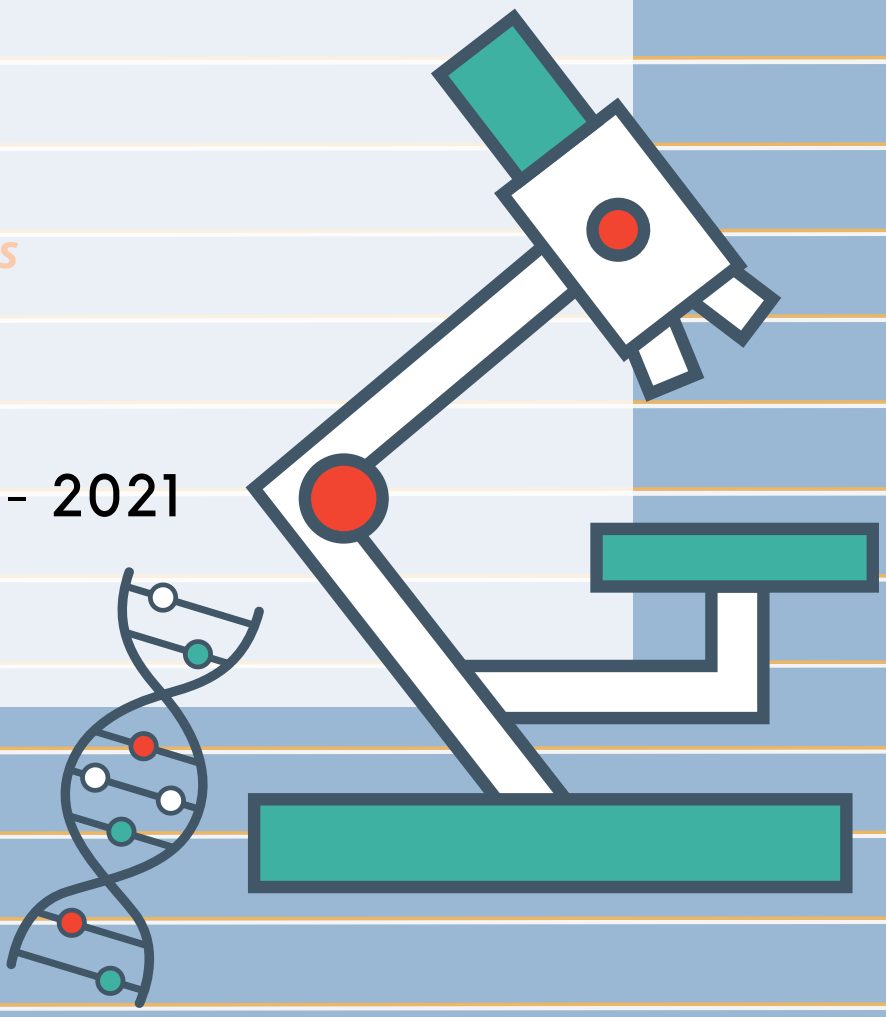
Genetics

Meeting Minutes

2021 APRIL - 2021 NOVEMBER



Genetics Meeting Minutes 2021

10/10/2021

- HARV experiments
    - Start Tuesday
        - Run SOD1 in HARV
        - Run same volume in petridish on side
        - protocol for clinosat, run this too
        - Clinostat testing protocol
        - Add cells with OD of 1.0, dilute 2 fold in media, run for like 5 hours
- Plan for the week
    - Tuesday - Autoclave tubes, start overnight cultures for limonene, and all the stressors - Amir, Paul films video
    - Wednesday - Start clinostat experiments try 5 hours - Brian and maybe Nat
    - Thursday - Try again in case it goes bad or do HARV
    - Friday - Try again in case it goes bad or do HARV

10/03/2021

- Clinostat and HARV work
    - Paul had the idea of petri dishes for HARV vessels (sterile)
- Limonene - transformation yesterday and ready to pick
- Tasks for the week
    - All week: **gravity stuff**
    - Sunday - picking colonies (2 tubes with 5 mL of LB media and add 5 uL of ampicillin) and maybe starting HARV and clinostat stuff (check with Paul first)
    - Monday - Miniprep plasmids
    - Tuesday - Transformation of yeast (get protocol running)
    - Wednesday - Do whatever
    - Thursday - Pick colonies
    - Friday - Make glycerol stocks

