic nature of electroelution, our team finally designed this set up. The design consists of 5 components, each of which are discussed in detail in the following subsections. These 5 components are:

1. Gel Holder
2. Cuvette Catch
3. Base
4. Boom
5. Cap
In designing this entire mechanical system, we have carefully considered where electrical components will mount and how the material and physical dimensions will affect the chemical system. We have 5 components to the design.

1. The Gel Holder, which stores the gel and negative electrode
2. The Catch Basin Cuvette, which we have fabricated out of quartz and is the compartment in which we elute DNA into
3. The Base is what holds the cuvette into place and has trenches for the UV-LED and Photodiode to run to the catch.
4. The Boom is a piece that holds the gel holder
5. The Cap is used to cap the gel holder in order to pour reagents into it.

The most important piece out of all of these is the quartz catch cuvette. It was number one priority in defining this mechanical design because as a team we would be fabricating it ourselves. The machine shop that we ordered from, FirstCUT, is unable to fabricate pieces in a material transparent to UV light, an essential aspect to our device. This led us to fabricate a 50 µl cuvette out of a 300 µl cylindrical cuvette that we were given by the Pourmand Lab. The rest of the design came from observations we made from biological verification and the electrical components.

*All parts that connect were designed with tolerances are based off of machining industry Standard Tolerances*
2.1. Gel Holder and its Heat Accumulation determined by the proportion of electrode distance to cross-sectional area

![Figure 23](image)

This part holds the gel and bath for the negative electrode. The opening was made to open up to fit the electrode and thermistor (heat sensor) that will be going into it. These dimensions were defined by the proportion of cross-sectional area of the cuvette to the length between the electrodes.

The gel holder is a conical tube that the agarose gel will sit in. The conical design prevents the gel from moving around and potentially mixing buffers. The gel is poured into this part of the apparatus and allowed to solidify for the gel to take shape of the tube itself. We will be fitting a micropore mesh onto the bottom of the tube (the smaller end) to help separate the gel from the catching buffer. This mesh does not have to be glued onto the apparatus but just pushed into place. Placing the gel holder on top of the cuvette will pinch the mesh between the gel and catching buffer.

The large opening has an outer diameter of 26.0 ± 0.13. The smaller end of the holder has a 3.3 ± 0.0165 mm inner diameter (The tolerances were defined by the calipers used) The bottom opening to the tube has a lip that will fit over the Catch Basin.

We have defined this particular part by the mathematical analyses of heat accumulation that we discussed in the preliminary assays.
2.2. Catch Basin

Figure 24
The cuvette is the most important piece of our apparatus. It is quartz glass. We took a 300 \( \mu l \) cuvette from a spectrophotometer and used a Dremel saw to cut the piece to approximately 60 \( \mu l \). We then took a Diamond tipped pencil and scored a pocket into the bottom of the cuvette. Afterward we drilled a pinhole through the bottom with a 1mm steel drill-bit for the positive electrode. We placed the electrode in and sealed the hole with epoxy.

When the DNA elutes from the gel, it elutes into the catch. Our catch is a cylindrical glass that we fabricated from a standard cuvette piece for a specific spectrophotometer. In order to detect the DNA when it elutes, we are emitting 260 nm light (ultraviolet light) through the catch. The cuvette is made of quartz and is transparent to this light, allowing us to detect the transmittance. Quartz is negatively charged and will not bind to DNA, keeping the sample in solution [8].

The Gel Holder fits directly onto this piece. The cuvette had a volume of 300 \( \mu l \), but it has been cut to roughly 60 \( \mu l \) by a diamond tip dremel saw. This amount was defined by our preliminary assays.
2.3. Base to hold the sensors and cuvette catch

Figure 25 shows the base piece. The base is support pad to hold the cuvette, allowing trenches to run all the way up to the catch piece. The trench holes were made $8.5 \pm 0.0425$ mm in diameter to account for the $8.0 \pm 0.04$ mm LED. The LED only has an active area of 0.25 in the middle of the piece but the light has a $40^\circ$ spread. The base only allows light to pass straight through the cuvette because the holder wraps around, cutting widespread light off.
2.4. Boom to hold Gel Holder in place

The boom is what holds the gel holder on top of the cuvette. It fits directly into the base.

The boom holds the gel holder in place when it is on top of the cuvette. It is made of ABS plastic. The hole for the gel holder has a $\pm 0.5\%$ tolerance taken into account to allow for the holder to pass through.

