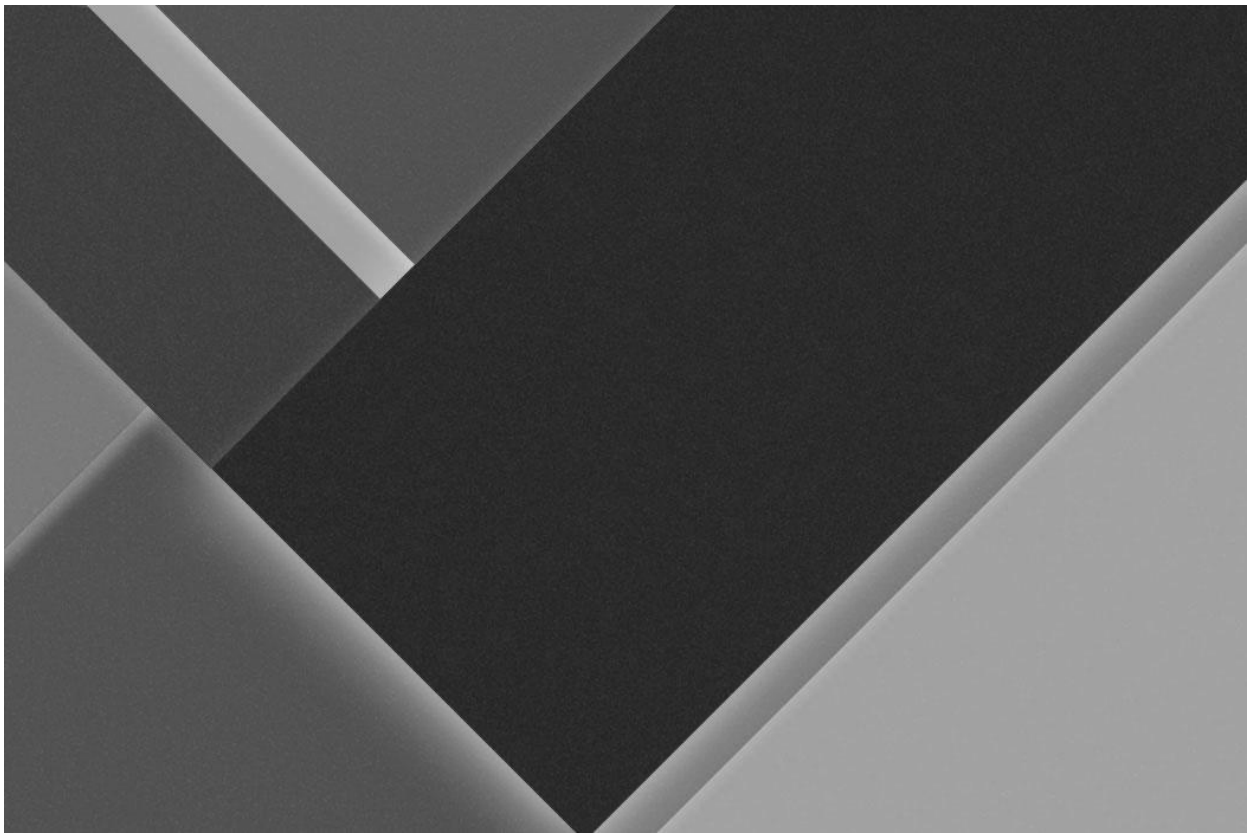
Title



Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Applied Science Project

—

Justin Nguyen

Dr. Heng Y.

Calendar

Calendar

MONTH NAME (TEMPLATE)

SUN	MON	TUE	WED	THU	FRI	SAT

September

SEPTEMBER

MON	TUE	WED	THU	FRI	SAT	SUN
1	2	3 ASP Block: Went through writing the logbook and research proposal	4	5 ASP Block: Went through private meetings and what to do in free time during ASP Individual: Emailed Youshan about reading research papers for next lab visit	6	7
8	9 ASP Block: Read through CRISPR-Cas9 research paper	10	11 ASP Block: Learned how to use PaperPile and began looking for papers related to my topic	12	13	14
15 ASP Block: Read through a research paper on cell mitigation and how PRPF4B can reduce the impact of cancer	16	17 Plan: need to find a time that is available, plan out the rest of the meeting times ASP Block: plan meetings and blocks a week ahead	18	19 Lab Plan: Go into the lab and RNA extraction used for reverse transcription and RNA level of PRPF4B after using CRISPR-Cas9 to generate PRPF4B heterozygous cells ASP Plan: Start writing the first topic of the introduction (brain fluid buildup).	20	21
22	23 ASP Plan: Finish writing and edit the background info on brain fluid buildup. Need to remember to highlight PURPOSE. Once complete, transition it to the PRPF4B gene ASP Block:	24	25 Lab Plan: Run transcription and reverse transcription for the cells (which will turn RNA into DNA and vise versa) ASP Plan: Edit the first two parts of the introduction and ensure everything	26	27 Own Time Plan: Complete and edit the introduction of the research paper	28

			makes sense. Connect the topics to RNA splicing and knockout efficiency of this gene and how CRISPR can be applied ASP Block: Read through the PCR paper again and finished the short and long term goals (still need to edit because not everything lines up), also started working on the variables part (not completely done but somewhat started), also had a meeting with Dr. Garcia			
29 ASP Plan: Work on the variables and objectives behind the project Lab Plan: Reverse Transcription PCR	30	● NEED A ROUGH DRAFT FOR RESEARCH PROPOSAL ○ Lots of research needed and outline of what topic we need to bring up				

October

OCTOBER

SUN	MON	TUE	WED	THU	FRI	SAT
			1 ASP Block Plan: - Write significance section - go through and edit Dr. Garcia's notes - finish RP rough draft - Dr. Garcia meeting Complete the Research Proposal by completing the significance and references	2 Lab Plan: -review data from previous lab visit - explore the significance	3 ASP Block Plan: - Edit RP for all of Youshan's edits - check for missing items and edit accordingly Go through research proposal and address all missing aspects is there are any	4
5	6 Lab Plan: - practice extracting RNA out of infant kidney cells	7 ASP Block Plan: - review sentence structure - format the whole research proposal - start methods	8	9 ASP Block Plan: -go through references - link it to the research proposal - meeting with Dr. Garcia Lab Plan: - practice creating stacking gels Double check the references part and link any info to any research papers if necessary	10	11
12	13	14 ASP Block Plan: - complete methods part of the RP	15 Complete oral presentation	16 ASP Block Plan: -edit everything as a whole -send a rough draft to Dr. Garcia	17 Practice oral presentation	18
19 Home: - go over Dr. Garcia edited RP - send it to Youshan	20 ASP Block Plan: -last minute edits for the research proposal - take and apply/reject all edits	21	22 ASP Block Plan: -edited october notes and calendar in logbook - work on oral presentation (find format and plan out every slide)	23 Lab Plan: -practice running a western blot and examine results	24 ASP Block Plan: -work on introduction, research question, variables and objectives on slideshow Prepare for lab	25

	Lab Plan: -run through and practice qtPCR - review RP with Youshan if extra time Listen to 4 other presentations				visit	
26	27 RP DUE Lab Plan: - practice create stacking gels - look through rp with Youshan	28 ASP Block Plan: - finish oral presentation, this includes variables, methodology, significance	29	30 PRESENTATION DUE ASP Block Plan: - Oral presentation - 10 minute presentation, 5 minute questions - participate in other 2 classmates presentations Lab Plan: - practice extracting rna out of cells	31	

Goals and Notes:

- Have a strong research proposal as that will allow for a very strong understanding of the topic
- Have all procedures understood and ran through so that we can start actually working on the project
    - Remember to review all procedures before going into the lab

**all comments were not removed but instead more was added (this is so I know what I did wrong)

November

NOVEMBER

SUN	MON	TUE	WED	THU	FRI	SAT
						1
2	3 ASP Plan: - enter info on CYSF - listen to 2 peoples oral presentation	4	5 ASP Plan: - review rt qpcr - watch youtube videos on rt qpcr procedure - find and read more about how rt qpcr works through research papers	6 Lab Plan: - extract RNA from cells with a Zymo Research kit	7 ASP Plan: - listen to other presentations because of curiosity (started taking notes but decided to just listen)	8
9	10	11 ASP Plan: - relearn how to analyze western blot results - watch youtube and read procedure - read papers about western blotting and its limitations	12	13 ASP Plan: - complete all CYSF forms - listen to Arvind's presentation	14 Lab Plan: - practice running a qt-pcr with WT, Homo and HT with Youshan	15
16	17 Lab Plan: - analyse the qt-pcr results and determine conclusions ASP Plan: - read the research paper on RT PCR (can be found in daily agenda) and summarize	18	19 ASP Plan: - go through extracting DNA procedure - learn why scientists use THAT specific method - look into the process of rt-pcr again	20 Lab Plan: - perform RT-PCR again as results from last lab day aren't significant - need to avoid the little mistakes when transferring materials into the PCR plate	21 ASP Plan: - go through procedure of extracting rna - look at results and determine whether knockout yesterday it is successful	22
23	24 Lab Plan: - do RT-PCR again in case something goes wrong	25 ASP Plan: - no class due to assembly	26	27 Lab Plan: - Analyze the rt-pcr results ASP Plan: - review converting RNA	28	29

				into cDNA - prepare the december calendar		
30						

Goals and Notes:

- By the end of this month, the process of gene knockout on iPSCs should be done
    - All procedures must be understood thoroughly
    - Perform rt qpcr on the iPSCs
- Completely understand all of the methodology
    - LOOK AT PROCEDURES BEFORE GOING INTO THE LAB

December

DECEMBER

SUN	MON	TUE	WED	THU	FRI	SAT
	1 Lab Plan: - Extract total protein from edited and control iPSCs - measure protein concentration ASP Block: - Meeting with Dr. Garcia - Write a finalized research question - begin writing introduction paragraph for final paper	2	3 ASP Block: - Write one introduction paragraph on the mutation and its role	4 Lab Plan: - Prepare gels and run SDS-PAGE (load equal amounts of protein) - then transfer to membrane	5 ASP Block: - Write one paragraph on PRPF4B gene and what is being researched - going to be used for introduction paragraph	6
7	8 Lab Plan: - Block membranes - perform primary antibody incubation for PRPF4B	9 ASP Block: - Rewrite procedure, basing it off of research proposal - focus on mRNA analysis - talk to Dr. Garcia	10	11 Lab Plan: - Complete secondary antibody incubation - develop/image the blot, and save raw images ASP Block: - rewrite western blotting procedure, basing it off of research proposal	12	13
14	15 Lab Plan: - Quantify band intensities (normalize PRPF4B to GAPDH) - compare to qPCR results ASP Block: - write about results section for band intensities and qPCR results	16	17 ASP Block: - research more into western blotting and rt-pcr - do not have the papers yet, will find them	18 Lab Plan: - Collect all figures - go through them and ask Youshan about interpreting them	19 ASP Block: - go through already written procedure and talk to Youhsan about accuracy and wording - look at biorender figures for western blotting	20

21	22 Lab Plan: - Analyze results and begin formatting graphs ASP Plan: - study for tests in other subjects - look at cooper's asp project and see how he formatted his poster - start a rough plan on canva of the poster formatting	23	24 ASP Plan: - study for tests in other subjects - look through biorender for rt-qpcr figures	25 Lab Plan: - Write methods section ASP Plan: - study for tests in other subjects - look through biorender for good allele representation	26	27
28	29	30	31			

Goals and Notes:

- Should be done a majority of the procedure of the project
    - Main thing next would be data analysis
    - Writing final research paper would be close
    - Science fair is close
- Plan out poster during winter break
- Going to be a very busy month but i have time during winter break
    - Preferably don't go into the lab during winter break but if it has to be done, it will be done

January

JANUARY

SUN	MON	TUE	WED	THU	FRI	SAT
				1	2	3
4	5	6 Study for Midterms	7	8 Study for Midterms	9 MIDTERMS	10
11	12 MIDTERMS	13 MIDTERMS	14 MIDTERMS	15 MIDTERMS	16 MIDTERMS	17
18	19 MIDTERMS	20 Will miss class due to dentist appointment	21	22 ASP Plan: - complete January and February calendar for logbook - meeting with Youshan to discuss results - meeting with Dr. Garcia	23	24
25	26 ASP Plan: - Listen to Dr. Garcia go through methodology writing	27	28 ASP Plan: - go through introduction section - write about the RT-qPCR portion - write about the value of using iPSCs	29	30 ASP Plan: - write about delta delta ct and the steps of RT-qPCR analysis	31

February

FEBRUARY

SUN	MON	TUE	WED	THU	FRI	SAT
1	2	3 ASP Plan: - create the graphs required for RT-PCR and Western Blot - finalize introduction on poster - write limitations for poster	4	5 ASP Plan: - create template on canva for poster - create figure titles for graphs - outline key parts of poster (intro, methodology, discussion, conclusion etc.)	6	7
8	9 ASP Plan: - Write about the iPSC model and cell culture - Write about CRISPR Cas-9 gene editing	10	11 ASP Plan: - Write about western blotting and protein extraction - make flowchart for poster	12	13 ASP Plan: - complete all citations necessary and lab equipment information for methodology - Send it to Dr. Garcia and Youshan to check	14
15	16	17 ASP Plan: - complete any final edits for the methodology section based of Youshan and Dr. Garcia's' suggestions	18	19 ASP Plan: - Methodology Due - finalize poster design and send to Staples to print	20	21
22	23 ASP Plan: - prepare for the oral presentation -practice the explaining part of introduction and methodology in particular	24	25 ASP Plan: - Oral Presentation (poster needed) - observe other presentations for participation mark	26	27 ASP Plan: - prepare for science fair by practicing the presentation - talk to Youhsan and ask him any final questions - could also present to Youshan if he is available	28

March

MARCH

SUN	MON	TUE	WED	THU	FRI	SAT
1	2 SCIENCE FAIR PRESENTATIONS	3 ASP: - science fair results - make all needed edits - complete CYSF portal if I make it - email Youshan about SF results - look at SF feedback	4	5 ASP: - incorporate Youshan's feedback into the methodology - incorporate Dr. Garcia's feedback into the methodology	6	7
8	9 ASP: - Format and cite everything for methodology - Run through everything and ensure no mistakes/final edits METHODOLOGY PAPER DUE	10	11 ASP: - Start writing the results paper - mainly focus on results for RT-qPCR and mRNA levels	12	13 ASP: - write the results for the Western Blot - write the applications for the results	14
15	16	17 ASP: - finalize paper and include all necessary citation - write about - grammar edits primarily - send paper to Youshan and Dr.	18	19 ASP: - Hand in result section paper - prepare for science fair through simplifying all background info for judges there	20	21
22	23 Spring break	24 Spring break	25 Spring break	26 Spring break	27 Spring break	28 Spring break

Daily Notes

Daily Notes

Template 1

Tasks To-do	Review	Notes

Tasks Completed

Summer Notes

Daily Notes

July 3, 2025

Tasks To-do	Review	Notes
☑ Plan an in person meeting with Dr. Heng ☑ Talk on a Google Meet individually to ask questions and learn about his work	I was on a call with Dr. Garcia and Dr. Heng. We discussed future steps and Dr. Garcia introduced the ASP program to him. I then went and planned both an in person meeting on July 18th and a google meet on July 5th.	July 18th: In-Person July 5th: Online

July 5, 2025

Tasks To-do	Review	Notes
☑ Make some questions to ask him in the lab ☐ Prepare to go to the lab	I had a one-on-one chat with my mentor. This was only around 15 minutes and he explained a little about his own project. We talked about the PRPF4B gene and how it impacts someone's brain development (by allowing extra brain fluid buildup). We also reviewed the main components of a human gene strand and how one mutation can impact future growth and development.	July 18th: In-Person July 5th: Online

July 18, 2025

Tasks To-do	Review	Notes
☐ Complete all of the 7 ethics and safety courses ☐ Come back into the lab at 10am on July 21st	I went into the University of Calgary Health Science building and got a tour around the lab with Isabella. I also met Youshan in-person for the first time. Because he was mid-experiment, Isabella worked with me on reading through all of the consent forms, safety quizzes and more. I completed 3 during the one hour I was there (which was on paper) and have to complete 7 more online. We also planned to meet July 21st to start the actual procedures (which at this time was running electrophoresis on DNA strands, more notes will be provided on the day I did the experiment).	Email Isabella when done all the tests Do not wear shorts

July 19, 2025

Tasks To-do	Review	Notes
☑ Complete all of the 7 ethics and safety courses ☐ Come back into the lab at 10am on July 25st	Today, I spent 4 hours working on the ethics and safety courses. Although I didn't take any notes, I memorized what I needed to in order to complete the quizzes and be prepared in case something happens in the lab.	Email Isabella when done all the tests July 25st: Lab Day

July 25, 2025

Tasks To-do	Review	Notes
☐ Come back tomorrow	While I was in the lab today, my professor and I worked on running electrophoresis for plasmid DNA to express the PRPF4B domain. While I originally thought this would be a really straightforward procedure, it turned out to be a lot more complicated than expected. Here are the notes I took: • Spent time learning how to make agarose gel to run electrophoresis for plasmid DNA to express the PRPF4B domain	July 26nd: Lab Day Review this procedure!!

- Main goal was to detect the presence of a particular DNA strand based on the length the stain goes
    - Preparing agarose gel
        - Take agarose powder (mainly made of seaweed) and combine with 1/50 of purple stain, and for every gram of agarose gel, use 100ml of a buffer
            - This creates a 0.8% - 2% solution
        - Heat up the agarose gel in the microwave in increments of 30 seconds until there is no precipitate
    - Preparing electrophoresis apparatus
        - Place a comb in between the middle structure to create wells to hold DNA
    - Pouring the agarose gel into the apparatus
        - Wait 20-30 minutes until the gel solidifies
    - Remove comb and ensure air tight seals
    - Dye DNA samples with 1/50 of green stain
    - Place DNA strands into singular wells
        - Wait 20-30 minutes
    - Remove gel and take it to the scanner machine in order to detect length through contrast

July 26, 2025

Tasks To-do	Review	Notes
☐ Come back tomorrow	Today, my professor and I worked on learning how to effectively transfer different mediums such as DNA into little test tubes in order to test them in the future. Transfusions felt pretty easy. They were basically done using pipettes under a fume hood. All that is required for this is sterile equipment (make sure to sanitize everything with the alcoholic spray before putting it into the fume hood), your samples, and many pipettes and pipette tips. Make sure to also replace all pipette tips after usage.	July 27rd: Lab Day

July 27, 2025

Tasks To-do	Review	Notes
☐ Come back tomorrow	Today, Youshan should an undergrad and I how to create a stacking gel. This was a lot harder because of all of the measurements we had to take. This was necessary to learn how to do a western blot. ● Creating stacking gels in order to run electrophoresis for protein rather than DNA ● Main goal was to detect the presence of a protein sample to detect the influence of the PRPF4B's influence on the synthesis of certain proteins ○ Preparing first gel solution ■ Use 2 glass plates and press them against each other ■ Apply green clamps on the panels in order to prevent leakage ■ Place them in the designated green machine in order to keep them upright for long periods of time ■ Create solution using the recipe provided on the paper for 10 mL ■ Mix it in the green test tube ○ Using gel solution in the clasp	July 28th: Lab Day

- Pour gel solution mostly to the top with a little bit of space between the top of the clasp and the created substance
- Keep some left in the flask in order to measure whether or not substance has solidified
- Wait 10-20 minutes

- Creating a secondary gel (or what is known as the stacking gel)
  - Follow the instructions in the bottom of the same sheet
- Apply the green comb, make sure it is the right size
- Pour the stacking gel into the apparatus and wait 20-30 minutes
- When done, take out the green comb
- Get out all of the necessary equipment to run electrophoresis
  - Includes the positive negative current plugs, plastic structures, foam pieces, blank pieces of paper
- Create a sandwich in the bottom of the plastic tray in order to prevent protein from escaping
- Run at 80 volts for 30 minutes and 100 volts for 30 minutes

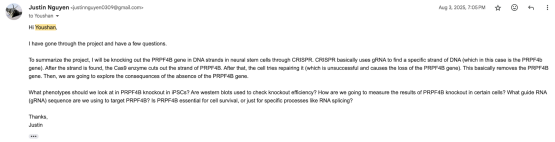
July 28, 2025

Tasks To-do	Review	Notes
☐ Come back tomorrow	We primarily worked on setting up a western blot, using the stacking gel we made from the previous day. This was very similar to the first experiment we did regarding the electrophoresis but instead, the western blot took place primarily with milk and in a cooler. Here is the process: ● Sample Preparation ○ Use lyse cells or tissues to extract proteins ■ Measure protein concentration ○ Mix protein samples with loading buffer and heat to denature proteins ● Gel Electrophoresis (SDS-PAGE) ○ Load protein samples and molecular weight markers into wells of an SDS-PAGE gel ■ Apply an electric current to separate proteins by size. ● Protein Transfer ○ Transfer separated proteins from gel to a membrane ○ Confirm successful transfer ● Blocking ○ Incubate the membrane with a blocking solution (milk in this case) to prevent non-specific antibody binding ○ Put into the freezer for 1.5 hours ● Compare band size in the printing machine	July 29rd: Lab Day

July 29, 2025

To-Do	Review	Notes
☐ Learn more about the project he gave me ☐ Ask questions where there is confusion	Today, I created a stacking gel myself. This process was very simple but I made several mistakes when taking precise measurements of the materials. In the future, this could be avoided just by reading the data sheet correctly. After creating the stacking gel, my professor introduced me to my project. This would be similar to something he is doing but on a very minor scale. The title the professor gave me was: the evaluation of PRPF4B knockout efficiency in iPSCs using CRISPR.	2 materials are located in the freezer Always use a labcoat and gloves

August 3, 2025

To-Do	Review	Notes
☐ Learn more about the project he gave me ☐ Ask questions where there is confusion	I went through the project more in-depth, mainly by googling it and reading through my professors research proposal. I emailed him all of my questions and my own interpretation of the project. Please see the attached questions below this. 	Go through his email more in-depth.

Justin Nguyen <justinnguyen0304@gmail.com>
to Youshan

Aug 3, 2025, 7:05 PM

Hi Youshan,

I have gone through the project and have a few questions.

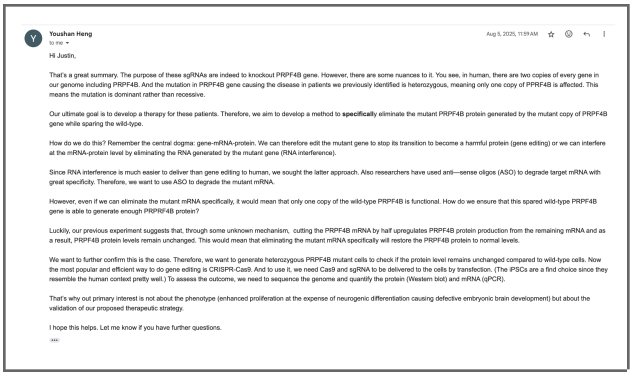
To summarize the project, I will be knocking out the PRPF4B gene in DNA strands in neural stem cells through CRISPR. CRISPR basically uses gRNA to find a specific strand of DNA (which in this case is the PRPF4b gene). After the strand is found, the Cas9 enzyme cuts out the strand of PRPF4B. After that, the cell tries repairing it (which is unsuccessful and causes the loss of the PRPF4B gene). This basically removes the PRPF4B gene. Then, we are going to explore the consequences of the absence of the PRPF4B gene.

What phenotypes should we look at in PRPF4B knockout in IPSCs? Are western blots used to check knockout efficiency? How are we going to measure the results of PRPF4B knockout in certain cells? What guide RNA (gRNA) sequence are we using to target PRPF4B? Is PRPF4B essential for cell survival, or just for specific processes like RNA splicing?

Thanks,
Justin

August 5, 2025

To-Do	Review	Notes
☑ Learn more about the project he gave me ☐ Ask questions where there is confusion	Youshan replied to my email and explained all of my questions more in-depth. I realized that most of my interpretation had been incorrect and the function of the PRPF4B gene was completely different. I also learned that the main goal of the project would be to test whether or not removing the mutant gene would allow the other “normal” gene to properly function at an extended speed. Please see the attached questions below this.	Go through his email more in-depth.



Youshan Heng
to me

Aug 5, 2025, 11:59 AM

Hi Justin,

That's a great summary. The purpose of these sgRNAs are indeed to knockout PRPF4B gene. However, there are some nuances to it. You see, in human, there are two copies of every gene in our genome including PRPF4B. And the mutation in PRPF4B gene causing the disease in patients we previously identified is heterozygous, meaning only one copy of PPRF4B is affected. This means the mutation is dominant rather than recessive.

Our ultimate goal is to develop a therapy for these patients. Therefore, we aim to develop a method to **specifically** eliminate the mutant PRPF4B protein generated by the mutant copy of PRPF4B gene while sparing the wild-type.