09/26/2021

- Strain selection
    - SOD1 ia the only one to be differently regulated in all conditions so we'll use it for the HARV
    - Overnight in HARV then check in flow (Thursday to Friday) (only add like 10 mL)
        - In wiki, do no explicitly say it's from microgravity stress because of other factors
    - Limonene primers came in
        - Monday: pcr of limonene (Paula) PCR cleanup of what Paul did for sequencing
        - Tuesday: restriction digest (3 hours) and ligation of limonene (overnight) ()
        - Wednesday: QC of ligation (3 hours) ()
        - Thursday: HARV stuff and transformation ()
        - Friday: Makeup day
- Wiki stuff

    - Editing

09/20/2021

- To do list for the week
    - Redo ethanol 20%
    - Order primer (they'll come in wednesday or thursday)
    - Build construct
    - Send strains out for sequencing
    - Wiki
- Second meeting this week on Tuesday for Wiki content at 6:30
    - Genetics wiki content doc

09/13/2021

- Data from flow looks clean (we're almost done stress)
    - Can start concluding which strains to focus on

09/06/2021

Stress Experiments

- Salt Stressors, Trials BTN2, SOD1 SOD2 is the more consistent in the salt stressors, but the fold change is mostly one which means that there is no change, meaning that they are barely upregulated. The values are also not consistent and this is concerning
- Amir's suggestion on stress experiments, using data points on one strain in one condition and not averaging values.
- The T-test is the best way to analyze the data.
- Heat experiments, very inconsistent.

08/23/2021

- Dry lab
    - Annotated the plasmid
    - Will be finishing the primers soon
    - Inserting two DNA comes in in particular
    - Small terminators added with primers, avoids two inserts
- Wiki
    - Generally not really started
    - Reminder of deadlines (see below)
    - Can use lab notebook, but take out links and names
- Wet lab
    - Try multiple experimental conditions for a system that has a known response and get more replicates that way
    - Grubbs test for one set of data

- Put HSP in the HARV
    - After a week or two ask a mentor to help us access a better microscope
    - Culture from Hsp from frozen stock and compare the budding of the two

- Team 1 - Stress data
    - Everyone clean up data split up
    - Find out stressors for our strains? Or no
    - Aim for more replicates in designing the protocol

- Team 2 - Microgravity Protocol development
    - Culturing times based on growth curve
    - Getting peoples set schedule set up

08/16/2021

- Dry lab
    - pYES2 vector in plasmid
        - Almost done
- Wiki
    - Content deadline
        - September 7th
    - Internal deadline October 1st
    - Assignments: on wiki doc
- Wet lab
    - All stressors were done once
        - Tuesday ethanol
        - Wednesday ethanol
        - Thursday peroxide - still put in incubator for consistency
        - Friday salt
    - Incorporate error bars into all graphs
    - Start graphing into Excel
    - Start graphing OD too (second y axis)
    - Heat stress
        - 3 temperatures: 42, 37, 30
        - Shaking for all of them
        - Plates in different temperature
    - Clean plates better to avoid crusty plates

- Tasks
    - Everyone
        - Wiki content: September 7th
        - Do some background research on stuff we do
    - Dry lab
        - Finish up with construct
        - Design primers
    - Wet lab
        - Testing heat
        - Repeating experiments
        - Play with HARV
        - Clinostat coming soon
- Next monday
    - Team for data, cleanup and error bars and stuff
    - Team goes over what's expected with ystrex
    - Team for clinostat and harv protocol

08/09/2021

-Growth curve

-Our Sc is no good

-Our media same as Mo's just with lower concentration of amino acids, must have been our YPD was prepared poorly

-Won't need SC with new stress protocol

-Some of our YPD is good others grew less, might be due to prep of it

-Stress experiments

-"Stress data analysis protocol" is to be followed for analyzing the data

-In the stress experiment don't fill in template but follow the template

-Put in standard deviations with error bars when analyzing the data to see outliers

-Change plate layout so each plate has each strain once and then do 3 plates for the triplicates as plates can be the source for error

-Do data analysis directly on google sheets

-**Dry lab**

-Mo Doesn't know about an entry vector

-Option from addgene but takes too long to order

-Maybe instead of vector we have FF sequences for homology for entry but also have the adapters (cut sites used in biobrick standard)

-Mo has pYES2 vector which is a standard vector for yeast

-Part: BBa_K801060 that produces limonene from yeast can be bought off IGEM and used

-Limonene is partially gaseous

-Other

-Brian will make the parts for sequencing, message if you want to help.