2.5. Cap for sealing the gel holder while pouring the agarose gel

The cap covers the small opening at the bottom of the gel holder. This is so when gel is put in, and more liquid gel is poured, it doesn’t leak out. When the agarose is dried, the cap is taken off.
3. **Control Unit – Microcontroller**

All of the electrical components are controlled by the microcontroller that we have selected, the Texas Instruments MSP-EXP430F5438 experimenter board. Each sensor circuit and the electrodes to the apparatus are connected to the microcontroller. The microcontroller has an LCD screen to interface with the user. When running an elution, the microcontroller runs controlled feedback loops with the sensors and electrodes to monitor the chemical system in the apparatus.

There are 3 sensors that feedback to the microcontroller:

- **The Ammeter** tells the microcontroller whether the controlled voltage across the gel has a connected current.
- **The Heat Sensor** is placed into the Gel Holder bath, and gives real-time thermodynamic readings to the microcontroller.
- **The Spectrophotometer**, or Concentration Sensor, quantitatively measures the transmittance of UV light signals through the cuvette catch as DNA elutes.

The microcontroller has 4 main components to interface with the sensors and the user. These are:

- Analog to Digital Converter
- Digital to Analog Converter
- Ability to interface with a computer
- User Interface via LCD / Buttons

**Figure 28** illustrates how the electrical components interface with the mechanical apparatus and the microcontroller.
This is a detailed diagram to illustrate how the sensors interface with the apparatus, microcontroller and user interface. The digital-to-analog converter outputs signals to the three components, the controlled voltage source/ammeter, the heat sensor and the concentration sensor. All of the signals that are detected are inputted into the analog-to-digital converter on the microcontroller to be processed. The flow of signals is stipulated by the arrows going into the microcontroller and leaving. As seen, all components are controlled by this microcontroller.

3.1. ADC and DAC for Sensor Feedback Control

For our sensors that will be outputting analog signals, the MSP has a 12-bit analog-to-digital converter as well as a 12-bit digital-to-analog converter capable of outputting 1.8 to 3.6 V. It has multiple analog inputs as well as outputs[7].

The thermistor, photodetector and ammeter output their conditioned signal to the analog-to-digital converter where the microcontroller can process the converted value. Algorithms programmed into the microcontroller take the voltage signals and relate them to their temperature, transmittance and amperage values.

3.2. User Interface connection with a Microcontroller

This microcontroller can interface with a computer via mini-USB UART connection. This has given us the capability of writing specific software for the iRAGE device that can be used for testing from a computer and saving the results of an elution.

The joystick and buttons, coupled with the LCD allow for independent control of the device without needing to know how to use a particular computer or program. The voltages can be set, the temperature monitored, and the results displayed when done.

The USB UART allows for computer-based control of the system as well as data transfers when it is done so any information can be archived or seen on a larger screen.
3.3. Microcontroller Pseudo Programming

The program will take user input using the LCD and joystick plus buttons for the maximum temperature, starting voltage, and maximum voltage. At any time the user can push the button labeled "Cancel" to end the process or "Pause" to pause it (which will then become "resume" to continue).

User Input:

- Set
  - Max Temperature
  - Start Voltage
  - Max Voltage

Set Default
- Max Temp: 55°C
- Start Volt: 15V
- Max Volt: 20V

Routine - Start Program

- Check Thermistor Voltage $V_{\text{therm}}$
- Store $V_{\text{therm}}$
- Convert $V_{\text{therm}}$ to $T_{\text{read}}$
- Check $T_{\text{read}} < 55^\circ$C
  - If not: Go to Start
  - If yes: Go to Stop Incomplete

- Check Specific Voltage $V_{\text{spec}}$
- Store $V_{\text{spec}}$
- Convert $V_{\text{spec}}$ to $C_{\text{read}}$
- Check $C_{\text{therm}} < C_{\text{read}}$
  - If not: Go to Start
  - If yes: Go to Stop Complete

- Check Ammeter Voltage $V_{\text{amp}}$
- Store $V_{\text{amp}}$
- Convert $V_{\text{amp}}$ to $I_{\text{read}}$
- Check $I_{\text{read}} = 0$ Amps
  - If not: Go to Start
  - If yes: Go to Stop Incomplete

Stop Incomplete

- Turn controlled voltage off
- Run Graph Algorithm on All values
- Alert User Fail

Stop Complete

- Turn controlled voltage off
- Run Graph Algorithm on All values
- Alert User Success
4. **Controlled Voltage Source and Ammeter**

The first electrical component is the controlled voltage source, which supplies current to the chemical system. The current from the apparatus is run into an ammeter circuit to alert the microcontroller whether current is actually going through the system.

To determine the voltage to apply over a gel, the distance between the electrodes is taken into account. Voltage to supply over an agarose gel is measured in *volts per centimeter*. The buffer and gel act as a resistor together and can be approximated to have similar resistances [2]. Most gels run 1-5 $V/cm$. Since our apparatus is going to be millimeters in size, it is best to realize that 5 $V/cm$ is 500 $mV/mm$. The distance between our electrodes is approximately 20 $mm$. This means that for our gels we will need a range of 2 to 20 *Volts*.

