Lab

Meeting Minutes

2021 APRIL - 2021 NOVEMBER

Oct. 17
Paul

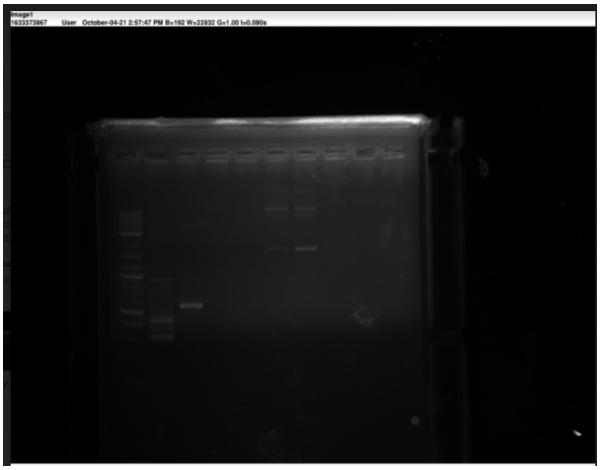
- Microgravity experiment Protocol: Clinostat testing protocol
    - Modifications: used black 96 well plate, filled 29uL liquid each well.
    - Stacked 2 plates on top of each other, with two yellow small rubber bands stuffed in the middle to assist air flow, then taped two plates together using green masking tape.
    - Control plates are marked with a black sharpie marker. You might see black dots on A1, A12, H1 or H12 (corners with no cells in them.)
    - Plate loading time: 4:00 pm
    - Clinostat starting time: 4:30pm
- Plate map: on Stress sheet 11. HSP30 and WT positions are ALREADY swapped in this plate map. The first column is HSP.

October 5th
Gabe

- Transformations
    - Protocol modifications
        - Added 1 uL of miniprepped DNA to yeast cells
    - Diluted yeast 20 fold as per protocol and began incubation at 8:25 am
    - Cells grew for 4 hours
    - Prepared 4 replicates of competent cells
        - Two transformed with Tube A of the DNA
        - Two transformed with Tube B of the DNA

October 4th 2021
Amir and Gabe

- Miniprepped 2.5 mL of overnight culture (1 prep per culture)
    - Tube A: 126.6 ng/uL, 260/280=1.87
    - Tube B: 146.3 ng/uL, 260/280=1.91
- Performed colony PCR on miniprep to determine if gene present
    - Used the following protocol: colony PCR
    - Used same primers as to amplify limonene in the first place
        - Modifications:
            - starting at step 6 because e.coli get lysed in the thermocycler
            - Poked colony and added to 20 uL of nuclease free water, then took 1 uL of that and added it to PCR mixture
            - 1 uL of minipreps used for PCR
            - Elongation step 30 seconds

- 
    - Ladder, random colony from 1, another random colony from 1, random colony from 2, another random colony from 2, miniprep A, miniprep B
  - Fragment size is 1.7 kb, can confirm that it's present in both tubes A and B

October 3rd 2021
Brian

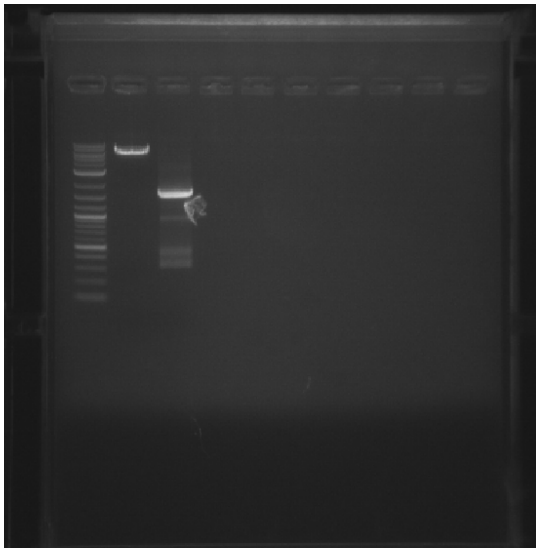
- Prepared overnight cultures of pYES2 plasmid + limonene synthase, 1 per plate

October 2nd 2021
Gabe and Natasha

- Transformation of pYES2 plasmid + limonene synthase into DH5alpha *E. coli*
  - Protocol: Bacterial transformation
    - Added 1 uL of DNA
    - Heat shock 45 seconds
  - Plates added to incubator at 8:25 pm, will be ready to pick after 12:30 pm tomorrow

October 1st 2021
Brian

- Performed restriction enzyme digest to insert the limonene synthase gene into the pYES2 plasmid
- Ran a gel to confirm



Insert not clean but decided best to catch it in colony pcr, unlikely integration anyways

- Performed PCR Purification
    - Vector DNA concentration = 55.7 ng/microL ratio = 1.98
    - Insert DNA concentration = 128.3 ng/microL ratio = 1.9
- Did ligase reaction

7 microL vector DNA = 389.9
- Need 78.18ng per 55.7ng vector DNA so 547.26ng

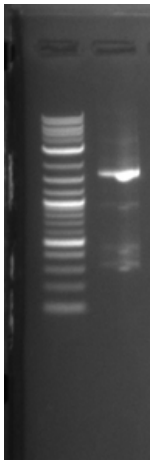
547.26ng / 128.3ng/microL = 4.26 microL
Total DNA = 937.15ng

5microL vector DNA = 278.5ng
- 78.18 x 5 = 390.9

390.9 / 128.18 = 3.04 microL
Total DNA = 669.4ng

September 28th 2021
Gabe

- PCR reaction for limonene synthase gene
    - 10 tubes og 50 uL reaction mixture (500 uL total)
- PCR purification
    - Ran pcr purification after pooling samples
    - Concentrated DNA into 40 uL
    - Yielded 261.6 ng/uL with 260/280 of 1.84 (good purity)

 Gel for the limonene synthase gene from the PCR reaction. Right size but some impurities.

September 27th 2021
Gabe and Paula

- Prepared primers for (l)-limonene synthase
    - FWD is labelled 18
    - REV is labelled 19
- Prepared PCR reaction for (l)-limonene synthase
    - Used protocols for Q5 reactions with annealing temp of 66 degrees Celsius

September 24th 2021
Gabe

- Stores pYES2 e. Coli as glycerol stock (3 mL of culture and 3 mL of 50% glycerol)
- Performed miniprep for pYES2 plasmid
    - Used 7 mL of e. Coli
    - 345 ng/ul
    - 1.84 ratio

September 23rd 2021
Gabe

- Prepared overnight culture of pYES2 e.coli for miniprep and storage
    - 10 mL of media inoculated at around 5 pm
    - 10 uL of 1000x ampicillin added to overnight culture

Paul, Jenol, Tiago

- HARV:
    - Tiago updated HARV code
    - Successfully restarted HARV
    - Paul: Sterilized HARV bioreactor, put in fresh LB media inside biosafety hood and put inside the incubator to test sterility
- Clinostat:

- Jenol: Re-checked clinostat code, added new variables
- Have some issue with coupler fitting, will put the motor back on another day.

September 22nd 2021
Amir
Performed ethanol stress experiment using protocol: new stress protocols.
Modifications made to the protocol is as the following:
First three rows was 20% ethanol triplicates and next three rows were control where 50% of the volume of the cells were autoclaved water. Also the reading is done using flow cytometer.
Data (20% ethanol tap)
Data shows upregulation for HSP30.
September 21st 2021
Brian

- Stress Experiment
  - 20% ethanol
    - Started seeing a response at 10% but nothing significant
  - Experiment failed, no control added to plate, must be redone
- Cultures started for next day to repeat

September 16th 2021
Paul
- PCR of the genomic DNA sample.
- Prepared overnight cultures for ethanol stress experiments for next day

September 13th 2021
Gabe and Amir
- Performed stress experiments with protocol involving flow
  - Hydrogen peroxide at 4mM

September 11th 2021
- Genome DNA extraction for sequencing
  - 1 tube for each of the 8 strains (200uL of each overnight culture)
  - Made more 0.2M LiOAc (0.2040g in 9mL ddH2O and 1mL of 10% SDS)
  - DNA quantification:
    -

Strain	Concentration 1 (ng/uL)	Concentration 2 (ng/uL)	Average concentration(ng/uL)
ARC19	164.6	156.2	160.4
BTN2	151.7	167.2	159.45
HSP30	174.5	183.9	179.2

OXR1	195.4	168.6	182.0
RAV2	173.7	153.1	163.4
SAF1	200.7	174.9	187.8
SOD1	204.7	162.7	183.7
SOD2	203.4	192.1	197.75

- Started cultures for Monday (all strains)

September 9th
PaulB, PaulA, Gabe

- First test of the clinostat. Protocol: Clinostate testing
- Performed salt stress experiment with 50 mM
    - Read using flow cytometer
    -

Brian

- Performed stress experiment with ethanol and gave it to mo for flow cytometry
- Prepared cultures for Friday (USED YPD SEPT 8th 'A')

September 5th
Natasha and Gabe

- Salt stress experiment for all strains at 0, 0.125 and 0.25M NaCl
    - Protocol: Salt stress
    - Data in salt stress sheet (9)

September 4th
Natasha and Gabe

- Salt stress experiment for all strains at 0, 0.125 and 0.25M NaCl
    - Protocol: Salt Stress
    - Data: salt stress (sheet 8)

September 3rd
Gabe

- Poor growth on some overnight cultures - no stress experiments performed
- Discussion with Mo about data and moving forward
    - Look through data we have and focus on consistent strains and strains that follow the trends that we're looking for
- Autoclaved some stuff

September 2nd
Brian

- Cultured for experiments tomorrow

- Picked up IDT sequencing primers
- Did research into the RFC10 assembly standard and how it may work with our pYES plasmid. It appears like we will need to do some adjustments to our plasmid by inserting cut sites as all of them are present but on the same side of our insert. Alternatively Order our insert premade with the cut sites and remove the other restriction sites
- Set up the microgravity experiments doc
    - On same doc made a list of everything we have left to do
    - Created a stress response summary doc where we can summarize our data to make pulling conclusions a little more clear

September 1st
Amir and Brian

- Picked up the chem store.
- Autoclaved all tips and gave back 12~13 boxes lent from Mo.
- Didn't do any stress assay but took the time to go over all the previous data with Brian to figure out where everything is headed.

August 31st
Amir & Brian & Paul

- Protocol: New stress protocol
- Data in ethanol stress sheet
- Paul: Clinostat is almost fixed!!!!! Still needs a little bit of tuning. I will come later this week DO NOT RUN IT USING THE CURRENT ARDUINO CODE. (Clinostat might break) Windows laptop is in the lab now. **August 30th**

Paula & Brian

- Performed triplicates of ethanol experiment each plate having 4 replicates of 10% ethanol and 4 replicates of 0% ethanol
- Data will be analyzed tonight
- Autoclaved tubes, tips, and 1.5mL tubes
- Prepared cultures for next day and washed plates

August 27th
Gabe and Amir

- Performed salt stress using new stress protocol but across 2 concentration (0.25 and 0.125M) instead of what is written in the protocol
- Data
- Notes: 0.125 plate column 9 diluted

August 26th
Amir & Brian

- Performed ethanol stress protocol using:
    - Protocol: New stress protocol
    - Data: Ethanol stress sheet10

- **Notes:**
  - Multiple strains did not achieve high enough growth possibly because they were cultured in still warm YPD?
  - Strains were thus plated lengthwise along the plate with strains WT, BTN2, OXR,

Gabe and Paula

- Prepared SC media.

August 25th
Paul & Brian

- Saw our YPD had growth in it confirming there was contamination
- Emptied cleaned and autoclaved the tubes, made and autoclaved new YPD
- Cultured new tubes with YPD 'B'
- Streaked all 9 samples and incubated to see if there's any contamination from our frozen stocks, might use these to make 'fresh' frozen stocks

August 24th
L & Brian & Paul

- Re-performed new stress experiment with new plate map having 4 replicates
  - Protocol: Stress protocol
  - Data: Ethanol stress
  - **Notes:**
    - 10% ethanol used 4 times and 0% ethanol used 4 times for all 9 strains
    - When doing the blank make sure you know which YPD was used to incubate the cultures the night prior! As the YPDs have dif colours.
    *** We used the YPD with no date and a happy face on it
  - Columns 1-3 of plate 10%-B were double pipetted. The rest of the samples were loaded onto plate 5% in the last three columns (it'll be more clear once I clean up the data in Google Sheets). Note that the second pipetting volumes< 50uL. This only affects plate B for 10% for SAF1, SOD1 and SOD2. We have 4 replicates in the other plate at 10% for these promoters.
  - I will clean up and analyze this data tomorrow, L
  - Mo ran our samples for 10% with control (plate A) in the Flow Cytometer

- Paul: Re-performed EtOH stress with 10%, 5% and 0% for WT, BTN and HSP, 8 samples each.
  - Data: sheet 5 Ethanol Stress (Updated)
  - Plate mapping in vertical (see sheet 5 plate map)
  - Start OD: WT-0.9935 BTN-1.0129 HSP-0.9557
  - Added 154uL water (instead of 100) at the end, may need to check data

- Prepared cultures for tomorrow.
    - Used YPD which ran out (was lighter than other batch that remains)

- Papers to investigate best concentrations to play with salt stress
    - Suggests 1M will kill a good amount in 90min incubation, the results showed best result for 1M so maybe do 2 plates tomorrow with 4 replicates each at this concentration and 0M and then 2 plates with 4 replicates each at half of this concentration and 0M
- Plate: 5% EtOH @ bottom of SAF

August 23rd
Brian

- Prepared cultures for ethanol stress experiments tomorrow
- Autoclaved culturing tubes

August 21st
Gabe and Natasha

- Did stress experiment for heat stress
    - Protocol: Stress protocol
    - Data: Heat stress sheet
- High variation in data

August 19th
Paula, Gabe, Brian, Amir

- Experiments
    - Ran two stress experiments as discussed in this protocol: stress protocol
        - Salt experiment, sheet 5 of Salt stressor data
        - Hydrogen peroxide experiment
- Other
    - Made new SC media because growth curve not the best

August 18th
Paula & Brain

- Autoclaved tips and tubes to get started
- Needed to use Q-tips and dry opaque plates there was a little bit of yeast in the bottoms
- Most cultures did not grow, 3 (including the WT) just hit 0.8 pre dilution and were used to try and troubleshoot peroxide experiment giving respect to the time yeast is in peroxide before being incubated
- Alternative procedure tested: Serial dilution was done with peroxide as normal (except someone (paula) didn't leave any well blank but given the extreme dilution we decided we could use the one with 0.0125% as our zero given the goal was troubleshooting.

Instead of adding the cultures directly one by one an excess was placed in PCR tubes and then a multichannel pipette was used to add all the culture at once
    - Pre-incubation time was able to be under 2 minutes for 3 strains
    - Technique added an extra step but seemed to work
    - That being said data remained inconsistent with no detectable pattern between these strains and peroxide concentration
    - OD's given they started at 0.8 ended up low after transfer clocking between 0.15-2 which is lower than desired
- Cultures were prepared for tomorrow and plates for all steps of the process were disinfected and dried so experiments will be ready to start as soon as people arrive tomorrow

August 17th
L

- **Heat stress.**
- Initial ODs for first heat stress experiment 50uL cells, 50uL YPD dilution in plates, 1hr 200rpm shaking 30, 37, 42 (ODs too low. To be repeated)

WT	ARC	BTN	HSP	OXR	RAV	SAF	SOD1	SOD2	
0.5496	0.5722	0.5796	0.6326	0.5748	0.6504	0.6292	0.5136	0.5054	
0.5844	0.5954	0.593	0.6802	0.6392	0.6738	0.6642	0.563	0.5446	

- second set of good ODs and heat stress experiment went well. Analysis to come

- started a H2O2 experiment but found pippetting to be taking too long for triplicates of all 9 samples with only a 10minute incubation time. Aka if pipetting the samples takes 5 minutes that's already ½ of the incubation them. Maybe better to prep for all promoters then perform in groups? Where all of one group are pipetted quickly…
- I left the H2O2 falcon tubes I used. You could use them to prep a new solution.
- Cleaned the 96 well plates quickly and left them soaking in 25% ethanol. I meant to make 75% but made it quickly and had already added the water. Please discard and replace with 75% EtOH in the am.
- I brought Qtips. They work. Important to be gentle and not scratch. Some of the plates are already scratched.
- Looks like we might need liquids autoclaved this week. H2O, YPD..?
- Cultures started from frozen stock and in 30C room.
- Our vortex and centrifuge and lab bench are soooooo cool. Multichannel pipette Omg! We're a really lucky and deserving team.
- Have a good one! Can't wait to get some cells in that HARV.

August 16th
Brian

- Did Peroxide stress experiment on all strains
    - Protocol: Stress experiment protocol
    - Peroxide stress sheet for results

- Autoclaved tubes for culturing

1. Are we sure 6 minutes at 3750rpms is enough

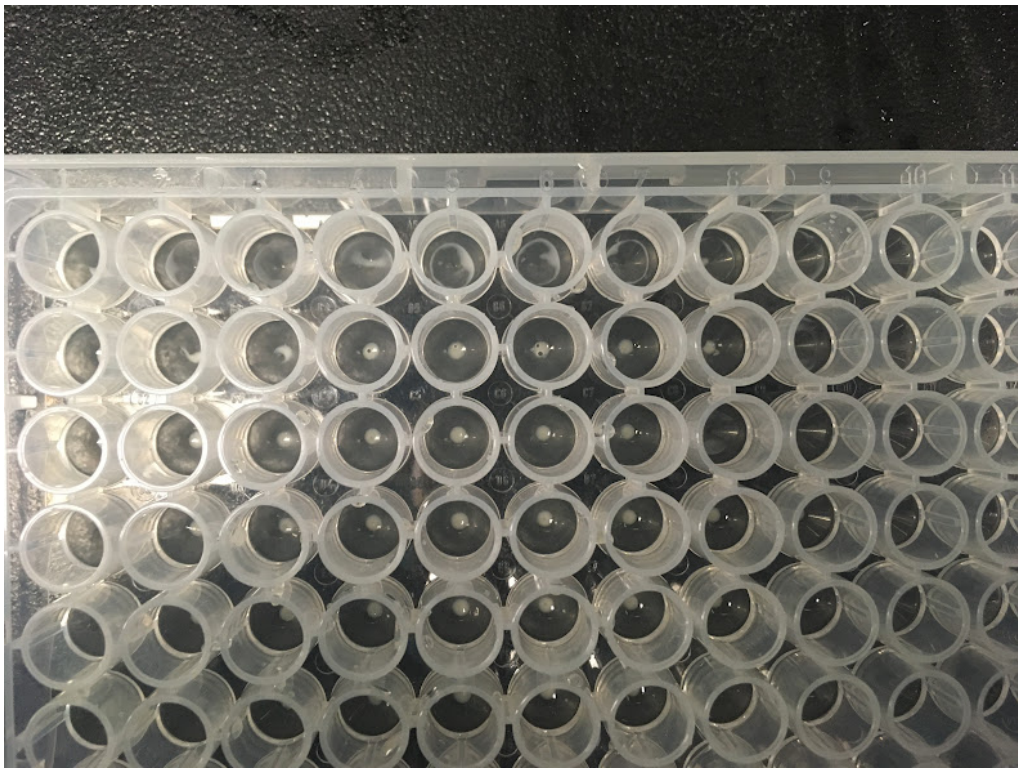


Plate 1

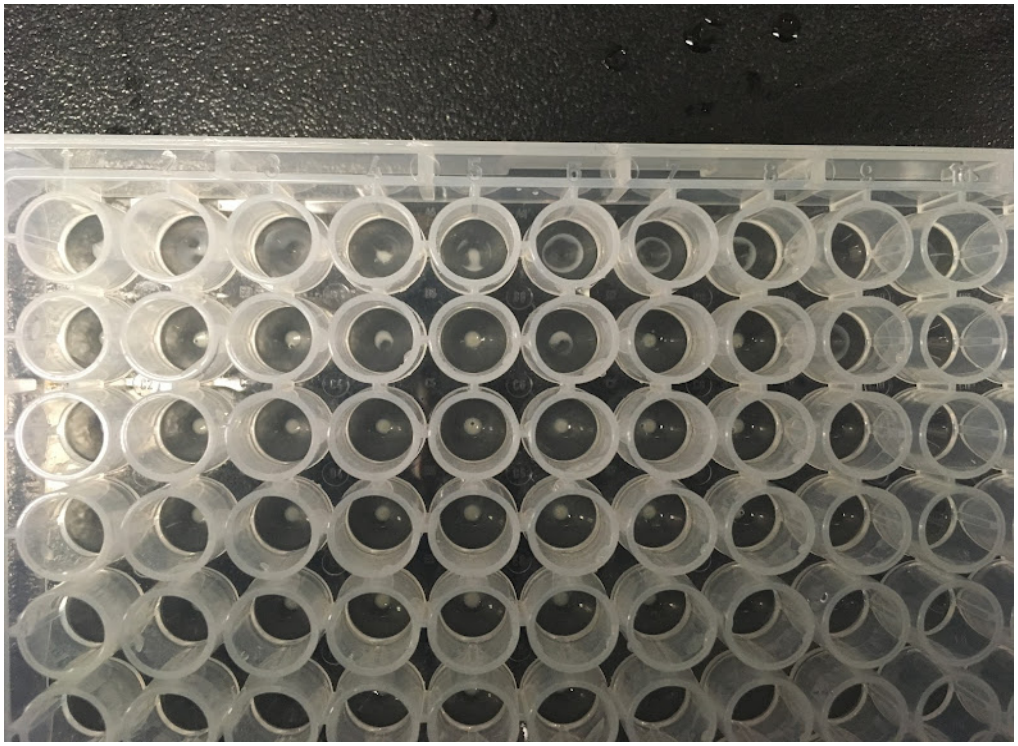


Plate 2

2. Templates are not set up for 3 plate 9 strain system

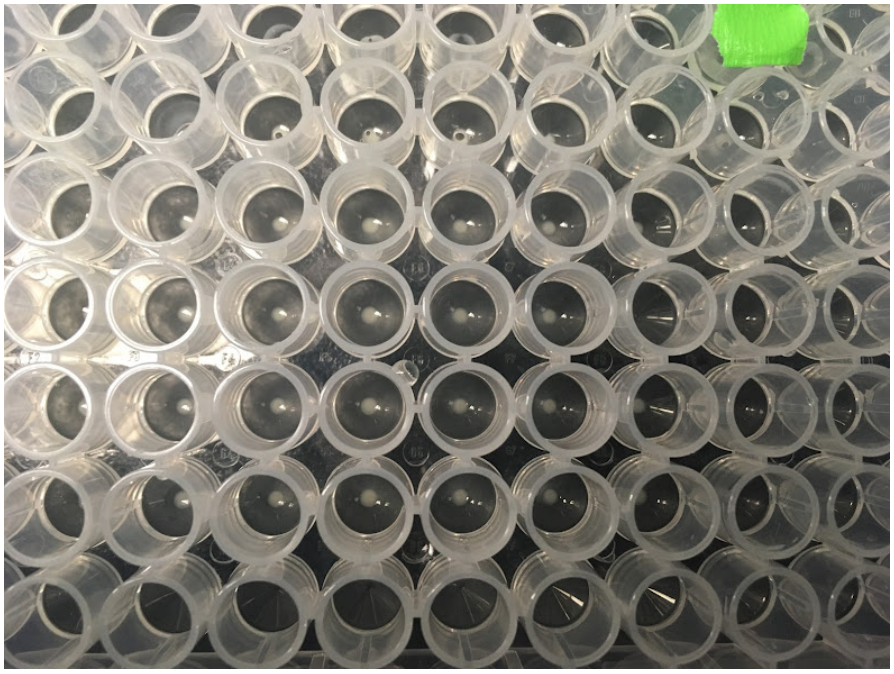
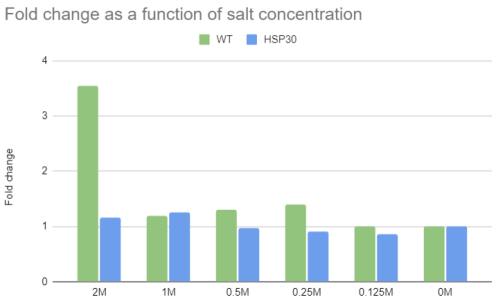


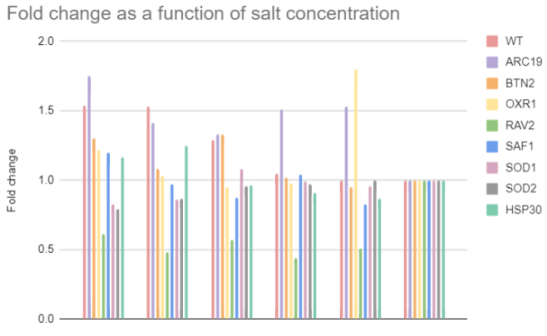
Plate 3

Paul:

- Connected the new motor with Jenol.
- Cleaned 96-well plates
- Prepared overnight cultures.

