

ME 450 Design Review 3

Team 3: Automated Gel Casting

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REVISED ABSTRACT

Polyacrylamide gels for electrophoresis can be bought precast or can be manually cast. These two options present a tradeoff between labor time and price. Our team plans to solve this dynamic through automation. Our pursuit is to remove the labor cost of in-lab casting, while maintaining the inexpensive nature of creating gels in-lab. Additionally, our team strives to use this automation to increase reproducibility and reduce error of the gel casting capabilities in the lab. Our current project goal is to produce a prototype that creates viable gels, while satisfying the previous two objectives on accuracy and economic cost.

PROJECT INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) is a trusted technique for separating proteins by molecular mass [1]. PAGE is a common technique used in labs worldwide to isolate proteins, study their composition, and compare their molecular weights. An essential component of the PAGE process is the polyacrylamide gel through which the proteins are separated. The success of a PAGE experiment is directly dependent on the quality of gel that is used and the consistency across experiments is also a factor of gel uniformity.

For a lab regularly conducting PAGE experiments, there are currently two options to access a supply of polyacrylamide gel. The lab can either make the gels onsite or order precast gels from a company that mass produces them. Precast gels offer a level of convenience and reliability that gels made onsite lack. This is because gels made onsite require up to two hours of preparation time from an experienced lab technician and demand precise measurements with little room for human error [2]. Still, the practice of producing gels onsite is widespread throughout the PAGE community. Hand cast gels are fully customizable and up to 36.59% less expensive than precast gels because of the lower material cost for gel production compared to manufacturing upcharges.

Inspired by the benefits of lab made gels, we and our sponsor Duane Day of Innovative Research Inc. seek to automate small scale gel production in order to improve its convenience and reliability. Mr. Day has been working with gel electrophoresis for over thirty years and this project is benefitting greatly from his long-time consideration of automating the gel making process. His previous work considering this problem has led to us taking the approach of designing an apparatus around current gel making equipment. We expect that automating the casting process, in-lab, may result in a cost reduction of 65.83% from precast gel prices without factoring in the cost of the machine.

The major objective of this project is to develop an automatic gel casting system that is competitive against precast gels in the areas of convenience, reliability and cost. The final deliverable will be a prototype that can produce a uniform gel with reduced human interaction beyond the initial setup. Engineering standards applicable to this project are primarily related to the safe chemical handling required by the gel making process. Safety guidance was provided by the following standards: the OSHA General Industry Standards (specifically the Hazard Communication, Hazardous Materials, Machinery and Machine Guarding, and Electrical specifications), the “NIOSH Guide to Chemical Hazards, and “Prudent Practices In The

Laboratory: Handling and Management of Chemical Hazards by the Board of Chemical Sciences and Technology.”

BACKGROUND

Polyacrylamide gel electrophoresis (PAGE) was introduced in the 1950’s as a reliable method of separating proteins by molecular mass [1]. It has been critical to studies in RNA, detecting pathogens, and forensics [3]. PAGE involves running a current through a gel, which draws charged proteins through the gel matrix. The distance that the proteins travel is logarithmically related to their molecular mass, with the smallest proteins traveling the farthest from the sample wells [4]. Figure 1A shows the polyacrylamide gel in its vertical electrophoresis stand with samples being loaded into its wells. The gel is placed in a running buffer that allows a current to flow through the gel from the anode to the cathode [5]. Figure 1B shows proteins with the smallest molecular mass traveling the fastest through the gel matrix, leading to protein separation by molecular weight.

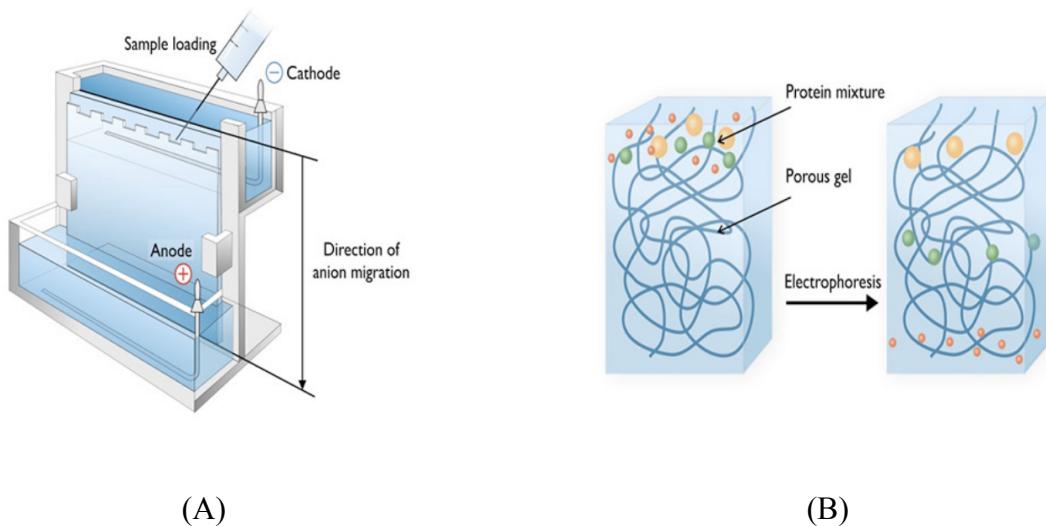


Figure 1: (A) polyacrylamide gel in its vertical electrophoresis stand, with samples being loaded into its wells. (B) proteins with the smallest molecular mass traveling the fastest through the gel matrix, forming bands [6].

Basic gels are made from polyacrylamide, gel buffer, sodium dodecyl sulfate (SDS), water, ammonium persulfate (APS), and tetramethylethylenediamine (TEMED) [2]. The percentage of polyacrylamide affects the density of the gel matrix, and therefore what ranges of protein sizes can be sorted on one gel [7]. Common percentages of polyacrylamide range between 7.5% and 20% [8]. Gradient gels vary in percentage of polyacrylamide along the direction of protein travel, most commonly varying from 4-20% or 8-16%. Gradient gels are used when samples containing a broad range of molecular weights must be separated. APS initiates the polymerization of acrylamide solution, with TEMED acting as a catalyst for the reaction. The polymerization reaction is exothermic, so its speed must be carefully limited by the concentrations of TEMED and APS in order to prevent rapid heating that risks the formation of non-uniform pore structures. The gel buffer prevents the gel pH from changing as protein

samples are added to it. Water and SDS are used to dilute the other reagents to the proper concentration [9].

The procedure for producing a polyacrylamide gel as we observed in a demonstration by our sponsor is highlighted in Figure 2. The gel mold is composed of two glass plates, spacers for the plates, a stand, and a rubber gasket to keep gel from leaking out of the base of the glass plates. Polyacrylamide is mixed with the previously mentioned reagents to make the separating gel solution, TEMED is the last reagent to be added because it kicks off the catalyzation reaction. Once mixed, the separating gel solution is carefully poured between the glass plates on the casting stand. This step is done using gloves because acrylamide is neurotoxic before it polymerizes. Isopropanol is added to the top of the separating gel solution immediately and the gel is left to fully polymerize over the period of 30 minutes to an hour. Leftover separating solution is used as an indicator of full polymerization. Once the separating gel has fully polymerized, the isopropanol is removed by tilting the casting stand to allow the isopropanol to run out of the mold and onto a paper towel. The stacking solution is mixed using the same reagents as the separating gel, but at different concentrations. The stacking solution is purposefully overflowed, out of the plates, in order to ensure the casting mold is full. The well-forming comb is laid on top of the stacking solution immediately. The comb must be laid in a way that does not cause bubbles to form beneath it; this is often done by inserting the comb at an angled direction. The stacking solution is left to polymerize for 15 - 30 minutes before the gel is ready to be used. The comb must be extracted before gel use to open up the sample wells for the proteins [2]. Gradient gels are produced in a similar fashion with the same reagents, deviating only in that the running gel is made by making a gradient between two running gel solutions of different polyacrylamide concentrations. A gradient former is a common laboratory tool used to make a gradient of the two running gel solutions as they are poured into the gel mold [2].

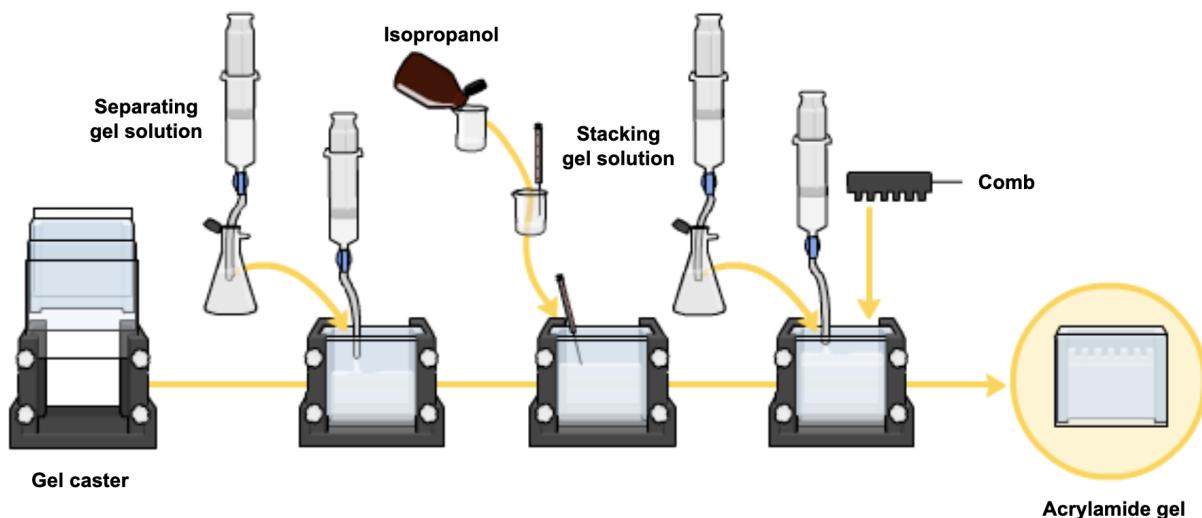


Figure 2: PAGE casting procedure, as conducted by Innovative Research Inc. [2][10]

Companies such as Bio-Rad and Invitrogen mass produce gels to distribute to laboratories. These gels cost within a range of \$12-\$20 and have a shelf life of up to 12 months [11]. These gels are convenient because they save lab technicians time but their limited shelf life, their high cost, and their inability to be customized make them imperfect solutions for many labs. Additionally, there are negative environmental impacts from this process as precast gels come in plastic casings which are discarded and must be shipped from the manufacturer to the lab [2].

Casting by hand requires extensive training (about 20-30 gels) and a lifetime of experience to perfect the process. The likelihood of inconsistencies in manually cast gels is higher compared to precast gels because of the human factor involved [8]. Casting manually requires up to two hours of lab technician attention, and requires the technicians to handle neurotoxic chemicals. Casting gels by hand also requires specialized casting equipment sold by many of the same companies that offer precast gels.

DESIGN PROCESS

The design process we decided to follow for this project is the Design Process Framework outlined by the ME 450 course, shown in Figure 3.

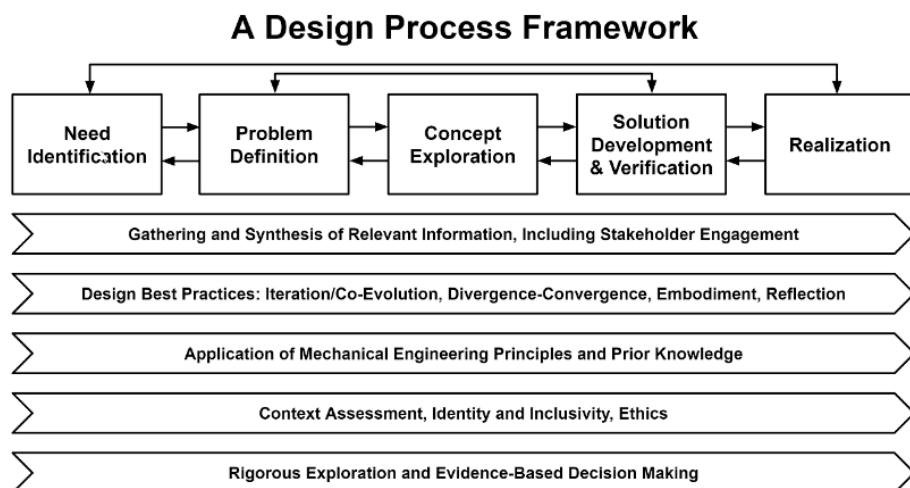


Figure 3: ME 450's Design Process Framework

This methodology is stage based and iterative – two components we wanted to ensure we had. We wanted a methodology that includes space for iteration. Another important feature to note are the arrows across the bottom of the framework. Out of all the methodologies analyzed in the course, this process was the only one to include features to be considered over the course of design. Our exact process to date has included multiple iterations through problem definition and need identification, and we expect to continue looping through the stages.

Another methodology considered was Pahl and Beitz's stage-based model (Appendix, A.1) for mechanical design [12]. It was similar to the one provided by the course, and included the side considerations for outside factors. However, the constraint of combining “process documentation” and “detailing” at the end of the process was the deciding factor against it. We

could not see how we could effectively merge the methodologies with the constraints of the course (and design report milestones), our team's documentation preferences (to detail everything as we go), and the ideology of Pahl and Beitz's method.

Our design process has mostly followed the design process from the ME 450 procedure. One new sub-step that our team added was a trial experiment to understand the process of casting a polyacrylamide gel. This step is inserted before the concept generation step. This step gave us insights into the gel casting process and helped us to focus our concept generation session afterwards. With an innate understanding of the process, we were able to break the procedure down into the individual sub functions our machine needed to complete. Other than the aforementioned change, our team has stayed within the stages of the chosen design methodology. After the trial, we began the concept generation phase, and it has proven to be quite iterative.

DESIGN CONTEXT

Stakeholders

The laboratory setting of our project is directly tied to the larger scientific community. Within the context of this course and our project scope, we have identified primary stakeholders to be the internal and external stakeholders. Internal primary stakeholders are Innovative Research, Inc., Our Team, the University of Michigan. Our external primary stakeholders are: Lab Technicians, Gel Running Labs, Chemical Providers, Precast Gel Manufacturers, and Underfunded Labs. These groups, along with the secondary and tertiary stakeholders, are visualized in Figure 4, our stakeholder map.

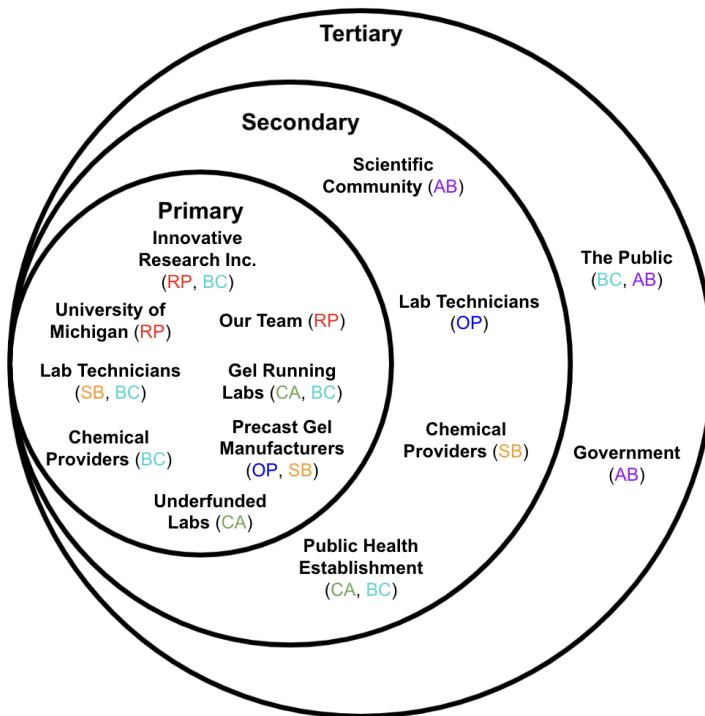


Figure 4: Target graphic of our primary, secondary, and tertiary stakeholders.

We assume that the internal group of stakeholders would positively benefit from the adoption of this technology. Within the external group of stakeholders, we believe lab technicians, underfunded labs, gel running labs, and chemical providers would all benefit from the technology, by reducing costs, increasing lab work efficiency, and minimizing lab time with menial tasks. However, if the technology becomes widespread, precast gel manufacturers and lab technicians could be negatively impacted. Precast gel manufacturers may lose customers if labs choose to cast gels in-house. Additionally, an automated system would reduce the scope of work or even replace lab technicians who primarily cast gels.

Gel electrophoresis is a standard laboratory practice that has aided medical and scientific exploration. Our process, PAGE, is used to separate proteins for further analysis and has wide ranging implications in applied biology. The effort to minimize menial lab work and make lab technicians more efficient is driven by the social desire to increase research productivity within biological labs.

As a business with economic responsibilities, our sponsor's profit is their priority. When considering our stakeholder ecosystem, our sponsor's focus on profit may limit the scope of social impacts our final design has, depending on how accessible they decide to make its market price. Their next focus area is the potential environmental and social impacts. The order of these priorities has already had an impact on the actual design of our project. This is because the functionality of our final design was clearly defined by our sponsor to meet their desires. The environmental and economic consequences we envision for our project are mostly a result of the overall functionality rather than the specific manner of implementation. Therefore, our freedom in making design decisions will not be greatly affected by the overall social impact of our project.

I.P. and Sustainability

As requested by Innovative Research Inc. our team has transferred our intellectual property rights to them within the extent of this project. Intellectual property rights play an important role in examining the social context, sustainability, and stakeholder environment as it will relate to who has access to the technology we will be developing. Since our sponsor has prioritized profitability, our team believes that the primary effect of our sponsor retaining the intellectual property rights will be to reduce the economic access to our device. The principal cost to acquire our final product will limit who has access to it, but if a lab has the needed capital it will likely be much more efficient for repeated use than buying precast gels. This has the potential to make the device favored over the current precast gel market, depending on the initial price set by our sponsor. That said, our sponsor will benefit from the price being as high as possible; therefore, our design may not actually impact who has access to gel electrophoresis.

Beyond accessibility, the effects of intellectual property are relatively minimal, as there is currently no market-available solution that is comparable. With a lab-bench casting machine not yet invented, the primary limitations on our design process will be on what processes we can use

to achieve our goals. Patents on mixing, measuring, fluid driving, and clamping will affect what solutions our team can use.

If the monopolization of our end-product by Innovative Research Inc. does not result in limited access to the machines but rather their widespread use, there may be significant sustainability effects. The increased ease of gel production will likely lead to a rebound effect, with gels being used less efficiently and more gels being produced [2]. This would increase the demand for materials and their shipment, increasing the carbon emissions associated with chemical manufacture, disposal, and transport. The machines themselves will also require electricity, which is a predominately fossil fuel powered industry. One possible solution to these environmental costs is to make the machine able to cast gels with variable lane-counts, so that users can create a gel with just as many lanes as needed, limiting excess waste. This, however, is a complex change that would require many resources in development and increase the end cost of our product.

Even though there are potential drawbacks in sustainability due to the rebound effect, power draw, and cost of manufacturing our device, we believe that our product will be beneficial in terms of sustainability. We believe that the impact of the rebound effect and increased electrical power use, will cancel to some extent with the reduction in packaging and shipping associated with the precast gel purchases. We believe that in the long term, the social benefits of increasing research productivity will be more impactful than the potential environmental trade-offs.

Ethics and Power

The main ethical dilemma we expect to face is if the automation of gel casting becomes too widespread. If this occurs, the decrease in the workload for lab technicians could potentially lead to fewer hours and fewer employment opportunities. As for the team's personal ethics, we believe that they align with the professional ethics we are expected to uphold by the University of Michigan and future employers.

The power dynamics among our teammates is strict equality. We all have provided, are providing, and will provide work and effort towards the project goal. Our project sponsors are our primary stakeholder and are the ones who have provided design requirements; they serve as our mentors. The end users, or lab technicians, have also contributed to the design requirements as they have the most experience with casting gels. For our project, Dave Ginsberg, a lab technician from Innovative Research Inc. will serve this role.

When considering the stakeholder requirements, engineering specification, and this project as a whole, our team only considered the opinions and technicians of a single lab technician, Dave Ginsberg. While Dave Ginsberg has extensive expertise, there may be efficiencies that other lab technicians may include in their own PAGE process. Because of this, our design may suffer from this lack of outside expertise. Inclusivity is also an issue when it comes to IP. Our team has assigned our IP to Innovative Research Inc. which means that they will maintain sole rights to our automated casting machine. This has the potential for Innovative

Research to create a temporary monopoly on the automated casting machine market, increasing the principal cost of the device. With a high principle cost, some labs may not be able to afford the device and may suffer exclusion from the benefits of our device.

USER REQUIREMENTS AND ENGINEERING SPECIFICATIONS

Specifications and Marketability

Stakeholder Requirements [2]	Engineering Specifications	Importance [14]
Functional Gels	95% of gels do not display a meniscus, warping, or separation	1
Accurate	Chemical measurements made with < 5% error	2
Minimal Human Intervention	< 3 human interventions per gel, taking < 5 minutes total per gel	1
System Compatibility	Compatible with systems < 14 x 11 x 1 cm in < 5 minutes	3
Less Expensive	Cost to produce a gel is < \$4.52	2
Easily Maintainable	< 5 minutes of maintenance per gel	1
Chemical Containment	0 chemical exposures to a pH outside of 6.5-8.5, temperatures outside of 45-85°F, and any organic material	1

Table 1: These requirements and specifications were generated by a stakeholder interview with our sponsor, a design consultant, and a lab technician from Innovative Research Inc. After their generation, the specifications and requirements were further refined by obtaining both an approval and a priority ranking in a second, follow up interview. Note that importance is ranked from 1 (most) to 3 (least).

After the generation of these requirements and following the feedback provided to us during design review 1 from our sponsor and peers, we added the additional priority importance metric to our specifications. In addition to the requirements and specifications above, our interviews also displayed a set of desires that were deemed to be outside the scope of our project. These desires included the ability to produce gradient gels and to have online or app compatibility. These desires are further developments of our project which we have ensured are possible additions to our design. While gradient gel creation remains outside of our project scope, we detail a possible expansion for gradient solution capabilities in the First Selected Concept section.

Focusing on the stakeholders chief priority, our team examined profitability first. Initially, our team did preliminary research to ascertain whether there is a large enough market to incorporate another gel casting solution. We found that the gel electrophoresis market was estimated at \$2.3 billion [15]. After establishing the market size, our team's next step was to perform a preliminary cost analysis, to discern whether our project would be able to produce an economically viable solution.

Cost Source	Precast Gel [16] [17]	Manual Casting [18]	Automated Casting
Materials	\$13.2	\$0.65	\$0.65
Labor [14]	\$0	\$7.72	\$3.86
Total Cost	\$13.2	\$8.37	\$4.51

Table 2: Using the gel creation protocol and material prices from Innovative Research Inc, alongside the average biolab technician wage from the Bureau of Labor Statistics and the prices of precast gels from Millipore, ThermoFisher, and Bio-Rad, our team was able to compile the cost calculations above.

Our preliminary expectation is that a final product price may be \$484 based on our bill of materials, a typical markup rate of ~50% [19], and a rough estimate for 3D printing costs. Using this estimate alongside the results of Table 2, we believe our device could return on its initial capital investment between 56-125 gels produced depending on whether a lab hand casts or buys precast gels. If a lab is using just one of these hydrogels a day, they could expect to begin making a profit on their investment as soon as 11-25 weeks after their purchase. Notably, Table 2 does not include the cost that is associated with training lab technicians (which can take up to 20-30 gels), failed gels, and shipping costs. Based on our survey of these factors, we believe our final product may return on investment earlier than we expect from this calculation.

If our automation were to gain gradient gel casting capabilities, the return on investment would be fair faster and the convenience much greater than expectations explained above. Gradient gels are substantially more tedious to manufacture and require nearly twice as many measurements from a technician [5]. This is the domain in which automation would have the greatest impact with reliability and convenience, it is for these reasons that we believe it would be substantially more economically successful with this capability. This is why our team has decided it is important to detail an avenue for using our device for gradient gel fabrication, even though this remains a stretch goal that is outside of our current scope.

Current Standards and Regulations

When researching standards, our team found a set of codes and standards that we believe following will improve the quality of our design. Because of the nature of our device, our team will need to consult the OSHA General Industry Standards [20] (specifically the Hazard Communication, Hazardous Materials, Machinery and Machine Guarding, and Electrical specifications). Additionally, our team plans to review the “NIOSH Guide to Chemical Hazards” [21] and “Prudent Practices In The Laboratory: Handling and Management of Chemical Hazards by the Board of Chemical Sciences and Technology” [22]. These standards and guidelines will continue to inform our design decisions as our team moves forward.

CONCEPT GENERATION

Process and Flow Chart

The main concept generation methods used were design heuristics and functional decomposition. By skimming through the list of the 77 design heuristics, relevant heuristics to our project were recorded to be used within functional decomposition (Appendix, A.3) [26]. We separated our design into the essential functions: chemical storage, measurement, chemical transport, mixing, comb insertion, leveling, polymerization check, and cleaning. Some of the ideas generated were applicable to multiple functions or would only be practical if the previous or subsequent function were complementary, which was taken into consideration while conceptualizing.

In order to brainstorm, we looked at each individual function and developed as many ideas as possible no matter how impractical they were; each team member did this on their own (Appendix B). After doing so individually, we held a team concept generation session. At this meeting, we came with our individually generated concepts and wrote down all of our ideas on several whiteboards, which were separated into the aforementioned essential functions. After everyone's ideas were written on the whiteboards, we began to discuss function by function. Each concept was discussed in different levels of detail and similar concepts were grouped together. The concept groups which we found effectively met the requirements were marked with a sticky note. This was determined through questions such as, "How practical is this? Does this accomplish the function? How easily does it function? How effective is this at completing this function?" We also had overarching goals: to minimize moving parts, condense functions, and condense components. We discussed each function iterating through this same process, producing multiple viable concepts for each function. This process flow is shown in Figure 5.

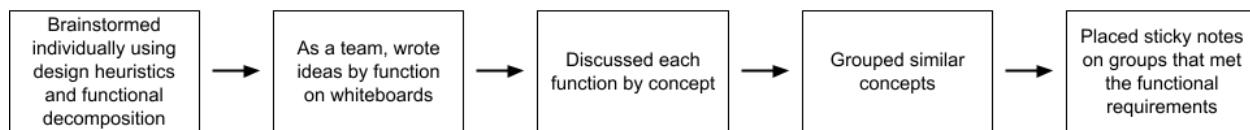


Figure 5: Concept Generation Flow Chart

Results

Chemical storage is vital to an automated casting machine as the chemicals need to be accessible while also being safely contained. The ideas produced through concept generation for chemical storage included two main designs. The first of which involved rotating cylindrical tanks that when the tanks were aligned properly the fluid would be allowed to flow, shown in Figure 6A. This design leaned heavily on Design Heuristic #57 Rotate (Appendix, A.3) [27]. The second concept is much simpler: stationary cylindrical containers, seen in Figure 6B. These containers would be accessed from the top.

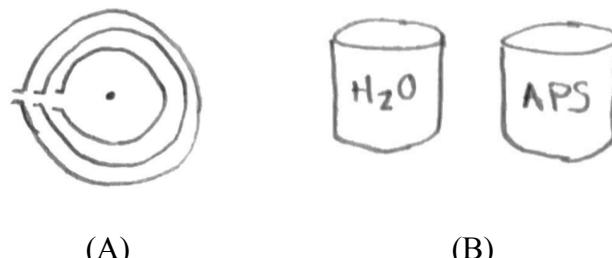


Figure 6: Chemical storage concepts

When making a gel, a protocol with specific measurements must be followed in order to ensure the functionality and viability of the gel. For measurement, the first concept for this function is pipetting, shown in Figure 7A. Manual pipetting is how a lab technician measures the chemicals during hand casting, so this would be implemented into our gel casting machine through automated pipetting. Another measurement concept is measuring through mass or weight, seen in Figure 7B. A fixed target weight would be set and liquid would be added until the target weight is met. A third concept of measuring is through flow, shown in Figure 7C. A mass flow meter could be installed to measure the fluid.

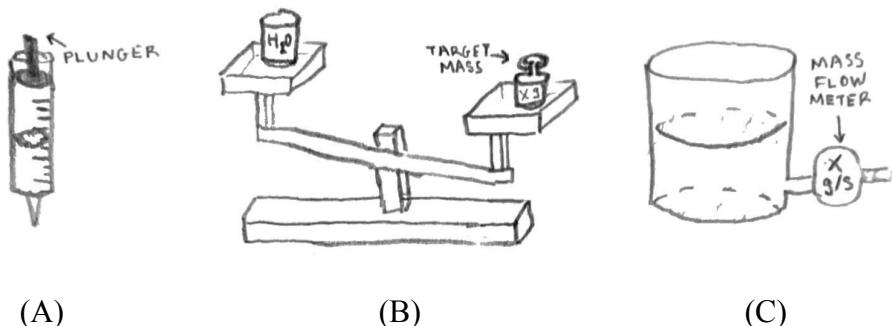


Figure 7: Measurement concepts

Chemicals must be moved in the correct amounts from the storage container to the mixing chamber and from the mixing chamber to the plates. Based on a lab technician's hand casting process, automated pipetting became a concept for chemical transport. Once the chemical has been measured, the pipette can be actuated to move to its next destination, shown in Figure 8A. With this method, the pipette serves multiple functions even though it is a single component. Another method of chemical transport is a container with a spigot or a hatch, seen in Figure 8B. When the fluid needs to move to the next section or container, the spigot or hatch can be opened. Using gravity to move the fluid was an additional proposed concept for chemical transport, seen in Figure 8C.

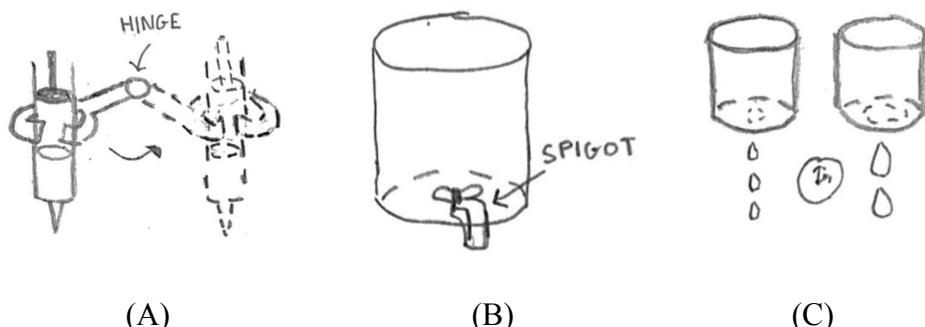


Figure 8: Chemical transport concepts

The chemicals must be mixed in order to achieve a homogenous solution. A pipette can also be used to mix the chemicals by pipetting four to five times, which is how a lab technician mixes the chemicals. However, for our design, the pipette would be automated, as shown in Figure 9A. Mixing can also be achieved by shaking the container, seen in Figure 9B, or rotating the container itself, seen in Figure 9C. Finally, the chemicals can be mixed with a paddle or a

whisk, shown in Figure 9Di and ii respectively. This is similar to a baking mixer or a drill attachment to mix paint.

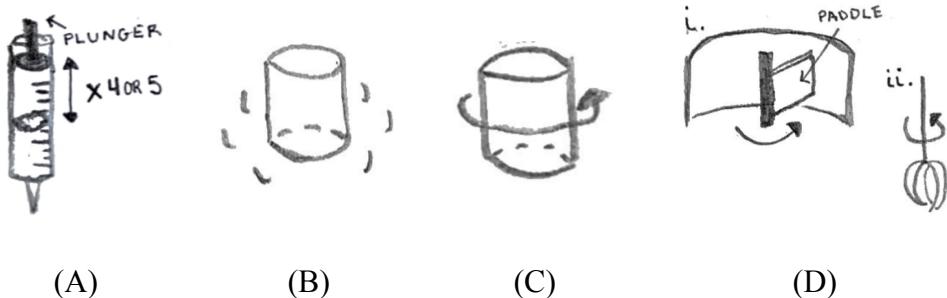


Figure 9: Mixing concepts

An essential aspect of a well-casted gel is a level running-stacking gel interface; otherwise, the gel interface is wavy and results in the proteins running wavy or crooked during electrophoresis. A lab technician achieves this by pipetting a small amount of water or isopropanol in between the plates on top of the running gel solution. Once the running gel has polymerized, the water or isopropanol is wicked up with a tissue. One concept involves automating this process through a swab. A second idea to achieve leveling is by having a hard boundary so that the running gel has a solid level interface producing a level gel, shown in Figure 10.

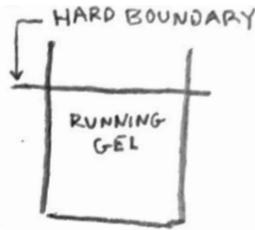


Figure 10: Leveling via hard boundary concept

Comb insertion is vital for the creation of the wells in which the proteins are inserted. Design heuristics were applied especially within the concept generation of comb insertion. Design heuristic #18 is to change the direction of access (Appendix, A.3) [26]. Instead of inserting the comb from the top in between the plates, the plates are inverted with the comb pre-inserted. In this way, the stacking gel is poured first with the running gel after instead of the traditional running and then stacking succession, shown in Figure 11A. Additional methods of comb insertion included one we dubbed “The Hole-y Comb.” This concept had the comb pre-inserted with holes in it; these holes allowed the liquid to be inserted between the plates without moving the comb, seen in Figure 11B. A method of cutting and removing the wells was developed, similar to a cookie cutter which is shown in Figure 11C. Finally, physical motion of the comb was also proposed whether the motion was through mechanical or magnetic means, shown in Figure 11D.

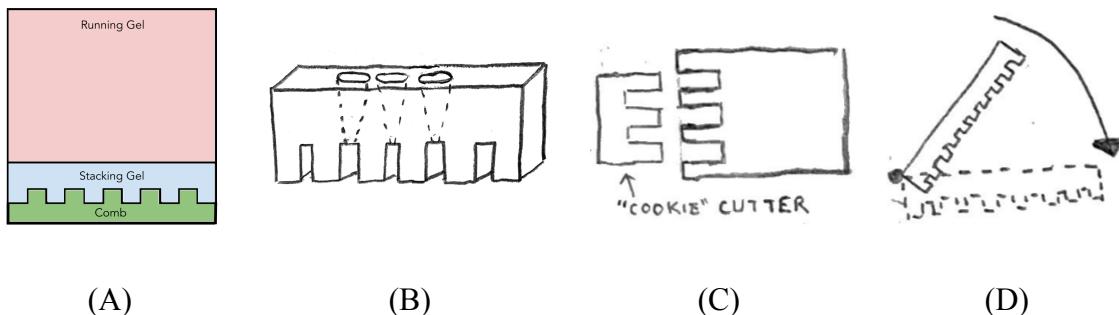


Figure 11: Comb insertion concepts

Before adding the stacking gel, the running gel must be polymerized; thus, its polymerization must be confirmed by our machine before proceeding with pouring the stacking gel. A lab technician does this by checking the excess gel to see if it has polymerized. One simple way for our device to check for polymerization would be to set a timer. Once this timer is finished, the next step proceeds. A second more interesting concept of checking for polymerization is through torque and force, shown in Figure 12. The gel solution could be put into a separate container that rotates. Depending on whether the gel is liquid or solid will determine whether the gel moves within the container. From this, the polymerization can be verified.

