Make sure to add your initials, time, and date to all entries in case of questions. Highlight tasks that have been started but not completed. Once completed, take the highlight off.

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**1/6/20**
Sequencing results are back for the following: R248C, T1122C, EPG-1, EPG-2, OPRM1 pGHE. Downloaded sequencing files saved in DNA and RNA inventory in G:Drive. Cleaned freezer C and set up new rocker, disposed of old ice packs. Disposed of Phenol:Chloroform waste and Ethanol waste. -TR

**1/7/20**
OPRM1 pGHE still in pANT7 plasmid re-think subcloning. Frog surgery. Moved all items back to freezer C made new MBSH +AB+AF. Made new AB+AF aliquots. Put charcoal filter in IMBX waste. Made alignments for blunt ended subcloning. -TR

**1/8/20**
L333C, Y109C and A120C DNA need to be purified.EPG protein primers will need to be made for pGHE, for now we can use genewiz universal primers until move if complete. 1X DS solution needs to be made. 5X MBSH needs to be made. DnpI digest of OPRM1, pGHE, KCNJ3. PCR for KCNJ9 started. Oocytes ordered for friday. -TR

**1/9/20**
OPRM1, pGHE, KCNJ3 placed in PCR rack. Autoclaved bottles and LB+agar. Ligated pGHE vector. Gel of KCNJ9 insert. -TR

**1/10/20**
Phosphorylation of OPRM1 (pGHE) and KCNJ3 (pGHE). Injected L102C RNA and R104C RNA (newly made found in TAAR1 box). -TR

**1/13/20**
Transformation of OPRM1(pGHE), KCNJ3 (pGHE), G103C, D113H. Ordered sutures. Checked expression of L102C oocytes.-TR

Read about, set up, and attempted to slow freeze a line of LM45 cells using the cryotstore media and Mr Frosty with Erika. Passaged LM45 cells. A video on passaging will be made next week. Autoclaved DI water for the cell incubator. - BS

Attempted to slow freeze a line of LM45 cells with the Mr. Frosty. Autoclaved 2L of DI water for cell incubator. -EY

**1/14/20**
Autoclaved transformation waste and cell culture waste. Checked expression levels of oocytes. Autoclaved pipette tips. Disposed of old RNA created a b2AR HiFi page in wiki. Labeled concentration of EPG. -TR

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Performed steps 6-8 of miniprep for L333C, A102C, and Y108C again to remove additional chloroform. Analyzed sequencing results for T1122C. Wrong portion was sequenced, meaning the primers are labelled wrong and must be discarded and new dilutions need to be made. -CF

Analyzed the re-run of KCNJ9_beginning-KCNJ9_R1. Updated the opioid project lab wiki. Updated the Box 18 key. Designed opioid project Hifi DNA assembly primers (saved in opioid project folder in G drive). Inoculated 3 OPRM1 colonies, 3 KCNJ3 colonies, and 1 colony of R104C/E116H, R104CE116C, D113H, and D103C for miniprep. -CK

**1/15/19**

Mini prep of blunt ended subcloning KCNJ3 and OPRM1, D103C, D113H, R104C/E116C and R104C/E116H. Made EPG HiFi primers. Ordered HIFI primers for magnetic and opioid projects. -TR

Moved the LM45 cells that were put in the Mr. Frosty to slow freeze on Monday into its own bag (labeled LM45 01/15/20) that is in the -80 freezer. The Mr. Frosty is now in the lab in the cabinet with the CRISPR dishes above the microscope. -EY

**1/16/20**

Frog surgery, autoclaved miniprep waste, made 1X DS. Looked at sequencing results for R248C, did not find another binding site for the primers. -TR

Finished Miniprep for Tina. Looked at R248C sequencing.Learned more about the opioid project. - LC

Learned SigmaPlot analysis. Helped change Ch. 1 parameters on the pulling station on the left. Tested Ch. 1 pipetted with FR test on WT oocytes that can be found on the Lab station 1 computer under the 2020 processed pclamp files. -- NV

Showed Ryan the basics of the lab and the CRISPR project. Replaced the water in the cell incubator and cleaned out the humidity pan as something appeared to be in the water. Also wiped down the incubator and checked the confluency of the cells. - BS

Blake showed me some of the Lab basics. We discussed information about CRISPR. Assisted in replacing the water in the cell culture incubator and cleaning the humidity pan. -RB

Performed inoculation and pulled pipettes. -CF

**1/17/20**

Miniprep of OPRM1-4-6 and KCNJ3-4-6. Autoclaved miniprep waste. Updated slack -TR

Pulled and broke new micropipettes, injected R104C and L102C. - ACH

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**1/21/20**

Rewrote procedure for CFTR mutant R248C/D363C. Cleaned out remaining R248C DNA in lab. Resuspended the Opioid Projects HIFI primers. - LC

Finished miniprep got rid of L333C, A120C and Y109C DNA would not pellett concentration most likely too low. KCNJ3, OPRM1, D113H, D103C , R104C/E116H and R104C/E116H. Autoclaved pyrex bottles and DI water. Made 5x MBSH and 1x MBSH. 1x MBSH needs osmol checked, pH checked and filtered.-TR

Read more about the CRISPR project, researched information about Lipofectamine, learned more about transfection and associated protocols. -RB

Performed transformation on A107C and T1122C. Alloquoted JM109 cells. Diluted hifi primers for opioid and magnetic project. -CF

Passaged LM45 cells and made a video of passaging with Erika. Preplated LM45 cells for attempting transfection of GFP and GFP+Cas9 tomorrow. - BS

Filmed footage of Blake passaging LM45 cells to make a video for the passaging protocol. Preated LM45 cells to attempt a transfection of GFP and GFP+Cas9 tomorrow. -EY

Finished HIFI Subcloning Protocol for Tina. Set up PCR reactions for OPRM1, KCNJ9, KCNJ3, EPG, OPRM1 pGHE, KCNJ9 pGHE, KCNJ3 pGHE, and EPG pGHE. Ran thermocycler for OPRM1, KCNJ9, and KCNJ3 and they need to be taken out and put in PCR box in freezer A in the morning. EPG needs to be run separately and OPRM1 pGHE, KCNJ9 pGHE, KCNJ3 pGHE, and EPG pGHE can be ran together. -CK

Injected oocytes R104C 50ppm .5% BAR, L102C .02% .5% BAR. Performed Electrophysiology experiment, efb012120, R104C GlyH 101 dose response w/ MTSET modification. Needs to be analyzed. Made video for electrophysiology experiment set up, during experiment, and clean up. Needs to be edited. Retrieved bottles from autoclave and put them away -EF.