How do we do this? Remember the central dogma: gene-mRNA-protein. We can therefore edit the mutant gene to stop its transition to become a harmful protein (gene editing) or we can interfere at the mRNA-protein level by eliminating the RNA generated by the mutant gene (RNA interference).

Since RNA interference is much easier to deliver than gene editing to human, we sought the latter approach. Also researchers have used anti—sense oligos (ASO) to degrade target mRNA with great specificity. Therefore, we want to use ASO to degrade the mutant mRNA.

However, even if we can eliminate the mutant mRNA specifically, it would mean that only one copy of the wild-type PRPF4B is functional. How do we ensure that this spared wild-type PRPF4B gene is able to generate enough PRPRF4B protein?

Luckily, our previous experiment suggests that, through some unknown mechanism, cutting the PRPF4B mRNA by half upregulates PRPF4B protein production from the remaining mRNA and as a result, PRPF4B protein levels remain unchanged. This would mean that eliminating the mutant mRNA specifically will restore the PRPF4B protein to normal levels.

We want to further confirm this is the case. Therefore, we want to generate heterozygous PRPF4B mutant cells to check if the protein level remains unchanged compared to wild-type cells. Now the most popular and efficient way to do gene editing is CRISPR-Cas9. And to use it, we need Cas9 and sgRNA to be delivered to the cells by transfection. (The iPSCs are a first choice since they resemble the human context pretty well.) To assess the outcome, we need to sequence the genome and quantify the protein (Western blot) and mRNA (qPCR).

That's why out primary interest is not about the phenotype (enhanced proliferation at the expense of neurogenic differentiation causing defective embryonic brain development) but about the validation of our proposed therapeutic strategy.

I hope this helps. Let me know if you have further questions.

-xxx-

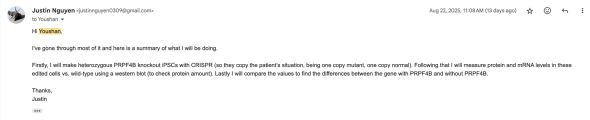
August 21, 2025

To-Do

- [ ] Plan when to come back to the lab

Review

I was on vacation during this period so I wasn't really thinking about the project. During a sudden realization in the airport terminal, I thought about emailing him back and ended up going through the project again. I once again, confirmed what I would be doing, this time in a single sentence. This summary allowed me to simply understand my goal and Youshan's goal for the experiment.



Justin Nguyen <justinnguyen03079@gmail.com>
to Youshan

Aug 22, 2025, 11:08 AM (13 days ago)

Hi Youshan,

I've gone through most of it and here is a summary of what I will be doing.

Firstly, I will make heterozygous PRPF4B knockout iPSCs with CRISPR (so they copy the patient's situation, being one copy mutant, one copy normal). Following that I will measure protein and mRNA levels in these edited cells vs. wild-type using a western blot (to check protein amount). Lastly I will compare the values to find the differences between the gene with PRPF4B and without PRPF4B.

Thanks,
Justin

-xxx-

Notes

Get ready to go back into the lab.

September Notes

Daily Notes

September 1, 2025

Tasks To-do

- [ ] Email Dr. Garcia the homework on what I have already done in ASP
- [ ] Decide which logbook to do

Tasks Completed

- [ ] Read through the research proposal document and the course outline

Notes

Need to start documenting everything

Review

Thanks to Arvind for showing me his notes as I wasn't here.

1. Planning
2. Time-Management
3. Task-Tracking
4. Communication
    a. CC ASP coordinators (Dr. Garcia + Ms. Kale) when emailing mentor + supervisor
    b. Reply ASAP (< 2 hours, preferably)
5. REALLY IMPORTANT:
    a. After a meeting with a mentor, email them regarding the tasks that you have set out for yourself (or that have been set out for you), and try to complete them by the next meeting or applicable due-date
        i. Ask "Have I missed anything?" at the end of the email, just in case
6. Calendar
    a. Note down all ASP classes a month in advance
    b. Note down all work to be done in those class blocks, a month in advance
        i. Setting deadlines for yourself – be kind, but not too kind
7. Top 15 Projects (JH/SH) admitted to CYSF
    a. Around 10 ASP projects admitted every year
    b. Won't make or break grade, but can impact it?
8. Logbook
    - Used to track notes (daily or with mentor), work, data-collection, basically everything ASP-related
    - Should retroactively include work done during the summer
9. Assessments every other week, regarding communication + schedule
10. Oral Presentation – 4 major presentations throughout the year, w/ different weightings (30% total)
    a. Research Proposal
    b. Preparation for Science Fair
    c. Science Fair Presentation
    d. Final
11. Written Work – Papers / write-ups (30% total)

e. Research Proposal
f. Introduction
g. Experimental Proposal
h. Results
i. Analysis
j. Final Edited Paper

DUE NEXT CLASS (Next Wednesday)

- Met with mentor?
- Where are you at? Topic?
- Literature been read?
- Meeting times established? Weekly, biweekly, otherwise?
- Mention Poster, Research, 7 AFTs, Systematic Review

September 3, 2025

Tasks To-do	Tasks Completed	Notes
☐ Email professor to tell him my schedule ☐ Plan out next meeting	☐ Emailed professor my schedule ☐ Started doing the logbook	Still need to work out a time with my mentor

Review

- Can use physical or digital notebook depending on preferences
  - Digital notebook allows it to be accessed anywhere at any time while physical is much more limited
  - Use tabs on google docs to organize the logbook
    - Can also use notion or another program to record as long as it can be accessed and written in
  - Full month laid out in advance – specific tasks for each class
    - Meetings laid out, as well
    - Example: for project proposal, list “edited project proposal, correcting grammatical errors and increasing brevity, then sent to mentor for back-and-forth editing”
- Background research
  - Must be very in-depth and record everything that has been provided
  - Need to record where the resource was found
  - Ask mentor for his any background information he may have
- Research proposal
  - Figure out whether or not your project will be an experiment, study, or innovation-type
  - Will be different for different projects
  - Research Proposals are important especially for research
    - Allows research to get funded, mainly through grants
    - Gets approval from peers

- Abstract isn't necessary
  - Only a minor summary of everything in the research proposal
- Will take lots of time and be stressful
  - Need to complete everything in the rubric
    - Title is very important and can change the course of your project
      - Needs to be simple and understandable but still needs to show what your project is about and its goals
- Main purpose is to answer how does using this technique answer the question in the best manner

September 5, 2025

Tasks To-do

- [ ] Read through literature and take notes
- [ ] Start working on research proposal
- [ ] Background research
- [ ] Research questions
- [ ] Short term goals and long term goals

Tasks Completed

- [ ] Had a private meeting with Dr. Garcia about my project
- [ ] Started reading through my first research paper
  bio20200034.pdf

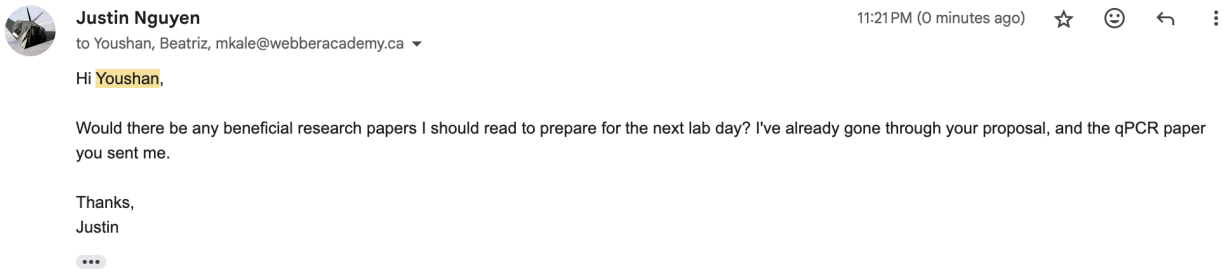
Notes

Still need to work out a time with my mentor

Review

- Need to record everything done in free time in the logbook
  - If you do not have a private meeting (around 20 minutes), do your own work
    - This could include reading literature, spending time writing on your logbook, make an outline on the research proposal, figure out research question and more
    - Can also read about individual parts that are important to your own research
      - Broad aspects to very specific in order to figure out exactly what you need to cover
      - Short term -> long term
        - Break it down into smaller steps
- When writing/submitting a research proposal or paper need to check in with mentor
  - Allow them a week in advance in order for them to go through it, clear any misconceptions and edit
    - Need to plan all of it
      - Start with your due date and break it down
- Need to have a plan with the mentor
  - Talk about regular meetings
    - Are you ok with this?
  - Research paper assignment
    - Topic

        - Research question
        - Goals
        - Outline intro
        - Background research online
    - Use pubmed with the NCBI to research
        - Very strong peer reviewed research papers
- Tuesday class
    - Web-based
    - Reference manager
    - Paper-Pile
- During my meeting with Dr. Garcia
    - Went through establishing long-term and short-term goals
    - Realize that that is what you are aiming for
    - Start making a schedule and starting working on research proposal
        - Have a very clear background question and do lots of supplemental background research
- During my spare time today, I started reading through my first paper
    - RT-PCR, qPCR and RT-qPCR
        - Many misconceptions between them because they are synonymous
            - Similarities often result in the incorrect use
    - RT-PCR is the process of reverse transcription PCR and not real-time PCR
        - Allows for the use of RNA to create a similar copy with DNA
            - Uses reverse transcriptase enzyme
    - qPCR is quantitative real-time PCR
        - Amplification of DNA in real time
            - Measured through a fluorescent probe created from a intercalating dye or a hydrolysis-based probe
                - Allows quantitation
    - RT-qPCR is used for reverse transcription quantitative real-time PCR
        - Is a combination of qPCR and RT-PCR
            - Enables the measurement of RNA levels through the use of cDNA
    - qPCR: detect pathogens, measure DNA copy numbers
    - RT-qPCR: study gene expression changes
- Emailed Youshan about reading more research papers
    - The email is attached below



Justin Nguyen 11:21 PM (0 minutes ago)

to Youshan, Beatriz, mkale@webberacademy.ca

Hi Youshan,

Would there be any beneficial research papers I should read to prepare for the next lab day? I've already gone through your proposal, and the qPCR paper you sent me.

Thanks,
Justin

September 9, 2025

Tasks To-Do	Tasks Completed	Notes
☐ Read through the research paper he attached	☐ Applied Science Project Class ☐ Read through research paper, link and more info in the background research portion jinek2012.pdf	

Review

- Installing PaperPile next class
- Goal of September
  - Rough draft of the research proposal
    - Strong background research
      - With the outline, intro and more
    - Need to be specific with the research questions
    - Need to have goals in mine
    - Have variables clearly defined
    - Methods must be outlined clearly
- CRISPR-Cas
  - Adaptive immunity against against viruses and plasmids
  - Key components
    - CRISPR arrays
    - Cas proteins
    - crRNA
  - Different phases of CRISPR immunity
    - Adaptation
    - Expression
    - Interference
- Focusing on Type II CRISPR
  - tracrRNA base pairs with pre-crRNA
  - Requires RNase III and Cas9
  - Cas9 is through to be the protein needed for DNA interference
- Key info
  - Cas9 needs dual RNAs (crRNA and tracrRNA)
    - crDNA alone is not good enough
    - tracrRNA pairs with crRNA
      - Activates Cas0 for site-specific DNA
  - Cas9 cleavage mechanism
    - Recognizes target DNA through crRNA sequence and a “protospacer adjacent motif (PAM)”
    - HNH domain cuts the strand
    - RuvC domain cuts the non-complementary strand
  - Requirements
    - Need a need region near the PAM to be recognized
    - PAM sequence is needed for separating self and non-self
  - Programmability
    - Dual-RNA system can be used to create a single chimeric RNA
      - Is a fusion of tracrRNA and crRNA

- Experiments that have already been done
    - Purified Cas9 has been tested with plasmid and linear DNA
    - Mutant cas9 proteins have shown the roles of HNH and RuvC domains
    - Truncated RNA experiments found very little requirements for RNA
    - A single-guide RNA has been engineered
        - It basically has the same function as dual RNAs
            - Can be used as a way to replace a single RNA if one set of the 2 become mutated or stop properly function
- Conclusions they have decided upon
    - Cas9 is a programmable RNA-guided DNA
        - Requires dual-DNA structure
    - Can be reprogrammed
    - It is a very simple, efficient, and versatile genome-editing tool
- How it has impacted the research area
    - Very important work that lead up to CRISPR-Cas9 genome editing
    - Found RNA programmability of Cas9
        - Can change the way we look at biotechnology, gene editing and medicine

September 9, 2025

Tasks To-Do	Tasks Completed	Notes
☐ Add 5 sources to your libraries ☐ Star google doc and add 5 sources using AMA	☐ Signed up for PaperPile	

Review

- Paperpile setup
    - Only works when not many people are using it
        - Need to make sure you upload the file to paperpile before citing it
        - Might take a lot of time to upload
    - Need to update the citation style everytime
        - ALWAYS check the box that allows you to paste the link inside
- Found 5 research papers and quickly skinned through it

September 15, 2025

Tasks To-Do	Tasks Completed	Notes
☐ Add 5 sources to your libraries ☐ Star google doc and add 5 sources using AMA	☐ Read through multiple papers Uncovering the signaling landscape contr… ☐ protocol.pdf ☐ Created a plan for the first meeting back in the lab	

Review

- Uncovering the signaling landscape controlling breast cancer cell migration identifies novel metastasis driver genes
    - Molecular drivers of triple-negative breast cancer
        - Three genes were the central regulators
            - They were PFPF4B, BUD31 and BPTF
                - PRPF4B: A splicing kinase that is essential for metastasis in vivo
                - BUD31: a spliceosome component
                - BPTF: a chromatin remodelling factor
                    - Both this and BUD31 allow for cell adhesion and migration
        - Knocking out these genes would lead to downregulation of focal adhesion and extracellular matrix (ECM)
            - Impairing migration and metastatic potential
            - high expression of these 3 genes would lead to tumors with poorer metastasis-free survival
                - This allows it to be used to other research in the future as there is proof that these 3 genes can be used
    - Suppressing PRPF4B in a mouse xenograft model reduced lung and organ metastases without affecting the tumor growth
    - Procedure
        - Cell lines chosen
            - Hs5781T and MDA-MB-231
        - RNAi-based imaging
            - Phagokinetic track assay, PKT
            - 4200 genes encoding signaling proteins were targeted in this experiment
                - Using siRNA pools
            - Cells were grown on fibronectin coated with beads
                - When they migrate, they leave tracks
            - Automated imaging quantified migration parameters
            - Very important for PCA grouped migration
        - Results
            - Found a total of 2807 hits (1501 in Hs578T, 1306 in MDA-MB-231)
    - This study is a map of TNBC cell migration and identifying important driver gene
        - PRPF4B, BUD31, and BPTF are important genes for therapeutic targets that prevent and treat metastatic breast cancer
            - Can be used for drug creation

- Protocol: a beginner's guide to the analysis of RNA-directed DNA methylation in plants
    - DNA methylation

- Plan for the lab visit (whenever it is)
    - Go through and confirm the procedures I've taken notes on
        - Western blot, stacking gel and electrophoresis
    - Work on creating a stacking gel
    - Learn more about the use of CRSIPR
        - How is it done in the lab?
        - Where does it happen?
        - How long would that take?
        - How long do I have to wait until it takes effect?

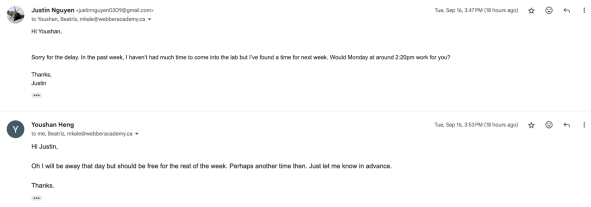
September 16, 2025

Tasks To-do

- [ ] Find another day that works

Review

I emailed Youshan my plans to come into the lab. Originally, I was only available Monday after school but it turned out he wasn't available. I need to find another data asap.



Justin Nguyen <justinnguyen0309@gmail.com> Tue, Sep 16, 3:47 PM (18 hours ago)
to Youshan, Beatriz, mikale@webberacademy.ca

Hi Youshan,

Sorry for the delay. In the past week, I haven't had much time to come into the lab but I've found a time for next week. Would Monday at around 2:20pm work for you?

Thanks,
Justin

Youshan Heng Tue, Sep 16, 3:53 PM (18 hours ago)
to me, Beatriz, mikale@webberacademy.ca

Hi Justin,

Oh I will be away that day but should be free for the rest of the week. Perhaps another time then. Just let me know in advance.

Thanks.

Notes

Email him back asap

September 17, 2025

Tasks To-do

- [ ] Find a date that works
- [ ] Separate each topic of the intro
- [ ] Create deadlines for everything
- [ ] Plan everything ahead of time

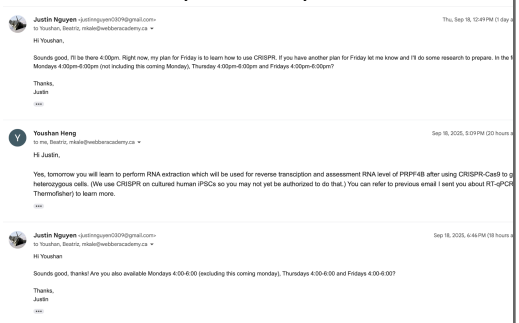
Review

- Emailed him if he was available this Friday
- Ran through dates
    - Friday everydays after school
        - Thursday maybe??
    - Monday everyday after school
- Separating topics in the research proposal
    - Starting off with Introduction
        - Brain fluid buildup
        - PRPF4B
        - RNA Splicing
        - Knockout efficiency of that gene in particular
        - Use of CRISPR
- Need to get research proposal done and send it to Youshan and Dr. Garcia to review
  Professor emailed me back saying that he was available

Notes

Need to separate different sections of the research proposal

- We also worked on what we were planning to do in the lab tomorrow
    - RNA extraction which will be used for reverse transcription and assessment RNA level of PRPF4B after using CRISPR-Cas9 to generate PRPF4B heterozygous cells.
    - Use CRISPR on cultured human iPSCs so I may not yet be authorized to do that.
- Planning to meet Mondays, Thursday and Fridays



Justin Nguyen <justinnguyen0309@gmail.com> Thu, Sep 18, 12:49 PM (1 day a
to Youshan, Beatriz, mkale@webberacademy.ca

Hi Youshan,

Sounds good, I'll be there 4:00pm. Right now, my plan for Friday is to learn how to use CRISPR. If you have another plan for Friday let me know and I'll do some research to prepare. In the f
Mondays 4:00pm-6:00pm (not including this coming Monday), Thursday 4:00pm-6:00pm and Fridays 4:00pm-6:00pm?

Thanks,
Justin

Youshan Heng Sep 18, 2025, 5:09 PM (20 hours a
to me, Beatriz, mkale@webberacademy.ca

Hi Justin,

Yes, tomorrow you will learn to perform RNA extraction which will be used for reverse transciption and assessment RNA level of PRPF4B after using CRISPR-Cas9 to g
heterozygous cells. (We use CRISPR on cultured human iPSCs so you may not yet be authorized to do that.) You can refer to previous email I sent you about RT-qPCR
Thermofisher) to learn more.

Justin Nguyen <justinnguyen0309@gmail.com> Sep 18, 2025, 6:44 PM (18 hours a
to Youshan, Beatriz, mkale@webberacademy.ca

Hi Youshan

Sounds good, thanks! Are you also available Mondays 4:00-6:00 (excluding this coming monday), Thursdays 4:00-6:00 and Fridays 4:00-6:00?

Thanks,
Justin

September 18, 2025

Tasks To-do

- [ ] Plan next meeting
- [ ] Read something to prepare

Review

- Spent time working on the introduction of the research proposal (mainly on the brain fluid buildup part)
- Read through the iPSCs research paper (can be found on background research tab)
- Went into the lab after school and started working on:
    - RNA extraction which will be used for reverse transcription and assessment RNA level of PRPF4B after using CRISPR-Cas9 to generate PRPF4B heterozygous cells.
    - Use CRISPR on cultured human iPSCs so I may not yet be authorized to do that.

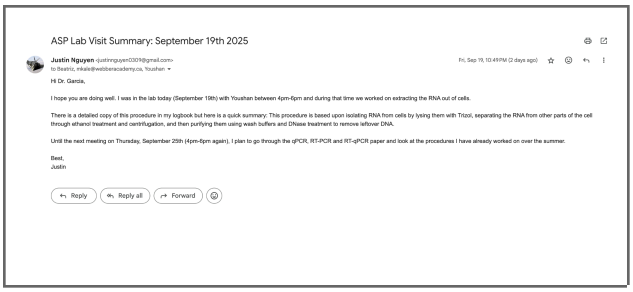
Extracting RNA from Cells

- In this case, start with cells from the fridge
    - Need to then transfer vials and place it into the centrifuge at 6000

Notes

times per minute for 60 seconds
- Get trizol from the fridge
    - Is neurotoxic
    - Make sure to use the fumehood when transfering
- Start with the cell sample and centrifuge it
    - After it is done, remove all of the liquid that isn't the white cells
- Use the menu to then add that much trizol into the bottle of cells
    - if there are too many cells, change the amount of trizol to be proportionate
    - Use the pipette to extract amount of trizol from the bottle and put it into the vial with the cells
    - Mix it by taking in and extracting the mixture
- Then mix the vial with equal volume of ethanol and mix it thoroughly
- Transfer it into a collection tube
    - Max collection limit is 700 microliters
    - Has a filter and a collector
    - Centrifuge it at 13000 per minute for one minute
        - What this will do it separate the RNA from the proteins and everything else, RNA kept in filter on the top “storage compartment”
- Take it out of the centrifuge
    - Remove all of the pink liquid and put it into the discard buffer
- DNase I treatment
    - Add 400 microliters of RNA wash buffer into the column and centrifuge again
        - May need to mix ethanol into it just follow the labels on the bottle
- Grab and RNase free tube and add 5 microliters of DNase I and 75 microliters of DNA Digestion Buffer
- Wait for 15 minutes at room temperature

- Emailed my daily lab summary and set a plan for next Thursday at 4:00



ASP Lab Visit Summary: September 19th 2025

Justin Nguyen <justinnguyen0309@gmail.com>
to Beatriz, mkale@webberacademy.ca, Youshan

Fri, Sep 19, 10:49 PM (2 days ago)

Hi Dr. Garcia,

I hope you are doing well. I was in the lab today (September 19th) with Youshan between 4pm-6pm and during that time we worked on extracting the RNA out of cells.

There is a detailed copy of this procedure in my logbook but here is a quick summary: This procedure is based upon isolating RNA from cells by lysing them with Trizol, separating the RNA from other parts of the cell through ethanol treatment and centrifugation, and then purifying them using wash buffers and DNase treatment to remove leftover DNA.

Until the next meeting on Thursday, September 25th (4pm-6pm again), I plan to go through the qPCR, RT-PCR and RT-qPCR paper and look at the procedures I have already worked on over the summer.