We tested what voltages are most efficient in eluting the DNA with an apparatus the size of our design. We found that 15 V was the magnitude that did not cause any gel melting, and roughly a 20-30 minute runtime. These are rough numbers In order to elute the DNA a voltage is applied across the gel. To apply this voltage we are using a controlled voltage source. The voltage is controlled by digital-to-analog signals outputted from our microcontroller.

4.1. **Voltage source and ammeter circuit**

The range for the controlled voltage source is 0-20$V$. The microcontroller is only capable of outputting at max 3.6$V$. In **Figure 29** we have implemented a noninverted amplifier with a 20 volt rail from our external power supply to boost the microcontroller DAC signal. The current travels through the gel and to a voltage divider R26. The voltage difference across R26 is supplied back to the microcontroller. If it reads zero volts, then there is zero current through the system and the microcontroller alerts the user.
Figure 29
This is the circuit that we have implemented for the controlled voltage source. It contains an input from the DAC on the microcontroller, an op amp for gain and a voltage divider to determine whether current is running through the gel.

5. **Heat Sensor – Thermistor**

5.1. Thermistor Properties

Our team has implemented a thermistor to detect the heat change in our apparatus. The thermistor has a temperature range of -50 to 130° C. It has a high precision in the range that we need.

Since the thermistor is going to bathe in a liquid buffer, it has been covered with epoxy to prevent the device from corrosion. Since the thermistor will be in the negative electrode bath, we will not have to worry about DNA sticking to the component since it is traveling to the positive end.
5.2. Thermistor Circuit and Calibration Curve

Figure 30
This is a graph of the Thermistor circuit's calibration test—the data table is shown in the appendix. It is a nonlinear relationship as our team expected.

![Thermistor Circuit Diagram](image)

**Figure 31 – Thermistor Circuit**
This is the circuit designed for our thermistor. The thermistor is a part of a Wheatstone bridge with $R_1$, $R_2$ and $R_0$. The node between $R_2$ and the thermistor is run as $V_{in}$ into an op amp with a negative feedback loop. This amplifies the signal.

In this schematic, we use a Wheatstone bridge, and an operational amplifier. The Wheatstone bridge follows the voltage regulator from the external power supply. There are four overall resistor values including the thermistor. We know that there will be no voltage difference between the nodes labeled plus and minus. Therefore,

\[ R_1 = R_2 \]
\[ R_0 = R_{thermistor} \]
if no voltage is measured. We also want high linearity which means that $R_2$ and $R_1$ are going to have to be large compared to the resistance of the thermistor. However, we want high sensitivity so that $R_2$ and $R_1$ cannot be too high. This brings us to use a well known engineering approximation,

$$R_2 = R_1 = 10 \text{thermistor}$$

where $\text{thermistor}$ is the highest resistance expected. Similarly, $R_0$ is going to be equal to the lowest resistance expected.

This circuit alone is not very sensitive and has a fairly high output impedance, which can be undesirable. The LM324 is an operational amplifier which provides the necessary buffering and amplifying characteristics. The Gain is equal to $(R_4/R_3)+1$ which gives us 11 times gain in this configuration.

The signal from the operational amplifier feeds into the ADC of the microcontroller where it is processed.

6. **Concentration Sensor - Spectrophotometer**

To quantify the elution of all DNA from the gel, the device has a concentration sensor to check when the DNA has stopped eluting. DNA absorbs light in the UV region, more specifically 260 nm wavelength. As DNA elutes into the catching buffer, our UV LED emits light into the catch. The transmittance through the cuvette is detected by our photodiode and we are able to verify whether DNA is done eluting or not.

6.1. **Monitoring change in Transmittance**

Our team is detecting the rate of change in transmittance. Once the elution process begins, the microcontroller is taking constant readings from the photodiode. Once DNA begins to enter the catch, a certain fraction of light will be absorbed, and the photodiode reacts to less light. More DNA will enter the buffer, lowering the transmittance of light. When the DNA has completely separated from the gel, the transmittance stays constant and the photodiode outputs a constant voltage. It is at this point that we can qualitatively say the process is done.

6.2. **Ultraviolet Light Emitting Diode**

The photoemitter is a UV-LED that emits light specifically at 260 nm wavelength. This is the exact wavelength of light that DNA absorbs. The LED that we have chosen has a flat lens that spreads the light at a wide angle (above 40°). The LED we are purchasing is expensive (as noted in the Budget section in the appendix).