August 15th
Natasha

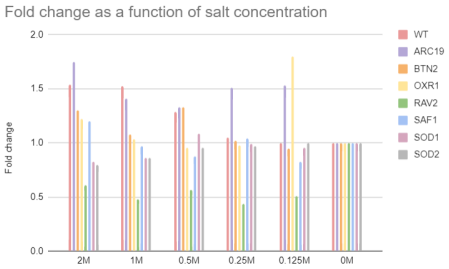
- Did salt stressor experiment on HSP30 in triplicates to complete the salt stress data
    - Protocol: Stress experiments
    - Results: Salt stressor (sheet 3)
        - This is the graph for fold change from todays experiment
        - 

        - The WT at 0M is higher than it should be because I accidentally pipetted the pellets when removing media.
        - In the graph below I added HSP30 to the graph from the experiment on August 14th:
        - 

- Started overnight culture for all strains (9 in total) for stress experiments tomorrow

August 14th
Gabe and Natasha

- Did salt stressor experiment on all strains in triplicates (except HSP30 since there was no growth)
    - Protocol: Stress experiments
    - Results: Salt stressor (sheet 2)



- Arc19 seems to be best (upregulated at every concentration)

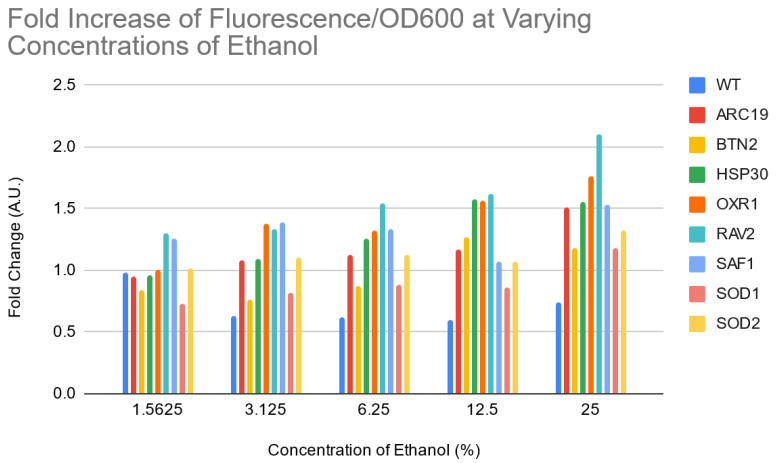
- Started overnight culture for WT and HSP30 for salt experiment tomorrow

August 13th
Gabe and Amir

wt	wt	wt	Arc19	arc	arc	BTN	btn	btn	OXR	oxr	oxr
200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2	200mM h2o2
100mM	100mM	100mM	100mM	100mM	100mM	100mM	100mM	100mM	100mM	100mM	100mM
50mM	50mM	50mM	50mM	50mM	50mM	50mM	50mM	50mM	50mM	50mM	50mM
25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM	25mM
12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM	12.5 mM
0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2	0mM h2o2
Blank (1;1) water and YPD	Blank (1;1) water and YPD	Blank (1;1) water and YPD									

August 12th
Gabe and Brian

- Did stress experiment with all strains with ethanol
    - Protocol used: Stress protocols
    - Top concentration: 25%
    - Results in sheet “3” of following sheet: Ethanol stress
    - Slight modification in the use of 50 uL, however, this may have led to some variation, will go back to 100 uL for next experiment



    -
    - RAV2 shown to be the most responsive, followed by OXR1
        - Makes sense based on literature
    - Data inconsistent with previous run in regards to relationship between BTN2, SOD1, and SOD2, may be due to variance from low volume
- Additional notes:
    - Do not shake plates using shake function on Tecan, causing aggregation of cells (almost like a minor pellet)
    - Be careful when aspirating media, easy to accidentally aspirate pellets

Note from Paul:
Jenol from hardware delivered the new motor but it was not installed. We will be installing it, fixing some other parts then running it for a while next Monday.

August 11th
Paula & Brian

1. Updated the measuring OD procedure which is not apart of the stress data analysis protocol: Stress protocols ETHANOL section
2. Performed **Ethanol** stress experiments using this protocol Stress protocols.
3. Paula spent a lot of time making sure our plates were properly clean for good OD measurements. Make sure to clean plates right away so they are easy to do!
4. Asked mentor for more triangle plates and we’re back up at 3

5. Autoclaved more large and medium tips along with culturing tubes for tomorrow's experiments
6. Prepared cultures of every sample for a proper run tomorrow

Notes:

a. Can't do a pre-incubation measurement with these plates as they are opaque
b. Stress experiments were performed with 50microL total volume rather than 50 in order to comply with the plate reader

August 10th
L, Brian and Paul

TO do:
Pick up autoclave. Yes
Make culture of every strain in YPD 7ml. → Paul (done)
Pick up UV plates---> half still dirty.

- Mo gave us a bag of 5 sterile reservoirs. Keep in mind only take from the bottom since the top one is not sterile after the bag is opened. Thanks Mo!

→ let's overnight culture more volume so we can account for making mistakes 7ml

- Ethanol experiment with WT, BTN2, RAV2, SOD2 is performed.
  - Altered Ethanol set up so that the highest concentration of ethanol after the addition of cells is 25% rather than 50%
  - Initial ODs before adding to the plates

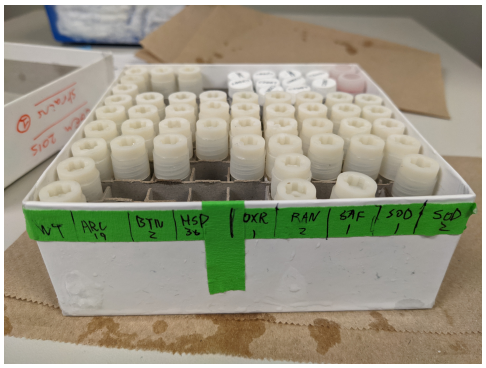
	BLK	WT	BTN	RAV	SOD
1:10	0.0507	0.1438	0.1571	0.1497	0.1432
MInus Blank		0.0931	0.1064	0.099	0.0925
x10		0.931	1.064	0.99	0.925

-

→ Later had ODs closer to 1.5…!!

- The 96 well we have isn't great for centrifuging so Mo gave us one that has V-wells for the removing media and resuspension step. It gets cleaned like the others and then UV'd. When taking out of the UV machine first cover with a breathable membrane.
- L: Ethanol experiment went long due to poor centrifuging and transfer to new 96 plate. I'll work the data later

- Paul: Salt stress experiments do not have enough data. I will not analyze it. Data: Salt stress
  Salt stress was done in the wrong plate so no OD was taken to normalize the data.
  Updated stress protocol: stress protocol
  Organized cell stocks in the freezer. Please respect the order!



August 9th

- Stress experiment with ethanol were not completed as there was:
  - No autoclaved water at all
  - The procedure was not yet vetted by Mo
  - One of the tubes were mysteriously spilled

- Investigate differences in our and advisor media

- Note: New YPD media is contaminated! As a result this was thrown out. Water was also contaminated and was thrown out too

- Large and medium tips were autoclaved as there were no more of the large ones

- Water and new YPD Media were also prepared and autoclaved

- Prepped cultures for incubation tomorrow

August 8th
PaulB

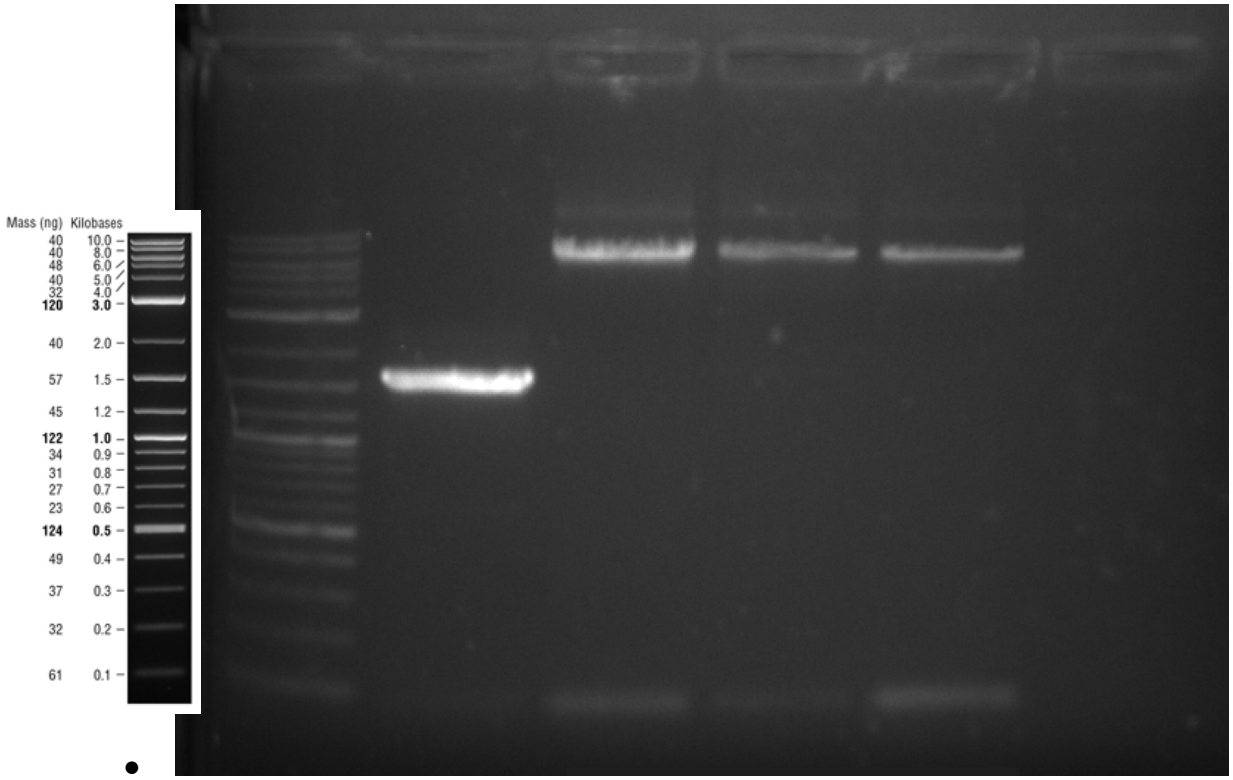
- Started overnight culture using YPD: SOD2, RAV2 and OXR1 with WT
- <u>We might be running low on OXR1, we have to double check our stock.</u>

August 5th

- Colony PCR for restreaked SAF1 to double check
- Colony PCR for 2nd restreak SAF1 all positive

Amir and PaulB August 5th

- Colony PCR for SAF1: 1kB Ladder, WT, SAF1: 1,2,3



- Confirms the previous SAF1 colonies are successful.
- Was put on culture to expand the colony. To be stored for future use.

- Made H_2O_2 stress assay using the peroxide stress protocol but didn't follow the steps fully. Firstly measured OD of overnight samples (which can be found) in the Paula section august 5th. Then I did a 2.33 fold dilution to change the media from YPD to SC (cause YPD interferes with the fluorescence data). The 2.33 fold dilution is done as the following: we want the final concentration of each well to be 100 uL thus we needed 30 uL of yeast in YPD grown overnight in 70 uL of the new media (SC here). However, this time I didn't add the yeast solution first and did the serial dilution prior to it (according to discussion Paul had with Mo concerning the changes that might occur in the concentration of the cells in each well). Thus, I added 70ul of SC media first to each well and then added 100uL of 30% H_2O_2 to the first well and then performed serial dilution (which means I took 100 uL from the first well and transferred to the second well, pipetted up and down for mixing and then take 100uL from the second well and transfer to the third well and do so until reaching the 5th well if you have 6 wells in total in each column because we want to leave the last well empty of stressor.) Then I added 30 uL of yeast cells solution to each well. Molarity of 30% H_2O_2 stock is 9.8 mM according to sigmaaldrich
  I have not added any blank to both plates.
- Wt SAF1 SOD1 Arc19 Rav2 SOD2

15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2
7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2
3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2
1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2
0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2
0	0	0	0	0	0	0	0	0	0	0	0

It should be noted that plate one in column 6 row E there is a possibility of contamination.

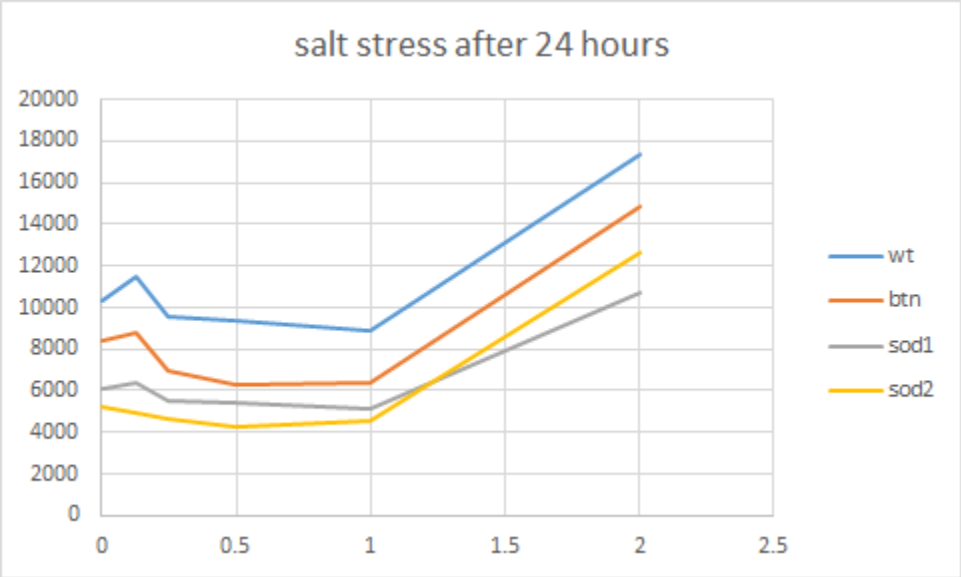
- Second plate

BTN2 OXR1 HSP30

15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2	15% H2O2
7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2	7.5% H2O2
3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2	3.75% H2O2
1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2	1.875% H2O2
0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2	0.9375% H2O2
0	0	0	0	0	0

- Data of plates collected from yesterday can be found here Stress experiment data

- Graph of salt experiment august 4th



Wild type Flu/OD is higher than the rest which is not what is expected.
Since the data is considered a failure, calculating fold change against 0 salt was not performed.

- Overnight cultures of SOD1 and WT prepared for tomorrow's stress experiment.
- SAF1 overnight cultured prepared upon successful colony PCR.
- 6 UV radiated 96 well plates are prepared.
- Please autoclave the long tubes plus refill yellow pipette tips and autoclave them.

Paula

Growth Curve : Protocol used, Growth curve protocol

- Setup
  - Cultures diluted in YPD
  - Cultures diluted in YNB
  - Mentor's SC

SOD1									HSP30		
SAF1									HSP30		
WT									HSP30		
SOD2											
RAV2											

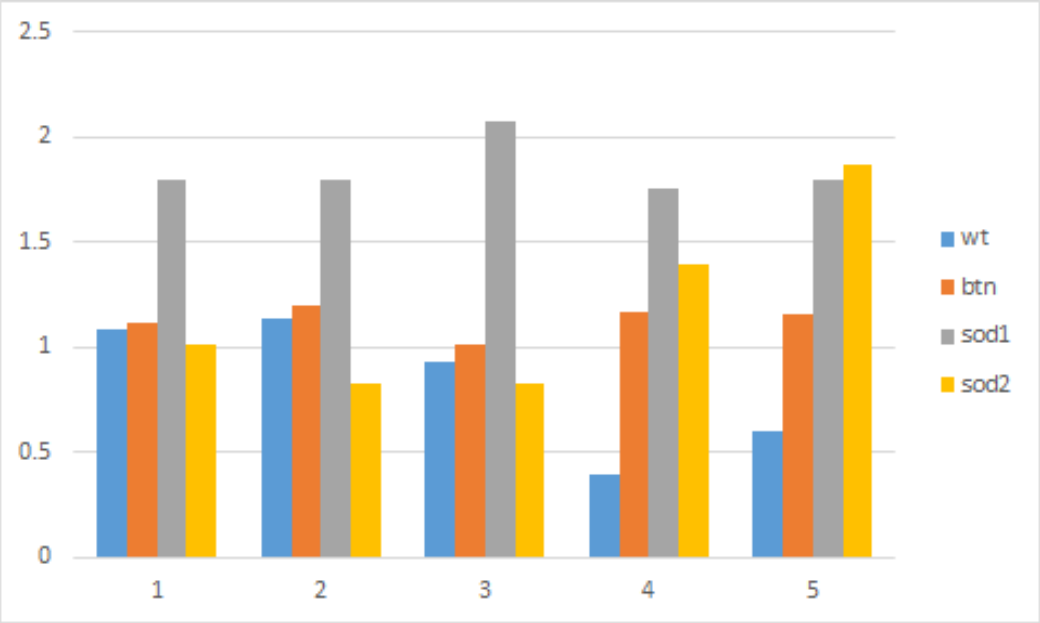
ARC19											
OXR1											
BTN2											

Overnight Cultures Initial OD BEFORE Dilution

WT	0.9037
SAF1	0.8064
SOD1	0.8409
ARC19	0.851
RAV2	0.7004
SOD2	0.8182
BTN2	0.8112
OXR1	0.8461
HSP30	0.7895

August 4th
Amir

- Overnight cultures of 9 different strains are inoculated (possibly for the growth curve experiment downstairs tomorrow?!)(not informed fully about this)
- Made three -80 cultures of SAF1 from the overnight inoculum
- Took absorbance and fluorescence of 2 different 96 well plate, data in Stress Experiment Results sheet. 1 was at a two day growth and made the measurement only to check the OD. Turns out the OD has increased from average of 0.3 to average of 0.6 for the first 9 columns (wt-oxr-arc) and decreased significantly for (one arc and RAV).
- This is the fold change from the 0% ethanol of the overnight plate made on august 3rd



1 to 5 is increasing concentration of ethanol

August 4th
Paula and Brian
Stress experiments with HSP30, WT, OXR1, ARC- Salt Stressor

- Overnight Culture Initial OD

HSP30	0.8293
Wild Type	0.986
OXR1	0.9198
ARC19	0.9634

- Plate organization
  - HSP30
  - WT
  - OXR1
  - ARC

2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M	2.0 M
1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M	1.0 M
0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M	0.5 M
0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M	0.25 M

0.125M	0.125M	0.125M	0.125M	0.125M	0.125M	0.125M	0.125M	0.125M	0.125M	0.125M	0.125M
0	0	0	0	0	0	0	0	0	0	0	0

- Recheck the stress experiments for the last two days. The Ethanol and Salt stress experiments were uploaded here : Stress experiment data
- Culture tubes were cleaned and autoclaved.
- Brian picked up the ChemStore Order.