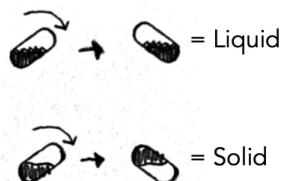


Figure 12: Polymerization check force/torque concept

As the polymerized gel will create a residue and cross-contamination needs to be avoided, cleaning is an essential part of our design. This could be accomplished by flushing the system with distilled water or the lab technician could hand wash the components between each gel.

CONCEPT SELECTION

Moving from the concept generation phase and into concept selection, we worked on combining our subfunctions into operational designs from which we selected our alpha design. Figure 13 shows the flowchart for our concept selection process. We combined the chemical mixing, measuring, and transport subfunctions from concepting into seven working designs that fulfilled those requirements (Figures 14-20). We then used a Pugh chart (Table 3) to compare the top seven designs and determine which one best met our design criteria. The Pugh chart design criteria include the requirements and engineering specifications we set at the beginning of this project (Table 1) as well as additional design and manufacturing considerations. Once we had selected an alpha design that combined the chemical mixing, measuring, and transport subfunctions, we conducted several sessions of brainstorming to determine how to incorporate the comb insertion, leveling of the gel interface, and ensuring polymerization subfunctions. We decided on these final three subfunctions separately from the designs discussed in the pugh chart

because they could be incorporated interchangeably into any alpha design, whereas the chemical mixing, measuring, and transport subfunctions were linked with one another.

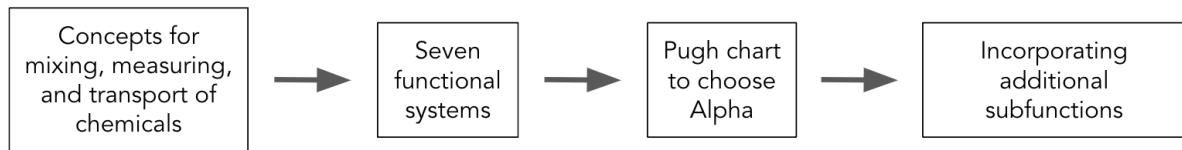


Figure 13: Flow chart describing our concept selection process.

Concept #1 (Figure 14) scored fifth on the pugh chart. It suffered mainly from the high amount of human intervention needed to work with the complicated reservoir system. However, it had strengths in expected actuator count and cost. The system could work with only three actuators and the cylindrical parts could be produced out of plastic for a low cost compared to components on other designs. Its compactness was quite appealing but came at the cost of high maintenance and a low simplicity score.

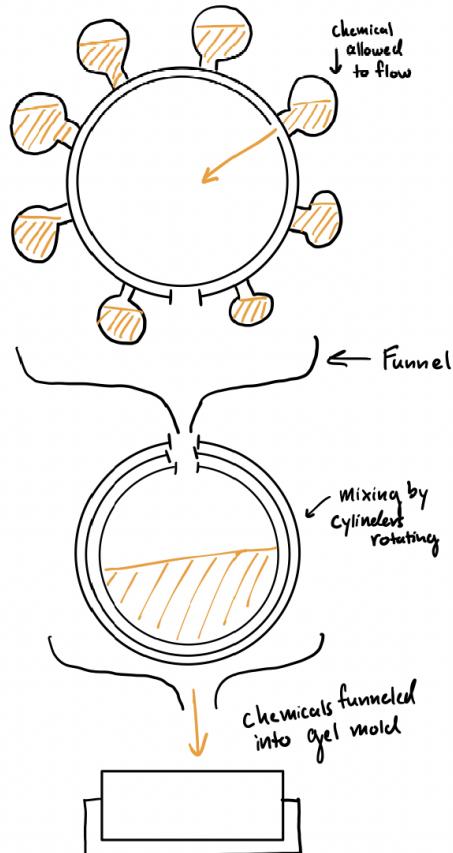


Figure 14: Concept #1, 'cylindrical storage and mixing'. Chemical reservoirs are attached to a rotating set of cylinders which control the flow of chemicals by aligning the opening of the inner cylinder with an opening in a chosen reservoir. Measurements are made using precise flow rate timing. The measured chemicals are funneled into a mixing chamber of rotating cylinders and finally funneled into the gel casting plates.

Concept #2 (Figure 15) was part of our goal to consider some out of the box ideas. It scored fourth on our Pugh chart. Concept #2 was strong in simplicity and ease of manufacturing but had trouble in the areas of measurement accuracy and safety features. This was definitely one of the simplest designs we had and the components required only basic methods to make. The volume displacement method of measuring liquid showed promise in its similarity to syringe pumps, but would be difficult to tune to microliter level measurements. The mixing bag was an entertaining idea, but would likely cause an unnecessary amount of chemical waste (due to buildup on the surface area of the bag) and was a safety concern due to the potential for leakage and spraying of the chemicals being mixed.

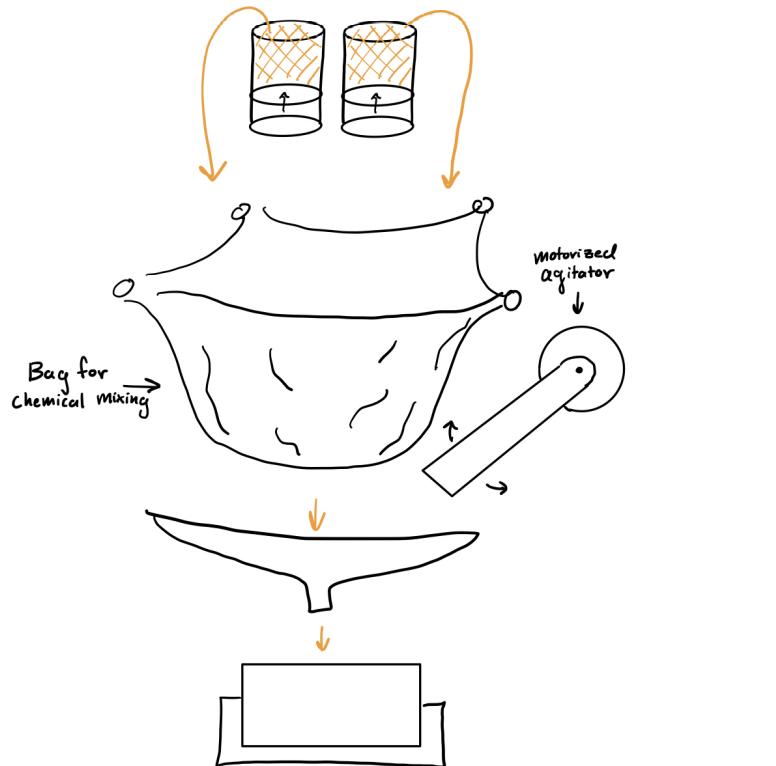


Figure 15: Concept #2, ‘slap bag’. Chemical reservoirs are overflowed using precise volume displacement (similar to how a syringe works), into a flexible mixing chamber. The mixing chamber is agitated by a motorized contacting device. The mixing chamber is then opened into a funnel that channels the mixed chemicals to the gel casting plates.

Concept #3 (Figure 16) was created with the goal of designing the most passive chemical mixing, measuring, and transport method. It scored seventh on the pugh chart due to its high maintenance requirements, expected difficulties in manufacturing, and expected actuator count. Its biggest downfall was that the premeasured reservoirs we designed ended up requiring far more actuators to function than we originally anticipated. Chemicals in their individual reservoirs needed an actuator for the appropriate latch to open in the premeasured reservoirs. This required an actuator for each graduation of the premeasured reservoirs - adding hundreds of actuators based on the range of measurements needed to make a variety of gels. The textured ‘river flow’ passive mixing design was aesthetically appealing but would have been complex to manufacture and difficult to clean.

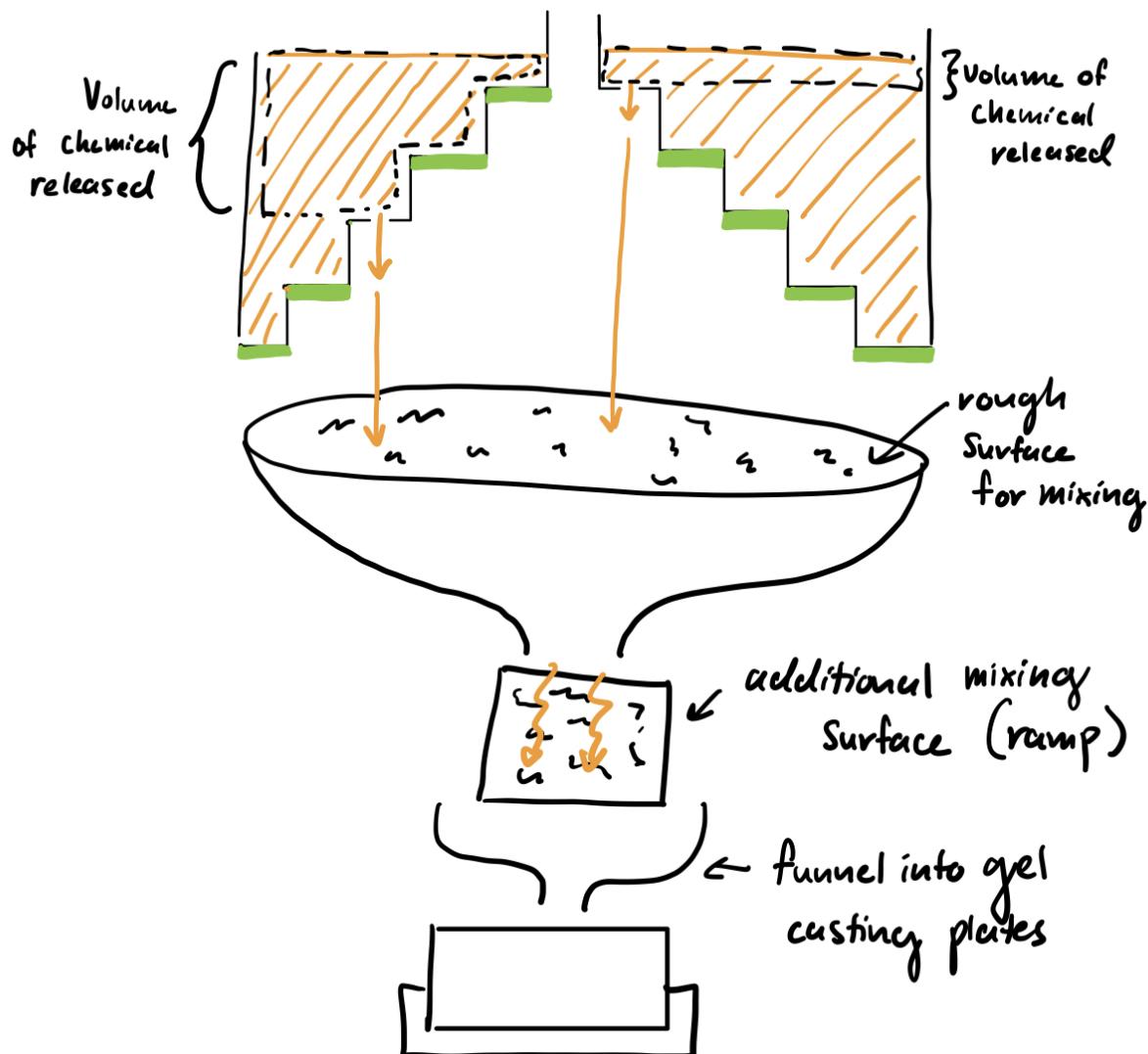


Figure 16: Concept #3, ‘premeasured reservoirs into river flow mixing’. A series of fixed-volume reservoirs release chemicals based on the volume required in the procedure. The reservoirs release into a funnel that utilizes a rough surface to passively mix the chemicals. Additional passive mixing is performed beyond the funnel. The mixed chemicals are then funneled into the gel casting plates.

Concept #4 (Figure 17) scored second on our Pugh chart, excelling in the areas of reliable measurements, simplicity, and manufacturability. The measurements for this design would require an initial calibration but would be quite repeatable because the process of forcing chemicals from the fixed volume tube would be simple to replicate. Decreasing the diameter of the tube would also help increase the resolution of the measurements, so the design is highly adaptable. The simple concept of pushing fluid out of tubes was inspired by the humble toothpaste tube. This simplicity was an advantage for group understanding of the design and also reflected positively on the manufacturability score. The biggest flaw of this design is the high expected number of actuators. Each chemical reservoir would require an actuator for pushing the liquid from the reservoir and an actuator to open and close the valve leading to the mixing

chamber. Two actuators per chemical adds up to at least twelve actuators for the measurement system alone.

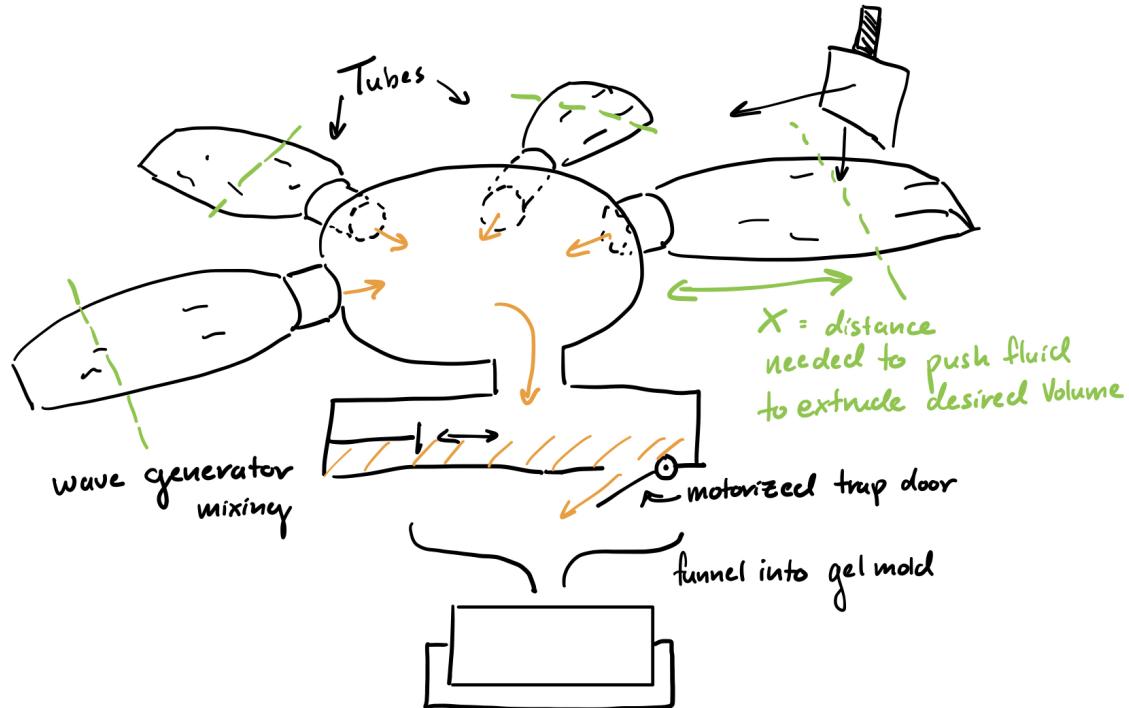


Figure 17: Concept #4, ‘toothpaste tube measurements with turbulent mixing’. Each chemical is stored in a separate flexible tube-shaped reservoir. The reservoirs each have one channel that can be opened to the mixing chamber. To measure a chemical, the channel is set to ‘closed’ and the flexible tube is clamped at an exact point on the tube. The clamping creates a compartment with the correct volume of chemical needed, the channel is then set to ‘open’ and the fluid flows into the mixing chamber. The mixing is done by generating waves in the fluid by oscillating a platform in the chamber. A hatch opens to allow the chemicals to funnel into the gel casting plates.

Concept #5 (Figure 18) was highly reflective of the steps a lab technician would take to create a gel. It scored first on the Pugh chart, with high scores in reliable and accurate measurements, easy to acquire components, and safety. Perhaps one of the most attractive features of Concept #5 is that micropipettes, which are standard measurement tools in the gel making process, are incorporated into the design. Using micropipettes means that we would not have to work from scratch to design a precision chemical measuring system. Micropipettes are also easy to acquire as they are standard laboratory equipment and our sponsor has already offered to provide us with micropipettes to use in a design. This design was rated highest in the Pugh chart safety category because the chemicals are not pressurized and lab technicians would be able to understand the machine best because it closely replicates a procedure they are familiar with using known tools. The biggest shortcoming of this design is the high cost of quality micropipettes [24]. We chose this design as our alpha design because it ultimately proved the most reliable in producing a quality gel by using a proven process, and it does not require manufacturing a precision measuring device.

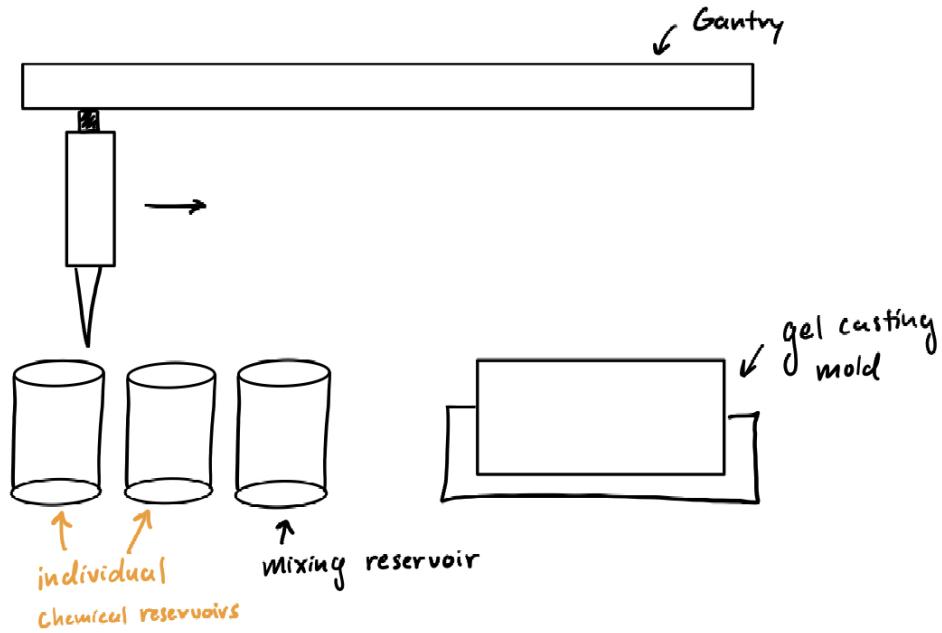


Figure 18: Concept #5, ‘pipetting’. Each chemical is stored in a separate reservoir that is opened during the setup time for the device. A micropipette on a linear gantry moves between the reservoirs and transports the chemicals to a mixing reservoir. To access the chemicals, the micropipette is driven in and out of the reservoirs (‘up and down’) by a linear actuator situated perpendicular to the gantry. Mixing is accomplished by agitating the gel fluid with the micropipette as a lab technician would. Once mixed, the chemicals are transported into the gel casting plates using the micropipette.

Concept #6 (Figure 19) scored sixth on the Pugh chart, struggling in the categories of stacking and running gel adhesion, simplicity, and compatibility with other casting systems. While ditching traditional vertical casting for the excitingly novel horizontal casting option would be revolutionary, there were many drawbacks to the newness of the design. The first drawback was that there is no literature on casting the stacking and running gels separately and then attempting to combine them. This gave way to a large amount of uncertainty in the area of adhesion between the two layers of gel, as any inconsistency in the boundary layer (such as air bubbles) could affect the results of electrophoresis. The design was also not simple to accomplish because it required modifying a current casting system to make it function horizontally. This lack of simplicity caused the design to lose points on compatibility because it would likely call for modifications of the casting kits. The design scored neutrally in most other categories of the pugh chart. Ultimately, we decided that there is not enough time in a single semester to develop such a novel design and make it functional. The concept confirmation alone would take a whole semester.

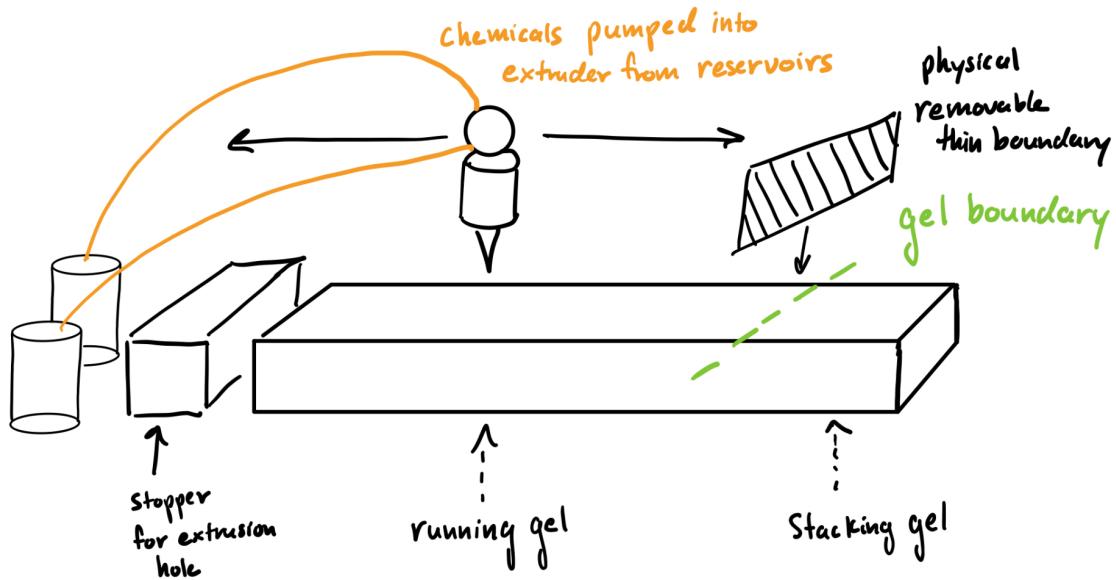


Figure 19: Concept #6, ‘horizontal casting’. To execute this method, the gel casting set must be laid horizontally. This requires a boundary to be set to from the top of the gel as well as between the running and stacking gels. The chemicals are stored in individual reservoirs which are pumped into an extruder that also functions as a mixing chamber. The running gel and stacking gel are formed within their respective chambers and the physical boundary that separates them is removed once both gels have polymerized.

Concept #7 (Figure 20) scored third on our Pugh chart, its strengths were in the areas of simplicity, build speed, and reliable measurements. The greatest appeal of Concept #7 is its simplicity, there are very few moving parts in the design because it relies primarily on pumps to move fluid through tubes. This makes the design easy to maintain and also means that it would likely need less frequent maintenance than designs with more moving parts. Building the design would likely be quicker than the other concepts because assembly would primarily involve building reservoirs and routing pumps. The downfall of this design was cost, peristaltic pumps with microliter precision cost upwards of \$1,000 - far beyond our \$400 budget [25]. Without the appropriate technology available to us within our budget, this solution became inaccessible.

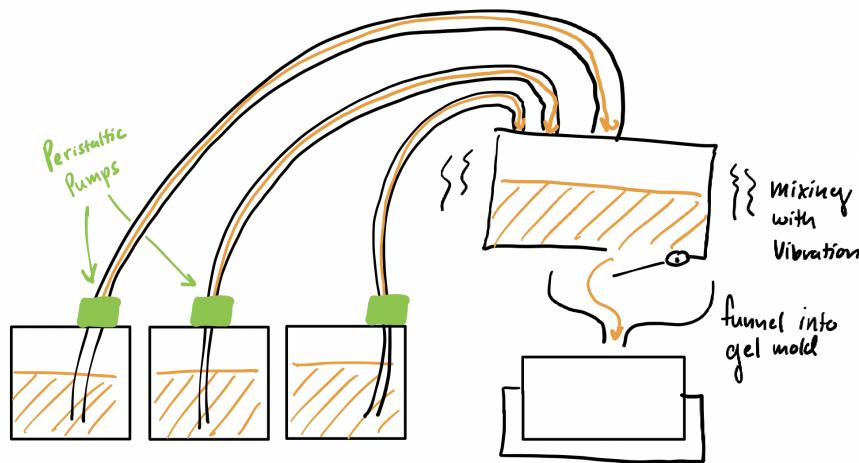


Figure 20: Concept #7, ‘peristaltic pump’. Chemicals are stored in individual reservoirs and are drawn into the mixing chamber by peristaltic pumps. The mixing chamber shakes the chemicals to mix them and then opens to let the mixture passively flow into the gel casting plates.

Concept	1	2	3	4	5	6	7
Design Criteria	Weight	Scores					
Functional Running Gel	4	0	0	0	0	0	0
Functional Stacking Gel	4	0	0	0	0	0	0
Stacking + Running gel adhesion	4	0	0	0	0	-1	0
Accurate measurements	3	0	-1	0	0	1	0
Reliable measurements	3	0	-1	-1	1	1	0
<3 human interventions per gel	3	0	0	0	0	0	0
<5 minutes human intervention per gel	3	0	1	1	1	1	-1
Compatible with other casting systems	2	0	0	0	0	0	-1
Less expensive	2	0	0	-1	1	-1	0
Easily maintainable	3	0	1	-1	0	1	0
Expected Actuator Count	1	0	-1	-2	-2	-1	-1
Simple	1	0	1	-2	1	1	-1
Quick to Build	1	0	1	-1	1	1	-1
Easy to Acquire Components	1	0	-1	0	0	1	0
Easy to Manufacture	2	0	1	-1	1	1	0
Proper chemical containment	4	0	1	0	0	1	0
Aesthetics + Compactness	1	0	-1	1	1	0	0
Safety features	4	0	-1	-1	0	1	0
Score	0	1	-15	11	22	-12	3

Table 3: Pugh chart for comparing chemical mixing, measuring, and transport designs.

Once Concept #5 was selected as the alpha design, we worked on incorporating additional subfunctions into the design. We decided that the best way to ensure that the gel was polymerized was to wait the maximum amount of time it could take gel to polymerize rather than incorporating a polymerization test. The advantage of a controlled polymerization check was that it could save up to 45 minutes. Ultimately though, the complicated controls and additional motors required to set up a polymerization checking mechanism offered little return on the quality of the gel, so we decided it was unnecessary to incorporate into the initial design.

For the comb insertion process, as well as the removal of the leveling fluid, we decided to take advantage of the motion that the pipette gantry was already required to make. The vertical motion needed for comb insertion was achieved by attaching the comb to the gantry head alongside the pipettes. Similarly, the tissue needed to swab the leveling fluid was attached to the gantry head to lower into the gel casting mold when required. Combining the vertical motion of the comb, leveling system, and pipettes ultimately saved on two independent actuators.

Overall, our concept selection process included a wide range of concepts for consideration and led to extensive strategic discussion. Having a diversity of concepts was a goal of ours throughout the concepting process as we wanted to explore as many unique solutions as possible. Our selected alpha concept is quite different from the pump-based design that our sponsor originally suggested at our first meeting [2]. Our chosen design has a satisfying foundation in the current proven gel casting procedure with the advantages and challenges of automation mixed in. It is a promising design that has objectively surpassed the other designs that we generated, and can be expected to perform well when built.

SELECTED CONCEPT DESCRIPTION

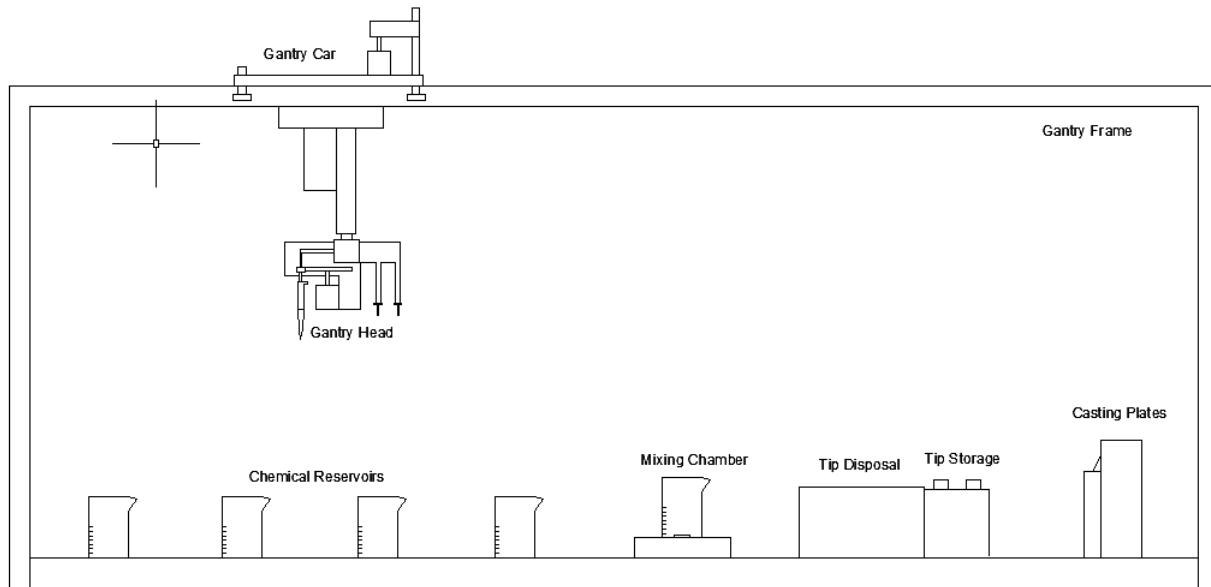


Figure 21: Alpha Concept Layout Drawing (general layout, not to scale).

Our alpha concept as seen in Figure 21, is a relatively simple design that relies on a two directional gantry system to manipulate the position of two pipettes, a swab, and a comb. The concept itself consists of four major elements: the frame, the base, the gantry car, and the gantry head. The base holds the chemical reservoirs, mixing chamber, tip disposal, tip storage, and casting system in known positions, so that the gantry can access them reliably. The gantry car is

responsible for moving the gantry head both vertically and horizontally such that it can accurately acquire and dispense both the pipette tips and the chemicals. The gantry head itself contains and operates a pair of linear displacement micropipettes, a swab, and the casting system's comb.

The user will be required to replace or maintain the mixing chamber after each use, refill the tip storage when it is empty, and empty the tip disposal when it is full. Beyond this, the user need only input the casting stand, input the desired gel concentration, and remove the stand when the gel is polymerized. In between the user's initial inputs and their retrieval of the casting stand, this concept will operate much like a 3D printer. It will travel back and forth using the gantry head to make measurements and dispense them in the mixing chamber and casting plates.

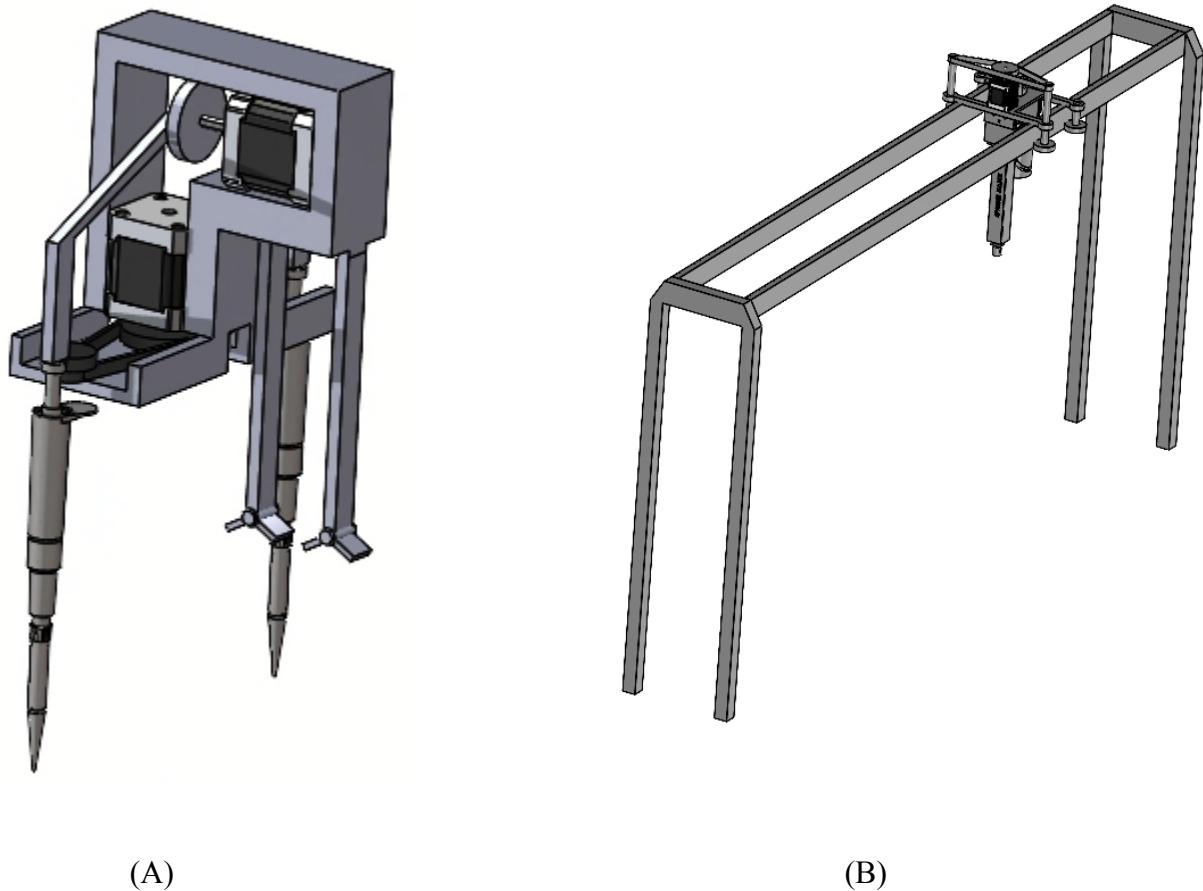


Figure 22 A & B: This figure depicts the preliminary models for gantry elements of the design. Section A shows the gantry head. The gantry frame and car can be seen in Section B. Note that in our concept the gantry head will be attached to the end of the linear actuator on the gantry car.