**1/22/20**

Made LB plates, ran PCR for EPG and HIFI pGHE vectors. Ran gel needs analyzed.Made 1X MBSH and MBSH +AB+AF. Sorted oocytes. Autoclaved old plates.-TR

Transfected the cells that were plated yesterday with Erika. Will observe for fluorescence on Friday. - BS

Transfected the cells that were plated yesterday with Blake. -EY

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**1/23/20**

Uploaded snapgene and ape files for magnetic project to slack. Autoclaved old plates and old MBSH +AB+AF. -TR

Made 2 gels and taught Ryan how to make them. Cleaned and put away dishes. Read the HIFI protocol. Ran both gels (the black with 10 DNA's and the yellow with 7 DNA's) In the black lane 2 looked very light before the gel was run. Need someone to analyze both gels. -LC

Made a Gel with Leah, learned these processes. Started Electrophysiology Experiment (Anion Substitution Unmodified with R104C) with Tina. Tina is continuing the experiment. Data to be analyzed on Tuesday. -RB

Worked on miniprep for KCNJ3-4, KCNJ3-5, KCNJ3-6, OPRM1-4, OPRM1-5, and OPRM1-6. Waiting overnight for pellets to form. Made a gel. Took pictures of gels lc011220 and 012320 and labeled them. Still need to be analyzed. -CF

Started PCR for N312C, needs to be retrieved in the morning. Analyzed efb012120. Disposed gels. Attempted to make salt bridges. -EF

**1/24/20**

Ran PCR for KCNJ9 HIFI. DpnI digest of pGHE HIFI reactions and OPRM1, KCNJ3 HIFI. Analyzed gels from 1/23. -TR

Attempted to observe transfected cells under fluorescence using the microscope in room 515, but no fluorescence was observed in the cells transfected with GFP or the cells transfected with GFP + Cas9. Checked the cells acquired from Dr. Baer's lab on thursday that are permanently fluorescent to observe under fluorescence and compare, but no fluorescence was observed. I will check for fluorescence in the line of cell's from Dr. Baer on Monday, and will consult Dr. Baer if no fluorescence is observed again. - BS

Attemped to observe the transfected cells with Blake. Began uploading unedited videos recorded 01/21 to make a Passaging video into the Unedited/Unlabeled CRISPR Videos folder under CRISPR Project. -EY

Pulled and broke injection micropipettes, began electrophysiology with R104C 50 ppm MTSES GlyH101. -AP

Injected 50 ppm R104C and 0.02% L102C (3x usual number of oocytes), made and tested new 1x MBSH solution, and began reorganization of lab chemical stock. - ACH

**1/27/20**

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Got rid of old injected oocytes. Ran gel of N312C and HIFI KCNJ9. KCNJ9 failed-retry.Prepped and ran KCNJ3, OPRM1 and EPG for HIFI assembly. DnpI digest N312C. L102C electrophys experiment. Made a gel.  -TR

Checked fluorescence of permanently fluorescent cells and of the transfected cells again with Dr. Norimatsu. Dr. Norimatsu adjusted the mercury lamp so that the fluorescence could be observed  properly. The transfection of eGFP showed to be successful, but the Cas9+eGFP transfection was inconclusive. Disposed of the transfected cells from last week. Will try another transfection next week. - BS

Checked fluorescence of the cells transfected last week in the shared equipment room. Began looking up microscope cameras like Dr. Baer's to purchase for the microscope in the shared equipment room to photograph the fluorescent cells in the future. -EY

**1/28/20**

Made gels and ran. DnpI digested KCNJ9 HIFI insert.-TR

| Needs to be run | Lane: | run:tr012820 |
|---|---|---|
| ladder | 1 | ladder |
| KCNJ3 pGHE | 2 | KCNJ3 pGHE |
| OPRM pGHE | 3 | OPRM pGHE |
| EPG pGHE | 4 | EPG pGHE |
| KCNJ9 HIFI | 5 | KCNJ9 HIFI |
| KCNJ9 | 6 | KCNJ9 |
| | 7 | |
| | 8 | |
| Needs to be run | Lane: | run:trb012820 |
| Ladder | 1 | Ladder |
| OPRM1-4 | 2 | OPRM1-4 |
| OPRM1-5 | 3 | OPRM1-5 |
| OPRM1-6 | 4 | OPRM1 |
| OPRM1 | 5 | OPRM1-6 |
| KCNJ3-4 | 6 | KCNJ3-4 |
| KCNJ3-5 | 7 | KCNJ3-5 |
| KCNJ3-6 | 8 | KCNJ3-6 |
| KCNJ3 | 9 | KCNJ3 |

Did the data analysis for R104C (Unmodified Anion Substitution) and learned how the associated programs worked. Assisted Blake and Erika with passaging cells. -RTB

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Performed transformation on KCNJ3, OPRM1, EPG, N312C, and I290C/D103H. Need to order more JM109 cells. Aliquoted Q5 and made gel. -CF

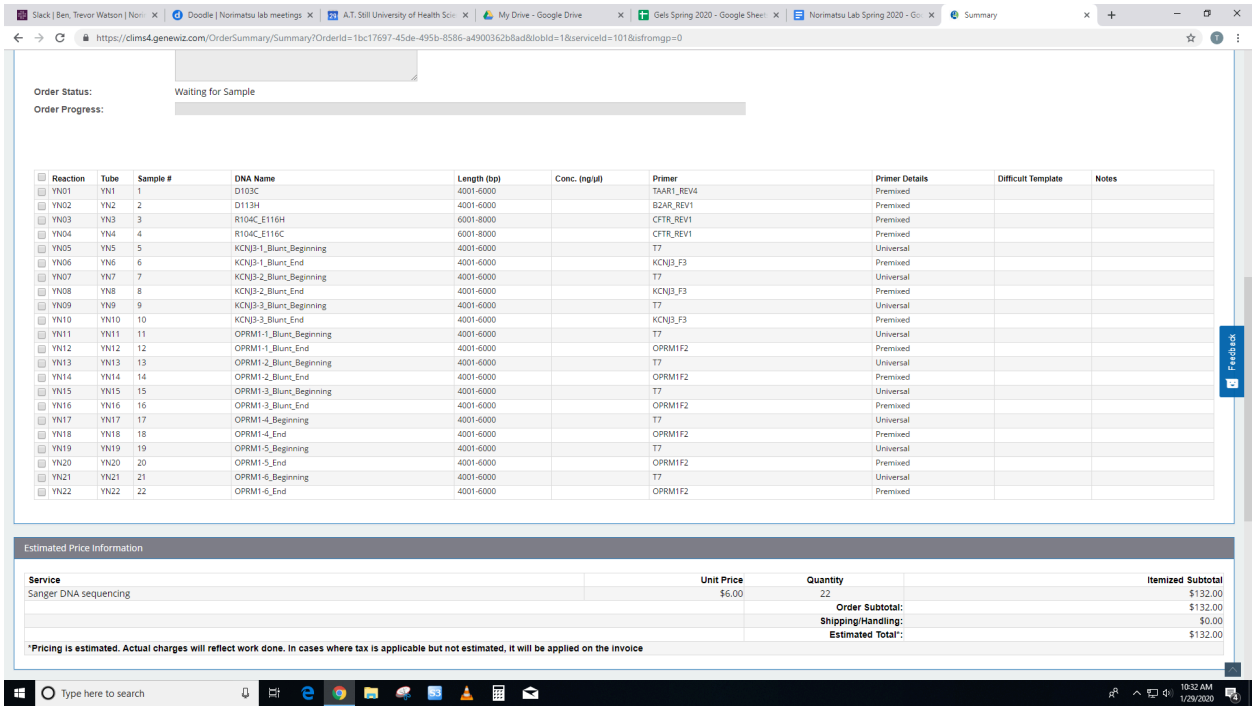
Helped Ryan passage LM45 cells. Passaged LM45-CD47 GFP cells into two 60 mm dishes for one of them to be frozen soon. Started writing a protocol on unthawing deep frozen cells on the google drive. Began updating freezing protocols under the CRISPR project on the google drive. - BS

