Stony Brook University 2022 PROS Team Notebook

JANUARY

Week 1: Jan 24 - Jan 30

- Team Creation
  - This week marked the creation of the 2022 official Stony Brook iGEM team. We picked all team members:
    - Isam Adnan; Class of 2024
    - Ya Jing Chen; Class of 2024
    - Stephanie Laderwager; Class of 2023
    - Maulik Masaliya; Class of 2023
    - Eva Paruch; Class of 2023
    - Ahmed Rehman; Class of 2023
    - Maira Riaz; Class of 2023
    - Lori Saxena; Class of 2025
    - Namrata Singh; Class of 2025
    - Divleen Singh; Class of 2025
    - Ziyin Zhang; Class of 2024
  - We started to get in touch with each other and set up a weekly meeting time. We also got in touch with our student advisors, and joined a group chat with them.

FEBRUARY

Week 2: Jan 31 - Feb 6

- Assignments and Project Development
  - Our assignments for the week included developing a basic understanding of synthetic biology by watching videos and reading various articles. Each individual on our team began brainstorming a list of potential project ideas. We also registered for the annual iGEM Spring Reading Course offered through our university.

Week 3: Feb 7 - Feb 13

- Project Development
  - Each individual team member wrote a list of possible projects, and shared them with the rest of the team. Everyone was responsible for verbally communicating their ideas, and the pros and cons of each topic. We actively worked to give each

other feedback and did extensive literature searches. We compiled all of our research ideas into a google document which summarized each of the ideas and provided links to related journal articles.
    - ➢ **https://docs.google.com/document/d/11KtH-bzM5lkPACJJUx5KNZnGIZ7RY_k0tBELI5zNUdg/edit**

Week 4: Feb 14 - Feb 20

- ❖ Assignments and Project Development
    - ➢ We continued working to further develop our project ideas and present them to each other as we gathered more information and updated our ideas. We also continued taking lessons on synthetic biology provided by our student advisors, working to establish a foundation of background knowledge in order to bring our project to life.
    - ➢ At the end of the week, we narrowed down our list of potential projects. The team split into three sub-teams, each of which was responsible for investigating and presenting a particular topic. The three sub-teams were as follows:
        - Team 1: Protein S Synthesis
            - Team members: Divleen, Maira, Lori, and Ahmed
        - Team 2: PET-ase as a Plastic Degradation Tool
            - Team members: Eva, Maulik, Isam, and Ziyin
        - Team 3: Space Bugs - Engineering fungi with manganese complexes to repair DNA damage from ionizing radiation to have them become a microbial cell factory in space.
            - Team members: Stephanie, Namrata, and Ya Jing

Week 5: Feb 21 - Feb 27

- ❖ Reading Assignments and Project Development
    - ➢ Everyone on the team watched a thorough tutorial on how to use SnapGene to design plasmids. We each practiced making our own plasmids using the software, and getting comfortable with the program.
    - ➢ We also worked to get more comfortable with addgene, the iGEM registry and NCBI.
    - ➢ Each sub-team was working on developing elevator pitches for their respective projects at this time

MARCH

Week 6: Feb 28 - Mar 6

- ❖ Project Development
  - ➢ Each team continued to research their chosen research topic and our student advisors taught us how to make an effective presentation in order to communicate each of our projects. Furthermore, we studied previous Stony Brook University iGEM teams' presentations.

Week 7: Mar 7 - Mar 13

- ❖ Project Development
  - ➢ Each sub-team presented their proposed projects to our student advisors and fellow iGEM team members, and received extensive feedback. In the presentations, each team made sure to answer the following questions in under 10 minutes:
    - ■ What are we tackling?
    - ■ Why is this issue important?
    - ■ What is our proposed solution?
    - ■ How will we use synthetic biology to solve the problem?
    - ■ How will we measure and verify our results (tentative assays)?
    - ■ What is the draft of our plasmid?
  - ➢ All the presentations were uploaded to YouTube for further review and discussion: **https://www.youtube.com/watch?v=-DhaOPps2Kg**

Week 8: Mar 14 - Mar 20

- ❖ We were on spring break!

Week 9: Mar 21 - Mar 27

- ❖ Project Development
  - ➢ We narrowed down our potential project topics from three to two. Every member on our team voted on the two projects they felt were the most viable. Ultimately, the following projects were continued, with the following, expanded sub-teams. Each team then proceeded in fine-tuning their presentations and project ideas based on the feedback they received. These presentations were created to present to our advisors the following week.
    - ■ Team 1: Protein S Synthesis
      - ● Team members: Divleen, Maira, Lori, Ahmed, Eva, and Maulik

        - Team 2: Space Bugs - Engineering fungi with manganese complexes to repair DNA damage from ionizing radiation to have them become a microbial cell factory in space.
            - Team members: Stephanie, Namrata, Ya Jing, Isam, and Ziyin

APRIL

Week 10: Mar 28 - Apr 3

- ❖ Project Development
    - ➢ Each sub-team presented their project to the rest of the team members, our student advisors, and faculty advisors. The advisors included
        - John Peter Gergen
        - Gabor Balazsi
        - Joshua Rest
        - Steven Glynn

      Each team fielded questions and received extensive feedback on the feasibility and technicalities of their project.
    - ➢ After the presentations, the team members discussed which of the projects would be more feasible. This helped the team conduct an informed vote.
    - ➢ Detailed notes were taken and shared with all team members: **https://docs.google.com/document/d/1PcSxi0B1KZG6Vpd-NMM-kjsshQ7qso49oqTkIW0iMEs/edit**

Week 11: Apr 4 - Apr 10

- ❖ Project Development
    - ➢ All of the team members voted on their top project. Factors such as advisor feedback, feasibility, cost-efficiency, etc. were used to determine which would ultimately become our final topic. The results of the vote were announced, and the synthesis of protein S was picked as the 2022 Stony Brook iGEM Team project.
    - ➢ We also had a vote for our two team leaders. Eva Paruch and Divleen Singh were chosen.
- ❖ Wet Lab
    - ➢ We started contacting Dr. Stephen Glynn and Dr. Michael Airola to discuss the technical implementation of our project. We started looking into potential cell lines and purification techniques.