Best,
Justin

Reply | Reply all | Forward

September 23, 2025

Tasks To-do	Review	Notes

☐ Prepare for Thursday meeting	• Finished the introduction part of the research proposal and the objectives category
	  ○ Read more into the rtPCR paper and more information behind reverse transcriptions and normal transcriptions
	  ■ All info added to the background research tab
	  ○ Still need to go through and edit it
	  ■ What is needed and what can I remove
	  ○ Check with Dr. Garcia if it is the right format and everything
	  ○ Objectives need to be more clear??
	  ■ Emphasized short and long term goals
	• Have a meeting next Thursday after school at 4:00
		Remember to read about the PCR and review transcriptions for next lab visit

September 25, 2025

Tasks To-do	Review	Notes

☐ Prepare for Thursday meeting	• Finished the objectives and long and short-term goals
	  ○ Still need to edit because it sounds really funky
	  ■ Need to be a lot more specific
	• Go through the introduction and review grammar
	• Need to get a research question done
		Remember to read about the PCR and review transcriptions for next lab visit
		
		Edit writing portions

- Meeting with Dr. Garcia
    - Went through lab visits and plans for the next couple dates
        - Need to give him a set of research questions and let him choose which one is most accurate
    - Need to do a research question and edit the variables and objectives

Cell Fractionation Protocol (in mice cortex), will separate the cytoplasm from the nucleus

- Required materials
    - 0.05% NP-40 Alternative in PBS (6mL PBS, 3uL NP-40 alternative)
        - Ice cold
    - Ice cold PBS
    - 4x Laemmli Buffer (0.25M Tris-HCl, pH=6.8, 8% SDS, 40% Glycerol, 0.05% Bromophenol Blue, 100M DDT (need to add fresh)

Reverse Transcription process
Performing Reverse Transcriptions

- Required materials
    - DNase, iScript RT Supermix, Nuclease-free water, DNase Buffer, incubator, iScript Reverse Transcription Supermix
- Procedure
    - Setup DNase master mix
        - Mix iScript DNase and iScript DNase Buffer (found in the mini fridge and mini freezer)
            - In ratio 0.5 microlitre DNase to 1.5 microlitre DNase Buffer
            - Add more or less depending on the amount of RNA samples
    - Add DNase master mix to RNA samples
        - 2 microlitre master mix to each 14 microlitre RNA sample
        - Mix using pipette up and down
    - Incubate
        - At 25C for 5 minutes and then 75C for 5 minutes
    - Add iScript Reverse Transcription Supermix to DNase-treated RNA sample

	■ 4 microliters supermix per 16 microliters of DNaser-treated RNA template ○ Incubate ■ 25C for 5 minutes then 46C for 20 minutes then 95C for 1 minute	

September 29, 2025

Tasks To-do	Review	Notes
☐ Submit the September Logbook	● Went through what I already had of the research proposal ○ Edited any weird formatting or sentence structure ○ Finished the rest of the variables section ■ Learned about confounding variables ● Created a primary research question ● Finished the significance portion of the research proposal Qt PCR ● Reverse transcription pcr ○ Creates multiple copies of DNA off of one strand ■ Through isolating it, heating it up to an intense temperature and then allow the DNA to split ■ Then giving it time to duplicate ● Measure the amount of PRPF4B mRNA in the samples and compare it to a reference (housekeeping) gene ● Household gene ○ GAPDH gene ■ Reference gene ● Looking for PRPF4B ● Add 30mL of DNAfree water to the samples ○ One negative control one prpf4b ● Prepare multiple “containers” to use to create the master mix ○ Follow the correct instructions and ensure no cross contamination and extremely careful ● Load the samples into the qPCR machine with the appropriate primers and master mix	Next Lab date is Thursday 4:00-6:00

	• Run the qPCR program, which amplifies the DNA in cycles and measures fluorescence • Compare PRPF4B expression to GAPDH expression	

October Notes

Daily Notes

October 1, 2025

Tasks To-do	Review	Notes

~~Talk to Youshan about the methods section~~
 	
~~Review clarity of the title portion of RP~~
	• Went through all of Dr. Garcia's comments and reviewed them to make sure mistakes wouldn't happen again
	• Added different papers to my paperpile on introduction topics for RP, included topics on iPSCs, gene editing using CRISPR and brain fluid (didn't take many notes because I just skimmed them so they could help me with the Background part of the project)
	• Fixed the title
	  ○ Was originally unclear but seems a lot more straightforward now
	  ○ Just need to talk to Youshan about it
		Finish RP by next week so I can submit it to Dr. Garcia for initial editing

October 2, 2025

Tasks To-do	Review	Notes

~~Remind Youshan about the methods section~~	• Went into the lab (analysis can also be found in procedure parts)
	  ○ Analyzed the significance of the qt PCR procedure we worked on on Monday
	  ○ X-axis
	  ■ Cycle number (PCR cycle), can be quantified to determine the ratio of actual part we are looking for
	  ■ Used primarily to compare data
	  ○ Y-axis
	  ■ Fluorescence intensity
	  • RFU
	  • Corresponds to the amount of amplified DNA
	  ○ Key point measured is the Ct (threshold cycle) or Cq
		Finish RP by next week so I can submit it to Dr. Garcia for initial editing
		
		Remind Youshan about methods section once it gets past next thursday

	■ PCR cycle number where the fluorescence crosses a certain threshold ● In our case, we used 5000 ■ A lower Ct means more starting template (more target DNA/RNA present) ■ A higher Ct means less starting template ● Got results 30+ for the GAPDH house gene and under 20 for the PRPF4B ● Youshan also said that he is working on the methods section and will send it to me once he finishes	

October 3, 2025

Tasks To-do	Review	Notes
~~Work on all sections except methods because I do not have it yet~~	● Reviewed logbooks with Dr. Garcia as a class ○ Need to separate everything and have both a monthly calendar and daily "agenda" ○ Make everything in calender a lot simpler ○ Be more specific with the Tasks To-do sections ● Took all of procedures out of the daily notes and created its own section called "Procedures" ● Added the part on the RP that connects the rest of the background to the role of heterozygous PRPF4B allele	Finish RP by next week so I can submit it to Dr. Garcia for initial editing Remind Youshan about methods section

October 6, 2025

Tasks To-do	Review	Notes

~~Ask for methods section~~	• Lab visit
	  ○ Worked on practicing extracting RNA out of human cells
	  ■ In this case, we were using baby kidney cells as they can easily reproduce and are very accessible
	  ○ The detailed procedure is in the procedure section but here are things I need to remember
	  ■ Need to follow instructions very carefully
	  ■ Cover the centrifuge machine during use with metal plate
	  ■ Trizol first (makes it pink) and then ethanol
	  ■ Can skip the DNase I treatment
		Finish RP by next week so I can submit it to Dr. Garcia for initial editing

October 7, 2025

Tasks To-do	Review	Notes

~~Put together the hypothesis section~~
 	
~~Add all citations to introduction paragraph~~
	• Completed the hypothesis section for the research proposal
	  ○ All of the key concepts and the justification parts are there it just needs to be edited for grammar errors
	• Looked through my history and found all the sources I used and prepared to in-line do citations for next class
	  ○ Had them all in a list in the RP
	• Worked on formatting all of the research proposal
		Thursday lab visit

October 9, 2025

Tasks To-do	Review	Notes

~~Lab visit today from 4-6pm~~
 	
~~Complete methodology section once received~~
	• Updated all of the citations in the right spots
	• Edited the hypothesis section for both grammatical and conceptual errors
	  ○ Need to make sure everything is "adding" up and makes logical sense
	• RP has been bumped back to October 22
	  ○ Gives me enough time to work on the methodology
	  ○ Also allows for editing from Dr. Garcia and Youshan
	
	Lab visit (more specificity can be found in procedure part)
	• Made a stacking gel that allows us to run tests to determine protein levels
	  ○ Need to remember to wait until the first gel fully dries before making the stacking gel
	  ■ May take around 20 minutes
	  ○ 10 mL 15% of the first one (because only using 1 holder)
	  ■ Ensure that the holder is not leaking AT ALL
	  ● May take a while but trial and error and switch around the clear plates
	  ○ 5 mL 5% on the stacking gel
		Youshan will be away from the lab next week, so avoid excessive emails
		
		Next meeting October 20th from 4-6

October 14, 2025

Tasks To-do	Review	Notes

~~Sent Youshan methodology section for editing~~	• Oral presentation will be on October the 30th
	  ○ Going second after Arvind
	  ○ Need to have around 9-10 slides
	  ■ Avoid having too much text and keep it simple for everyone to understand
	  ○ Can use figures but don't add anything distracting to the project
	• Taking notes on other peoples presentations and asking question is necessary to get participation marks
	• Worked on the methodology section of the RP
	  ○ Finished a rough draft and sent it to Youshan for him to go through
	  ○ Youshan emailed me back within the same day and said it was good to go
		No lab visits this week

October 16, 2025

Tasks To-do	Review	Notes

~~Send Dr. Garcia RP rough draft for her to edit~~	• Spent majority of the time going through the research paper as a whole
	  ○ Read through individual sections various times and changed a lot as some of it was unclear
	  ○ Simplified certain terms so that readability and flow would be better
	• Created a new tab and sent Dr. Garcia the research proposal for her to edit
	  ○ Also went through rubric again and double checked that I had everything needed
		No lab visits this week

October 20, 2025

Tasks To-do	Review	Notes

~~Go through Dr. Garcia's edits~~

~~Read entire paper one more time and do not edit anything yet, just highlight unclarity~~

- Reviewed through the research proposal with Dr. Garcia
    - Many unclear points such as confusing PRPF4B protein levels with overall protein levels
    - Fix the title because "to assess mutant mRNA Blocking" is not clear enough
        - Need to specify
    - Need to add a paragraph on what is currently known about the PRPF4B gene
    - Many grammar errors and being way too general
    - Specify knockout or wild type or KOKO throughout the proposal
    - Went through each part of the introduction and edited all of the grammar mistakes
    - Also updated everything unclear
- Lab work (4:00-5:30)
    - Went through making stacking gels again because Youshan needed it for his work tomorrow
        - Failed twice…
            - Need to be more careful when setting up the apparatus and mixing the gels
        - Everything from the procedure felt well rehearsed and a lot better than last time
            - I've gotten much better at these procedures I think from practicing them
    - Send Youshan RP on Thursday or Friday

Go into the lab today between 4 and 6

October 22, 2025

Tasks To-do	Review	Notes

~~Fix all edits by Dr. Garcia~~
 	
Send to Youshan
 	
~~Talk to Youshan about all misunderstandings and questions on proteins and variables~~
	• Went through my logbook and edited some of the october notes so that I could be ready when the logbook is due
	• Spent majority of the time reading through Dr. Garcia's edits
	  ○ REALLY focus on PRPF4B
	  ○ CRISPR part is unclear and need to make it more general and not about my project
	  ○ Added the PRPF4B background information paragraph
	  ■ Took lots of time to research and find sources that actually gave valuable information
	  ■ Also did the citations for that paragraph and added it to the references part
	  ○ Still have lots to edit especially with the references, significance, methodology, hypothesis and variables
	  ■ Problem is most of them are informational errors and understanding errors
	  ■ Need to talk to youshan about misunderstandings
		Into the lab tomorrow

October 23, 2025

Tasks To-do	Review	Notes

Send RP to Youshan by tomorrow after ASP block	• Went through analyzing western blot analysis
	  ○ Need to put it into the machine, analyze color and darkness
	  ○ Inverting the image is very beneficial to seeing the protein bands
	  ■ In the lab, we looked at nuclear proteins
	  ○ Specific antibodies are used to detect the target protein
	  ○ There is also a wash that is required before putting it in the machine	

	■ Two white bottles in the fridge, mix equal volume of each in a rectangular petri dish ■ Right before putting membrane on the machine, need to soak the membrane in the rectangular petri dish ■ Do that with both membranes	

October 27, 2025

Tasks To-do	Review	Notes
~~Submit RP!!~~	● Was in the lab again today between 4:00 and 5:30 ○ Spent 30 mins creating 2 stacking gels for something Youshan was doing tomorrow ■ Need to be especially careful when pouring the resolving gel into the apparatus ● If it pours out, might need to redo the process of creating the gel ■ Everything else with the procedure went really smoothly ● Remember when setting up apparatus to constantly test leakage ○ Spent some time going through the research proposal and the introduction section ■ Edited everything that was wrong or even the slightest weird ○ Worked on the presentation for October 30 ■ Planned out all of the slides and what they would have ● Still don't know how much text to add on each slide	Need to finish the oral presentation Practice oral presentation

	• Finished the introduction part, the variables, the objectives and the hypothesis	

October 28, 2025

Tasks To-do	Review	Notes

~~Practice!!~~	• Reformatted the presentation for the oral presentation
	  ○ Made it mostly pictures instead of text, especially the introduction part
	  ○ Also went onto Biorender and created all of the images needed
	  ○ Practiced 3 times through the presentation and realized I need some notes
	  ■ Finished writing a plan/what to talk about for each slide
	• Remember to listen and take notes on other peoples presentations
		Go into the lab Thursday

October 30, 2025

Tasks To-do	Review	Notes
~~Review extracting rna before going into the lab~~	• Finished presentation at the beginning of project ○ Went alright except for how I skipped a slide… • Listened to other peoples presentations • Down below are my notes Andi: - Sarcasm is easy for humans but not for computers	Go into the lab today

- Avoid giving overly negative comments
- Different ways to find
  - Called prosotic features
  - Lower/higher in pitch (statistically significant average)
  - Facial expressions
  - Noisier sounding sentence
  - Different intonation
  - Pitch accents
  - Elongation
- Spectograms
  - Y axis shows frequency in hertz
- Applications
  - Finding emotion in business
    - Medicine (sampling patients)
- Analyzing images from datasets, neural networks
  - A need to develop something like this
- Can a simple logistic regression model preform at an equal level as neural networks when detecting sarcasm from audio?
  - Turn it into a computer application
  - Can cause misinterpretation
- What machine learning model are you using and are you taking into account other factors or
  - Are the datasets coming from the uofc?
  - Mustard++ dataset
- Are you analyzing the sound or converting it into an image or quantifying it
  - Looking at the sound directly

Kinjal:

- Impact of LBNP on activity and hemodynamic responses
  - Blood flow
  - Gravity
- Can pots be measured while using LBNP
  - Change in posture
    - Pots is measured through an a graph (time and heart rate)
    - Restricted blood flow throughout the body

- Symptoms
    - Nausea, rapid heart beat, brain fog and chest pain
- Flowchart for diagnosis
    - Roman josi
    - Anyone can get pots regardless of age or gender
- Process of measuring LBNP
    - Valsalva maneuver, sinus arrhythmia, hyperventilation
- LBNP = lower body negative pressure
    - Simulates the effect of gravity
- How do hemodynamic responses differ between baseline and lbnp in individuals with POTS
    - Should differ
- How does LBNP induced orthostatic stress impact parasympathetic activity in POTS patients
    - Activity will decrease
- Independent: lbnp induced orthostatic stress
- Dependent: hemodynamic responses and parasympathetic activity
- Controlled: supine position
- Significance: ease of patient measurement, mri machines

How many patients are you going to be testing

Lab visit:

- Went through extracting rna
    - Took cells that Youshan was already using
    - Broke down cells and released RNA with trizol and ethanol
    - Filtered it multiple times and centrifuged between adding trizol and ethanol
    - RNA will be used later in RT-qPCR to measure PRPF4B mRNA levels
- Placed into wells with urea
    - helps break open the cells and release their contents
        - unwanted liquid and cell debris were washed away

November Notes

Daily Notes

November 3, 2025

Tasks To-do	Review	Notes

~~Listen to 2-3 other presentations~~	Elise
	- Using machine learning to improve low earth orbit prediction using tle and gps data
	  - Improve prediction accuracy
	  - What % are you aiming for and what was the avg prediction data before
	- Main issue is # of orbital object is growing
	  - Especially in low earth orbit
	  - 34000 objects, need to avoid every single one when leaving and entering earth
	  - Increased exponentially
	- Current methods
	  - Physics based SGP4 algorithm
	  - Many dropbacks as it is only physics based
	  - Many many variables
	  - Errors grow quickly
	- Nothing has been employed globally
	  - Can a ML correction model significantly reduce multi-day or extended orbital prediction error for LEO objects?
	- spacetrack.org
	
	Shaayaan
	- Developing alloy coating that helps thermal stability for aerospace applications
	  - How will you test the extreme conditions in space and how will you know that it works?
	  - Are you trial and error for alloy compositions?
	  - 3-6 metals in near-equal proportions
	  - High strength, oxidation resistance
	- Face thermal and mechanical stress, HEAs have improved durability and performance
	- Testing compositions impact on oxidation resistance, adhesion strength and structural integrity
	  - Confound variables??
	- A strong coating = less coating required	

- Would be more effective and can reduce maintenance costs

Samir

- Analyzing kidney tissues between healthy and type 2 diabetic female rats
  - Diabetes
    - Undiagnosed diabetic, how are there stats on undiagnosed? Is it just a prediction
  - Diabetic kidney disease
    - Most common complication
  - Microscopic injury
    - Thickened GBM
- Animal models allow for a controlled study of diabetic kidney disease
  - Rats are very similar to human kidneys
    - Easily samplable
  - Golo-kakizaki rats are used because they “naturally” have diabetes
    - Don't have to give them diabetes
- How does the kidney tissue differ between healthy and type 2 diabetic female rates when compared using histological analysis
- Look at high resolution digital images of the stained tissue
  - Analyze how diabetes alters kidney tissue structure in female rats over time
    - Female > male, more similar and controlled results
- Healthy vs control rats
  - Looking at cross-sectional area
  - Membrane thickness
  - Collagen content
- Methodology
  - Kidney tissues collected and analyzed
  - Tissues then get processed and embedded into wax and then sectioned
  - Stained again and analyzed
- You said imagej was for analyzing images but what does it actually look for

November 5, 2025

Tasks To-do	Review	Notes

	• Go to Dr. Garcia to review any writings, presentations
	  ○ Plan everything ahead of time (go for around a week before the deadline)
	• Logbook needs to be updated regularly
	• Everything done in the logbook MUST be put in the actual daily logbook entry
	• Need to send weekly email to mentor and Dr. Garcia
	  ○ Progress reports
	  ○ Summaries of meeting
	  ○ Plan next week
	
	https://nccid.ca/publications/understanding-rt-pcr-tests-and-results/
	• Genetic material and pathogens
	  ○ Genetic material are the instructions inside cells or viruses
	  ○ Viruses like SARS use RNA
	  ■ RNA can be used as a test target because viruses like SARS target RNA
	• RT-PCR
	  ○ Real time polymerase chain reaction
	  ■ Amplifies and makes many copies of a DNA segment
	  ■ Real time means the version that works for RNA viruses (which uses reverse transcriptase to convert RNA to DNA)
	  ● Looks at fluorescence
	  ○ Very sensitive to light
	• How testing works
	  ○ Sample collection
	  ■ Prepare it by extracting RNA
	  ■ Add it to a reaction mixture with reverse transcriptase, DNA polymerase, primers and probes
	  ○ Reverse transcription
	  ■ Convert RNA to cDNA
	  ○ Amplify PCR
	  ■ Measure amplification through fluorescent probes
	• Looking at the values and test results	

	○ Lower Ct value means a higher amount of genetic material ○ Higher Ct value means a lower amount of genetic material ○ Ct values help people look at disease state, transmissibility risk and recovery ● Usage and limits ○ Can be used for diagnosing infections, looking for treatments and analyzing diseases ○ Limits ■ Tests genetic material but RNA does not mean there is a live virus ■ Sample quality and timing can impact accuracy ■ Ct values can also be influenced by many other factors like sample type and assay ■ A Ct value does not give enough context and a conclusion	

November 6, 2025

Tasks To-do	Review	Notes
	● RNA extraction is a complex procedure with a lot of tedious steps ○ Need to mix multiple solutions provided by the Zymo Research kit ■ I don't exactly remember the procedure so it can be found in the pamphlet in the kit ○ Need to be careful between steps to ensure I remember which step im on ○ Centrifuging it between steps is required ■ The caps (when open) need to be facing counter clockwise in the centrifuge ● This ensures that the caps don't snap off ○ Label all of the tubes before transferring ■ This allows you to know	Next meeting November 13 at 4:00 till 5:30

	exactly what mixture is in each tube ■ Avoids the chance that they get mixed up and results get skewed ○ Pipette tips when adding any buffers don't need to be changed between uses AS LONG as they don't touch the tube in any way ■ When using small amounts of fluids in the procedure, need to change tips because all of the fluid needs to cover the white filter	

November 7, 2025

Tasks To-do	Review	Notes
	Ronald - Sticky board varroa mite detection and counting using a machine learning model - Trying to help bees as they are extremely important to Alberta's ecosystem - Varroa Mites eat bees, which reduces the population - Object detection with machine learning - How effective is synthetic data generation from limited real samples for developing accurate varroa mite detection - Generate a synthetic dataset Shicheng - Evaluating the impact of 2-phage and 5-phage bacteria against e coli Dr. Garcia ● Twice to the lab weekly ● Need to be weekly emails ○ Need to let them know when you are coming and what is happening ○ Logbook and calendar need to be very up to date ● Need to look at the big picture when going to the lab ○ Where will I be in several week? ○ Whats the plan for the future?	Need to review Dr. Garcia notes on logbook for next month

November 13, 2025

Tasks To-do	Review	Notes

~~Ethnics 2A~~	Arvind
	- Stretch induced syncope
	  - Basically fainting
	  - Call transient hypotension
	- Is orthostatic stress necessary for induced hypotension?
	  - Orthostatic stress is necessary for induced hypotension
	  - Orthostatic stress is observed in standing subjects, no cases of SIS have been reported in supine subjects nor has presyncope
	- Testing protocol
	  - Stretching with a shoulder abduction
	- 20 control 20 SS
	
	CYSF
	● Finished the ethics form
	  ○ “Human iPSCs, which come from patients in Foothills Hospital, will be grown under lab conditions. Using CRISPR-Cas9, gene editing, a single allele of the PRPF4B gene will be edited to form heterozygous knockout cells. RNA and protein levels will be measured in both the edited and unedited cells. The levels of PRPF4B mRNA and protein will be measured through procedures such as RT-qPCR and Western blot to compare gene expression and protein production. Studying the implications of knocking out an allele can determine whether gene knockout can be used to remove a mutant PRPF4B allele. Mutations in the PRPF4B gene often cause either a buildup or lack of cerebrospinal fluid in the brain, which can lead nutrient level fluctuations. The human iPSCs used in this project were already collected by medical professionals for research purposes, and no new samples are being taken. All of the lab work, which include RNA extraction, RT-qPCR, Western blot, electrophoresis and	

more will be done under the supervision of Youshan Heng (youshan.heng@ucalgary.ca), a postdoc at the University of Calgary. This is one of the subtopics of his primary project on the PRPF4B gene."

Dr. Garcia and Mrs. Kale

- Science Fair poster and presentation is very specific and has a bunch of requirements
    - From a hypothesis to logbook, everything is necessary to getting the point
    - Need to practice lots so no information is forgotten
- Don't have much time at all for ASP project (if unplanned/not planned well)
    - Need to divide tasks up throughout the span of the next couple months
        - Have a rough idea when each tasks need to be done
            - Contact mentor if unsure
        - Check how long task will take
    - Don't have time during winter break or midterm break
        - Have lots of stuff and very very busy
        - There are many tradeoffs if you choose to focus on one specific goal
    - Presentations and writing information takes up to two weeks
    - Should go into the lab two-three times a week
    - Plan EVERYTHING
        - Not a week can go by without communication and a summary
            - Get more work done soon rather than later if the mentor has time

November 14, 2025

Tasks To-do	Review	Notes
	Lab Visit ● Worked on rt pcr ○ Compared wild type, homozygous and heterozygous with GAPDH household gene ● Takes the amount of mRNA in the sample ○ Copies that DNA over and over again while dye glows brighter as more copies appear ○ How bright it is = the amount of PRPF4B mRNA ○ Uses the PRPF4B forward and reverse primers ■ Tells exactly which gene needs to be copied ● Procedure overview ○ In the lab, we switched the company providing the buffers ■ New one requires a yellow sample buffer and a blue sample buffer, which are used in two different steps ○ Use ratios to your advantage when creating master mix ○ Can dilute the PRPF4B samples before (this time diluted it 20ul)	Monday will analyze results, should take about 30 mins Includes data analysis and processing

1. *(Optional)* Add the Yellow Sample Buffer (40X) to the amoun

Final reaction volume
20 µL
10 µL

The Yellow Sample Buffer is diluted to 1X in the final reactio

2. *(Optional)* Vortex, then centrifuge the DNA and Yellow Samp
3. Combine the master mix, the primers, and nuclease-free wa
4. Combine the master mix, the primers, and nuclease-free wa tables.

Note: If the Yellow Sample Buffer is not used, add nuclease

Table 1 20–µL reaction

Component	Stock concentration	Final
Yellow Sample Buffer and DNA (step 1)		
DNA[2]	5 ng/µL	
Yellow Sample Buffer	40X	
Master mix, primers, and nuclease-free water (step 3)		
PowerTrack™ SYBR™ Green Master Mix	2X	
Forward and reverse primers[4]	8,000 nM	
Nuclease-free water	—	
Total PCR volume	—	

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 8.5 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentra

Table 2 10–µL reaction

Component	Stock concentration	Final
Yellow Sample Buffer and DNA (step 1)		
DNA[2]	5 ng/µL	
Yellow Sample Buffer	40X	
Master mix, primers, and nuclease-free water (step 3)		
PowerTrack™ SYBR™ Green Master Mix	2X	
Forward and reverse primers[4]	8,000 nM	
Nuclease-free water	—	
Total PCR volume	—	

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 4.25 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentra

IMPORTANT! The reaction turns green due to the Yellow S

Mix the components thoroughly, then centrifuge briefly to co

6. Transfer the appropriate volume of each reaction to each wel

7. Seal the plate with an optical adhesive cover, then centrifuge any air bubbles.

PCR can be performed on the reaction plate up to 8 hours after co light.

Set up and run the real-time PCR instrument

1. Set up the thermal protocol according to one of the following

   Note: Standard cycling conditions are recommended for gen

   Table 3 Fast cycling mode

Step	Ter
Enzyme activation	
Denature	
Anneal/extend	

   Table 4 Standard cycling mode

Step	Ter
Enzyme activation	
Denature	
Anneal/extend	

2. Set the instrument to perform a default dissociation step, acc

   Table 5 Fast cycling mode

Step	Ramp rate[1]
1	1.99°C/second
2	1.77°C/second
3 (Dissociation)	0.075°C/second

   [1] Use the default ramp rate for the StepOnePlus™ Instrument.