6.3. **Photodiode properties**

The other sensor that we are using is a UV photodiode. The one we have chosen is SiC, silicon carbide and is engineered to respond only to a wavelength of approximately 260 nm. The spectral response is shown in Figure 32.
This is the spectral response of the diode provided by the company Electro Optics. The range of response is around 275 nm. We are emitting light at exactly 260 nm. The x-axis is wavelength and the y-axis is spectral responsivity.

The photodiode has a range of 210 to 380 nm. The maximum spectral response is at 275 nm. Since we are emitting a specific wavelength of light, we

6.4. Circuit for a photodiode sensor

We are detecting very small concentrations of DNA, so we have implemented some basic filters to help regulate the signals to the LED and microcontroller.

![Diagram of UV LED circuit](image)

Figure 33

The UV LED circuit is powered by the 5 volt regulated external power supply. This feeds into the LED. The photodiode is connected to a regulated op amp.

Input to the UV LED is from the 5 volt regulator from the external power supply. The 5 volt feeds into a low-pass filter. We implemented this filter since we want to supply a
specific direct current to the LED. If any small frequency signals disrupt the current to
the LED, it will output UV frequencies different than 260 nm light.

When we supply UV light to the photodiode, a current is induced into the operational
amplifier.

7. **External Power Source**

![Figure 34](image)

Our external power source consists of a 20V AC adaptor and a 5 volt regulator. These
two lines supply power to all op amps and sensors.

The external power supply that we are using is an HP AC adaptor that outputs 20 V. We
have placed two wires to the supply to feed out into other circuits. We divide the
voltage and regulate one of the 20 V lines with a 5 volt regulator.

The regulated voltage supply is Nationals LM317 configured in the following
manner. This gives us a $V_{out}$ of a little more than 5 Volts as long as $V_{in}$ is greater than 7V
and less than 28. This gives us a highly stable output for the following circuits which are
sensitive to the input voltage. Since the microcontroller can not output more than 3.6V,
the supply voltage will come from the extra power source we have.
Elution Efficiency and Consistency

Our team did a range of elution trials to test the efficiency, consistency and yield of our method of electro-elution. We ran 17 trials specifically for the purpose of elution. All effort and technique was applied to monitor the elution process. We got an average of 16.21% yield with a standard deviation of ± 10.02%. The results are show in Figure 35.

Our trials showed that it is possible to successfully extract DNA from the agarose gel. We trials gave us nearly consistent results. Some of the trials did contain human error and missteps that may have led to low yield. Our next step is to test our finalized mechanical design to see if it has a higher efficiency. While doing this, our team will troubleshoot how to increase the yield of the iRAGE device.
Figure 36
This diagram shows an overview of the electrical and mechanical components and how they interact with each other. A picture of the real life integration is in Figure 37.
The project status is as follows:
1. All the electrical sensors and circuits are built and calibrated
2. User Interface of the microcontroller is programmed
3. Algorithms for sensor feedback are programmed
4. Mechanical design is finalized and ordered

The next portion of this paper illustrates what the next steps are in order to finish integrating all components.

**Testing the final mechanical design**

When the final mechanical design comes in we will be testing it to get it all set up for the final integration. We will be checking heat accumulation through the system, elution time, elution yield and any flaws in the design that prevent the device from working at its fullest.

**Optimization of sensors interface with microcontroller**

Each of the 3 sensors, the ammeter, concentration sensor and heat sensor must feedback to the microcontroller smoothly. The Microcontroller must then be able to process these signals and output them to the user. We want to make sure that all components interface without any difficulties. To do this we are going to test each sensor separately.

**Controlled voltage and ammeter test**

The controlled voltage source and ammeter will be run through a chemical system like the ones we fabricated and run into the ADC. We will be testing the voltage divider in the circuit that determines whether a current is going across the gel or not.

**Concentration sensor test**

The concentration sensor will be tested with our catch cuvette. The test will involve the same type of calibration protocols we ran. This time the concentration sensor will be monitoring a DNA sample that changes in concentration. This will test the microcontrollers ‘completion’ routine when transmittance stops changing over time.

**Heat sensor test**

The heat sensor will be tested in a bath of water on a hot plate. This will test the shutdown routine that the microcontroller runs when the bath gets too hot.

**Interfacing sensors/microcontroller system with the final mechanical fabrication**

This is the final step in creating the iRAGE device. Once all of the sensors are interfacing with the microcontroller properly and the mechanical design has been analyzed, the last step is to have the sensors interface with the design. We will be running
elution trials with the device and monitoring all of the sensors to see if the device works together.

**Contingency Plan**

**Independent Study**

**Plan**

The goal is to apply the systems integration process next quarter. By the end of the quarter we hope to have a functional microcontroller that interfaces with the sensors, user and mechanical apparatus. We also hope to efficiently and consistently DNA like we have but at a higher level. Since we have been delayed by multiple things the plan is to work with Nader Pourmand next quarter for an independent study to get a functional device for his lab.