Week 12: Apr 11 - Apr 17

- ❖ Assignments

- ➢ All of our members attended a lecture on splicing, which was conducted by our student advisors. We took detailed notes about what we learned, and we studied synthetic biology techniques in more depth throughout the week. This helped give us a greater understanding of how we would conduct our project and successfully synthesize protein S.
- ➢ A summary of what we learned can be read at this link: **https://docs.google.com/document/d/1lsxckKR8zcu3bmOI8hqeS3tv73u-_pxQ/edit**

❖ Fundraising
- ➢ We also started researching possible grants we could apply to in order to secure the funding necessary for our project. Specifically, we looked into the Promega 2022 iGEM grant.
- ➢ 10 out of our 11 members applied for individual funding in order to procure stipends that would enable each member to devote their summer to our project without financial burden.

❖ Wet Lab
- ➢ We continued researching the following cell lines' ability to perform post translational modifications, splicing, and folding: CHO (hamster line), HEK293 (human line) and S2 (insect line).
- ➢ We continued discussing with Dr. Stephen Glynn about choosing between the listed cell lines and the purification techniques.

Week 13: Apr 18 - Apr 24

❖ Project Development
- ➢ Members on our team began familiarizing themselves with project details, the problem statement, and goals. We did a thorough literature search about protein S deficiency and associated disorders.

❖ Fundraising
- ➢ Every member on our team who applied for summer stipends received money through our university that greatly contributed to allowing us to focus on our project without financial worries. Among 10 members, we received a total of $38,700 for housing and transportation costs.
- ➢ Steve Galson provided $20,000 for registration fees and Jamboree participation

❖ Team Assignments
- ➢ We divided the team into sub-teams and assigned each member to a specific aspect of the project. The assignments are as follows:
  - ■ Outreach
    - Team Lead: Lori
    - Members: Stephanie, Ya Jing, and Nami

- Wet Lab
  - Team Leads: Maulik and Eva
  - Members: Maira, Nami, Ya Jing, Stephanie, Lori, and Divleen
- Dry Lab
  - Team Lead: Stephanie
  - Members: Lori, Maulik, and Divleen
- Wiki Production
  - Team Lead: Ahmed
  - Members: Isam and Ziyin

MAY

Week 14: Apr 25 - May 1

- Outreach
  - We began writing our individual bios, and designing our team brochure, which we sent out to various organizations, individuals, and companies in order to procure funds, materials, and support for our project.
- Fundraising
  - We created a WeSci account which allowed us to create a crowdfunding fundraiser targeted towards scientific communities.
  - We first pitched the idea of contacting ThermoFisher about sponsoring our laboratory reagents and expendables.
- Wet Lab
  - We continued researching cell lines. We were leaning towards HEK human cell line since it would allow a high fidelity to the human protein S we were attempting to produce. We did not yet exclude the choices of CHO or insect cells as well. We excluded yeast since it did not offer sufficient post-translational modifications and other complex processing.
  - In our continued search for a cost effective cell line, we began comparing expression kits and asking the graduate students of Ed Luk Laboratory, Leonidas Perrakeas and Cindy Converso.
  - We began researching cell-free expression as an alternative to the cell lines.
  - We began seeking a company to order the gene of interest from.

Week 15: May 2 - May 8

- Outreach

- ➢ We began actively designing content and posting on our team Instagram in order to spread the word about iGEM and our project. We also started brainstorming possible logos and team names.

❖ Competition Initiatives
- ➢ We attended the Opening Day Kickoff Webinar

❖ Wet Lab
- ➢ We did an inventory check of all supplies that we had available.
- ➢ We contacted Dr. Daniel Moloney who could have offered a donation of CHO and/or HEK cell lines.
- ➢ Dr. Glynn had helped us clarify a misconception that we needed a pilot TALON pull-down as one of the pre-purification steps. We learned that SDS-PAGE and probing with anti-His antibody should suffice instead.
- ➢ We found a reasonably priced cell-free expression kit from Promega.
- ➢ We considered using mammalian tissue culture and cell-free expression in parallel. We still have not excluded SF9 cells.

Week 16: May 9 - May 15

❖ Outreach
- ➢ We reached out to various professionals and organizations about our project. We sent them our brochure and our proposed project solution. The individuals we reached out to included:
  - ■ Professor Kim Jones at Suffolk Community College
  - ■ Professor Camilo Salazar at Suffolk Community College
  - ■ Biology Department at Suffolk Community College
  - ■ Brookhaven National Laboratory Leader Doon Gibbs
  - ■ iGEM: NYCU-Taipei (2021)
  - ■ iGEM: Oneonta (2022)
  - ■ iGEM: Tas Taipei (2021)

❖ Fundraising
- ➢ We continued reaching out to various corporations, requesting funding and support for our project. Contract Pharmaceuticals offered us a donation of $1,000.