   Table 6 Standard cycling mode

Step	Ramp rate[1]
1	1.6°C/second
2	1.6°C/second
3 (Dissociation)	0.075°C/second

   [1] Use the default ramp rate for the StepOnePlus™ Instrument.

   Note: A dissociation step must be performed immediately afte

3. Set up the options.
   - Experiment type: Standard curve
   - Reagent: SYBR™ Green reagents
   - Reporter: SYBR™ Green
   - Quencher: None
   - Passive reference dye: ROX™ dye
   - Ramp speed: Standard or fast
   - Melt curve ramp increment (all instruments, except Step(

November 17, 2025

Tasks To-do	Review	Notes
	Rebrikov and Trofimov 2006 - Applied biochemi… ● What RT-qPCR measures? ○ RT-qPCR measures how much mRNA a gene is making inside the cell ○ mRNA is the first step before proteins are made ■ More mRNA means more proteins being made ○ Tells how much PRPF4B mRNA the cell is making after one copy of the gene is knocked out ● Why does RT-qPCR matter? ○ RT-qPCR shows whether the remaining PRPF4B allele is making ■ The same amount of mRNA ■ More mRNA (which is for compensation) ■ Less mRNA (which is a problem for the body) ○ If PRPF4B mRNA stays normal, it suggests the wild-type allele is compensating ● How does RT-qPCR work? ○ Extract RNA from cells ■ RNA represents the active genes inside the cell ■ Extract from ● Wild-type cells ● Heterozygous cells ● Knockout cells ■ Helps compare PRPF4B activity between the groups ○ Remove DNA contamination ■ DNase treatment removes any DNA that could screw up the results ■ Ensures signal only represents mRNA not DNA ○ Convert RNA -> cDNA ■ Not necessary in this project as it was previously done ■ Reverse transcriptase turns mRNA into cDNA ■ The amount of cDNA made directly correlates to how much mRNA was originally in the sample	Lab work tonight to analyse the rt qpcr results

- Use of primers
  - Use a forward primer and reverse primer that match only PRPF4B
    - Primers stick to the start and end of the PRPF4B cDNA
  - Basically tells the machine to only read the PRPF4B gene
- Do same for household gene
  - GAPDH
- PCR copies the PRPF4B DNA
  - Machine heats and cools the sample over and over again
  - Each cycle doubles the amount of target DNA
  - As DNA goes up, the fluorescence glows brighter
- Measuring fluorescence
  - The machine measures the exact PCR cycle when the sample is bright enough
  - Cycle number is the Ct value
    - A lower Ct means more starting mRNA
    - A higher Ct means less starting mRNA
- Comparing PRPF4B to GAPDH
  - Tells you whether PRPF4B mRNA is
    - Normal
    - Reduced
    - Increased

Lab Work

- Results weren't as expected and did not match past tests
  - Will redo again on Thursday
  - Main problem was human error and DNA staying inside the pipette when loading samples
- Had significant outliers that were off by nearly 0.5 from the other 2 tests
  - Two would be close and the other one would be very far
  - Results are practically only accurate when everything is within a margin of 0.1
- Everything needed for analysis is inside the document Youshan changed on the lab

	computer for rt-qpcr ○ Contains a lot of averaging out and subtracting (as of now, I do not remember exactly what he did but when I am back in the lab, I will check) ● Next time, need to be more careful during pipetting process and making sure nothing gets stuck in the tip ○ Need to eject all of it	

November 19, 2025

Tasks To-do	Review	Notes
	● Meeting with Dr. Garcia ○ Understanding the concept is equally as important as understanding how the procedure works ■ Going to be grilled during the science fair ■ Lots of specificity required and sometimes, research papers are confusing for that ○ Need to know why rt-qpcr or a western blot and exactly how it works ■ Can use biorender in the future to explain these in simpler context ○ Delta delta Ct is very very important in the CYSF, need to go very indepth into it ● Looked at Cooper's ASP project ○ Goes very detailed into the double antibodies and how one of them is a stainer ■ From biorender ● Textbooks and reading more deeply into Campbell biology and genetics can help a lot with understanding	

November 20, 2025

Tasks To-do	Review	Notes

	• Was in the lab with Youshan between 4:00-4:30
	  ○ Mainly just watched him sort iPSCs
	  ○ FACS (Fluorescence-Activated Cell Sorting)
	  ■ The cells are given a fluorescent tag that sticks only to the kind of cells wanted
	  ■ Cells flow one-by-one through a laser beam.
	  ■ The machine detects how bright each cell glows.
	  ■ It separates them by charging droplets and flicking them into different tubes.
	  ○ You can sort only the edited cells (CRISPR-positive).
	  ○ You can sort live iPSCs without damaging them.
	  ■ Very very precise
	• Very important part that ensures CRISPR-Cas9 allele knockout is successful
	  ○ CRISPR isn't 100% of the time successful
	  ○ Sorting picks only the cells with the correct PRPF4B knockout	

November 21, 2025

Tasks To-do	Review	Notes

~~Ethics 2A~~	• Studied for physics for a majority of the time
	• Ms. Kale talked about presenting for CYSF
	  ○ Need to avoid looking at the screen when presenting
	  ○ Only needs to be done if made it into the science fair
	  ○ Risk form needs to be completed ASAP
	  ○ Timing needs to be perfected before presenting
	  ■ Cannot rush any slides and need to thoroughly explain everything	

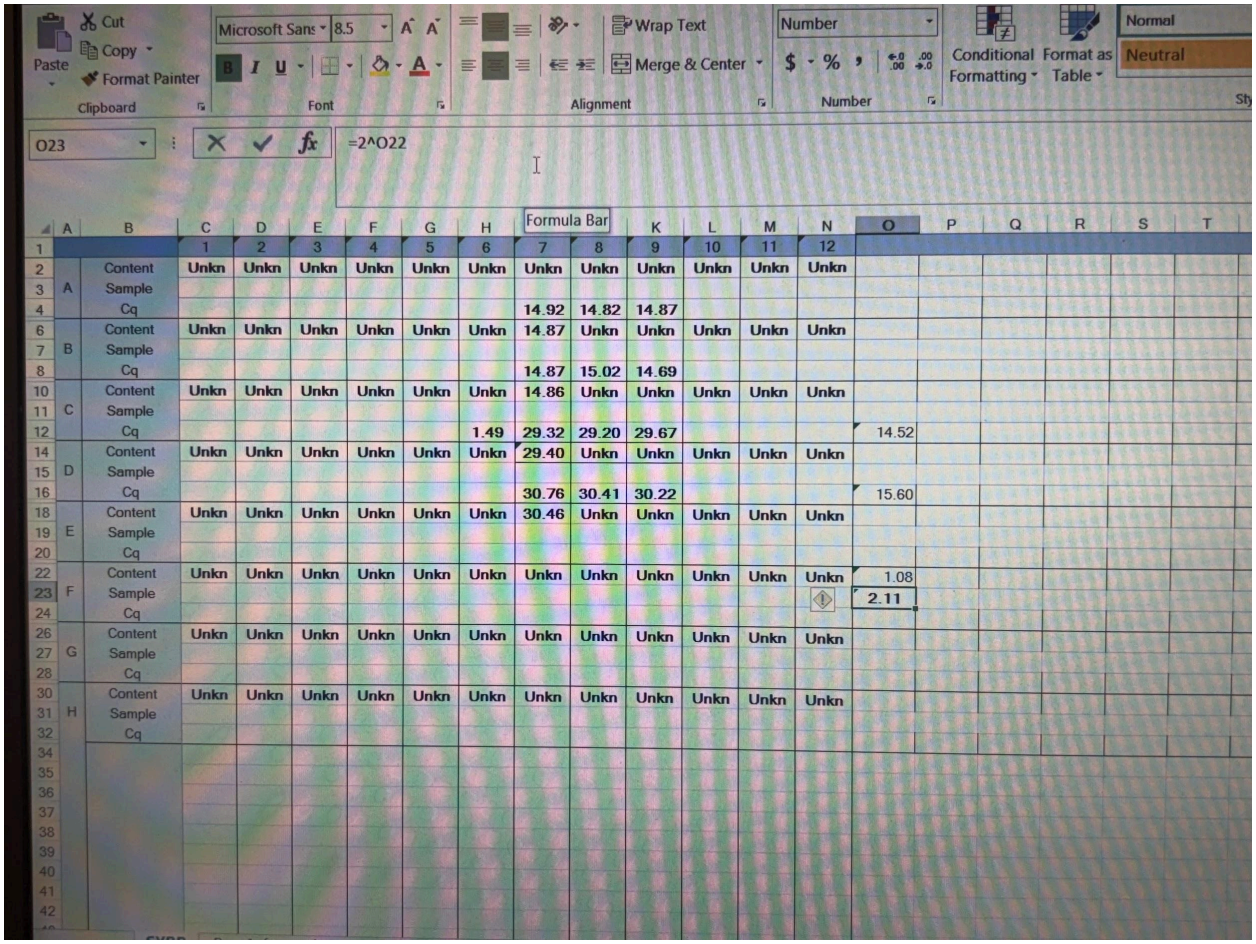
November 24, 2025

Tasks To-do	Review	Notes
~~Lab summary~~	● Lab visit with Youshan ○ Redid the rt-qpcr ■ This trial didn't really feel very accurate ■ Seemed like there was a lot of human error but will see on Thursday ● There was always substances left in the pipettes that would not go out ○ Youshan said next time that its ok as long as the rest of the procedure is consistent ■ The rest of the procedure was the same as the previous time (November 14th) except we didn't make enough master mix for all three (homo, het and WT) ● We decided to ignore the homogenous case completely and instead focus on heterozygous and wild-type as those are the most important	Meet with Youhsan on Thursday Logbook due Thursday

November 27, 2025

Tasks To-do	Review	Notes

~~Hand in logbook~~	• Finished the logbook
	  ○ Worked on the december calender
	  ■ Can be found on the december tab
	• Lab
	  ○ Analyzed the rt-qpcr results from Monday's lab visit
	  ○ Found that the data was actually a lot more significant than the first time, meaning the experiment was done relatively well
	  ■ The deviation was relatively minor in each of the tests
	  ○ Analysis portion
	  ■ Average out each of the plate values
	  ■ Subtract PRPF4B from GAPDH test with homo and wt
	  ■ Subtract the homo from wt
	  ● This tells you how many PCR cycles apart the two samples are
	  ■ 2^of the subtraction
	  ■ ΔΔCt method
	  ● turns cycle difference into a real "how many times more?" number
	  ● PCR doubles DNA every cycle so thats why its 2^
	  ○ Image of results can be found below
	  ○ Created a stacking and resolving gel for protein analysis next Monday
		Meet with Youhsan today
		
		Logbook due today

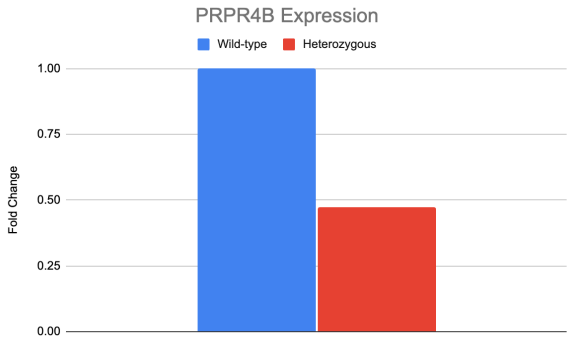


			1	2	3	4	5	6	7	8	9	10	11	12	O
2		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	
3	A	Sample													
4		Cq							14.92	14.82	14.87				
6		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	14.87	Unkn	Unkn	Unkn	Unkn	Unkn	
7	B	Sample													
8		Cq							14.87	15.02	14.69				
10		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	14.86	Unkn	Unkn	Unkn	Unkn	Unkn	
11	C	Sample													
12		Cq						1.49	29.32	29.20	29.67				14.52
14		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	29.40	Unkn	Unkn	Unkn	Unkn	Unkn	
15	D	Sample													
16		Cq							30.76	30.41	30.22				15.60
18		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	30.46	Unkn	Unkn	Unkn	Unkn	Unkn	
19	E	Sample													
20		Cq													
22		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	1.08
23	F	Sample													2.11
24		Cq													
26		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	
27	G	Sample													
28		Cq													
30		Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	
31	H	Sample													
32		Cq													

December Notes

December 1, 2025

Tasks To-do	Review	Notes
☑ CYSF 2B	ASP Class ● Finished the ethics 2B form ○ "No human participants were recruited for this project. The human induced pluripotent stem cells (iPSCs) used in this study were not collected by me and were not collected for the purpose of this science fair project." ○ "There are no risks to people because this project uses previously collected human iPSC samples that were obtained by medical professionals and are only used for research." ○ "This project works to understand whether cells can stay healthy and produce enough PRPF4B proteins when one PRPF4B allele is missing. This would be important for studying the causes of PRPF4B-related brain disorders." Lab Work ● Visualizing the rt-pcr results from the previous day ○ Did not do western blot because Youshan was busy ■ Plan to do it next Thursday ■ Instead we visualized the results using a bar graph RT-PCR Results	Go into the lab today at 4 Next meeting thursday



December 3, 2025

Tasks To-do	Review	Notes
	ASP Class ● Project is getting more rushed and there are more assignments going into the next several weeks ● Introduction due end of december ○ Take the research proposal introduction and expand based on feedback from teachers ○ Ask for feedback after writing drafts from Dr. Garcia ● Need to get cysf ethics approval ● Talked to Dr. Garcia 1 on 1 for a little bit ○ In the introduction, these need to be covered ■ Connection between brain function and gene ■ More about gene ■ Does it have a role in other parts of the body ● Edited half of the introduction paragraph… still lots and lots to go but at least 3 paragraphs are fully complete ○ A balance of brain fluid is necessary for proper neurological development and function for humans.1,2 Cerebrospinal fluid (CSF) circulates within the brain's ventricular system and allows for nutrient delivery, waste removal, and the protection of neural tissues.3 Disruptions or constant changes in the regulation of CSF, whether its an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.4 These conditions have been known to cause developmental problems like speech disorders and in some cases, neurological development disorders.5 Understanding the molecular mechanism that play a role in CSF dynamics is important to preventing this disease and creating a proper treatment for people who are already affected.6-9 ○ Recent research in genetic biology has found that RNA splicing in	

	neurological development has been directly linked to CSF homeostasis.10 One gene in particular, PRPF4B, is being investigated.11 The PRPF4B gene encodes a serine/threonine kinase protein that is involved in the regulation of pre-mRNA splicing.12 Splicing is the process of removing non-coding introns and joining exons together to create the mature mRNA that cells use to make proteins. ○ More specifically, the PRPF4B gene plays an important role in the spliceosome complex, which is a group of molecules that removes noncoding parts of RNA to make correct mRNA for protein production.13,14 The PRPF4B gene helps in the synthesis of the necessary proteins for neuron growth by maintaining correct mRNA splicing.14-16 Incorrect splicing caused by changes in PRPF4B activity can lead to incorrect protein production.17 When this happens in the brain, it can cause fluid inconsistencies and cause abnormal CSF buildup.	

December 4, 2025

Tasks To-do	Review	Notes
	Lab Work ● Applied the second antibody on the paper with the protein samples ○ Washed it twice with buffers after 30 mins ■ Then rinsed it twice every 5 minutes for 15 minutes ○ Binding the detection antibody of the PRPF4B protein ■ Then removes the excess antibodies so only the signals from the correct bands show up ○ Need to ensure the paper always stays wet and never dries out	Next meeting Monday

	• Analyzed the results
	  ○ Protein levels in HET were the same as in WT
	  ■ Don't really know why yet
	  ■ Band size and length were the exact same between HET and WT
	  ○ Still need to compare it to GAPDH household gene	

December 5, 2025

Tasks To-do	Review	Notes

	• Spent the entire time writing another paragraph which expands on the impact of the PRPF4B gene on the body
	  ○ Read through Youshan's research proposal
	  ○ "The PRPF4B gene plays an important role in the spliceosome complex, which is a group of molecules that removes noncoding parts of RNA.13,14 Incorrect splicing due to variations within PRPF4B can cause incorrectly expressed proteins and variability within bodily fluids.17 In the brain, splicing inconsistencies can lead to inaccurate expression of proteins needed for the regulation of fluid transport, ion channels, and cilia function within the ventricular system. This leads to an abnormal accumulation of cerebrospinal fluid and, as a result, variability within intracranial pressures. In addition to the brain, PRPF4B splicing errors can impact other organ systems dependent on fluid regulation and correct protein synthesis. While these are significantly less minor than CSF regulation in the brain, PRPF4B mutations in other parts of the body can still impact human health. The kidneys, which are responsible for the balance of fluids within the body, may be affected if the incorrect proteins are produced. Protein misregulation in the kidneys
		Next meeting Monday

	can affect the functioning of the endothelial cells, which often causes inflammation, oxidative stress, and impaired blood flow in the kidneys." ● Talked to Dr. Garcia about project ○ This intro needs to have a lot more detail and needs to cover this genes role in different parts of the body ○ More specificity ○ Can have extension if needed	

December 8, 2025

Tasks To-do	Review	Notes
	● Lab visit ○ Brought USB drive and loaded the data and images from the western blot on it ■ Edited the images on a powerpoint slide so that the band intensities could be directly comparable ■ Band sizes for the GAPDH were uneven meaning there could be some inconsistencies but it is highly unlikely ■ Made a stacking gel to rerun the experiment and ensure the starting protein levels were the same	Next meeting Thursday

December 9, 2025

Tasks To-do	Review	Notes
	● Meeting with Dr. Garcia ○ Decide with Youshan when to meet because results are completed ○ Zoom meetings? ■ Discuss the results and do more research into the processes ● Edited the last two paragraphs according to Dr. Garcia's suggested edits on the research proposal	Next meeting Thursday

	○ "To understand how disruptions in the PRPF4B gene affect human cells and mRNA splicing, gene editing is required. CRISPR-Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, allows researchers to create targeted gene knockouts. This includes creating heterozygous knockouts of PRPF4B to study how reduced protein levels impact cell function and cerebrospinal fluid. ○ The current study therefore focuses on evaluating PRPF4B protein levels in iPSCs after CRISPR-Cas9–mediated gene removal and measuring its impact on mRNA and protein production. Understanding whether cells can tolerate the loss of a single PRPF4B allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development. This research also provides a basis for future work towards a treatment that reduces the impact of PRPF4B gene mutations in the development of neurodevelopmental disorders."	

December 11, 2025

Tasks To-do	Review	Notes
	● Was gone half the class for a dentist appointment ● Can get extension on introduction paper if needed ● Edited the middle paragraph and made it so that it aligns more with what I need to talk about ○ "Because patients with PRPF4B-linked neurodevelopmental disorders often have a dominant heterozygous mutation, understanding how reduced PRPF4B function affects fluid production is necessary. iPSCs, which are adult cells that have been reprogrammed into a stem-cell-like state, allow researchers to model neural	Next meeting Thursday

	development without brain cells. It will help researchers understand if a single copy of a functional PRPF4B gene can maintain normal mRNA splicing and protein levels." ● Lab visit ○ Ran electrophoresis ○ Learned about the process of transferring proteins from a sds-page onto a membrane ○ This allows for the antibodies to bond ○ Happens through the transfer of proteins because they are negatively charged to a positive side ○ Proteins are negatively charged because acidic amino acid side chains (like aspartic acid, glutamic acid) losing protons (H+) at physiological pH ○ Still need to go more in depth into western blot, why its done and why it works	

December 15, 2025

Tasks To-do	Review	Notes
	● Added another paragraph to the introduction section ○ This covers the rubric (which requires the research question and hypothesis) ■ "Having this background, the primary research question of this study is whether human induced pluripotent stem cells (iPSCs) can maintain the normal levels of PRPF4B protein and mRNA expression (normal levels found in wild-type cells) after one allele of the PRPF4B gene is knocked out using CRISPR-Cas9. It is hypothesized that the knockout of a single PRPF4B allele will not affect the level of PRPF4B protein	Next meeting Thursday

	or PRPF4B mRNA compared with non-edited, wild-type PRPF4B cells. This is because the remaining wild-type PRPF4B allele is expected to compensate for the loss by maintaining transcription levels and translation to maintain normal PRPF4B mRNA and protein levels. If this were to not happen within the human body, important organs could be impacted, which could cause development disorders and fluid maintenance problems." ○ Mostly paraphrased from the research paper ○ Also added this paragraph ■ Don't know how strong or understandable it is, will look into more next class because ran out of time ■ "Not all genes will respond the same way to the loss of a single allele.23 Some genes are dosage-sensitive. This means that a single functional copy is not enough to maintain normal mRNA and protein levels.24 Others are dosage-tolerant and can compensate through the remaining allele.25 Whether PRPF4B is dosage-sensitive or dosage-tolerant is currently undetermined. Understanding if cells can maintain normal PRPF4B expression with only one functional allele is therefore required for understanding how heterozygous PRPF4B mutations contribute to diseases."	

December 17, 2025

Tasks To-do	Review	Notes
	● Completed all the citations ○ Citations can be found at the end of the Introduction section ● Looked over the grammar and sentence structure ○ Here is the full intro section ○ “A balance of brain fluid is necessary for neurological development and function in humans.1,2 Cerebrospinal fluid (CSF) circulates within the brain’s ventricular system and performs the functions of nutrient delivery, waste removal, and the protection of neural tissues.3 Disruptions or constant changes in the regulation of CSF, whether its an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.4 These conditions have been known to cause a range of developmental problems such as speech disorders and cognitive disorders.5 Understanding the molecular mechanisms that impact cerebrospinal fluid regulation is important for preventing these conditions and creating an effective treatment for people who are already affected.6-9 ○ Recent research has found that RNA splicing in neurological development has been linked to CSF homeostasis.10 One gene, PRPF4B, is being investigated.11 This gene codes for a serine/threonine kinase protein that plays a role within pre-mRNA splicing.12 RNA splicing is the removal of non-coding introns from pre-mRNA and the joining of exons together to create the mRNA that cells use to make proteins. 13 ○ The PRPF4B gene plays an important role in the spliceosome complex, which is a group of molecules that removes noncoding introns of RNA. 14 PRPF4B acts as a serine/threonine kinase within the spliceosome. It adds phosphate	Next meeting Thursday

groups to specific splicing proteins to regulate their activity. This is required for proper spliceosome assembly, timing and disassembly during the pre-mRNA processing. By doing this, PRPF4B helps to ensure that the introns are correctly removed and the exons are correctly joined. When the PRPF4B kinase activity is changed, it can cause discrepancies in splice-site selections and mRNA creation transcripts. Even though these transcripts could still be expressed at normal levels, it could also encode proteins with impaired function.

- Incorrect splicing due to variations within PRPF4B can cause improperly expressed proteins that could disrupt cellular processes that play a role in fluid regulation.15 In the brain, splicing inconsistencies can lead to inaccurate expression of proteins needed for the regulation of fluid transport, ion channels, and cilia function within the ventricular system. 16 This leads to an abnormal accumulation of cerebrospinal fluid and, as a result, variability within intracranial pressures.17 In addition to the brain, PRPF4B splicing errors can impact other organ systems dependent on fluid regulation and correct protein synthesis.18 While these are significantly less minor than CSF regulation in the brain, PRPF4B mutations in other parts of the body can still impact human health. The kidneys, which are responsible for the balance of fluids within the body, may be affected if the incorrect proteins are produced.19, 20 Protein misregulation in the kidneys can affect the functioning of the endothelial cells, which can cause inflammation, oxidative stress, and impaired blood flow in the kidneys.21, 22
- Not all genes will respond the same way to the loss of a single allele.23 Some genes are dosage-sensitive. This means that a single functional copy is not enough to maintain

normal mRNA and protein levels.24 Others are dosage-tolerant and can compensate through the remaining allele.25 Whether PRPF4B is dosage-sensitive or dosage-tolerant is currently undetermined. Understanding if cells can maintain normal PRPF4B expression with only one functional allele is therefore required for understanding how heterozygous PRPF4B mutations contribute to diseases.

- Because patients with PRPF4B-linked neurodevelopmental disorders often have a dominant heterozygous mutation, understanding how reduced PRPF4B function affects fluid production is necessary.26 iPSCs, which are adult cells that have been reprogrammed into a stem-cell-like state, allow researchers to model neural development without needing actual brain tissue from patients.27 It will help researchers understand if a single copy of a functional PRPF4B gene can maintain normal mRNA splicing and protein levels.
- Although gene expression is often measured using mRNA levels, lots of mRNA does not always directly correlate to protein levels.28 Post-transcriptional regulation, translation efficiency and protein stability can all impact how much functional protein is synthesized.29 For the PRPF4B gene, mutations could change RNA splicing without changing transcript abundance. Removing the uncertainty through measuring both PRPF4B mRNA levels and PRPF4B protein levels is required to determine whether the loss of an allele affects transcription, protein production or both. This is important for determining whether cells can maintain normal PRPF4B protein levels even with reduced gene expression, which is important for evaluating and understanding the effectiveness of therapeutic strategies that selectively block mutant PRPF4B mRNA.
- To understand how disruptions in the PRPF4B gene affect human cells and mRNA splicing, gene editing

provides a direct approach.30, 31 CRISPR-Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, allows researchers to create targeted gene knockouts.32 This includes creating heterozygous knockouts of PRPF4B to study how reduced protein levels impact cell function and cerebrospinal fluid.