**Conclusion**

The iRAGE device has further designing and troubleshooting, but results have shown that this device is on its way to becoming fully functional. We are able to consistently elute 16.21% of DNA samples from our device. Our circuits are calibrated and working. The Microcontroller is programmed. The next steps are to interface the microcontroller and sensors with the mechanical apparatus and the chemical system inside. The successful integration of the finalized mechanical device, the electrical sensors and microcontroller will make iRAGE fully functional, saving the users valuable time in the laboratory.

**Acknowledgements**

We would like to acknowledge our professor, Nader Pourmand, for coming up with this project and funding it. Also to all the 123 seniors that have helped us on the way and our professors.
References


Appendices
Appendix 1

Project Charter

Team Organization and Responsibilities:

The first step is to research possible components. Sahil will research thermometers, Raj will research spectrophotometers, and Scott will research controllable voltage sources.

The second step is to create a prototype. Scott is using the software with specification and dimensional guidance from Raj and Sahil. Raj and Sahil are in charge of the testing prototypes and wet lab management. The integration of all components will be a group effort. Scott is in charge of programming our device to read outputs of concentration, temperature and voltage.

Everyone needs to keep a timesheet with what they worked on and when. There is a mandatory group meeting per week – tentatively scheduled for Wednesday afternoons 4pm-6pm. Each member should have 30 hours documented time spent on the project per week during BME123b quarter. Each member should have 15 hours documented time spent on the project per week during BME123a quarter. All data should have an electronic component stored online in the dropbox associated with the group project.

This project is sponsored by Nader Pourmand – during group meetings, an update email will be sent to Professor Pourmand.

Guidelines of Conduct:

1. Be respectful of teammates.
2. Do not interrupt during meetings.
3. Do not disparage another’s ideas.
4. Do encourage new ideas and methods.

Conflict Resolution:

If there is an issue with another team member, it needs to be recorded and brought up during the next team meeting or an email must be sent to ALL team members about it. Everyone doesn’t need to respond, but all members should be aware of any and all conflicts. Conflict resolution will be a group endeavor.

Procedure for Firing:

Issues must be addressed in a weekly meeting and via group email. The group will discuss situation and possible solutions. If the member in question continues to be uncooperative, or not available, then all notes will be brought to the professors. Since all data are online, records of effort should not be difficult to compile.
**Appendix 2**

**Work Breakdown**

The following breakdown structure corresponds to the Gantt Chart in the appendices.

<table>
<thead>
<tr>
<th>Task</th>
<th>Raj</th>
<th>Sahil</th>
<th>Scott</th>
<th>Brandon</th>
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<tr>
<td>Electrical Sensor Research</td>
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<tr>
<td>Microcontroller Research</td>
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<tr>
<td>Biological Preliminary Research</td>
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<tr>
<td>Preliminary device mechanical fabrications</td>
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<tr>
<td>Prototyping</td>
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<td>Biological Preliminary Experimentation</td>
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<tr>
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<td>Electrical Sensor/Microcontroller Integration</td>
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<tr>
<td>Group management and organization</td>
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Appendix 3

Wet-Lab Experimental Data for figures

All of the experiments were done in the Pourmand Lab. The

**Resistance Data**

*Figure 9 Data*

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Cross-Section Area (mm²)</th>
<th>Mesh</th>
<th>Resistance (Ω)</th>
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<td>Yes</td>
<td>13450</td>
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**Heat Accumulation Data**

*Figure 12 Data*

<table>
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<th>Apparatus</th>
<th>Length (mm)</th>
<th>Cross-Section Area (mm²)</th>
<th>Mass (g)</th>
<th>Voltage (V)</th>
<th>Resistance (Ω)</th>
<th>Time Run (S)</th>
<th>Total Temperature Celcius</th>
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**Figure 14 Data**

**Heat Experiment with Final Design Mimic**

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**Elution Efficiency Data**

**Figure 35**

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<th>Elution time minutes</th>
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<td>20</td>
<td>20</td>
<td>30.4</td>
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<tr>
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Various Data Not Mentioned

First resistance tests

Trial 1

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Current (mA)</th>
<th>Time (min)</th>
<th>Resistance of Gel (Ohms)</th>
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<tbody>
<tr>
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<td>5</td>
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Trial 2

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<th>Resistance of Gel (Ohms)</th>
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<td>10</td>
<td>3937.0</td>
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<td>10</td>
<td>6159</td>
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Trial 3

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<th>Voltage (V)</th>
<th>Current (mA)</th>
<th>Time (min)</th>
<th>Resistance of Gel (Ohms)</th>
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</thead>
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<td>5434.7</td>
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<td>10</td>
<td>7142.8</td>
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<td>10</td>
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Appendix 4

**Calibration Tables**

**Thermistor Calibration Data Figure**

The thermistor was tested in different baths of liquid with a known temperature. The thermistors resistance was recorded with the temperature.