❖ Wet Lab
- ➢ Ed Luk Laboratory offered to donate BL21 E. coli cells for cloning.
- ➢ We began working on several plasmid drafts for different cell lines.

❖ Wiki
- ➢ Our Wiki team had no previous coding experience, so they started teaching themselves how to design and construct the website

Week 17: May 16 - May 22

❖ Outreach

- ➢ We met with the EmpireGene team from NYC to discuss a possible collaboration between our two teams. We each discussed our projects and project goals, and how we could possibly collaborate over the course of the summer.
- ➢ We met with Dr. Francis Farraye, the alumni speaker for the Biology and Biochemistry Convocation at Stony Brook University. We introduced ourselves and our project.
- ➢ We drafted an outreach email template to send to clubs and stakeholders about our project.

❖ Fundraising
- ➢ We learned about the Team Impact Grant and started working on our application.

❖ Wet Lab
- ➢ Everyone on our team started doing the required safety trainings in order to participate in wet lab procedures. We all completed courses provided by Stony Brook University:
  - ■ ELS 003 - Laboratory Safety - Safe Handling of Biological Hazards
  - ■ ELS 002 - Lab Safety Chemical Hazards
  - ■ ENV 001 - Hazardous Waste Management
- ➢ We received approval from our university.
- ➢ We began talking to Dr. Balazsi and Rafal Krzyszton about plasmid design.
- ➢ We eliminated HEK cells from our choices, and focused on researching CHO and SF9 cells as our cell line of choice.
- ➢ We decided to use a gene of interest for protein S (PROS) that has removed all introns for ease of expression.

❖ Wiki
- ➢ The Wiki team drafted a crude version of the final website, playing around with code and creating a barebones draft.

Week 18: May 23 - May 29

❖ Outreach
- ➢ We continued doing a thorough literature review about the communities impacted by protein S deficiency. By this point we decided to specifically focus on African American and Indigenous populations due to undercharacterization of protein S deficiency in these communities.
  - ■ We began creating a list of possible stakeholders we could reach out to.
- ➢ We brainstormed possible educational and communication initiatives. We wanted to make synthetic biology accessible to and understandable by a larger audience, mainly the general public. In order to accomplish this we decided to create a journal that describeS iGEM projects and how they address social and health inequities using synthetic biology.

❖ Fundraising

- ➢ We continued working on the Team Impact Grant.
- ➢ We continued working on the Research Support Request.
- ➢ We continued working with WeSci, fine-tuning our project campaign, and writing up descriptions about the societal implications of our project.

❖ Wet Lab

- ➢ We kept having extensive conversations with Dr. Gabor Balazsi and Dr. Rafał Krzysztoń on plasmid design. He offered to donate a plasmid compatible with CHO cells, which at the time we were still considering. He also helped us detail what steps we should take towards the end of the experiment, such as SDS-PAGE provided we find if there are any other proteins with a similar weight expressed in our cell line of choice. If time permits, we were planning to do FPLC (fast protein liquid chromatography).
- ➢ We continued researching and comparing SF9 and CHO cells for cell expression, time constraints, costs, and feasibility for our team of undergraduate students.
- ➢ We decided on using a 6xHis tag on the C terminal of the protein, and the His tag would be included in our plasmid or ordered with the protein sequence.
- ➢ We work with Dr. Rafał Krzysztoń to make sure that any possible plasmids for mammalian cells have the correct promoters.
- ➢ We were deciding between transient and stable expression for the cell lines. We kept discussing this with Dr. Gabor Balazsi.
- ➢ We started looking for UV-equipped hoods to perform any possible experiments on CHO cells.
- ➢ We spoke with Katarzyna Jankowska who recommended we stick to SF9 cells since for our set of skills, time and money, they are a more appropriate fit. She also recommended we stop considering cell-free expression, since it is far above our skillset. On stable vs. transient transfection, she recommended we start with transient first of all because it is easier, and second of all because it will give us the information on what we need, so then we can decide if we should proceed with one or the other. She gave us ideas on how to assess PTMs (post translational modifications) via mass spectrometry, and how to assess function and binding via an assessment with the protein's binding partner (protein C), namely binding assay or kinetics assay. She also suggested checking if the protein has a function that is more complex than binding, and if that is the case, we could run an ELISA. Whichever is the case, however, she recommended we use commercially available assays to lower the amount of variables for us to take care of. We spoke with Katarzyna Jankowska on multiple occasions throughout the project, and we used her advice to modify our project design. Because of her input (and because of Dr. Glynn's partially) we in time decided not to use cell-free expression, and her input influenced us to use SF9 cell line as opposed to CHO.

- ➢ We got in contact with Douglass Marr who for the remainder of the project would remain our advisor on the SF9 aspect of the wet lab of our project. He offered to donate the protocols used in his lab (Dr. Michael Airola lab at Stony Brook University), and he offered to advise us through the entire process. He offered help growing the cells in suspension, which influenced us to plan to grow the cells in suspension as well. Furthermore, growing cells in suspension would not require us to use Trypsin diluents and bovine serum for the acquisition of which he had no resources.
- ➢ **We made the decision to use SF9 cells and not CHO.**
- ➢ We kept seeking cheap ways to acquire laboratory expendables and reagents since our resources were limited. We made lists of reagents to order.
- ➢ We met with Nabeel Farukh, a previous iGEM team member from Stony Brook to help us with primer design, adding His-tags, restriction enzymes and calculating annealing temperatures (Tm).
- ➢ We met with Dr. Balazsi on plasmid design for SF9 cells.
- ➢ We applied for a Promega grant.