- The current study therefore focuses on evaluating PRPF4B protein levels in iPSCs after CRISPR-Cas9–mediated gene removal and measuring its impact on mRNA and protein production. Understanding whether cells can tolerate the loss of a single PRPF4B allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development and kidney function. This research also provides a basis for future work towards a treatment that reduces the impact of PRPF4B gene mutations in the development of neurodevelopmental disorders.
- Having this background, the primary research question of this study is whether human induced pluripotent stem cells (iPSCs) can maintain the normal levels of PRPF4B protein and mRNA expression (normal levels found in wild-type cells) after one allele of the PRPF4B gene is knocked out using CRISPR-Cas9. It is hypothesized that the knockout of a single PRPF4B allele will not affect the level of PRPF4B protein or PRPF4B mRNA compared with non-edited, wild-type PRPF4B cells. This is because the remaining wild-type PRPF4B allele is expected to compensate for the loss by maintaining transcription levels and translation to maintain normal PRPF4B mRNA and protein levels. If this were to not happen within the human body, important organs could be impacted, which could cause development disorders and fluid maintenance problems."

December 19, 2025

Tasks To-do	Review	Notes
	● Studied for physics ● Submitted final introduction to Dr. Garcia ○ Didn't have enough time to send it to Youshan or Dr. Garcia to edit ○ Final copy ■ A balance of brain fluid is necessary for neurological development and function in humans.1,2 Cerebrospinal fluid (CSF) circulates within the brain's ventricular system and performs the functions of nutrient delivery, waste removal, and the protection of neural tissues.3 Disruptions or constant changes in the regulation of CSF, whether its an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.4 These conditions have been known to cause a range of developmental problems such as speech disorders and cognitive disorders.5 Understanding the molecular mechanisms that impact cerebrospinal fluid regulation is important for preventing these conditions and creating an effective treatment for people who are already affected.6-9 ■ Recent research has found that RNA splicing in neurological development has been linked to CSF homeostasis.10 One gene, PRPF4B, is being investigated.11 This gene codes for a serine/threonine kinase protein that plays a role within pre-mRNA	Over the winter break, plan out ASP

splicing.12 RNA splicing is the removal of non-coding introns from pre-mRNA and the joining of exons together to create the mRNA that cells use to make proteins. 13

-
- The PRPF4B gene plays an important role in the spliceosome complex, which is a group of molecules that removes noncoding introns of RNA. 14 PRPF4B acts as a serine/threonine kinase within the spliceosome. It adds phosphate groups to specific splicing proteins to regulate their activity. This is required for proper spliceosome assembly, timing and disassembly during the pre-mRNA processing. By doing this, PRPF4B helps to ensure that the introns are correctly removed and the exons are correctly joined. When the PRPF4B kinase activity is changed, it can cause discrepancies in splice-site selections and mRNA creation transcripts. Even though these transcripts could still be expressed at normal levels, it could also encode proteins with impaired function.
-
- Incorrect splicing due to variations within PRPF4B can cause improperly expressed proteins that could disrupt cellular processes that play a role in fluid regulation.15 In the brain, splicing inconsistencies can lead to inaccurate expression of proteins needed for the regulation of fluid transport, ion channels, and cilia function within the ventricular system. 16

This leads to an abnormal accumulation of cerebrospinal fluid and, as a result, variability within intracranial pressures.17 In addition to the brain, PRPF4B splicing errors can impact other organ systems dependent on fluid regulation and correct protein synthesis.18 While these are significantly less minor than CSF regulation in the brain, PRPF4B mutations in other parts of the body can still impact human health. The kidneys, which are responsible for the balance of fluids within the body, may be affected if the incorrect proteins are produced.19, 20 Protein misregulation in the kidneys can affect the functioning of the endothelial cells, which can cause inflammation, oxidative stress, and impaired blood flow in the kidneys.21, 22

- Not all genes will respond the same way to the loss of a single allele.23 Some genes are dosage-sensitive. This means that a single functional copy is not enough to maintain normal mRNA and protein levels.24 Others are dosage-tolerant and can compensate through the remaining allele.25 Whether PRPF4B is dosage-sensitive or dosage-tolerant is currently undetermined. Understanding if cells can maintain normal PRPF4B expression with only one functional allele is therefore required for understanding how heterozygous PRPF4B mutations contribute to diseases.
- Because patients with

	PRPF4B-linked neurodevelopmental disorders often have a dominant heterozygous mutation, understanding how reduced PRPF4B function affects fluid production is necessary.26 iPSCs, which are adult cells that have been reprogrammed into a stem-cell-like state, allow researchers to model neural development without needing actual brain tissue from patients.27 It will help researchers understand if a single copy of a functional PRPF4B gene can maintain normal mRNA splicing and protein levels. ■ Although gene expression is often measured using mRNA levels, lots of mRNA does not always directly correlate to protein levels.28 Post-transcriptional regulation, translation efficiency and protein stability can all impact how much functional protein is synthesized.29 For the PRPF4B gene, mutations could change RNA splicing without changing transcript abundance. Removing the uncertainty through measuring both PRPF4B mRNA levels and PRPF4B protein levels is required to determine whether the loss of an allele affects transcription, protein production or both. This is important for determining whether cells can maintain normal PRPF4B protein levels even with reduced gene expression, which is important for evaluating and understanding the effectiveness of therapeutic strategies that selectively block mutant	

PRPF4B mRNA.

- To understand how disruptions in the PRPF4B gene affect human cells and mRNA splicing, gene editing provides a direct approach.30, 31 CRISPR-Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, allows researchers to create targeted gene knockouts.32 This includes creating heterozygous knockouts of PRPF4B to study how reduced protein levels impact cell function and cerebrospinal fluid.
- The current study therefore focuses on evaluating PRPF4B protein levels in iPSCs after CRISPR-Cas9–mediated gene removal and measuring its impact on mRNA and protein production. Understanding whether cells can tolerate the loss of a single PRPF4B allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development and kidney function. This research also provides a basis for future work towards a treatment that reduces the impact of PRPF4B gene mutations in the development of neurodevelopmental disorders.
- Having this background, the primary research question of this study is whether human induced pluripotent stem cells (iPSCs) can maintain the normal levels of PRPF4B protein and mRNA expression (normal levels found in wild-type cells) after one allele of the PRPF4B gene is knocked

		out using CRISPR-Cas9. It is hypothesized that the knockout of a single PRPF4B allele will not affect the level of PRPF4B protein or PRPF4B mRNA compared with non-edited, wild-type PRPF4B cells. This is because the remaining wild-type PRPF4B allele is expected to compensate for the loss by maintaining transcription levels and translation to maintain normal PRPF4B mRNA and protein levels. If this were to not happen within the human body, important organs could be impacted, which could cause development disorders and fluid maintenance problems.	

January Notes

January 6, 2026

Tasks To-do	Review	Notes
ASP Logbook	• Used this as primarily a study day for physics	Need to contact Youshan to set up a meeting when possible

January 8, 2026

Tasks To-do	Review	Notes
ASP Logbook	• Used this as primarily a study day for physics	Need to contact Youshan to set up a meeting when possible

January 20, 2026

Tasks To-do	Review	Notes

~~Contact Youshan and ask him if he is available on Thursday~~	• Notes from Arvind as I wasn't here
	  ○ Methodology Section Due Date: February 19th
	  ■ Specifics of how to write the Methodology Section paper will be described during a later class-block (2 classes in the future)
	  ○ Need to research the precise methodology of the studies that I have observed
	  ○ Send an email to Dr. Garcia before
		Meeting Thursday with Youshan at 10:30am

	February 12th, with a draft of the Methodology Section attached, in order to receive feedback ○ Pre-Science Fair Oral Presentation Date (For Me): February 23rd ■ This oral presentation is intended to prepare us for our real CYSF presentations ○ Webber Science Fair: March 2nd ■ Should have poster / presentation prepared and ready ○ Can either use a large, school-provided Trifold, or a flat poster, that would need to be manually printed out ○ CYSF Portal: March 4th ■ Needs to be completed by the deadline in order to reach Top 15 status in the city-wide Science Fair	

January 20, 2026

Tasks To-do	Review	Notes
	● Spent a majority of the time going more detailed into RT-PCR and working on logbook ○ Main takeaways for RT-PCR ■ Basically duplicates a DNA strand until it reaches a certain threshold ● First, the conversion from RNA to cDNA is required ● cDNA is complimentary DNA and is used because its significantly more stable ■ Denaturation ● Heats up the current amount of cDNA to around 95 degrees celsius ● DNA strand splits into 2 pieces ■ Annealing ● Cool to about	

	50–65°C. ● Forward and reverse primers will bond to the complimentary DNA sequence ■ Extension ● Heat to about 72°C ● Taq polymerase adds DNA nucleotides ● Doubles the DNA sequences ○ Main takeaways for delta delta Ct ■ Because every single time the amount of cDNA doubles, it is a logarithmic function ■ Find Ct values for both wt and het ■ Minus GAPDH from Ct values to ensure consistency ● Removes all variability ○	

January 22, 2026

Tasks To-do	Review	Notes
	● Completed February calendar and planed out every day for logbook, can be found in the subsection “Calendar” ● Talked to Youshan about the results section ○ He said that they are redoing the experiment right now to ensure the results are the way they are ○ Any further questions I can just email him	

January 26, 2026

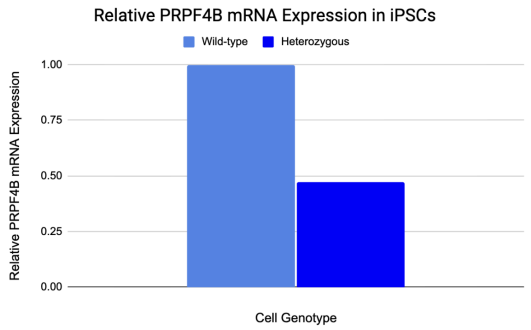
Tasks To-do	Review	Notes

- Methodology paper advice
    - Can base it off of other papers and their methodologies
- Don't get too specific but needs some specificity still
    - No need to reference exact amounts
- Need to know where the antibodies and everything comes from
    - Display a deep understanding that is farther than just what the professor told
    - Needs to be in paragraph format and not
- PAST TENSE REQUIRED
    - Will complete by the time the paper is done so everything needs to be in past
    - Do not need to cite the research equipment
- Figure is always going to be labeled by Figure 1, Figure 2 etc.
- Refer everything in a parathetic format
    - Reference the source
        - Needs peer-reviewed sources
- Need to explain exactly what was involved
- Finish with aims and goals
- Avoid using “then”
- Need to reference formulas
- Need to cite all equipment
- When were things harvest? Before or after certain conditions
- Immunobiochemistry
    - Tagging of antibodies
        - Where is it coming from
        - Which animal
        - What protein does it target
        - Where does it come from
- Steps
    - What do i need to cover?
        - Intro
        - Animal car/patients
        - How the people were treated, how it was grown etc.
        - Enter everything you know, just start writing
        - Identify what is not known
- Worked on creating the graph for RT-qPCR for the poster

Table 1. Raw RT-qPCR Cq values for PRPF4B and GAPDH in iPSCs

	Gene	Trial 1	Trial 2	Trial 3	Average Ct
Wild Type (WT)	GAPDH	14.92	14.82	14.87	**14.87**
Heterozygous KO	GAPDH	14.87	15.02	14.69	**14.86**
Wild Type (WT)	PRPF4B	29.32	29.23	29.67	**29.41**
Heterozygous KO	PRPF4B	30.76	30.41	30.22	**30.46**

Figure 1. Relative PRPF4B mRNA expression normalized to GAPDH.



January 28, 2026

Tasks To-do

Review

- Emailed Youshan a quick message asking him to check the graphs I have made
    - Put out due dates and asked if he could check methodology and poster once it finishes
- Began writing about the use of iPSCs in the project
    - “Human induced pluripotent stem cells (iPSCs) were used as a model to evaluate PRPF4B’s dosage sensitivity after heterozygous gene knockout. Both wild-type and heterozygous PRPF4B knockout lines started as single-celled colonies and were incubated for 2 weeks under similar conditions. “
- Notes on Science Fair meeting at lunch
    - Meet at 8:30am on March 2nd
    - Will take the entire day
    - 2 separate judging sessions
        - Different judges will judge at different times, it is dependent on the judge
        - Going to bring in experts for your project
    - CYSF needs to be entered by February 6th
    - March 3rd results

Notes

Methodology section do by feb. 13 for Youshan and Dr. Garcia to check

January 30, 2026

Tasks To-do	Review	Notes

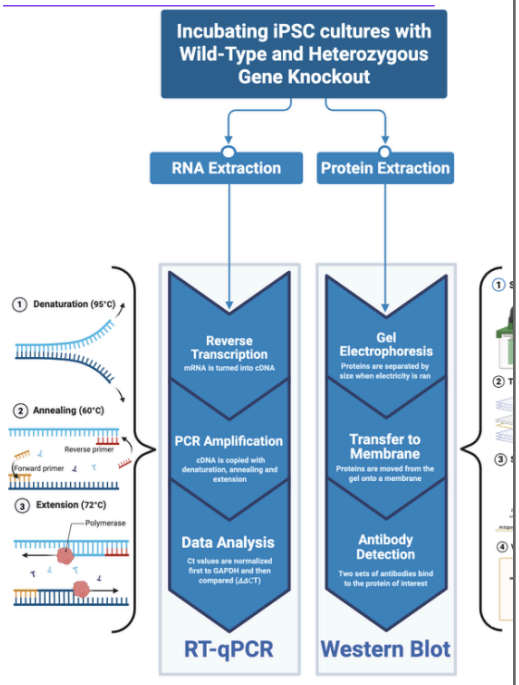
	• Spent time writing about RT-qPCR
	  ○ "Human induced pluripotent stem cells (iPSCs) were used as a model to test PRPF4B's dosage sensitivity after heterozygous gene knockout. Both wild-type and heterozygous PRPF4B knockout lines both started as single-celled colonies and were incubated for 2 weeks under the exact same conditions.
	  ○ Total RNA was then lysed from each cell population with a commercial RNA extraction kit (Zymo Research), following the manufacturer's protocol.
	  ○ To allow for quantification of gene expression, RNA was then reverse-transcribed into complementary DNA (cDNA) using a commercially available cDNA synthesis kit (Zymo Research). This uses reverse transcriptase enzymes to create single stranded cDNA from mRNA templates by matching nucleotide base pairs in a separate strand. The cDNA starts as a single strand but is duplicated during QPCR. This step is required as RNA is extremely unstable and cannot be consistently amplified by DNA polymerase during PCR.
	  ○ The created cDNA samples were then used as temples for real-time quantitative polymerase chain reaction (QPCR). PRPF4B forward and reverse primers were then added to selectively amplify the amount of PRPF4B DNA in the samples. Primers targeting the GAPDH housekeeping sample were also added because of its stable expression between different experimental conditions.
	  ○ During qPCR, DNA was amplified by repeating cycles of denaturation, annealing and extension. The denaturation step, which heats up
		Want to ask Dr. Garcia what needs to be stated in the presentation
		
		For example, do I need to explain why the temperature has to be at 60C to allow for primers to attach

	the cDNA to 95 degrees celsius, breaks the hydrogen bonds between complementary base pairs. This step is required in order for primers to access the target DNA sequence. During annealing, the temperature was lowered to 65 degrees celsius to allow forward and reverse primers to bind to the sequences directly around the PRPF4B or GAPDH target spot. Primers determine which part of the DNA strand gets copied and bonds through hydrogen bonds. This temperature must be maintained as it is the perfect temperature in which bonds between the primers and the DNA strands will form at the perfect location for base pairing. These primers fall off when the DNA gets re-amplified at the start of every cycle when the bonds break. Lastly, in the process of extension (at 72 degrees celsius), DNA polymerase extended from the bound primers synthesizes new DNA strands by adding complementary nucleotides in the 5’ to 3’ direction. This happens to every single place where the primers can be found and creates double-stranded DNA copies of the target region. SYBR Green then bonds to any dsDNA (double-stranded DNA) that is created by the end of extension. Because double-stranded DNA has stacked base pairs, a major groove and a minor groove. SYBR Green is correct width for the minor groove and has complementary charge distribution, making it able to perfectly slide between the adjacent base pairs. In a process called intercalation, the dye is stabilized through LDFs. This allows for fluorescence and a measurement of the amount of DNA strands after each cycle. These three steps were repeated until it reached a certain threshold.”	

February Notes

February 3, 2026

Tasks To-do	Review	Notes

	• Began reformatting my flowchart for the poster
	  ○ Made it possible to fit in the small square on the poster
	 
	•
	• Finished writing the limitations on poster
	  ○ “Induced pluripotent stem cells may not fully represent real human brain tissue. Protein levels were analyzed rather than protein functionality”
		Start methodology asap

February 5, 2026

Tasks To-do	Review	Notes

	• Started writing the rt-qpcr portion of the methodology
	• Still need to get to writing the equations and what they mean
	  ○ "Human induced pluripotent stem cells (iPSCs) were used as a model to test PRPF4B's dosage sensitivity after heterozygous gene knockout. Both wild-type and heterozygous PRPF4B knockout lines started as single-celled colonies and were incubated for 2 weeks under the exact same conditions. (what conditions)
	  ○ Cells (specify what amount) were lysed from each cell population with a commercial RNA extraction kit (Zymo Research, Irvine, CA), following the manufacturer's protocol to separate RNA. (explain normalization) (be more specific with lysing process, and how much, how, what happened to the cells before)
	  ○ To allow for quantification of gene expression, RNA was then reverse-transcribed into complementary DNA (cDNA) using a commercially available cDNA synthesis kit (Zymo Research).
	  ○ This step uses the enzyme reverse transcriptase to create single-stranded complementary DNA (cDNA) from mRNA templates by complementary base pairing. The cDNA starts as a single strand but is duplicated during quantitative PCR. This step is required as RNA is extremely unstable and cannot be consistently amplified by DNA polymerase during PCR.
	  ○ The obtained cDNA samples were then used as templates for real-time quantitative polymerase chain reaction (qPCR). PRPF4B forward and reverse primers (add primer info) were then added to selectively amplify the amount of PRPF4B DNA in the samples. Primers targeting	

the GAPDH housekeeping gene were also added because of its stable expression between different experimental conditions.

- During qPCR, DNA was amplified by repeating cycles of denaturation, annealing and extension using a CFX96 Real-Time PCR Detection System (Bio-Rad, place). The denaturation step, which heats up the cDNA to 95 degrees celsius, breaks the hydrogen bonds between complementary base pairs. This step is required in order for primers to access the target DNA sequence. During annealing, the temperature was lowered to 65 degrees celsius to allow forward and reverse primers to bind to the sequences directly around the PRPF4B or GAPDH target spot. Primers determine which part of the DNA strand gets copied and bonds through hydrogen bonds. This temperature must be maintained as it is the temperature in which bonds between the primers and the DNA strands will form at the perfect location for base pairing. These primers dissociate at the beginning of each cycle during the denaturation step when the hydrogen bonds between each DNA strand is broken. Lastly, in the process of extension (at 72 degrees celsius), DNA polymerase extended from the bound primers synthesizes new DNA strands by adding complementary nucleotides in the 5’ to 3’ direction. This happens to every single place where the primers can be found and creates double-stranded DNA copies of the target region. SYBR Green then bonds to any dsDNA (double-stranded DNA) that is created by the end of extension. Because double-stranded DNA has stacked base pairs, a major groove and a minor groove. SYBR Green is correct width for the minor groove and has complementary charge distribution, making it able to slide between the adjacent base pairs. In a process called intercalation, the dye is stabilized through LDFs. This

	allows for fluorescence and a measurement of the amount of DNA strands after each cycle. These three steps were repeated until it reached a certain threshold." ● Still need to finish citations ○ And the citing the equipment that is used (company, where it is)	

February 9, 2026

Tasks To-do	Review	Notes
	● Started the qPCR data analysis portion, still need to complete the delta delta ct but currently have the main equations ○ "RT-qPCR Data Analysis: ○ As PCR progressed, greater amounts of double-stranded DNA gets produced, which allows SYBR green dye molecules to emit greater fluorescence. SYBR bonds to the grooves in double-stranded DNA through bouncing around at immense concentrations. These molecules are positively charged compare to the negatively charged DNA strands and fit perfectly in the grooves. When being read by the PCR machine, they jump to a different energy level and transmit small amounts of light. This is then detected by the machine and recorded after every single cycle. ○ A fluorescence threshold was set in order to determine cycle threshold (Ct) value for each sample. The Ct value represents the number of amplification cycles for the fluorescence to pass this threshold. A sample with higher amounts of starting PRPF4B mRNA would have a lower Ct value while samples with less starting mRNA have a higher Ct value. ○ To control for differences in RNA input and reverse transcription efficiency, PRPF4B Ct values were normalized to Ct values of GAPDH, the housekeeping gene. This process of normalization was done through	

	the ΔCt equation with both wild-type and heterozygous: ○ ΔCt = Average Ct (PRPF4B) - Average Ct (GAPDH) ○ To further compare gene expression between heterozygous PRPF4B knockout cells and wild-type, ΔCt values were normalized again using the ΔΔCt method: ○ ΔΔCt = ΔCt (PRPF4B Heterozygous) - ΔCt (PRPF4B Wild-Type)	

February 11, 2026

Tasks To-do	Review	Notes
	● Completed the rt-qpcr portion (still need to complete all citations and referencing to materials) ● Finished the writing portion for rt-qpcr for the last step of delta delta ct ○ Because PCR amplification doubles each cycle and therefore, is exponential, differences in Ct values mean differences in the starting amount of PRPF4B mRNA. Due to Ct values being the amount of cycles needed to reach a threshold, directly comparing Ct values cannot be done. The following equation turns Ct differences into relative expression: ○ 2-ΔΔCt ○ The values represent the relative PRPF4B mRNA expression of heterozygous knockout cells compared to wild-type cells, having wild-type expression as the reference value. A value of 1 means there was no change found in expression relative to wild-type,	Email Youhsan and ask him about more specific information on the machines used

	values less than 1 show less PRPF4B expression in heterozygous cells and values greater than 1 mean increased PRPF4B expression relative to wild-type. Because wild-type samples are used as the control, they represent the maximum normalized expression level, and all heterozygous values are a proportion of wild-type expression.	

February 13, 2026

Tasks To-do	Review	Notes

	- Wrote all the western blotting procedures for the methodology (spent full ASP class as well as time after school)
	  - "To determine whether PRPF4B protein levels remain constant after heterozygous gene knockout, quantitative Western blotting was used on wild-type and heterozygous PRPF4B knockout human induced pluripotent stem cell (iPSC) populations. After the identical processes of incubation, the cells were harvested and lysed using a protein extraction buffer with detergents and protease inhibitors. This step destroyed cellular membranes and released intracellular proteins, avoiding enzymatic degradation in the process.
	  - After lysis, protein lysates were centrifuged to remove cellular debris. Equal amounts of total protein lysate from each sample was then prepared for electrophoresis. Any variation in protein loading was controlled during data analysis by normalizing PRPF4B protein expression to GAPDH protein expression.
	  - Protein samples were mixed with sodium dodecyl sulfate (SDS) sample buffer and heated to	

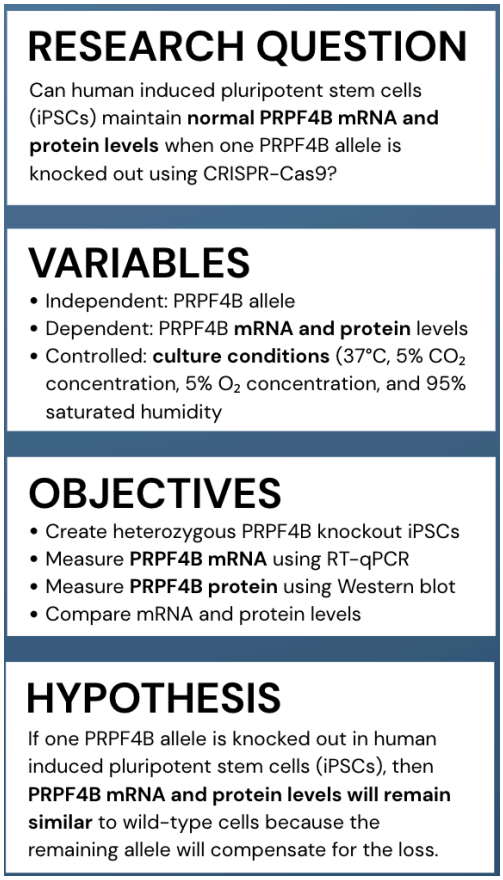
denature protein secondary and tertiary structures. SDS binds to proteins and gives them a consistently negative charge, which allows proteins to migrate toward the positive electrode during electrophoresis in the following step. Denaturation makes sure all proteins are separated only on molecular weight.