The gantry frame and car are composed of a set of frames and a pair of actuators. One is a traditional motor which uses a belt to drive the wheels that transfer the car in the horizontal direction. The other is a linear actuator that adjusts the vertical position of the gantry head to which it is attached. Inside the gantry head, there is the pipetting system which uses a motor and linkage to depress and release the pipette plungers. Additionally, the gantry cart uses another

motor to manipulate a belt or gear mesh which adjusts the pipette measurements by twisting the ends of their plungers.

To accomplish our accuracy requirements, it's necessary for our design to allow for the precise measurement and dispensing of the chemical ingredients for our gels. Our alpha concept achieves this by using lab-grade pipettes attached to our gantry shown in Figure 23 below. The pipette measurements are adjusted through rotating the button caps. To automate this process, we plan to embed gears into the caps and mesh them to another gear driven by a motor to rotate the caps. When an individual pipette needs to be actuated, the linkage presses down on the button cap which temporarily decouples the gear before it returns to its original position.

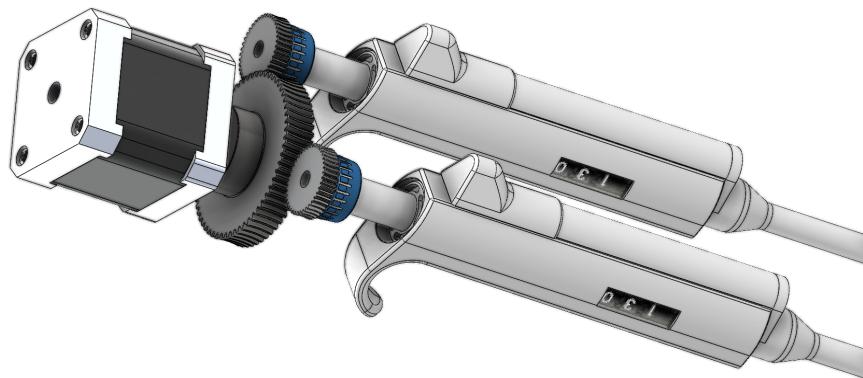


Figure 23: The pipette measurements are adjusted through rotating the button caps via embedded gears.

The gantry assembly operates on a workspace shown below in Figure 24 that includes all the materials required for mixing and casting the gel. It includes two rows of reservoirs that hold all the gel ingredients for the gantry pipettes to draw from and measure. The pipettes then deposit the ingredients into a mixing chamber powered by a magnetic stirrer. After the ingredients have been mixed, the pipettes draw up the solution and dispense it into the casting kit which is placed at a space at the end. Throughout this process, the pipettes will be changing their tips using the tip holder and tip disposal bin in order to prevent cross contamination.

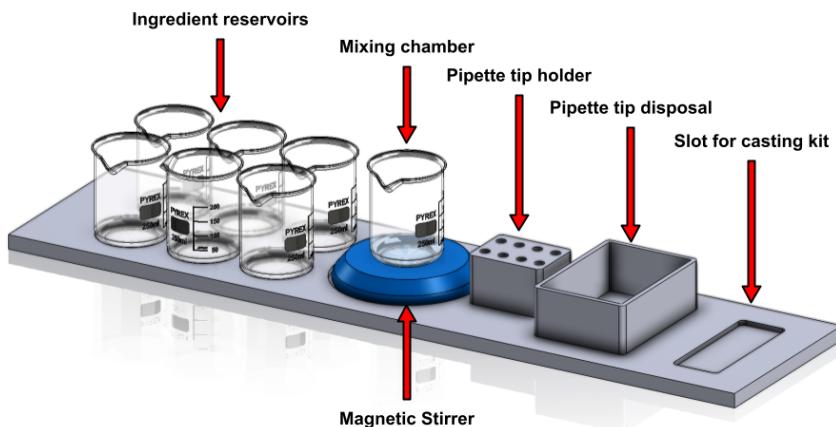


Figure 24: This layout of the workspace the gantry operates on, including the reservoirs, mixing chamber, pipette stations, and casting kit placement

Bill of Materials

Part #	Description	Quantity	Units	Price	Subsystem
1	Multipurpose 6061 Aluminum Stock	4.50	m	\$14.19	Gantry Frame
2	Synthetic Rubber Belt	0.50	m	\$12.89	Gantry Car and Head
3	Stepper Motor	3	ea	\$29.97	Gantry Car and Head
4	3D Printed Plunging Apparatus	1	ea	\$15.00	Gantry Head
5	Gears	3	ea	\$31.98	Gantry Head
6	Linear Displacement Micropipettes	2	ea	\$0.00	Gantry Head
7	Disposable Micropipette Tips	2	ea	\$0.00	Gantry Head
8	3D Printed Gantry Car Frame	1	ea	\$15.00	Gantry Car
9	Wheels	4	ea	\$12.99	Gantry Car
10	Linear Actuator	1	ea	\$15.99	Gantry Car
11	Glass Beakers	7	ea	\$20.00	Base
12	3D Printed Custom Base Platform	1	ea	\$30.00	Base
13	Arduino Uno (Controller)	1	ea	\$28.50	Controls
14	Wiring	5	m	\$40.00	Controls

Table 4: Initial bill of materials for our alpha concept. Please note that the phrase “ea” is used as shorthand for “each” to indicate a unit value. Additionally, it is worth noting that the price column is given in terms of total cost instead of unit cost. Embedded in each price is a URL linked to the location our team used to identify the price given in the table.

Preliminary Acquisition and Assembly Plan

Our team plans to develop our CAD models so that we can create final versions of and print our gantry car frame, plunging apparatus, and custom base platform. Our team currently plans to use a combination of McMaster-Carr, Amazon, and ThermoScientific to supply our aluminum stock, wheels, gears, stepper motors, beakers, stir bar, and stirrer. Much to our appreciation, Innovative Research Inc, has already supplied us with a set of pipettes, as well as chemicals and a casting stand for testing our device.

As for assembly, we plan to first cut and fasten our aluminum stock to create the gantry frame. After the frame is assembled, our team will mesh it to the base and ensure that they fit in a satisfactory manner. Then, we will fasten the wheels, belt, drive, and linear actuator to the gantry car before placing them onto the gantry frame. Next, we will input the remaining motors, gears, and belts into the gantry head alongside our modified pipettes. The gantry head will then be fastened to the end of the linear actuator on the gantry car. Finally, the reservoirs and mixing chamber will be placed into the base after everything has been checked for stability.

Possible Expansion For Gradient Gel Fabrication

The benefits of automation would contribute most effectively to the production of gradient gels, as discussed in the User Requirement and Specification section. Our team has taken the time to design a possible expansion to our alpha concept that would bring gradient gel capabilities to our device.

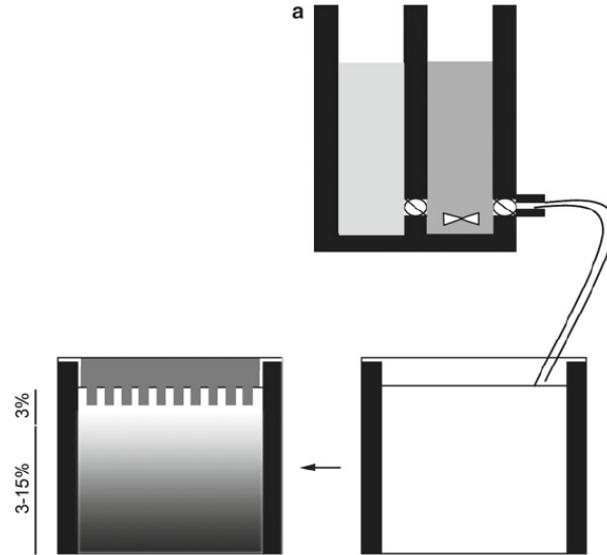


Figure 25: Diagram outlining a proven and current method of mixing of gradient gels [23].

As seen in Figure 25, gradient gels can be produced using a pair of reservoirs connected through a valve. By placing a pair of such reservoirs into the base of our current design, the gantry system could be used to automatically measure and create the needed concentrations in these reservoirs. Then through the addition of a peristaltic pump and a motorized linkage, our device could automatically insert the exit tube into the casting plates and fill them. Then the tube could be removed by the linkage in order to allow for the gantry to level, lay the stacking gel, and then insert the comb as normal.

We have decided, however, to maintain this as an avenue for further expansion because of the time constraints our team will be under. Within the current time constraints, our team does not believe that this development is feasible, but that it is extremely viable as a future development.

Problem Analysis and Iteration

When analyzing our alpha design, we can extrapolate direct correlations between our engineering specifications and design aspects that address them. A handful of the specifications are best addressed through empirical testing. They are: the physical quality of the completed gel, the time a human will need to spend on our machine, and the time it takes for maintenance. The others can be addressed conceptually or through a combination of methods.

To ensure the accuracy of chemical measurements, we chose to use current micropipetting systems. These pipettes all have $< 5\%$ error [24]. In order to confirm that our measurements from the final procedure are within the acceptable bounds, we will extrapolate the approximate measurement error data to match the final measurements. To create a compatible system, we will design our machine base with enough space for the largest system, and verify by placing the glass plates in the base. Making a cheap gel will depend on a variety of factors:

current market prices for materials, production volume, and the cost of the machine. These can be calculated, but the production volume will be dependent on how long the machine takes to make a gel. Our last specification including environment and materials chosen for the machine can all be handled by correct design choices.

Verifying the results from empirical testing will involve a variety of other methods. We will have to analyze the gels made by the machine visually to test the physical gel quality. This is the most efficient method because there are too many variables that can affect the quality of a gel- and attempting to model the quality would be extremely complex. Minimizing human interaction time (a goal of two of our specifications) is also empirically tested, as there are no easy ways to estimate how long it takes someone to clean, setup, or pour chemicals without data.

Below, in Table 5, we summarize the correlations between the engineering specifications and the alpha design aspect that will address it.

Engineering Specification	Alpha Design Relation
95% of gels do not display a meniscus, warping, or separation	→ Direct testing [Empirical]
Chemical measurements made with < 5% error	→ Current pipette systems <5% error [Conceptual] [24]
< 3 human interventions per gel, < 5 minutes total per gel	→ Minimal setup [Empirical]
Compatible with systems < 14 x 11 x 1 cm in < 5 minutes	→ Design can handle [Conceptual]
Cost to produce a gel is < \$4.52	→ Use of final volumes and market prices [Hybrid]
6 < 5 minutes of maintenance per gel	→ Anticipated maintenance challenge [Empirical]
0 chemical exposures to a pH outside of 6.5-8.5, temperatures outside of 45-85°F, and any organic material	→ Lab environment, material choice [Conceptual]

Table 5: Engineering Specifications and the Alpha Design Concepts that address it. Note that the method of verification is also listed in brackets.

Due to the automated nature of our machine, we expect to work most with controls, electronics, gear ratio calculations, and statics for a majority of our gantry design. Specifically, making the controls with programming and understanding the electrical system and design for all of the anticipated actuators and movements will be our largest challenge. However, with the addition of the gradient gel expansion or potential pump driven designs, we will have to use fluid dynamic calculations to understand the pump flow from the gradient mixer and how to automate that process through linear actuation or automated tests.

ENGINEERING ANALYSIS

A variety of engineering analysis methods were used to develop, evaluate, refine, and optimize our design with respect to the requirements and specifications. We used the simplest models possible to give the accuracy needed for our decision, this saved time and resources. Most of our engineering decisions are built on each other, so this section is presented in the order that our analyses were conducted.

Syringe Pump Analysis

The syringe pump system is relied upon to fulfill the chemical measurement engineering specifications and secondarily involved with the chemical containment, cost, and gel accuracy specifications. We chose to assess an open source syringe pump called “Poseidon” designed by Pachter Lab at Caltech [27]. In terms of analysis, we relied on the documentation of previous testing by Pachter Lab and our own empirical testing done by assembling the syringe pump to determine if it was the right fit for our design.

An initial budget analysis using the bill of materials (Table 9) given by Pachter Lab confirmed that the design, with a cost of \$136.97 to produce, would be a good choice considering the budget of our project. Previous difficulties in finding quality precision measurement systems within our budget made us keen to learn the accuracy limitations of this design. The accuracy limitations of the syringe displacement were tested by Pachter Lab and presented along with the design instructions. The data from Pachter Lab showed an average position error of $\pm 1.44\%$, which translates to volume error using calculations shown in Appendix C.1. The results of these accuracy calculations are summarized in Table 6. The errors for the 10 mL and 10 μL pump are within the $\pm 5\%$ range of our chemical measurement accuracy specification, so we concluded that the Poseidon system would be capable of making the measurements we needed and proceeded to build a prototype of the design.

Syringe Volume	Stroke Length [28]	Inner Radius [28]	Calculated Volume Error	Calculated Percent Volume Error
10 mL	80 mm	14.5 mm	$\pm .003 \text{ mL}$	$\pm .03\%$
10 μL	60 mm	.245 mm	$\pm .375 \mu\text{L}$	$\pm 3.75 \%$

Table 6: The errors shown are the maximum errors attainable for the respective syringes being operated with the Poseidon syringe pump.

The prototype we built, shown in Figure 26 gave us useful insight into the challenges we would encounter incorporating the syringe pump into our final design, provided further information on its behavior and properties not listed in the Pachter Lab documentation, and clarified the manufacturing process for the syringe pump.

The first issue we encountered with the prototype was the flimsiness of the frame. The pliability of the 3D printed material upon touching it indicated that the 3D print did not have enough fill, rather than a structural design flaw. There was a distinct “crunch” to the material when compressed, revealing that the open pockets in the print were being crushed. As a result of this observation, we are increasing the fill from the original 20% to 30% in our next print. Additional modifications include redesigning the clamp that secures the syringe which we found inadequate when jogging the motor, and modifying the interface between the syringe handle and

the machine so that the syringe can be fully depressed. Our protocol requires full depression of the syringe so that chemicals are not transferred into other reservoirs as the syringe is reused for measurements. In terms of controls, we were able to control the stepper motor that drives the pump using custom code and found that the motor could be successfully driven by our code. However, we had errors in motor rotation direction and stalling of the motor. Once we receive the CNC shield we ordered we will be incorporating the code from Pachter lab and testing its ability to control the stepper motor with greater precision and reliability

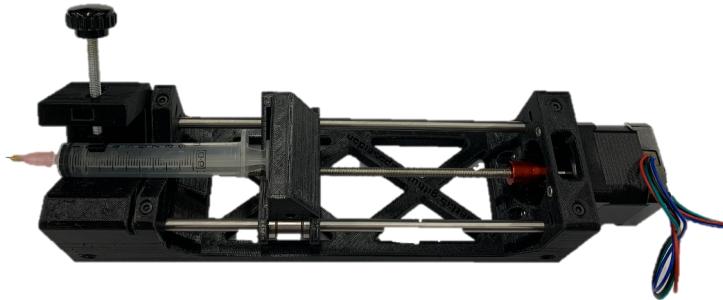


Figure 26: our prototype syringe pump.

Chemical Dilution Analysis

The standard gel casting procedure calls for measurements ranging from $3.8 \mu\text{L}$ to 5.43 mL , as shown in Table 7 and Table 8. The microliter measurements posed a major design challenge due to the precision they required becoming costly in execution. Additionally, making measurements with such a range added new levels of complexity to our design as we found the only way to make milliliter and microliter measurements effectively was to have a dedicated system for each measurement range. Our sponsors at Innovative Research had the idea to dilute the microliter volume reagents with water to make them milliliter measurements. The solution is actually best labeled as ‘redistribution’ because Innovative Research proposed that the reagents only be diluted with water that was already going to be added to the gel (so the reagent concentrations in the overall gel do not change). Table 7 and Table 8 show measurements of the standard gel procedure compared to the redistributed gel procedure. The redistributed method causes the range of measurements to fall within the capabilities of a single syringe pump. Although this change had the potential to greatly simplify our design, we kept in the original track of using the standard procedure for the time that Innovative Research was in the process of evaluating the new method.

Resolving Gel Method	H ₂ O	AM	RGB	SDS	APS	TEMED	Total Volume
Standard	5.43 mL	2.81 mL	2.81 mL	112.5 μ L	60 μ L	3.8 μ L	11.23 mL
Redistributed	2.43 mL	2.81 mL	2.81 mL	<i>1.0 mL</i>	<i>1.0 mL</i>	<i>1.0 mL</i>	11.23 mL

Table 7: Standard and redistributed methods of mixing the resolving gel, where AM is acrylamide monomer, RGB is running gel buffer, SDS is sodium dodecyl sulfate, APS is ammonium persulfate, and TEMED is Tetramethylethylenediamine. The measurements in italics have been diluted using the water reserved for the resolving gel.

Stacking Gel Method	H ₂ O	AM	SGB	SDS	APS	TEMED	Total Volume
Standard	3.2 mL	0.5 mL	1.25 mL	50 μ L	40 μ L	4 μ L	5.04 mL
Redistributed	.2 mL	0.5 mL	1.25 mL	<i>1.0 mL</i>	<i>1.0 mL</i>	<i>1.0 mL</i>	4.95 mL

Table 8: Standard and redistributed methods of mixing the stacking gel, where AM is acrylamide monomer, RGB is running gel buffer, SDS is sodium dodecyl sulfate, APS is ammonium persulfate, and TEMED is Tetramethylethylenediamine. The measurements in italics have been diluted using the water reserved for the stacking gel. Note that there have been changes made to the chemical concentration for the redistributed stacking gel, these concentrations are how the redistributed method for the stacking gel was tested.

Innovative Research performed tests using the redistributed chemicals to analyze the effectiveness of the new method. Their main concern was that the gel would lose catalytic activity, leading to delayed polymerization times and unusual runs with molecular weight standards if the cross linking was aberrant. They began by mixing concentrations of the reagents for ten gels using the concentrations from Tables 7 and 8. The polymerization time of the first test gel was reported as normal (normal meaning: identical to a gel using the standard procedure) and the gel performed normally in a PCR test run with molecular weight standards. In order to simulate the environment within the gel casting device, the remaining reagents were kept at 37 °C for seven days, and after that period another gel was made. The one week gel passed the polymerization and molecular weight tests, confirming that the redistributed chemicals could sit for a week. The reagents in our device have a required turnover time of once per week that is built into the allotted maintenance time (under the easily maintainable engineering specification in Table 1). Since the lifespan of the redistributed chemicals was proven, we were content moving on with that method. Innovative research continued to test the gels until they failed at 3 weeks of the chemical sitting, the failure was determined to be that the APS had expired. This was anticipated, as mixing fresh APS weekly in the lab is already standard for hand casting gels.

From the success of this experiment, we have decided to move forward with the redistributed method of gel casting. This simplified our design from using two syringe pumps to cover the range of measurements to just one. Our only additional worry had been the potential contamination of the reservoirs with the single syringe accessing every reservoir. We consulted Innovative Research about the potential of flushing the syringe with water to cut down on contamination and they believe that will be a valid solution to the contamination problem.

Chemical Reservoir Selection

The dimensions of the chemical reservoirs are critical to determining many of our design parameters. This short discussion will cover the selection of the reservoirs and their dimensions to be referenced in upcoming analysis sections.

Reagent refills are scheduled as part of weekly maintenance and Innovative Research has approved a production goal of 10 gels per week, which gives the total volumes of chemicals needed to be stored in the device. The range of the reagent volumes for 10 gels is 20 mL to 26.3 mL. In order to keep the reagents contained under the rotation of the carousel, we opted for 50 mL chemical reservoirs because they are about twice the volume of the required reagents. Figure 27 shows the dimensions of the selected chemical reservoir as labeled by the distributor. We have not received the reservoirs yet but we will be confirming these dimensions as they arrive.



Figure 27: The dimensions of the selected 50 mL reservoir. [29]

Carousel Base

The carousel base required strategic procedural discussions to determine the optimal layout of the gel mold, chemical reservoirs, and potential space for expansion. The most valuable analysis we did was to walk through the gel casting protocol and work out exactly how our syringe pump will access the reservoirs. Figure 28 shows a snapshot of the base arrangement during the process of procedural prototyping. This analysis gave us an approximate base diameter of 240 mm.

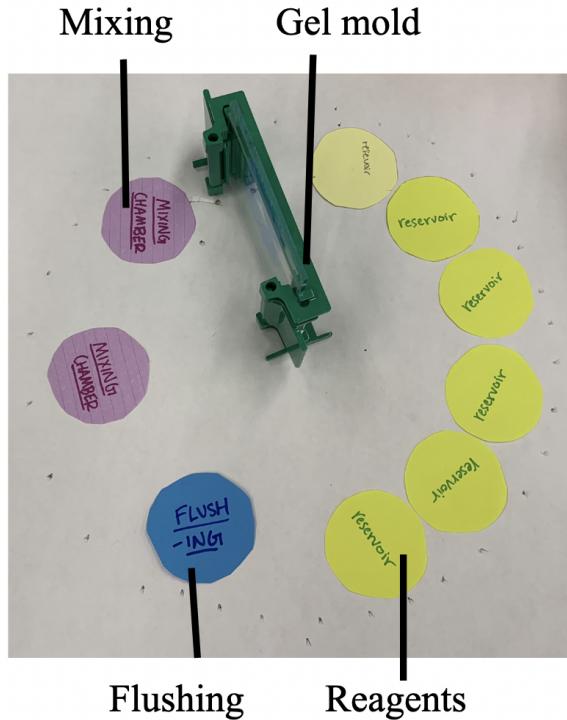


Figure 28: prototyping the carousel base configuration

Using the base diameter determined from prototyping, we conducted a beam bending analysis on a section of the carousel base plate to determine the thickness of acrylic we need to build our base with. Acrylic was chosen as the carousel base material for its cost and accessibility benefits. The bending calculations for the acrylic carousel are recorded in Appendix C.2. The results of the bending calculations are that a 1mm thick piece of acrylic should be able to hold the maximum loads from the reservoirs with a 5% deflection. This means we can use a sheet of 5mm thick acrylic to reach a safety factor of 5, which is our minimum safety factor for this critical base component.

The thickness of the acrylic was used to find the minimum torque to move the carousel base plate, in order to choose a motor with the right specifications to rotate the base plate. Maximum motor speed is not a specification that we are considering because the base will be moving at a slow pace to accommodate the open chemical reservoirs without spilling them. Our engineering judgment tells us that the moment needed to move the reservoirs on the outer radius of the carousel is our biggest concern for this part of the design. The calculations for the torque on the motor are shown in Appendix C.3. The results of the torque calculations are that the motor we use requires a torque of .005 N/m.

Syringe Pump Linear Actuator

A linear actuator translates the syringe pump in and out of the reservoirs and into the gel casting kit. It will need to be able to carry the load of the syringe pump and associated supports as well as translate from the lowest point on the base to above the highest point on the base. The lowest point on the base will be the bottom of the chemical reservoirs and the highest point on the base will be the top of the gel casting plates. The chemical reservoirs will be secured halfway beneath the plane of the acrylic plate and the gel mold will sit just on top of the acrylic plate so

the total distance linear actuator travels will be half the length of the reservoir plus the full length of the gel. This travel distance comes to 113 mm. The mass of the syringe pump assembly is .56 kg as shown in Figure 29. With a safety factor of three, the load that the linear actuator must be able to transport is 1.68 kg.

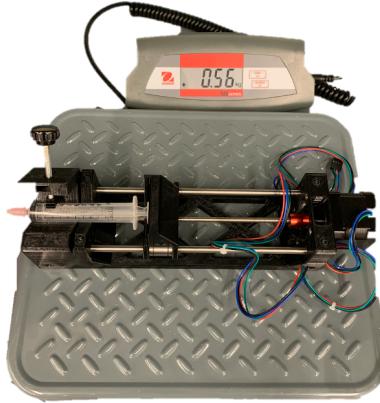


Figure 29: mass of the syringe pump assembly.

Comb & Swab insertion

While swab insertion to remove the leveling fluid was tested empirically when we made gels as a team, we have decided through discussion with our sponsor that it may not be necessary to design a subsystem for this task. Our sponsors at innovative research believe it is likely the leveling solution could remain in the gel mold as long as we use water (and not isopropanol) as our leveling fluid. Further analysis needs to be done in this area by casting gels without removing the leveling fluid.

There were three main solutions that we tested for comb insertion: comb inserted from the bottom, comb half-inserted with a mechanized full insertion stage, and comb half-inserted with a passive insertion stage. After passing the gel casting system around and trying to insert the .98 mm comb into the 1 mm gap of the casting mold, we decided that the comb should either be pre inserted or partially inserted at the start of the casting procedure due to the tight tolerances of the fit. We found that physical prototyping was an excellent way to analyze the successes and failures of our designs.

Figure 30 shows the main challenge of inserting the comb from the bottom and casting in reverse order - the base with the comb inserted instead of a gasket is very hard to seal from leaks. After unsuccessfully attempting to seal the comb/gel mold interface with electrical tape, plastic wrap, clay, foam, and two gaskets in a vice, we decided that sealing the comb was not an attainable solution. This was a good decision considering our chemical safety engineering specification because sealing the comb properly in repeated uses would have been a safety risk.



Figure 30: the foam seal on the gel leaking.

Figure 31 shows a working prototype for a half-inserted comb that is pushed into the casting plates by a rotating arm driven by a DC motor. Having the comb half inserted at the start of the casting procedure confirms the proper position of the comb and allows reagents to be poured between the gaps in the comb. This was quick to assemble and code, and proved the validity of such an approach as well as the potential space-related complications we might experience with such a device being incorporated into the carousel base.

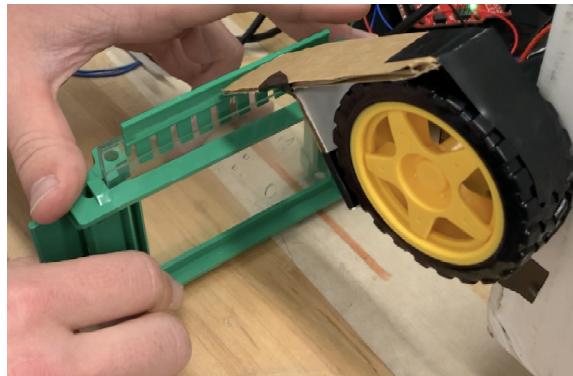


Figure 31: comb half-inserted with a mechanized full insertion stage.

Figure 32 shows the approach of having the comb half-inserted with a passive insertion stage. The passive insertion works by securing the comb half-inserted into the gel mold with a clip that can be pushed on by the syringe pump linear actuator to fully insert the comb. A specialized clip (not the clothespin shown) has been developed for this design and is currently in the process of being 3D printed. Further analysis will prove the validity of this design.



Figure 32: comb half-inserted with a passive full insertion stage.

Next Steps

With all of the parameters of our design worked out, there is now an opportunity to analyze the functionality of our subsystems more closely. We will be testing the Poseidon code for full functionality once the CNC shield arrives, and this will give us more freedom in our functionality analysis of the syringe pump as well. Now that the method of handling the chemicals has been confirmed, we will need to test how contaminated the reservoirs get when using the same syringe pump. This can be done using colored dye to track contamination. We will also need to do a slosh test of the chemical reservoirs in the carousel base to determine what the safe acceleration of the system is. Further testing of our comb insertion subsystem will complete our engineering analysis up to the final design.

BUILD DESCRIPTION

One of the most critical elements of our design is the measurement of each chemical. As shown in our final design, the measurement is done through the use of syringe pumps. Therefore, our team's build for prototyping purposes was a physical build of our syringe pump – a necessary component for our eventual gel-casting machine. The syringe pump controls the measurements of each chemical, and accurate measurements are vital in creating a gel (as specified in our engineering specifications). For this design, it was necessary to revisit the design phase. After doing research, we determined that buying a syringe pump would cost more than \$1000 and thus be too expensive for our budget. With this design idea eliminated, we explored building our own syringe pump. We found the Poseidon project by Pachter Lab at Caltech, which provided open-source documentation of the design, materials, hardware, and software on a syringe pump design for less than \$400 [27]. An image of our syringe pump build is shown below in Figure 33. The validity of this design was tested in-house by Pachter Lab at Caltech as well as Harvard, increasing our confidence in building our own syringe pump.

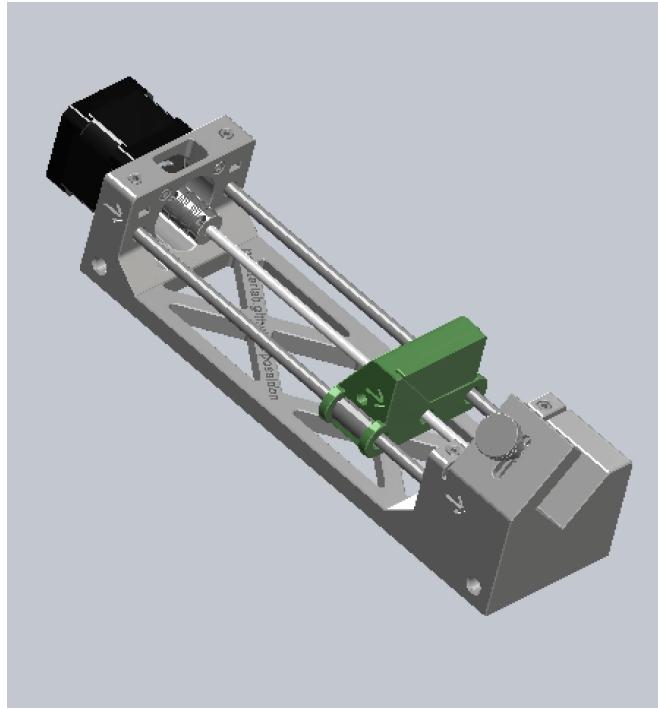


Figure 33: A detailed CAD of our ‘build’, the syringe pump. This CAD includes 3D printed parts and items purchased online in a final assembly.

The materials and parts used for our syringe pump are listed below in Table 9. The Poseidon project listed a majority of these items for us, but some of the items were no longer available for purchase through the sources provided by Poseidon, so our team researched adequate replacements for the necessary parts. The costs were pulled from online resources, mainly Amazon, to complete the BOM. The 3D printed parts were outsourced to a friend of one of our team members, so we were able to print these parts at no cost. Our build totaled to \$136.97.

Part No.	Part/Material	Manufacturer	Cost
1	Nema 17 Stepper Motor (Bipolar, 40mm, 45Ncm)	StepperOnline	\$12.99
2	5mm to 5mm Motor Shaft Coupling	uxcell	\$5.65
3	6mm Steel Rod with Length 200mm (2 Pack)	uxcell	\$6.59
4	6mm Linear Bearing (12 Pack)	Shutao	\$15.99
5	M5x0.8 170mm Threaded Rod	uxcell	\$9.85
6	M5x0.8 Nut (50 pack)	SpzcdZa	\$7.99

7	M3x0.5 20mm Socket Head Screws (50 Pack)	McMaster-Carr	\$7.95
8	M3x0.5 10mm Socket Head Screws (50 Pack)	McMaster-Carr	\$8.55
9	M3 Nut (100 Pack)	McMaster-Carr	\$6.94
10	M5 knob (10 Pack)	uxcell	\$14.49
11	12V power unit with AC Adapter	Kastar	\$13.99
12	UNO R3, Stepper Motor Driver, and CNC Shield Pack	kuman	\$25.99
13	Pump Base	-	\$0.00
14	Pump Carriage Sled	-	\$0.00
15	Pump Syringe Base	-	\$0.00
-	TOTAL	-	\$136.97

Table 9. The Bill of Materials for one syringe pump.

Our manufacturing plan for the syringe pump is fairly simple. Only three pieces needed to be manufactured by 3D printing, shown below in Table 10. All pieces were printed on a Creality CR-10 V2 printer with 15% infill. For these parts, our critical surfaces include the holes for the threaded rod and the slots for the nuts. In order to allow for rotation of the threaded rod, the tolerances must be tight so that the design allows the rod to rotate while also keeping it secured. Additionally, slots were created in the part so that a nut can be inserted to help hold the bolts more securely. These slots must have tight tolerances, within ± 0.05 - 0.08 mm, to insert the nut. Referencing the assembly video by Poseideon, the builder uses an allen wrench to forcibly press the nut into the slot. Having too small of a hole will make this building process extremely difficult and potentially impossible, and too large will not correctly secure the bolts. Most other surfaces and structures serve as support and are not as dependent on tolerances. If tolerances were a major concern, then we would not proceed with 3D printing our parts and opt to use a manufacturing process that is more accurate. The precision of the chosen printer was ± 0.1 mm and therefore suitable for our parts. Upon delivery, the parts underwent basic physical evaluation and testing and were found to be dimensioned and manufactured correctly for our purposes. Some conclusions from those tests were to increase the infill to prevent flexing and to modify the CAD to more accurately match our syringe.

Part/Material	Machine	Operation
Pump Base	Creality CR-10 V2 3	Fused Deposition Modeling (FDM)
Pump Carriage Sled	Creality CR-10 V2	FDM
Pump Syringe Base	Creality CR-10 V2	FDM

Table 10: Manufactured parts and their corresponding manufacturing process

Our build of the syringe pump is a critical component of our overall final design. As the syringe pump is responsible for measuring all of the chemicals and the correct measurements of each chemical is vital for gel creation, the success of this syringe pump build directly correlates with the success of our final design. By testing the syringe pump as an individual component, we can better diagnose problems and troubleshoot. Once the syringe pump is functioning properly, it can be assumed that its implementation in the final design will function properly as well. Engineering the syringe pump well will translate to the overall engineering value of the final design, as more thought and testing has been done initially to prevent later issues.

FINAL DESIGN DESCRIPTION

After conducting engineering analysis and verification/build tests, our team revised our final design from the alpha design mentioned previously to integrate the results of our analysis to streamline our machine. Our new final design is composed of two separate subsystems: the carousel base and syringe pump stand. The final assembly and manufacturing plans can be found in Appendix E.

The carousel base is designed to contain the ingredient reservoirs as well as the casting kit—it serves as the workspace that the syringe pump operates on. The design consists of a plate created from stacked pieces of laser-cut acrylic where the casting kit and reservoirs are placed (the casting kit and reservoirs are pre-purchased). The plate is coupled to a NEMA 23 stepper motor which is housed by a base made from 3D printed plastic. The assembly is supported by a variety of off-the-shelf bearings and fasteners to hold the components together. The carousel works by rotating the plate to allow the syringe pump to access all the different components of gel-making. Our team is confident in its functionality because the critical rotation functionality is powered by a NEMA stepper motor which we have already thoroughly tested and verified our ability to operate during our syringe pump investigation (as outlined in the build description section). Our engineering analysis also suggests that our motor has more than enough power to rotate the workspace (as shown in our engineering analysis section).