Did calculations for and picked primers for sequencing. -CK

Downloaded gel imager to back computer. Pulled pipettes, still need to be checked. Made tubes for sequencing. Autoclaved flask. -EF

**1/29/20**

Removed KCNJ3, OPRM1, EPG, N312C, and I290C/D103H from Ingrid. Sent samples for sequencing.



Slack | Ben, Trevor Watson | Nori | Doodle | Norimatsu lab meetings | A.T. Still University of Health Sci | My Drive - Google Drive | Gels Spring 2020 - Google Sheet | Norimatsu Lab Spring 2020 - Go | Summary

https://clims4.genewiz.com/OrderSummary/Summary?OrderId=1bc17697-45de-495b-8586-a4900362b8ad&JobId=1&serviceId=101&isfromgp=0

Order Status: Waiting for Sample

Order Progress:

| Reaction | Tube | Sample # | DNA Name | Length (bp) | Conc. (ng/µl) | Primer | Primer Details | Difficult Template | Notes |
|---|---|---|---|---|---|---|---|---|---|
| YN01 | YN1 | 1 | D103C | 4001-6000 | | TAAR1_REV4 | Premixed | | |
| YN02 | YN2 | 2 | D113H | 4001-6000 | | B2AR_REV1 | Premixed | | |
| YN03 | YN3 | 3 | R104C_E116H | 6001-8000 | | CFTR_REV1 | Premixed | | |
| YN04 | YN4 | 4 | R104C_E116C | 6001-8000 | | CFTR_REV1 | Premixed | | |
| YN05 | YN5 | 5 | KCNJ3-1_Blunt_Beginning | 4001-6000 | | T7 | Universal | | |
| YN06 | YN6 | 6 | KCNJ3-1_Blunt_End | 4001-6000 | | KCNJ3_F3 | Premixed | | |
| YN07 | YN7 | 7 | KCNJ3-2_Blunt_Beginning | 4001-6000 | | T7 | Universal | | |
| YN08 | YN8 | 8 | KCNJ3-2_Blunt_End | 4001-6000 | | KCNJ3_F3 | Premixed | | |
| YN09 | YN9 | 9 | KCNJ3-3_Blunt_Beginning | 4001-6000 | | T7 | Universal | | |
| YN10 | YN10 | 10 | KCNJ3-3_Blunt_End | 4001-6000 | | KCNJ3_F3 | Premixed | | |
| YN11 | YN11 | 11 | OPRM1-1_Blunt_Beginning | 4001-6000 | | T7 | Universal | | |
| YN12 | YN12 | 12 | OPRM1-1_Blunt_End | 4001-6000 | | OPRM1F2 | Premixed | | |
| YN13 | YN13 | 13 | OPRM1-2_Blunt_Beginning | 4001-6000 | | T7 | Universal | | |
| YN14 | YN14 | 14 | OPRM1-2_Blunt_End | 4001-6000 | | OPRM1F2 | Premixed | | |
| YN15 | YN15 | 15 | OPRM1-3_Blunt_Beginning | 4001-6000 | | T7 | Universal | | |
| YN16 | YN16 | 16 | OPRM1-3_Blunt_End | 4001-6000 | | OPRM1F2 | Premixed | | |
| YN17 | YN17 | 17 | OPRM1-4_Beginning | 4001-6000 | | T7 | Universal | | |
| YN18 | YN18 | 18 | OPRM1-4_End | 4001-6000 | | OPRM1F2 | Premixed | | |
| YN19 | YN19 | 19 | OPRM1-5_Beginning | 4001-6000 | | T7 | Universal | | |
| YN20 | YN20 | 20 | OPRM1-5_End | 4001-6000 | | OPRM1F2 | Premixed | | |
| YN21 | YN21 | 21 | OPRM1-6_Beginning | 4001-6000 | | T7 | Universal | | |
| YN22 | YN22 | 22 | OPRM1-6_End | 4001-6000 | | OPRM1F2 | Premixed | | |

Feedback

Estimated Price Information

| Service | Unit Price | Quantity | Itemized Subtotal |
|---|---|---|---|
| Sanger DNA sequencing | $6.00 | 22 | $132.00 |
| | | Order Subtotal: | $132.00 |
| | | Shipping/Handling: | $0.00 |
| | | Estimated Total*: | $132.00 |

*Pricing is estimated. Actual charges will reflect work done. In cases where tax is applicable but not estimated, it will be applied on the invoice

Type here to search

10:32 AM 1/29/2020

Ordered more JM109 cells. -TR

**1/30/20**

Began miniprep of I290C/D103H, EPG, OPRM1, KCNJ3, T1122C, and N312C .-TR

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Ran PCR on CFTR open mutants R248C, P111C, S118C, and L219C. Need someone to retrieve the 4 PCR tubes in the morning. Pulled pipettes with Ryan. Made TB broth and agar. Both need to be removed from autoclave at about 4:10pm. - LC

Unthawed the LM45 cells we froze 01/14 with Blake and Ryan; the 700 microliters of cells were split between two new dishes that are now in the incubator of the shared equipment room. I will be passaging both dishes tomorrow, and we eventually will use them to freeze again. -EY