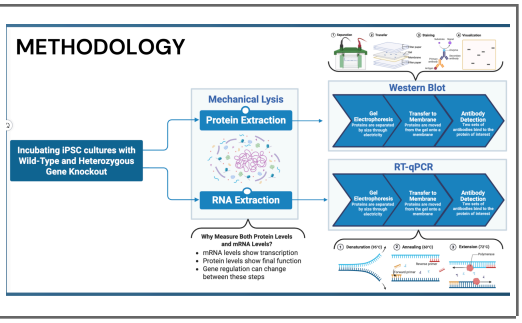
- Denatured protein samples were separated in a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A stacking gel and resolving gel were employed to allow for consistent protein separation. The stacking gel concentrated proteins into narrow bands, which allowed for uniform protein migration. The resolving gel separated proteins based on molecular weight.
- After electrophoresis, separated proteins where transferred from the gel onto a polyvinylidene difluoride (PVDF) membrane through electrotransfer. This process stopped the movement of proteins on the membrane and allowed for antibody-based quantification of these proteins. The membrane was then incubated into a blocking solution containing non-fat milk proteins to prevent non-specific antibody binding.
- A Novus Biologicals NB100-86997 primary antibody was then incubated with the membrane to bind with PRPF4B proteins while a Sigma G9545 rabbit antibody that targeted GAPDH was used to detect GAPDH proteins as a housekeeping protein. These antibodies selectively bind to their target protein because of antigen-antibody specificity.
- After washing to remove unbound primary antibodies, the membranes were incubated with a secondary antibody. The second antibody was conjugated to a chemiluminescent enzyme reporter. When exposed to chemiluminescent substrate, the enzyme creates a light-producing reaction at antibody binding sites. The emitted light signal was seen and measured using an imaging

	system. Because light intensity corresponds to protein amount, the band intensities could be directly compared. “	

February 17, 2026

Tasks To-do	Review	Notes
	- Fixed the methodology so that it would fit in the new section for the poster - Added the research question, variables, objectives, and hypothesis section to the poster - Formatting and writing can be found in the images below -	





February 19, 2026

Tasks To-do

Review

- Finished discussion and conclusion on the poster
  - Discussion
    - Heterozygous PRPF4B knockout led to reduced PRPF4B mRNA and protein levels.
    - Means that one functional allele cannot maintain normal expression.
    - PRPF4B is dosage-sensitive, meaning gene expression depends on having two functional copies
    - Less mRNA likely means disruption occurs at that transcription stage
    - Remaining allele cannot compensate
  - Conclusion
    - PRPF4B-linked disorders may be because of insufficient gene expression
    - A potential strategy could be restoring PRPF4B expression, such as enhancing transcription from the remaining allele
    - Activating the PRPF4B promoter
    - Using transcription-enhancing drugs

Notes

DISCUSSION

- Heterozygous PRPF4B knockout led to **reduced PRPF4B mRNA and protein levels**.
  - Means that one functional allele **cannot** maintain normal expression.
- PRPF4B is **dosage-sensitive**, meaning gene expression depends on having two functional copies
- Less mRNA likely means **disruption occurs at that transcription** stage
- Remaining allele **cannot compensate**

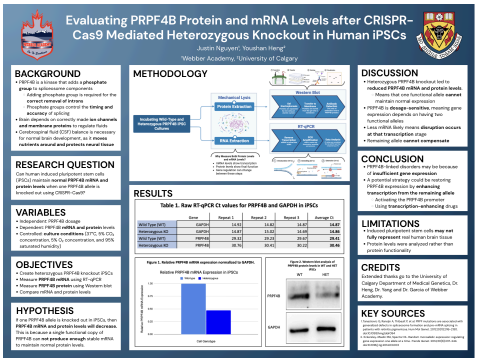
CONCLUSION

- PRPF4B-linked disorders may be because of **insufficient gene expression**
- A potential strategy could be restoring PRPF4B expression, such as **enhancing transcription from the remaining allele**
  - Activating the PRPF4B promoter
  - Using **transcription-enhancing** drugs

-

February 22, 2026

Tasks To-do	Review	Notes

	- Finished the entire poster so it could be ready to print
	  - Rewrote the introduction part because it sounded too mechanic and didn't flow well enough
	  - “PRPF4B is a kinase that adds a phosphate group to spliceosome components
	  - Adding phosphate group is required for the correct removal of introns
	  - Phosphate groups control the timing and accuracy of splicing
	  - Brain depends on correctly made ion channels and membrane proteins to regulate fluids
	  - Cerebrospinal fluid (CSF) balance is necessary for normal brain development, as it moves nutrients around and protects neural tissue”
	- Fixed the final formatting for poster
	 	

February 23, 2026

Tasks To-do	Review	Notes

Implement Dr. Garcia's changes tonight to get ready for oral presentation	- Did an oral presentation with Dr. Garcia to practice for tomorrow
	- Feedback:
	  - Background needs to be more clear
	  - Even though the starting parts are functional, the resultant bedframe isn't. Similarly in biology, when small splicing errors occur, the final proteins become non-functional
	  - The PRPF4B gene in this case, is responsible for making sure that there aren't mistakes in the instruction manual. It is practically responsible for correctly producing proteins.
	  - My interest in this project began…reference connection from PRPF4B and speech disorders.
	  - Mention title and iPSCs
	  - Explain dosage
	  - Objectives
	  - Creating heterozygous knockouts
	  - Methodology
	  - Dont talk about pcr
	  - Leave graph and data analysis for the results
	  - Western blot
	  - Importance of membrane
	  - Stops proteins from moving
	  - Results
	  - The meaning of GAPDH and why it was used
	  - Delta delta CT
	  - Meaning of the table
	  - Relative and normalization meaning	

February 24, 2026

Tasks To-do	Review	Notes

	- Practiced oral presentation several times during class
	- Attended multiple presentations but didn't take notes
	  - Planning to get the contributions in on Friday
	- Completed the Oral Presentation
	  - Feedback from Dr. Garcia
	  - Pacing was good
	  - Left 4 minutes on the table
	  - Need to talk about the role of GAPDH even more
	  - Talk about the difference between heterozygous and wild type
	  - Heterozygous means 2 different alleles, Wild type means 2 of the same alleles
	- Need to change everything in oral presentation before monday	

February 25, 2026

Tasks To-do	Review	Notes

	- Practiced oral presentation several times during class
	- Attended multiple presentations but didn't take notes
	  - Planning to get the contributions in on Friday
	- Completed the Oral Presentation
	  - Feedback from Dr. Garcia
	  - Pacing was good
	  - Left 4 minutes on the table
	  - Need to talk about the role of GAPDH even more
	  - Talk about the difference between heterozygous and wild type
	  - Heterozygous means 2 different	

	alleles, Wild type means 2 of the same alleles - Need to change everything in oral presentation before monday	

February 27, 2026

Tasks To-do	Review	Notes

	- Shicheng
	  - Ascertaining the genomic composition
	  - Most prokaryotic bacteria can be treated with antibodies
	  - But are slowly starting to adapt to these antibiotics
	  - Using bioinformatic processing wants to analyze AMR and antiviral defenses in P multocida isolates
	  - Uses Whole Genome Sequencing
	  - What AMR genes are present
	  - Presence of antiviral defense systems in five isolate observed
	  - What bioinformatic tools are used to look at AMR genes
	  - Abricate in galaxy
	  - AMR genes aad and aph were resisting aminogly coside antibodies
	  - AMR Tet h3 showed tetracycline resistance
	  - P multicoide is understudied and studying can help understand how antibodies impact strains of the bacteria
	- Richard Gao is a GB
	  - Nuclear power
	  - Trying to find more efficient heating
	  - Heat exchange is not effective at all now
	  - High temp, high pressure and experiences a lot stress
	  - Nanocriss material
	  - Arrange themselves in a certain way
	  - Prevent any defects
	  - Copper is one of the most
		autoimmune disease of the central nervous system where the immune system damages the protective myelin sheath covering nerves

conductive materials
        - However is weak and soft
        - Cannot use pure copper
    - Used an alloy instead
        - Copper chromium
            - Exactly a 2:1 ratio
        - Harden alloy
    - How can this type of copper act as a heat exchanger without being too weak
    - Uses xray diffraction
    - Simulates radiation
        - In past studies, how has copper generally reacted in a nuclear reactor
    - Absorbs a lot of energy
    - Limitation did not consider Laives and general structure
        - Phonons will probably be less
    - Neutrons were sent like bullets at the GR Copper to determine strength and nuclear reaction capabilities
- Emma
    - Child abuse with and without a domestic violence component
    - Comparing them
        - Both very prevalent health issues
    - Short term affects and long term affects especially with forming trust
        - Can influence intergenerational abuse
    - Chi squared test
    - P value
    - Standard residuals
    - How would analyzing the other types of abuses affect your findings
    - She found that child abuse involveing biological fathers and other family members had higher rates of domestic violence
- Jessica
    - Investigating changes in grey matter associated with cognitive difficulties in Multiple Sclerosis
        - autoimmune disease of the central nervous system where the immune system damages the protective myelin sheath covering nerves
    - Affects many many people and costs 4-4.5 million dollars per person in the US

- Can cause loss of balance, memory loss
- How does gene expression change grey matter
- Firstly, collect data
    - Used a gene expression database
    - Ran through a metascape and looked at the pathways the gene is involved in
        - Heatmap used
    - Then compared findings to validate/invalidate
- Cerebrospinal fluid protein
    - How did you measure your proteins
- Blue means lowly expressed, grey means pretty similar
- Why does damage to the myelin sheath in Multiple Sclerosis disrupt brain signaling?

- Lara
    - Mapping and evaluating the quality of intrauterine device information accessed by adolescents
    - IUD
        - Is put in the uterus
        - 70% experience painful while 37% experience heavy menstruation
    - Hormonal contraceptives can reduce blood loss, alleviate dysmenorrhea, improve quality of life
    - One of the most effect long acting reversible contraceptives
    - Global cumulative pregnancy rate with IUD 2%
        - Only 10.6% of Canadian adolescence use IUDS
            - Low uptake despite its effectiveness
    - What do adolescents know about IUDs
    - Want to give doctors an overview about contraception
    - Ran a survey
    - What were the most common misconceptions found
        - Lack of knowledge
    - If nobody has a negative view of IUDs, why is the usage rate so low?
        - How would learning about IUDs in schools impact the way that students view the

	use? - Why does low awareness persist even for well-studied contraceptive methods - Even though IUDs have been well researched, why is there still low awareness? - Because you've only looked at Social media, how would having a bigger sample size impact	

March Notes

March 3, 2026

Tasks To-do	Review	Notes

	• Started writing a thank you email with SF results to Youshan
	  ○ Hi Youshan,
	  ○ This recent Monday (March 2nd), I had my school wide science fair, where the top 15 projects out of the 50 go to the Calgary Wide Science Fair in April. Along with winning gold, I was also one of those top 15 projects.
	  ○ I want to thank you for the opportunity to research in your lab and learn from your project. I still have several papers to write unfortunately so I may need some help with that.
	  ○ Best,
	  ○ Justin
	• Main comments
	  ○ Very strong personal connection and very knowledgeable about the background
	  ○ Strong presentation
	  ○ Need to expand on limitations and future directions more
	  ○ Make sure to not skip some parts of the background
	  ○ Clearer description of the GAPDH control
	  ○ Connection between raw data and normalized data was not immediately obvious
	  ○ Need to simplify everything because judges will have different backgrounds
	•	

Background Research

Research Articles

Research Article: A beginner's guide to RT-PCR, qPCR and RT-qPCR - September 15

- RT-PCR, qPCR and RT-qPCR
    - Many misconceptions between them because they are synonymous
        - Similarities often result in the incorrect use
    - RT-PCR is the process of reverse transcription PCR and not real-time PCR
        - Allows for the use of RNA to create a similar copy with DNA
            - Uses reverse transcriptase enzyme
    - qPCR is quantitative real-time PCR
        - Amplification of DNA in real time
            - Measured through a fluorescent probe created from a intercalating dye or a hydrolysis-based probe
                - Allows quantitation
    - RT-qPCR is used for reverse transcription quantitative real-time PCR
        - Is a combination of qPCR and RT-PCR
            - Enables the measurement of RNA levels through the use of cDNA
    - qPCR: detect pathogens, measure DNA copy numbers
    - RT-qPCR: study gene expression changes
- A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity
- Transcription
    - The process where a cell makes an RNA copy of DNA
        - DNA -> RNA
    - Uses the RNA polymerase enzyme
    - Purpose
        - In prokaryotes and eukaryotes, transcription is the first step of gene expression
        - Creates messenger RNA, which carries the instructions to make proteins
        - Also creates non-coding RNA
- Reverse Transcription
    - The process where an RNA template is copied back into the DNA form
        - RNA -> DNA
    - Enzyme needed is reverse transcriptase
    - Purpose
        - Used by retroviruses like HIV to put their RNA genome into host DNA
        - Use reverse transcription to make cDNA (complementary DNA)

Research Article: A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity - September 9

- CRISPR-Cas
    - Adaptive immunity against against viruses and plasmids
    - Key components
        - CRISPR arrays
        - Cas proteins
        - crRNA
    - Different phases of CRISPR immunity
        - Adaptation
        - Expression

        - Interference
- Focusing on Type II CRISPR
    - tracrRNA base pairs with pre-crRNA
    - Requires RNase III and Cas9
    - Cas9 is through to be the protein needed for DNA interference
- Key info
    - Cas9 needs dual RNAs (crRNA and tracrRNA)
        - crDNA alone is not good enough
        - tracrRNA pairs with crRNA
            - Activates Cas0 for site-specific DNA
    - Cas9 cleavage mechanism
        - Recognizes target DNA through crRNA sequence and a “protospacer adjacent motif (PAM)”
        - HNH domain cuts the strand
        - RuvC domain cuts the non-complementary strand
    - Requirements
        - Need a need region near the PAM to be recognized
        - PAM sequence is needed for separating self and non-self
    - Programmability
        - Dual-RNA system can be used to create a single chimeric RNA
            - Is a fusion of tracrRNA and crRNA
- Experiments that have already been done
    - Purified Cas9 has been tested with plasmid and linear DNA
    - Mutant cas9 proteins have shown the roles of HNH and RuvC domains
    - Truncated RNA experiments found very little requirements for RNA
    - A single-guide RNA has been engineered
        - It basically has the same function as dual RNAs
            - Can be used as a way to replace a single RNA if one set of the 2 become mutated or stop properly function
- Conclusions they have decided upon
    - Cas9 is a programmable RNA-guided DNA
        - Requires dual-DNA structure
    - Can be reprogrammed
    - It is a very simple, efficient, and versatile genome-editing tool
- How it has impacted the research area
    - Very important work that lead up to CRISPR-Cas9 genome editing
    - Found RNA programmability of Cas9
        - Can change the way we look at biotechnology, gene editing and medicine

Research Article: Uncovering the signaling landscape controlling breast cancer cell migration identifies novel metastasis driver genes - September 15

- Uncovering the signaling landscape controlling breast cancer cell migration identifies novel metastasis driver genes
    - Molecular drivers of triple-negative breast cancer
        - Three genes were the central regulators
            - They were PFPF4B, BUD31 and BPTF
                - PRPF4B: A splicing kinase that is essential for metastasis in vivo
                - BUD31: a spliceosome component
                - BPTF: a chromatin remodelling factor
                    - Both this and BUD31 allow for cell adhesion and migration
        - Knocking out these genes would lead to downregulation of focal adhesion and

extracellular matrix (ECM)
- Impairing migration and metastatic potential
- high expression of these 3 genes would lead to tumors with poorer metastasis-free survival
  - This allows it to be used to other research in the future as there is proof that these 3 genes can be used

- Suppressing PRPF4B in a mouse xenograft model reduced lung and organ metastases without affecting the tumor growth
- Procedure
  - Cell lines chosen
    - Hs5781T and MDA-MB-231
  - RNAi-based imaging
    - Phagokinetic track assay, PKT
    - 4200 genes encoding signaling proteins were targeted in this experiment
      - Using siRNA pools
    - Cells were grown on fibronectin coated with beads
      - When they migrate, they leave tracks
    - Automated imaging quantified migration parameters
    - Very important for PCA grouped migration
  - Results
    - Found a total of 2807 hits (1501 in Hs578T, 1306 in MDA-MB-231)
- This study is a map of TNBC cell migration and identifying important driver gene
  - PRPF4B, BUD31, and BPTF are important genes for therapeutic targets that prevent and treat metastatic breast cancer
    - Can be used for drug creation

Research Article: Induced Pluripotent Stem Cells and Their Potential for Basic and Clinical Sciences - September 18

- Are a type of pluripotent stem cell that come from adult somatic cells
  - Genetically reprogrammed to an embryonic stem cell-like state
    - Forced expression of genes and factors
- Regular adult cells (like skin or blood cells)
  - Scientists however reprogram them back into a stem cell-like state
  - They can develop into almost any type of cell
- Used because they are easier to grow and manipulate then actual brain tissue
  - Also have behaviors that can mimic early developmental processes, which are useful for studying neurodevelopmental genes
    - Can grow indefinitely and can be turned into brain-related cells
    - Good model for studying genes
- Using iPSCs in this experiment because they can grow indefinitely and can be turned into brain-related cells
  - Good model for studying genes in neurodevelopment because they are easier to grow and manipulate, basically more maneuverable and accessible cell compared to brain cells

Research Article:
Understanding RT-PCR Tests and Results - November 5

- Genetic material and pathogens
    - Genetic material are the instructions inside cells or viruses
    - Viruses like SARS use RNA
        - RNA can be used as a test target because viruses like SARS target RNA
- RT-PCR
    - Real time polymerase chain reaction
        - Amplifies and makes many copies of a DNA segment
        - Real time means the version that works for RNA viruses (which uses reverse transcriptase to convert RNA to DNA)
            - Looks at fluorescence
    - Very sensitive to light
- How testing works
    - Sample collection
        - Prepare it by extracting RNA
        - Add it to a reaction mixture with reverse transcriptase, DNA polymerase, primers and probes
    - Reverse transcription
        - Convert RNA to cDNA
    - Amplify PCR
        - Measure amplification through fluorescent probes
- Looking at the values and test results
    - Lower Ct value means a higher amount of genetic material
    - Higher Ct value means a lower amount of genetic material
    - Ct values help people look at disease state, transmissibility risk and recovery
- Usage and limits
    - Can be used for diagnosing infections, looking for treatments and analyzing diseases
    - Limits
        - Tests genetic material but RNA does not mean there is a live virus
        - Sample quality and timing can impact accuracy
        - Ct values can also be influenced by many other factors like sample type and assay
        - A Ct value does not give enough context and a conclusion

Citations

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Research Proposal

Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Project Proposal
Applied Science Project

Justin Nguyen

Dr. Heng Y.

First Draft

Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Research Proposal
Applied Science Project

Justin Nguyen
Dr. Heng Y.

Introduction (fix citations, period before citation):

A balance of brain fluid is a necessity for proper neurological development and function for humans.[1] Cerebrospinal fluid (CSF) circulates within the ventricular system of the brain and allows for nutrient delivery, waste extraction, and the protection of neural tissues.[2–3] Disruptions/constant changes in the regulation of CSF, whether an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.[4] These conditions have often been known to cause developmental problems like speech disorders and, in some cases, neurological development disorders.[5] Understanding the molecular mechanism that play a role in CSF dynamics is important to avoiding future cases and creating a treatment for the people already affected.[6-9]

Recent research in genetic biology has found that RNA splicing in neurological development has been directly linked to CSF homeostasis.[10] One gene in particular, PRPF4B, is being watched. The PRPF4B gene encodes a serine/threonine kinase protein that is involved in the regulation of pre-mRNA splicing.[11-12] For this process, removing the non coding parts of RNA and joining the coding parts together to form the correct mRNA needed to make proteins is required.

Because proper splicing controls the accuracy of the protein produced, even small changes in splicing can have extreme consequences downstream on brain development and brain function.[13] Patients who have heterozygous mutations in the PRPF4B gene often also have severe neurodevelopmental disorders. This implies that mutations in this particular splicing regular could be the cause of abnormal neuronal differentiation and a lack of CSF balance in the brain.[14]

Additionally, because patients with PRPF4B-linked neurodevelopment disorders often only carry the heterozygous mutation (where only one of the two copies of the gene is impacted), understanding how other cells react is neccessary.[15] Simulating the effects of a dominant mutation can be done through creating heterozygous PRPF4B mutations in iPSCs. This can be used to test whether the wild-type allele can maintain protein production levels when the mutated allele is removed.

To confirm PRPF4B's role in these processes, understanding how human cells react to gene disruptions is required. New genome editing technologies now allows scientists to target the PRPF4B gene.[16] CRISPR-Cas9 is one of these. Standing for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, this gene editing tool makes it possible to target specific mutations, allowing researchers to delete or modify their target gene with high precision.[17] By using CRISPR-Cas9 to create heterozygous knockouts of the PRPF4B gene in induced pluripotent stem cells (iPSCs), researchers can directly test the situation portrayed in the human body and whether the loss of one allele changes gene expression and more importantly, whether it impacts protein production.[18-22] This approach tests the overall effectiveness of blocking mutant PRPF4B mRNA for patients.

The current study therefore focuses on evaluating the knockout efficiency of PRPF4B in iPSCs using CRISPR-Cas9 and measuring its downstream impact on the protein level. Understanding whether cells can tolerate the loss of a single allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development. Furthermore, this research also provides a basis for future work towards a treatment that

reduces the impact of the PRPF4B gene mutations in the development of neurodevelopmental disorder.

Research Question:
Can human induced pluripotent stem cells (iPSCs) maintain normal protein levels when one allele of the PRPF4B gene is knocked out using CRISPR-Cas9?

Objectives:
The short-term goal of this project is to find out whether human induced pluripotent stem cells (iPSCs) can handle the loss of a single PRPF4B allele without affecting normal protein levels in the cell. To test this, CRISPR-Cas9 will be used to knockout a single PRPF4B allele. PRPF4B mRNA and protein levels will be measured afterward.

This experiment's long-term goal is to contribute to the development of therapeutic strategies that could reduce the influence of PRPF4B mutations on neurodevelopmental disorders.

Question/Hypothesis:
If one allele of the PRPF4B gene is knocked out through CRISPR-Cas9 in human induced pluripotent stem cells (iPSCs), the PRPF4B protein levels would remain the same. This is because the remaining wild-type allele needs to and would be expected to produce more proteins in order to maintain normal protein levels. If this were not to happen, the body would not be able perform the required functions to survive.

Variables:
Independent variable: The independent variable in this experiment is the PRPF4B genotype present in iPSCs: one wild-type (has no edit) and one knocked out using CRISPR-Cas9. This makes it so that only one copy of the PRPF4B gene is present and allows us to test the impact of losing one allele on protein production.

Dependent variable: The dependent variable is the protein levels that happen as a result of knocking out a single PRPF4B allele. Measured through a western blot band sizes, the protein levels show us the cell's ability to continue producing enough of the proper proteins from the singular remaining PRPF4B gene.

Controlled variables: The controlled variables in this experiment are the same parental iPSC line for all protein comparisons, having identical cell culture conditions (same medium, incubator time and temperature, CO2 level and passage number), using the same CRISPR delivery method and keeping reagent concentrations similar. Furthermore, when analyzing protein levels, conditions must also remain constant. This includes identical sample preparation, the same protein loading volumes, the same gel and transfer settings and the same qPCR primer sets and cycling conditions.

Confounding variable: Confounding variables for this project include off-target CRISPR edits, RNA or protein degradation during handling, inconsistent sample being loaded on gels, antibody cross-reactivity and differences in image exposure.

Methodology:

This project will use human induced pluripotent stem cells (iPSCs) as a model. This is because they can divide indefinitely and differentiate into many different cell types, which makes them useful for studying gene function. The main materials would include: CRISPR-Cas9 gene editing reactants (Cas9 enzyme and PRPF4B-specific single guide DNA), a commercial RNA extraction kit, a cDNA synthesis kit, RT-qPCR reagents, Western blot materials (antibodies, gels, buffers, and detection reagents), cell culture supplies and standard lab equipment like pipettes, imaging computers, centrifuges and thermocyclers.