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Resistance (Ohms)</th>
<th>Voltage (mV)</th>
<th>After amplification (V)</th>
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</thead>
<tbody>
<tr>
<td>100</td>
<td>724</td>
<td>0</td>
<td>0.057</td>
</tr>
<tr>
<td>94.4</td>
<td>848</td>
<td>1.88</td>
<td>0.063</td>
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<tr>
<td>88.89</td>
<td>998</td>
<td>4.16</td>
<td>0.071</td>
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<tr>
<td>83.3</td>
<td>1180</td>
<td>5.88</td>
<td>0.083</td>
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<tr>
<td>77.7</td>
<td>1403</td>
<td>10.2</td>
<td>0.100</td>
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<tr>
<td>72.2</td>
<td>1677</td>
<td>14.3</td>
<td>0.124</td>
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<td>66.6</td>
<td>2016</td>
<td>19.3</td>
<td>0.156</td>
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<td>61.1</td>
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<td>55.5</td>
<td>2969</td>
<td>33</td>
<td>0.251</td>
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<td>50</td>
<td>3641</td>
<td>43.4</td>
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<td>56.1</td>
<td>0.401</td>
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<td>0.661</td>
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<td>119</td>
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<td>22.2</td>
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<td>154.9</td>
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<td>1.380</td>
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<td>18930</td>
<td>258.7</td>
<td>1.800</td>
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<td>2.337</td>
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<tr>
<td>0</td>
<td>33048</td>
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<td>3.047</td>
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</tbody>
</table>

Data Table of measured Resistance and Voltage.
Appendix 5

Coding for Mathematica graphs

Concentration Sensor Calibration

Thermistor Calibration
data = List[ {724, 100}, {848, 94.4}, {998, 88.89}, {1180, 83.3}, {1403, 77.7}, {1677, 72.2},
{2016, 66.6}, {2439, 61.1}, {2969, 55.5}, {3641, 50}, {4496, 44.4}, {5591, 38.8}, {7011, 33.3},
{8863, 27.7}, {11308, 22.2}, {14555, 16.6}, {18930, 11.1}, {24867, 5.5}, {33048, 0}]

ListPlot[data, PlotRange -> {{500, 34000}, {0, 100}}, AxesLabel -> "Resistance Ohms", "Voltage of Thermistor, Volts"]

Heat Plots

3D Graph
Plot3D[3.68389 x/y^2, {x, 0, 10}, {y, 0, 1}, AxesLabel -> "Time Seconds", "Cross-sectional Area" m^2, "Temperature Celsius"], TicksStyle -> Directive[10], LabelStyle -> Directive[Bold, 15]

Bar Chart

Scatter + Fitted Line
data = List[ {1, 45}, {2, 52}, {3, 43}, {4, 46}, {5, 44}, {6, 46}, {7, 56}, {8, 45}, {9, 44},
{10, 49}]
Fit[data, {1, 1}, x]
P = ListPlot[data, PlotRange -> {{0, 10}, {0, 65}}, AxesLabel -> "Resistance Ohms", "Voltage Volts"]
P1 = Plot[47, {x, 0, 10}]
Show[P, P1]

Time versus length
Plot[{2263.2/(x)}, {x, 0, 10}, PlotRange -> {0, 900}, AxesLabel -> "Length between electrodes", "Time in Seconds"]

Elution Plots
data = List[ {1, 0}, {2, 9.82}, {3, 6.87}, {4, 13.53}, {5, 20.1}, {6, 15.6}, {7, 34.2}, {8, 17.4},
{9, 11.2}, {10, 6.35}, {11, 0}, {12, 13.27}, {13, 24.1}, {14, 30.4}, {15, 25.6}, {16, 26.2}, {17, 21}]
Fit[data, {1, 1}, x]
P = ListPlot[data, PlotRange -> {{0, 17}, {0, 100}}, AxesLabel -> "Trial", "%Yield"]
P1 = Plot[16.2141, {x, 0, 17}]
Show[P, P1]
Appendix 7

*Mechanical - Apparatus Design 2*

![Figure 14](image1)

> Figure 14
> This is a horizontal apparatus with 3 different pieces. The large box part is what holds the TAE buffer. The middle part that is conical is what holds the gel and DNA. The small box at the end is the catch and holds the elution buffer.

![Figure 15](image2)

> Figure 15
> This piece holds the gel with the DNA. The small opening is capped when the gel is added. When the gel is placed in the middle conical tube, the catch is fit onto the small end. The large end is 25 mm in inner diameter and 26 mm in outer diameter.