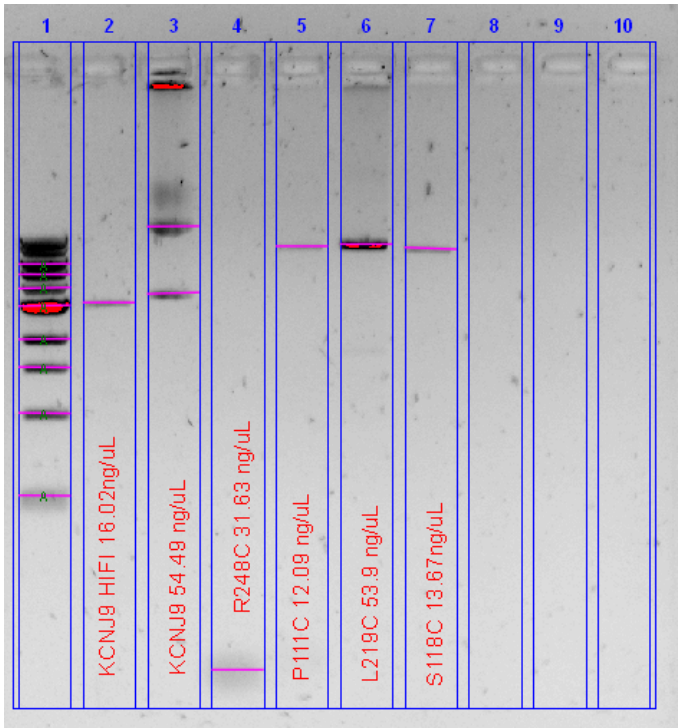
Read about Transfection and different ways to perform transfection including Lipofection and electroporation. Pulled Pipettes with Leah. Unthawed LM45 cells with Blake and Erika, split cells. Helped get the electrophysiology tubing in order with Tina. -RTB

Unthawed Frozen LM45 cells. 2 more LM45 cell tubes will be frozen next week. Verified unthawing cells protocol and began adding freezing cells protocol to the google sites. Ordererd 35 mm cell culture dishes. - BS

Made new KCl bridges and aliquoted new JM109 cells. -CF

**1/31/20**

Ran gel. Saved as tr013119. R248C failed tube disposed of. DnpI digest P111C, L219C, S118C-will be done at 2:30.




-TR

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Passaged the two plates of cells that were unthawed yesterday each into their own new plate that will be frozen next week. The plates are in the incubator in the shared equipment room. -EY

Made IMBX. Helped Justin with electrophysiology and cleaned up experiment. Updated chemical list classifications and colored. -EF

Continued work on chemical list classifications and color coding, made 0.01% L102C solution, attempted R104C and L102C injection (failed due to micropipette issues). - ACH

Sorted oocytes, made salt bridges, pulled and broke injection pipettes, updated hazards on chemical list, and circularized L219C, P111C, and S118C (still need to be transformed). -AP

**2/3/20**

Finished miniprep of EPG, KCNJ3, OPRM1, T1122C, I290C/D103H, and N312C. Disposed of bacterial waste. Amazon and ebay orders for new computers and micromanipulator. Took pictures of oocytes from frog M updated the oocyte health sheet. Transformed L219C, P111C, and S118C. Inoculated KCNJ9-1 and -2. Cleaned micropipette holders and electrodes. -TR

Passaged LM45 cells. Passaged LM45-CD47 cells (GFP) into two dishes. Two plates of LM45 cells will be frozen tomorrow, as well as one plate of LM45-CD47 cells. A second plate of LM45-CD47 will be frozen next week. Preplated LM45 cells for transfection of eGFP and Cas9+eGFP tomorrow. - BS

The plates passaged on 01/31 appeared to be alive, and will be frozen tomorrow. Preplated LM45 cells for transfection with eGFP and Cas9+eGFP tomorrow. -EY

**2/4/20**

Began miniprep of KCNJ9-1 and -2, Stopped after RNase step. Disposed of PCI tips and tubes. Disposed of bacterial waste. Linearize R104C/E116C, D113C, D113H, will be done at 3pm. Retrieved D113A from thermocycler placed in PCR box. Electrophys L102C. -TR

Started electrophysiology experiment with L102C Anion Unmodified. Emma F. took over the experiment for the final solution and data processing. Helped with Blake to do transfection of LM45 cells of eGFP and Cas9+eGFP, but found a substance in the media. Decided to throw out the sample with the advice of Dr. Norimatsu. Cleaned the wells with bleach and disposed of cells. Cleaned the work area to limit the spread of unwanted substances. Observed Erika freeze cells. -RTB

Attempted to do a transfection of eGFP and eGFP+Cas9, but found a substance growing in the media and decided not to transfect. Filled the pre-plated wells with bleach and threw them away to disinfect. Disinfected the equipment room and the inside of the cell incubator. Helped Erika freeze one tube of LM45-CD47 GFP cells, and two tubes of LM45 cells. The tubes are sitting in

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the -80 degree freezer in the Mr. Frosty but need to be removed and put in labeled plastic bags for long term storage. Refer to steps 16 and 17 in the “Slow Freezing Cells Protocol” on the google drive under CRISPR project. - BS

Finished and analyzed RTB02042020; moved to G drive. Put away dishes. Recleaned PCI bottles. Removed agar from autoclave and made Lb +amp plates. Took picture of gel, ef020420. -EF

Froze the two LM45 tubes and one LM45-CD47 tube. All three are in the -80 freezer on the second floor. -EY

Analyzed 01/31/20 sequencing results. Blunt ended subcloning did not work for KCNJ3 1-3 and OPRM1 1-6. D103C mutagenesis was successful. For blunt ended subcloning in the future, purify PCR products with Purelink PCR purification kit on the bottom shelf of Cabinet A to avoid the unwanted insertion of primer dimers. -CK

**2/5/20**

Made .5X TBE. Ran gels. Inoculation of L219C, S118C, P111C.  Removed tubes from Mr. Frosty. - TR

| Lane: | run: tr020520 |
|---|---|
| 1 | Ladder |
| 2 | OPRM1-1 |
| 3 | OPRM1-2D113H |
| 4 | KCNJ3-1 |
| 5 | KCNJ3-2 |
| 6 | T1122C-1 |
| 7 | T1122C-2 |
| 8 | EPG-1 |
| 9 | EPG-2 |
| 10 | I290C/D103H-1 |

| Lane: | run: trb020520 |
|---|---|
| 1 | ladder |
| 2 | R104C/E116H LIN |
| 3 | CFTR |
| 4 | D113C LIN |
| 5 | D113H LIN |