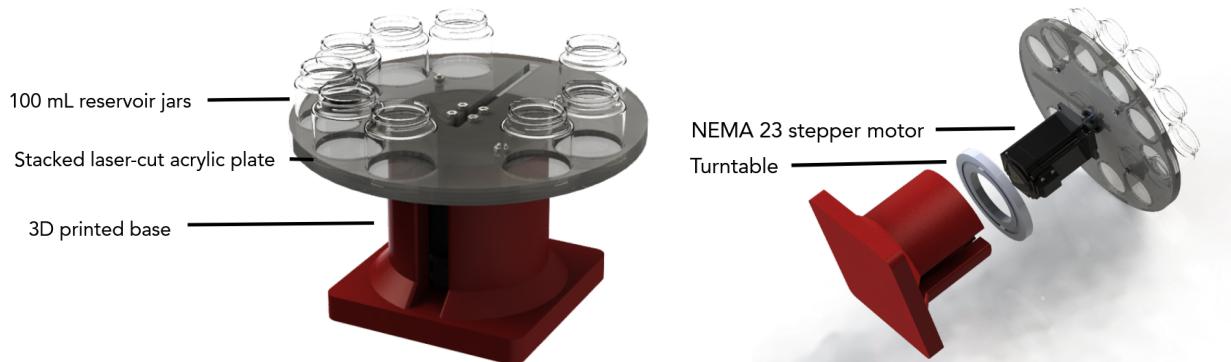


Figure 34: The carousel assembly of our device houses and rotates the reservoirs and casting plate, powered by a stepper motor

The syringe pump stand is designed to house the syringe pumps and allow them to access the reservoirs/casting kit on the carousel. The design consists of a 3D printed syringe pump holder and 3D printed base connected together using an off-the-shelf linear actuator. Again, the

assembly is held together with various fasteners. The stand raises and lowers the syringe pumps in order to facilitate the extraction and dispensing of liquids contained on the carousel via the linear actuator. The syringe pumps operate by powering a motor to rotate a lead screw which supplies the linear displacement used to drive the syringe. This subassembly of our device for drawing and mixing the fluids presents a significant departure from our alpha design from DR2. We have gained confidence in this design as a superior alternative due to the verification testing conducted on the syringe pumps.

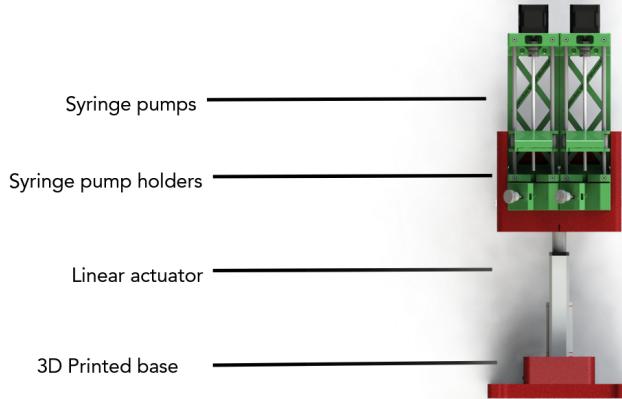


Figure 35: The syringe stand uses syringe pumps and a linear actuator to work with the fluids on the carousel

The electronics and controls for the machine are simple. Each stepper motor is wired through a CNC Shield using one Arduino Uno board, according to the Poseidon Project build video [32]; this is documented in Appendix F. The motors are controlled individually using the AccelStepper Library [33]. The code is shown in Appendix G and H.

Put together, the two subassemblies of our device work in tandem to facilitate the gel casting process, as instructed through the code. For each ingredient:

- The carousel rotates to the ingredient reservoir while the syringe stand lowers the syringe pumps into the ingredient reservoir, draws up the ingredient, and raises the pumps out of the reservoir
- The carousel rotates to the mixing chamber and the syringes dispense the ingredients into the chamber
- The carousel rotates to the rinsing reservoir while the syringe stand lowers the syringe pumps into the reservoir and repeatedly draw/dispense water to flush out the syringes

After all the ingredients are dispensed into the mixing chamber, the syringe pumps are once again lowered into the solution and used to mix them into the liquid gel. The gel is finally drawn up and dispensed into the casting plates. In short, the operation of the linear actuator, syringe pump, and motorized base all work in tandem to replicate the actions of a human creating gels manually. Our testing and engineering analysis has given us confidence in our design's ability to do so. One notable component of our final design that has yet to be finalized is the mechanism for insertion of the comb. Possible solutions are being evaluated as of the writing of this report, but all options involve at most small modifications to the certain parts of the current final design. Thus the overall mechanisms described in this section have been finalized and manufacturing has begun for many of these components.

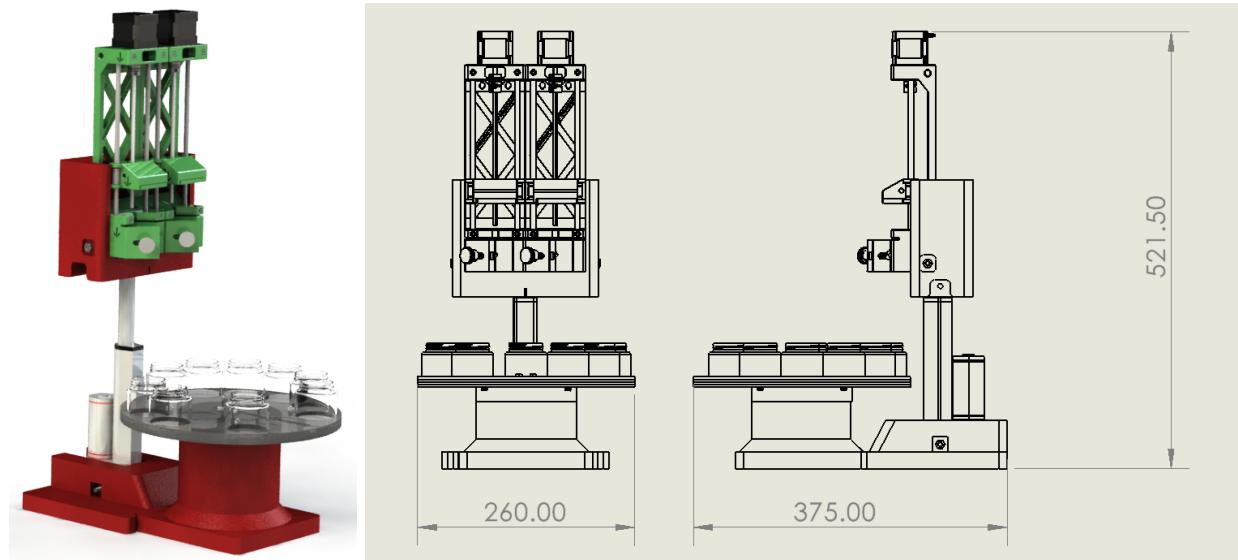


Figure 36: The final design set up in its operational state, with both the carousel and syringe pump stand properly positioned. The drawings on the right show dimensions in millimeters (mm) for a sense of scale of how much space the device takes up on a workbench

VERIFICATION AND VALIDATION PLAN

Due to the number and diversity of our engineering specifications and user requirements, we will be using multiple verification methods. These methods include empirical testing, inspection, and mathematical analysis.

Most significantly, our team will be employing empirical testing to verify our designs ability to meet our physical success rate, human interaction, maintenance time, and chemical containment specifications. Empirical testing was chosen for the maintenance time and human interaction specifications because our other verification options would have required us to model human behavior, which is clearly outside of our technical area. For our chemical containment and physical success rate specifications, empirical testing was chosen because these specifications are far too important to the overall usability of our device to leave to theoretical methods or to a single demonstration.

Since all four of these specifications are not mutually exclusive and will require multiple iterations of testing, our team planned to perform all of these empirical trials synchronously in order to reduce the number of trials required to verify these four specifications. Each of these trials would have consisted of a team member operating our device to create a hydrogel. Before each trial our team would have taken the time to carefully coat our device and lab bench in a thin layer of absorbing paper. Then, during the operation of our device, each of their interactions would have been recorded - with our team taking care to detail the time and the type of interaction. This detailed recording would have continued after the hydrogel was complete, as we would have continued to document the user going through the maintenance process of fully resetting the device. These records would be the basis on which we would verify our ability to meet the human interaction and maintenance time requirements. As the gel is polymerized, our team would have inspected the absorbing paper for any spotting in order to help verify our chemical containment specification. Finally, after the gel was fully polymerized we would examine it - looking for any signs of warping along the casting plates, separation between the layers or plates, and any distortions in the layer boundary. It is through this repeated inspection

that we would verify that we are generating physically successful gels at a satisfactory rate. Due to the time constraints and certain corrupting factors, it is important to note that this ideal empirical verification could not be achieved. In the end our human interaction and maintenance specifications were verified through demonstration, much as detailed above, however, the chemical containment and physical success rate specifications were not. The liquid supports used in the 3D printing of our syringe pump ruined the viability of the using spotting paper, as our pump leaked liquid supports for multiple days during our testing period. Because of this, this specification had to be verified through direct observation, which it passed, throughout all of our repeated observations. Similarly, our lack of access to the chemicals required for a successful casting as well as the limited time frame where we had a fully functional prototype, meant that we do not have the data to make a satisfactory claim on verification for the physical success rate. For now, we can consider this specification failed until further research is done.

Inspection will be the method used to validate the system compatibility requirement. This method was chosen because of the ease with which it can verify the specification, without our team being forced to purchase an expensive array of casting kits. The verification process begins with continued research documentation of the currently available casting kits for mini-gel systems. After this process is complete, the next step will be to inspect our final prototype and compare whether the locations, systems, and fit of these casting systems are able to mesh successfully with our device. Specific emphasis will be given to the viability of our liquid insertion, comb insertion, and gel boundary leveling systems. After our examination and discussion with the sponsor it became clear that this specification was met by our replaceable and reconfigurable casting plate gasket.

For our final specifications, which are focused on cost and chemical measurement error, our team has performed a pair of mathematical analyses. This methodology was chosen because of clear dependence on other engineering specifications or on our component parts. The cost per gel is obviously dependent on our human interaction and maintenance time results. Therefore, we used the highest labor time that would satisfy those requirements. Using the following equation, we found that the cost specifications were by definition met if our time specifications were.

$$\Sigma(c \times V) + L \times (t \div 60) = C$$

Note that in the equation above, c is the cost per ounce of a chemical component, V is the number of ounces required to produce a single gel, L is the average hourly rate for a lab technician (as specified by the US Bureau of Labor Statistics), and t is the time in minutes required by a lab technician to produce a gel [14] [18].

To verify the measurement error specification, our team used the following set of calculations to determine the maximum allowable motor error. First, the pitch of our syringe pump's threaded rod and the motors rotational step distance was used to determine the linear step of our syringe pump.

$$p \times (s \div 360) = \delta$$

Please note that p represents the rod's pitch in millimeters, s represents the motor's step distance in degrees, and δ is the resulting linear step in millimeters. Next, our syringe itself was examined to find its cross-sectional area. The equation below uses the syringes volume (V) and length (L) in order to calculate the cross sectional area.

$$V \div L = A$$

This cross sectional area then is used alongside the linear step in order to calculate the volume metric step, which is represented in these equations by Δ .

$$A \times \delta = \Delta$$

This volumetric step then allows us to compare our maximum allowable volumetric error directly to our volumetric steps. Note that m is the volume of our smallest measurement and that ϵ represents the maximum allowable error for our measurements in motor steps.

$$0.05 \times m \div \Delta = \epsilon$$

The results of these calculations showed that for our smallest measurement, our pump motor would need to be off by a total of 89 steps for our 10 milliliter syringe pump and a total of 107 steps for our 10 microliter syringe pump. The motor's specifications stipulate that due to the sensors onboard the motor, its position error is non-accumulative with a magnitude of ± 0.05 steps. Since our required error in steps is orders of magnitude larger than the maximum magnitude of error allowable by our motor it is clear that design is able to produce a measurement error much less than what is mandated by our engineering specifications.

Controls Verification

One empirical test we have conducted is the calibration of millimeter to milliliter measurements for the syringe pump. Using the specifications given for the motor and a target volume, we were able to measure the correct amount of liquid (water was used for testing), shown in Appendix D.1. To verify that this was the correct volume, we measured with a micropipette 5.62 mL of water and measured the same amount with our syringe pump design and then weighed them using a scale. Our tests led to the syringe pump accurately drawing the same amount of fluid, seen in Figure 37A and 37B, showing that our syringe pump was calibrated correctly.

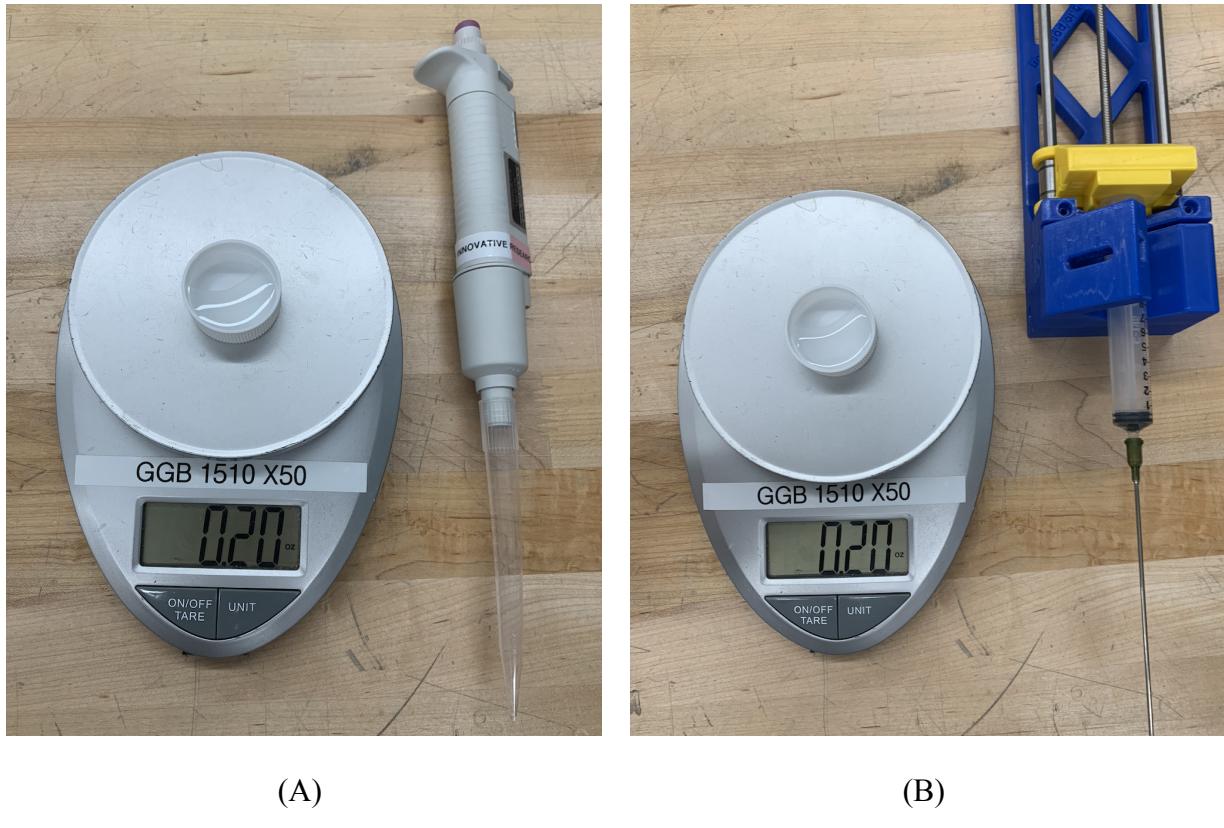


Figure 37: (A) Micropipette measuring 5.62 mL of water weighing 0.2 oz (B) Our syringe pump measuring the same amount of water, which also weighed 0.2 oz

The second empirical test was for the linear actuator that controlled the up and down movement of the entire syringe pump and its housing. This was done empirically by testing different step counts to move the linear actuator 20 mm (measured using calipers). The specifications for this motor proved to be unreliable, resulting in the need for extensive empirical testing through trial and error; however, as the tip of the syringe only needs to be under the surface of the chemical and needs to clear the height of each reservoir, very precise measurements were unnecessary. To avoid precision error with dispensing the chemical mixture in between the plates, the entire system was initialized at the plate opening and the linear actuator increased and decreased the same amount, thus returning to its original position. This test and method proved successful, as shown in Figure 38A and 38B.

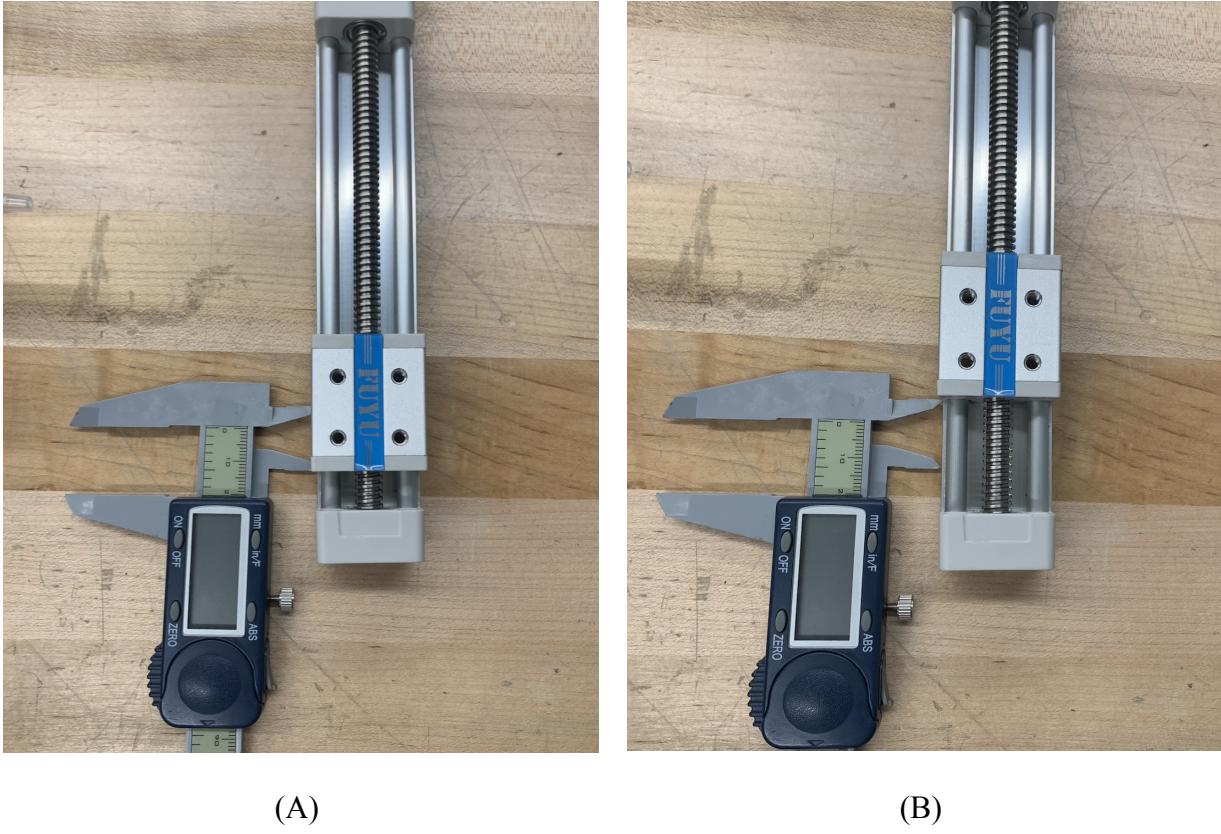


Figure 38: (A) Calipers measuring 20 mm for which the linear actuator is expected to move (B) Calipers showing that the linear actuator moved 20 mm

The last empirical test for controls involved the base motor, which controlled the plate's position. Within the CAD files, the exact angles between the casting plates and the flushing (first reservoir), between the flushing reservoir and the first mixing reservoir, and between two of the normal reservoirs. These were all of the angles needed to calculate all of the positions for the base plate. Using the motor specifications for the base motor and these angle measurements, the proper motor step count was calculated through mathematical analysis, shown in Appendix D.2. Finally, the base motor was tested empirically by inputting these angles and checking to see if the base moved to the correct location.

Validation

Beyond our verification, our team performed a pair of validation meetings with our sponsor to ensure that the results of our work are meeting the end-user's needs. This process consisted of an initial interview, a demonstration and investigation period, as well as an exit interview. The initial interview was primarily focused on introducing the final prototype, discussing its price, critical components, and a demonstration of its functionality. After this interview, the testing and investigation period began, where our sponsors got hands-on experience with the device. Once the sponsors had watched and interacted with the prototype, we had an exit interview focused on receiving feedback and exploring our sponsor's thoughts, feelings, impressions, reactions to their time with the device, as well as their findings on the validity of our project.

Through this process we found that our sponsor was quite happy with the end product

that we delivered. While we could not conclusively verify all of the specifications our team generated from the user-requirements, our sponsor was appreciative of how well we had managed to meet the specifications under the constraints we operated within. Among those specifications, three in particular stood out during our exit interview. Those were our cost and chemical safety. The cost metrics our team was able to meet, as well as our ROI calculations, made this the requirement with which the sponsor was most pleased. Notably, the physical success rate and chemical safety were the specification the sponsor was most concerned about. They plan to investigate the physical success further and have us increase the depth of the carousel plate to reduce the risk of reservoir tipping. Ultimately, our sponsor was happy with our work and excited to internally develop it further, but failed to validate our design as the physical success rate had not been concretely determined.

PROBLEM DOMAIN ANALYSIS AND REFLECTION

While trying to invent a gel casting machine, we will likely encounter problems with getting the gel to polymerize properly and getting our measurements to be precise enough to achieve the necessary concentration of chemicals. We plan to tackle these challenges by consulting with our sponsor, biolab technicians, labs, and current industry figures in order to gather ideas and gain industry knowledge necessary to design our product. We will need this industry knowledge in order to accurately predict the amount of time for polymerization to occur and to predict the concentration ranges that will produce a viable gel. One method that we plan to use to address some of these issues is to gain experience casting the gel ourselves manually to provide us with some insight and general familiarity with the process. We also need special equipment such as the casting panels and lab-grade pipettes to dispense our gel ingredients accurately. Our sponsor has agreed to provide us with these specialized equipment.

We expect to have difficulties with finding certain details to drive our specifications. The overall goal of our project is to create a replacement for the precast gel industry, so we wanted to base our specifications on the standards of how precast gels are created. Unfortunately, the process of making precast gels appears to be a closely guarded industry secret from our preliminary research, and it's difficult for us to find their specifications. These information gaps will make it harder for us to benchmark our product against the incumbent in order to accurately quantify tradeoffs and advantages for our product. We can try to make up for this information by reaching out to other labs that regularly use precast gels so we can at least understand their usages and advantages better.

Our final deliverable at the end of the semester is our completed prototype gel casting machine in a fully operational state along with a demonstration to our sponsor of how to use it. This would fulfill our sponsor's goals of proving that the gel casting process can be successfully automated, and having an actual device to be readily used in his labs. Since our current alpha concept is fairly complex, we anticipate running a tight schedule in order to produce the prototype in time with only the remaining half semester left.

A key design “driver” of our alpha concept that is immediately relevant is the layout of the workspace. This is because the layout determines the length and dimensions of our gantry which allows us to start quantifying and purchasing materials for the start of our manufacturing

process, which we want to do early in the process to account for lead times. Another key feature is the measurement-adjusting subsystem outlined in the Selected Concept Description section of this report. We anticipate that it will be difficult to get perfectly accurate measurements so we intend to address this by dedicating lots of time to experiment with different stepper motors and gear ratios to find the optimal combination for accurate tuning of the pipettes.

At this point, we have mitigated most of our knowledge and experience gaps concerning gel casting after conducting numerous meetings with our lab sponsors and casting a batch of gels ourselves to familiarize ourselves with the process. We do require special equipment in the form of lab-grade pipettes which our sponsor has agreed to provide to us. Now that we are close to beginning work on manufacturing, we are working to bridge knowledge gaps regarding specific design choices. For example, we want to know the best ways to move the gantry, whether that be using a rack and pinion or motorized belt. We will find the lacking information by looking up existing implementations of similar devices (such as 3D printers) and consulting with professors about best design practices.

ANTICIPATED CHALLENGES

Currently, we are facing multiple manufacturing and design choice challenges. For the design of the machine, we are reevaluating a variety of measurement systems. It will be tricky ensuring the correct precision and accuracy that our specifications need. Additionally, in order to get a working prototype, we know that our design may evolve around the budget we have. This budget, however, is changing and we are redesigning as we receive more information. We plan on performing research to create preliminary BOM's per design, meeting more with our sponsor to confirm the budget, and researching and discussing the best option for our specifications.

Some anticipated challenges farther down the road include adding the following functionalities to the machine: creating the controls for the specific measurements, removing and absorbing the water used in the gel leveling, machine maintenance through cleaning, timing and confirmation of polymerization, and handling chemical safety. Incorporating all of these functions will require extensive precise designing, prototyping, and testing work due the complexity of our project. This is a challenge to accomplish given the limited amount of time our team has to work on this project, but we believe that our high standards for organization and communication will aid us in completing these tasks successfully.

PROJECT TIMELINE AND PLAN

The original scope of this project as proposed by the sponsors included an automated casting machine that could be remotely operated via an app downloaded on one's phone. After discussing needs, determining the functional requirements, and consulting with our sponsor, we have reduced the scope from the original request. The revised design scope is an automated gel casting machine that is initially setup by the user; thus, our scope no longer includes remote start-up capabilities. Because of these alterations, we believe we have narrowed the scope to what is achievable for one semester. In order to track our progress throughout the semester, we have created a Gantt chart which is located in the appendix.

Initial tasks and milestones for our project include continuing background research, which will allow us to better understand our project and functional requirements. Research will be conducted by all team members. This research will include patent information, component design, and specifications of pre-built components. Additionally, lab certification and lab access is vital for gaining experience in producing gels. While we have user feedback from our sponsors, our sponsor and our team agreed that our own firsthand experience will be extremely valuable in producing a design. After completing the EHS_BLS025w Chemical Laboratory Safety course, each member casted a gel under supervision of an experienced lab tech. Taking what we have learned by casting gels ourselves, our team now plans on working through concept generation to determine approaches to automating the process and what restraints may be limiting our design.

These tasks contribute to research and concepting, our initial steps on our critical path. The critical path in order of precedence is research, concepting, solution selection, solution refinement, prototyping, and testing. We believe that by following these steps the number of design obstacles will be minimized while also maximizing work efficiency within the span of this semester.

According to ME450, our budget is \$400 for the physical prototype. Our sponsor is providing a Bio-Rad casting kit and gel ingredients as well as pipettes, so that we are able to gain insight from casting gels ourselves. Additionally, we must consider the tradeoffs between precision and cost effectiveness. After narrowing our scope, we believe that our project is achievable within one semester, with this budget, and number of people.

DISCUSSION

Problem Definition

Given more time, it would have been useful to have had more hands on experience with the gel casting procedure. This would have helped our understanding of the details of the process and the precision required in each step. Additionally, in our research and our time interviewing our sponsor we learned about many modifications to the process we would have liked to try. For example, Bio-Rad has a gel casting procedure that does not use a leveling solution [16]. Instead, the procedure requires that the stacking gel be poured on the liquid running gel, utilizing the density difference between the stacking gel and the running gel to keep them separate. This modified process would have been useful to develop because incorporating a leveling solution and its removal was one of the more challenging elements of the procedure to automate, and it eliminates that step completely.

The initial problem definition could have benefitted from a wider understanding of how labs other than Innovative Research choose to maintain their polyacrylamide gel supply. In our research, we could not find statistics on the percentage of labs that choose hand cast or pre-cast gels. If time had allowed, a survey of labs at the University of Michigan or even a wider area could have been used to give us a better understanding of the potential users of our device. Consulting multiple labs on their polyacrylamide gel making procedures would have also helped us eliminate bias towards the procedure of a single lab.

In our benchmarking process, it would have been valuable to have taken a deeper dive into chemical mixing and measuring technologies. We had the time to study the mechanics of several pumps and micropipettes, but taking a look at a wider range of devices would have given us more variety in our designs. We could not find a device within our budget capable of making microliter measurements, although we believed such devices might exist in current drug delivery technology such as automated insulin injectors. Further research into these devices may have yielded a solution to our microliter measurement challenges.

Design Critique

Our design has a few key factors that contribute to its effectiveness, including its functional separation, reprogrammability, and flexibility. The separation of the syringe pump, lift, carousel, and chemical reservoirs allows for each to be diagnosed separately, used separately, and even discretely improved or repaired. Our use of the Arduino IDE and open source libraries allows for the device's controls to be updated continually across all users and use cases. The combination of this reprogrammability and component separation result in the huge flexibility that our prototype provides our end-users. Ultimately our device can be easily reconfigured, reused in multiple ways, and reprogrammed to operate outside of the original design scope.

While our design has many benefits, it also has a number of issues. The current form has limited cable management, a deficit of safety features, no protective housing for the controls hardware, and a limited UI. The cable management is currently limited to the utilization of slots for cable pathing in both the lift-stand and carousel-base, but these do not have cable ties to prevent unwanted cable movement. More problematically, our device has no feedback or control system that prevents itself from damaging either itself or the user if the wrong code is uploaded or if a foreign object is brought into its space during operation. Additionally, there is a safety hazard with the possibility of chemical reservoirs tipping over. Similarly, our device does not have a housing for its electrical hardware, which is problematic as ideally they would be protected from the potentially hazardous environment of the wet lab they are meant to operate in. Finally, the current UI is simply the Arduino IDE, which is unideal for widespread use as it forces the user to be familiar with Arduino as well as the fact that it provides a rather unsatisfying user experience. Specifically, the user must manually initialize the machine, and the initialization process isn't inherently intuitive if the user does not have coding background.

Risks

There were a variety of challenges present during the design process, each with their own constraints and solutions. Generally, we found there was not enough time budgeted for prototyping. When unexpected errors occurred, there was just enough time to recover. In order to counter this, we implemented sub teams to work on the machine in parallel from two perspectives: electronics & controls, and structures. To work more efficiently, we asked for help from experts when needed and used calculations to estimate dimensions and optimize them empirically. One of our largest setbacks was a failed 3D print of our custom linear actuator/syringe frame. The 3D printer was unable to print the complex geometry, and time was

running short to provide a working machine. We overcame this by fabricating a base with excess material in the lab. A flexible design was used to allow for continual adjustments- velcro attachments of the syringe allowed simple angle adjustments, and a sliding base for the frame allowed displacement adjustments between the carousel base and frame. Our system also utilized a predesigned, open source syringe pump, but the dimensions and code were not optimized for our exact application. The CAD was modified according to calculations to match it to the dimensions of our 10mL syringe, and the code was almost completely scrapped. However, it provided a good baseline and open source motor function library to use.

Another major challenge was finding the calibration specifications for each motor and the controls. There was inadequate documentation about each motor (as they were ordered off Amazon), and it made accurate control more difficult. The issue was managed by determining the conversions empirically, as explained in the verification section. This was also a subproblem of lack of time- if there was more time working with the machine, these calibrations would have easily been completed.

After a semester's worth of work, we acknowledge that the machine is still not completed according to our design specifications and requirements, and pose a handful of risks to the end user. The risks are explained in the Design Critique section above and all impact the end user.

REFLECTION

Relevant Factors and Their Impacts

One feature we were not able to include in our design due to time constraints is a safety feature. We were able to seal our chemicals to prevent chemical contamination; however, our current prototype lacks important safety features, such as a hood to prevent access to the device while it is in operation. While highly relevant to our project at the beginning of our project, time constraints did not allow us to include this in our final design.

As gel electrophoresis is a technique used in labs worldwide, we have continued to see our machine providing great benefit in the global marketplace. With our machine, lab technicians will have more time to do other research and gels will be able to be procured more quickly and easily. This will significantly increase productivity. This belief has been held throughout the project as we see great potential for future development for this machine.

When speaking to our sponsors initially, it was made known that profit was of prime importance. Thus, throughout our decision making process social impacts were limited in our discussion. One potential social impact would be the accessibility of this machine, but again this was not specified as a priority by our sponsors. Yet because the social impact is important to our team, we have strived to design a device with as accessible a price tag as possible.

One possible side effect we foresaw was if this machine became so widespread that gel production would increase to the extent of inefficiency. The demand for materials would increase, increasing carbon emissions, and more electricity would be required. In relation to the social impacts of increasing research productivity, we still believe that this will outweigh the environmental costs. Notably these environmental issues are also mitigated by the reduction of shipping and package for precast gels.

The main tools we used to characterize potential social impacts were an ecosystem diagram and cost analysis. The ecosystem diagram was helpful throughout in reminding us that lab technicians will be the end user. The cost analysis was very relevant throughout our project, especially with our final design built, as cost was of prime importance to our sponsors.

Cultural and Stylistic Similarities and Differences

Our team worked very well together despite our differences; we appreciated those differences and we celebrated our similarities. One minor difference that resulted in discussion was stylistic differences in organization. When organization techniques did differ, then we discussed and created compromises that promoted rather than deterred efficiency and productivity.

One major difference between our team and our sponsors is our identities. Our team consists of mechanical engineering students while our sponsors were research employees. However, this did not majorly affect our design processes or the final design. Our sponsors have had this idea for decades and wanted to see if their idea was even possible. To them it was a fun project and an opportunity for our team to grow.

Inclusion and Equity

At the beginning of this project, our team decided that the power dynamics among our teammates would be strict equality – the work would be shared equally. As our time on this project comes to an end, we believe that we upheld these power dynamics. Whenever someone had a busy week and asked if they could do less, they volunteered to compensate for this in the next assignment. As for the power relationship between our team and our sponsors, this has not changed. Our sponsors served as our mentors and lab technicians served as the end users.

With any design project, multiple viewpoints will be shared and often they differ from another's viewpoint. Our sponsors, one of which was a lab technician or our end user, were clear on their specifications and informed us that they did not want their previous visions of this machine to affect our design and decision making process. When multiple opinions were proposed for the design during team meetings, we would listen to each idea fully and ask questions when necessary. We would discuss and we would create quick sketches so that everyone would understand. With further discussion of the pros and cons of each design, we were able to make group decisions efficiently.

Ethics

An ethical issue that may arise from marketing this device is the potential of Innovative research Inc. creating a temporary monopoly, increasing the principal cost of the device and limiting the accessibility of the device. We foresaw this as a possible ethical issue and still believe that this outcome is possible.