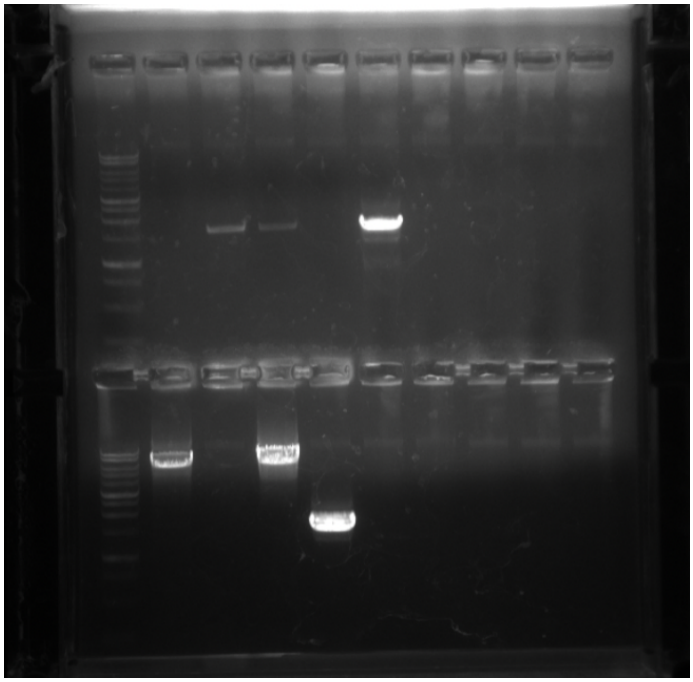
August 3rd
Paula and Brian
Stress experiments with WT, SOD1, SOD2, BTN2

1. Used triplicates again
2. Measured initial
3. Did serial dilution with ethanol and water before filling the rest of it with cells
4. Cleaned up the data and moved to our ethanol doc in “shared with me”

Yesterdays stress experiment

1. Checked the data this morning at 9:50am
2. Checked the data again at 11:50
   Note: more growth observed in later check

Colony PCR SAF1 first Restreak : SAF1 Success. Colony 3 plate A was used for a restreak and an overnight culture. The overnight culture is for glycerol stocks

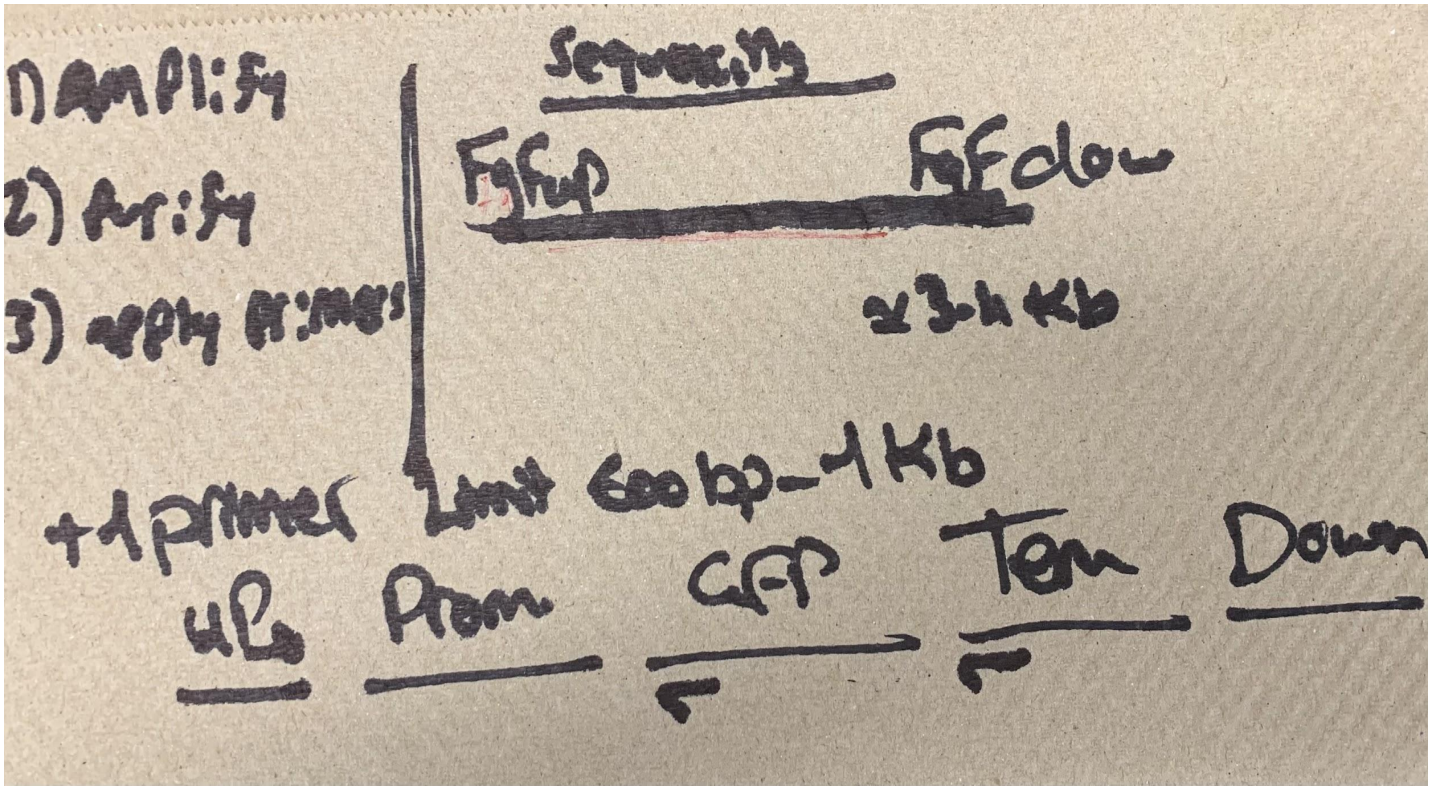


Row 1 Colonies from plate B SAF1 : Ladder, colony 1, colony 2 , colony 3, colony 4, WT.
Row 2 Colonies from plate A SAF1 : Ladder, colony 1, colony 2 , colony 3, WT.

Cultured frozen strains for tomorrow's experiments

1. Cultured strains for salt stress experiments tomorrow

Sequencing Procedure



L AUGUST 3rd

- Growth Curve Protocol : Growth curve protocol
    1. Prepare media
    2. Dilute and plate cultures

- (5min) Check plate from August 2nd (in back room at buttom in 30 degree incubator)
    1. Click application on bottom tab
    2. Click plate, part of plate (all columns and all rows except last one), absorbance (600), fluorescence (470-510nm)
    3. Save to usb in top drawer (the purple one)

Tasks completed
- Prepared YNB media from recipe with aseptic techniques
- OD600 of liquid overnight cultures

Plate label		OD600 Initial

A	ARC	0.9941
B	BTN	1.0264
C	HSP	1.04
D	OXR	1.2552
E	RAV	1.0981
F	SOD1	1.055
G	SOD2	1.0587
H	WT	1.1407
I	YPD	0.1543

Plate Index- triplicates YPD and triplicates YNB

	YPD	YPD	YPD	-	YNB	YNB	YNB
ARC	A1	A2	A3		A5	A6	A7
BTN	B1	B2	B3		B5	B6	B7
HSP	C1	C2	C3		C5	C6	C7
OXR	You get it	:)					
RAV							
SOD1							
SOD2							
WT	H1	H2	H3		H5	H6	H7

Wells A1→ C3 may have discrepancies due to me tipping the plate like a fool lol

Put into TECAN for Growth curve readings at ~5:15pm. Check in the morning to see if the readings make sense. If not we can repeat it. We have it reserved for 3 days. But I have faith ! :)

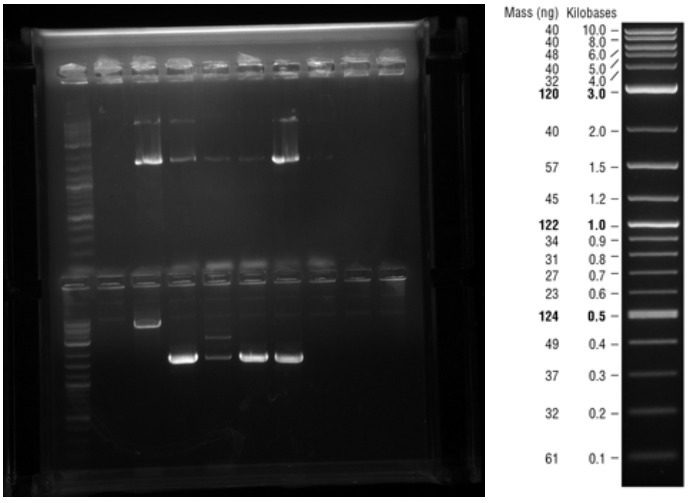
→ overnight cultures are on the bench top in case we still needed them? Rinsed the 96 well plates with H2O and ethanol but they are not properly cleaned or sterilized.

August 2nd
Gabe and Brian

- Stress experiments
    - Prepared overnight experiment for WT, ARC19, OXR1, and RAV2
    - Read at t=0
        - Data: Ethanol august 2nd
    - Consistent data across all samples and concentrations, suggesting that there is little stress response before incubation
- Updated recipe document with YNB recipe

August 1st

Gabe

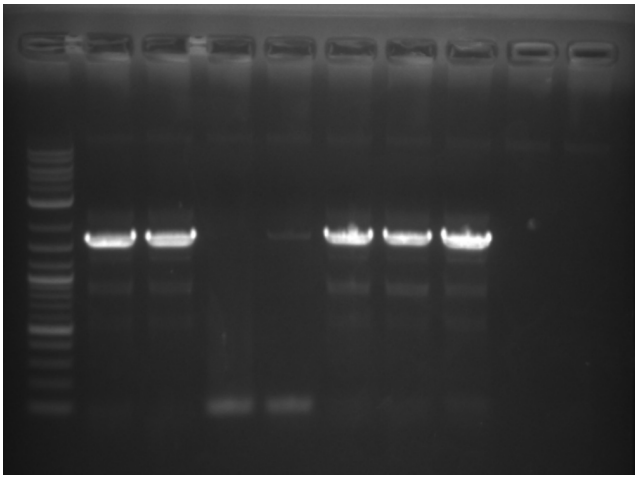
- Colony PCR on SAF1
    - Colony 8 promising, restreaked (SAF B)
    - Colony 6 strange but may work, restreaked as well in case (SAF A)
    - Both samples will need to be colony PCRd in case but likely only that of 8 will be positive
    - 

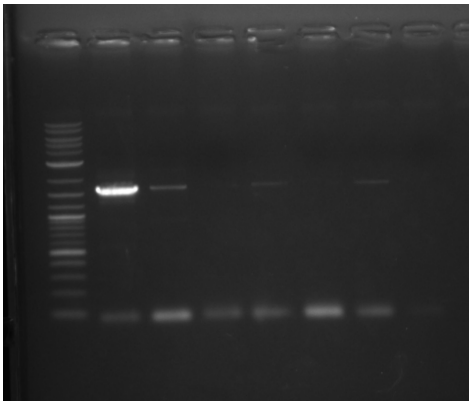
    - Important note: the SAF1 construct is 4 kb, not 3 kb like the other constructs
- Stress experiment
    - WT, HSP, SOD2, SOD1, RAV2, BTN2
    - Data: Salt stress August 1st sheet
    - Underwent salt stress for 4 hours
    - Strange trends likely due to lack of growth in 4 hours

July 31st

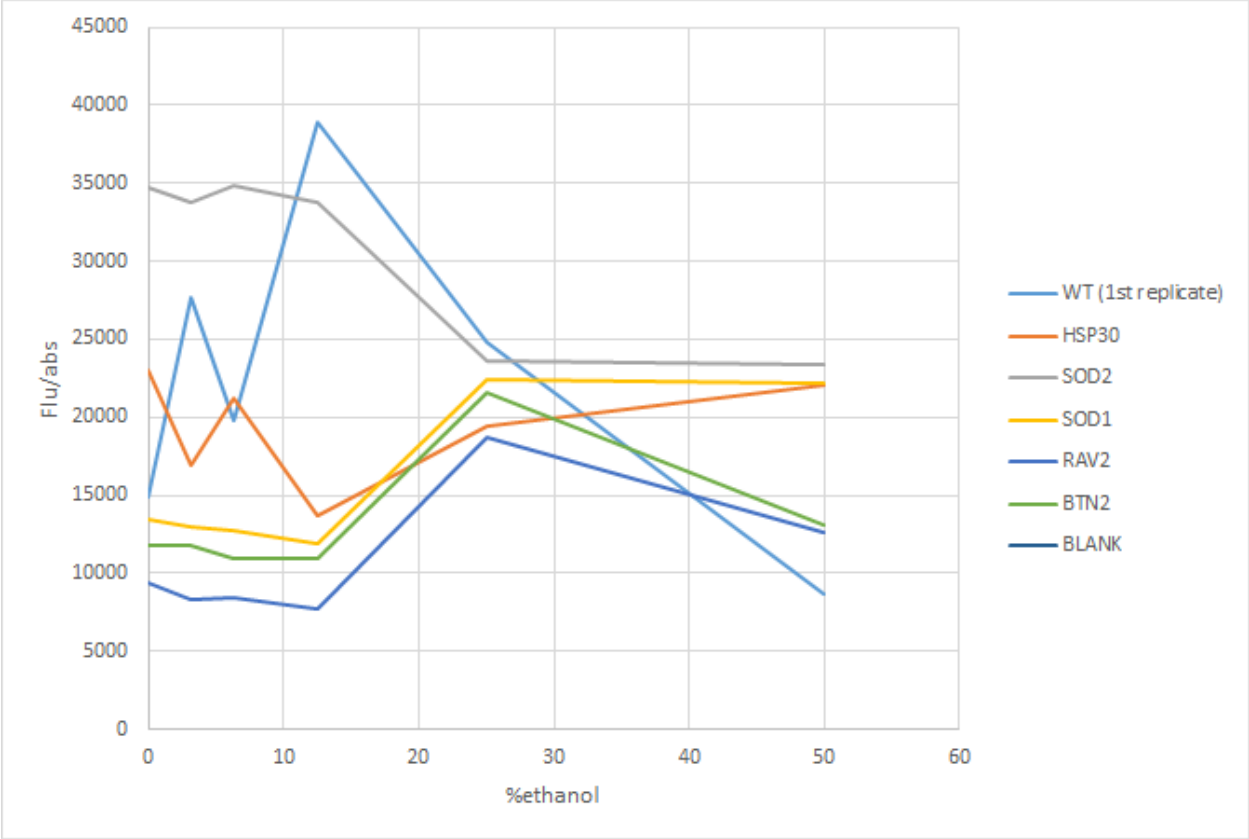
Gabe, Paul and Natasha

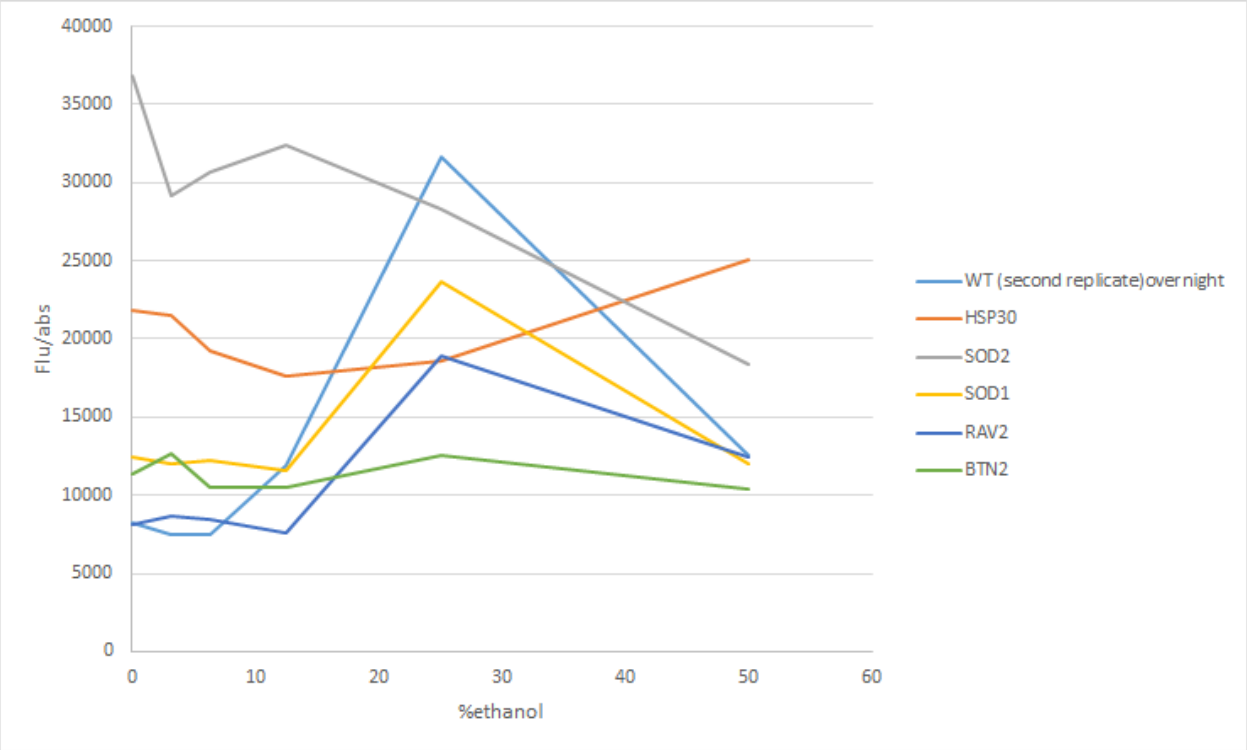
- Tested the stress protocol for salt stress
    - Strains: WT, HSP30, SOD1, SOD2, BTN2, Rav2
    - NaCl concentrations: 2M, 1M, 0.5M, 0.25M, 0.125M and 0M
    - Incubation: 2:15-6:15pm
    - Protocol: Stress protocol
    - Data: Ethanol stress sheet
- Colony PCR for SAF from first restreak (6 replicates)
    - Protocol: Updated colony pcr protocol

- Gel
  - 
  - Order: Ladder, WT, SAF 1-6
  - SAF1, 4, 5 and 6 all were not transformed, SAF 2 and 3 PCR failed (no band on the gel)
- Colony PCR to redo SAF 2 and 3
- Protocol: Updated colony PCR

- Gel:
  - 
  - Order: Ladder, WT, SAF 2 (3 rows), SAF 3 (3 rows)
  - Both SAF 2 and SAF 3 are not transformed

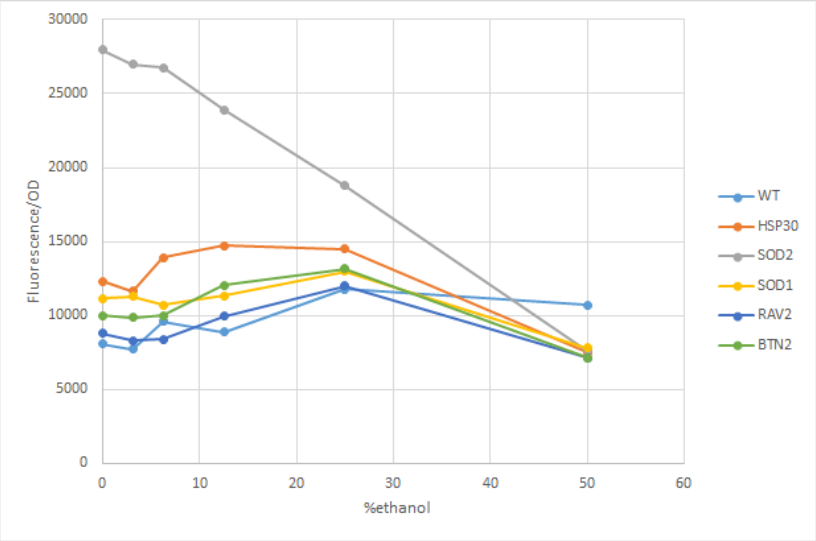
Results of the overnight ethanol stress experiment of the strains of the July 30th experiment:





Gabe and Amir July 30th

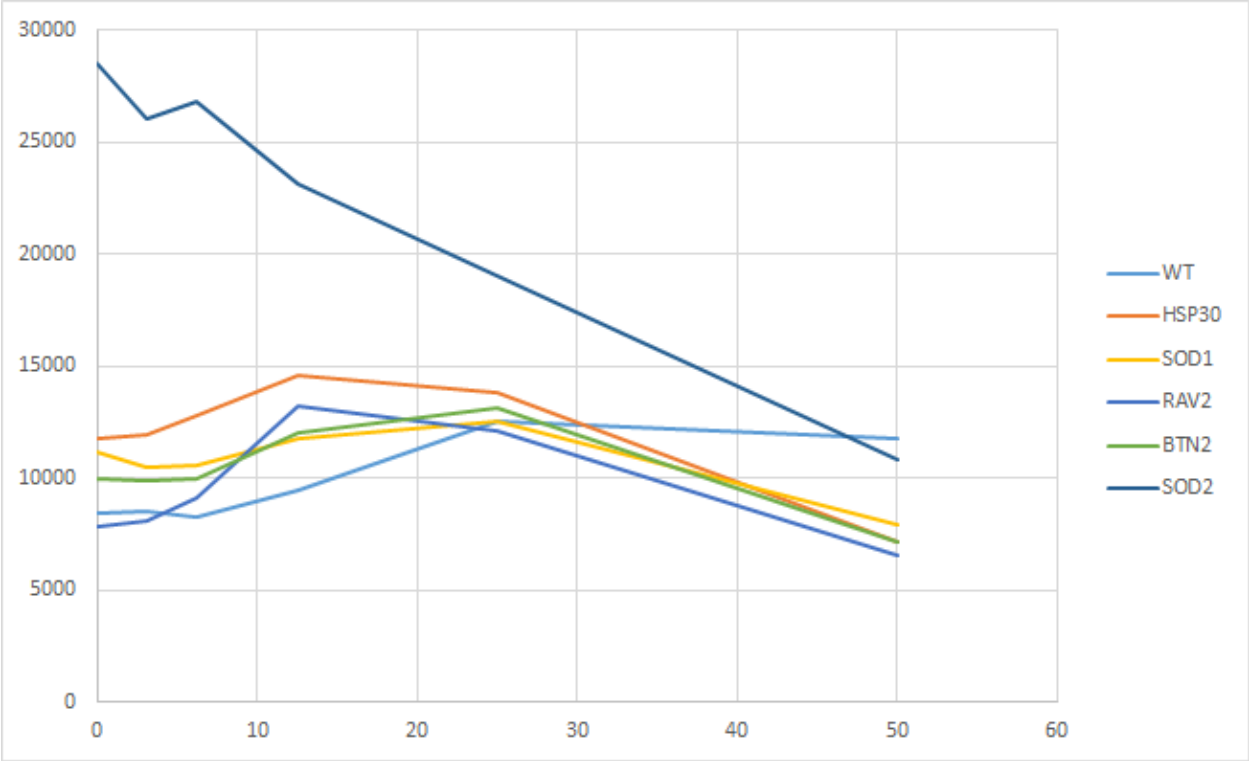
- Autoclaved tips and tubes
- Picked up orders from chem store /NEB/thermo
- Results of the overnight culture stress experiment can be found here: ethanol stressors
- Comparing the results of the 4 hours with the overnight showed that the overnight was not reliable. In total the data was inconclusive. BTN increased more but also its starting point was higher.
- Today we did the ethanol stress experiment of 4 hours and the results can be found here under the sheet 4 tab: Ethanol stressors
- We didn't follow the protocol fully: Stress protocols version 2., but used the same concept. Firstly measure the OD. For us they were all in the same range of 1.5 to 1.6. Thus we decided to do a 5 fold dilution in the YNB. The way it works is that we want to reach 100uL so for a 5 fold dilution there will be 20ul of yeast in YPD plus 80ul of YNB. we did a duplicate so 6 strains filled 12 columns (in a 96 well plate). We need 6 rows. To the first one we add 100ul of ethanol and pipette up and down a couple of times then take 100uL from that well and add it to the row underneath. Do it for all the wells in the column except for the last one because we want the concentration of ethanol to be 0% in the last well.
- Overnight cultures for Saturday's stress experiment are made and left in the hot room shaker.
- Below is the graph of the ethanol stress experiment following 4hours incubation.