To assess the impact of knocking out a single PRPF4B allele on protein expression, transcripts and protein levels will both be measured. To analyze the transcription levels, the total RNA would be extracted using a commercial RNA kit and then be reverse-transcribed into complementary DNA (cDNA). This will then be used as a template for real-time quantitative PCR (RT-qPCR). Afterward, exon-spanning primers will be used to measure the total PRPF4B mRNA expression. Where possible, allele-specific assays (ex. SNP-informative primers or probe-based assays) will be used to separate wild-type from edited transcripts. GAPDH will be the reference housekeeping gene and the ΔΔCt method will be used for data analysis.

To assess protein expression, quantitative immunoblotting (or a western blot) will be used. Proteins will be isolated from both wild-type and heterozygous knockout iPSCs and will be separated by gel electrophoresis. They then will be transferred onto membranes to be searched for antibodies. Band intensities corresponding to PRPF4B will be quantified by background-subtracted densitometry and normalized against stable loading controls, which allows for a direct comparison.

Quantification of PRPF4B transcript and protein level/

Transcript level.

- Total RNA extraction, reverse transcription and real-time quantitative PCR (RT-qPCR) will be done using commercial kit. RT-qPCR with exon-spanning primers to measure total PRPF4B mRNA. Where possible, allele-specific assays (e.g., SNP-informative primers or probe-based assays) will distinguish wild type from edited transcripts. Primers targeting GAPDH amplicon will be used as reference. ΔΔCt method will be used for data analysis.

Protein level.

- Quantitative immunoblotting (i.e., Western blot) to measure PRPF4B protein, normalized to stable loading controls; band intensities will be quantified with background-subtracted densitometry.

Significance:

This project matters because understanding whether induced pluripotent stem cells (iPSCs) can function normally under the loss of a single allele will determine if removing the mutant copy in patients can be a safe treatment option. If cells can maintain normal proteins levels with only

one functional allele, blocking/knocking out the mutant PRPF4B gene becomes an option. This could prevent harmful protein production without affecting the body's ability to produce the necessary proteins.

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Dr. Garcia Edits

Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Research Proposal
Applied Science Project

Justin Nguyen
Dr. Heng Y.

Introduction:

A balance of brain fluid is necessary for proper neurological development and function for humans.[1,2] Cerebrospinal fluid (CSF) circulates within the brain's ventricular system and allows for nutrient delivery, waste removal, and the protection of neural tissues.[3] Disruptions or constant changes in the regulation of CSF, whether an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.[4] These conditions have been known to cause developmental problems like speech disorders and in some cases, neurological development disorders.[5] Understanding the molecular mechanism that play a role in CSF dynamics is important to avoiding future cases and creating a proper treatment for already affected people.[6-9]

Recent research in genetic biology has found that RNA splicing in neurological development has been directly linked to CSF homeostasis.[10] One gene in particular, PRPF4B, is being investigated. The PRPF4B gene encodes a serine/threonine kinase protein that is involved in the regulation of pre-mRNA splicing.[11,12] For this process, removing the non-coding introns of the pre-mRNA and joining the exons together to form the mature mRNA needed to make proteins is required.

More specifically, the PRPF4B gene plays an important role in the splicesome complex, which is a group of molecules that removes noncoding parts of RNA to make correct mRNA for protein production.[13,14] The PRPF4B gene helps in the synthesis of the necessary proteins for neuron growth by maintaining proper mRNA splicing.[14-16] Incorrect splicing of transcripts due to changes in PRPF4B activity can cause incorrect protein creation.[17] In the brain, incorrect protein creation can impact fluid regulation and can cause abnormal CSF buildup.

Because proper splicing controls the accuracy of the protein produced, even small changes in splicing can have serious effects on brain development and brain function.[18] Patients who have heterozygous mutations in the PRPF4B gene often also have severe neurodevelopmental disorders. This suggests that mutations in the PRPF4B gene could lower PRPF4B protein levels, which could be the cause of abnormal neuronal differentiation and a lack of CSF balance in the brain.[19]

Additionally, because patients with PRPF4B-linked neurodevelopmental disorders often carry a dominant heterozygous mutation, understanding how human induced pluripotent stem cells (iPSCs) respond to a reduced PRPF4B gene expression is neccessary.[20] Simulating the effects of a dominant mutation can be done through creating heterozygous PRPF4B mutations in iPSCs. This can be used to test whether the wild-type allele can maintain normal PRPF4B kinase mRNA and PRPF4B protein levels when one allele is non-functional.

To confirm the role of PRPF4B in regulating mRNA splicing and maintaining proper PRPF4B protein levels, understanding how human cells behave to gene disruptions is required. New genome editing technologies now allows scientists to target the PRPF4B gene.[21] CRISPR-Cas9 is one of these. Standing for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, this gene editing tool makes it possible to target specific mutations, allowing researchers to delete or modify their target gene with high precision.[22] CRISPR-Cas9 can also be used to create gene knockouts, which allows researchers to study how specific genes function and how their loss affects cells.[23-27]

The current study therefore focuses on evaluating the knockout efficiency of PRPF4B in iPSCs using CRISPR-Cas9 and measuring its downstream impact on PRP4B protein levels. Understanding whether cells can tolerate the loss of a single allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development. Furthermore, this research also provides a basis for future work towards a treatment that reduces the impact of PRPF4B gene mutations in the development of neurodevelopmental disorders.

Research Question:

Can human induced pluripotent stem cells (iPSCs) maintain normal PRPF4B protein levels when one allele of the PRPF4B gene is knocked out using CRISPR-Cas9?

Objectives:

The short-term goal of this project is to find out whether human induced pluripotent stem cells (iPSCs) can maintain normal PRPF4B protein levels when one allele of the PRPF4B gene is knocked out. To test this, CRISPR-Cas9 will be used to create iPSCs with only a single PRPF4B allele. PRPF4B protein levels will then be measured in these knockout cells and compared to unchanged control cells with both functional alleles.

This experiment's long-term goal is to contribute to the development of therapeutic strategies that could reduce the influence of PRPF4B mutations on neurodevelopmental disorders.

Hypothesis:

If one allele of the PRPF4B gene is knocked out through CRISPR-Cas9 in human induced pluripotent stem cells (iPSCs), the PRPF4B protein levels would remain the same as those in unedited, control cells. This is because the remaining wild-type allele would compensate by producing enough PRPF4B proteins in order to maintain normal PRP4B protein levels. This would lead to the same PRPF4B protein levels as those found in unedited wild-type iPSCs.

Variables:

Independent variable: The independent variable is the PRPF4B genotype in the iPSCs. This would either be homozygous wild-type (both alleles are unedited) or heterozygous (one wild-type allele and one knockout allele, which is done through CRISPR-Cas9). This makes it so that only one copy of the PRPF4B gene is functional, which allows the PRPF4B protein production to be tested.

Dependent variable: The dependent variable is the PRP4B protein levels in the cells as a result of knocking out a single PRPF4B allele. Measured through a western blot band sizes, the PRP4B protein levels show us the cell's ability to continue producing enough PRPF4B proteins from the singular remaining PRPF4B allele.

Controlled variables: The controlled variables in this experiment are the same use of parental iPSC line for all protein comparisons, having identical cell culture conditions (same medium, incubator time and temperature, and CO2 level), using the same CRISPR delivery method and keeping reagent concentrations the same across all samples. Furthermore, when analyzing PRP4B protein levels, the environmental conditions must also remain constant. This includes identical sample preparation, the same protein loading volumes, the same gel and transfer settings and the same qPCR primer sets and cycling conditions.

Confounding variable: Confounding variables for this project include off-target CRISPR edits, RNA or protein degradation during handling, inconsistent sample being loaded on gels, antibody cross-reactivity and differences in image exposure.

Methodology:

This project will use human induced pluripotent stem cells (iPSCs) as a model. These cells can divide indefinitely and differentiate into many different cell types, which makes them useful for studying gene function. The main materials would include: CRISPR-Cas9 gene editing reactants (Cas9 enzyme and PRPF4B-specific single guide DNA), a commercial RNA extraction kit (provided by Zymo Research), a cDNA synthesis kit (provided by Zymo Research), RT-qPCR reagents (provided by Zymo Research), Western blot materials (PRPF4B antibodies, gels, buffers, and detection reagents), cell culture supplies and standard lab equipment like pipettes, imaging computers, centrifuges and thermocyclers.

To assess the impact of knocking out a single PRPF4B allele on protein expression, transcripts and PRP4B protein levels will both be measured. While PRP4B protein levels are the primary area of study, measuring transcript levels can determine if any changes in protein expression are caused by mRNA production.

To analyze the transcription levels, the total RNA would be extracted using a commercial RNA kit and then be reverse-transcribed into complementary DNA (cDNA). This will then be used as

a template for real-time quantitative PCR (RT-qPCR). Afterward, exon-spanning primers will be used to measure the total PRPF4B transcription levels (mRNA expression). Where possible, allele-specific assays (ex. SNP-informative primers or probe-based assays) will be used to separate wild-type from edited transcripts. GAPDH will be the reference housekeeping gene and the ΔΔCt method will be used for data analysis.

To assess PRPF4B protein expression, quantitative immunoblotting (or a western blot) will be used. PRP4B proteins will be isolated from both wild-type and heterozygous knockout iPSCs and will be separated by gel electrophoresis. They then will be transferred onto membranes to be bound to PRPF4B antibodies. Band intensities corresponding to PRPF4B will be quantified by background-subtracted densitometry and normalized against stable loading controls, GAPDH, which allows for a direct comparison.

Significance:

This study will contribute to the current understanding through determining whether induced pluripotent stem cells (iPSCs) can function normally (produce the same amount of PRP4B proteins as a homogenous PRP4B gene) after the loss of a single PRPF4B allele will determine if removing the mutant copy in patients can be a safe treatment option. If cells can maintain normal PRPF4B proteins levels with only one functional allele, blocking/knocking out the mutant PRPF4B gene becomes an option. This could prevent harmful protein production without affecting the body's ability to produce the necessary proteins.

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Youshan Edits

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Research Proposal
Applied Science Project

Justin Nguyen
Dr. Heng Y.

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Additionally, because patients with PRPF4B-linked neurodevelopmental disorders often carry a dominant heterozygous mutation, understanding how human induced pluripotent stem cells (iPSCs) respond to a reduced PRPF4B gene expression is neccessary.[19] iPSCs, which are reprogrammed adult cells what have been turned back into a stem cell-like state, allow researchers to simulate brain cells without actually going through the difficulty of obtaining actual neural tissue.[20] The effects of a dominant mutation can also be simulated through creating heterozygous PRPF4B mutations in iPSCs. This can be used to test whether the wild-type allele can maintain normal PRPF4B kinase mRNA and PRPF4B protein levels when one allele is non-functional.

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FINAL!!

Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Research Proposal
Applied Science Project

Justin Nguyen
Dr. Heng Y.

Introduction:

A balance of brain fluid is necessary for proper neurological development and function for humans.[1,2] Cerebrospinal fluid (CSF) circulates within the brain's ventricular system and allows for nutrient delivery, waste removal, and the protection of neural tissues.[3] Disruptions or constant changes in the regulation of CSF, whether its an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.[4] These conditions have been known to cause developmental problems like speech disorders and in some cases, neurological development disorders.[5] Understanding the molecular mechanism that play a role in CSF dynamics is important to avoiding future cases and creating a proper treatment people who are already affected.[6-9]

Recent research in genetic biology has found that RNA splicing in neurological development has been directly linked to CSF homeostasis.[10] One gene in particular, PRPF4B, is being investigated. The PRPF4B gene encodes a serine/threonine kinase protein that is involved in the regulation of pre-mRNA splicing.[11,12] For this process, removing the non-coding introns of the pre-mRNA and joining the exons together to form the mature mRNA needed to make proteins is required.

More specifically, the PRPF4B gene plays an important role in the spliceosome complex, which is a group of molecules that removes noncoding parts of RNA to make correct mRNA for protein production.[13,14] The PRPF4B gene helps in the synthesis of the necessary proteins for neuron growth by maintaining correct mRNA splicing.[14-16] Incorrect splicing of transcripts because of changes in PRPF4B activity can cause incorrect protein creation.[17] In the brain, incorrect protein creation can impact fluid regulation and can cause abnormal CSF buildup.

Because proper splicing controls the accuracy of the protein produced, even small changes in splicing can have serious effects on brain development and brain function.[18] Patients who have heterozygous mutations in the PRPF4B gene often also have severe neurodevelopmental disorders. This suggests that mutations in the PRPF4B gene could lower PRPF4B protein levels, which could be the cause of abnormal CSF in the brain.

Additionally, because patients with PRPF4B-linked neurodevelopmental disorders often carry a dominant heterozygous mutation, understanding how human induced pluripotent stem cells (iPSCs) respond to a reduced PRPF4B gene expression is neccessary.[19] iPSCs, which are reprogrammed adult cells what have been turned back into a stem cell-like state, allow researchers to simulate brain cells without actually going through the difficulty of obtaining actual neural tissue.[20] The effects of a dominant mutation can also be simulated through creating heterozygous PRPF4B mutations in iPSCs. This can be used to test whether the wild-type allele can maintain normal PRPF4B kinase mRNA and PRPF4B protein levels when one allele is non-functional.

To confirm the role of PRPF4B in regulating mRNA splicing and maintaining proper PRPF4B protein levels, understanding how human cells respond to gene disruptions is required. New genome editing technologies now allows scientists to target the PRPF4B gene.[21] CRISPR-Cas9 is one of these. Standing for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, this gene editing tool makes it possible to target specific mutations, allowing researchers to delete or modify their target gene with high precision.[22] CRISPR-Cas9 can also be used to create gene knockouts, which allows researchers to study how specific genes function and how their loss affects cells.[23-27]

The current study therefore focuses on evaluating the knockout efficiency of PRPF4B in iPSCs using CRISPR-Cas9 and measuring its impact on PRP4B protein levels. Understanding whether cells can tolerate the loss of a single allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development. This research also provides a basis for future work towards a treatment that reduces the impact of PRPF4B gene mutations in the development of neurodevelopmental disorders.

Research Question:

Can human induced pluripotent stem cells (iPSCs) maintain normal PRPF4B protein levels when one allele of the PRPF4B gene is knocked out using CRISPR-Cas9?

Objectives:

The short-term goal of this project is to find out whether human induced pluripotent stem cells (iPSCs) can maintain normal PRPF4B protein levels when one allele of the PRPF4B gene is knocked out. To test this, CRISPR-Cas9 will be used to create iPSCs with only a single PRPF4B allele. PRPF4B protein levels will then be measured in these knockout cells and compared to unchanged control cells with both functional alleles.

This experiment's long-term goal is to contribute to the development of therapeutic strategies that could reduce the influence of PRPF4B mutations on neurodevelopmental disorders.

Hypothesis:

If one allele of the PRPF4B gene is knocked out through CRISPR-Cas9 in human induced pluripotent stem cells (iPSCs), the PRPF4B protein levels would remain the same as those in unedited, control cells. This is because the remaining wild-type allele would compensate by producing enough PRPF4B proteins in order to maintain normal PRP4B protein levels. This would lead to the same PRPF4B protein levels as those found in unedited homogeneous wild-type iPSCs.

Variables:

Independent variable: The independent variable is the PRPF4B genotype in the iPSCs. This would either be homozygous wild-type (both alleles are unedited) or heterozygous (one wild-type allele and one knockout allele, which would be done through CRISPR-Cas9). This makes it so that only one copy of the PRPF4B gene is functional, which allows the PRPF4B protein production to be tested.

Dependent variable: The dependent variable is the PRP4B protein levels in the cells as a result of knocking out a single PRPF4B allele. Measured through a western blot band intensity, the PRP4B protein levels show us the cell's ability to continue producing enough PRPF4B proteins from the singular remaining PRPF4B allele.

Controlled variables: The controlled variables in this experiment are the same use of parental iPSC line for all protein comparisons, having identical cell culture conditions (same medium, incubator time and temperature, and CO2 level), using the same CRISPR delivery method and keeping reagent concentrations the same across all samples. Furthermore, when analyzing PRP4B protein levels, the environmental conditions must also remain constant. This includes identical sample preparation, the same protein loading volumes, the same gel and transfer settings and the same qPCR primer sets and cycling conditions.

Confounding variable: Confounding variables for this project include off-target CRISPR edits, RNA or protein degradation during handling, inconsistent sample being loaded on gels, antibody cross-reactivity and differences in image exposure.

Methodology:

This project will use human induced pluripotent stem cells (iPSCs) as a model. Because these cells can divide indefinitely and differentiate into different cell types, they are useful for studying gene function. The main materials would include: CRISPR-Cas9 gene editing reactants (Cas9 enzyme and PRPF4B-specific single guide DNA), a RNA extraction kit (provided by Zymo Research), a cDNA synthesis kit (provided by Zymo Research), RT-qPCR reagents, Western blot materials (PRPF4B antibodies, gels, buffers, and detection reagents), cell culture supplies and lab equipment like pipettes, imaging computers, centrifuges and thermocyclers.

To assess the impact of knocking out a single PRPF4B allele on protein expression, transcripts and PRP4B protein levels will both be measured. While PRP4B protein levels are the primary area of study, measuring transcript levels can determine if any changes in protein expression are caused by mRNA production.

To analyze the transcription levels, the total RNA would be extracted using a commercial RNA kit and then be reverse-transcribed into complementary DNA (cDNA). This will then be used as a template for real-time quantitative PCR (RT-qPCR). Afterward, exon-spanning primers will be used to measure the total PRPF4B transcription levels (mRNA expression). Where possible,

allele-specific assays (ex. SNP-informative primers or probe-based assays) will be used to separate wild-type from edited transcripts. GAPDH will be the reference housekeeping gene and the ΔΔCt method will be used for data analysis.

To assess PRPF4B protein expression, quantitative immunoblotting (or a western blot) will be used. PRP4B proteins will be isolated from both wild-type and heterozygous knockout iPSCs and will be separated by gel electrophoresis. They then will be transferred onto membranes to be bound to PRPF4B antibodies. Band intensities corresponding to PRPF4B will be quantified by background-subtracted densitometry and normalized against stable loading controls, GAPDH, which allows for a direct comparison.

Significance:

This study will contribute to the current understanding through determining whether induced pluripotent stem cells (iPSCs) can function normally (produce the same amount of PRP4B proteins as a homogenous wild-type PRP4B gene) after the loss of a single PRPF4B allele, determining if removing the mutant copy in patients can be a safe treatment option. If cells can maintain normal PRPF4B proteins levels with only one functional allele, blocking/knocking out the mutant PRPF4B gene becomes an option. This could prevent harmful protein production without affecting the body's ability to produce the necessary proteins.

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Procedures

Procedures

Making agarose gel and run electrophoresis for plasmid DNA designed to express PRPF4B kinase domain- July 25th

- Main goal was to detect the presence of a particular DNA strand based on the length the stain goes
    - Preparing agarose gel
        - Take agarose powder (mainly made of seaweed) and combine with 1/50 of purple stain, and for every gram of agarose gel, use 100ml of a buffer
            - This creates a 0.8% - 2% solution
        - Heat up the agarose gel in the microwave in increments of 30 seconds until there is no precipitate
    - Preparing electrophoresis apparatus
        - Place a comb in between the middle structure to create wells to hold DNA
    - Pouring the agarose gel into the apparatus
        - Wait 20-30 minutes until the gel solidifies
    - Remove comb and ensure air tight seals
    - Dye DNA samples with 1/50 of green stain
    - Place DNA strands into singular wells
        - Wait 20-30 minutes
    - Remove gel and take it to the scanner machine in order to detect length through contrast

Creating stacking gels- July 27th

- Creating stacking gels in order to run electrophoresis for protein rather than DNA
- Main goal was to detect the presence of a protein sample to detect the influence of the PRPF4B's influence on the synthesis of certain proteins
    - Preparing first gel solution
        - Use 2 glass plates and press them against each other
        - Apply green clamps on the panels in order to prevent leakage
        - Place them in the designated green machine in order to keep them upright for long periods of time
        - Create solution using the recipe provided on the paper for 10 mL
        - Mix it in the green test tube
    - Using gel solution in the clasp
        - Pour gel solution mostly to the top with a little bit of space between the top of the clasp and the created substance
        - Keep some left in the flask in order to measure whether or not substance has solidified
        - Wait 10-20 minutes
    - Creating a secondary gel (or what is known as the stacking gel)
        - Follow the instructions in the bottom of the same sheet
    - Apply the green comb, make sure it is the right size
    - Pour the stacking gel into the apparatus and wait 20-30 minutes
    - When done, take out the green comb

- Get out all of the necessary equipment to run electrophoresis
  - Includes the positive negative current plugs, plastic structures, foam pieces, blank pieces of paper
- Create a sandwich in the bottom of the plastic tray in order to prevent protein from escaping
- Run at 80 volts for 30 minutes and 100 volts for 30 minutes

Running a Western Blot- July 28th

- Sample Preparation
  - Use lyse cells or tissues to extract proteins
    - Measure protein concentration
  - Mix protein samples with loading buffer and heat to denature proteins
- Gel Electrophoresis (SDS-PAGE)
  - Load protein samples and molecular weight markers into wells of an SDS-PAGE gel
    - Apply an electric current to separate proteins by size.
- Protein Transfer
  - Transfer separated proteins from gel to a membrane
  - Confirm successful transfer
- Blocking
  - Incubate the membrane with a blocking solution (milk in this case) to prevent non-specific antibody binding
  - Put into the freezer for 1.5 hours
- Compare band size in the printing machine

Extracting RNA out of cells- September 18th

Extracting RNA from Cells

- In this case, start with cells from the fridge
  - Need to then transfer vials and place it into the centrifuge at 6000 times per minute for 60 seconds
- Get trizol from the fridge
  - Is neurotoxic
  - Make sure to use the fumehood when transfering
- Start with the cell sample and centrifuge it
  - After it is done, remove all of the liquid that isn't the white cells
- Use the menu to then add that much trizol into the bottle of cells
  - if there are too many cells, change the amount of trizol to be proportionate
  - Use the pipette to extract amount of trizol from the bottle and put it into the vial with the cells
  - Mix it by taking in and extracting the mixture
- Then mix the vial with equal volume of ethanol and mix it thoroughly
- Transfer it into a collection tube
  - Max collection limit is 700 microliters
  - Has a filter and a collector
  - Centrifuge it at 13000 per minute for one minute
    - What this will do it separate the RNA from the proteins and everything else, RNA

kept in filter on the top “storage compartment”

- Take it out of the centrifuge
    - Remove all of the pink liquid and put it into the discard buffer
- DNase I treatment
    - Add 400 microliters of RNA wash buffer into the column and centrifuge again
        - May need to mix ethanol into it just follow the labels on the bottle
- Grab and RNase free tube and add 5 microliters of DNase I and 75 microliters of DNA Digestion Buffer
- Wait for 15 minutes at room temperature

Cell Fractionation Protocol in Mice Cortexs- September 25th

Cell Fractionation Protocol (in mice cortex), will separate the cytoplasm from the nucleus

- Required materials
    - 0.05% NP-40 Alternative in PBS (6mL PBS, 3uL NP-40 alternative)
        - Ice cold
    - Ice cold PBS
    - 4x Laemmli Buffer (0.25M Tris-HCl, pH=6.8, 8% SDS, 40% Glycerol, 0.05% Bromophenol Blue, 100M DDT (need to add fresh)

Note: Not fully completed

Reverse Transcription- September 25th

Reverse Transcription process
Performing Reverse Transcriptions

- Required materials
    - DNase, iScript RT Supermix, Nuclease-free water, DNase Buffer, incubator, iScript Reverse Transcription Supermix
- Procedure
    - Setup DNase master mix
        - Mix iScript DNase and iScript DNase Buffer (found in the mini fridge and mini freezer)
            - In ratio 0.5 microlitre DNase to 1.5 microlitre DNase Buffer
            - Add more or less depending on the amount of RNA samples
    - Add DNase master mix to RNA samples
        - 2 microlitre master mix to each 14 microlitre RNA sample
        - Mix using pipette up and down
    - Incubate
        - At 25C for 5 minutes and then 75C for 5 minutes
    - Add iScript Reverse Transcription Supermix to DNase-treated RNA sample
        - 4 microliters supermix per 16 microliters of DNaser-treated RNA template
    - Incubate
        - 25C for 5 minutes then 46C for 20 minutes then 95C for 1 minute

QT-PCR- November 5th

- Reverse transcription pcr
    - Creates multiple copies of DNA off of one strand
        - Through isolating it, heating it up to an intense temperature and then allow the DNA to split
        - Then giving it time to duplicate
    - Measure the amount of PRPF4B mRNA in the samples and compare it to a reference (housekeeping) gene
    - Household gene
        - GAPDH gene
            - Reference gene
    - Looking for PRPF4B
    - Add 30mL of DNAfree water to the samples
        - One negative control one prpf4b
    - Prepare multiple “containers” to use to create the master mix
        - Follow the correct instructions and ensure no cross contamination and extremely careful
    - Load the samples into the qPCR machine with the appropriate primers and master mix
    - Run the qPCR program, which amplifies the DNA in cycles and measures fluorescence
    - Compare PRPF4B expression to GAPDH expression
- Analyzing Significance
    - Analyzed the significance of the qt PCR procedure we worked on on Monday
    - X-axis
        - Cycle number (PCR cycle), can be quantified to determine the ratio of actual part we are looking for
        - Used primarily to compare data
    - Y-axis
        - Fluorescence intensity
            - RFU
            - Corresponds to the amount of amplified DNA
    - Key point measured is the Ct (threshold cycle) or Cq
        - PCR cycle number where the fluorescence crosses a certain threshold
            - In our case, we used 5000
        - A lower Ct means more starting template (more target DNA/RNA present)
        - A higher Ct means less starting template
            - Got results 30+ for the GAPDH house gene and under 20 for the PRPF4B

- Worked on rt pcr
    - Compared wild type, homozygous and heterozygous with GAPDH household gene
- Takes the amount of mRNA in the sample
    - Copies that DNA over and over again while dye glows brighter as more copies appear
    - How bright it is = the amount of PRPF4B mRNA
    - Uses the PRPF4B forward and reverse primers
        - Tells exactly which gene needs to be copied
- Procedure overview
    - In the lab, we switched the company providing the buffers
        - New one requires a yellow sample buffer and a blue sample buffer, which are used in two different steps
    - Use ratios to your advantage when creating master mix
    - Can dilute the PRPF4B samples before (this time diluted it 20ul)

1. *(Optional)* Add the Yellow Sample Buffer (40X) to the amount of DNA that is used in the PCR.