RECOMMENDATIONS

As discussed in previous sections, our device has problematic limitations in cable management, automation safety, protecting electrical hardware, and its UI. Because of these issues, we would encourage our sponsor to continue the development of our prototype. Specifically, we believe that the implementation of a “case” would be immensely beneficial. Our team foresees this case as the most likely solution, as it could solve our issues through the combination of a security gate, built in cable ties, an electrical compartment, and a personalized UI. The security gate is a common solution for automation safety, wiring the power to the system through such a gate means that the device can only operate while the door is closed, preventing a user from interacting with the device during its operation. The electrical compartment could easily be designed as waterproof and externally accessible, allowing the user to access it while mitigating the risk to the electrical components. Finally the case could come with a built in UI, that is used rather than the Arduino IDE, picking options off of a touch screen, or similar device, eliminates the risk of a user uploading and implementing a faulty piece of code.

CONCLUSIONS

Within the current market, labs can purchase precast gels or create them manually. These two options present a tradeoff between labor time and price, and our team plans to solve this dynamic through automation. Ultimately our objective is to reduce the time, cost, and error involved in polyacrylamide gel casting, while furthering the reproducibility, viability, and common access of gel electrophoresis. Our current target is to produce a prototype that produces viable gels, while optimizing the previously stated objectives. While we are producing this prototype, it is important to our team to consider accessibility, inclusivity, as well as social and environmental sustainability. As discussed earlier, we are concerned about our problem space’s potential for the rebound effect, monopolization, and exclusivity. As we move forward, our team will be making the mitigation of these possible negative effects a design priority, as our stakeholders are not limited to our sponsor and our responsibility is to all of them as well as society. With the background research we conducted at the beginning of the semester, hands on gel casting experience, and extensive strategic discussion, we entered our design concepting phase. Using tools such as design heuristics and a shared motivation to think outside of the box, we considered a diverse range of concepts for our final design. Our chosen alpha design reflects extensive discussion and has high potential to meet our design requirements and specifications. After further review of our design space, research into the Poseidon project, and considerations of cost, our team moved from our initial alpha design to a final design. This final design is more economically inclusive due to its lower cost and simpler build requirements. Ultimately, our sponsor was happy with our work and excited to internally develop it further, but failed to validate our design as the physical success rate had not been concretely determined.

ACKNOWLEDGEMENTS

Very special thanks to Duane Day, our company sponsor, for always providing us with extra resources, insider information, support, and for checking through our work to ensure

we were always on track. Thank you to Professor Fu, for allowing us to use his lab space, his materials, and meeting with us so frequently. Thanks to David Ginsberg and Doug Necci, for also helping to guide our design process and concepting, by listening to our ideas, providing input, and doing extra work to ensure that our machine would work. We really appreciated all your help and guidance this semester.

REFERENCES

- [1] Wilson, Ian D., and P. J. Wirth. "Detection of Proteins in Electrophoresis." Essay. In Encyclopedia of Separation Science, 1233–39. Acad. Press, 2000.
- [2] Duane D and Ginsberg D. "Sponsor Meeting with Innovative Research Inc. #1". Interview by Andrews H, Kuske N, Newton S, Santos E, and Zhu J. ME 450, September 15th, 2022.
- [3] Zahra, Andleeb, Bilal Hussain, Amer Jamil, Z. Ahmed, and Shahid Mahboob. "Forensic STR Profiling Based Smart Barcode, a Highly Efficient and Cost Effective Human Identification System." Saudi Journal of Biological Sciences 25, no. 8 (December 2018): 1720–23. <https://doi.org/10.1016/j.sjbs.2018.10.001>.
- [4] Caprette, David R. "Measuring Relative Mobility of Protein Bands." Rice University, January 7, 2007. <https://www.ruf.rice.edu/~bioslabs/studies/sds-page/rf.html>.
- [5] "A Guide to Polyacrylamide Gel Electrophoresis and Detection." Hercules, CA: Bio-Rad, n.d.
- [6] Aryal, Sagar, Moorthy, and Sahana ko. "Polyacrylamide Gel Electrophoresis (Page)." Microbe Notes, September 5, 2022. <https://microbenotes.com/polyacrylamide-gel-electrophoresis-page/>.
- [7] "Protein Gel Electrophoresis Technical Handbook." Thermo Fisher Scientific Inc., 2015.
- [8] "Introduction to Polyacrylamide Gels." Bio-Rad Laboratories Inc. Accessed October 5, 2022. <https://www.bio-rad.com/en-us/applications-technologies/introduction-polyacrylamide-gels?ID=LUSPBRM5B>.
- [9] "Hand Casting Polyacrylamide Gels." Bio-Rad Laboratories Inc. Accessed October 5, 2022. <https://www.bio-rad.com/en-us/applications-technologies/hand-casting-polyacrylamide-gels?ID=LUSPDTO62>.
- [10] *Polyacrylamide Gel Casting Process. Polyacrylamide Gel Electrophoresis*. Wikimedia Foundation Inc., September 25, 2022. Polyacrylamide Gel Casting Process.
- [11] "Polyacrylamide Reagents and Precast Gels." Bio-Rad Laboratories Inc. Accessed October 5, 2022. <https://www.bio-rad.com/en-us/product/polyacrylamide-reagents-precast-gels?ID=020f84c9-81a0-4540-a476-f2c707f7a385>.
- [12] Kannengiesser, Udo, and John S. Gero. "Can Pahl and Beitz' Systematic Approach Be a Predictive Model of Designing?" *Design Science* 3 (2017): e24. doi:10.1017/dsj.2017.24.

- [13] Fu J. “Project meeting to discuss design progress #3”. Interview by Andrews H, Kuske N, Newton S, Santos E, and Zhu J. ME 450, September 29th, 2022.
- [14] Duane D and Ginsberg D. “Sponsor Meeting with Innovative Research Inc. #2”. Interview by Andrews H, Kuske N, Newton S, Santos E, and Zhu J. ME 450, September 22th, 2022.
- [15] Electrophoresis Market Size & Growth Statistics: By 2031. Allied Market Research. (n.d.). Retrieved 2022, from <https://www.alliedmarketresearch.com/electrophoresis-market>
- [16] “Mini-PROTEAN® TGX™ Precast Gels.” Bio-Rad. Accessed 2022. <https://www.bio-rad.com/en-us/product/mini-protean-tgx-precast-gels?ID=N3GRW04VY>
- [17] “Precast Electrophoresis Gels.” Precast Electrophoresis Gels | Thermo Fisher Scientific. Accessed 2022. <https://www.thermofisher.com/search/browse/category/us/en/90155190/Precast%20Electrophoresis%20Gels#:~:text=Precast%20gels%20in%20a%20variety,native%20and%20for%20denaturing%20conditions>.
- [18] “19-4021 Biological Technicians.” U.S. Bureau of Labor Statistics. U.S. Bureau of Labor Statistics, March 31, 2022. <https://www.bls.gov/oes/current/oes194021.html>.
- [19] “Laboratory Analytical Instruments Industry Profitability.” Laboratory Analytical Instruments Industry Profitability by quarter, Gross, Operating and Net Margin from 2 Q 2022. Accessed October 5, 2022. https://csimarket.com/Industry/industry_Profitability_Ratios.php?ind=806.
- [20] “Regulations (Standards - 29 CFR).” United States Department of Labor. Occupational Safety and Health Administration, n.d. <https://www.osha.gov/laws-regulations/standardnumber/ 1910>.
- [21] “Pocket Guide to Chemical Hazards.” Centers for Disease Control and Prevention. Centers for Disease Control and Prevention, February 18, 2020. <https://www.cdc.gov/niosh/npg/default.html>.
- [22] Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards. Washington, D.C.: National Academies Press, 2011.
- [23] “The Tris–Acetate Polyacrylamide Gel. (a) Schematic Representation of ...” Accessed October 27, 2022. https://researchgate.net/figure/The-Tris-acetate-polyacrylamide-gel-a-Schematic-representation-of-how-to-make-a_fig1_224958213.

- [24] "Pipette Accuracy & Precision." Pipette Precision & Accuracy | Hamilton Pipettes | Hamilton Company,
<https://www.hamiltoncompany.com/laboratory-products/pipette-knowledge/pipette-accuracy-precision>.
- [25] "Ultra-Small Peristaltic Pump - BCP/RCP Series - Takasago Fluidic Systems." TFS Takasago Fluidic Systems. Accessed October 31, 2022.
<https://www.takasago-fluidics.com/products/ultra-small-peristaltic-pump-micro-peristaltic-pump>.
- [26] Christian, J.L, S.R. Daly, S. Yilmaz, C. Seifert, and R. Gonzalez. (2012). Design heuristic support two modes of idea generation: Initiating ideas and transitioning among concepts. *American Society for Engineering Education*. 101(4): 601-629.
- [27] Pachterlab. "Pachterlab/Poseidon: Poseidon System - Open Source Syringe Pumps and Microscope for Laboratories." GitHub, January 28, 2019.
<https://github.com/pachterlab/poseidon>.
- [28] Chaimowitz, Matthew. "700 Series Microliter Syringes." Thomas Scientific - Lab Supplies, Lab Equipment, Lab Chemicals, & Lab Safety. Accessed November 22, 2022.
https://www.thomassci.com/Laboratory-Supplies/Chromatography-Syringes/_/700-Series-Microliter-Syringes?q=Microliter+Syringe.
- [29] "OTHMRO Plastic White Translucent Lab Chemical Reagent Bottle 50ml Wide ..." Accessed November 22, 2022.
<https://www.amazon.com/Othmro-Plastic-Bottles-Translucent-Bottle/dp/B082NLC2J8>.
- [30] Dym, Clive L. *Solid Mechanics*. Springer, 2015.
- [31] Overview of materials for acrylic, cast. Accessed November 22, 2022.
<https://www.matweb.com/search/datasheet.aspx?bassnum=O1303>.
- [32] Booeshagi, Sina. "Poseidon Build Videos: Arduino." YouTube. YouTube, October 27, 2018.
<https://www.youtube.com/watch?v=Xl02fsRCJ7U>.
- [33] "AccelStepper Class Reference." Accelstepper: Accelstepper class reference. Accessed December 15, 2022.
<https://www.airspace.com/mikem/arduino/AccelStepper/classAccelStepper.html>.

BIOS

Sophia Newton



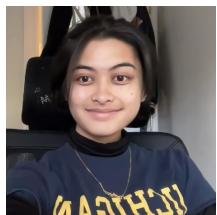
Sophia is a senior finishing her B.S.E. in mechanical engineering with a minor in creative writing. She has enjoyed working with robotics in space, surgical, and ocean applications. Sophia plans to study robotics further in a masters degree or pursue a full time position in space exploration technology - there's still time to choose! Whenever she isn't working on the 450 capstone project, Sophia enjoys being outside and writing silly stories.

Halia Andrews



Halia is a senior pursuing her B.S.E. in Mechanical Engineering. Halia has spent several years working as a Technology Intern, but plans on eventually expanding her career to robotics. After graduation, Halia plans on commuting from her home in Frankenmuth, MI, to Nexteer Automotive based in Saginaw, MI. In her freetime, Halia enjoys crocheting and playing with her cat, Chai.

Erica Santos



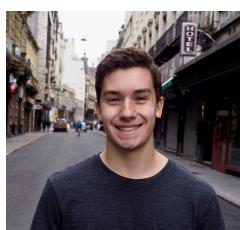
Erica is a senior finishing her B.S.E. in Mechanical Engineering, Minor in Computer Science, and concentration in the Program in Sustainable Engineering. To merge these interests and experiences, she is planning on going back to school for a Master's in Robotics. Outside of academics, she competes in ballroom dancing and loves to rollerblade, read, and sketch. Next summer she will be working an internship in Fetch Robotics, a startup in CA.

Jason Zhu



Jason is a 5th year student finishing his B.S.E. in Mechanical Engineering and Computer Science who has a strong interest in technology and robotics. Throughout college, he has done internships in the automotive and consumer electronics industry and spent two years on the Michigan Solar Car team as an aerodynamics engineer. After graduating in December, he will be joining a Bay Area startup focusing on software for developing autonomous vehicles.

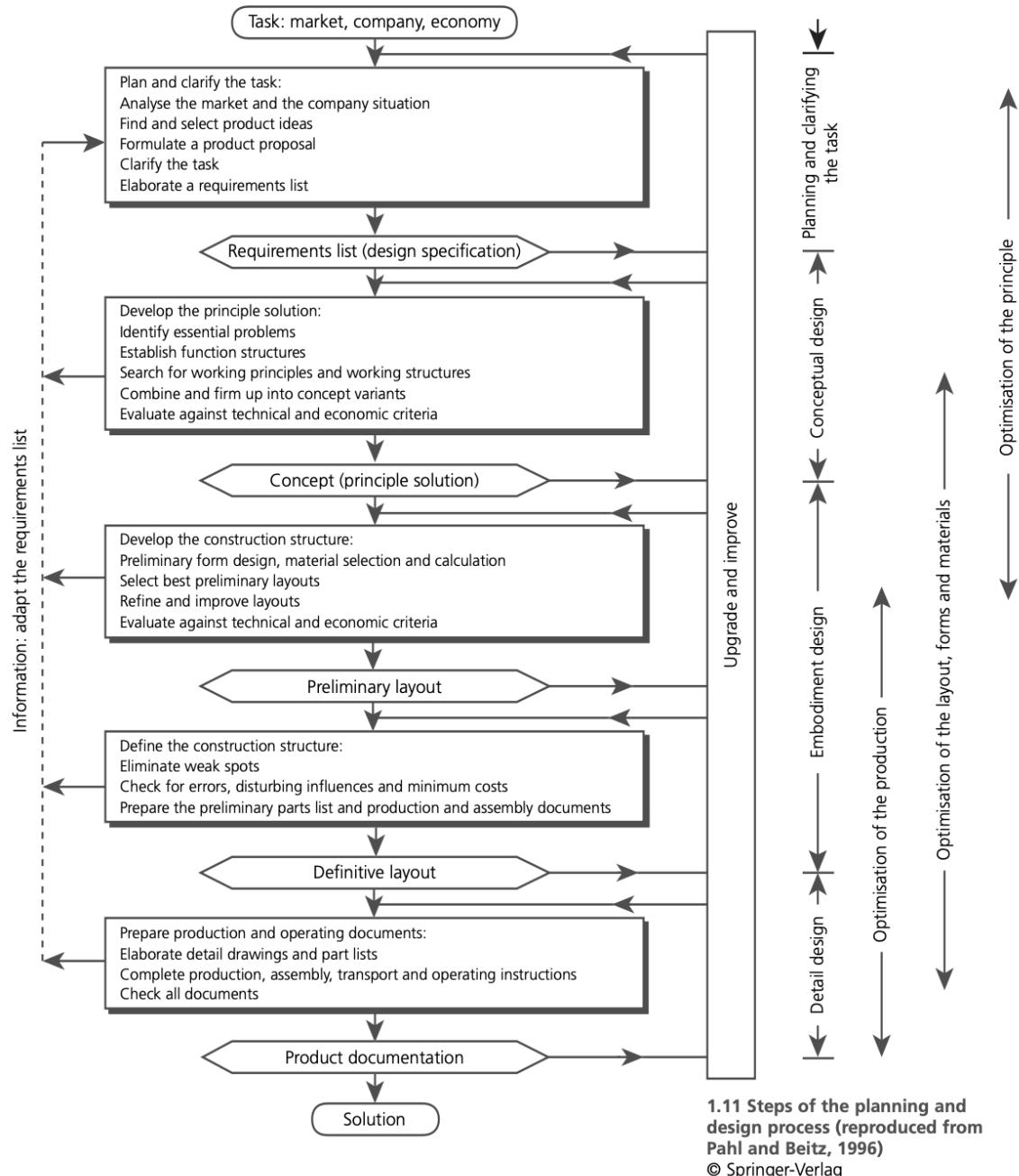
Nicholas Kuske



Nicholas is a senior finishing his B.S.E. in Mechanical Engineering as well as a Minor in History. While growing up in Grand Rapids, Nicholas spent his summers working in distribution centers. After interning at TGW, he has agreed to return to Grand Rapids and to continue working as an Applications Engineer. Nicholas plans to eventually move from engineering into primary education.

APPENDIX A

A.1: Pahl and Beitz's Design Process



A.2: Gantt Chart

Cast-o-matic Project Gantt Chart

PROJECT TITLE		COMPANY NAME		Design Report/Presentation Due Dates															
		DATE		9/18/22															
TASK NUMBER	TASK TITLE	START DATE	DUE DATE	% OF TASK COMPLETE	WEEK 1 (9/18)				WEEK 2 (9/26)				WEEK 3 (10/3)						
					M	T	W	R	F	M	T	W	R	F	M	T	W	R	F
1	Design Report 1		10/04																
1.1	Problem Definition	9/18/22	10/4/22	100%															
1.2	Background Research and Benchmarking	9/20/22	10/4/22	100%															
1.3	Stakeholder Analysis	9/18/22	9/20/22	100%															
1.4	User Requirements and Engineering Specifications	9/18/22	9/26/22	100%															
1.5	Problem Domain Analysis	9/22/22	9/28/22	100%															
1.6	Project Plan and Timeline	9/18/22	9/21/22	100%															
						WEEK 4 (10/10)				WEEK 5 (10/17)				WEEK 6 (10/24)					
2	Design Report 2		10/25																
2.1	Concepting	10/4/22	10/11/22	100%															
2.2	Cast a second demo gel with sponsors	10/10/22	10/10/22	100%															
2.3	Each team member runs a gel	10/7/22	10/13/22	100%															
2.4	Develop top 3 concepts	10/13/22	10/18/22	100%															
2.5	Sponsor Concept Evaluation Meetings	10/18/22	10/26/22	100%															
2.6	Final Concept Selection	10/19/22	10/20/22	100%															
						WEEK 7 (10/31)				WEEK 8 (11/7)				WEEK 9 (11/14)					
3																			
3.1	Test hardware components as it is received	10/27/22	11/1/22	0%															
3.2	Functional Component Prototypes	11/1/22	11/3/22	0%															
3.3	Functional Component Prototype Testing (mixing, measureing, and transport)	11/2/22	11/5/22	0%															
3.4	Functional Component Evaluation With Segmented Gel Casting	11/3/22	11/9/22	0%															
3.5	Design Change List	11/9/22	11/14/22	0%															
3.6	Second FC Prototypes	11/14/22	11/17/22	0%															
3.7	Second FC Prototype Evaluation With Full Gel Casting	11/15/22	11/21/22	0%															
						WEEK 10 (11/21)				WEEK 11 (11/28)				WEEK 12 (12/5)					
4	Final Design Report		12/08																
4.1	Final Prototype	11/22/22	11/25/22	0%															
4.2	Final Prototype Evaluation	11/25/22	12/1/22	0%															
4.3	Design Expo Poster	12/1/22	12/5/22	0%															
4.4	Sponsor Handover	12/5/22	12/8/22	0%															

A.3: Design Heuristics [26]

1. Add features from nature	27. Distinguish functions visually	52. Reduce material
2. Add gradations	28. Divide continuous surface	53. Reorient
3. Add motion	29. Elevate or lower	54. Repeat
4. Add to existing product	30. Expand or collapse	55. Repurpose packaging
5. Adjust function through movement	31. Expose interior	56. Reverse direction or change angle
6. Adjust functions for specific users	32. Extend surface	57. Roll
7. Align components around center	33. Extrude	58. Rotate
8. Allow user to assemble	34. Flatten	59. Scale up or down
9. Allow user to customize	35. Fold	60. Separate parts
10. Allow user to reconfigure	36. Hollow out	61. Slide components
11. Animate	37. Impose hierarchy on functions	62. Stack
12. Apply existing mechanism in new way	38. Incorporate environment	63. Substitute
13. Attach independent functional components	39. Incorporate user input	64. Synthesize functions
14. Attach product to user	40. Layer	65. Telescope
15. Bend	41. Make component multifunctional	66. Texturize
16. Build user community	42. Make components attachable or detachable	67. Twist
17. Change contact surface	43. Make product reusable or recyclable	68. Unify
18. Change direction of access	44. Merge functions with same energy source	69. Use alternative energy source
19. Change flexibility	45. Merge surfaces	70. Use common base to hold components
20. Change geometry	46. Mirror or array	71. Use continuous material
21. Compartmentalize	47. Nest	72. Use human-generated power
22. Convert 2-D to 3-D	48. Offer optional components	73. Use multiple components for one function
23. Convert for second function	49. Provide sensory feedback	74. Use packaging as functional component
24. Cover or remove joints	50. Reconfigure	75. Use recycled or recyclable materials
25. Cover or wrap	51. Recycle to manufacturer	76. Utilize inner space
26. Create system		77. Utilize opposite surface

A.4: Final Design, Bill of Materials

Part #	Item Description	Quantity	Supplier	Cost	Contact	System	Notes
1	Nema 17 Stepper Motor 45 Ncm	1	StepperOnline	\$12.99	omc-stepperonline.com	Syringe Pump	Bipolar, 40mm
2	5-5mm Shaft Coupling	1	Uxcell	\$5.65	uxcell.com	Syringe Pump	2 pack
3	6X200mm Steel Rod	2	Uxcell	\$6.59	uxcell.com	Syringe Pump	2 pack
4	6mm Linear Bearing	2	Shutao	\$15.99	unavailable	Syringe Pump	12 pack
5	M5x0.8 Threaded Rod	1	uxcell	\$9.85	uxcell.com	Syringe Pump	170mm
6	M5x0.8 Nut	1	SpzcdZa	\$7.99	unavailable	Syringe Pump	50 pack

7	M3x0.5 20mm	4	McMaster-Carr	\$7.95	mcmaster.com	Syringe Pump	50 pack, socket head
8	M3x0.5 10m	4	McMaster-Carr	\$8.55	mcmaster.com	Syringe Pump	50 pack, socket head
9	M3 Nut	6	McMaster-Carr	\$6.94	mcmaster.com	Syringe Pump	100 pack
10	M5 knob	2	Uxcell	\$14.49	uxcell.com	Syringe Pump	10 pack
11	10ml Syringe	1	Dr. Jianping Fu	\$3.88*	me-web.engin.umich.edu/ibbl	Syringe Pump	
12	Pump Carriage	1	Pachter Lab	\$0*	pachterlab.github.io/poseidon	Syringe Pump	3D Print
13	Pump Carriage Sled	1	Pachter Lab	\$0*	pachterlab.github.io/poseidon	Syringe Pump	3D Print
14	12V power unit	1	Kastar	\$13.99	mykastar.com	Controls	AC Adapter
15	UNO R3, Stepper Motor Driver, and CNC Shield Pack	1	Kuman	\$25.99	kumantech.com	Controls	
16	Spacers	12	Agaros3	\$0*	github.com/Pewps/ME450-CA D	Lift Stand	3D Print
17	100m Linear Actuator	1	FUYU	\$82.31	fuyumotion.com	Lift Stand	
18	Lift Base	1	Agaros3	\$0*	github.com/Pewps/ME450-CA D	Lift Stand	3D Print
19	Lift/Pump Bracket	1	Agaros3	\$0*	github.com/Pewps/ME450-CA D	Lift Stand	3D Print
20	Lift/Pump Clamp	1	495 Team 3 '22	\$0*	github.com/Pewps/ME450-CA D	Lift Stand	3D Print
21	Carousel Base	1	Agaros3	\$0*	github.com/Pewps/ME450-CA D	Lift Stand	3D Print
22	Carousel Gasket	1	Agaros3	\$0*	github.com/Pewps/ME450-CA D	Lift Stand	3D Print
23	M4 Screws	20	HobbyKing	\$1.28	hobbyking.com	Lift Stand & Carousel	2 sets of 10, socket head
24	M4 Nut	16	Harfington	\$4.88	harfington.com	Carousel	50 pack

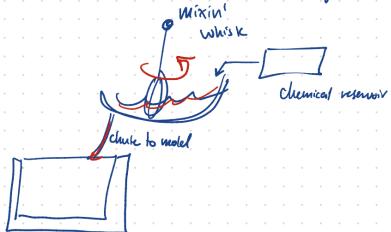
25	Nema 17 Stepper Motor, 59 Ncm	1	StepperOnline	\$13.99	omc-stepperonline.com	Carousel	Bipolar, 48mm
26	Acrylic Sheets	4	Walmart	\$17.53	walmart.com	Carousel	
27	Rubber Bands	2	Amazon Basics	\$6.29	amazon.com	Carousel	
28	50ml Bottles	9	Walmart	\$10.98	walmart.com	Carousel	10 pack
29	100ml Bottles	1	Uxcell	\$11.49	uxcell.com	Carousel	5 pack
30	Flange Coupler	1	Amazon	\$13.49	amazon.com	Carousel	2 pack
Total	30	100	15	\$299.21		4	

APPENDIX B

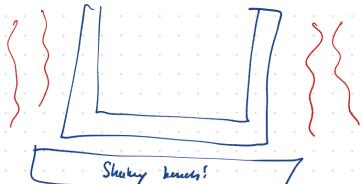
B.1: Sophia Newton

Part I

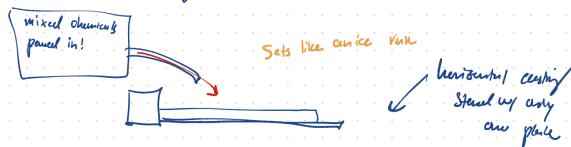
- ① Mix everything and pour it down a tube into the gel mold



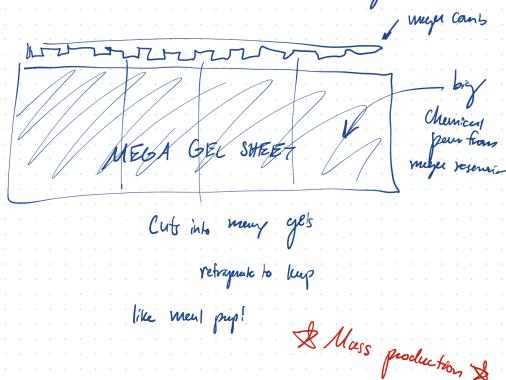
- ② Dump everything into the mold! Wait there who needs a Shaky gel?



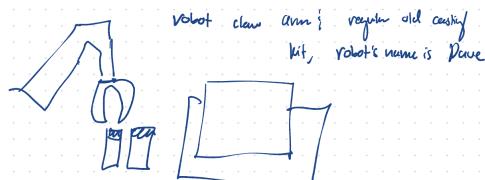
- ③ Make gel horizontally!



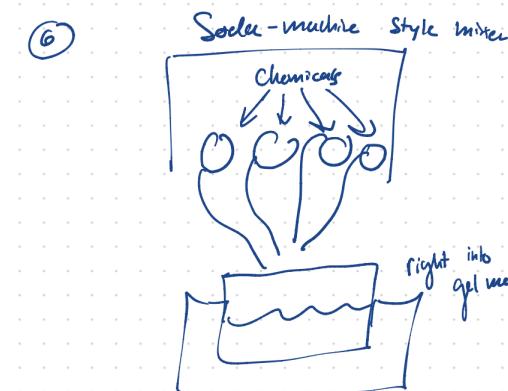
- ④ Ditch the mold and cookie sheet the thing!

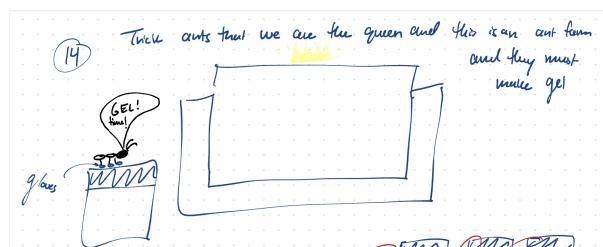
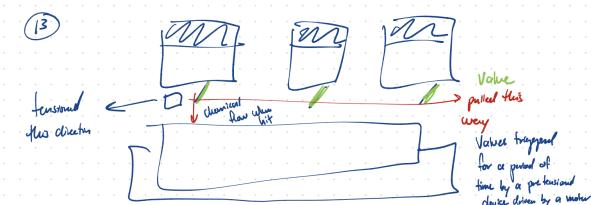
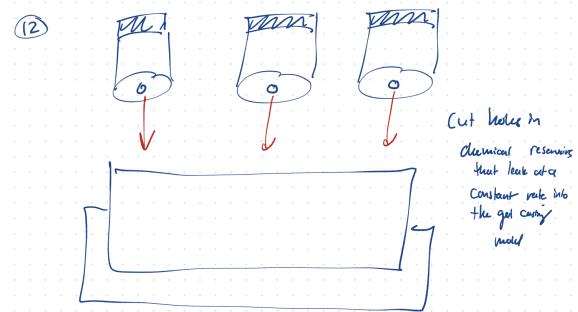
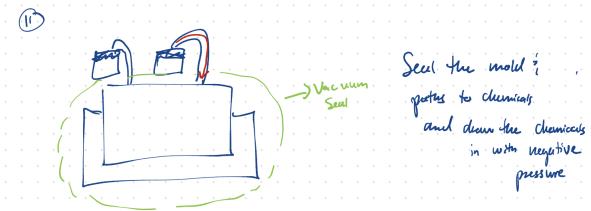
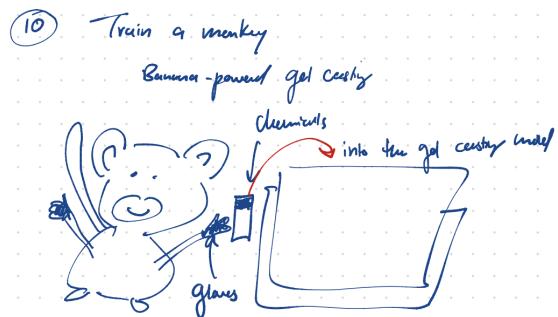
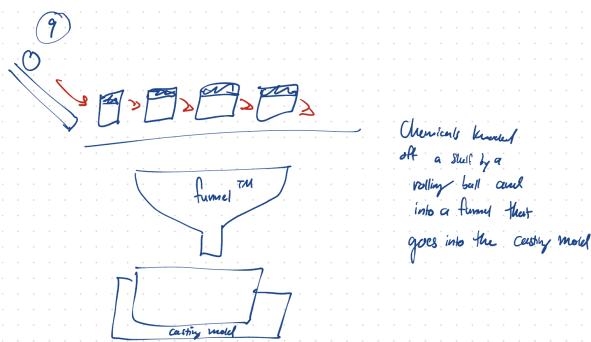
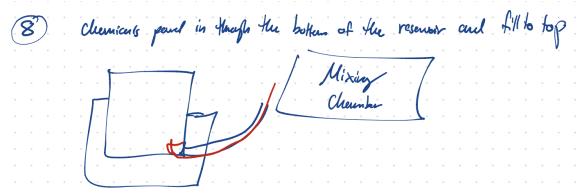
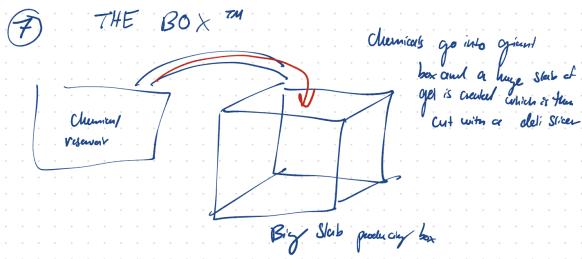


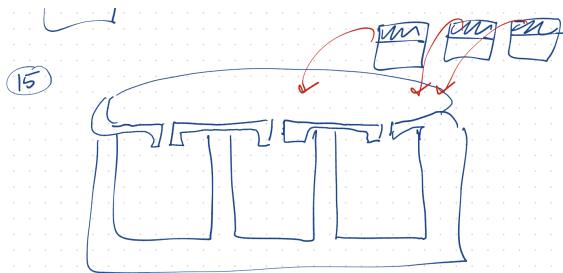
- ⑤



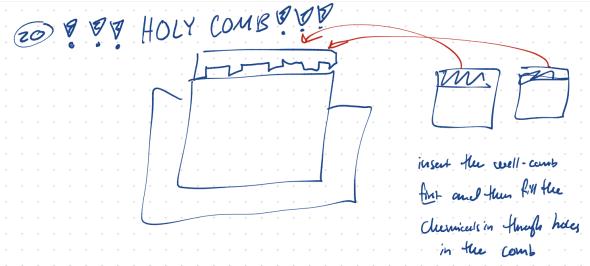
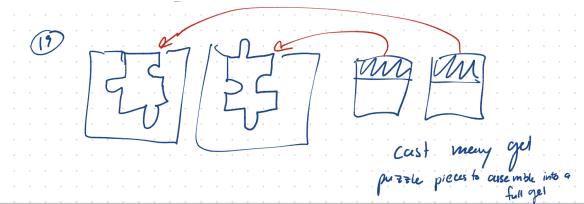
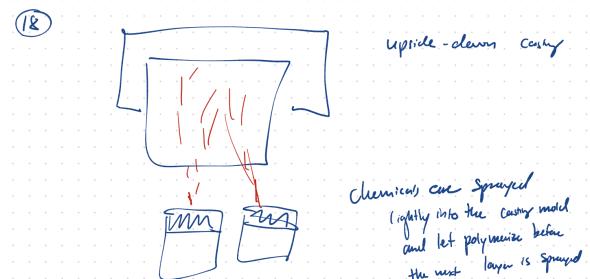
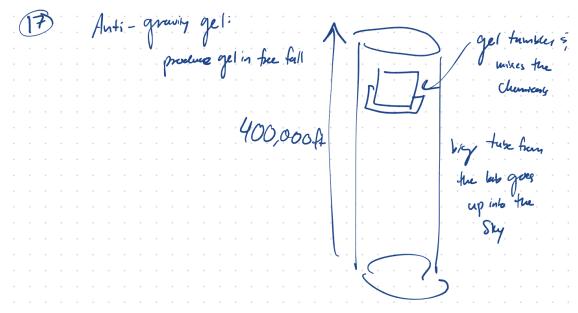
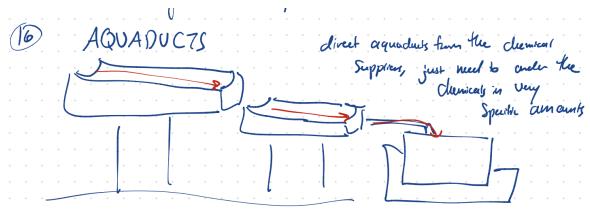
- ⑥