My interpretation of graph (Amir) (it might be wrong, pls check with Mo when in doubt):
1- Wild type strain is almost the lowest line which is a positive indication (yeast might glow naturally but we expect that our strains will glow more because of our insertion which is kinda the case here) 2- for all other strains (except for SOD2), there is an increase in fluorescence (when controlled for OD) at around 12-20% ethanol and then decreases. Looked up some papers. It seems like BTN2 is not specifically upregulated under ethanol stress (which means other promoters can become upregulated as well as BTN2) which is consistent with our data today. 3- it seems like our SOD2 was either already under stress or just tends to glow more than others. To be confirmed later.

Stress induction of HSP30, the plasma membrane heat shock protein gene of Saccharomyces cerevisiae, appears not to use known stress-regulated transcription factors

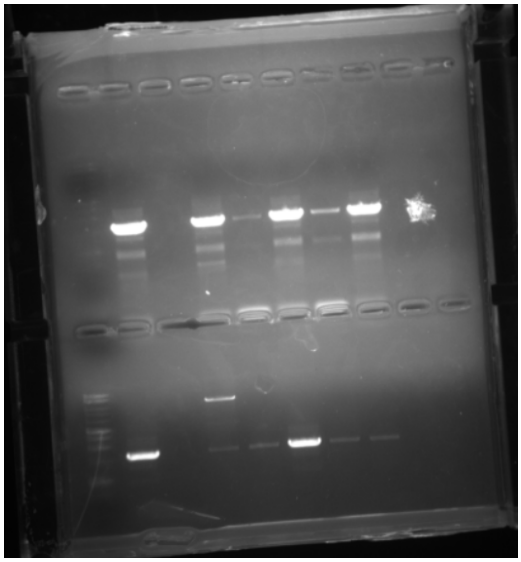
: this paper indicates HSP30 is upregulated under some stresses other than heat including ethanol consistent with our data.
However, expression of RAV2 is comparable to wild type suggesting ethanol might not be a strong stressor. To be confirmed with more data.
The following is the replicate graph:



All the observations of the first graph apply to the replicate. However, RAV2 seems to be upregulated at 12% (in contrast to the first one which was getting upregulated only the same as WT.)
Last observation similar to that of the first graph is that WT gets upregulated slightly starting 10% ethanol till 25% ethanol which I don't have any explanation for why this happens.
The most responsive strain to my point of view based on today's data is HSP30.

Thursday, July 29th
Amir and Brian

Colony PCR



Row 1: ladder - Wt - SAF1 (1th to 5th)
Row 2: ladder - Wt - SAF1 (6th to 10th)
SAF1 deemed successful and restreaked

Ran the gel

- UNDERWAY

Stress Experiments

- Data in lab book
- Calculations in lab book mainly C1V1=C2V2

- <u>This table is for the measurements after 4 hours. We need to measure it again tomorrow in another table.</u>

Sample	**OD 600**	**Fluorescence**
Blank	0.0363	232
Blank 2		
Wild type 50%	0.0475	403
Wild type 25%	0.0482	571
Wild type 12.5%	0.0507	630
Wild type 6.25%	0.0496	705
Wild type 3.125%	0.0508	496
Wild type 0% ethanol	0.1245	217

BTN2 50% ethanol	0.0415	376

BTN2 25%	0.0464	600
BTN2 12.5%	0.042	717
BTN2 6.25%	0.0471	726
BTN2 0% ethanol	0.0924	799
HSP30 50%	0.0563	413
HSP30 25%	0.0535	603
HSP30 12.5%	0.0526	805
HSP30 6.25%	0.0573	754
HSP30 0% ethanol	0.0596	673
SOD1 50%	0.0474	399
SOD1 25%	0.0451	594

-

SOD1 12.5%	0.0482	688
SOD1 6.25%	0.0459	550
SOD1 3.125% ethanol	0.049	347
SOD1 0% ethanol	0.0798	603

-

Weighing

- Did not dry overnight
- Instead vacuum filtered the cells and measured that way
- **Total weight was 11.1187 grams**

Autoclave waste

- DONE
- Added a water too, autoclave tape was folded over and got into the water

Prepare for frozen stocks of ARC and OXR

- DONE

Prepare liquid culture for stress experiments tomorrow

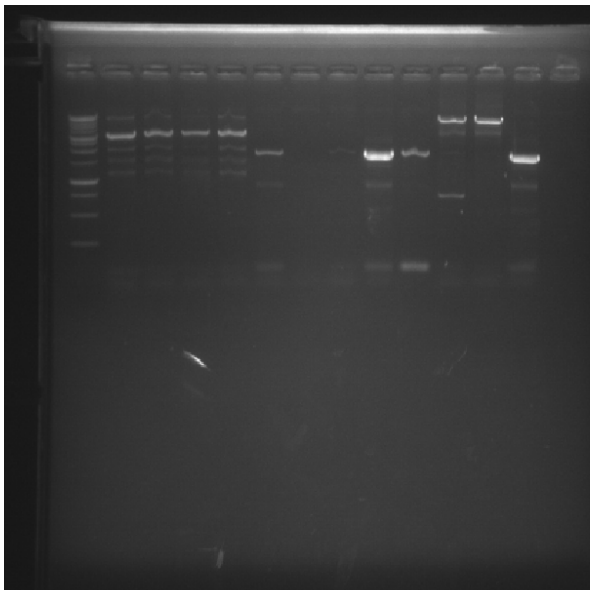
- DONE

Pick up from chem store
- They are apparently closed on Thursdays

Sterilized
- 96 well plates are now UV and ethanol sterilized

Wednesday, July 28th
Paula and Brian

Colony PCR:
- Ran Wt, 2 of 2nd restreak ARC19, 4 of 2nd restreak OXR, 5 colonies SAF
- ARC19 , OXR1 were not restreak because they are not cleaner than the previous one. The next step will be to send them to be sequence .



Row 1 : ladder , oxr1 colony 1 , oxr1 colony 2, oxr1 colony 3, oxr1 colony 5 , saf1 colony 1, saf1 colony 2, saf1 colony 3 saf1 colony 4, saf1 colony 5, arc19 colony 2, arc19 colony 3, wt .

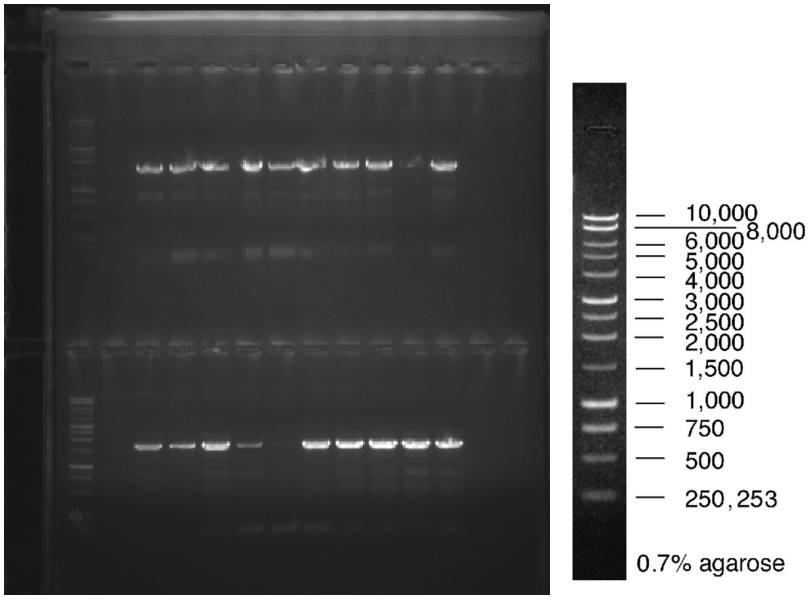
Stress experiments:
- Colonies did not grow. The machine was turned off overnight but additionally too research shows that they should be cultured in YPD the night before which should not have an effect on fluorescence due to the degree which it gets diluted the next day.
- Cultures were prepared for the experiments tomorrow
- New procedure was prepared for stress experiments and put into the the doc “Stress experiments July 18th”

Weighing of cells CSA:

- The large culture was divided into tubes and centrifuged at 3750rpm for 10 minutes
- The Cells were kept and the media poured out and left to dry overnight

Other:
- Autoclaved more water
- Cleaned and autoclaved culturing tubes

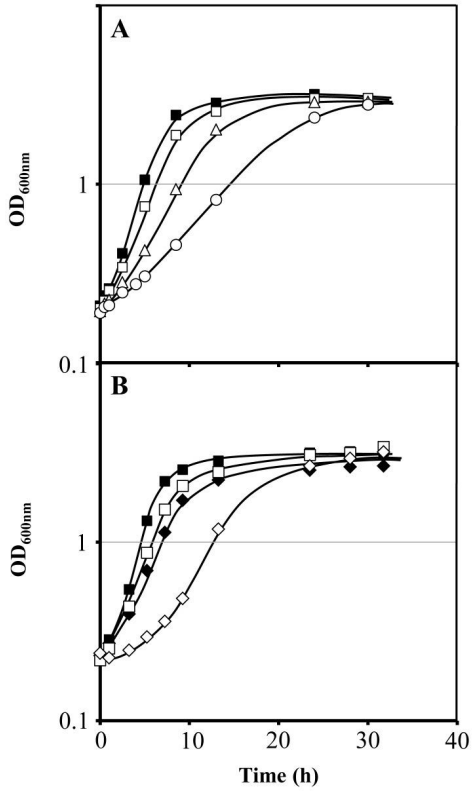
Tuesday, July 27th
L and Brian
- Ran colony PCR for SAF1→ FAIL
- <u>Row one</u>
  Ladder WTA SAF1 (1-9)
  <u>Row two</u>
  Ladder WTB SAF1 (10-18)
  GEL (promega 1kb ladder)



-

- Repeated ethanol stress experiments
  *** Started a **folder** called ‘Stressors Data’ under WetLab under Genetics on the Drive for stressors data. Make spreadsheets as needed. Link to EtOH data: https://docs.google.com/spreadsheets/d/1pbHSMqAKADzu1vsrllCTZb23QkJHoeCNzLk6

p8I9UJ0/edit?usp=sharing



- ***Quantitative 1H-NMR-Metabolomics Reveals Extensive Metabolic Reprogramming and the Effect of the Aquaglyceroporin FPS1 in Ethanol-Stressed Yeast Cells***
-
- Weird results. We think that we need to culture longer to reach higher OD where there is less error. The above image (the triangle curve) is BY4741 in 6%v/v ethanol giving us an idea of what to expect
- Talked to Mo
    - Cultures need 5x the head space****** can cheat it down to 2x, 10ml culture in 20ml flask.
    - ***Have to shake
- EtOH new protocol via Mo→ also in stressors doc
    - Frozen overnight cultures, make liquid culture
    - Set to same OD600, 0.001 or 0.01
    - culture into 96 well plate
    - Every row is a culture, one row for wild type, 100uL of culture total
    - Highest concentration serial dilute across rows. Series of 11 concentrations of EtOH and zero.
    - Place in incubator on shaker overnight with the cover

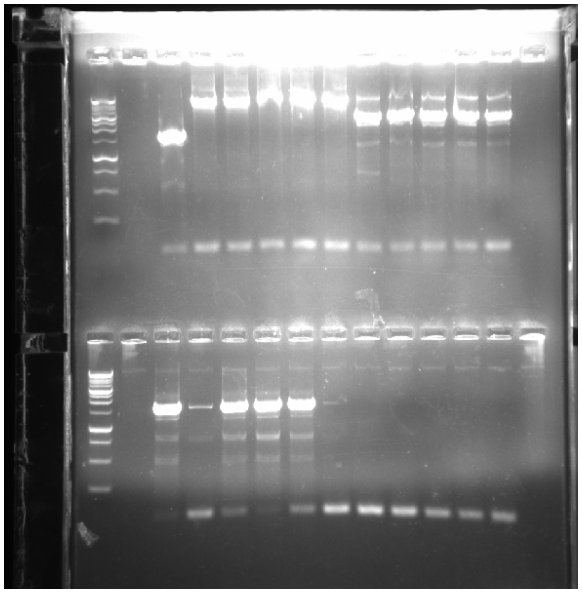
Sample	**OD 600**	**Fluorescence**
Blank	0.0397	324

Blank 2	0.0379	318
Wild type	0.0478	2692
Wild type ethanol	0.1252	4126
BTN2	0.0491	2854
BTN2 ethanol	0.0453	964
HSP30	0.1043	3043
HSP30 ethanol	0.0492	1745
SOD1	0.0913	2198
SOD1 ethanol	0.2126	12538

-

Monday July 26th
Gabe and Brian

COLONY PCR:



Order: ladder, wt, ARC, OXR. Second row: ladder, wt, SAF, SLT2

Stress experiment

- 10% ethanol
-

Sample	OD 600	Fluorescence	Fluorescence/OD 600
Blank	0.068	348	5118
Blank 2	0.0389	340	8740
Wild type	0.5889	2489	4227
Wild type ethanol	0.1886	1887	10005
BTN2	0.6297	4994	7931
BTN2 ethanol	0.5785	4299	7431
HSP30	0.5202	5095	9794
HSP30 ethanol	0.314	10434	33229
SOD1	0.0802	5462	68105
SOD1 ethanol	0.0856	4080	47664

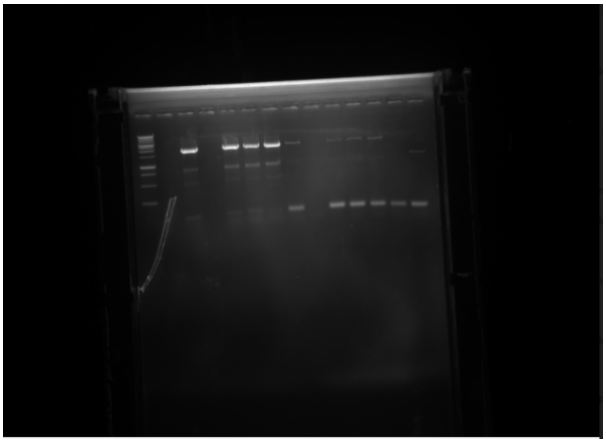
-

Sunday July 25th
Gabe and Paul

- Stress experiments
    - Cells removed at 10 am, (16 and a half hours)
    - 5% ethanol

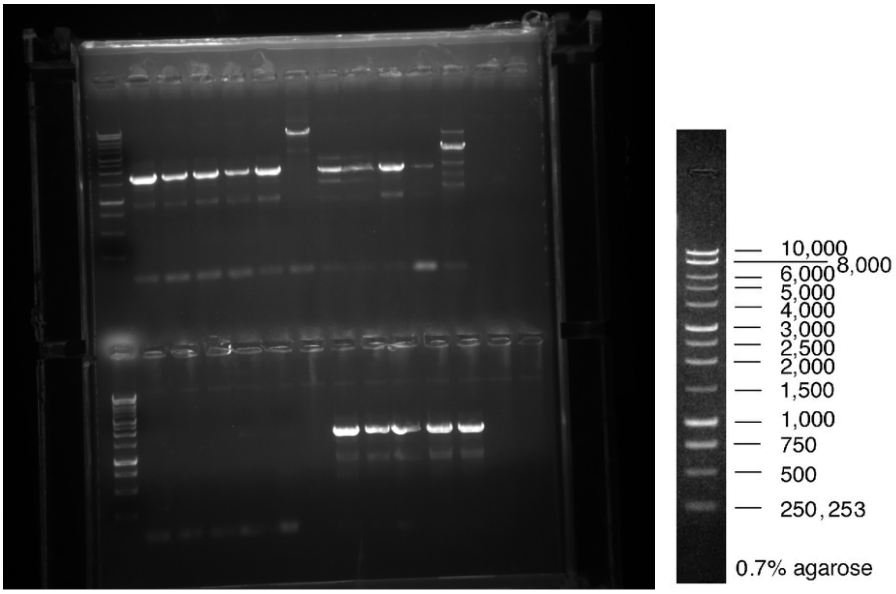
Sample	OD 600	Fluorescence	Fluorescence/OD 600
Blank	0.0392	324	8265
Blank 2	0.0397	322	8111
Wild type	0.1198	2009	16770
Wild type ethanol	0.1262	2211	17520
SOD1	0.1661	3817	32877
SOD1 ethanol	0.1826	4505	24671
BTN2	0.2004	4663	23268

BTN2 ethanol	0.2052	4688	22846

- Colony PCR
  - Did colony PCR on SLT2 and SAF1
    - Only two that needed to be tested
  - Gel
    - 
    - Ladder, blank, WT, SAF1 (1-5), blank, SLT2 (1-5)
- Maintenance
  - Set up HSP30, BTN2, SOD1, and WT overnight culture with 10%
  - Prepared 4 M NaCl for future stress experiments

Saturday July 24th

Gabe, Natasha, and Amir

- Colony PCR for Arc19, OXR1, SAF1 (transformation) and SLT2 (1st restreak)
  - Protocol: Colony PCR
  - Gel:
    - 

    - Order:

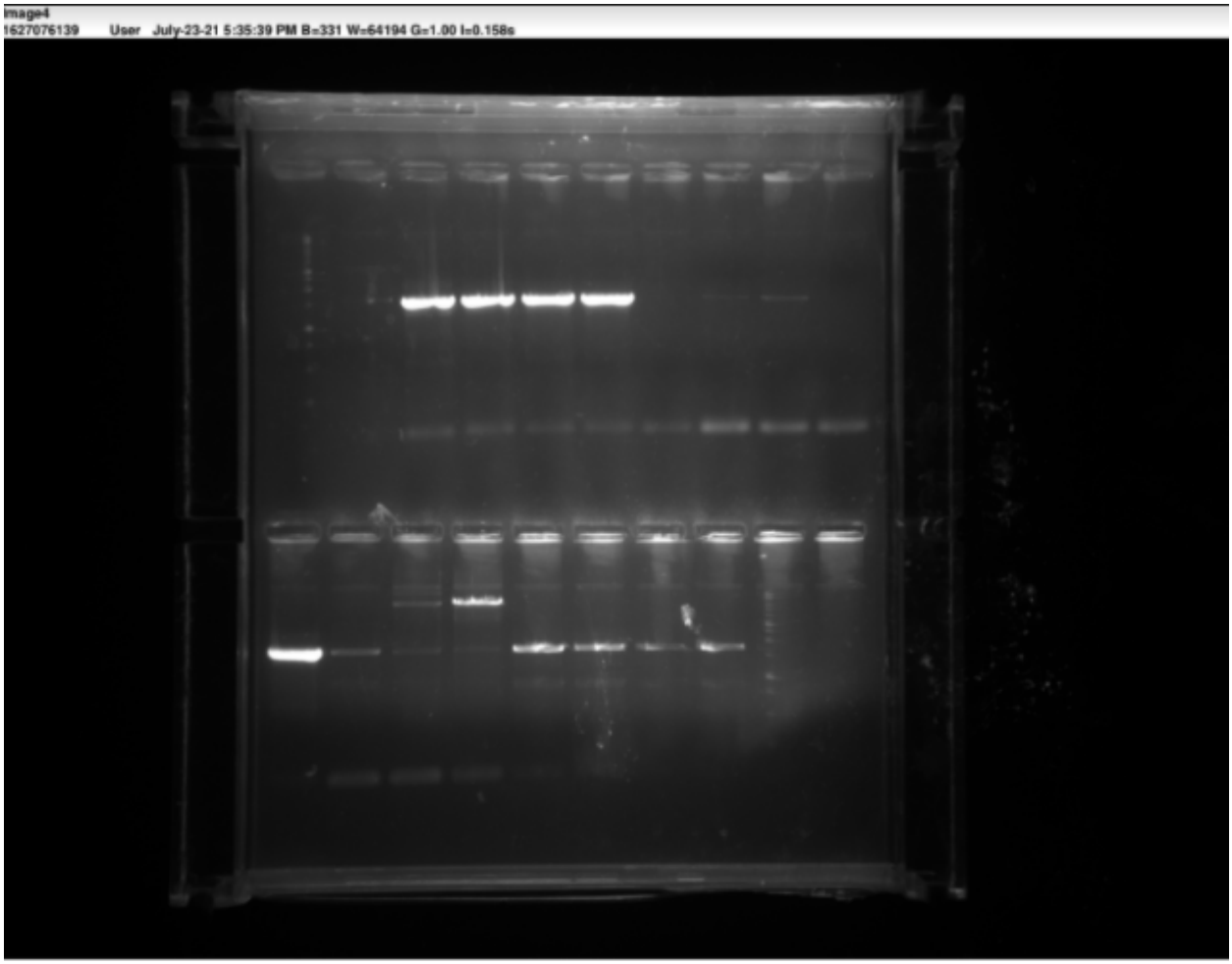
        - Top row = Ladder, WT, Arc19 1-5, OXR1 1-5
        - Bottom row = Ladder, SLT2 1-5, SAF1 1-5
    - Arc19 5 and OXR1 5 both worked, so they were restreaked (1st restreak)
- Testing stress experiments (5% ethanol on wild type, SOD1 and BTN2)
    - 5% initial yeast content in 500 uL of YNB media (4 hours)
        - Adaptation of the following protocol: Stress protocols version 2
    -