Final reaction volume	Amount of Yellow Sample Buffer
20 µL	0.5 µL
10 µL	0.25 µL

The Yellow Sample Buffer is diluted to 1X in the final reaction. See the following tables.

2. *(Optional)* Vortex, then centrifuge the DNA and Yellow Sample Buffer.
3. Combine the master mix, the primers, and nuclease-free water according to the following tables.
4. Combine the master mix, the primers, and nuclease-free water with the DNA and Yellow Sample Buffer according to the following tables.

Note: If the Yellow Sample Buffer is not used, add nuclease-free water to achieve the total PCR volume.

Table 1 20–µL reaction

Component	Stock concentration	Final concentration	Volume for 1 reaction (20–µL reaction)	Volume for 4 reactions with 10% overage (20–µL reaction)[1]
Yellow Sample Buffer and DNA (step 1)				
DNA[2]	5 ng/µL	0.5 ng/µL	2 µL[3]	8.8 µL
Yellow Sample Buffer	40X	1X	0.5 µL	2.2 µL
Master mix, primers, and nuclease-free water (step 3)				
PowerTrack™ SYBR™ Green Master Mix	2X	1X	10 µL	44.0 µL
Forward and reverse primers[4]	8,000 nM	400 nM	1 µL	4.4 µL
Nuclease-free water	—	—	6.5 µL	28.6 µL
Total PCR volume	**—**	**—**	**20 µL**	**88 µL**

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 8.5 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a Tm of 55°C.

Table 2 10–µL reaction

Component	Stock concentration	Final concentration	Volume for 1 reaction (10–µL reaction)	Volume for 4 reactions with 10% overage (10–µL reaction)[1]
Yellow Sample Buffer and DNA (step 1)				
DNA[2]	5 ng/µL	0.5 ng/µL	1 µL[3]	4.4 µL
Yellow Sample Buffer	40X	1X	0.25 µL	1.1 µL
Master mix, primers, and nuclease-free water (step 3)				
PowerTrack™ SYBR™ Green Master Mix	2X	1X	5 µL	22.0 µL
Forward and reverse primers[4]	8,000 nM	400 nM	0.5 µL	2.2 µL
Nuclease-free water	—	—	3.25 µL	14.3 µL
Total PCR volume	**—**	**—**	**10 µL**	**44 µL**

[1] 10% overage is recommended for pipetting variations.
[2] Use 1–10ng of cDNA.
[3] Does not exceed 4.25 µL.
[4] The final primer concentration can vary from 300–800 nM. A final concentration of 400 nM is recommended for primers with a Tm of 55°C.

IMPORTANT! The reaction turns green due to the Yellow Sample Buffer added to the DNA and the inert blue dye in the master mix

5. Mix the components thoroughly, then centrifuge briefly to collect the contents at the bottom of the tube.
6. Transfer the appropriate volume of each reaction to each well of an optical plate.
7. Seal the plate with an optical adhesive cover, then centrifuge briefly to collect the contents at the bottom of each well and eliminate any air bubbles.

PCR can be performed on the reaction plate up to 8 hours after completing the set-up, when stored at room temperature protected from light.

Set up and run the real-time PCR instrument

1. Set up the thermal protocol according to one of the following tables.

   Note: Standard cycling conditions are recommended for genomic DNA templates or long amplicons.

   Table 3 Fast cycling mode

Step	Temperature	Duration	Cycles
Enzyme activation	95°C	2 minutes	1
Denature	95°C	5 seconds	40
Anneal/extend	60°C	30 seconds	

   Table 4 Standard cycling mode

Step	Temperature	Duration	Cycles
Enzyme activation	95°C	2 minutes	1
Denature	95°C	15 seconds	40
Anneal/extend	60°C	60 seconds	

2. Set the instrument to perform a default dissociation step, according to one of the following tables.

   Table 5 Fast cycling mode

Step	Ramp rate[1]	Temperature	Time
1	1.99°C/second	95°C	15 seconds
2	1.77°C/second	60°C	1 minute
3 (Dissociation)	0.075°C/second	95°C	15 seconds

   [1] Use the default ramp rate for the StepOnePlus™ Instrument.

   Table 6 Standard cycling mode

Step	Ramp rate[1]	Temperature	Time
1	1.6°C/second	95°C	15 seconds
2	1.6°C/second	60°C	1 minute
3 (Dissociation)	0.075°C/second	95°C	15 seconds

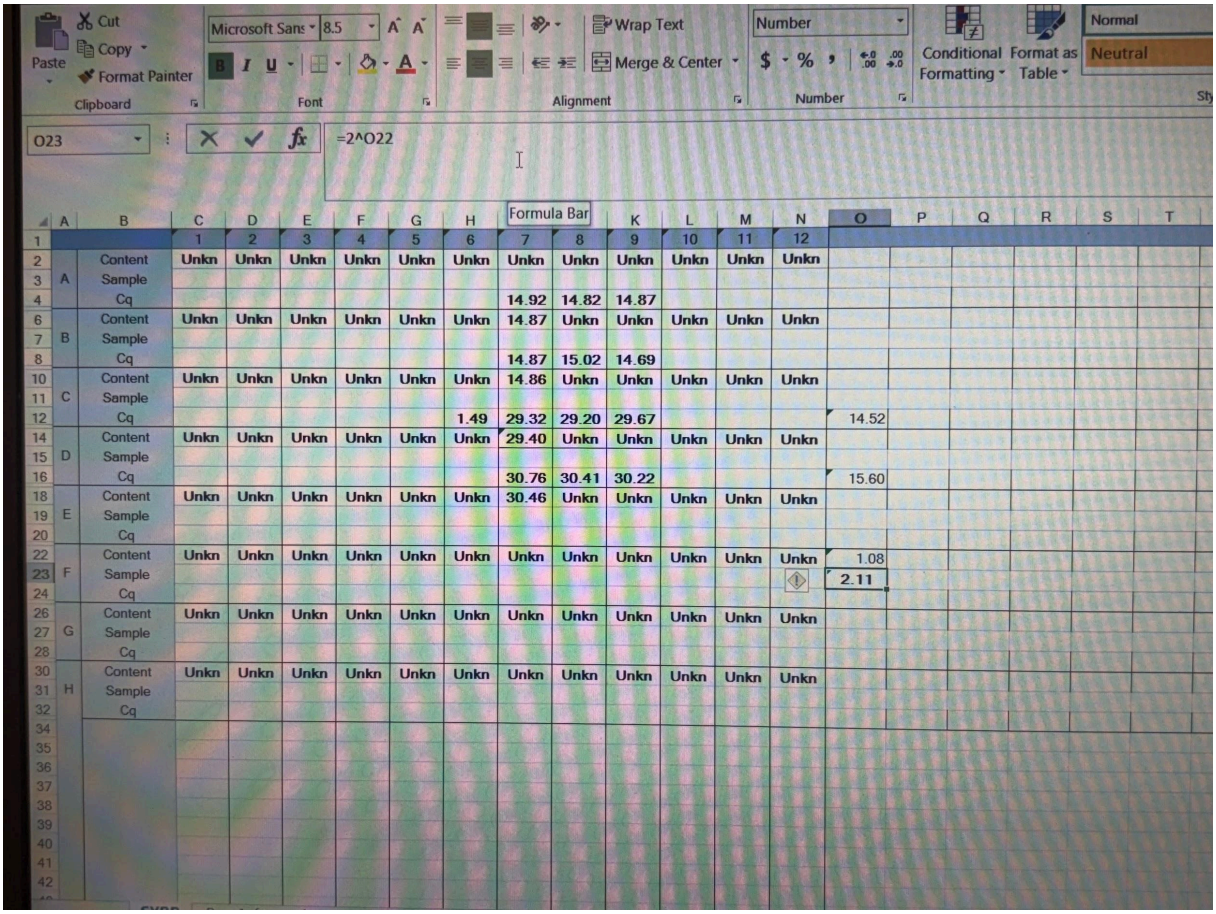
   [1] Use the default ramp rate for the StepOnePlus™ Instrument.

   Note: A dissociation step must be performed immediately after the real-time PCR run with PowerTrack™ SYBR™ Green Master Mix.

3. Set up the options.
   - Experiment type: Standard curve
   - Reagent: SYBR™ Green reagents
   - Reporter: SYBR™ Green
   - Quencher: None
   - Passive reference dye: ROX™ dye
   - Ramp speed: Standard or fast
   - Melt curve ramp increment (all instruments, except StepOnePlus™ instrument): Continuous

Analyzing rt-qpcr results on spreadsheet- November 27th

- Average out values on the palate
  - Minus the prpf4b one from the household gene (or in this case GAPDH)
  - Subtract wt from hetero
  - Delta delta CC
    - 2^ the result from the previous subtraction



			1	2	3	4	5	6	7	8	9	10	11	12	O
2	A	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	
3		Sample													
4		Cq							14.92	14.82	14.87				
6	B	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	14.87	Unkn	Unkn	Unkn	Unkn	Unkn	
7		Sample													
8		Cq							14.87	15.02	14.69				
10	C	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	14.86	Unkn	Unkn	Unkn	Unkn	Unkn	
11		Sample													
12		Cq						1.49	29.32	29.20	29.67				14.52
14	D	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	29.40	Unkn	Unkn	Unkn	Unkn	Unkn	
15		Sample													
16		Cq							30.76	30.41	30.22				15.60
18	E	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	30.46	Unkn	Unkn	Unkn	Unkn	Unkn	
19		Sample													
20		Cq													
22	F	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	1.08
23		Sample													2.11
24		Cq													
26	G	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	
27		Sample													
28		Cq													
30	H	Content	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	Unkn	
31		Sample													
32		Cq													

Introduction Section

Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Introduction Section
Applied Science Project

Justin Nguyen
Dr. Heng Y.

A balance of brain fluid is necessary for neurological development and function in humans.[1,2] Cerebrospinal fluid (CSF) circulates within the brain's ventricular system and performs the functions of nutrient delivery, waste removal, and the protection of neural tissues.[3] Disruptions or constant changes in the regulation of CSF, whether its an overproduction, blocked fluid flow, or even a lack of fluid can create pathological fluid buildup.[4] These conditions have been known to cause a range of developmental problems such as speech disorders and cognitive disorders.[5] Understanding the molecular mechanisms that impact cerebrospinal fluid regulation is important for preventing these conditions and creating an effective treatment for people who are already affected.[6-9]

Recent research has found that RNA splicing in neurological development has been linked to CSF homeostasis.[10] One gene, PRPF4B, is being investigated.[11] This gene codes for a serine/threonine kinase protein that plays a role within pre-mRNA splicing.[12] RNA splicing is the removal of non-coding introns from pre-mRNA and the joining of exons together to create the mRNA that cells use to make proteins. [13]

The PRPF4B gene plays an important role in the spliceosome complex, which is a group of molecules that removes noncoding introns of RNA. [14] PRPF4B acts as a serine/threonine kinase within the spliceosome. It adds phosphate groups to specific splicing proteins to regulate their activity. This is required for proper spliceosome assembly, timing and disassembly during the pre-mRNA processing. By doing this, PRPF4B helps to ensure that the introns are correctly removed and the exons are correctly joined. When the PRPF4B kinase activity is changed, it can cause discrepancies in splice-site selections and mRNA creation transcripts. Even though these transcripts could still be expressed at normal levels, it could also encode proteins with impaired function.

Incorrect splicing due to variations within PRPF4B can cause improperly expressed proteins that could disrupt cellular processes that play a role in fluid regulation.[15] In the brain, splicing inconsistencies can lead to inaccurate expression of proteins needed for the regulation of fluid transport, ion channels, and cilia function within the ventricular system. [16] This leads to an abnormal accumulation of cerebrospinal fluid and, as a result, variability within intracranial

pressures.[17] In addition to the brain, PRPF4B splicing errors can impact other organ systems dependent on fluid regulation and correct protein synthesis.[18] While these are significantly less minor than CSF regulation in the brain, PRPF4B mutations in other parts of the body can still impact human health. The kidneys, which are responsible for the balance of fluids within the body, may be affected if the incorrect proteins are produced.[19, 20] Protein misregulation in the kidneys can affect the functioning of the endothelial cells, which can cause inflammation, oxidative stress, and impaired blood flow in the kidneys.[21, 22]

Not all genes will respond the same way to the loss of a single allele.[23] Some genes are dosage-sensitive. This means that a single functional copy is not enough to maintain normal mRNA and protein levels.[24] Others are dosage-tolerant and can compensate through the remaining allele.[25] Whether PRPF4B is dosage-sensitive or dosage-tolerant is currently undetermined. Understanding if cells can maintain normal PRPF4B expression with only one functional allele is therefore required for understanding how heterozygous PRPF4B mutations contribute to diseases.

Because patients with PRPF4B-linked neurodevelopmental disorders often have a dominant heterozygous mutation, understanding how reduced PRPF4B function affects fluid production is necessary.[26] iPSCs, which are adult cells that have been reprogrammed into a stem-cell-like state, allow researchers to model neural development without needing actual brain tissue from patients.[27] It will help researchers understand if a single copy of a functional PRPF4B gene can maintain normal mRNA splicing and protein levels.

Although gene expression is often measured using mRNA levels, lots of mRNA does not always directly correlate to protein levels.[28] Post-transcriptional regulation, translation efficiency and protein stability can all impact how much functional protein is synthesized.[29] For the PRPF4B gene, mutations could change RNA splicing without changing transcript abundance. Removing the uncertainty through measuring both PRPF4B mRNA levels and PRPF4B protein levels is required to determine whether the loss of an allele affects transcription, protein production or both. This is important for determining whether cells can maintain normal PRPF4B protein

levels even with reduced gene expression, which is important for evaluating and understanding the effectiveness of therapeutic strategies that selectively block mutant PRPF4B mRNA.

To understand how disruptions in the PRPF4B gene affect human cells and mRNA splicing, gene editing provides a direct approach.[30, 31] CRISPR-Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats Cas9, allows researchers to create targeted gene knockouts.[32] This includes creating heterozygous knockouts of PRPF4B to study how reduced protein levels impact cell function and cerebrospinal fluid.

The current study therefore focuses on evaluating PRPF4B protein levels in iPSCs after CRISPR-Cas9–mediated gene removal and measuring its impact on mRNA and protein production. Understanding whether cells can tolerate the loss of a single PRPF4B allele without losing the required protein functions is important to understanding the impact of PRPF4B in brain development and kidney function. This research also provides a basis for future work towards a treatment that reduces the impact of PRPF4B gene mutations in the development of neurodevelopmental disorders.

Having this background, the primary research question of this study is whether human induced pluripotent stem cells (iPSCs) can maintain the normal levels of PRPF4B protein and mRNA expression (normal levels found in wild-type cells) after one allele of the PRPF4B gene is knocked out using CRISPR-Cas9. It is hypothesized that the knockout of a single PRPF4B allele will not affect the level of PRPF4B protein or PRPF4B mRNA compared with non-edited, wild-type PRPF4B cells. This is because the remaining wild-type PRPF4B allele is expected to compensate for the loss by maintaining transcription levels and translation to maintain normal PRPF4B mRNA and protein levels. If this were to not happen within the human body, important organs could be impacted, which could cause development disorders and fluid maintenance problems.

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Methodology Section

Evaluating PRPF4B Protein Levels after CRISPR-Cas9 Mediated Heterozygous Knockout in Human iPSCs

Methodology Section
Applied Science Project

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Human induced pluripotent stem cells (iPSCs) were used as a model to test PRPF4B's dosage sensitivity after heterozygous gene knockout. Both wild-type and heterozygous PRPF4B knockout lines started as single-celled colonies and were incubated for 2 weeks under the exact same conditions. They were kept in defined, serum-free and feeder-free media, with Essential 8 (Thermo Fisher Scientific, Waltham, Massachusetts), at a temperature of 37°C, 5% CO_2 concentration, 5% O_2 concentration, and 95% saturated humidity.

RT-qPCR:

Cells were cultured to between 70–90% confluency, where point culture media was removed and cells were washed with phosphate-buffered saline (PBS) to remove residual proteins and debris. Cells were then lysed using a guanidinium-based lysis buffer provided in a commercial RNA extraction kit (Zymo Research, Irvine, CA). This buffer disrupts cell membranes and inactivates RNases. The total RNA is preserved and released. The resulting lysate was processed following the manufacturer's protocol to separate RNA. To account for differences in cell colony numbers and RNA differences, gene expression was normalized to GAPDH.

To allow for quantification of gene expression, the resulting RNA was reverse-transcribed into complementary DNA (cDNA) using a commercially available cDNA synthesis kit (Zymo Research). This step uses the enzyme reverse transcriptase to create single-stranded complementary DNA (cDNA) from mRNA templates by complementary base pairing. The cDNA starts as a single strand but is duplicated during quantitative PCR. This step is required as RNA is extremely unstable and cannot be consistently amplified by DNA polymerase during PCR.

The obtained cDNA samples were used as templates for real-time quantitative polymerase chain reaction (qPCR). PRPF4B forward (GACTCTTCAGGCACTTCTATCAC) and reverse primers (ACACCTCTCGTAAGTTCATGC) were then added to selectively amplify the amount of PRPF4B DNA in the samples. Primers targeting the GAPDH housekeeping gene (forward: GTCTCCTCTGACTTCAACAGCG, reverse: ACCACCCTGTTGCTGTAGCCAA) were also added because

of its stable expression between different experimental conditions.

During qPCR, DNA was amplified by repeating cycles of denaturation, annealing and extension using a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California). The denaturation step, which heats up the cDNA to 95°C, breaks the hydrogen bonds between complementary base pairs. This step is required in order for primers to access the target DNA sequence. During annealing, the temperature was lowered to 65°C to allow forward and reverse primers to bind to the sequences directly around the PRPF4B or GAPDH target spot. Primers determine which part of the DNA strand gets copied and bonds through hydrogen bonds. This temperature must be maintained as it is the temperature in which bonds between the primers and the DNA strands will form at the perfect location for base pairing. These primers dissociate at the beginning of each cycle during the denaturation step when the hydrogen bonds between each DNA strand is broken. Lastly, in the process of extension (at 72°C), DNA polymerase extended from the bound primers synthesizes new DNA strands by adding complementary nucleotides in the 5’ to 3’ direction. This happens to every single place where the primers can be found and creates double-stranded DNA copies of the target region. PowerTrack SYBR Green Master Mix for qPCR (Thermofisher, Waltham, MA) bonds to any dsDNA (double-stranded DNA) that is created by the end of extension. Because double-stranded DNA has stacked base pairs, a major groove and a minor groove. SYBR Green is correct width for the minor groove and has complementary charge distribution, making it able to slide between the adjacent base pairs. In a process called intercalation, the dye is stabilized through LDFs. This allows for fluorescence to be an accurate a measurement of the amount of DNA strands after each cycle. These three steps were repeated until fluorescence reached a certain threshold. The amount of cycles required was recorded.

RT-qPCR Data Analysis:

As PCR progressed, greater amounts of double-stranded DNA gets produced, which allows SYBR green dye molecules to emit greater fluorescence. SYBR bonds to the grooves in double-stranded DNA through bouncing around at immense concentrations. These molecules are positively charged compare to the negatively charged DNA strands and fit perfectly in the

grooves. When being read by the PCR machine, they jump to a different energy level and transmit small amounts of light. This is then detected by the machine and recorded after every single cycle.

A fluorescence threshold was set in order to determine cycle threshold (Ct) value for each sample. The Ct value represents the number of amplification cycles for the fluorescence to pass this threshold. A sample with higher amounts of starting PRPF4B mRNA would have a lower Ct value while samples with less starting mRNA have a higher Ct value.

To control for differences in RNA input and reverse transcription efficiency, PRPF4B Ct values were normalized to Ct values of GAPDH, the housekeeping gene. This process of normalization was done through the ΔCt equation with both wild-type and heterozygous:

ΔCt = Average Ct (PRPF4B) - Average Ct (GAPDH)

To further compare gene expression between heterozygous PRPF4B knockout cells and wild-type, ΔCt values were normalized again using the $\Delta\Delta$Ct method:

$\Delta\Delta$Ct = ΔCt (PRPF4B Heterozygous) - ΔCt (PRPF4B Wild-Type)

Because PCR amplification doubles each cycle and therefore, is exponential, differences in Ct values mean differences in the starting amount of PRPF4B mRNA. Due to Ct values being the amount of cycles needed to reach a threshold, directly comparing Ct values cannot be done. The following equation turns Ct differences into relative expression:

$2^{-\Delta\Delta Ct}$

The values represent the relative PRPF4B mRNA expression of heterozygous knockout cells compared to wild-type cells, having wild-type expression as the reference value. A value of 1 means there was no change found in expression relative to wild-type, values less than 1 show less PRPF4B expression in heterozygous cells and values greater than 1 mean increased PRPF4B expression relative to wild-type. Because wild-type samples are used as the control, they represent the maximum normalized expression level, and all heterozygous values are a proportion of wild-type expression.

Western Blotting:

To determine whether PRPF4B protein levels remain constant after heterozygous gene knockout, quantitative Western blotting was used on wild-type and heterozygous PRPF4B knockout human induced pluripotent stem cell (iPSC) populations. After the identical processes of incubation, the cells were harvested and lysed using a protein extraction buffer with detergents and protease inhibitors. This step destroyed cellular membranes and released intracellular proteins, avoiding enzymatic degradation in the process.

After lysis, protein lysates were centrifuged to remove cellular debris. Equal amounts of total protein lysate from each sample was then prepared for electrophoresis. Any variation in protein loading was controlled during data analysis by normalizing PRPF4B protein expression to GAPDH protein expression.

Protein samples were mixed with sodium dodecyl sulfate (SDS) sample buffer and heated to denature protein secondary and tertiary structures. SDS binds to proteins and gives them a consistently negative charge, which allows proteins to migrate toward the positive electrode during electrophoresis in the following step. Denaturation makes sure all proteins are separated only on molecular weight.

Denatured protein samples were separated in a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A stacking gel and resolving gel were employed to allow for consistent protein separation. The stacking gel concentrated proteins into narrow bands, which allowed for uniform protein migration. The resolving gel separated proteins based on molecular weight.

After electrophoresis, separated proteins where transferred from the gel onto a polyvinylidene difluoride (PVDF) membrane through electrotransfer. This process stopped the movement of proteins on the membrane and allowed for antibody-based quantification of these proteins. The membrane was then incubated into a blocking solution containing non-fat milk proteins to prevent non-specific antibody binding.

A Novus Biologicals NB100-86997 primary antibody was then incubated with the membrane to bind with PRPF4B proteins while a Sigma G9545 rabbit antibody that targeted

GAPDH was used to detect GAPDH proteins as a housekeeping protein. These antibodies selectively bind to their target protein because of antigen-antibody specificity.

After washing to remove unbound primary antibodies, the membranes were incubated with a secondary antibody. The second antibody was conjugated to a chemiluminescent enzyme reporter. When exposed to chemiluminescent substrate, the enzyme creates a light-producing reaction at antibody binding sites. The emitted light signal was seen and measured using an imaging system. Because light intensity corresponds to protein amount, the band intensities could be directly compared.

Final Paper