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| | |
|---|---|
| 6 | D113C |
| 7 | D113A PCR |
| 8 | N312C-1 |
| 9 | N312C-2 |
| 10 | I290C/D103H-2 |

Remade D113A and set up PCR, good to take out tomorrow. Performed chloroform extraction for D113C, D113H, R104C/E116C, no pellets seen so leaving overnight. -MH

**2/6/20**

Finished KCNJ9 miniprep drying on counter. Began miniprep of L219C, S118C, P111C. Sent N312C, OPRM1, EPG, KCNJ3, T1122C, and R104C/E116H for sequencing. -TR

Pulled pipettes. Attempted anion substitution experiment for L102C da020620, bubbles showed up halfway through and had to end the experiment early. Put away dishes. Made new I+I -DA

Ran PCR of CFTR R248C. Need someone to retrieve PCR product in the morning. Did steps 5-7 of miniprep and Cassadi will finish the rest. Autoclaved glass bottles with Hannah and they need to be picked up. -LC

Completed hazardous waste training and pipette training. Autoclaved glass bottles with Leah. -HS

Performed electrophysiology experiment ef020620, 50pp, R104C. Updated lab wiki. Worked on lab meeting stuff. Retrieved Leah's PCR (R248C) and put in the PCR rack in freezer A. Put away autoclaved bottles. -EF

**2/7/20**

Got rid of miniprep waste. -TR

Resuspended KCNJ 1 and KNCJ 2. Made a gel. Ran a gel for D113A PCR, R248C PCR, KCNJ1, and KCNJ 2. Gel can be analyzed by 2pm. -LC

Learned how to resuspend KCNJ 1 and KNCJ 2. Learned how to make a gel. Learned how to run a gel. -HS

Pulled channel 1 pipettes, pulled and broke injection pipettes, analysed a gel, and made a video to clean the oocyte incubator (Video needs to be edited). -AP

Read about prime editing and the version of the Cas9 protein that we are working with. I posted a link to the paper explaining this on the google drive under CRISPR Project → Background.

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Talked to Dr. Norimatsu about the status of the CRISPR project and outlined future goals. Began looking into sequencing our eGFP + Cas9 to confirm that our Cas9 sequence in the plasmid is correct. - BS

**2/10/20**

Analyzed sequencing results. EPG, N312C confirmed, KCNJ3 and OPRM1 beginning confirmed. Finished ethanol steps of D113C, D113H, and R104C/E116C. Analyzed 3 electrophys experiments. Linearized EPG, OPRM1, KCNJ3. -TR

Watched video on different methods of Electrophysiology. Followed Purelink Quick PCR Purification protocol for OPRM1 and pGHE blunt ended subcloning PCR product that CK discussed on 02/04/2020. The products are in Freezer B on the orange rack top shelf. -NV

Learned how to sequence DNA from Tina. Diluted Cas+eGFP DNA to 321.47 ng/uL and figured out/added Cas9_end to sequencing list. Will work on designing a primer for the beginning of Cas9 soon. - BS

Passaged two LM45-CD47 plates and one LM45 plate. Pre-plated LM45 cells in order to attempt a transfection tomorrow. -EY

**2/11/20**

Discussed with Norimatsu about the lab and how to go about the CFTR R248C mutant. Ran PCR for R248C (using pfultra) and D363C (using Q5). Needs to be retrieved at around 4:30 pm. -LC

Ran gel for EPG+LIN, EPG, OPRM1+LIN, OPRM1, KCNJ3+LIN, and KCNJ3. Gel can be analyzed by 12:30 pm. - HS

Made D113C, D113H, and R104C/E116C RNA. -TR,

Practiced setting up electrophysiology experiments, including placing and puncturing the oocyte. Helped Erika with Transfection of LM45 cells, unfortunately they had some form of unknown contamination. need to follow up on procedure and possible sources of error- RB.

Attempted to make sodium acetate buffer. Read sequencing results for T1122C. Appears to be primer dimers. Requested to be resequenced and prepped another PCR for it. Needs to be ran. -CF

Looked at cells that were plated yesterday to attempt a transfection today; one well had the same contamination as in the wells that were attempted to transfect last week. We decided to not attempt at transfection to avoid the possible spread of the contamination, and cleaned

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the wells with bleach before disposing of the tray. Looked at transfection protocols to find a better pre-plating protocol. -EY

Did phenol chloroform cleanup of linearized KCNJ3 and OPRM1. Updated Opioid Project lab wiki. Got cell culture media waste out of autoclave and disposed of. -CK

**2/12/20**

Began making RNA for EPG, KCNJ3, OPRM1. Sent DNA for sequencing:

| |
|---|
| I290C/D103H-1 |
| I290C/D103H-2 |
| KCNJ3-4 Blunt_Beg |
| KCNJ3-4 Blunt_End |
| KCNJ3-5 Blunt_Beg |
| KCNJ3-5 Blunt_End |
| KCNJ3-6 Blunt_Beg |
| KCNJ3-6 Blunt_End |
| KCNJ9-1_Beg |
| KCNJ9-1_End |
| KCNJ9-2_Beg |
| KCNJ9-2_End |
| Cas9_end |
| KCNJ3-1_End |
| KCNJ3-2_End |
| OPRM1-1_End |
| OPRM1-2_End |

-TR

Restocked 200uL tips. Initiated Phenol-chloroform extraction for D113C, D113H, R104C/E116H, FPC4, KCNJ3, ODRM1. All of these were left in Freezer A after finishing step 4 of the procedure. -MH

**2/13/20**

Finished RNA phenol-chloroform extraction of D113C, D113H, R104C/E116H, EPG, OPRM1. Updated oocyte health tracking sheet. -TR

Discussed CRISPR with Dr. Norimatsu, cleaned CRISPR cell culture incubator due to issues with Transfection, made a gel for Tina, read about primers. -RB

Make sure to add your initials, time, and date to all entries in case of questions. Highlight tasks that have been started but not completed. Once completed, take the highlight off.