	Blank	WT	SOD1	BTN2
OD600 (no EtOH)	0.0389	0.0408	0.0392	0.0476
OD600 (5% EtOH)	0.0407	0.0413	0.0441	0.0517
Fluorescence (no EtOH)	349	383	441	441
Fluorescence (5% EtOH)	337	389	368	537
Ratio for no EtOH (Fluorescence/OD600)	8971.72	9387.25	11250	9264.71
Ratio for 5% EtOH (Fluorescence/OD600)	8280.10	9418.89	8344.67	10386.85

    -
- Ran the clinostat (1:55pm-2:55pm)
    - The motor got very hot (could barely touch it)
    - Very noisey (only test it if no one is here or its in another room)
- Started overnight culture for stress experiments
    - WT, BTN2 and SOD1 (1 replicate each without EtOH (control), 1 replicate each with 5% EtOH)
    - 450uL YNB media, 25uL of yeast (frozen stock), and 25uL water (controls) or 25uL of 5% EtOH
    - Grown in hot room overnight (start = 5:30pm)

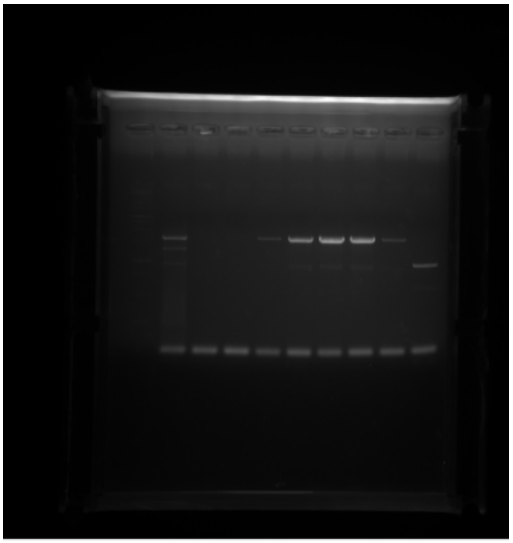
Friday July 23rd
Gabe and Amir

- Second colony PCR: Colony PCR protocol

- 

Row one: Ladder, WT, SAF(1-4), SLT(1-4)
Row two: OXR(1-4), Arc(1-4), Ladder :))))
I used 10 uL for loading the ladder
All are negative - OXR4 is showing some weird thing but not what we are looking for as it is too large -- ALSO the ladder is not showing which means we have to use more than 10 ul (or maybe adding loading dye to it (not sure about it))

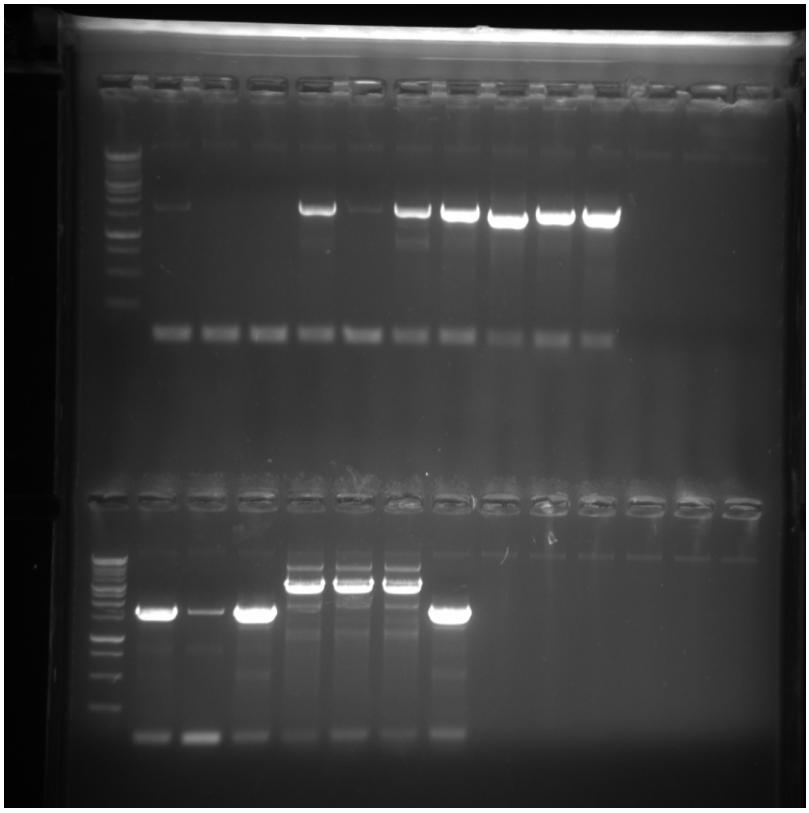
- Colony PCR protocol,
    - Protocol adjusted to minimize need for pcr tubes
        - Microwaved for 2 minutes and 45 seconds in microcentrifuge tubes

- 
    - Ladder, WT, SLT2 (1-2), SAF2 (1-2), OXR2 (1-2), ARC19 (1-2)
    - SLT2 failed
    - All other samples negative
        - Redo colony PCR with new colonies
  - Used new ladder, bands not clear enough with 6 uL, trying again with 10 uL
- Harvested culture for hardware
  - Last OD measurement at 9:32
    - OD blank: 0.0674
    - OD sample: 1.3521
  - 10 tubes with yeast in them
  - Letting sit to get rid of water content which could affect mass
- Maintenance
  - Autoclaved a buttload of stuff
  - Picked up chemstore order
    - PCR tubes
    - Glycerol
    - Flint
    - Small tips
  - Added missing media components to YNB
    - 7 mg uracil
    - 40 mg leucine
    - Overnight culture ran and will hopefully yield some data

Thursday July 22th

Paula

- Colony PCR
  - SLT2 (13-07-2021) 1st Restreak plate.
  - ARC19 (17-07-2021) Transformation plate
  - OXR1 (03-06-2021) Transformation plate
  - SAF1 (17-07-2021) Transformation plate
  - RAV (20-07-2021) 4th Restreak plate



Row one :

- Ladder
- SLT2 colony 1
- SLT2 colony 2
- SLT2 colony 3
- ARC19 colony 1
- ARC19 colony 2
- ARC19 colony 3
- OXR1 colony 1
- OXR1 colony 2
- OXR1 colony 3
- Wild Type

Row two :

- Ladder
- SAF1 colony 1
- SAF1 colony 2
- SAF1 colony 3
- RAV2 colony 1
- RAV2 colony 2
- RAV2 colony 3
- Wild Type

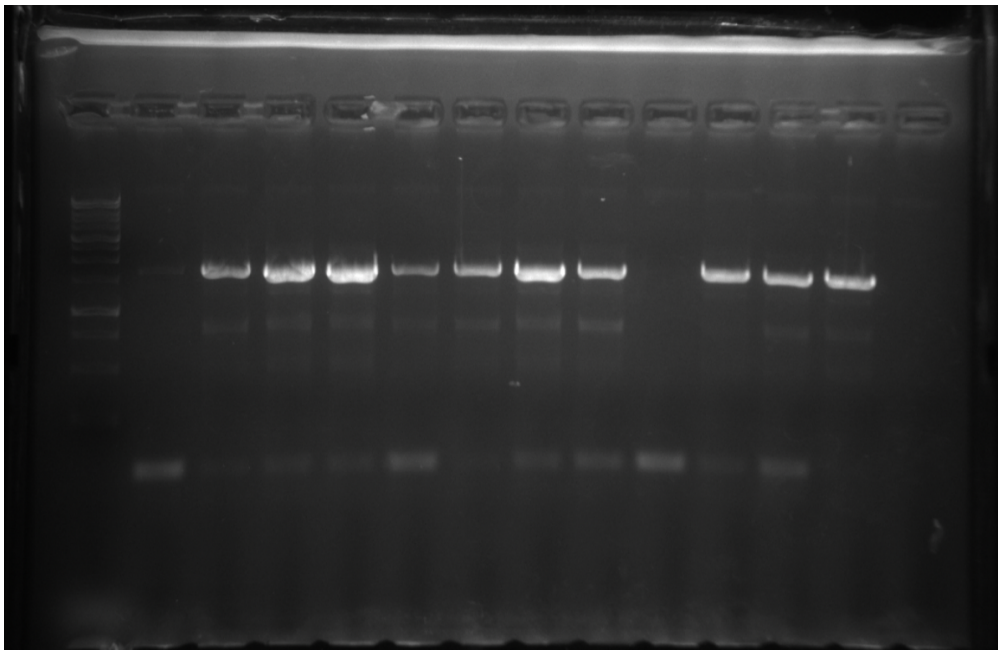
- Made overnight culture for wild type, SOD1 and BTN2
- OD measurements of the Liquid yeast culture for hardware

Time	Value	Blank	Difference
12:51 pm	1.6694	0.1089	1.5605
3:22 pm	1.6922	0.1123	1.5799
5:00 pm	1.7077	0.1079	1.5998

Wednesday July 21th
Paula and Brian

1. Checked OD of overnight cultures, had values of:
   blank value = 0.0305, SOD1 = 0.0256, 0.0272
   This shows no growth, Found out with the yeast nitrogen base media it is missing leucine and uracil

2. Ran colony PCR with 10 samples of SAF1 and 2 samples of wild type DNA



   a. Row 1 : Ladder ,SAF1 Colony 1, SAF1 Colony 2, SAF1 Colony 3 , SAF1 Colony 4 , SAF1 Colony 5 , SAF1 Colony 6 , SAF1 Colony 7 , SAF1 Colony 8 , SAF1 Colony 9 , SAF1 Colony 10, WT, WT.

Note: we ran out of purple 1 Kb Ladder, I used the blue loading dye with the 1kb transparent ladder to run the gel. Need to order the purple 1Kb ladder or more loading dye cause is low on stock .

3. Washed waste containers and made 1000ml YPD media was made
4. Made glycerol stocks of the RAV2 Cultures, they have been properly labeled but also added a yellow tape to show that this might not be the final stocks depending on the restreak done on Tuesday which has to be analyzed further in the week.
5. Prepare yeast overnight culture and put them on the shaker.

Tuesday July 20th
Paula and Brian

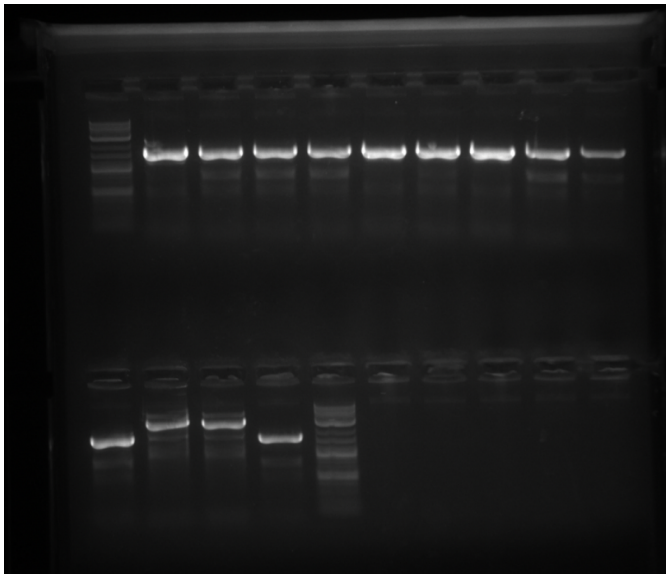
- Stress experiments
    - BTN2 in 5% ethanol

Sample	Absorbance (600nm)	Fluorescence intensity	Intensity/ OD600
5% ethanol	● 0.0442 ● 0.0432	● 311 ● 313	● ●
BTN2 control	● 0.0427 ● 0.0414	● 314 ● 305	● ●
YNB blank	● 0.0408 ● 0.0419	● 339 ● 320	● ●

    - No significant growth despite incubating directly on the plates
    - Will test the OD of the cultures being grown overnight to make sure they are growing properly in the first place.

<u>Colony PCR</u>

- Colony PCR for tuesday RAV restreaks and SAF1
- RAV worked and SAF1 failed.



- Row 1 : Ladder ,SAF1 Colony 1, SAF1 Colony 3 , SAF1 Colony 4 , SAF1 Colony 5 , SAF1 Colony 6 , SAF1 Colony 7 , SAF1 Colony 8 , SAF1 Colony 9 , WT.
- Row 2 : SAF1 Colony 10, RAV2 Colony 1 , RAV2 Colony 2 ,Wild Type, Ladder.

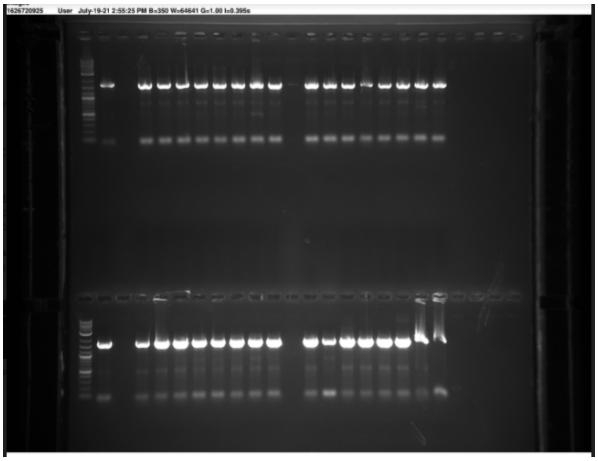
Made liquid cultures of SOD1, BTN2 in YNB, and Wild type for hardware in YPD

Restreaked RAV2 because it was not clean, plate came out more of a lawn than as individual colonies.

Stress Test

- Diluted cultures for the ethanol stress test of BTN2
  - 90 ml of YNB
  - 5 ml of Yeast
  - 5 ml of ethanol
  - 5 ml of H2O
- Control and media blank was made
  - Control : 90 ml of YNB,5 ml of Yeast , 5 ml of H2O
  - Blank : 95 ml media YNB and 5ml of H2O
- Overnight liquid Cultures of RAV2 were made.

Monday July 19th
Gabe and Brian

- Colony PCR on SLT2, SAF1, OXR2, and ARC19
    - All PCR reactions were negative
    - 
    - Top row: ladder, blank, SLT2, blank, SAF1
    - Bottom row: ladder, blank, ARC19, blank, OXR2

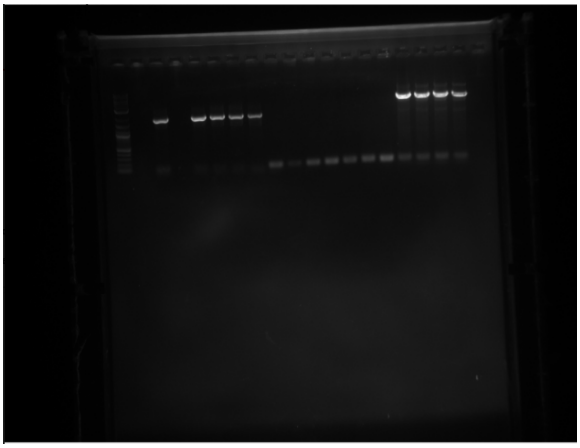
- Stress experiments
    - BTN2 in 5% ethanol

Sample	Absorbance (600nm)	Fluorescence intensity	Intensity/ OD600
YNB Blank	• 0.0529 • 0.0361	• 1408 • 1306	• •
BTN2 control	• 0.0393 • 0.0442	• 1392 • 1412	• •
5% ethanol	• 0.0405 • 0.0380	• 1329 • 1356	• •

    - No significant growth despite adding glucose. May be due to not enough headroom in the microcentrifuge tube. Will try again tomorrow with a 96 well plate

Sunday July 18th
Gabe and Amir

- Colony PCR
    - Samples: wild type control, RAV2 second restreak, OXR2 transformations, SLT2 first restreak

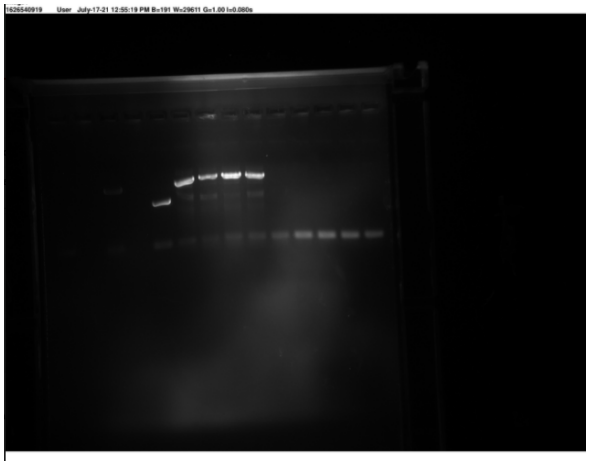
- 
  - Ladder, blank, wt, blank, OXR2 (1-5), SLT2 (1-5), RAV2 (1-5)
    - All RAV samples were positive but since there is a faint WT band, it will be restreaked for a third time to ensure purity
    - All OXR2 samples were negative
    - All SLT2 reactions failed
      - Will redo PCR with same reactions
- BTN2 Ethanol stress experiment
  - Following newly updated protocol, except using culture tubes instead of centrifuge tubes: Protool - Stress protocol version 2
  - Tested BTN2 control with no ethanol and with 5% ethanol
  - We read the

Sample	Absorbance (600nm)	Fluorescence intensity	Intensity/ OD600
YNB Blank	• 0.0369 • 0.0364	• 2529 • 2510	• 68537 • 68956
BTN2 control	• 0.0414 • 0.0407	• 2603 • 2650	• 62874 • 65110
5% ethanol	• 0.0478 • 0.0407	• 2374 • 2363	• 49665 • 68059

  - Not enough growth occurred over the span of 4 hours in YNB media for this to work properly
    - Apparently glucose not part of YNB mixture, will have to be added and reattempted tomorrow
- General observations
  - Removed 96 well plate from clinostat for measurements, found that water had not yet evaporated
    - Suggests that we are only going to have to change the media for dilutions, not because media is evaporated

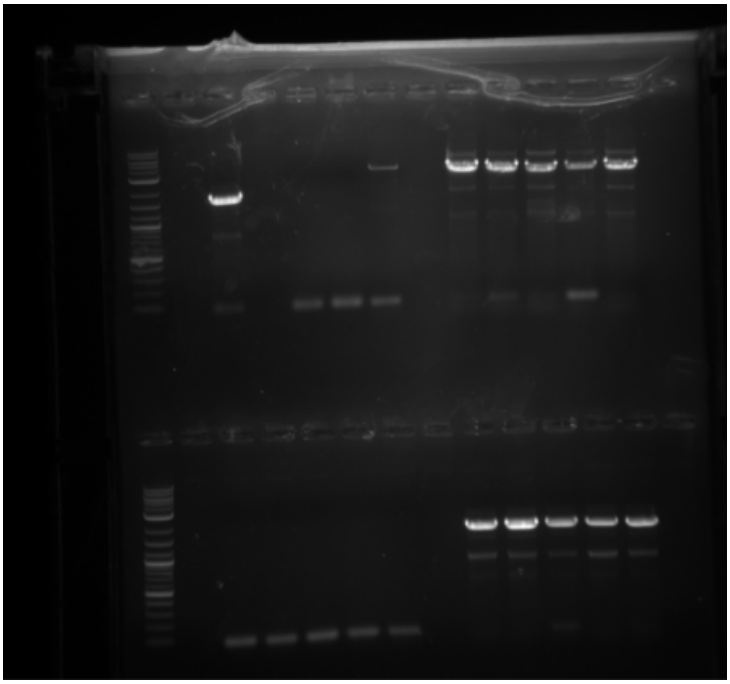
Saturday July 17th

Gabe, Natasha, Amir

- Colony PCR for OXR1 (transformation) and SLT2 (1st restreak), 5 replicates of each
    - Protocol: Colony PCR
    - Gel picture:
        - 
            - Ladder, blank, WT, blank, OXR1 (1-5), SLT2 (1-5)
    - Results inconclusive
        - SLT PCR failed
        - OXR PCR likely negative but difficult to determine due to gel quality
        - Both samples will be redone tomorrow
- Plated transformation from July 16th (Arc19 and SAF1)
    - Stored in the 30ºC room
- Made glycerol stock of SOD2
    - Stored at -80ºC
- Prepared overnight culture of BTN2 in YNB media

Friday July 16th
Gabe, Paula, Nhi

- Colony PCR
    - Plate SOD2 A thrown out because of redundancy
    - SOD 2 A and B were glowing entirely
    - 3 samples SOD 2, 5 samples OXR 1, 5 SLT 2, 5 RAV 2, and 1 WT
    - Protocol used: Colony PCR



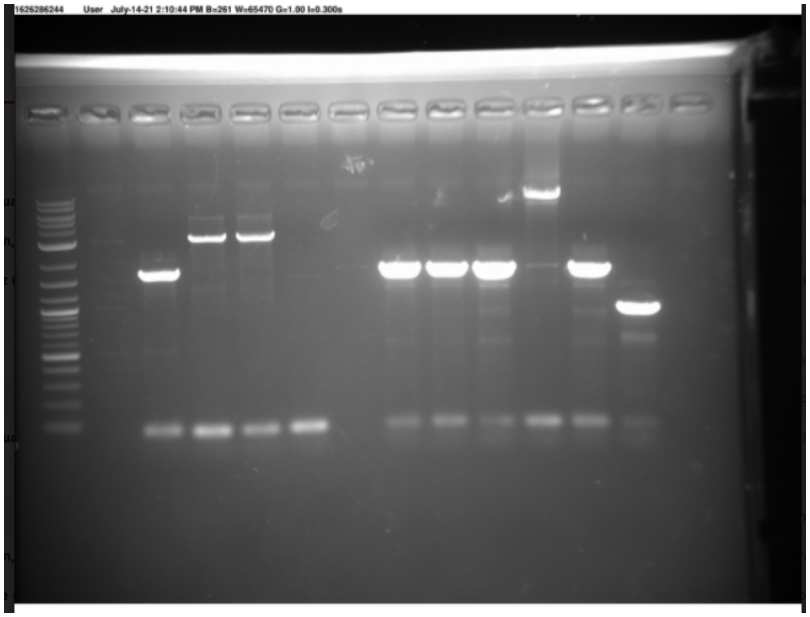
    - SOD sample 3 positive
        - Second restreak so liquid culture will be made (3 mL)
    - All RAV samples positive
        - Will be restreaked
        - Restreaked on YPD agar plate, split into 5 sections, one per sample
    - All SLT PCRs failed
        - Inconclusive, must repeat
    - All OXR samples negative
        - Must repeat
- Transformations
    - Cells diluted at 10 am

- Maintenance
    - Prepared YNB media
    - Resuspended IDT order (colony pcr REV and FWD)
    - Autoclaved water

Thursday July 15th
Paula

- Prepared SAF 1 primers for tomorrow's transformation
- Checked that all the needed parts for the transformation were ready
- Prepared glycerol stock for HSP30 colony and stored in the -80 °C.
- SAF 1 purified and prepared for tomorrow's transformation, concentration is 133.3 ng/u
- Colony PCR was not run, plates that were in the warm room were moved to the cool room in the iGEM box .
- YNB Media was not made.