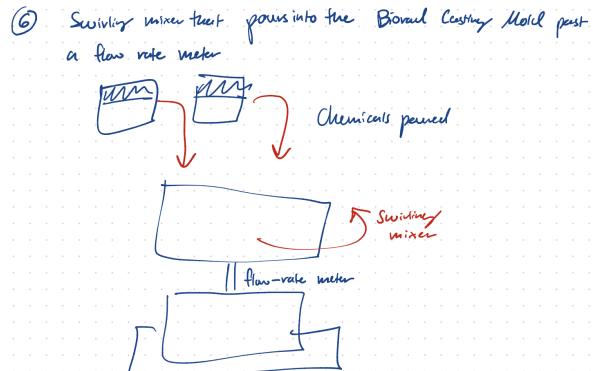
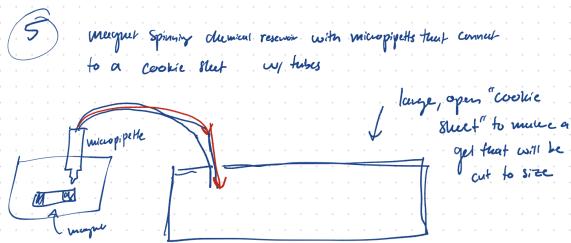
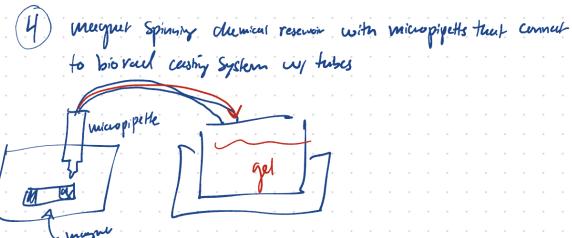
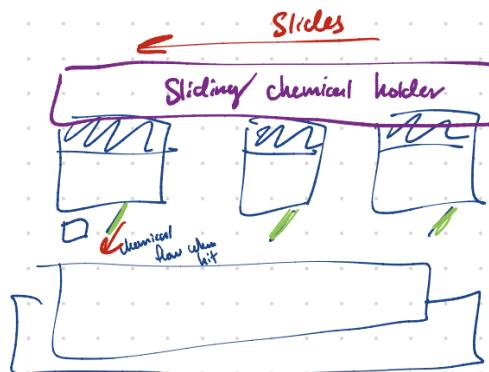
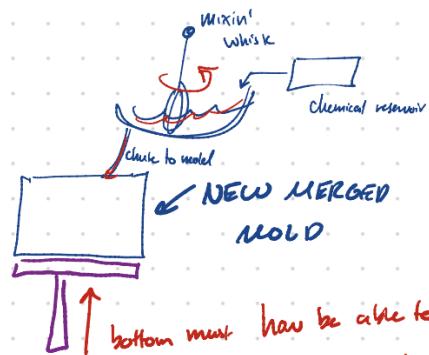
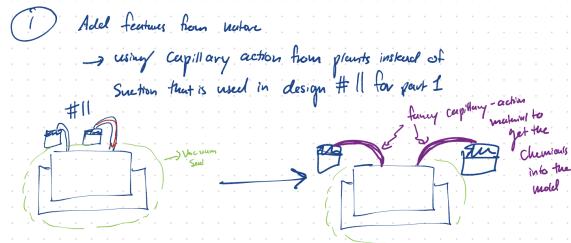


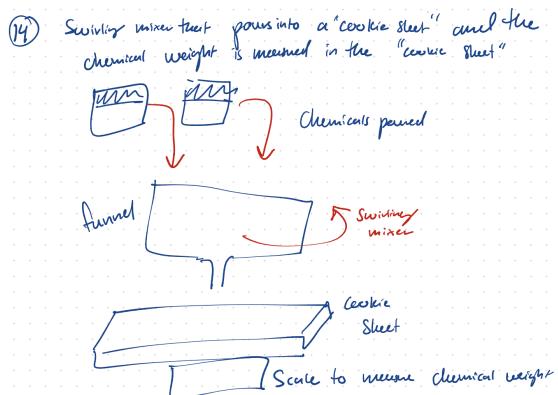
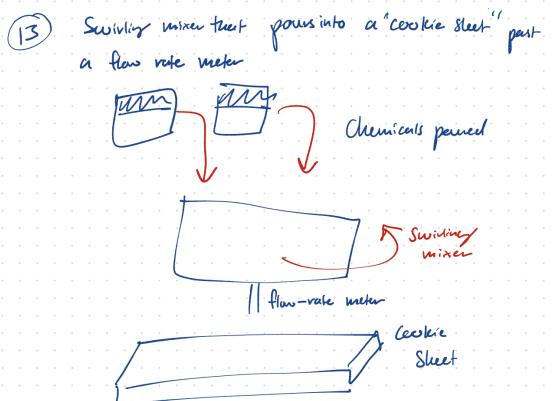
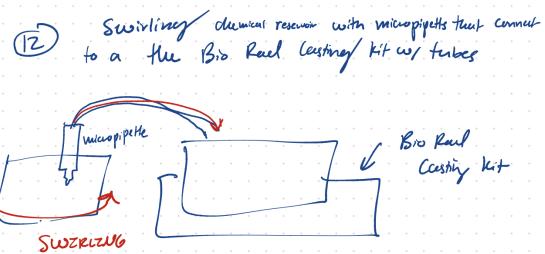
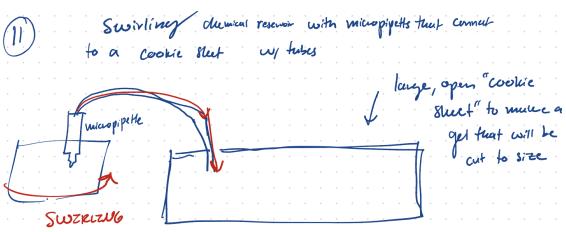
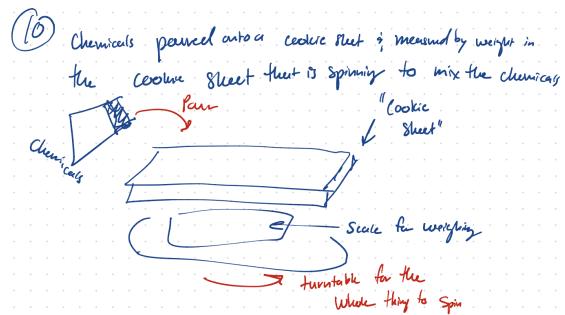
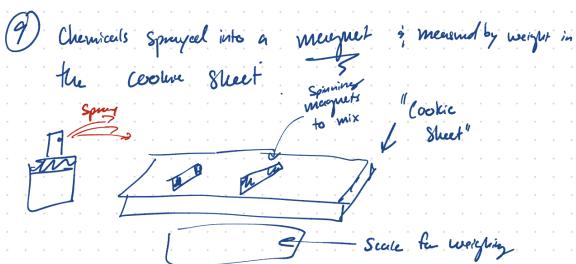
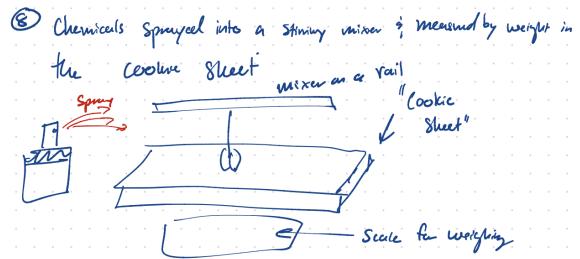
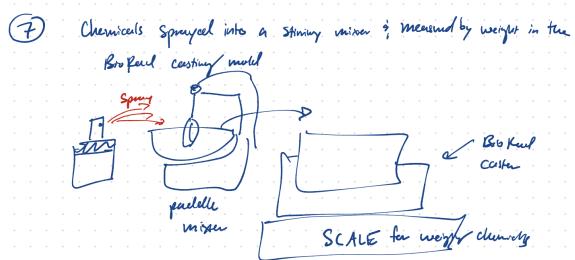


Still use Dave; mix the chemicals and run them into MANY gel spots. Chemical research leads into many molds to make much gel w/ same steps

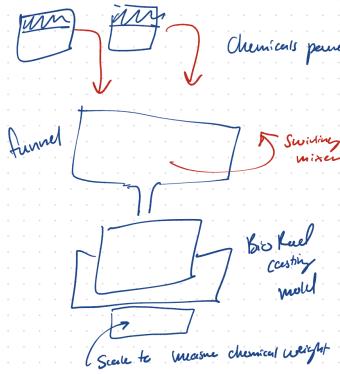


Part II

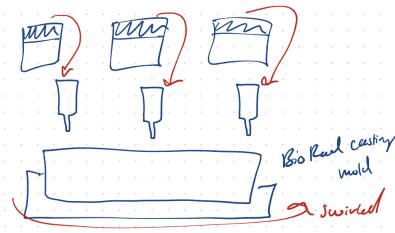




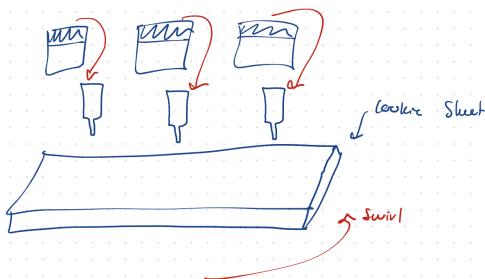
- 15 Swirling mixer tray pours into a BioReel casting mold and the chemical weight is measured in the "Cookie Sheet"



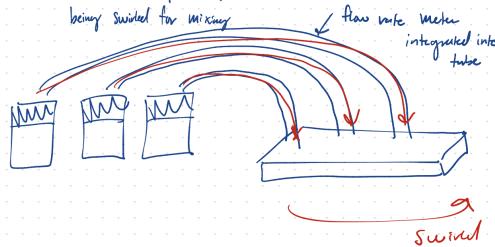
- 16 Chemicals are poured into separate pipettes and expelled into the BioReel casting mold which is swirled to mix the chemicals



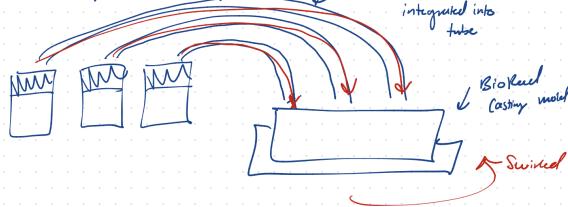
- 17 Chemicals are poured into separate pipettes and expelled into the "Cookie Sheet" which is swirled to mix the chemicals



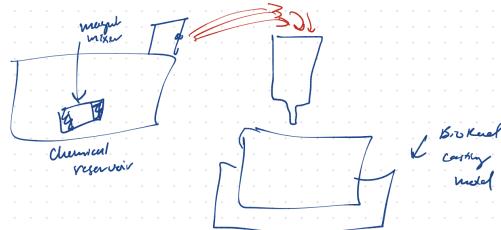
- 18 Tubes transport the chemicals and the flow rate is measured through the tubes as they carry the chemicals to a "Cookie Sheet" that is being swirled for mixing



- 19 Tubes transport the chemicals and the flow rate is measured through the tubes as they carry the chemicals to a "Cookie Sheet" that is being swirled for mixing

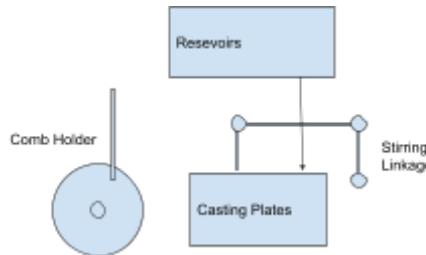


- 20 Chemicals are mixed by magnets and then sprayed into a pipette that distributes them into a BioReel casting mold

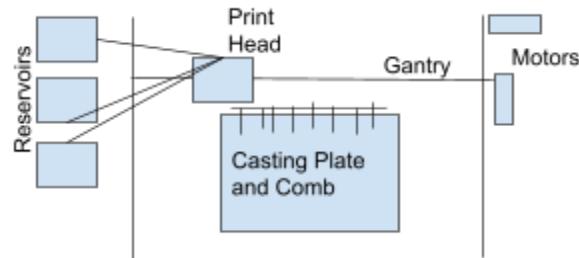


Part I

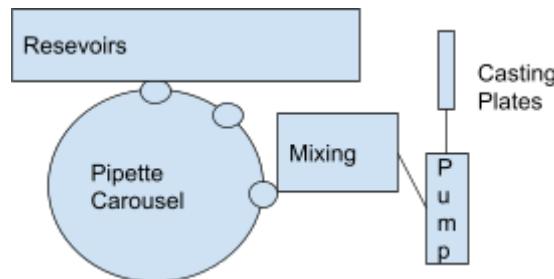
1. Reservoir storage system that pours directly into the casting plates, with a stick that comes down and mixes the chemicals in the casting plates, and a motorized wheel-arm combo that inserts the comb after.



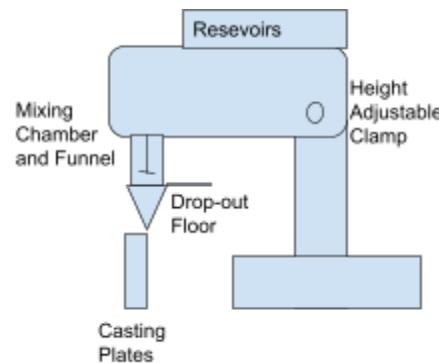
2. A gantry robot that 3D prints a gel onto the casting plates and comb.



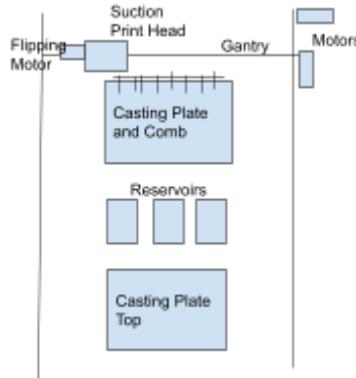
3. A reservoir system with a pipette carousel that pours into a vortexing chamber, which is pumped after mixing into the casting plates. A linkage then swings in from the front to insert the comb.



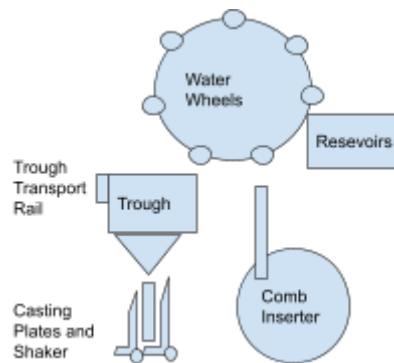
4. A “kitchen mixer” style head with height adjustability, where the floor of the mixer pulls out dropping the chemicals into a funnel that channels them into the casting plates.



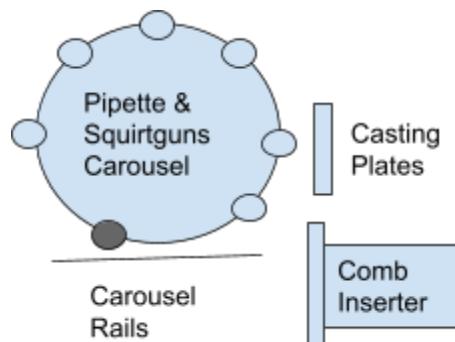
5. A gantry vacuum sucker that sucks up each chemical from its storage, swings in a circle around the gantry bar to mix the materials, then spits them back out into a horizontal casting plate. Next it suctions up the top casting plate and presses it down on top of the first.



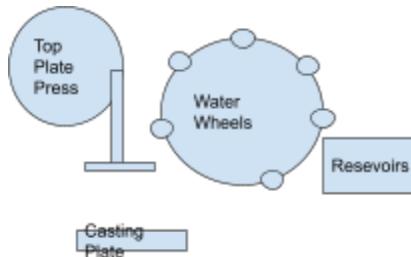
6. A set of water wheels that scoops up the chemicals and drops them into a trough that deposits them into the casting plates, which are then sealed and shaken. The comb is then inserted into the plates by a geared down wheel that clips onto the comb.



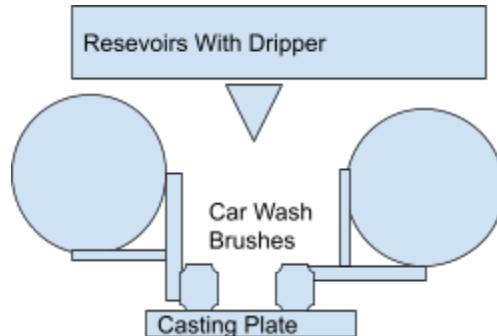
7. A set squirt gun style reservoirs on a carousel, that are each triggered by a little lever, each time they are positioned over the casting plates. The carousel rotates one last time, where a pipette is used to mix the chemicals, and it moves to the side to allow a linkage to sweep in the comb.



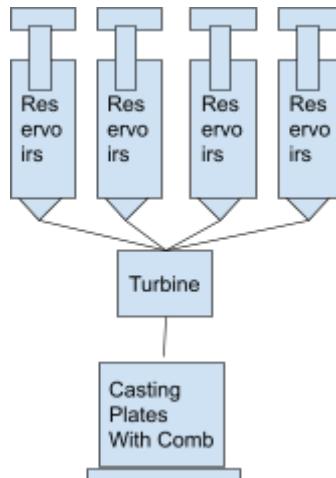
8. A set of water wheels drop the chemicals directly onto a horizontal casting plate with the comb in it. Then the top plate is mashed down on it and the whole thing is shaken.



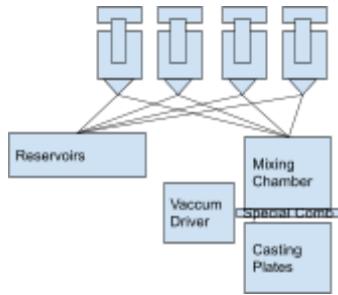
9. A set of reservoirs drip chemicals onto 2 different car wash brushes which then deposits them layer by layer onto the open plates.



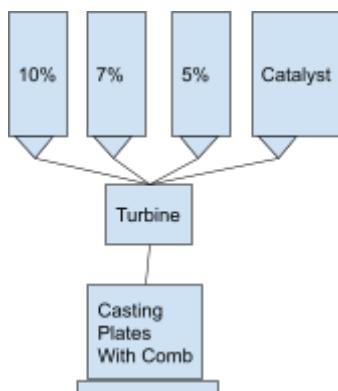
10. Set of reservoirs that are actually giant syringes, deposit the liquid chemicals into pipes with a small set of turbines in it. This pipe then shoots the liquid into a set of upside down casting plates that already have a comb in it.



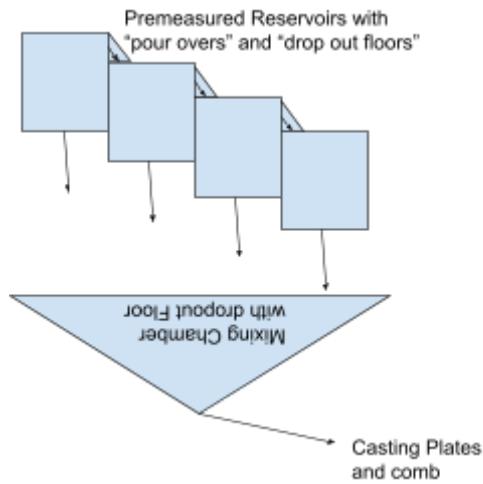
11. A system that uses a syringe pump to put chemicals into a mixing chamber, the bottom of which is a modified comb. The modified comb has openings to release liquid from the mixing chamber and a vacuum to suck out leveling solution.



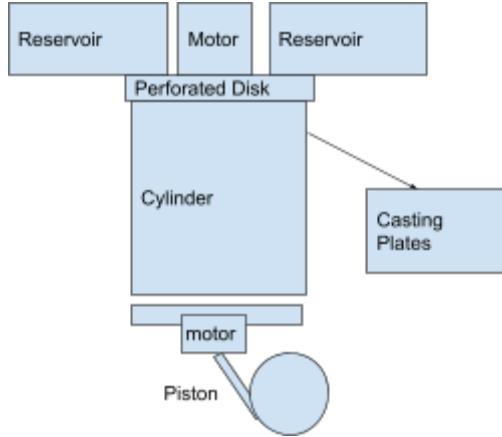
12. A device where you pre-measure concentrations for the running gel and stacking gel variants into separate reservoirs and it pours these into the plates thru a turbine alongside the catalyst.



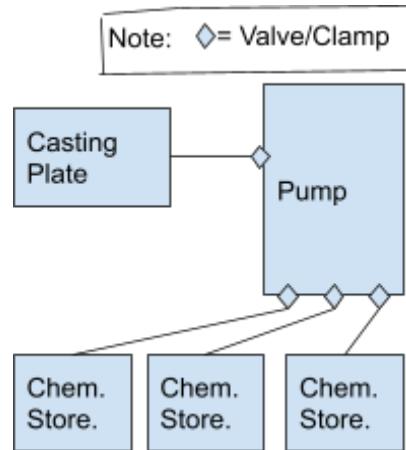
13. A set of storage chambers with “drop-offs” that funnel chemicals into other reservoirs when one is full. Using this volume control to make measurements. Floors drop out of these premeasured reservoirs, into the mixing chamber, and then its floor drops out, into the casting plates.



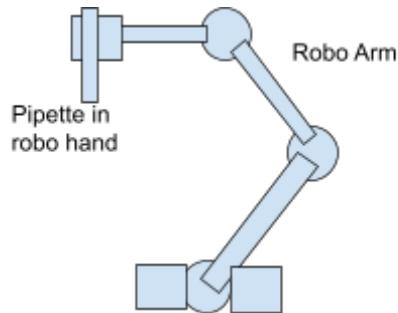
14. A big piston-cylinder with a disk lid with holes cut in it, where the inlets sit on the top of the disk. Using a stepper motor to create the right sized holes and a volume sensor to check when the cylinder is full to the desired volume. Then the piston spins, mixing the liquid, as it pumps it out into the casting plates.



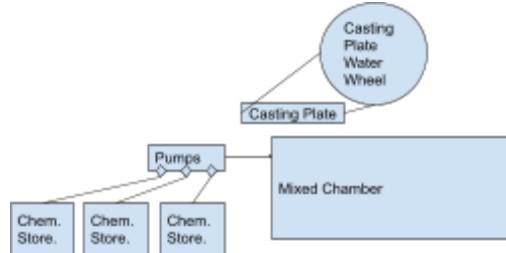
15. A pump with different diameter tubes going reservoirs (volume rate control), which pumps in the desired volume. Then clamps shut off the inlets and open the outlet to the casting plates.



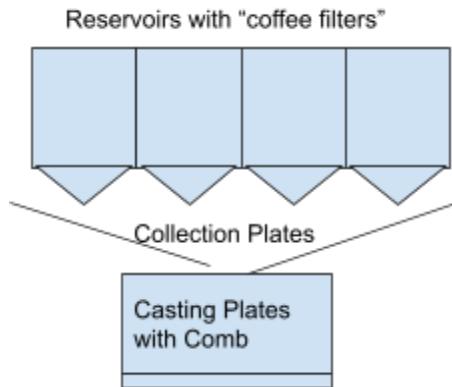
16. A robotic arm that uses pipettes to replicate the manual casting process exactly.



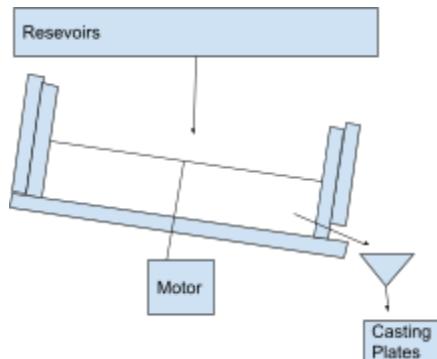
17. Inlets with their own set of peristaltic stepper pumps that measure the incoming volume into premixed reservoirs. A linkage, scoops the casting plates thru the mixture like a water wheel.



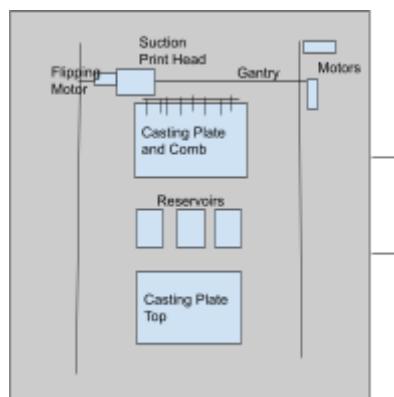
18. A system that uses a “filter-paper” to measure by dripping for a set amount of time into the bottom of an upside-down casting plate, comb combination.



19. A reservoir system that connects with a whirling cylinder that fits into the mixing chamber and retracts, relieving holes to pour the liquid out into a funnel that leads to the casting plates.

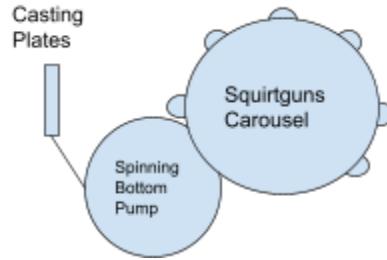


20. A gantry-style vacuum system that folds down into a briefcase for portability!

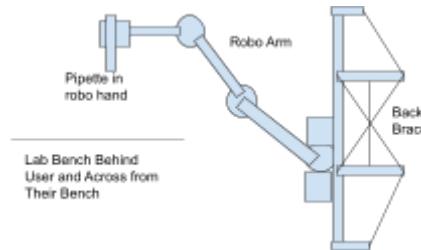


Part II

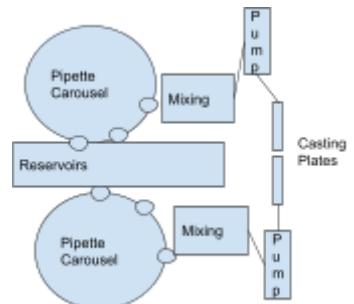
1. Morphology: 7 & 14 - Spinning piston and squirt gun carousel



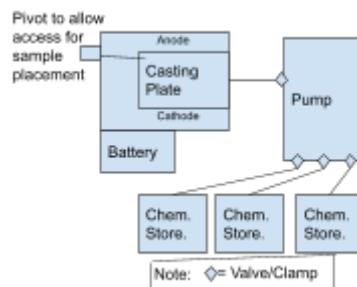
2. Design Heuristics 14: Attach product to user - backpack robo-arm



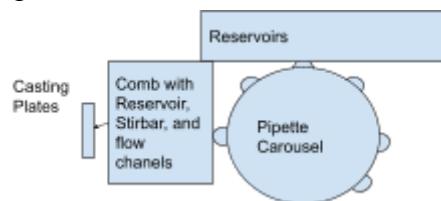
3. Design Heuristics 47: Mirror or Array - multi-casting!



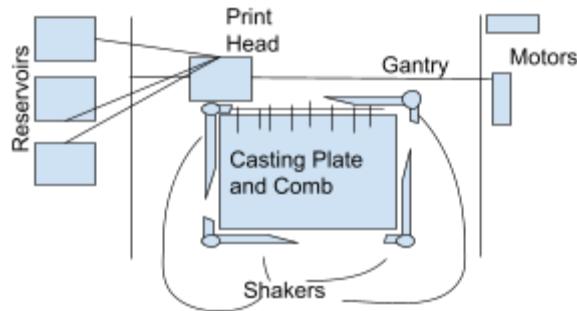
4. Design Heuristics 4: Add to Existing Product - electrophoresis machine



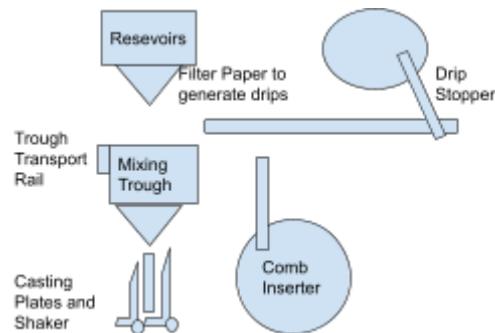
5. Morphology: 3 & 11 - Pipette Carousel and Modified Comb



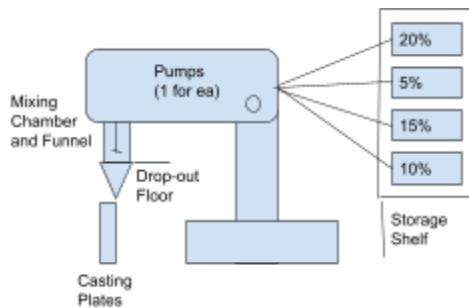
6. Morphology: 2 & 6 - Gantry Robot with Shaker



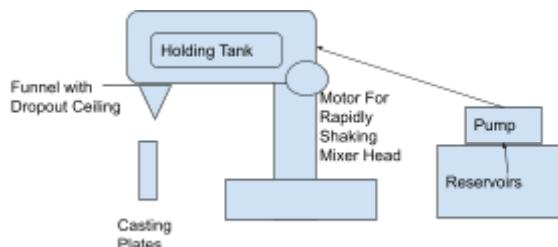
7. Morphology: 6 & 18 - Filter Paper and Shaker



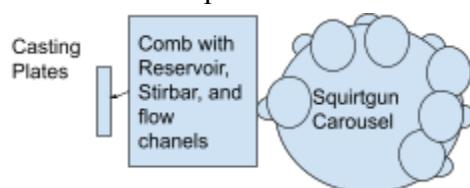
8. Morphology: 4 & 12 - Kitchen Mixer and Premeasured Concentrations



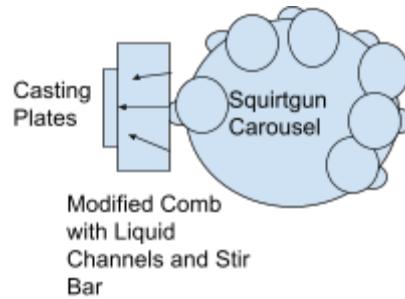
9. Morphology: 4 & 6 - Kitchen Mixer and Shaker



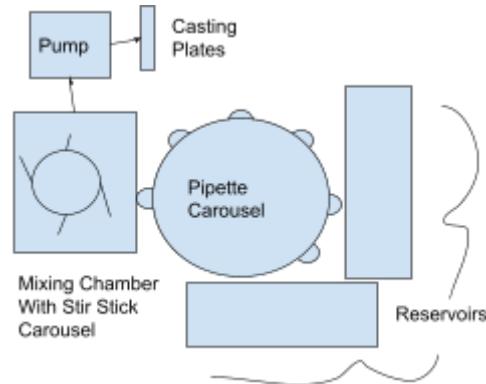
10. Morphology: 1 & 7 - Stir Stick and Squirt Gun Carousel



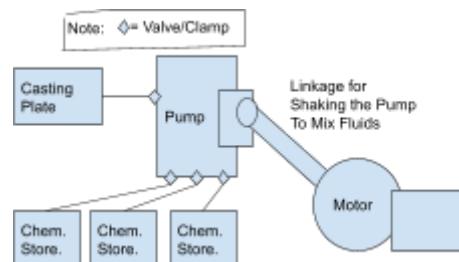
11. Morphology: 7 & 11 - Squirt Gun Carousel and Modified Comb



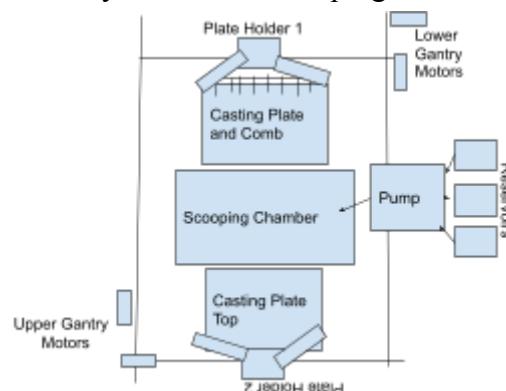
12. Morphology: 1 & 3 - Stir Stick and Pipette Carousel



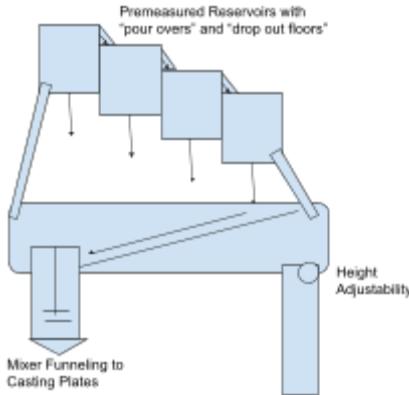
13. Morphology: 6 & 15 - Shaker and Volume Flow Control Pump



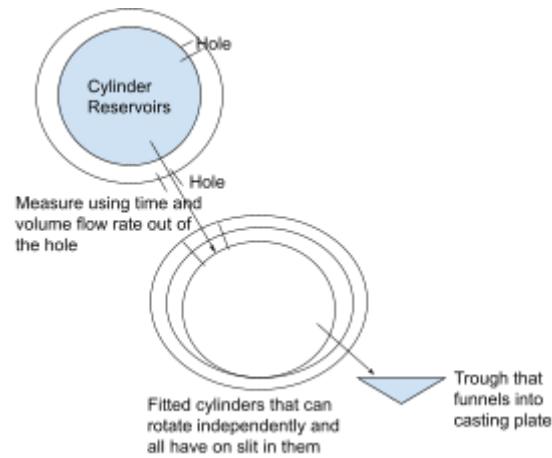
14. Morphology: 2 & 17 - Gantry Robot and Scooping with Casting Plates



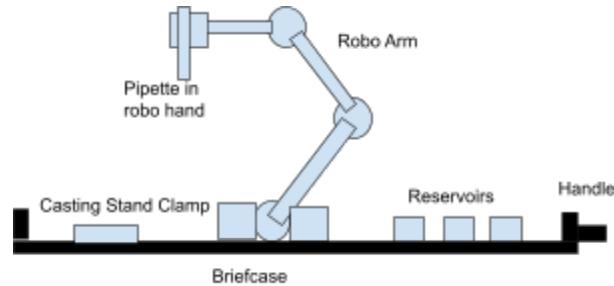
15. Morphology: 4 & 13 - Kitchen Mixer and “drop-off” Storage Chambers



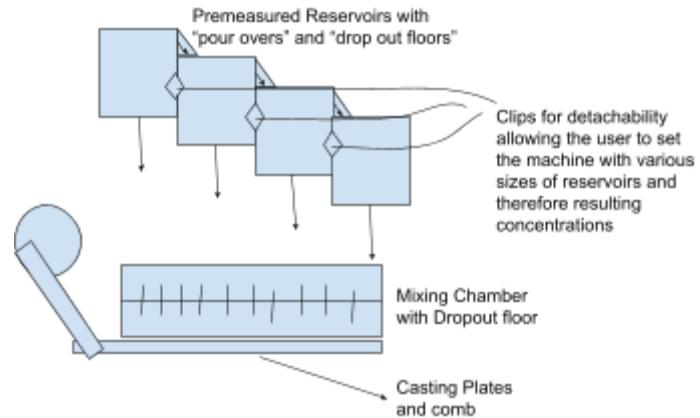
16. Design Heuristics: 56 - Roll



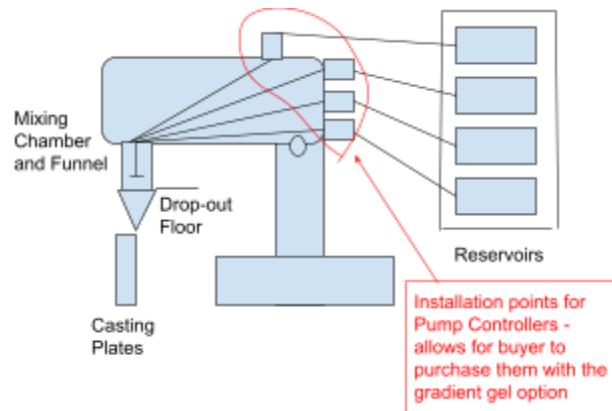
17. Morphology: 17 & 20 - Portage Gel Casting Robo Arm