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Ran a gel labeled lc021320.

| |
|---|
| D103C+ LIN |
| D103C |
| N312C+ LIN |
| N312C |
| P111C |
| S118C |
| L219C |
| D363C |
| R248C |

Discussed with Emma and Tina about the lab meeting. Prepared my transformation powerpoint for the meeting. - LC

Worked on lab meeting. Ran PCR of D113h/N312C, needs to be retrieved in the morning. Updated lab wiki progress. -EF

**2/14/20**

Uploaded picture of frog O oocytes. Ran gel with D103C and N312C linearization products on it. Re-linearized N312C and D103C-TR

Analyzed sequencing results of D103H. Updated lab wiki. Learned secondary analysis. Recorded a video on secondary analysis. Dumped FR. Retrieved PCR. -EF

Injected R104C, L102C, and R104C/E116C, worked on chemical list, diluted, labeled, and organized RNA stock and injection solutions. - ACH

Looked up transfection protocols to use as a guide to edit our transfection protocol. -EY

Read about refining our plating for “transfection protocol”, as we’ve been having trouble the past two attempts. The refined protocol will be attempted on monday. - BS

Pulled channel 1 pipettes and attempted electrophysiology experiment on R104C oocyte but did not express. -AP

**2/17/20**

Confirmed ends of KCNJ3 and OPRM1. PCR of pGHE HiFi KCNJ9. Made Gels. Ran gel.

| Lane: | run: tr021720 |
|---|---|
| 1 | Ladder |

Make sure to add your initials, time, and date to all entries in case of questions. Highlight tasks that have been started but not completed. Once completed, take the highlight off.

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| | |
|---|---|
| 2 | D103C+LIN |
| 3 | D103C |
| 4 | N312C+ LIN |
| 5 | N312C |
| 6 | pGHE KCNJ9 HIFI I |
| 7 | pGHE KCNJ9 HIFI L |
| 8 | KCNJ9 HIFI K |
| 9 | KCNJ9 HIFI J |
| 10 | KCNJ9 |

-TR

Plated LM45 cells for transfection tomorrow. Used a slightly modified pre-plating protocol due to overgrowth of cell clumps in the past two attempts. Will update the protocol later if it works. Tina showed me how to analyze the sequencing results. The sequencing of the end of the Cas9 region of the Cas9 + eGFP plasmid was a perfect match. Started looking at designing a primer for the beginning of Cas9.- BS

Froze one Cryotube of LM45-CD47 cells that are currently in the Mr. Frosty in the -80 freezer; tomorrow I will remove the tube and place it in its own bag that will remain in the -80 freezer until we thaw it. Looked into making a trypan blue solution from the powder trypan blue we already have in the lab to help count cells to improve accuracy for transfection. -EY

**2/18/20f**

Learned how to purify PCR products with an invitrogen kit. Purified KCNJ-1 with B2 and B3 from PCR purification kit. Checked with a nanodrop machine and got 0.014 µg/µL, 1.81 A260/A280, 1.86 A260/A230. Checked the KCNJ-1 DNA before purification on nanodrop machine and got 1.152 µg/µL, 2.05 A260/A280, 2.46 A260/A230 -LC and HS

Attempted to purify KCNJ9-1 with genejet. Ran KCNJ9-1 samples on gel (cf021820). -CF

Transfected eGFP and eGFP+Cas9 into LM45 cells. The plated cells looked good, so the I plan to use the slightly altered pre-plating protocol in the future, and will change the protocol on the google drive to reflect it - BS

Transferred the Cryotube of LM45-CD47 cells from the Mr. Frosty into a ziploc bag labeled “LM45-CD47 02/18/20” in the -80 freezer. Autoclaved 1.5L of water for the CO2 incubator in the shared equipment room. Assisted Blake with the transfection. -EY

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Purify KCNJ9-1 analyzed gel. -TR

**2/19/20**

Secondary analysis of anion data. Made gels. Ran KCNJ9 HiFi inserts on a gel.-TR

Phenol Chloroform extraction of N312C + LIN and D103C + LIN; accidentally skipped chloroform step; stopped at EtOH step. Made 90% NaSCN and filtered. -EF

**2/20/20**

Purified KCNJ9-1. PCR of KCNJ9 insert. Analyzed lc022020 gel. -TR

Surgery on Frog P, oocytes placed on rocker at ~12:00 PM -DA

Pulled channel 1 pipettes. Researched solutions for PCR of R248C and looked at the plasmid and the primers. Ran a gel labeled lc022020.

| Lane: | run: lc022020 |
|---|---|
| 1 | Ladder |
| 2 | KCNJ9-1P |
| 3 | KCNJ9-1P HiFi PCR |
| 4 | pGHE blunt pur |
| 5 | OPRM1 blunt pur |
| 6 | D113A (D113H) PCR |
| 7 | D113A (D113C) PCR |
| 8 | D113A (WT) PCR |
| 9 | CFTR (WT) |
| 10 | KCNJ9-1 HiFi PCR |

Autoclaved MBSH. Retrieved the bottles and cleaned them. Made 2 gels. Learned how to make Frogringer. Cleaned dishes and put some away. Retrieved KCNJ 9-1 PCR product. -LC

Made 3M sodium acetate buffer. Made 16L FR solution in middle jug. The pH was taken at 7.29, but the pH probe should be recalibrated and then checked again. The osmolarity also needs to be run. Instructions for both can be found at G:\Physiology Lab\Norimatsu Lab\Lab Protocols\Housekeeping\Videos - NV

Purified T1122C. Needs to be ran on a gel. Calibrated pH meter and confirmed pH of 3M Sodium acetate buffer and FR. -CF

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Analyzed sequencing results. Made .5X TBE. Put gel in fridge. Measured osmolarity of FR (203 mmol/kg). Retrieved LB from autoclave and added Amp; poured plates. -EF

**2/21/20**

PCR for KNCJ9 HiFi. Ordered molecular linker for R104C/E116C. -TR

Worked on lab meeting stuff. Taught Justin secondary analysis. Talked to Norimatsu. -EF

Learned how to do secondary analysis from Emma. Secondary Analysis completed on all wtCFTR “good” electrophysiology experiments.-JB

Observed transfected cells of eGFP and Cas9 transfections. The eGFP transfected cells were fluorescing. On monday, I will start working on a page detailing what we have/haven’t accomplished with the CRISPR project so far and what protocols were used. Talked to Dr. Norimatsu about the status of the project - BS

Observed the transfected cells from Tuesday with Blake. Autoclaved the waste from the transfection. -EY

Performed PCR on A107C, R248C, D363C, A120C, G103C, K114C, L219C, and R104C/E116C (can someone retrieve these from the thermocycler), performed phosphorylation and ligation of vector and insert, and researched RNA and gels. -AP

Injected 0.01% L102C, 0.1% R104C/E116C, 0.01% R104C/E116C, and R104C/OPRM, relabelled chemicals in Cabinet A, updated Chemical List spreadsheet. - ACH

**2/24/20**

Ran a gel. Spun KCNJ9 bacterial pellet. Disposed of bacterial waste and old LB plates. Put Away dishes.Attempted electrophys experiment but channel 1 pipettes were the wrong size. DpnI digested L219C, D363C, K114C, G103C, A120C, R104C/E116H, and D113A please retrieve from Ingrid in the morning, and circularize.  Pur T1122C PCR product placed in green rack freezer A. pGHE OPRM1 Blunt placed in green rack freezer A. Updated Lab wiki to-do lists. -TR

| Lane: | run: tr022420 |
|---|---|
| 1 | Ladder |
| 2 | R248C PCR |
| 3 | L219C PCR |
| 4 | D363C PCR |
| 5 | K114C PCR |

Make sure to add your initials, time, and date to all entries in case of questions. Highlight tasks that have been started but not completed. Once completed, take the highlight off.