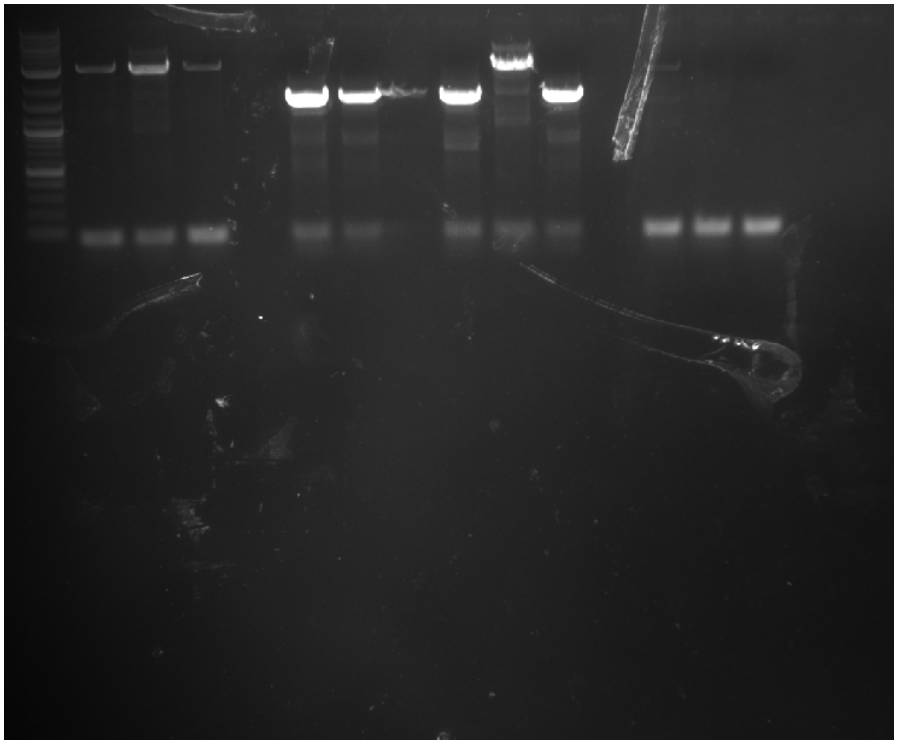
Wednesday July 14th
Gabe, Amir
Ran colony PCR with following protocol: Colony PCR



- Ladder, HSP 30 (1,2,3), Blank, OXR1 (1-6)
- OXR1 sample 4 has a higher band but since this is too high for the insert, it can be considered anomalous
- Stress experiments on hold until YNB media made as YPD is fluorescent
- HSP30 incubated in liquid media for tomorrow to be frozen

Tuesday July 13th
L & Brian

1. Ran colony PCR with the 3 glowing SOD2 colonies again, all 5 RAV sample possibilities, and 3 SLT colonies.
    - We limited SLT to 3 colonies due to shortage of PCR tubes, some red circled colonies without numbers went unused due to this
    - All colonies taken were circled with numbers
2. Ran a gel

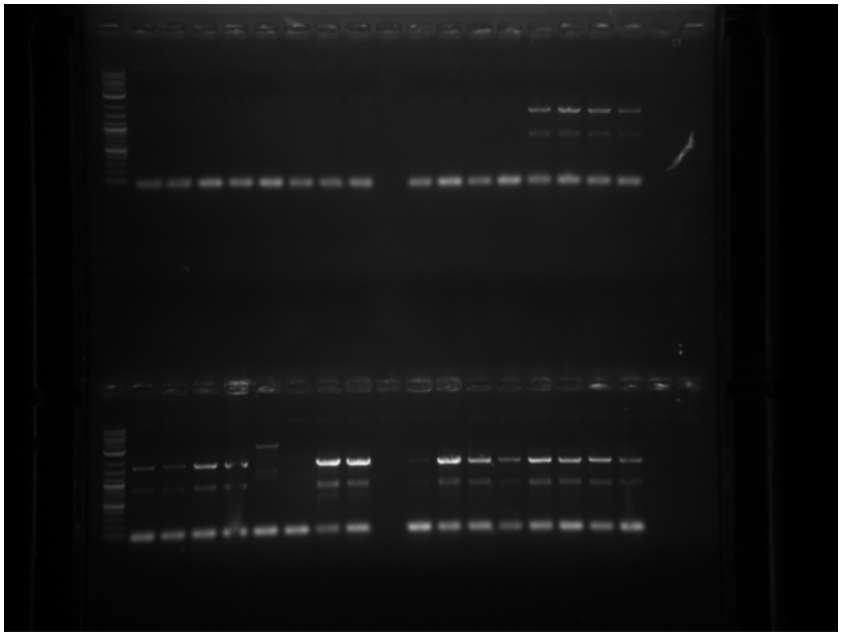


3. Restreaked success'
   RAV2 (#4)
   SOD2 (#2 & #3 → relabelled as A and B), third seems a little cleaner
   SLT2 (#1)
   In bag with our other plates in culture room

4. Prepared more TAE buffer
5. Autoclaved more PCR tubes (now out) and 1.5mL eppendorf tubes along with multiples 200microL pipette tips
6. Added preparing liquid cultures and making a gel to the recipes doc

Monday July 12th
Gabe and Brian

- Ran colony PCR of SOD2 and HSP30 restreaks and remaining transformations



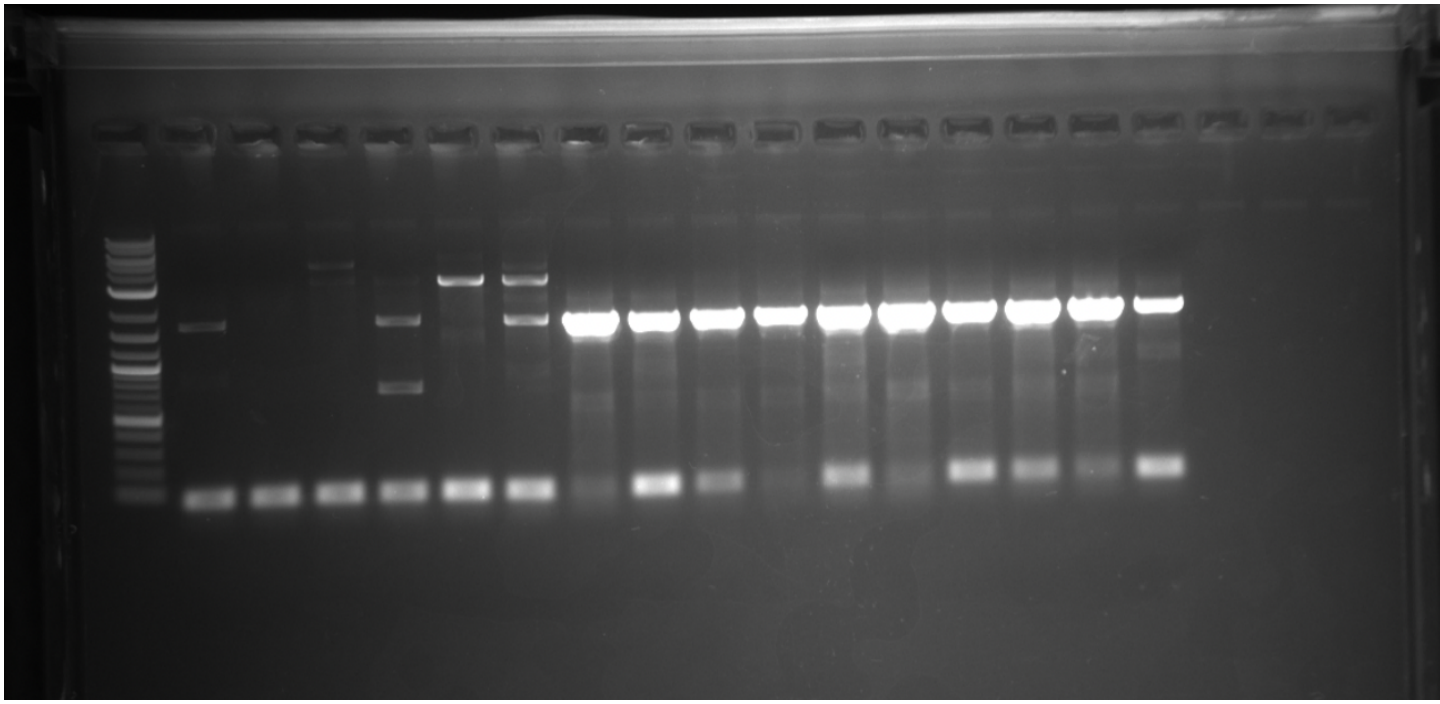
Top row: ladder SOD2 (1-3), HSP30 #2 (1-3), HSP30 #3 (1-2), blank, HSP30 #3 (3), SLT2 (1-3), Gal10 (1-4)

Bottom row: RAV2 (1-5), ARC19 (1-3), blank, WT, Saf1, OXR1 (1-6)

- While sample RAV 5 was mixed, some was not put aside for restreaking so that must be redone
- All samples must be redone, other than Gal10, which we will stop culturing
- Restreaked HSP30 sample 2 from Saturday as a pure colony was found on the transformation plate
- RAV samples must be run again in order to obtain culture with gene of interest
-

Saturday July 10th
Paula and Brian

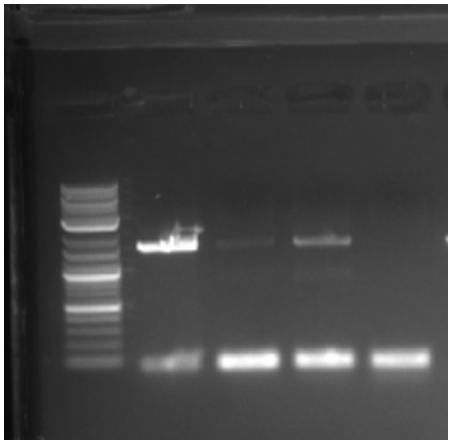
- Colony PCR ran from transformation plates,
    - 4 Colonies picked for HSP30
    - 2 Colonies picked for SLT2
    - 1 Colonie picked for Wild Type
    - 1 Colonie picked for SOD2
    - 8 ARC19
- We also prepared water and tip boxes to be autoclave, we don't have small tips anymore.
- Ran the Gel for the PCR and got the following results :



- Ladder
- SLT2 Colony 1
- SLT2 Colony 2
- SOD2
- HSP30 1
- HSP30 2
- HSP30 3
- HSP30 4
- ARC19 1
- ARC19 2
- ARC19 3
- ARC19 4
- ARC19 5
- ARC19 6
- ARC19 7
- ARC19 8
- Wild Type

Friday July 9th
Paula and Brian

- Colony PCR ran from the restreak of SLT2,
- Ran the gel, and got the following results

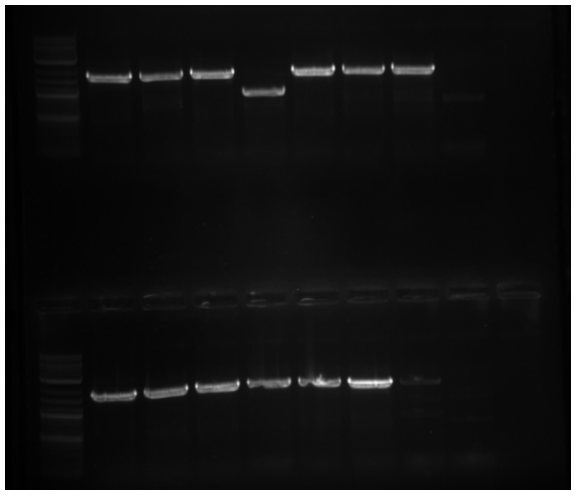


1- Ladder
2- Wild Type
3- SLT2 1
4- SLT2 2
5- SLT2 3

- We made culturing plates

Note **:** two orders were picked up. Microscope slides, hydrogen peroxide, two bags of 25 plates and M/L gloves.

Thursday July 8th
Gabe and Natasha

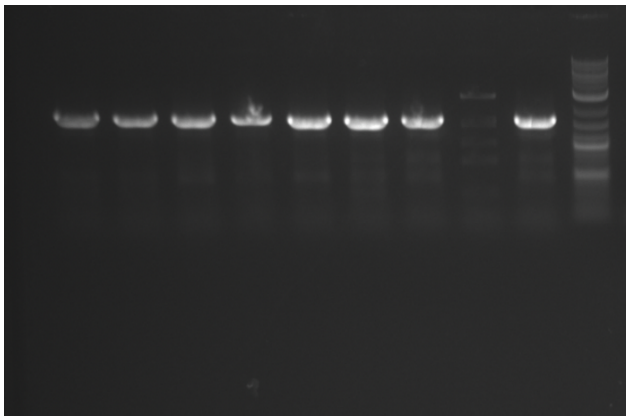
- In the morning an advisor helped run a colony PCR for Arc19, Gal10, Hsp30, Oxr1, Rav2, Saf1, Sod2 transformation (in duplicates)
    - Protocol: Colony PCr
- We ran the gel for that PCR
    - Picture:
        - 
    - Row 1 (duplicate 1): ladder Arc19, Gal10, Hsp30, Oxr1, Rav2, Saf1, Sod2

    - Row 2 (duplicate 2): ladder Arc19, Gal10, Hsp30, Oxr1, Rav2, Saf1, Sod2
    - All worked (were transformed) except duplicate 1 of Oxr1
- Did restreak 1 (2uL) or the samples that worked (stored in cold room)
    - For Arc19 and Gal10 = used duplicate 1 on one half, and duplicate 2 on the other half
    - For Oxr1 = used duplicate 2 (2uL on each half of the plate)
    - For Hsp30, Rav2, Saf1 and Sod2 = used duplicate 1 (2uL on each half of the plate)
- Made 1L of YPD media

Wednesday July 7th - Brian and Paula

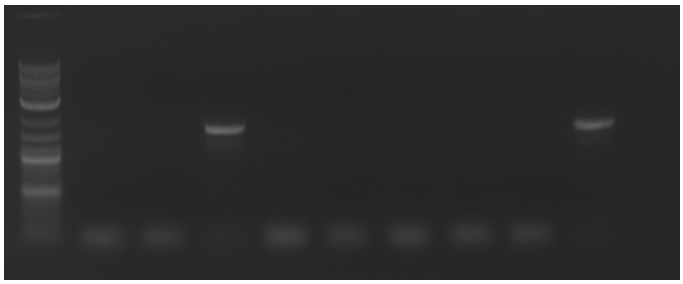
- We ran the gel for the PCR plate map, SLT2 Triplicates were the only sample that partially worked. Mixed colonies, needs restreaking
- Purpose is to verify both positive colonies and to compare the efficiency of the GoTaq polymerase and the phire polymerase

Mo's PCR (using Phire polymerase)



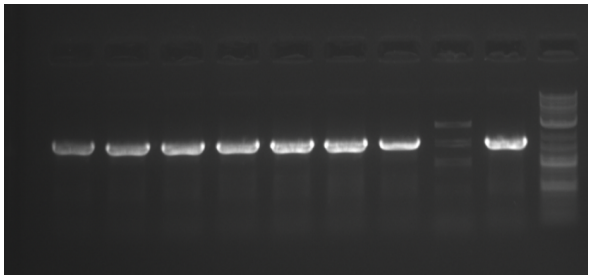
1- HSP30 1
2-ARC19 1
3- OXR1 1
4- SAF1 1
5- RAV2 1
6- SOD2 1
7- GAL10 1
8- SLT2 1
9- Wild Type
10 - Ladder

GoTaq polymerase Samples (our polymerase)



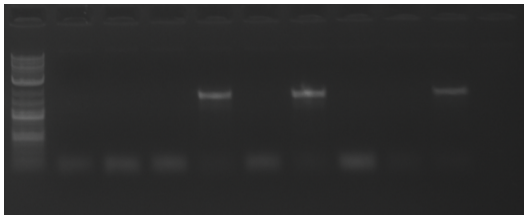
1 - Ladder
2- HSP30 1
3-ARC19 1
4- OXR1   1
5- SAF1   1
6- RAV2   1
7- SOD2   1
8- GAL10 1
9- SLT2 1
10- Wild Type

Mo’s PCR (Phire polymerase)



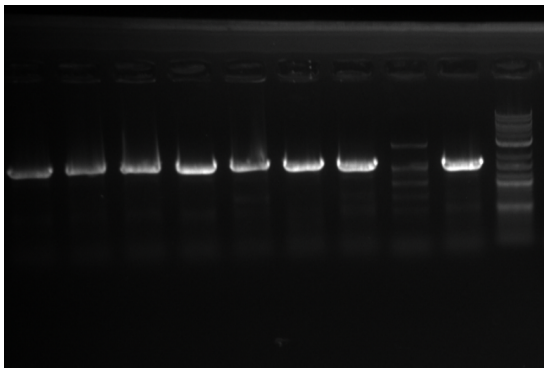
1- HSP30 2
2-ARC19 2
3- OXR1   2
4- SAF1   2
5- RAV2   2
6- SOD2   2
7- GAL10 2
8- SLT2 2
9- Wild Type
10 - Ladder

GoTaq polymerase Samples (our polymerase)



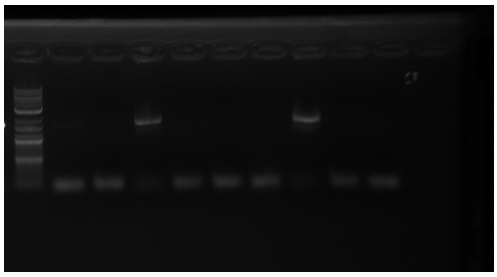
1- Ladder
2-HSP30 2
3-ARC19 2
4- OXR1   2
5- SAF1   2
6- RAV2   2
7- SOD2   2
8- GAL10 2
9- SLT2 2
10- Wild Type

Mo's Samples (Phire polymerase)



1- HSP30 3
2-ARC19 3
3- OXR1   3
4- SAF1   3
5- RAV2   3
6- SOD2   3
7- GAL10 3
8- SLT2 3
9- Wild Type
10 - Ladder

GoTaq polymerase Sample (our polymerase)



1- HSP30 2
2-ARC19 2
3- OXR1   2

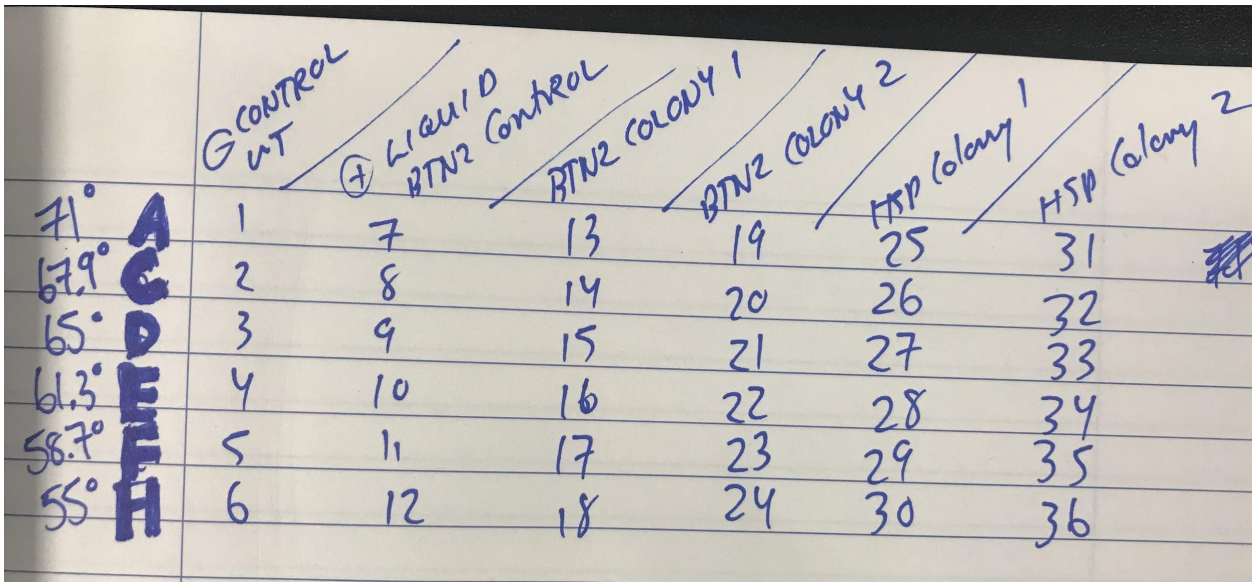
4- SAF1 2
5- RAV2 2
6- SOD2 2
7- GAL10 2
8- SLT2 2
9- Wild Type
10 - Ladder

- Sample were restreak SLT2 triplicates
- We also made some TAE
- Our polymerase does not work well ...