08/02/2021

-Culturing

-7/9 with arc and oxr

-Saf 1st restreak

-Cut SLT2

-Stressors

-Purpose is to see if we can get strong signal from reporter

-Assumption is that there will not be external stressors not introduced by microgravity

-Doubling time 3 hours

-Triplicates with measurements

-The blank has some YPD dissolved in it

-Measure time before incubation

-Write what time we started the incubations at

-Use flow cytometer for data perhaps

-Should still have replicates

-Growth curves to find the right time to read stuff at

-Want to read it past lag phase and before death phase

-Wiki

-Assigning content to wiki: wiki content doc

Questions for hardware

Convection temperature of clinostat

Clinostat troubleshooting, including vibrations

Random stuff

Phone pictures of yeast with oil and adaptor thing for budding

Throw HSP in the HARV for now and get budding pictures and fluorescence
Talk to mo about sequencing
Build plasmid for limonene
Gal10 promoter
Yeast backbone from the igem kit (preferrably integrative)
Limonene
` Selection marker
Primer design
Get this done quick
For next week

07/26/2021
-Simulator
-Noisy and hot
-Stress experiment
-Our reporter is working
-CSA report
-Found mass from one culture
-Redoing experiment to get better idea
-Nutritional output
-Progress
-OXR and ARC 1st restreak successful
-Wiki stuff
-Plan out outline for wiki
-Things to buy
-oil from amazon

07/19/2021
-Simulator
-We have a space for it
-Start testing it for a while soon
-Run it for a full lab shift while having people keep and eye on it
-CSA report
-Grow yeast in 500 mL of YPD
-2 days should be enough

-Innoculate overnight culture then add it to the 500 mL and let grow two days

-Take OD readings in the mornings and other times

-Centrifuge down and weigh yeast

-HSP30 frozen

-RAV2 last restreak before overnight culture

-Start troubleshooting stress

-YPD is fluorescent which the YNB media

-Dry lab

-Sent email to HQ about part

-Looking at improving existing part

-Tuesday

- [x] ~~-Colony PCR, SAF and RAV (in hot room)~~
- [x] ~~-Maybe liquid culture for RAV~~
- [x] ~~-Stress protocol for BTN2~~
  - [x] ~~-Dilute it in YNB~~
  - [x] ~~-On plate~~
  - [x] ~~-Just to see if it grows properly~~
- [ ] -Running clinostat
- [x] ~~-Overnight culture of wild type for hardware and control for stress, 5 mL~~
- [x] ~~-Overnight culture of SOD1~~
- [x] ~~-Overnight culture of BTN2~~
  - [x] ~~-Overnight culture~~
  - [x] ~~-100 uL of frozen stock in 5 mL of YNB~~

-Wednesday

- [x] ~~-Colony PCR SAF2~~
- [ ] -Colony PCR SLT2, ARC19, OXR1
- [ ] -Stress protocol
  - [ ] -5% ethanol
  - [ ] -WT, SOD1, BTN2
- [x] ~~-Innoculate 500 mL for hardware~~
  - [x] ~~-make YPD media in the waste flask and autoclave them~~
  - [x] ~~-Add the overnight culture~~
- [ ] -Overnight culture (missing amino acids)

- [ ] -WT, SOD1, BTN2

-Thursday

- [ ] -Colony PCR
- [ ] -Stress experiments
- [ ] -OD measurements of the flask for hardware
  - [ ] -Remove a few mL with serological pipette
  - [ ] -Take OD measurements (morning and afternoon, write in notebook)
- [ ] -Make overnight culture of SOD1, BTN2, WT