JUNE

Week 19: May 30 - June 5

- ❖ Outreach
  - ➢ We continued doing a thorough literature review about the communities impacted by protein S deficiency.
    - ■ We continued adding to the list of possible stakeholders we could reach out to.
  - ➢ We reached out to multiple iGEM teams who could contribute to our journal, and began setting up meetings with teams from around the world.
  - ➢ We began considering planning a 5K to bring awareness to protein S deficiency. We reached out to fundraising committees at our university to bring this idea to light.
- ❖ Fundraising
  - ➢ We continued working on the Team Impact Grant.
  - ➢ We continued working on the Research Support Request.
  - ➢ We continued working with WeSci
- ❖ Wet Lab
  - ➢ We asked Thermo Fisher to give us a discount on products or to donate laboratory products needed for our project (this very influential idea on our project was pitched by Maulik Masaliya).
  - ➢ We found the specific products we would need for SF9 expression.

- ➢ We met with Dr. Balazsi and Dr. Krzysztoń, and we decided to find simpler plasmids than the ones they had to offer, since we would not be able to use restriction enzymes on their plasmids.
- ➢ We met with Lingshuang Wu, Ph. D. Candidate (PI: Airola). She explained to us how the SF9 procedure would work in detail and she gave us the opportunity to meet Dr. Airola and ask for the donated plasmid for SF9 (bacmid). We learned from Lingshuang that regular plasmids don't work for SP9 because they are insect cells. Insect cells don not accept plasmid like mammalian cells do so bacmid is how scientists can go around that.
- ➢ We met with Dr. Airola and created a basic summary of the steps we would take with SF9 cells all the way until expression.
- ➢ We met with Dr. Glynn, and he recommended we keep going with SF9, and he recommended that *E. coli* would not give the protein its necessary modifications, and out project may lose credibility. However, he also said that *E. coli* may be necessary as a baseline for comparison with SF9, and a cheaper and easier alternative for expression, which we should go through with. We listened to his advice and later **decided to go with SF9 and *E. coli* expressions. We also decided for sure not to use cell-free expression** based on his advice and Dr. Jankowska's advice.
- ➢ Dr. Glynn recommended we research the following: proteolysis (that is, make sure if there is a maturation step). If it's the second option then it may be difficult to get a credible protein that resembles the original. Secondly, we were to check what cells secrete protein S (and double check if its secreted). This mattered because we needed to know what transcription factors were needed for this protein.
- ➢ We registered for iLab to order the needed supplies.
- ➢ We considered buying a ready plasmid from Vector Builder website.
- ➢ We kept talking with Thermo Fisher representatives to acquire a donation of supplies. We continued working on the list of supplies to order using the protocols donated by Douglass Marr.

Week 20: June 6 - June 12

- ❖ Outreach
  - ➢ We continued doing a thorough literature review about the communities impacted by protein S deficiency.
    - ■ We started reaching out to stakeholders who could give us direction with our project. These included government agencies and entities who could guide us on how to better engage and address minority communities.
  - ➢ We reached out to multiple iGEM teams who could contribute to our journal, and began setting up meetings with teams from around the world.

        - Met with iGEM Maastricht
        - Met with iGEM IIT Roorkee
            - Agreed to collaborate on the journal!
        - Met with iGEM EmpireGene
            - Agreed to collaborate and translate on the journal!
- Fundraising
    - We reviewed and submitted the Research Support Request
    - We continued working on the Team Impact Grant.
    - We continued working with WeSci, i.e. made a plan of what deliverables to create for social media.
- Wet Lab
    - **Thermo Fisher agreed to donate supplies to us!**
    - We met with Dr. Glynn as a team.
    - We checked whether it agrees with iGEM rules to order a finished vector from Vector Builder, and the answer was yes.
    - We kept in contact with Nabeel Farrukh on plasmid design.
    - We contacted Vector Builder asking for sponsorship.
    - **We made the first draft of SF9 plasmid** using the vector donated by Airola lab. We confirmed the draft with Dr. Airola.
    - We awaited the plasmid sequences from Dr. Glynn for *E. coli* expression.
    - Maulik Masaliya so far was the team membered who worked on plasmid design, and Eva Paruch worked on cell line choices, and protocols.
    - **We made a draft of *E. coli* plasmid.**
    - All team members finished laboratory safety training at this point.
    - We decided which restriction enzymes to use: BamHI and HindIII but ordered only from NEB due to compatibility issues.

Week 21: June 13 - June 19

- Outreach
    - We continued doing a thorough literature review about the communities impacted by protein S deficiency.
        - We set up meetings with stakeholders that included:
            - Dr. Gregson Pigott, the Suffolk County Health Commissioner
            - The Office of Minority Health
            - A representative of Senator Chuck Schumer
    - We continued reaching out to and meeting with multiple iGEM teams about the journal collaboration
        - Met with iGEM Queens
            - Agreed to collaborate on the journal!