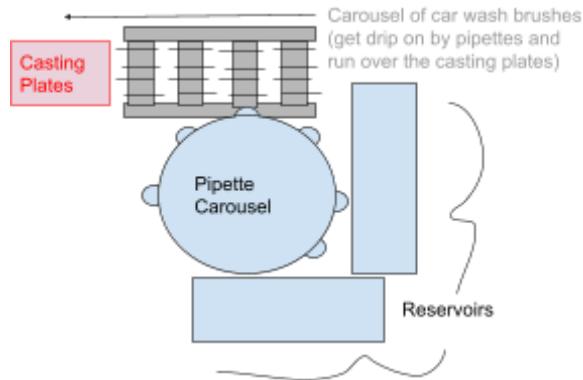
18. Design Heuristics: 42 - Make Components Attachable/Detachable



19. Design Heuristics: 49 - Offer Optional Components



20. Morphology: 3 & 9 - Pipette Carousel and Car Wash Brushes



B.3: Erica Santos

Part 1

	Chemical Measure	Mix	Hold	Pour	Clean
1	Syringe	**Bucket to bucket tilting into each reservoir	*Hanging buckets under each measurement	\$ Reservoirs connected by merging tube system (think roots)	Manually (wipe)
2	*Micro pipette	*Pipette up and down	Buckets on pedestal	Pump out from top	Swiper blade alone surfaces
3	Pre-measured tubes (operator fills to top)	Shake reservoir side to side	Big wide bucket	Paddle opening at bottom	Pipe cleaner
4 \$*	Pump (time based)	*Nothing-reservoir sideways	* Stopper at the edge of wide reservoir	*pour on opposite end , all tubes at once (controlled)	Water jet to flush
5	Mass flow meter	**Tornado spiral system; each chemical inserted in as the mass moves past its opening	*N/A, sorta	Slides w controlled stoppers	*Air jet immediately after
6	*Weight	magnetic vortexer	* Weighted plates	Tilt plates into funnel -> separating glasses	Water immediately after
7	Filtration; let the number of drops and time measure how much is added	** Mix within the glass plates w physical movement requires leveling afterwards	** none	** Slide latches, put catalyst in own reservoir and poured directly into	** Tubes don't have catalyst so no solidification
8	Volume measured smaller compartments; auto fills from main reservoir until full	Blender blade inside	Blender shaped U	\$ Root system slides -> reservoir for all chems but catalyst; stopper at bottom of U	Chemical to dissolve (ethanol??)
9	**Blade inserted to cut by volume	Rotate mixing chamber with motor	*Measured containers; neet to know exact sizes	*remove wall	Wipe to absorb

10	Weight, but hanging	*Rotate with belts	*Closed, capped circle w pivot point	Uncap, pour	Suction
11	Pressure measurements, mass flow calculations	*Air bubbles	Large reservoir w holes for air bubbling at bottom	*Air jet	Material does not allow stick :) (self cleaning?)
12	Ice tray with measurements	Flip upside down	Flexible chamber, like rubber or something	WIGGLE the flexible reservoir chambers	plunger/syringe like item to push all surface edges at once
13	Measuring cups to scoop	*Centrifugal force; ridges on the walls to hold it, then release it, as its spinning	* Cylindrical reservoir w holes connected to catchers, root system down into sheets	*Centrifugal force; holes on edges of cabin to push it out	Heat
14	*Measure length along tubing; inject air bubble	Wave generator	aquarium glass	*Tubing	UV/Laser scan to zap chemicals
15	*Volume, filling, then push w wiper	Same wiper in adjacent holder, back and forth movement	foldable compartment	*wiper push	*Entire system can flatten out (think origami or sheet metal) and then one wiper pushes off chemicals
16	**Stepper motor w/ incremental volume measurements	*Nothing; same reservoir	*reservoir	Flap opening at bottom	*Single wiper
17	degradable items that will disintegrate with time	Aquarium filtering system	3D printed	Pick up and move the plastic lining in each chem reservoir	Plastic lining; removed each use
18	pump the time	tilt	\$outside of motor, slides	under	drip, time
19	Robotic arm does exactly what human operator does	-	-	-	-
20	push up voluming push pop	one reservoir-nothing	same	tilt	air dry upside down

- ① Pump each ingredient into a mixer that pours into the plates



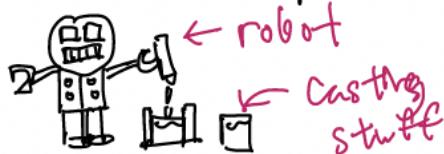
- ③ Use a gantry (like a 3D printer) apparatus to pipette the ingredients



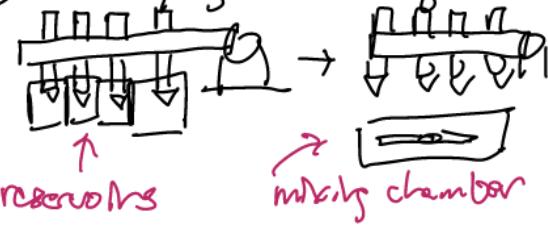
- ⑤ Robotic arm to replace humans



- ⑦ Enter the robot to replace humans



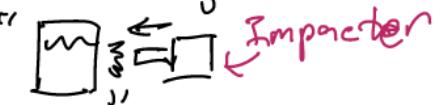
- ② Use a syringe for each ingredient



- ④ Vibrate a container for easy mixing of ingredients



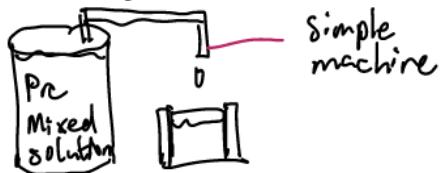
- ⑥ Impact a container ("flicking") to mix ingredients



- ⑧ Squirt the ingredients into the plates



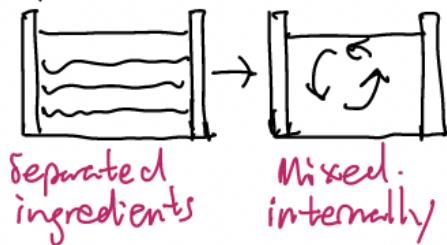
- ⑨ Mix a vat of solution to use for a long time



- ⑩ Flip the comb upside down to eliminate need for insertion



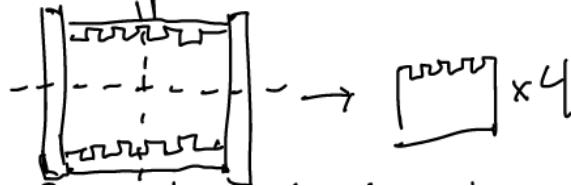
- ⑪ Mix the ingredients inside the plate



- ⑫ Use an opening in the comb to inject ingredients



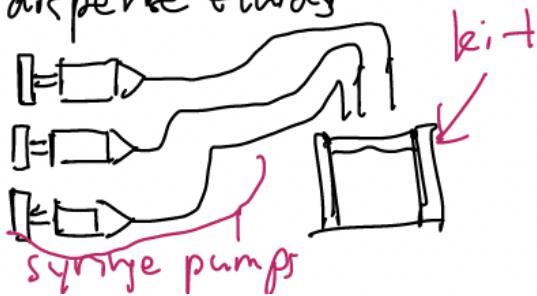
- ⑬ Make a single gel that's cut up into smaller ones



- ⑭ Stick plates to make multiple gels at once



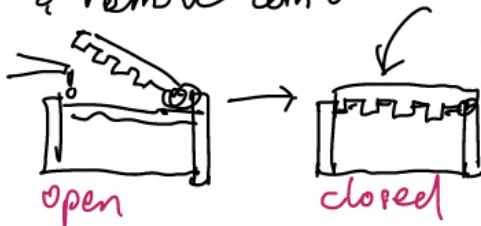
- ⑮ Use syringe pumps to dispense fluids



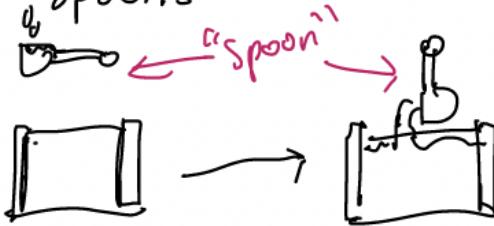
- ⑯ Mix with a magnetic stirring bar



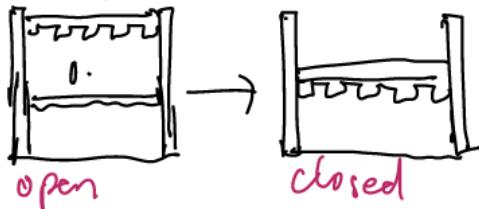
⑦ Use a notational
& remove comb



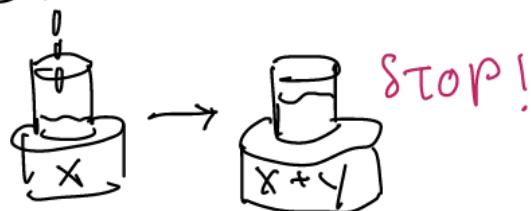
⑧ Measure with "measuring spoons"



⑯ Use translateral motion to add & remove comb

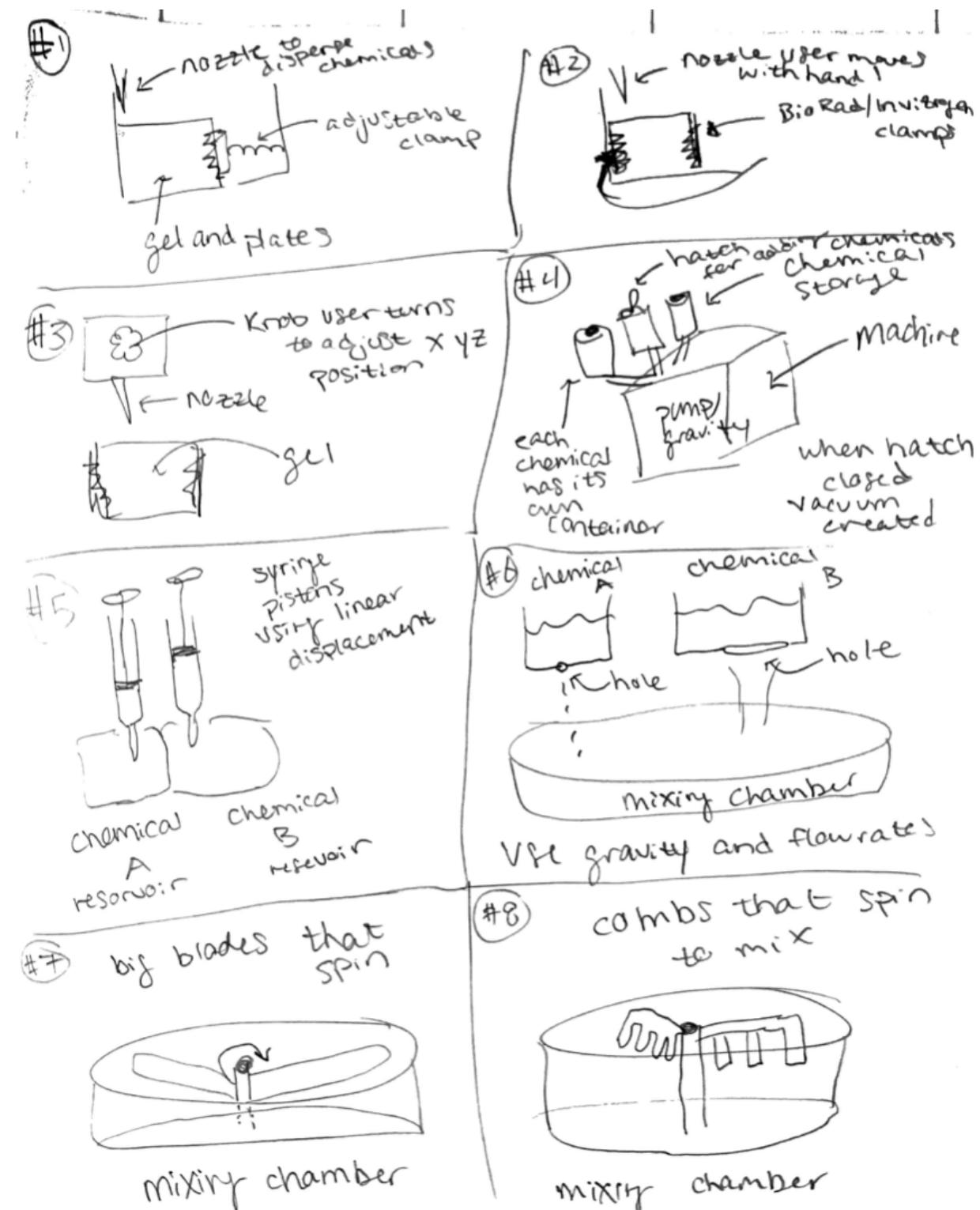


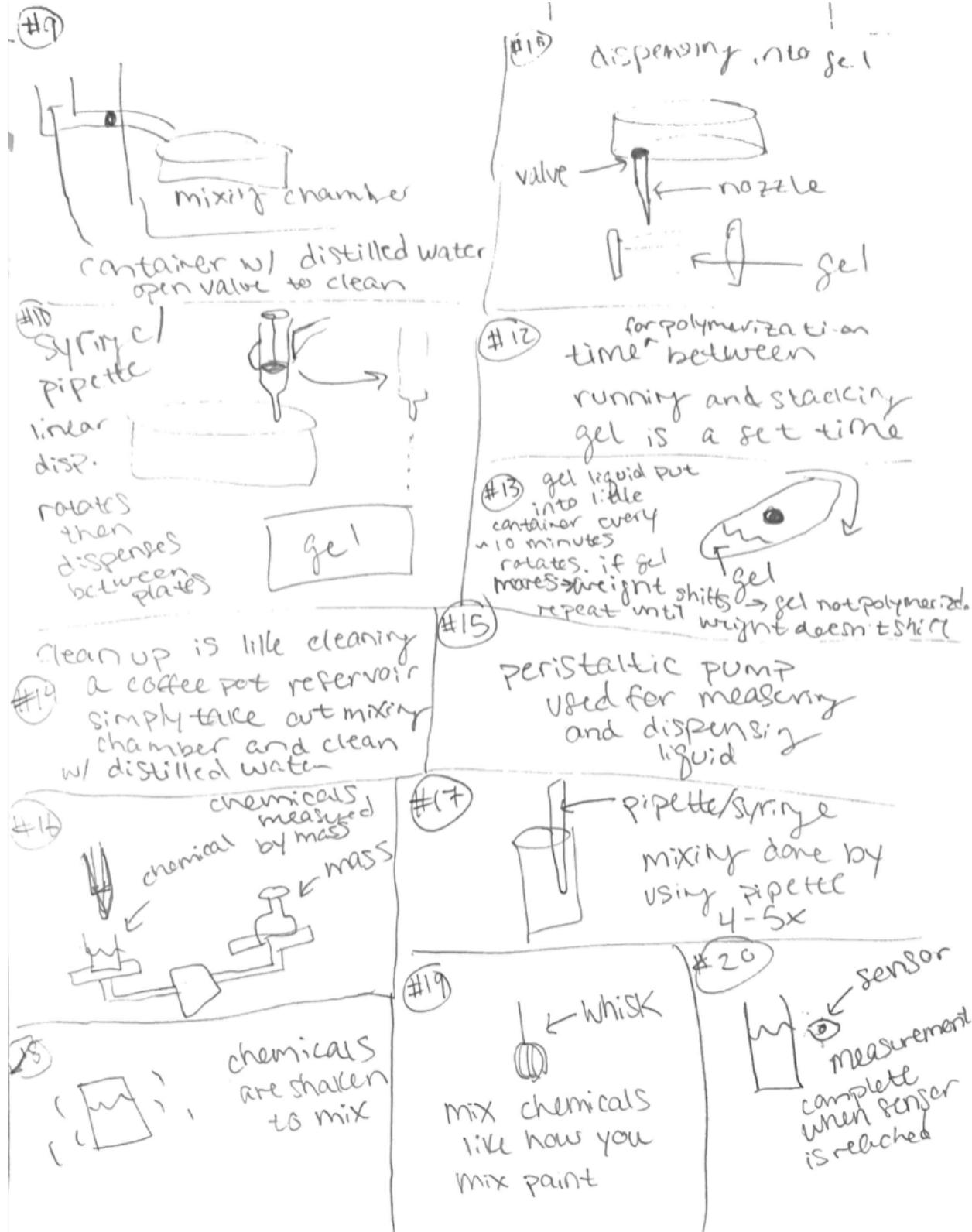
② Measure with a scale



Part II

Subsystem	Option 1	Option 2	Option 3
Measuring	Pump the exact amount using a syringe or peristaltic pump	Automate the adjusting of pipette measurements	Measure the mass of the ingredients to be added instead of using volume
Mixing	Use a magnetic stirring machine	Impact the container to deliver an excitation to mix the chemicals	Vibrate the container to mix the chemicals
Comb Insertion	Leave a small opening in the comb to pour the solution into the casting kit, removing the need to automate comb insertion	Use a motor to insert and remove the comb using rotational motion	Have the comb pre inserted on the bottom and the solution poured in from the top, removing the need to automate comb insertion





Part 2

Mixing	#15 big blades	#16 combs	#17 shake	#18 whisk	#19 pipette
measuring	#20 nozzle by mass	#21 by sensor	#22 peristaltic pump	#23 displacement	#24 flowrate/gravity
dispensing into cuvettes			#25 peristaltic pump		
dispensing into plates	#26 rotate valve gel		#27 peristaltic pump		
nozzle placement	#28 adjust cable clamp	#29 Bio-Rad/ Invitrogen nozzle by hand placed	#30 xyz position via knob		
chemical Storage	#31 hatches				
clean up	#32 valve distilled water flush	#33 manual clean up in sink			
gel polymer- ization check	#34 set time coded	#35 rotates weight shifts then not ready			

APPENDIX C: Engineering Analysis

C.1: Calculating the error of the volume measurements in the Posiedon syringe pump

We used the following mathematical calculations to determine the expression for volumetric error in the Posiedon syringe pump based on displacement error data provided by Pachter Lab [27].

Multiplication

To calculate the effect of the errors when multiplying two numbers, $Z = AB$, one obtains:

$Z \pm e_Z = AB \pm \sqrt{e_A^2 B^2 + e_B^2 A^2}$. Dividing both sides of the error part of this equation by AB produces a simple result in terms of fractional errors: $t_Z = \sqrt{t_A^2 + t_B^2}$. Hence the actual fractional error can be determined as the root of the sum of squares of the individual fractional errors.

$$\text{Volume} = \pi \cdot R^2 \cdot \text{displacement}$$

where R is the inner radius of the syringe.

For these calculations, we assume the radius of the syringe is errorless and that $\pi \cdot R^2$ represents an errorless constant. This is a valid assumption because we are using precision manufactured syringes, so the error in dimensions given by the manufacturer should not be significant. This gives that the error of the volume is:

$$\text{Volume error} = \pm \sqrt{(.0144 * \text{displacement})^2 \cdot (\pi \cdot R^2)}$$

This shows that the largest potential volume error for a syringe is a factor of the largest displacement value (the stroke length) of the syringe and the radius of the syringe. Using this data, we can calculate the largest volume error of the 10 mL and 10 μL syringes we are considering using. Table C.1 shows the values used and the results of these calculations:

Syringe Volume	Stroke Length [28]	Inner Radius [28]	Calculated Volume Error	Calculated Percent Volume Error
10 mL	80 mm	14.5 mm	$\pm .003 \text{ mL}$	$\pm .03\%$
10 μL	60 mm	.245 mm	$\pm .375 \mu\text{L}$	$\pm 3.75\%$

Table C.1: the errors shown are the maximum errors attainable for the respective syringes being operated with the Poseidon syringe pump.

C.2: Bending calculations for the carousel base

A section of the circular carousel base plate was modeled as a beam to determine the thickness of acrylic needed to support the weight of the chemical reservoirs. Although this is not an exact model of the circular base plate, the beam bending calculations will give a ballpark overestimate of the thickness of acrylic required. This is an overestimate because the beam used

does not have the structural advantage of support in the radial direction as a segment of the circular base does. As a structurally inferior element, the beam will require a greater thickness of acrylic than needed for the circular geometry, providing a safety factor to our calculations.

The beam loading case is shown in the diagram below [30], we are able to rearrange the maximum deflection equation to get the thickness of the beam if we substitute in the expression for the second moment area of a beam as shown below. We chose this beam loading case because we are modeling a segment of the base beyond where the base is fixed to the turntable.

Beam and load cases	Maximum Beam Deflection
	$\delta_{max} = \frac{PL^3}{3EI}$

Rearrange the values:

$$\delta_{max} = \frac{PL^3}{3EI} \rightarrow \delta_{max} = \frac{PL^3}{3E \left(\frac{w \cdot t^3}{12} \right)} \rightarrow t = \sqrt[3]{\frac{12 \cdot PL^3}{3 \cdot E \cdot w \cdot \delta_{max}}}$$

Where δ_{max} is the maximum deflection of the beam [m], P is the load [N], L is the length of the beam [m], E is the young's modulus of the beam material [Pa], w is the width of the beam (into the page on the diagram) [m], and t is the thickness of the beam [m].

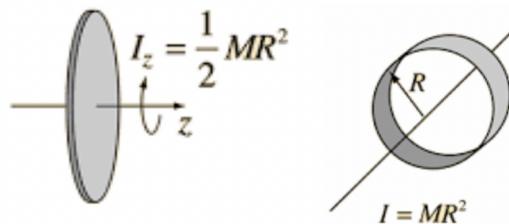
Using the rearranged values and the following table of values, the thickness of the acrylic can be solved for. Note that the length of the beam in the following table is obtained by measuring the length of the beam that extends past the turntable because it is assumed to be fixed to the turntable. The maximum displacement was obtained using 5% of the length of the beam. The force, P [N], on the beam is estimated using the weight of a reservoir filled with water, to account for the potential overfilling of the reservoirs. The width of the beam, w [m], was determined using the diameter of the chemical reservoir to account for the footprint.

Parameter	Value
P	.5 N
L	.06 m
$E_{acrylic}$ [31]	$2.76 * 10^9$ Pa
w	.042 m
δ_{max}	.003 m

Using the parameter values mentioned above and the rearranged equation for thickness, the minimum thickness of the acrylic is calculated as to be $t = 1.07$ mm to prevent a deflection of 5%.

C.3: Inertia calculations of the base plate to determine motor torque

The inertia of the carousel base was calculated by combining the inertias of a disk and a ring, with the disk representing the acrylic and the ring representing the mass added by the reservoirs. Although the acrylic will be laser cut to accommodate the reservoirs, the material is still modeled as a disk due to the much simpler inertia calculations that can be used for a disk and the fact that the disk will give an overestimate of the inertia because it has more distributed mass than the same shape with reservoir holes cut into it. The figure below shows the inertia of both a disk and a ring [30].



Using the equation for angular torque $\tau = I \cdot \alpha$, we calculated the torque required to turn the base where τ is the torque required to move the base [Nm], I is the total inertia of the base [$\text{kg} \cdot \text{m}^2$], and α [rad/sec^2] is the angular acceleration of the base. The desired acceleration was determined to be $.06 \text{ rad/sec}^2$ using kinematics for a situation where the base takes 10 seconds to travel 180° from rest, based on our prior experiences with liquid transport we believe this is an acceptable acceleration.

The values in the following table were used to calculate the inertia from the equations in the figure above. M_{Disk} was found using the density of acrylic, 1190 kg/m^3 [31], and the volume of the disk. $M_{Reservoirs}$ was determined by adding the mass of ten 50 mL reservoirs filled with water, all of the reagents have a density similar to water so this is a fair estimate for if all of the reservoirs were filled, including the mixing and flushing reservoirs.

Parameter	Value
M_{Disk}	.27 kg
$M_{Reservoirs}$.5 kg
R_{Disk}	.12 m
$R_{Reservoirs}$.10 m

Using the parameters from the above table and the equations provided, the minimum torque required from the motor is $\tau = .005 \text{ Nm}$.

APPENDIX D: Verification

D.1: Calibration from volume to displacement to steps for the syringe pump

According to the specifications for the threaded rod, the pitch was 0.8mm/rev with 3200 steps/rev. Measuring using calipers, 6 mL on the syringe corresponds to 1 mm of linear displacement. Using the conversion below, we were able to calculate the distance to plunge and the steps to do so.

$$volume = displacement \times 6 \frac{mL}{mm}$$

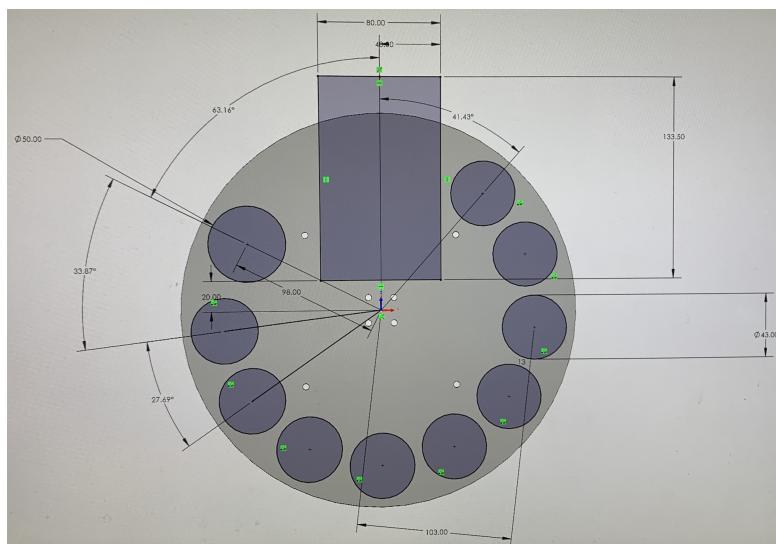
$$steps = distance \times \left(\frac{steps}{rev} / pitch \right)$$

While these measurements seemed correct at first, we began to accumulate error as the syringe performed more measurements. We empirically through trial and error determined better and more accurate values for the number of steps.

D.2: Calibration for the base position based on angle and step size

In CAD, the angles between the plates and the flushing reservoir, between the flushing reservoir and the normal reservoir, and between two reservoirs were determined (starting at the 12 o'clock position and moving counter clockwise, which is shown in the figure below). Using the below equation, the number of steps for these three angles was calculated and from here all other angle measurements were able to be determined.

$$steps = angle \times \left(3200 \frac{steps}{rev} / 360 \text{ deg} \right)$$



APPENDIX E: Manufacturing and Fabrication Plan

Manufacturing Plan:

Our prototype was designed to be easy to manufacture by relying mostly on 3D printed parts and off-the-shelf components with minimal needs for machining. The manufacturing plan for custom made parts is included below for reproducibility.

The circular acrylic plates used for the base were laser cut using a vector image of the CAD file loaded to an Epilog Helix Laser Cutter. These plates were cut from four 30x30cm acrylic sheets.

The larger 3D printed parts (with any dimension greater than 18 cm) were printed using a BigRep STUDIO G2 FDM 3D Printer. These parts were: the carousel base, the syringe pump base, and the linear actuator stand. The fill settings were 60% infill with water soluble support material used. This support material was difficult to fully dissolve from the syringe pump base so we would not recommend using that support material for that piece in the future. The linear actuator base failed printing multiple times, it may be printed more efficiently if the design is simplified.

Smaller 3D printed parts (with all dimensions less than 18cm) were printed using an Ultimaker 3 FDM printer with 60% infill and water soluble supports. These parts were: the gel mold base, syringe pump clamp, syringe pump sled, and syringe pump carriage sled. All parts were printed successfully on the first attempt.

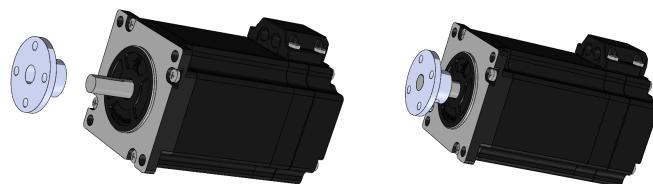
The comb clamp was 3D printed using a Form 3 SLA 3D Printer. To ensure that the fit with the gel mold was snug, the plane of the comb clamp in contact with the faces of the gel mold was sanded until the clamp had the appropriate grip. Changes to the CAD model have been made to reflect this post-print modification.

Assembly Plan:

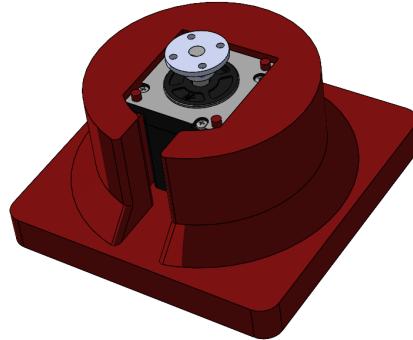
The assembly plan presented is separated into the two subassemblies: the base and the syringe pump stand. Assembly plans for the syringe pump itself can be accessed through the Pachter Lab's GitHub page [27].

BASE

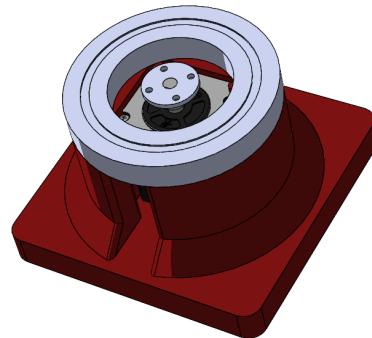
1. Attach the flange coupler to the motor shaft of the NEMA 23 motor and fasten the coupler with the included set screw.



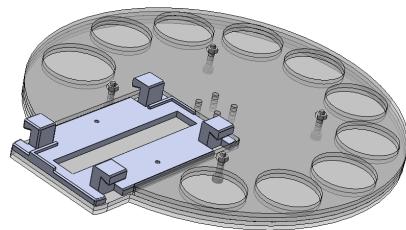
2. Place the motor into the 3D Printed base enclosure. Orient it in such a way that the motor cables extend out of the slot in the enclosure. Depending on the tolerances of the 3D printed enclosure and the exact model of the motor, the enclosure pegs may not fit into the motor holes. In this scenario, it may be necessary to break off the pegs so that the motor can sit in the enclosure properly.



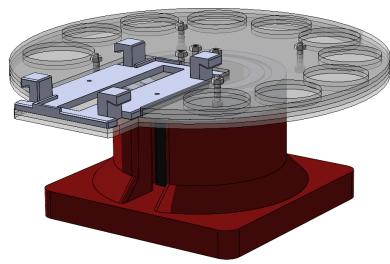
3. Adhere the carousel bearing to the upper face of the base housing using super glue.



4. Assemble the four acrylic plates using 4X M4 20mm bolts with 4X 3cmm spacers between the top plate and the next base plate. The bolts should be secured with M4 lock nuts at the top of the plate assembly.
5. Attach the Bio-Rad gel mold base to the acrylic plate assembly using 2X M3 nuts and locknuts.

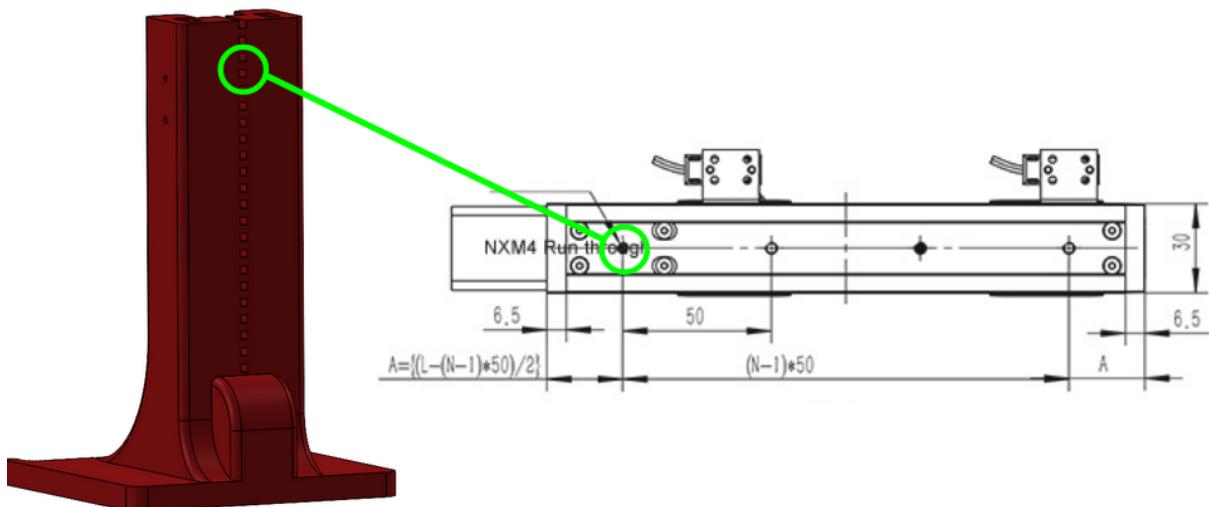


6. Attach the bottom acrylic plate to the flange coupler using 4X M4 nuts and locknuts.
7. add the gel mold plate (4 m4 bolts)

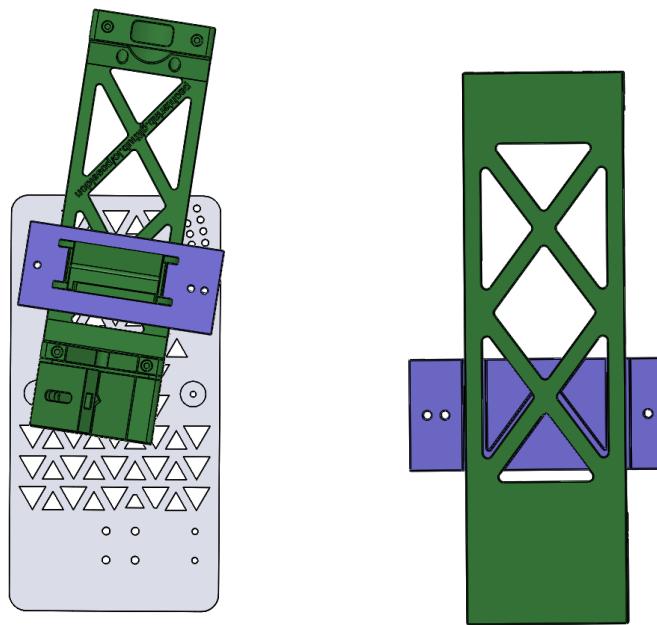


SYRINGE PUMP STAND

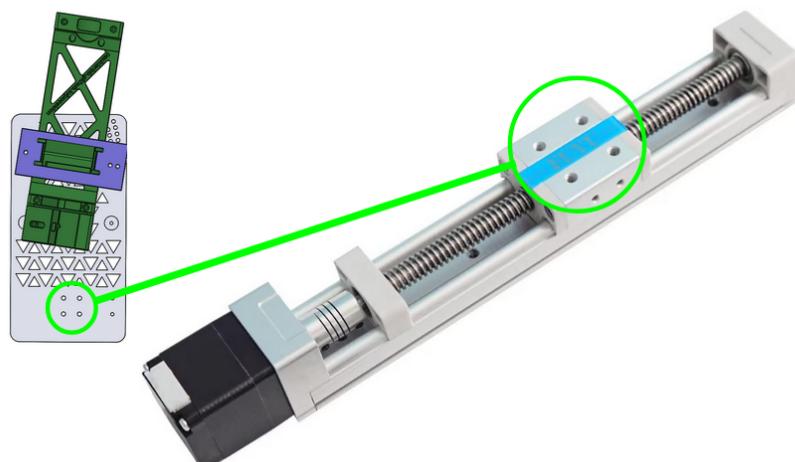
1. Attach the linear actuator to the stand using 2X M4 nuts with the motor end down and the carriage facing outwards. The lowest point of the linear actuator (end of the motor) should sit 210 mm off the ground.