-Friday

-Colony PCR

-Harvest yeast for hardware

-Stress protocols

-Saturday

-Colony PCR

-Harvest yeast for hardware

-Stress protocols

-Sunday

-Colony PCR

-Harvest yeast for hardware

-Stress protocols

07/12/2021

-5 strains working

-RAV2 and SOD2 in progress

-BTN2, SOD1, HSP30 worked

-GAL10 dropped

-Colony PCR with phire works well

-Stress experiments

-Heat, acid, salt, oxidative stress, pH, ethanol

-Look at pH changes for hardware

-Innoculate a 1 L culture of YPD

-Yeast grows faster at lower pH (around 4)

-Simulator stress tests

-Tuesday

-Brian and L

-Colony PCR

-No Gal 10, HSP30 redo everything else
-BTN2 liquid colony from frozen stocks

-Wednesday
-BTN2 ethanol experiments
-Colony PCR on HSP30 and anything else that needs it

-Thursday
-Colony PCR again
-SOD expression tests - hydrogen peroxide

-Friday
-Colony PCR
-HSP30 with heat (50 degrees)

-Saturday
-Colony PCR
-HSP30 with salt

-Sunday
-Colony PCR
-HSP30 acid

-Do we want to retransform the stuff that isn't working or move on?

-Dry lab
-Talk to HQ about part stuff
-Improving on an existing part

07/03/2021

-GFP signals get stronger in the fridge

-New members in the lab selected soon

-New meeting time

-Theory session tomorrow at 11
-Go over some successful experiments from papers

-Clean up boxes

06/26/2021

-Transformations works but colony pcr failing

-Not whole colony, just touch tip - very small amount

-Troubleshoot with colony
-50 to 65 gradient, 4 replicates of any of the 2nd restreak colonies

-Do control duplicate with WT

-Genome shuffling

06/19/2021

-Meeting for just lab people (new time)

-When to meet

-Make sure next day has enough to work with

-Throw out stuff that doesn't work

-Better note taking

-Throw out stuff if doesn't work

-Much more explicit about labelling

-Restreak into different segments of gel

-We have yeast

-Colony PCR side to side with Mo's

-Genomic DNA control

-Simulator in soon

-More lab people (from the team, not recruitment)

-Two days a week (12 hours ish)

-Availability and skill

-Dry lab

- proof of concept research continued

06/12/2021

-Wet lab

- Transformations done
- Colony PCR to be done monday
  - Mo has a procedure
- Set up earlier plans and timelines
  - Monday get a plan of experiments
  - Potentially split shifts
- Wear Proper lab attire! There have been warnings

-Dry lab

- Met with mentor, will prepare some candidate plasmids
- POC should be as simple and ready to implement as possible

06/05/2021

-Wet lab

-PCR is done for everything but Saf1

-Transformation

-Takes about 4-5 hours (not constant work)

-Do PCRs and cutting of the plasmid at this time

-Do one as a way to troubleshoot

-25 - 150 uL transformation

-Do colony PCR and troubleshoot it too

-Start thinking about parts

-By next meeting: get the colonies present

-Figure out mutation experiments

- Dry lab

-Taking parts and putting into database

-Define what a part is in the first place

05/29/2021

-Progress

-Wet lab

-PCR pretty much done (troubleshooting terminator)

-Next week: transformation and selection

-Dry lab

- Proof of Concept Constructs
  - Vit A
  - Limonene, geraniol
  - Get sequences off of addgene and make using twist
- Deadline: July 3rd - Will look over and finalize before buying, and get primer ready (transform into e.coli and then miniprep for yeast)
  - Beta carotene - addgene one and toulouse one for twist hopefully
  - Evelyn, Samman
- Media
  - Different vitamins, pathways, nutrients

05/22/2021

-Progress
    -Wet lab
        -Meeting timeline
        -Simulators coming into the lab
        -Next week: PCR and insertion of genes
        -Put in times for the schedule
    -Dry lab
        -Get collaboration with other team
        -A team that is working on space microbes or yeast biomanufacturing
-Felipe's project
    -What do we want to know before voting?
        -We have the priority
        -Anything damaged they will be responsible
-Reflection on first week of lab work
    -Labeling
        -Put label in doc
        -Sticker with labels in lab
    -Evernote
        -Using that now
        -Check in on using oneNote
        -Look into other note services