        - Met with iGEM Monterrey
            - Agreed to collaborate on the journal!
        - Met with iGEM ASU
            - Agreed to collaborate on the journal!
        - Met with iGEM Montreal
            - Agreed to collaborate on the journal!
    - ➢ Started reaching out to clubs and organizations with whom we could plan educational events
    - ➢ Started reaching out to clubs and organizations that could help us increase inclusivity in STEM and science more generally
    - ➢ Brainstormed how to increase diversity in STEM, did literature search about why it was important
- ❖ Fundraising
    - ➢ We reviewed and submitted the Research Support Request
    - ➢ We continued working on the Team Impact Grant.
    - ➢ We scripted and filmed a promotional video for the WeSci campaign
- ❖ Wet Lab
    - ➢ Getting ready to order the gene of interest, we considered deleting the 40 amino acid end-piece of PROS that gets deleted post-translationally. However, we decided not to delete it because it is a signaling peptide. We hope that this basic maturation step can be carried out by the cell lines of our choice. We think that without that peptide, our produced protein S would not be secreted extracellularly as it ought to be.
    - ➢ We continued ordering using Thermo Fisher donations and our own resources.
    - ➢ We ordered the gene sequence optimized and with primers attached, but the primers were not optimized to make sure the primers work with the plasmid.
    - ➢ Dr. Glynn offered to donate supplies to us for use for *E. coli* expression.
- ❖ Dry Lab
    - ➢ We reached out to Dr. Mei Lin Chan in order to get an idea of how to proceed with the dry lab.
    - ➢ We read many iGEM past projects to understand how to construct a mathematical model, and did a literature review to learn more about the theory behind modeling. All these sources are listed under the dry lab references.
    - ➢ We brainstormed possible ways to model our protein
        - Ab-initio modeling
        - Homology modeling
        - Threading/Fold Recognition

Week 22: June 20 - June 26

- ❖ Outreach

- ➢ Designed and distributed an infographic to stakeholders about our project and how protein S deficiency affects minority communities
- ➢ We had meetings with stakeholders that included:
  - Dr. Gregson Pigott, the Suffolk County Health Commissioner
  - The Office of Minority Health
  - A representative of Senator Chuck Schumer
- ➢ We reached out to and met with Dr. Deborah Zelizer, a professor at our university who could help us create a survey that would gauge how protein S deficiency affects minority communities
  - Based on Dr. Pigott's feedback that African American and Indigenous communities are often distrustful of their healthcare providers, we also wanted to include this aspect in our survey
- ➢ We started creating diagrams to model the framework of our human practices work
- ➢ We wrote up our takeaways and feedback from the stakeholders we met with, as well as the scientific experts we had contacted in previous months.
- ➢ We met with the CSTEP club, which aims to increase the number of minority students in STEM fields, and set up a workshop with them.
- ➢ We met with a representative from the campus newspaper, *The Statesman,* who gave us insight into how journalists typically approach researchers, and how we could be better at communicating our work
  - Established a collaboration to create podcasts about STEM research to distribute to the general public
- ➢ We met with the Pre-Genetic Counseling Society and started designing educational workshops to be held in the fall
- ➢ We met with the Society of Women Engineers to host workshops aimed at increasing the participation of women in STEM fields
- ➢ We met with the University of Rochester iGEM team and decided to collaborate through a children's workbook to communicate synthetic biology to younger kids.
- ➢ Met with iGEM Yale

❖ Fundraising
- ➢ We reviewed and submitted the Team Impact Grant.
- ➢ We took team headshots and pictures for the WeSci campaign

❖ Wet Lab
- ➢ Thanks to Dr. Glynn, we realized that the ordered gene of interest with primers has incorrect primers. We redesigned the primers, and ordered new ones to get ready to re-PCR them onto the gene of interest.

❖ Dry Lab
- ➢ We met with Christopher Helenek, a graduate student who gave us insight into mathematical modeling

- ➢ We determined what we wanted to accomplish through our mathematical modeling
    - Decided on ODE models
        - Decided to construct models for both E.coli and SF9 cell lines
        - Found the parameter values for E.coli for our ODE models
        - Downloaded MATLAB
- ➢ Received and analyzed ab-initio and threading/fold recognition protein models

Week 23: June 27 - July 3

- ❖ Outreach
    - ➢ We met with the Society of Asian Scientists and Engineers and started designing educational workshops to be held in the fall
    - ➢ Met with Dr. Zelizer to discuss survey questions
        - Compiled a literature review for survey questions
    - ➢ Developed infographic about how protein S deficiency is related to COVID-19
    - ➢ Reached out to representatives of the Shinnecock Indian Reservation about receiving feedback on how to better tailor our project to address their needs
    - ➢ Met with iGEM Seoul Korea
        - Agreed to collaborate on the journal!
    - ➢ Continued adding to the writeup for the website for Human Practices
- ❖ Fundraising
    - ➢ Determined campaign kickoff date with WeSci
    - ➢ We started organizing fundraising events
        - Tabling in our campus, selling iGEM charms and distributing infographics
        - Started planning for the 5K on campus in the fall
- ❖ Wet Lab
    - ➢ We continued ordering supplies.
    - ➢ Our primers arrived, and we diluted them into the needed concentrations.
    - ➢ **We began wet laboratory work for SF9.**
    - ➢ We PCR-ed new primers onto the ends of the gene of interest
    - ➢ PCR-ed new primers onto the ends of the YMBac II
    - ➢ Ran a DNA gel with both products of the PCR reaction (modified YmBac II and modified GOI) to see if they have been amplified and have correct weight.
- ❖ Dry Lab
    - ➢ Found rate parameter values for SF9 cells
        - Created ODE system for mathematical model
    - ➢ Wrote up the dry lab portion of the wiki that we completed, including an introduction to mathematical modeling and what we hoped to accomplish through our models
    - ➢ Continued homology protein modeling

- ➢ Started learning how to use MATLAB and create code to model our system
- ➢ Brainstormed possible diagnostic assays to model for type II protein S deficiency