![Figure 16](image3)

> Figure 16
> This is the catch. This is 100 microliters in size. It holds the elution buffer. It is placed onto the main tube by a tight fit.

This is a horizontal apparatus. Our team designed this in light of how complex the other design is. In design 1, the tube is held by the boom. Since the apparatus is small it may be very fragile. For the user it will be difficult to mount the tube on the catch and between the boom and pad. This second design overcomes those issues.

There are three parts. The large box shape in **Figure 16** is fit around the conical middle part making up what is essentially the tube in design 1. The gel will sit in the conical shape. The small catch is fit around the conical middle as well. This works the same as the vertical tube.

This quarter, however, our team will not be implementing this design because of our limited time.
Appendix 8

Specialized functions for TI-MSP-EXP430F5438 Experimenter Board

/*
 * setVariables.c
 *
 * Created by Scott Azarnoff on 2/26/10.
 * copied and modified from clock.c from TI code
 * examples SLAC227d
 *
 */

#include "setVariables.h"
#include <stdlib.h>
#define ITOA_BASE 10
#define MAX ITOA BITS 33
#define MAX_CURSOR_POS 4
#define FOURTHfromEND 14
#define THIRDfromEND 15
#define SECONDfromEND 16
#define LASTcolumn 17

enum {THRESH TEMP10, THRESH TEMP01, THRESH TEMP_DEC, THRESH TEMP_TENTH,
 MELT TEMP10, MELT TEMP01, MELT TEMP_DEC, MELT TEMP_TENTH,
 MAX TIME10, MAX TIME01, MAX TIME DEC MAX TIME_TENTH,
 START_VOLT10, START_VOLT_01, START_VOLT_DEC, START_VOLT_TENTH} 
enum {HOUR10, HOUR01, COLON2, MINUTE10, MINUTE01, COLON1, SECOND10,
 SECOND01} 
enum {THRESH TEMP, MELT TEMP, MAX TIME, START_VOLT} 
enum {TENS, ONES, POINT, DECIMAL}

char variables[17] =
{0, 0, '.', 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0};
char time[9] = {0, 0, ':', 3, 0, ':', 0, 0, 0};
char viewThreshTemp[5] = {'5', '0', '.', '0', '0'};
char viewMeltTemp[5] = {'6', '0', '.', '0', '0'};
char viewStartVolt[5] = {'2', '0', '.', '0', '0'};

int second = 0, minute = 30, hour = 0;
int secondOld, minuteOld, hourOld;

/*
 // this will later help get data from flash memory,
 // for now just returns the global variable from UserExperience
 unsigned int getThreshTemp( void )
 { 
  return threshTemp;
 }
 */

unsigned int threshTempAdd( void )
unsigned int Temp = getThreshTemp();

// unsigned int secondBCD = GetRTCSEC();

if ( (tempBCD & 0x0F) == 9 )
{
    secondBCD &= 0xF0;                      // low digit = 0
    if ( (secondBCD & 0xF0) == 0x90 )       // if high digit = 9
    {
        SetRTCSEC(0x00);                      // reset to 0
    }
    else
    {
        secondBCD += 0x10;
        SetRTCSEC(secondBCD);                 // add 1 to high digit
    }
}
else
{
    secondBCD++;
    SetRTCSEC(secondBCD);
}

// set a variable to be the maximum time:
// when the elution timer reaches this time, the elution will stop.
// these are functions modified from clock.c which will, along with the
// modified UI,
// allow for the time to be set
*/

int setVar( int maximumVar )
// REQUIRES int to be passed for current max var - returns integer for
// user set max temp
// Displays maximumTemp on LCD and allows joystick to modify
{
    char newVarStr[MAX ITOA_BITS];
    int newVar = maximumVar;
    halLcdClearScreen();
    while (!(buttonsPressed & BUTTON_S2))
    {
        itoa(newVar, newVarStr, ITOA_BASE);
        printLcdAcceptCancel();
        if ((buttonsPressed & BUTTON_S1) != 0) return maximumVar;

        if ((buttonsPressed & BUTTON_RIGHT) != 0) newVar++;
        if ((buttonsPressed & BUTTON_LEFT) != 0) newVar--;
    }
    buttonsPressed = 0;
    return newVar;
}
/**
 * @brief Executes the user interface for setting the variables.
 * Uses the D-pad to move between variables as well as
 * increments or decrements their set values.
 * @param none
 * @return none
**/
void setVariables( void )
{
    unsigned char cursorPosition = 0;
    unsigned char verticalCursor = 0;
    unsigned int threshTempLOCAL = threshTemp ;
    unsigned int meltTempLOCAL = meltTemp ;
    unsigned int maxTimeLOCAL = maxTime ;
    unsigned int startVoltLOCAL = startVolt ;