2. Assemble the syringe pump clamp using 3X M4 20mm bolts and locknuts. The angle of the clamp should be set to 15° as shown in the assembly diagram. The 15° setting corresponds to the 8th set of radial holes from the top of the mounting plate.

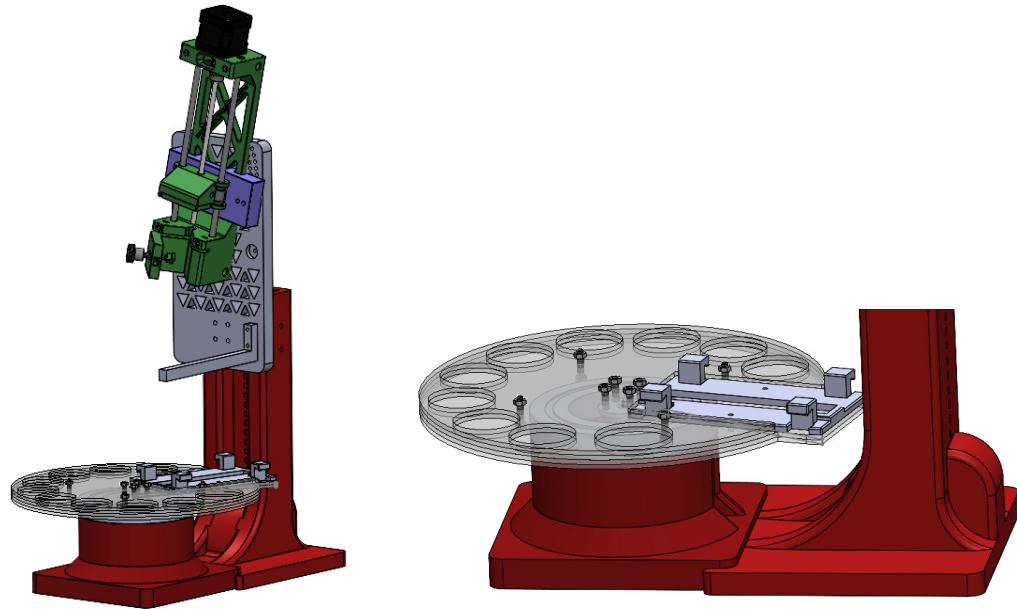


3. Attach the linear actuator mounting plate to the linear actuator glide using 4X M4 20mm bolts.



THE FULL ASSEMBLY

To combine the base and the syringe pump stand, the base must be located against the syringe pump stand as shown in the figure to the right. This ensures that the entire system is in alignment.

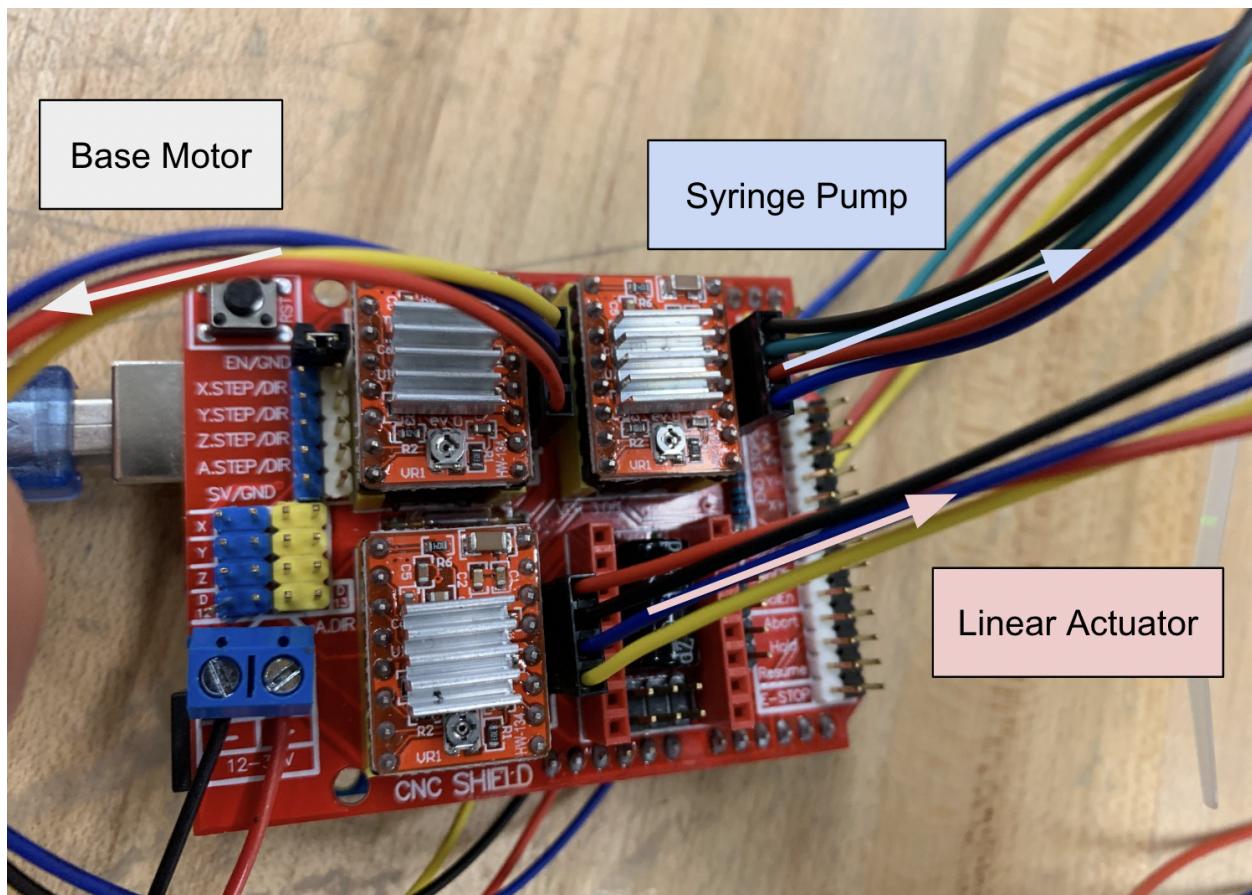


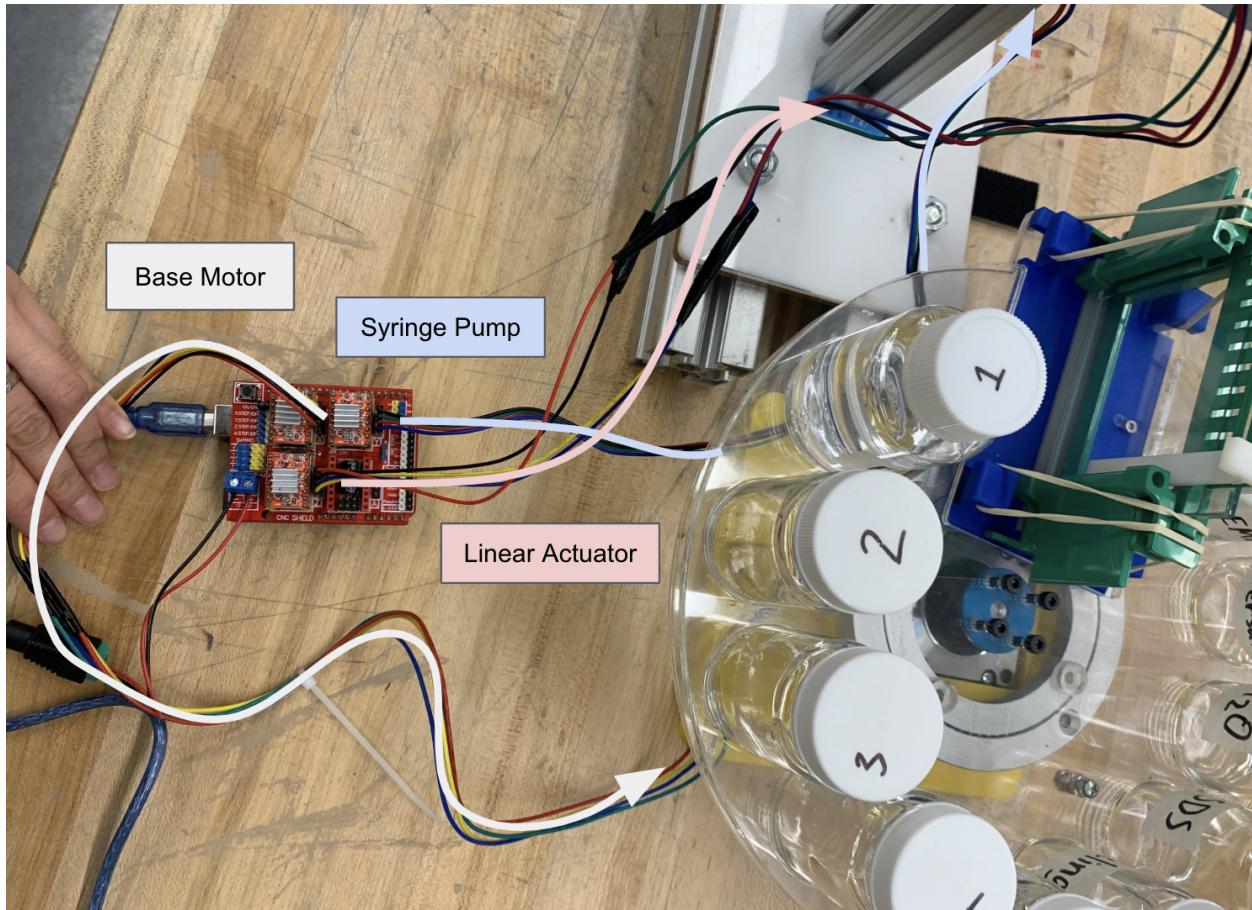
APPENDIX F: Electrical Setup

Arduino Board

Wire orientation and corresponding machines shown below. Ensure the groups are oriented correctly. Code is written to control the motors when connected to XYZ in this order.

Motor Name	Corresponding Location on Board	Wire color on top
Base	X	Yellow
Syringe	Y	Black
Linear Actuator	Z	Red





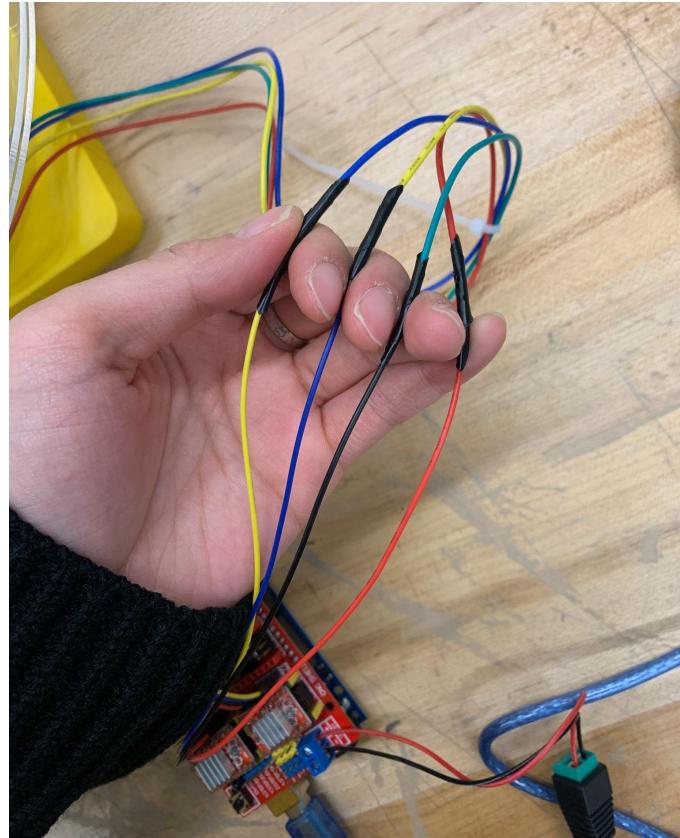
In case wires are cut, or changed, here are the colors they match with currently:

Syringe Pump

(No color changes, plug in straight. Keep Black on top)

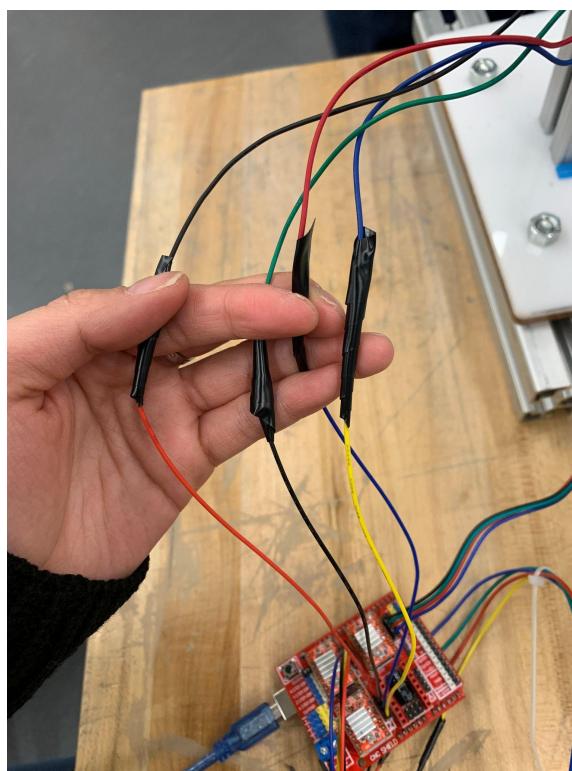
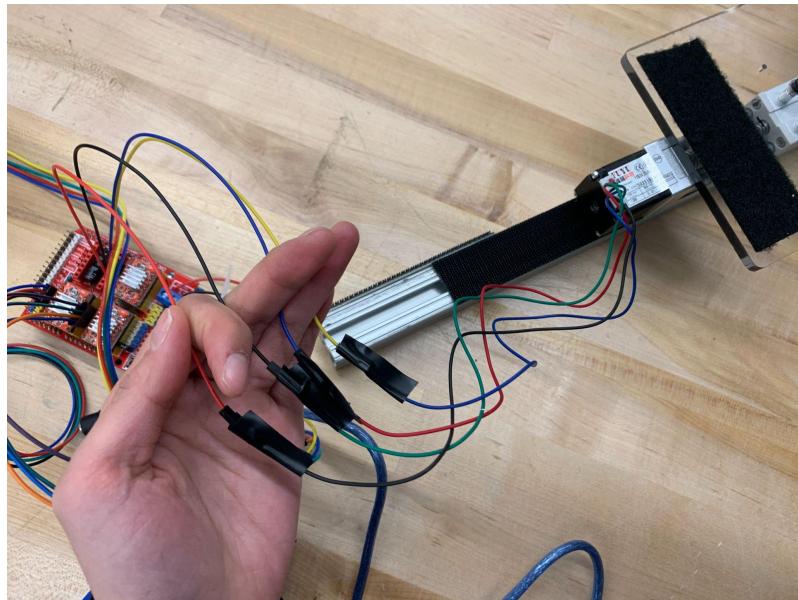
Base

Wire from Motor	Wire to Board
Blue	Yellow
Yellow	Blue
Green	Black
Red	Red



Linear Actuator

Wire from Motor	Wire to Board
Black	Red
Green	Black
Red	Blue
Blue	Yellow



APPENDIX G: Code Run Down

Overview

The code I've written is broken down into 4 main sections.

1. Variable Definition
2. Initialization (motor specifications and manual)
3. Main Code (the gel recipe)
 - a. Demo
 - b. Gel Making Process
4. Function Definition

At the end I also talk about emergency stops.

You'll have to comment in/ out sections to get it to run properly. I'll go into more detail with each section, but essentially:

- Variable Def and function definitions do not change
- Motor Initialization stays
- Manual Initialization is run on its own (Main Code should be commented out)
- Main Code runs on its own (Manual Initialization lines must be commented out)

Variable Definition

Shouldn't need to worry about this too much, but these variables are used to specify motor speeds, acceleration, and keep track of current position.

```
#include <AccelStepper.h>

// Variable Definition =====
#define MOTOR_STEPS 200
#define MICROSTEPS 32
#define TOTAL_STEPS 6400

#define X_SPEED 60000 // X steps per second (syringe)
#define Y_SPEED 300 // Y (base)
#define Z_SPEED 50000 // Z (LA)

#define X_ACCEL 50000.0 // X steps per second per second
#define Y_ACCEL 3000.0 // Y
#define Z_ACCEL 3000.0 // Z

#define EN 8 // stepper motor enable, low level effective (note put

#define X_DIR 5 // X axis, direction pin
#define Y_DIR 6 // Y
#define Z_DIR 7 // Z

#define X_STP 2 // X axis, step pin
#define Y_STP 3 // Y
#define Z_STP 4 // Z

// Function Variables =====
int Res_Num = 0;
int rise = 80;
int plate_rise = 10;
// 70 + plate_rise = rise
```

An important line at the top is the library we use to create the functions: AccelStepper.h.

Documentation here:

<http://www.airspayce.com/mikem/arduino/AccelStepper/classAccelStepper.html>

Other variables to note, that I change:

```
int rise = 80;  
int plate_rise = 10;  
// 70 + plate_rise = rise
```

And, as the comment says, $\text{rise} = 70 + \text{plate_rise}$. It's all math to travel the 70 mm of the reservoir, and ensures that my functions will return to 0.

Initialization

Motor

Again, don't worry too much about these, but it essentially tells the motor what speeds/accelerations they should be moving at.

```
void setup() {  
    Serial.begin(9600);  
  
    // Max Speed  
    syringe.setMaxSpeed(X_SPEED);  
    base.setMaxSpeed(Y_SPEED);  
    LA.setMaxSpeed(Z_SPEED);  
  
    // Acceleration  
    syringe.setAcceleration(X_ACCEL);  
    base.setAcceleration(Y_ACCEL);  
    LA.setAcceleration(Z_ACCEL);
```

Manual

To manually initialize the machine, essentially we move each motor individually in increments

Main Code

The code I wrote here follows this sequence:

1. Header Initialization
2. Pulls from AM, puts appropriate amount into mixing chamber 1(M1) and Mixing chamber 2 (M2)
3. Getting running buffer
4. Stacking buffer

5. H2O
6. SDS
7. AP
8. TEMED to just M1
9. Mixes M1
10. Pulls everything out from M1 into the stacking plates
11. Adds a small amount of leveling H2O (currently 0.5mL)
12. Waits an hour
13. Adds TEMED to M2
14. Mixes
15. Pulls from M2 to plates
16. Pushes the comb down
17. Waits 30 minutes
18. Footer Initialization (returns to 0)

Important! Even if the code changes inside, always keep the header/footer code.

```
base.setCurrentPosition(0);
syringe.setCurrentPosition(0);
LA.setCurrentPosition(0);

BaseTo(-1);
up(plate_rise);

// END initialization
BaseTo(-1);
down(33);
BaseTo(0);
```

** Note about the footer: right now it's set to go down 33, because the comb insertion had it go up 30. To set it back to 0, it goes down the 30 it raised. I calculate everything off of a 0 from the top of the plates.

This will simplify the debugging process by bringing it back to the *initial 0 state* at the very end of the code so you won't have to re-initialize all from scratch.

Function Definition

I'm not sure how important it is to run through every function I created but here's the gist of it. There are functions controlling the syringe, base, and LA.

Syringe Functions

Function Name	Input(s)	Description
measureAmt(float mL)	Measurement, in mL	Will measure the amount (mL) *

plungeAmt(float mL)	“	‘’ but expelling fluid.
mix(int amt)	A measurement, in mL, of how large the mixing volume should be	Will mix, internally, the volume specified (mL), twice. Ends above the reservoir
flush()	N/A	Essentially has a water mix, rises, then air flushes.
plunge(float mL, int res)	Measurement, in mL, and the reservoir number	Moves to a reservoir (res) and expels (mL), ends above that reservoir
suck(float mL, int res)	“	‘’ as plunge, but measuring
reservoir(float m1, float m2, int res)	m1 specifies the amount measured into mixing chamber 1 (currently set as running gel), m2 for mixing chamber 2 (stacking). res specifies which chemical reservoir to get from	Handles traveling to a reservoir, measuring m1+m2 from that chemical, moving to m2, outputting, then m1, outputting, and then flushes. It will end above flush
buffer(float mL, int res, int M)	Amount, in mL Res reservoir number M is a mixing chamber, either 1 or 2 .	Similar to reservoir, but it will only move to (res), measure (mL), and then output it into the mixing chamber you specify (M)

* If you notice, the functions have a commented out line where we add 12000 steps. This was while we were trying to handle the error volume or air inside the syringe tip. Thought it was messing with measurements. But we were unsure. AKA, still needs fixing

LA Functions

Function Name	Input(s)	Description
up(float mm)	Mm measurement to travel	Moves up the input (mm)
down(float mm)	“	‘’, but down

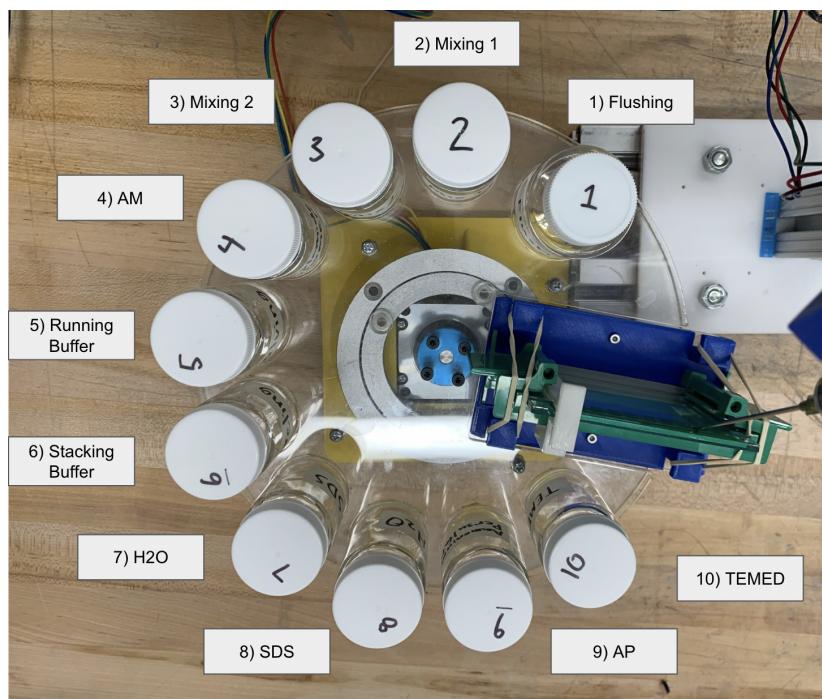
Base Functions

Function Name	Input(s)	Description

BaseTo(int Res_Num)	The number of the reservoir you'd like to travel to	Moves the carousel to the specified reservoir (Res_Num)
----------------------------	---	---

Reservoir Positions

1. Flushing
2. Mixing Chamber 2
3. Mixing Chamber 1
4. Acrylamide Monomer
5. Running Buffer
6. Stacking Buffer
7. H₂O
8. SDS
9. AP
10. TEMED



Emergency Stop(s)

There is no emergency stop or cancellation through the code end, but what I ended up doing:

- Unplugging the power
 - Essentially, unplug this connection from the 12 V adaptor to the arduino board
 - However, the code is still running, and commands are being sent to the motors. They just don't have power to move. You'll have to estimate when the code has finished before plugging back the power, or else it will resume moving.

- Uploading blank code
 - As soon as I uploaded code, I commented out all the active lines of code within the setup() section, and was ready to re-upload if I saw unexpected behavior
 - (pro tip: command + / is a fast way to comment out a line, or multiple lines if you've selected them)

APPENDIX H: Code

```
// AccelStepper is the class we use to run all of the motors in a parallel fashion
// Documentation can be found here:
http://www.airspayce.com/mikem/arduino/AccelStepper/classAccelStepper.html
#include <AccelStepper.h>

// Variable Definition =====
#define MOTOR_STEPS 200
#define MICROSTEPS 32
#define TOTAL_STEPS 6400

#define X_SPEED 600000 // X steps per second (syringe)
#define Y_SPEED 300 // Y (base)
#define Z_SPEED 50000 // Z (LA)

#define X_ACCEL 500000.0 // X steps per second per second
#define Y_ACCEL 3000.0 // Y
#define Z_ACCEL 3000.0 // Z

#define EN 8 // stepper motor enable, low level effective (note put jumper so automatic)

#define X_DIR 5 // X axis, direction pin
#define Y_DIR 6 // Y
#define Z_DIR 7 // Z

#define X_STP 2 // X axis, step pin
#define Y_STP 3 // Y
#define Z_STP 4 // Z

// Function Variables
int Res_Num = 0;
int rise = 80;
int plate_rise = 10;
// 70 + plate_rise = rise

// Defining Motor objects
AccelStepper base(AccelStepper::DRIVER, X_STP, X_DIR);
AccelStepper syringe(AccelStepper::DRIVER, Y_STP, Y_DIR);
AccelStepper LA(AccelStepper::DRIVER, Z_STP, Z_DIR);
```

```

void setup() {
  Serial.begin(9600);

  // Motor Specs
  syringe.setMaxSpeed(X_SPEED);
  base.setMaxSpeed(Y_SPEED);
  LA.setMaxSpeed(Z_SPEED);

  syringe.setAcceleration(X_ACCEL);
  base.setAcceleration(Y_ACCEL);
  LA.setAcceleration(Z_ACCEL);

  // Manual Initialization =====
  // (must comment in to initialize to 0 manually)
  // Notes: Syringe +Push -Pull | Linear Actuator (LA) use up/down() | Base +CCW -CW
  // Measurements: Syringe(~1mL-24000 steps) | LA: up/down(mm) | Base(~550 from
  plate-flush, ~250 res-res)
  Serial.println("start!!!!!!!!!!!!!!");

  // syringe.runToNewPosition(-1000);
  // up(10);
  // down(1);
  // base.runToNewPosition(5);
  // down(3);

  // DEMO CODE =====
  /*
  base.setCurrentPosition(0);
  syringe.setCurrentPosition(0);
  LA.setCurrentPosition(0);

  BaseTo(-1);
  up(plate_rise);

  // Reservoir (from 1, flush)
  reservoir(1.0, 1.0, 4); // From AM

  // From Flush -> Plates
  BaseTo(1);
  down(rise);

```

```
measureAmt(1.0);
up(rise);
BaseTo(-1);
down(plate_rise);
BaseTo(0);
plungeAmt(1.0);
```

```
// Comb
BaseTo(-1);
up(33);
BaseTo(-2);
down(95);
up(95);
```

```
BaseTo(-1);
down(33);
BaseTo(0);
*/
```

```
// CODE TO RUN =====
```

```
base.setCurrentPosition(0);
syringe.setCurrentPosition(0);
LA.setCurrentPosition(0);
```

```
BaseTo(-1);
up(plate_rise);
```

```
// AM
reservoir(2.81, 0.5,4);
```

```
// Running Buffer (Mixing Chamber 1)
buffer(2.81,5,1);
// Stacking Buffer (Mixing chamber 2)
buffer(1.25,6,2);
```

```
// H20
reservoir(2.43,0.2,7);
```

```

// SDS
reservoir(1.00,1.00,8);
//AP
reservoir(1.00,1.00,9);

// TEMED ; Running @ M1
buffer(1.0,10,1);

mix(6.0);

measureAmt(5.5); // gather first 1/2 running
BaseTo(-1);
down(plate_rise);
BaseTo(0);
plungeAmt(5.5);
BaseTo(-1);
up(plate_rise);

suck(6.0,2); // gather last 1/2 running
BaseTo(-1);
down(plate_rise);
BaseTo(0);
plungeAmt(6.0);
BaseTo(-1);
up(plate_rise);

// Leveling (H2O)
suck(0.5,7);
BaseTo(-1);
down(plate_rise);
BaseTo(0);
plungeAmt(0.5);
BaseTo(-1);
up(plate_rise);

// Solidify
delay(3600000); // Should be 30-60mins, 3600000

// TEMED ; Stacking @ M2
buffer(1.0,10,2);

```

```

mix(6.0);

measureAmt(5); // gather stacking
BaseTo(-1);
down(plate_rise);
BaseTo(0);
plungeAmt(5);
BaseTo(-1);

// Push Comb
up(33);
BaseTo(-2);
down(95);
up(95);

delay(1800000); // should be 15-30mins

// END initialization
BaseTo(-1);
down(33);
BaseTo(0);

Serial.println("Finished Code");

}

void loop() {
  // put your main code here, to run repeatedly:
}

// Function Definitions =====
// SYRINGE FUNCTIONS
// measureAmt(_mL_) // Will use syringe to measure inputted amount in mL
void measureAmt(float mL) {
  Serial.println("inside measure-----");
  float steps = mL * 24000; // mL * 6mm/mL * (3200/.8 steps/rev) [steps/rev] / [mm/rev]
  // steps = steps + 12000;
}

```

```

syringe.runToNewPosition(-steps); // negative to pull
syringe.setCurrentPosition(0);
}
void plungeAmt(float mL) { // Will use syringe to expel the inputted amount in mL
delay(1000);
Serial.println("inside plunge-----");
float steps = mL * 24000;
// steps = steps + 12000;
syringe.runToNewPosition(steps);
syringe.setCurrentPosition(0);
}

void mix(int amt) { // Up and down in place mixing, will fill and expel twice.
down(rise);
measureAmt(amt);
plungeAmt(amt);
measureAmt(amt);
plungeAmt(amt);
up(rise);
}

void flush() { // Manually measures + expels 1mL of water + an air flush when run
Serial.println("Flushing-----");

// Water
BaseTo(1);
mix(1.0);

// Air
measureAmt(1.0);
plungeAmt(1.0);
}

void plunge(float mL, int res) { // Will move to res, and output mL; ends above res
BaseTo(res); // M2
down(rise);
plungeAmt(mL);
up(rise);
}

```

```

void suck(float mL, int res) { // Will go to res, then measure mL; ends above res
    BaseTo(res);
    down(rise);
    measureAmt(mL);
    up(rise);
}

void reservoir(float m1, float m2, int res) {
    // Handles travelling to a reservoir, measuring m1+m2 from that chemical, moving to m2,
    outputting,
    // then m1, and outputting. Then flushes. Ends above flush
    BaseTo(res);
    // float amt = m1+m2;
    // suck(amt, res);
    down(rise);
    measureAmt(m1);
    measureAmt(m2);
    up(rise);

    // float amt2 = m2;
    plunge(m2, 3); // Deposit in Mixing 2
    plunge(m1, 2); // Deposit in Mixing 1

    flush();
}

void buffer(float mL, int res, int M) { // Will measure mL from res, into M.
    suck(mL, res);
    if (M == 1) {
        M = 2;
    } else if (M == 2) {
        M = 3;
    } else {
        Serial.print("Buffer error");
        return;
    }
    plunge(mL, M);

    flush();
}

```

```

// LA FUNCTIONS
void up(float mm) { // Moves up mm
    float steps = mm * 1579.3;
    LA.runToNewPosition(steps);
    LA.setCurrentPosition(0);
}

void down(float mm) { // Moves down mm
    up(-mm);
}

// BASE FUNCTION
void BaseTo(int Res_Num) { // Will travel to that base position number
    if (Res_Num == 0) {
        base.runToNewPosition(0);
        base.setCurrentPosition(0);
    }
    if (Res_Num == 1) { //flushing
        base.runToNewPosition(-551);
        base.setCurrentPosition(-551);
    }
    if (Res_Num == 2) {
        base.runToNewPosition(-837);
        base.setCurrentPosition(-837);
    }
    if (Res_Num == 3) {
        base.runToNewPosition(-1080);
        base.setCurrentPosition(-1080);
    }
    if (Res_Num == 4) {
        base.runToNewPosition(-1325);
        base.setCurrentPosition(-1325);
    }
    if (Res_Num == 5) {
        base.runToNewPosition(-1561);
        base.setCurrentPosition(-1561);
    }
    if (Res_Num == 6) {
        base.runToNewPosition(-1817);
    }
}

```

```

base.setCurrentPosition(-1817);
}
if (Res_Num == 7) {
  base.runToNewPosition(-2070);
  base.setCurrentPosition(-2070);
}
if (Res_Num == 8) {
  base.runToNewPosition(-2309);
  base.setCurrentPosition(-2309);
}
if (Res_Num == 9) {
  base.runToNewPosition(-2560);
  base.setCurrentPosition(-2560);
}
if (Res_Num == 10) {
  base.runToNewPosition(-2802);
  base.setCurrentPosition(-2802);
}
if (Res_Num == -1) { // half way between plates and flushing
  base.runToNewPosition(-271);
  base.setCurrentPosition(-271);
}
if (Res_Num == -2) { // between plates and positon 10 - ps. beware plate height
  base.runToNewPosition(130);
  base.setCurrentPosition(130);
}
}
}

```



Love, Team Agaros3