Tuesday July 6th
Gabe
-prepared samples for advisor to run colony pcr.
Nhi

- Run the gel: PCR failed
-

L & Brian



→ **Colony PCR details**

- **Lanes**
  - Negative Wild Type Control
  - Positive Liquid BTN2 Control
  - BTN2 Colony 1

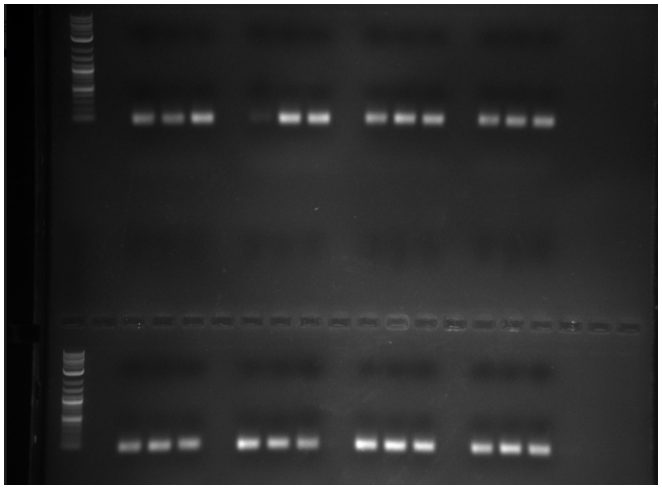
    - BTN2 Colony 2
    - HSP Colony 1
    - HSP Colony
- **Colonies were taken from the first restreak**
- **Used GoTaq, autoclaved water, Mo's primers and boiled cells in thermocycler**
- **Different temperatures for rows (see above)**
- **Liquid PCR protocol used for BTN2 liquid control**

PCR procedure: 95C (2min), Repeat 40x: 95 (30sec), [Temps above (1min), 72 (1min), After 40x 72 (5min), 4 (hold)

Glycerol stocks of SOD1 and BTN2 were made hopefully??

Monday July 5th
Gabe

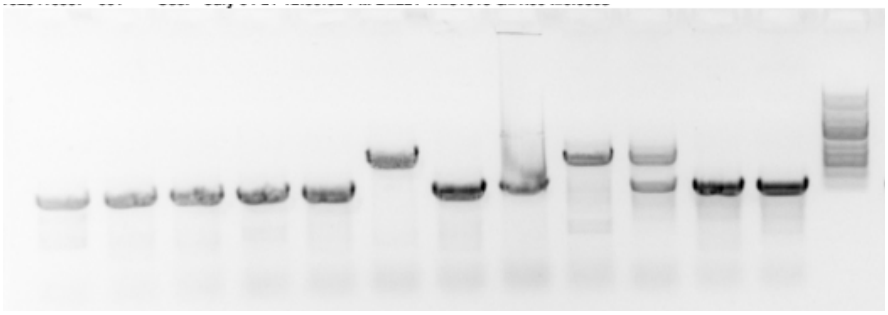
- Ran colony PCR for hsp30, arc19, rav2, saf1, gal10, sod2, slt2, oxr1
    - Used protocol as stated here with no alterations: Colony PCR
    - All preparations failed



- Prepared fresh YPD media
- Filtered glycerol

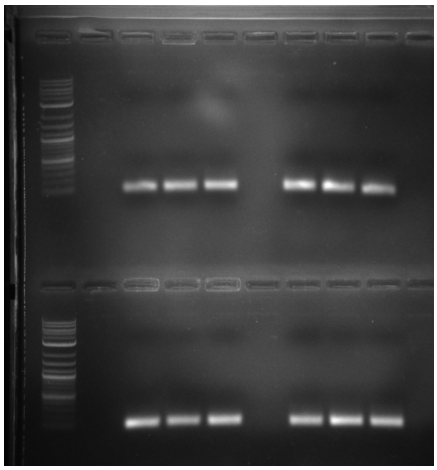
Sunday July 4th
Gabe, Natasha, Nhi

- Mentor did colony PCR for triplicates of SOD1, BTN2, OXR1 and SAF1 (using liquid cultures started on July 3rd)
    - Protocol: Colony PCR
        - Mo did not use this program for the thermocycler, he used a normal PCR (not touchdown). He uses a different polymerase, and the annealing temperature used was 72^{o}C (same as extension).

- Gel:
  - 
  - From left to right: BTN2 1, SOD1 1, SAF1 1, OXR1 1, BTN2 2, SOD1 2, SAF1 2, OXR1 2, BTN2 3, SOD1 3, SAF1 3, OXR1 3, ladder
  - SOD1 2 and BTN2 3 both worked and are pure (no wt band)
- SOD1 2, and BTN2 3 worked, so made glycerol stock of each and stored at -80°C
- Started liquid culture for: HSP30, ACR19, OXR1, SAF1, RAV2, SOD2, GAL10, SLT2 (each in triplicate) … in 30°C shaking incubator

Saturday July 3rd
Gabe

-Cultured BTN2-restreak-2-group 3
-overnight culture
-1:20 dilution in 5 mL of YPD (two sets)
-After 3 hours, 400 uL of 6 M HCl added to one to try to induce glowing
-Non-acidic colony began to glow (perhaps pH was too low and killed yeast)
-Ran gel for previous colony PCR (shown below)
-Top row: SOD1 at 65, 60, and 55 degrees, and duplicate
-Bottom row: WT and genomic DNA at 65, 60, and 55 degrees



Prepared liquid cultures for colony PCR
-From second restreaks of BTN2, SOD1, SAF1, OXR1
-Plate map here: Colony PCR plate map

Friday July 2nd

To do:

-Run colony PCR (touchdown protocol)
-Prepare with just water
-Preps to do:
WT and normal volumes for one and the other 0.75 uL of each primer
SOD1 (the glowing colony) normal prep and with 0.75 uL of each primer for the other prep
One prep using normal protocol and genomic DNA
-5 preps total (two WT and two SOD1)

L

1- Ran colony PCR without NaOH (only water and varying primer volumes):

Prep A: 'Normal Prep'
(A-W) Wild type
(A-S) SOD1- glowing
(A-G) Genomic DNA

Prep B: dif primer volume→ 0.75ul
(B-W)
(B-S) SOD1-glowing
(B-G)

→ "1,2,3" is ID of colony picked, ie #1 is the same colony and A/B are dif protocols
*** No NaOH, only H2O
*** used TouchDown Setting on Thremocycler

- Did two genomic DNA and 3 each of WT/ SOD protocol A and B.
- PCR tubes of cooked and non- cooked labelled in walk in fridge in falcon tubes

2 -put two of the falcon tubes in a bag in our bin in the walk-in so they're not always falling on the floor when we open the door (plasmids and….one other)

3- Prepped and ran gel for COLONY PCR from Wednesday Gel (1%), photo

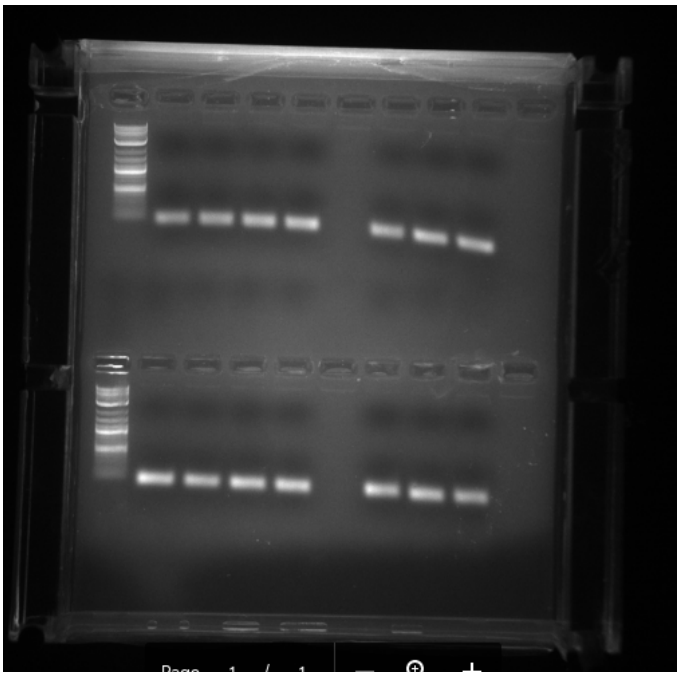
- Used1kB purple ladder (I used way too much though 30ul was 3ul oops),
  Lane order: Ladder-Genomic-Sod1 (1000bp)-WT
- **Fail**, bands ran off. End of gel looked melted?

Notes:

→ order small gloves please
→ Need to make TAE, can we please put a protocol in the folder under the Protocol titled (dilute it 40x)
→ Only one culturing plate left in the cold room box

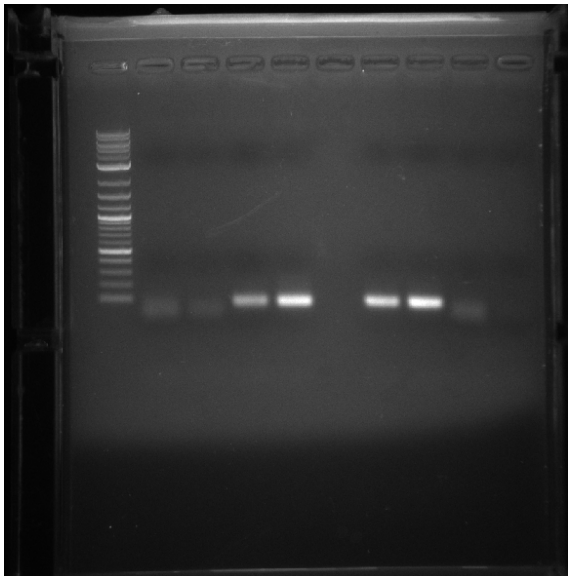
Gabe

Top: A series of replicates (Genomic, WT triplicates, SOD1 triplicates)
Bottom: B series (Genomic, WT triplicates, SOD1 triplicates)
Prepared culture of BTN2 for verification tomorrow
Colony PCR to verify proper temperatures
    Wild type, SOD1, and genomic DNA at 65, 59, and 55 degrees
    Microwaved for 2 minutes 45 seconds instead of in thermocycler



Wednesday June 30th

- New PCR Colony started. Adjustment to the procedure includes removing NaOH and just boiling in water. Ran with SOD1 , wild type and genomic DNA.
- PCR Colony samples from yesterday were run and the gel was a ...

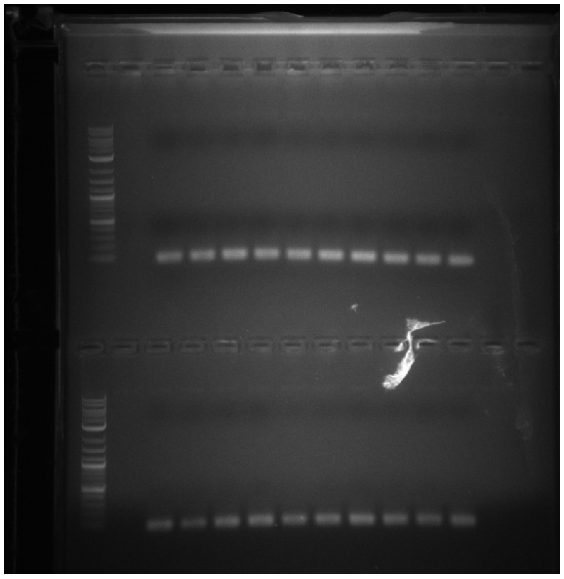


- Gabe's tube basic culture was inconclusive and needs to be cultured in minimum media and needs to be compared to some control .

Tuesday June 29th
Gabe and Brian

-SOD1 works (green colonies), still need to confirm with colony PCR
-Overnight culture of BTN2 incubated with 100 uL of 6M HCl, check tomorrow to see if it glows under UV

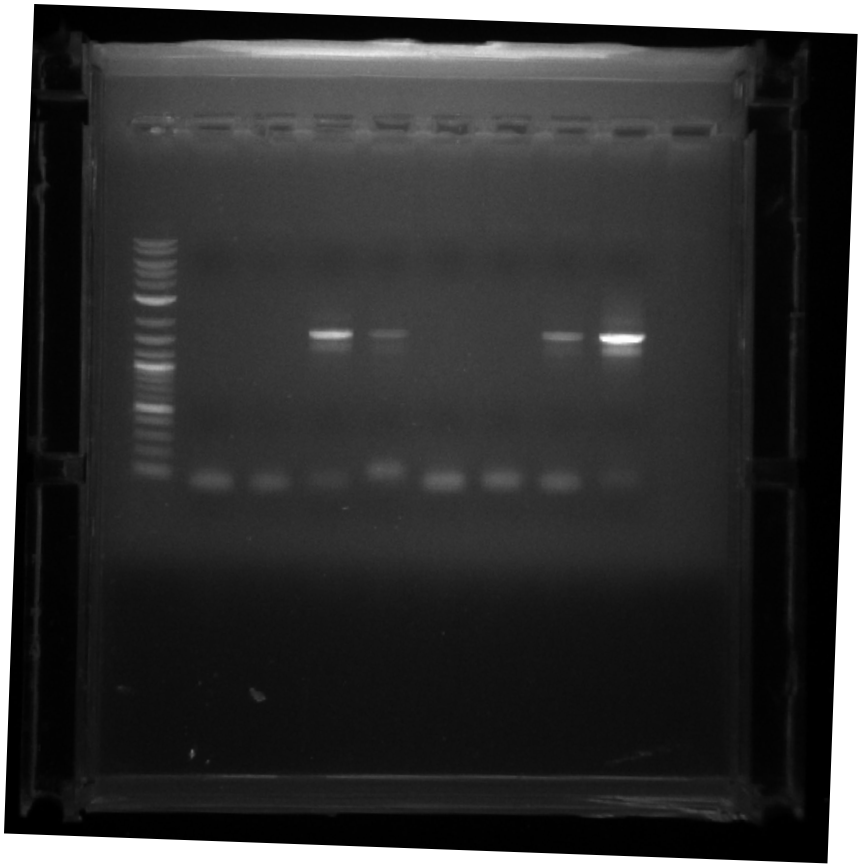
Slt
SOD1
BTN
RAV
SOD2
ARC
OXR
Gal
HSP
SAF



Colony PCR with adjusted protocol
-Do with 4 mM NaOH and 0.2 mM LiOAc
-SOD1 (since we know it works) and WT as a control

Monday June 27th
Brian

1. Prepared Gel and loaded samples
    - Used standard 1microL loading dye and 5microL DNA as there was not enough sample to double as suggested in the colony PCR procedure
    - Messed up a gel so got the pleasure of repeating it, they are <u>NOT</u> as bendy as you might think



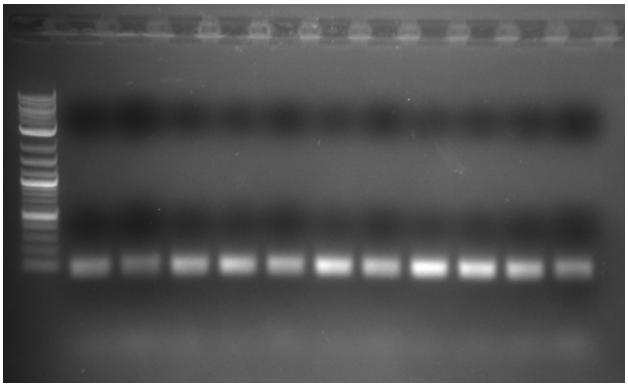
2. Procedure worked the proper transformation not so much
3. Did the colony PCR procedure again for all of the first restreaks from the successful transformations
    - Left overnight after PCR to be run in the gel tomorrow
    - Did duplicates of each
    - Kept a water culture in green rack in the freezer in case colony PCR was a success
    - Kept the NaOH culture in the same green rack in case the gel was somehow messed up before results could be obtained.

Sunday June 26th
Natasha

- Troubleshoot the colony PCR protocol
    - Used 4 colonies on the 2nd restreak plate for SAF1 (#2)
        - Circled on plate which colonies used
    - For control, did 2 with colonies from the WT (BY7471) plate, and 2 using genomic DNA
    - Used our primers and mentor's primers
    - Samples/tube labelling

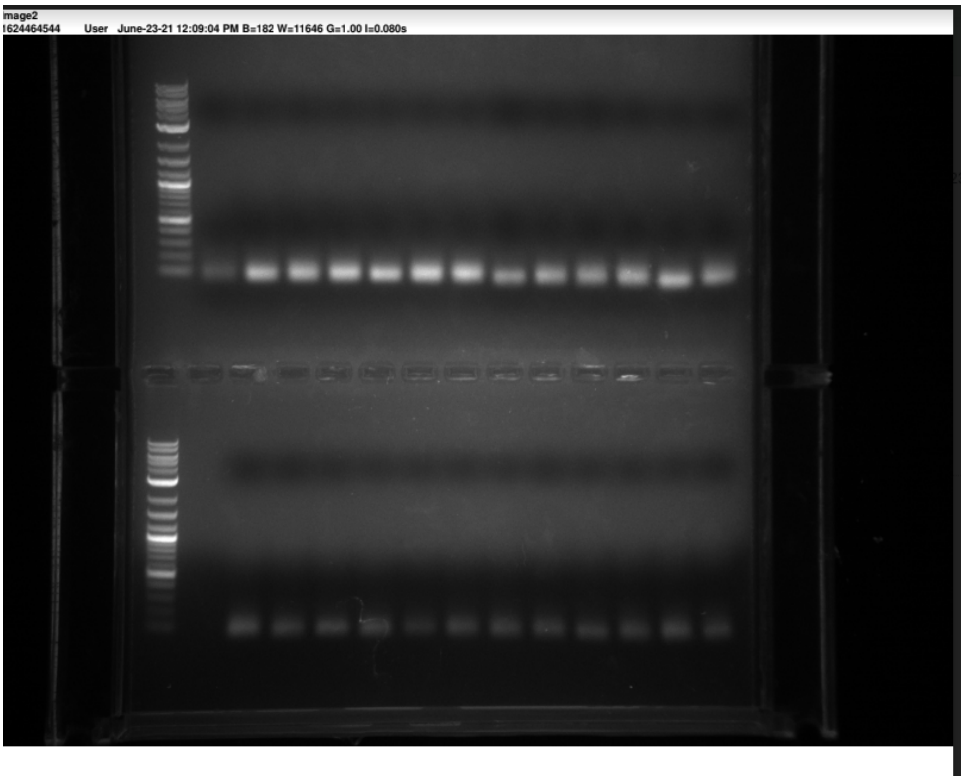
        - 1 = first replicate, using our primers
        - 2 = second replicate, using our primers
        - 3 mentor = third replicate, using Mo's primers
        - 4 mentor = fourth replicate, using Mo's primers
        - 1 crtl = control using WT colonies, using our primers
        - 2 ctrl mentor = control using WT colonies, using Mo's primers
        - 1 ctrl DNA = control using genomic DNA, using our primers
        - 2 ctrl DNA mentor = control using genomic DNA, using Mo's primers
    - Protocol used:
        - Colony PCR
        - I did not restreak the samples since this is just a troubleshoot
        - I just did up to step 7 (I did not do the gel)
        - Note: what Is written in this protocol for the PCR is slightly different than what is saved on the thermocycler for touchdown, I used what is written on this protocol.

Thursday June 24th
Gabe and Natasha

- SLT2 did not grow well (lawn vs individual colonies)
    - Restreaked SLT2 streaked on another G418 plate (from June 22) to try to remove the lawn/clean it up
- Did colony PCR for Rav2, Gal10, SOD2, Hsp30 and Arc19
    - Included control which consisted of a colony from wild type yeast (instead of genomic DNA)
    - Protocol: Colony PCR
        - For PCR, did 40 cycles instead of 35 cycles
        - Used 4uL of orange blye 6X loading dye instead of Anza red buffer
    - Gel Picture:
        - Ladder, control, Arc19 1-2, SOD2 1-2, Hsp30 1-2, Gal10 1-2, Rav2 1-2
        - 
- First restreak of Rav2, Gal10, Sod2, Hsp30, and Arc19 on antibiotic free plates

Wednesday June 23rd
Gabe

-Plates checked - all plates seem to be growing (no need for more transformations)
-Colony PCR gel run
    -4 uL of blue orange dye
    -10 uL DNA
    -8 uL water
-Colony PCR failed, try again tomorrow



-Restreaked “first pick” of SAF1, SOD1, OXR1, BTN2

Tuesday June 22nd
L

Gabe to order:

- Reagent Reservoirs- 50ml for multichannel pipetting, the pic I sent on Facebook.

Completed: Streaked transformation and did colony PCR (samples put away by Mo in two falcon tubes labelled as “Transformations Colony PCR Products”)

- Streaked transformations SOD2, HSP30, GAL10, RAV2, ARC19 and SLT2 on G418 plate
- Put in 30C in bag
- Colony PCR of restreaks: SOD1 (8), OXR1 (3), SAF1 (13), BTN2 (7)
    - Used GoTaq 5uL, Mo’s primers (FFUP) 0.5ul, Mo’s primers (FFDown) 0.5ul, 3.5ul nuclease free H2O and 0.5ul cooked cells from streaked plates.