❖ Wiki

JULY

Week 24: July 4th - July 10

❖ Outreach
- ➢ Created Inclusive Research Guide
  - ■ Emailed Inclusive Research Guide to The NIHR Centre for Engagement and Dissemination (CED)
- ➢ Met with Professor Zelizer to go over Protein S deficiency survey adjustments
- ➢ Met with iGEM UCSC to talk about contributing to the journal
- ➢ Met with iGEM Taiwan to talk about contributing to the journal
- ➢ Created infographics for *All Things STEM* and *Diversity, Equity, and Inclusion in STEM* podcasts

❖ Wet Lab - SF9
- ➢ Step 6 of the LIC cloning protocol: "Use the PCR clean up kit to remove the digested DNA and do the wash step twice. The double wash is to thoroughly remove dNTPs that could otherwise interfere with the T4 resection reaction." : PCR product clean up
- ➢ Nanodrop to determine the concentration of the cleaned product.
- ➢ Calculations to find the volume of cleaned up PCR product needed for LIC
- ➢ Making Ampicillin LB agar plates to grow the transformants on
- ➢ Cloning and plating the Nova Blue E. coli with the plasmid+gene of interest cloned into them
- ➢ Moving the experimental plate, and the positive and negative control plates from 24 C dark incubator into 37 C dark incubator

❖ Wet Lab - *E. coli*
- ➢ The gene of interest PROS was amplified using PCR. We ran a gel to check if the PCR was successful. Cultures of DH5-alpha *E. coli* cells containing 2Bc-T plasmid were grown overnight and miniprepped. Concentrations of the gene and plasmid were recorded.

❖ Dry Lab
- ➢ Analyzed the graphs that were produced from our mathematical model
- ➢ Brainstormed ideas for our next mathematical model
  - ■ Using a for loop in MATLAB to alter initial conditions
  - ■ Possibly construct an ROC curve for type II deficiency assay
  - ■ Stochastic Model
- ➢ Developed mathematical modeling guide

- ❖ Wiki

Week 25: July 11th - 17th July

- ❖ Outreach
  - ➢ Met with iGEM Copenhagen to discuss collaborating on the journal
  - ➢ Met with iGEM Rochester to discuss wet lab collaboration
  - ➢ Began writing Protein S deficiency assessment guide
  - ➢ Emailed Jill Santiago at the Center for Social Justice and Human Understanding
- ❖ Fundraising
  - ➢ Met with WeSci to discuss fundraising campaign
- ❖ Wet Lab - SF9
  - ➢ Running a positive control using intact YMBacII plasmid: transforming Nova Blue with intact plasmid and plating them
  - ➢ We redid adding primers to the gene and to the vector by PCR
  - ➢ Redo PCR the vector using a new annealing temperature 62 C
  - ➢ Redo the vector PCR with 55 and 62 annealing temperatures and several corrected steps and new vector from stock
  - ➢ Running all 90 ul of PCR product left (the vector) on 0.7 % agarose gel. Then performing a gel extraction
- ❖ Wet Lab - *E. coli*
  - ➢ The miniprepped 2Bc-T plasmid was then amplified by PCR and we ran a gel to check if the PCR was successful. This process was repeated and troubleshooted multiple times.
- ❖ Dry Lab
  - ➢ Decided to use a for loop in MATLAB to alter initial DNA concentration
  - ➢ Wrote the script for our second mathematical model
  - ➢ Began the wiki write up for the altered concentration mathematical model
  - ➢ Started distributing mathematical modeling guide to various iGEM teams
- ❖ Wiki

Week 26: July 18th - 24th July

- ❖ Outreach
  - ➢ Met with iGEM EmpireGene to discuss collaboration
  - ➢ Met with iGEM UNAM to discuss the journal, wet lab, and dry lab collaboration
  - ➢ Met with SBU Society of Women Engineers
  - ➢ Worked on editing journal submissions
- ❖ Wet Lab - SF9
  - ➢ Running PCR of the Gel extraction sample of the Vector and the gene, both are at very low concentrations than the ones required for LIC cloning and performing a nanodrop of the product.

- ➢ We performed another PCR for both the gene and the vector, both at two different annealing temperatures: 66 and 72 degrees Celsius. We ran the products on a gel.
- ➢ Another PCR experiment runs for the gene and the vector. Then, 0.8% agarose gel of the PCR products. We tested several different conditions yet again.
- ➢ Run the gel for evaluating the PCR results from 7/19 (using Luk Lab Typhoon scanner and SYBR Gold)
- ➢ Concentrating DNA from gel extraction by ethanol precipitation (protocol provided by Cindy Converso, Luk Lab)
- ➢ PCR reactions of gene and vector in more conditions and with and without GC enhancer. We then ran the gel of the results. We performed the PCR again in higher volume, did a gel extraction and a nanodrop.
- ➢ PCR reaction of the gene at favorable conditions to prepare enough samples for gel extraction.