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| | |
|---|---|
| 6 | A107C PCR |
| 7 | G103C PCR |
| 8 | A120C PCR |
| 9 | R104C/E116H PCR |
| 10 | T1122C Pur PCR |

Passaged LM45 and LM45-CD47 cells into one plate each. Began looking into thiol-reactive fluorophores to use if we ultimately create a mutant CFTR and a new Lipofectamine reagent. -EY

**2/25/20**

Autoclaved bottles. Retrieved DpnI digested L219C, D363C, K114C, G103C, A120C, R104C/E116H, and D113A from Ingrid (Incubator). Started circularization for L219C, D363C, K114C, G103C, A120C, R104C/E116H, and D113A. Retrieve L219C, D363C, K114C, G103C, A120C, R104C/E116H, and D113A from incubator and ligate (second half of circularization). Retrieved DI water. Updated Lab Wiki for R248C/D363C. -LC

Autoclaved bottles. Picked up bottles from the autoclave and cleaned them. Made a new gel and left in fridge A. Learned how to circularize DNA. Retrieved more DI water. - HS

Researched thiol-reactive fluorophores for the new direction of the CRISPR project. Researched how to make a primer for the BRAF (`GRCh38`) in melanoma cells. Cleaned up around the workspace. -RB

Began transformation of A107C, Y109C, L333C, and D103C. CK is plating. Moved Lucas's plasmids to the -80 freezer in the Opioid Box. -CF

Plated A107C, Y109C, L333C, and D103C for CF. Need to be taken out of Ingrid in the morning. Ligated A120C, D113A, D363C, R104C/E116H, G103C, K114C, and L219C and placed in box 14. -CK

Injected oocytes (old ones look infected); these seemed very soft and not great but are definitely healthier than the ones that are already injected. Made video about how to use new light at electrophys set up #1. -EF

**2/27/20**

Changed the pre-plating transfection protocol in the on both the google drive and google site to reflect the new protocol I used last week. I moved and labeled the old pre-plating protocol to the bottom of the document in case it is needed for reference. Started adding

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Deep-freezing protocol to the google site under CRISPR project protocols. Talked with Ryan about the state of the project. - BS

Analyzed expression test of .1% R104C/E116C, da022720. Created new folder in CFTR called Electrophysiology Data_Open, and da022720 is located there. Pulled pipettes-CF

Attempted .01% R104C/E116C, .5% BAR, but expression was too low. Cleaned lab. Printed out chemical list and posted on bulletin board. Worked on lab meeting stuff. -EF

Transformed A120C, D113A, D363C, R104C/Ell6H, G103C, K114C, L219C and left in the incubator (Ingrid). Resuspended KCNJ9 + pGHE. Ran a gel and analyzed it under lc022720. -LC

Mini-prepped KCNJ9_pGHE 2. -CK

Attempted .1% R104C/E116C, .5%  BAR Anion sub w/ MTSET but ran into blocks and could not get them resolved. Stopped after 10uM MTSET solution. Wiped down electrophysiology machine and data computer-JB

Continued adding to the CRISPR page on the wiki. Updated all protocols to be consistent with the google drive. Finished adding the Deep Freezing of Cells protocol, and uploaded the Unthawing cells protocol. Added/organized links to respective protocols/pages on the CRISPR page on the wiki. Began editing and updating the Background page for the CRISPR project, but will resume later. Created a page to display what has been accomplished with the CRISPR project so far, but will add information to it/finish it next week. Read about guide RNA, the Harvard Cas9 enzyme, and B-Raf inhibitors. - BS

**2/28/20**

Injected 50 ppm R104C, 0.01% L102C, R104C + OPRM1, 0.1% R104C/E116C, and 0.01% R104C/E116C, provided training on oocyte injection. - ACH

**3/2/20**

Moved all plates in the incubator to Fridge B:  A107C, Y109C, L333C, and D103C, and A120C, D113A, D363C, R104C/Ell6H, G103C, K114C, L219C. Disposed of bacterial waste. Electrophysiology R104C, ET mod. -TR

Passaged LM45 and LM45-CD47 cells each into one plate. Read primary lit articles about vemurafenib and melanoma cells. -EY

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**3/3/20**

Put away dishes. Updated mutant lab wikis and others. Set up miniprep for Y109C, L333C, D103C, and K114C. A107C failed transformation so no miniprep will be performed. The set up Falcon tubes are in fridge A top shelf. Pulled channel 1 pipettes. -LC

Put away dishes. Set up miniprep for Y109C, L333C, D103C, and K114C. The set up Falcon tubes are in fridge A top shelf. Someone needs to run a miniprep for Y109C, L333C, D103C, and K114C this afternoon. Pulled channel 1 pipettes. - HS

Analyzed Electrophysiology Data for SCN with MTSETof R104C. This is in the unprocessed data. Started analysis of Anion Sub with Mod of R104C with MTSET. Experienced issues with the program, will try again next time. Trained new lab member with making a gel, navigating the google drive, etc -RB

Received the Cas9 DNA ordered from DNAsu (as well as CRN1 for the opioid project). Placed both types of DNA in separate labeled bag in the -80 Freezer. I will begin plating the Cas9 containing bacteria the week I return from spring break. Mostly finished updating the "Current State of the CRISPR Project" page on the lab wiki under CRISPR project. I tried to put what we have done so far in regards to cell culture/transfections, and what our current goals are. I also started an inventory list for the project on this page. Inoculated Y333C, K114C, Y109C, and D103C colonies. - BS

Electrophys experiments.-TR

**3/4/20**

Electrophysiology experiment. Spun bacterial pellets, L333C, K114C, Y109C. Made 90% NaI, 90% NaSCN, ran gel:

| Lane: | run: tr030420 |
|---|---|
| 1 | Ladder |
| 2 | OPRM1+ pGHE |
| 3 | KCNJ9-1 |
| 4 | KCNJ9-2 |

-TR

**3/5/20**

Helped prep for frog surgery and oocyte digestion. Spun bacterial pellets for D113A, L219C, D363C, A120C. Sorted oocytes. Filtered 1X DS can someone check osm and pH? -TR.