-Promotional video

05/15/2021

-Proof of concept
    -Objectives
        -Create a small molecule proof of concept by making a molecule of interest
        -Create necessary gene circuitry (see reporter circuitry for inspiration)
        -Focus on one or two for iGEM and if we pass phase 1 of CSA, then we go ham
    -Ideas
        -Simple vitamins
        -Flavour molecules
        -Basic hormones (see constraints)
        - Look at pre-existing plasmids

-Constraints
- Must be a gene insertion (we're proving that our chassis works) (or plasmid)
- Minimal insertions (2 to 3 max) (keep in mind monocistronic)
- Maximize use of native pathways
- Don't worry about it being novel, our chassis is the focus

-Timeline
-End of June - Proposed pathways
-Target, genes, how to insert (CRISPR vs plasmid)
-Middle of July - Final transcriptional unit
-Benchling

-Wet lab plans
-Flowchart/roadmap
-Scheduling
-First few weeks fill in availabilities until we get settled
-Experiments for week 1 and week 2
-Plasmid purification and QC
-Amplification of circuit parts
-Testing experiments - maybe look at budding
- Announcements
-Keycards: Get them at the front desk before 2:30
-Access: Come in if you've done your trainings
-Trainings: In contact with Tina for autoclave training
- Will talk to Dr. Brett about GFP collection (might not use)
-Look into how his library is set up

-Random stuff
-Ask Mo about BsaI on Benchling
-While on Benchling, go over virtual digest

-Hardware
What criteria, specifications and design elements are important for a yeast bioreactor (*S. cerevisiae*) in microgravity?
-Aerobic conditions
-Glucose and other inputs
-Predict output based on similar strains
-pH (4.5), temperature (22-36)

-Agitation speed
-Airflow rate
-Fed batch vs continuous vs the other one
-Toxic byproducts
-Strains **BY4741** and CEN.P.K
-Answer when2meet

05/01/2021

-Schedule document
-Report document
-New meeting time

04/24/2021

-Gantt chart for schedule
-Transcriptomics maybe (look into feasibility)

******Thank you video for Dr. Kharma******

Notes

- SMG testing
    - Source GFP yeast cells.
- Get benchtop cooler:

04/10/2021
-Discussion of protocol comments
-Possible 30 degree room to grow stuff in
-Possible media for fluorescence measurements: YNB, YNB and amino acids (SC)(grow but transfer them into this for measurements
-Finishing ordering with Orly

04/03/2021
-Lab access: Brian, L, Nhi, Natasha, Paula, Gabriel
-Dry lab project: Proof of concept - lycopene and beta carotene or other stuff

-Due middle of July

-Two phase project: Phase 1 May to July - Microgravity resistance

Phase 2 - Proof of concept integration

-Ask mentors about trainings

03/27/2021

-Leadership

-In-person meeting

-Ordering

03/27/2021

Present: Gabe, Brian, Nhi, Amir, Evelyn, Natasha, L

Absent: Paula, Samman

-Leadership

- -Potential Co-leads: Brian

-In-person meeting

- -Picnic when the weather gets nicer

-Ordering

- -

-Training

- -Biosafety
- -WHMIS for laboratory personnel - 2015 and 1988 in the same session (Prereq for the others)
- -Hazardous waste disposal

-Proof of concept and plan B

- -Proof of concept: should be the insertion of a gene
- -Consistency in space of our strain (vitamin D)
- -Selling point
- -Cholesterol as a precursor to steroids (uptake and increased metabolism)
- -Lysine production
- -Check Concordia (Dr. Martin possibly)
- -Is the metabolite production affected in space? Is it altered and a change needed?
- - fertilizer, nutritional yeast, vanillin (aspirin)
- - co-culture
- -Hairloss potentially?

-Plan B
-Generic stress reporter
-might have to move away from space (heat, acid, salt resistance etc.)

- Contact Dr. Nislow, Dr. Brett (GFP)
- Standardization of the Simulators in parallel with Bacterial Work