❖ Dry Lab

- ➢ Continued to distribute mathematical modeling guide to various iGEM teams
- ➢ Continued to work on mathematical modeling wiki write up
- ➢ Began protein modeling of *PROS1 V510M*, a genetic mutation typically seen in African Americans with protein S deficiency
  - ■ Will hopefully serve as a foundation for additional research and characterization of this variant

Week 27: July 25th - 31st

❖ Outreach

- ➢ Discussed possible fall semester collaboration with SBU Synthetic Biology Society
- ➢ Met with iGEM UNAM to discuss translating our assessment guide
- ➢ Met with iGEM Rochester to go over the progress on the children's science workbook
- ➢ Continued to work on Protein S deficiency Assessment guide
- ➢ Met with Dr. Gergen and started to discuss working with non-traditional students for inclusivity aspect of project
- ➢ Translated iGEM Ashesi's journal article in Polish
- ➢ Continued to work on editing journal submissions

❖ Fundraising

- ➢ Brainstormed additional fundraising ideas
  - ■ Virtual 5k
  - ■ Jazz Loft
  - ■ Selling samosas

❖ Wet Lab - SF9

- ➢ Pilot gel of PCR products from 7/23/22

- ➢ Repeat of PCR reaction (7/23) of the gene for more product to use in DNA extraction
- ➢ Pilot gel of PCR products (repeat of 7/23 PCR) above
- ➢ Gel of PCR products (repeat of 7/23 PCR) above in preparation for gel extraction. Then gel extraction and nanodrop of the gel-extracted DNA.
- ➢ LIC (cloning insert into the vector)
- ➢ Transforming the LIC assembled vector into *E. coli* NovaBlue cells
- ➢ Repeating LIC with new conditions to troubleshoot the lack of cells on the experimental plate
- ➢ Do a new LIC reaction for both insert and the vector, increase the concentrations for both
- ➢ Repeat Gene and Vector PCR reactions for gel extraction. Then, gel of PCR products above in preparation for gel extraction.
- ➢ Looking at plated transformants from July 28 transformation
- ➢ Gel extraction of Vector
- ➢ Repeat Gene PCR reactions for gel extraction. Running a gel of these PCR products.

❖ Wet Lab - *E. coli*

- ➢ Restriction Digest of Vector - The 2Bc-T plasmid was linearized using the HpaI restriction enzyme and confirmed by a gel.
- ➢ LIC Reaction for Insert - A LIC reaction was performed for our PROS insert and concentration was confirmed.
- ➢ Vector Growth and Purification - Cultures of DH5-alpha E. coli cells containing 2Bc-T plasmid were grown overnight and miniprepped. Concentrations of the plasmid were recorded.

❖ Dry Lab

- ➢ Met with iGEM Ashesi to discuss partnership
  - ■ Went over their gold biosensor
  - ■ Possible model for UV kill switch
  - ■ ROC curve to detect the fluorescence threshold
  - ■ ODE for transcription
  - ■ Could model ion intake in soil
  - ■ Discussed possible issues with their hydrogel
  - ■ Shared our mathematical modeling guide with their team
  - ■ Asked them if they could design a protein S biosensor
- ➢ Located the protein S type II deficiency patent and started working on the ROC curve
- ➢ Started working on the outline for the stochastic model script
- ➢ Continued to work on protein modeling wiki write up

AUGUST

Week 28: August 1st - 7th

- Outreach
  - Finished protein S deficiency assessment guide
  - Analyzed numbers for diagnostic algorithm
  - Met with iGEM UCSC to discuss dry lab collaboration
    - Modeling yeast in our first mathematical model
    - UCSC to model E.coli in their system
  - Met with Dr. Gergen and got contact information for Suffolk County Community College contact for VIP research program
  - Emailed Veronica Treadwell, member of the Unkechaug Nation, about Protein S deficiency research in Indigenous communities
- Wet Lab - SF9
  - Nanodrop of July 29 gene gel extraction.
  - Gel extraction of the Vector Sample and nanodrop
  - PCR gene and insert from Doug's lab, the sample he uses for LIC. Running a DNA gel of the PCR results.
  - LIC reaction with gel extracted insert and vector samples.
  - Culturing one of the transformants from the plate
  - PCR-ing primers onto Doug's vector (to be used as positive control of our entire LIC protocol)
  - DNA gel of the above PCR product
  - Colony PCR
  - DNA gel of the above colony PCR product and the PCR of the control vector provided by Douglass Marr
  - **We decided to stop focusing on SF9 cells and to perform *E. coli* expression solely.**
- Wet Lab - *E. coli*
  - Restriction Digest of Vector - The 2Bc-T plasmid was linearized using the HpaI restriction enzyme and confirmed by a gel. This process was repeated and troubleshooted to ensure linearization took place.
  - Extraction of Linearized Vector - The linearized 2Bc-T vector was extracted from the gel using gel extraction and concentration was confirmed. A LIC reaction was performed for the gel extracted and linearized vector.
  - Annealing of Vector and Insert - The linearized 2Bc-T vector and PROS gene of interest were annealed to produce a recombinant vector.
  - Transformation into DH5-alpha E. coli Cells - The recombinant vector and a positive control vector (only plasmid) were transformed into DH5-alpha E. coli cells to determine if the recombinant vector was made successfully. This was

initially unsuccessful so all of the previous processes entailing making the recombinant plasmid were repeated.

- ❖ Dry Lab
  - ➢ Found paper on on the mathematical modeling of the parameter estimations of the baculovirus infection process and went through it to see how it could help with our model
  - ➢ Went over iGEM UNAM's mathematical model and emailed them corrections
  - ➢ Continued to work on the stochastic model script
  - ➢ Continued to work on the mathematical model wiki write up
    - ■ Added all of the plots from the second model altering DNA concentration
    - ■ Decided to model the *E.Coli* Lac Operon inducible gene network and the Sf9 gene regulatory network
  - ➢ Decided not to continue with the ROC curve
- ❖ Wiki