    halLcdClearScreen();

    halLcdPrintLine("iRage Variables", LCD_LINE_1, OVERWRITE_TEXT);
    halLcdPrintLine("Threshold Temp", LCD_LINE_3, OVERWRITE_TEXT);
    halLcdPrintLine("Melting Temp :", LCD_LINE_4, OVERWRITE_TEXT);
    halLcdPrintLine("Max Min:", LCD_LINE_5, OVERWRITE_TEXT);
    halLcdPrintLine("Start Voltage:", LCD_LINE_6, OVERWRITE_TEXT);

    halLcdPrintLineCol(viewThreshTemp, LCD_LINE_3, FOURTHfromEND, INVERT_TEXT | OVERWRITE_TEXT);
    halLcdPrintLineCol(viewMeltTemp, LCD_LINE_4, FOURTHfromEND, OVERWRITE_TEXT);
    halLcdPrintLineCol(&time[HOUR10], LCD_LINE_5, 7, INVERT_TEXT | OVERWRITE_TEXT);
    halLcdPrintLineCol(&time[COLON2], LCD_LINE_5, 9, OVERWRITE_TEXT);

    // 6 char wide boxes around Accept and Cancel
    halLcdLine(6, 94, 58, 94, PIXEL_ON);
    halLcdLine(6, 94, 6, 109, PIXEL_ON);
    halLcdLine(58, 94, 58, 109, PIXEL_ON);
    halLcdLine(6, 109, 58, 109, PIXEL_ON);
    halLcdLine(76, 94, 128, 94, PIXEL_ON);
    halLcdLine(128, 94, 128, 109, PIXEL_ON);
    halLcdLine(76, 94, 76, 109, PIXEL_ON);
    halLcdLine(76, 109, 128, 109, PIXEL_ON);
halButtonsInterruptEnable(BUTTON_ALL);

while (!quit)
{
    buttonsPressed = 0;
    __bis_SR_register(LPM3_bits + GIE);
    __no_operation();

    switch (buttonsPressed)
    {
    case BUTTON_UP:   if (cursorPosition == 0)
        cursorPosition = MAX_CURSOR_POS;
    else
        cursorPosition--; break;
    case BUTTON_DOWN: if (cursorPosition == MAX_CURSOR_POS)
        cursorPosition = 0;
    else
        cursorPosition++; break;

    case BUTTON_RIGHT:    switch(cursorPosition)
    {
    case 0: threshTempLOCAL++; break;
        // use itoa to convert to string, then move 4th item to 5th and insert '\'.'
        halLcdPrintLineCol(   // use itoa to convert
            // use itoa to convert
            break;
        case 1: meltTempLOCAL++; break;
        case 2: maxTimeLOCAL++; break;
        case 3: startVoltLOCAL++; break;
    }
    break;
    case BUTTON_LEFT:    switch(cursorPosition)
    {
    case 0: threshTempLOCAL--; break;
    case 1: meltTempLOCAL--; break;
    case 2: maxTimeLOCAL--; break;
    case 3: startVoltLOCAL--; break;
    }
    break;
    }

    switch (verticalCursor) {
    case THRESH_TEMP:
    case MELT_TEMP:
    case MAX_TIME:
        switch(cursorPosition)
case 0:
  halLcdPrintLineCol(&time[HOUR10],
  5, 5, INVERT_TEXT | OVERWRITE_TEXT);
  halLcdPrintLineCol(&time[COLON2],
  5, 7, OVERWRITE_TEXT);
  break;

case 1:
  halLcdPrintLineCol(&time[HOUR10],
  5, 5, OVERWRITE_TEXT);
  halLcdPrintLineCol(&time[MINUTE10],
  5, 8, INVERT_TEXT | OVERWRITE_TEXT);
  halLcdPrintLineCol(&time[COLON1],
  5, 10, OVERWRITE_TEXT);
  break;

case 2:
  halLcdPrintLineCol(&time[HOUR10],
  5, 5, OVERWRITE_TEXT);
  halLcdPrintLineCol(&time[SECOND10],
  5, 11, INVERT_TEXT | OVERWRITE_TEXT);
  break;

if (buttonsPressed & BUTTON_S2)
  quit = 1;
buttonsPressed = 0;
}
## Budget and Resources

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<th>Quantity</th>
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<td>Gift</td>
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**Total:** 9 $887.95

**Budget:** $10,000

This project budget is $10,000. Most of the money will be going to the electrical components. Reagents for the experiments will be a gift from the Pourmand Lab and are not included in the budget.

In this budget money has been allotted for all parts even if they are not specified yet. The apparatus price is not available at this moment until our principal investigator gets a quote with our designs. For each part, the quantity is a little over the needed in case of human error or broken devices.