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Completed surgery on Frog R, washed surgery tools, replaced CLS on 7th floor and brought down empty bottles, filled a few of those bottles. -DA

Performed an anion substitution (L102C) unmodified electrophysiology experiment using 0.01% L102C, 0.5% BAR, 2-28 AH, sorted on 3-4. Found in folder 03/05/20 under ww030520. Experiment still needs to be analyzed.

Completed steps 1-7 of miniprep of K114C, Y109C, L333C, A120C, D113A, D363C, and L219C. Left in red rack labeled mutant +c (for chloroform). The tubes they need to be transformed to in step 8 are in the rack labeled as just the mutant. Please complete miniprep. -LC

Completed LC's miniprep through 100% ethanol step. Waiting for pellet to form.

Attempted electrophysiology experiments but oocytes were not healthy enough. Talked with Norimatsu and LCI and manuscripts. -EF

**3/6/20**

Spun miniprep K114C, Y109C, L333C, D113A, D363C, and L219C. No pellet formation. -TR
Pulled channel 1 pipettes

Attempted electrophysiology experiments, but oocytes were not good enough. Made a gel and prepared samples. Tina will run the gel on Monday.- EF

Pulled channel 1 pipettes, pulled/broke injection pipettes, autoclaved frog surgery tools, filtered frog ringer+IBMX, practiced impaling. -AP

Injected 50 ppm R104C, 0.01% L102C, and OPRM1 and made new MBSH 1x and DS 1x. DS 1x made 3/6 in Fridge A needs to be checked for osmolarity, both MBSH 1x and DS 1x made 3/6 need to be checked for pH. - ACH

**3/9/20**

Put away dishes. Checked pH of MBSH. Electrophysiology experiment checking NaSCN and NaI solutions. Pulled channel 1 pipettes, ran a gel-.

| |
|---|
| Ladder |
| N312C/D113H PCR |
| KCNJ9-10 HiFi PCR |
| L219C PCR |
| P111C PCR |

Make sure to add your initials, time, and date to all entries in case of questions. Highlight tasks that have been started but not completed. Once completed, take the highlight off.

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| |
|---|
| R248C PCR |
| D363C PCR |
| KCNJ9 PCR |

- TR

**3/12/20**

Frog surgery. Prepped samples for sequencing, add primers tomorrow.

| |
|---|
| I290C/D103H-1 |
| I290C/D103H-2 |
| KCNJ9-1_ beg |
| KCNJ9-1_ end |
| KCNJ9-2_beg |
| KCNJ9-2_end |
| L219C |
| P111C |
| S118C |
| CRN1_Beg |

-TR

**3/13/20**

Sent samples for sequencing. Injected RNA, R104C, L102C, OPRM1+R104C. -TR

| Sample number | DNA_sample |
|---|---|
| 1 | KCNJ9-1_ beg |
| 2 | KCNJ9-1_ end |
| 3 | KCNJ9-2_beg |
| 4 | KCNJ9-2_end |
| 5 | I290C/D103H-1 |
| 6 | I290C/D103H-2 |
| 7 | P111C |
| 8 | L219C |
| 9 | S118C |
| 10 | CRN1_Beg |
| 11 | ADRBK1_Beg |
| 12 | ADRBK1_End |

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| | |
|---|---|
| 13 | ARRB2_Beg |
| 14 | ARRB2_End |

**3/16/20**

Created a Zoom account and scheduled a meeting with Tina. Took the LCI and recorded my results in the Slack thread. Read about the Learning Connections Inventory. Watched the video over optimising communication. -LC

Met with Tina via Zoom, took LCI and recorded results, read provided article and video on LCI and communication, watched video on COVID-19, installed and activated PyMOL. -ACH

**3/17/20**

Uploaded sequencing results for 3/13/20 to Google Drive. -TR

Worked on LCI stuff. Performed and analyzed electrophysiology experiment, .02% L102C .5%BAR. There seems to be something wrong with the oocyte or I+I, will troubleshoot tomorrow. Started order for autoclave tape and 600mL cups. -EF

Got access to Google Sites and created an updated, more detailed lab wiki entry for oocyte injection protocol. - ACH

**3/23/20**

Read COVID_19 information posted on slack. Enjoyed history of Influenza A. Completed RET 2020. Updated current experiment count. -TR

**3/26/20**

Updated tables on COVID-19 page of the wiki. Can someone double check that the entries are correct? I used the paper in Science to record important amino acid pairings. Used BLAST to align sequences of 6ACG(Trypsin-cleaves and low-pH treated SARS-CoV spike glycoprotein and ACE2 complex, ACE2-bound conformation) vs 6M17 (2019-nCoV RBD/ACE2-B0AT1); results found here: https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_Query_58273 , https://www.ncbi.nlm.nih.gov/projects/sviewer/?RID=7U80XZ7D114&id=lcl|Query_58273&tracks=[key:sequence_track,name:Sequence,display_name:Sequence,id:STD1,category:Sequence,annots:Sequence,ShowLabel:true][key:gene_model_track,CDSProductFeats:false][key:alignment_track,name:other%20alignments,annots:NG%20Alignments|Refseq%20Alignments|Gnomon%20Alignments|Unnamed,shown:false]&v=432:472&appname=ncbiblast&link_loc=fromHSP. Can someone look over this to make sure it is correct? -EF

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**3/27/20**

Completed RET. Attended Zoom call. Worked with Pymol to continue filling out tables. -EF

**4/1/20**

Continued working on side chain interaction tables on lab wiki. -EF

Renumbered Decoy1 labeled “In.pdb” in google drive folder “decoy1”.
https://drive.google.com/drive/u/1/folders/18QWvx4LG57y1miwVzEHQ6B4-3tJ3OLJp - LC

**4/2/20**

Continued working on side chain interaction tables on lab wiki. Started coordinating with Leah, Matthew, Alec, and Jianxiu. May have a zoom meeting tomorrow if anyone wants to join. -EF

**4/3/20**

Added the viral RBD amino acids to the alignment file labeled “complex1.ali” in google drive folder “complex1”.
https://drive.google.com/open?id=1mcsrsdvba17YbwjwoOjrdiiuZDDoOeyc -LC

**8/17/20**

Started adding more documents to the CRISPR project (cell culture) on the lab wiki. Updated the CRISPR/Cell Culture powerpoint from last fall to share with new students about the project. - BS

Continued to edit/add to the CRISPR project/cell culture section on the lab wiki. Added a sterile technique page with corresponding youtube video, pre-plating for transfection page, and edited the transfecting/pre-plating for transfection pages. Will need to go in person to test edited protocols at some point. I plan to also film videos for these protocols in the future as well. - BS