Week 29: August 8th - 14th

- ❖ Outreach
  - ➢ Weekly meeting with iGEM UNAM, EmpireGene, and UCSC
  - ➢ Emailed the NAACP to discuss inclusive clinical research
  - ➢ Emailed SCCC contacts about the VIP program
  - ➢ Continued to work on the integrated human practices wiki write up
  - ➢ Decided to continue with a virtual 5k when the fall semester starts
    - ■ Search for virtual 5k platforms
- ❖ Wet Lab - *E. coli*
  - ➢ Transformation into DH5-alpha E. coli Cells - On the second try, there were colonies present, indicating successful transformation.
- ❖ Dry Lab
  - ➢ Went over baculovirus infection process script
    - ■ Adjusted the script to fit our system
    - ■ Analyzed the MATLAB generated plots
  - ➢ Continued to work on mathematical modeling wiki write up
  - ➢ Worked specifically on the code for the DNA, mRNA, and protein histograms for the stochastic model
- ❖ Wiki

Week 30: August 15th - 21st

- ❖ Outreach
  - ➢ Began formatting the journal
  - ➢ Weekly meeting with iGEM UCSC and iGEM UNAM
  - ➢ Continued to work on integrated human practices and inclusivity wiki writeup

- Wet Lab - *E. coli*
  - Transformed Cells' Growth & Purification - The colonies from the transformation were grown overnight and miniprepped. The concentration was measured.
  - Digestion of Recombinant Vector - We performed a restriction digest of the recombinant vector with Xho1 restriction enzyme. We ran a DNA gel with the product to ensure annealing took place, which yielded positive results and indication that the recombinant vector was present in the samples.
  - Amplification of Recombinant Vector - A PCR of the miniprepped recombinant vector was performed. We ran a DNA gel to ensure that the samples contained the recombinant vector, which was successful.
  - Transformation of BL21 and Origami E. coli Cells - The recombinant vector was transformed into BL21 and Origami E. coli cells for protein expression. The transformations were successful.
- Dry Lab
  - iGEM Ashesi emailed us our biosensor using Aptazyme!
  - Did a literature search for gold biosensor parameters for Ashesi circuit model
  - Searched for rate constants for the gene regulatory models
  - Began writing gold circuit script in MATLAB
  - Finalized the stochastic model script!
- Wiki

Week 31: August 22nd - 28th

- Outreach
  - Decided not to continue with the virtual 5k due to logistical issues
  - Continued to discuss the VIP research program between SBU and SCCC
  - Continued to work on integrated human practices and inclusivity wiki writeup
  - Weekly meeting with iGEM UNAM, EmpireGene, and UCSC
- Wet Lab - *E. coli*
  - Growth of Transformed Cells - Cultures of Origami and BL21 E. coli cells containing the recombinant vector were grown overnight.
- Dry Lab
  - Continued to work on wiki writeup for mathematical modeling
  - Met with iGEM Ashesi to go over finalized gold circuit model
  - Continued to work on troubleshooting rate constants for gene regulatory models
- Wiki

Week 35: August 29th - September 4th

- Outreach
  - Strawberry DNA extraction event with SWE

- ➢ Met with Alexander Knight, industrial stakeholder, to discuss future plans for our project
- ➢ Continued to work on formatting the journal

❖ Wet Lab - *E. coli*

- ➢ SDS-PAGE and Western Blot (August 27 - September 3)
- ➢ Analytical techniques such as running an SDS-PAGE gel and Western Blot were performed to ensure that Protein S was made. The HRP anti-His 6 antibody successfully detected the Protein S target band at about 80 kD.
- ➢ **The *E. coli* expression part of the project was finished.**

❖ Dry Lab

- ➢ Continued to work on wiki writeup for mathematical modeling
- ➢ Did a literature search to find rate constants for iGEM Ashesi gold circuit script

❖ Wiki

Week 36: September 5th - 11th

❖ Outreach

- ➢ Completed the journal!
- ➢ Alexander Knight, industrial stakeholder, contacted the following individuals on behalf of our team
  - ■ Sr. Manager of Clinical Trial Diversity & Inclusion for Moderna
  - ■ VP of business development for Aetion
  - ■ Sr. Corporate Counsel of Diversity Clinical Development for Pfizer

❖ Dry Lab

- ➢ Started working on the mathematical modeling conclusion
- ➢ Finalized parameters for the gene regulatory models
- ➢ Learned that our histograms were displaying negative concentration values, tried to troubleshoot the issue

❖ Wiki

Week 37: September 12th - 18th

❖ Outreach

- ➢ Began writing the conclusion for integrated human practices wiki write up
- ➢ Met with Dr. Gergen to go over the logistics of the VIP program
- ➢ Continued to distribute the journal to all the iGEM teams who contributed

❖ Dry Lab

- ➢ Adjusted moving histogram code to reflect only positive concentration values
- ➢ Uploaded the moving histograms to the wiki write up

❖ Wiki

Week 38: September 19th - 25th

- Outreach
  - Finalized the inclusivity wiki write up
  - Finalized integrated human practices writeup
- Fundraising
- Wet Lab
- Dry Lab
  - Had to troubleshoot the parameters of the gene regulatory models
  - Edited the mathematical modeling wiki write up
- Wiki

Week 39: September 26th - October 2nd

- Outreach
  - Met with SBU's Red Cross Club
  - Finished integrated human practices wiki write up!
- Dry Lab
  - Finished mathematical modeling wiki write up!
  - Finalized all mathematical model scripts
    - Uploaded to the wiki write-up

Week 40: October 3rd - 9th

- Outreach
  - Finished!
- Fundraising
  - Finished!
- Wet Lab SF9 and *E. coli*
  - Finished!
- Dry Lab
  - Finished!

Week 41: October 10th - 16th

- Wiki
  - Finished!