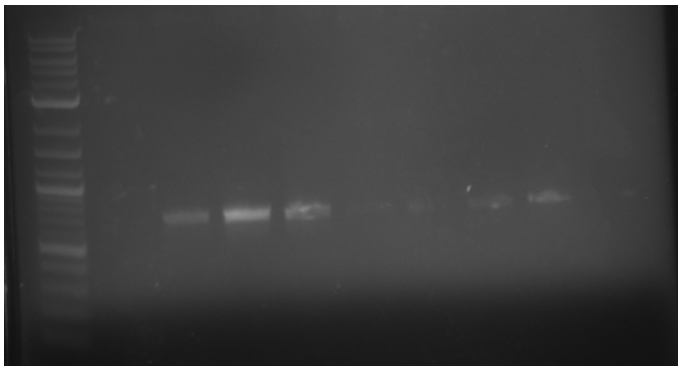
        - Thermocycler Protocol saved under iGEM folder as “iGEM Touchdo”, called the “Touchdown” protocol
        - 95, 2min
        - REpeat x12
            - 95, 30s
            - 72, 30s
            - 72, 5min
        - Repeat x23
            - 95, 30s
            - 60, 30s
            - 72, 5min
        - 72, 5min
        - Hold
- Samples: two colonies from each streak were taken. 3 streaks for each promoter. So 12 colonies, 2 samples each. The labelling is a little weird sorry. But SAF1 (1-3), SOD1 (4-6), BTN2 (7-9) and OXR1 (10-12). The ones that have a ‘dot’ are the ‘second picked transformations’.
- The H2O and NaOH samples are in the walkin fridge in falcon tubes. Streaked plates too.

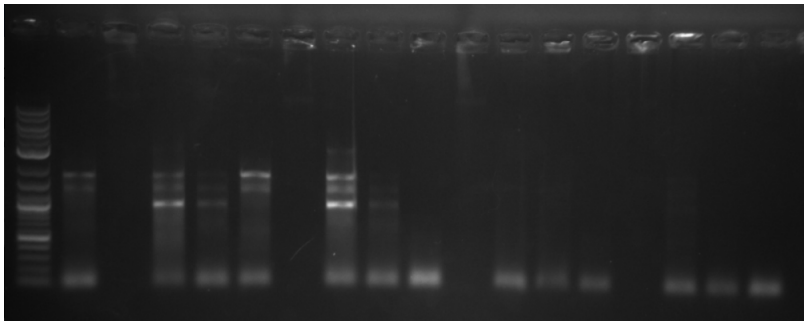
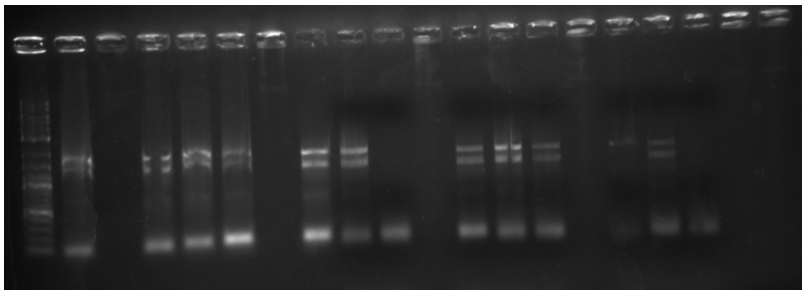
Monday June 21st
Morning Gabe
-Diluted yeast for transformation

Afternoon Paula
- OD for the yeast dilutions
    - Tube 1: 0.96, this one was used for the transformations.
    - Tube 2: 0.77
- Yeast transformations for the following promoters were done:
    - HSP30
    - SOD2
    - SLT2
    - ARC19
    - RAV2
    - GAL10
- DNA samples were made using the table date June 21st, on the following spreadsheet: Yeast transformation concentrations sheet
- Protocol used for Yeast transformation: Yeast transformation protocol updated
- Transformed samples were stored in the 30 degree celsius room on a pink rack label iGEM to growth overnight
- A YDP stock was put on the waste because it was contaminated

Sunday, June 20th

Natasha and Nhi

- Amplified more GFP
    - PCR
        - Reaction mixture:
            - GoTaq (25uL)
            - GFP-envy DNA template (1uL)
            - Autoclaved water (19uL)
            - Primers (2.5uL each)
        - Thermocycle program
            - Denature: 98^{0}C 30s, 98^{0}C 10s,
            - Annealing: gradient (2 at 54^{0}C, 2 at 57^{0}C, 2 at 59^{0}C)
            - Extension: 72^{0}C 30s
            - Final extension: 72^{0}C 5min
            - Hold: 20^{0}C
    - PCR clean-up
        - Protocol on drive
        - Started with “B. Processing PCR Amplifications”
        - Eluted DNA with 20uL water (not 50uL)
    - Ran gel
        - 5uL DNA + 1uL 6X loading dye (the green one)
        - Photo of the gel:
            - Ladder, 2 lanes are samples with annealing temp of 54^{0}C, 1 lane with 57^{0}C and then 2 lanes with 59^{0}C
            - 
            - Had difficulties loading the gel (the samples kept mysteriously coming out of the well), which is why the gel doesn’t look the nicest. All samples amplified GFP, so they were all pooled
    - DNA quantification
        - 300ng/uL (stored in box #1 in -20^{0}C)
- Ran gel for colony PCR done on June 19th
    - 10uL DNA + 2uL Anza red buffer + 8uL water
    - For samples SOD1 2+3, OXR1 1-3, BTN2 1-3 (all with Mos primers) used 4uL of 6X loading dye (green) since we ran out of the red dye
    - Picture of gel:
        - Both pictures: ladder, negative control (genomic DNA), SAF1 1-3, SOD1 1-3, OXR1 1-3, BTN2 1-3

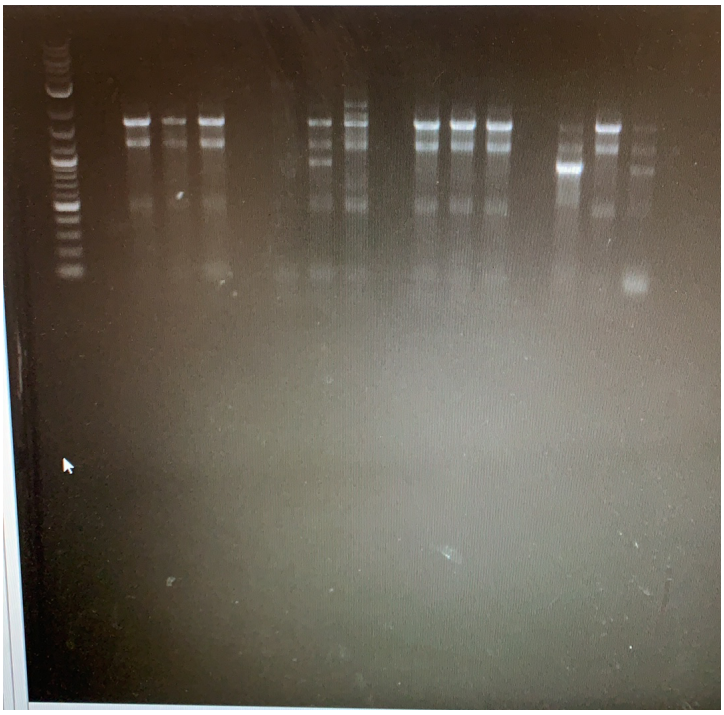
        - With our primers:
        - 
        - With Mentor's primers:
        - 
        - Does not seem to have worked (no bands larger than what is seen in control)...
- Started 2 tubes of yeast culture
    - 2 tubes, 5mL YPD media and 250uL yeast in each tube
    - In 30^0C room on shaker

Saturday, June 19th
Gabe and Natasha

- Did colony PCR on SAF1, SOD1, OXR1 and BTN2 (from plates streaked on Wednesday June 16th), in triplicates
    - Protocol: Colony PCR
    - Did PCR (step 6 of protocol) with our primers (annealing temp = 60°C, and with Mo's primers (annealing temp = 66°C)
    - Included a negative control (for both sets of primers), which included genomic DNA instead of boiled resuspension
- Restreaked 2uL of each of the unboiled resuspensions (in the 30°C room)

Friday, June 18th
L and Paula

- Checking colony growth on plates SAF1 (no antibiotic), BTN2, OXR1, SOD1 are all successful; HSP30 failed
- Performing colony PCR on SAF1, BTN2, OXR1, SOD1
    - Check via gel

- Plate on non-antibiotic plates
- Put away SAF1 plates (+ the one in the 30C room) and others in ziploc bag in fridge
- 20ul sterile H2O, 20ul 40mM NaOH 98C 5min
    - Then 5uL GoTaq, 0.5uL FFUP forward, 0.5uL FFDown reverse, 0.5uL cooked cells (genomic), 3.5 uL nuclease H2O
    - 95C (5min)
      Repeat: 95 (30sec), 60 (1min), 72 (1min)]
      72 (5min)
      hold 4C
- PAULA
    - After PCR run a gel to confirm size of products
    - 
        - A-SAF1
        - B-BTN2
        - C- OXR1
        - D-SOD1
        - If successful, pick colonies from plates in fridge (which you put away earlier) and suspend in 40uL autoclaved water. Streak on NO G418 plates. Place in ziploc in 30C

- Do GFP PCR
    - Use GFP template (instead of genomic DNA), green tube
    - #6 GFP Envy forward & #6 GFP Reverse (1:10 dilution autoclaved water)
    - Use volumes here: PCR fragment checklist
    - Use 3 temperatures. 5 below melting temp, 2 below melting temp and melting temp = 54C, 57C, & 59C

    - Tomorrow: PCR cleanup use ‘not gel’ (use 20uL of water per tube for elution, 1 tube per PCR tube)
    - Quantify DNA

To be done:

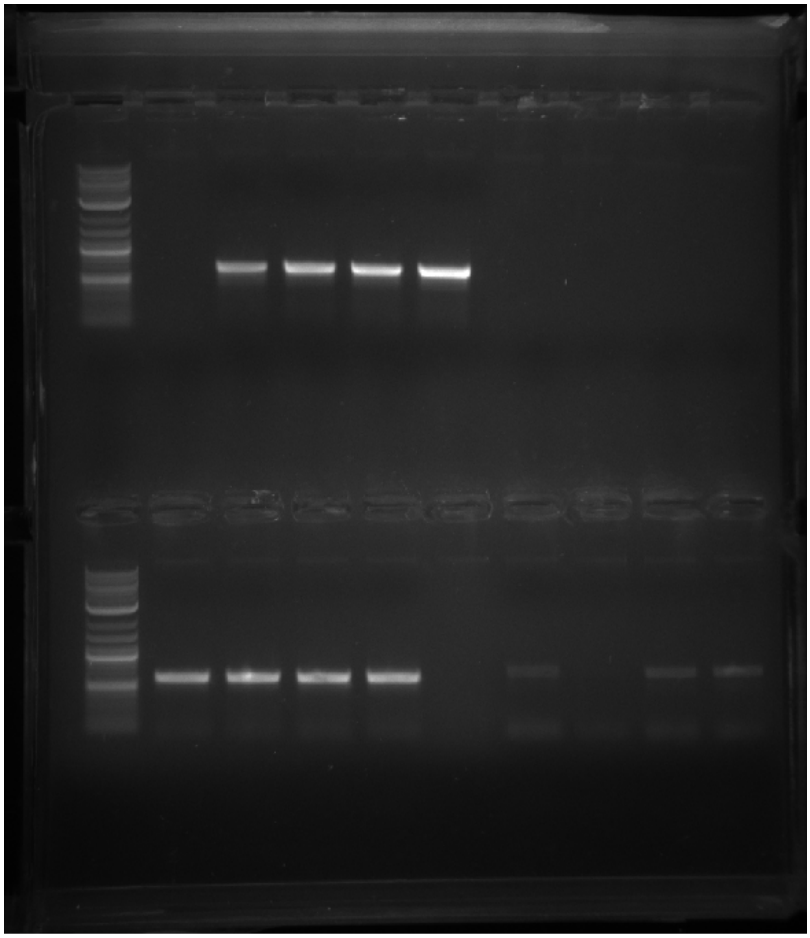
- Do colony PCR of SAF1 on antibiotic-free plate (use 60 degrees), FFup and FFdown primers
    - Colony PCR protocol
- Put both SAF1 plates (one on bench and one in 30 degree in zip lock and put in fridge) (seal with parafilm and put in a ziplock and put in the cold room)--> We couldn’t find the one in the 30C room - found it on the bench also
- GFP PCR (dilute GFP template 10x from stock in a separate tube with nuclease free or autoclaved water and use that (100 ng/uL)
- Check plates for growth
- Colony PCR any that grew and restreak on antibiotic free plates

Thursday June 17th
Brian

Objectives:

- Run gel for 12 PCR samples from yesterday
- Perform DNA purification of PCR samples
- Quantify DNA concentration
- Prepare genomic DNA extraction
- Check plates for growth (takes 2 days so if empty, don’t throw out)

1. Decided not to do colony PCR as tomorrow we will likely have more plates and the procedure doesn’t add a lot of work with the more samples

2. Gel: 
3. Gel purification is performed. GFP was only seen in 3 of 4 samples still all were pooled together in case small amounts of DNA were there that can be obtained through the purification process. Samples were diluted into 22.5 microL rather than 50 microL in order to get higher concentration

4. DNA quantification.
   Sample 2 (FFd) - 554.5 ng/microL, 1.83, 20 microL after quantification
   Sample 5 (FFu) - 404.5 ng/microL, 1.83, 20 microL after quantification
   Sample 6 (gfp) - 145.2 ng/microL, 1.82, 20 microL after quantification

5. Yeast genome DNA: Found 19 microL of genomic yeast DNA 54 ng/microL and placed in box 1, this is enough for another 19 PCR's so decided not to continue with the yeast genome extraction

6. Maaaaaybe I was looking at little colonies on the transformation plates, we will have to see tomorrow.

Wednesday June 16th

L

- No more yeast genomic DNA
- Did PCR for FFUP (5), FFDown (2) and GFP (6)
    - Used GoTaq- protocol: Promega goTAQ protocol
    - Gotaq 100uL, Genomic 6uL, Nuclease free H2O 74uL, Primer 1 10uL, Primer 2 10uL
      For each sample
    - 95C (2min),
      Repeat 40x: 95 (30sec), 54 (1min), 72 (1min)
      72 (5min)
      4 (hold)
    - PCR gel:
- Plated 3-OXR1,4-HSP30,7-BTN2 & 8-SOD1 on +G418 YPD

Tuesday June 15th
Paula, Gabe & Brian

- Culture was diluted for 3.5 hours
- Measurement of OD was done with a 1/10 dilution of our culture which has been incubating for 3 hours, the blank was incubated with 9 parts YPD and 1 part water.
    - OD Value: 0.1731
    - Blank : 0.0861
    - Diff: 0.087
        - Actual OD : 0.87

Yeast Transformation

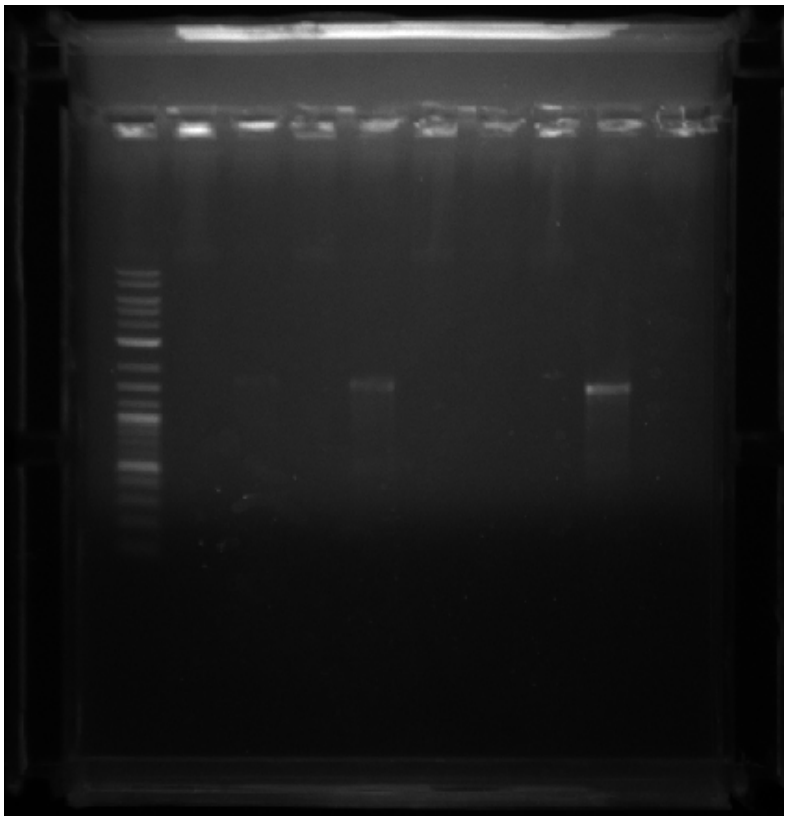
- We did the transformations for promoters
    - 3- OXR1
    - 4-HSP30
    - 7-BTN2
    - 8-SOD1
- To Prepare Promoter samples we used the table under the date June 15th:
    - Yeast transformation concentrations sheet
- To Prepare Yeast Cells:
    - Yeast transformation protocol
- YPD Culture was prepared and TAE stock was also made

Note: GFP, FFup and FFdown are done. Need to make more

Monday June 14th
-No colonies grown

Colony PCR protocol attempted with WT yeast

- -resuspend colony in 40 ul of water
- -Remove 20 ul and add 20 uL of 40 mM NaOH
- -Place in thermocycler at 98 degrees for 5 minutes
- -Prepare PCR (10 uL reaction)
  - -5 uL of gotaq
  - -0.5 uL of FFup and FFdown
  - -0.5 uL of cooked cells in NaOH (last PCR reaction)
  - -3.5 uL water
- -95 for 5 minutes
- -95 for 30 seconds
- -5 degrees below TM but make a few with a gradient going up
- -72 for 1 minute
- -72 for 5 minutes

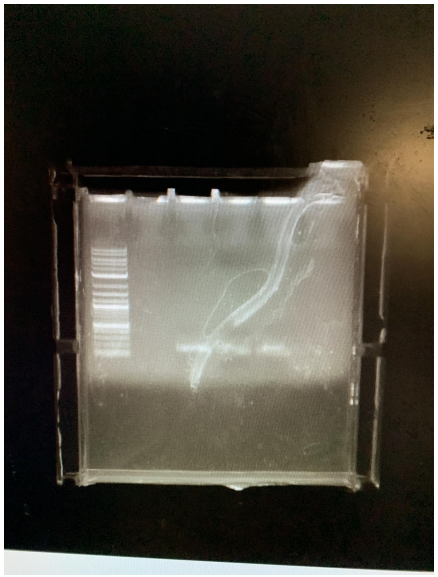


Gel

- -1: 54 degrees
- -2: 58.1 degrees
- -3: 59.3 degrees
- -4: 60 degrees

We ran a gel for SAF1 at three different temperature, no more because we ran out of primers.

1 - 54 degrees, 2- 57 degrees, 3- 60 degrees



Let half (20microL) of terminator open to air and it become more concentrated from 76.6 ng/mL to 97.6 ng/microL with 1.82 ratio.

- Side note out of primers for terminator

Saturday June 12th
-All promoters plated

Friday June 11th

- Prepared new sgRNA dimer - higher concentration so more cutting efficiency
    - Concentration not measured (not required)
- Salmon sperm DNA boiled and 11 100 uL aliquots made
    - If thawed, please reboil before use
- All promoters transformed into yeast
- Promoter 13 may have to be redone (considered possibly defective)
- 4 hour 45 minute

Thursday, June 10th
Brian and L

- ★ Yeast transformation!!!
- ★ Yeast Transformation Calculations sheet for DNA mix
    - Yeast transformation concentration sheet
    - Make total volume ~6.67
- ★ We cultured in water. OD600 0.0245

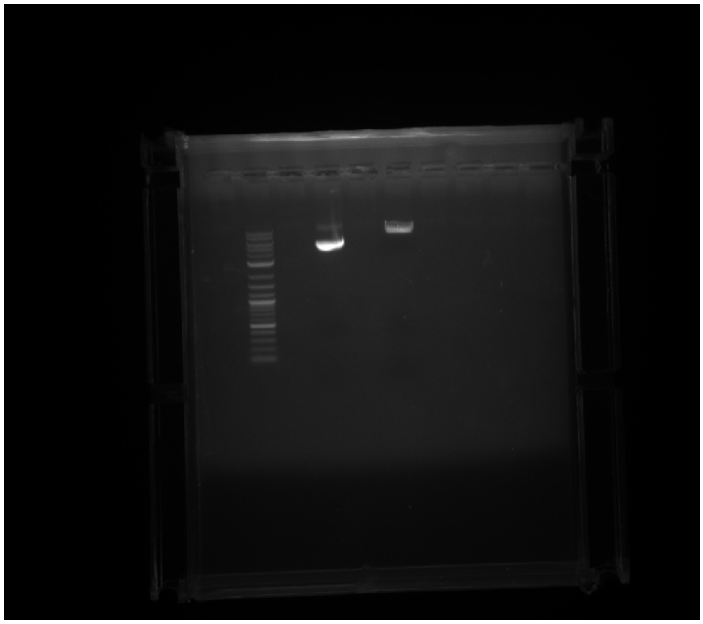
TECAN (cuvette):

- Know path length, 1cm
- 1ml of sample. If measuring proteins do triplicate
- Keep OD<1.0 or dilute if higher (inaccurate measurements above 1 due to scattering)

Wednesday June 9th
Brian and L

Did:

- Isolate Cas9 plasmid DNA purification Retained one tube of isolated plasmid undigested
    - Sample CRISPR plasmid DNA 277ng/uL ratio 1.85
        - One sample in 1.5mL tube in sample box. Other in a pre-mix in falcon tube in fridge door (labelled)
            - Falcon tube has ~250uL plasmid (277 ng/uL), 100 TANGO buffer, no enzymes*, 625 autoclaved H2O
    -
- Ran restriction digest with successful CRISPR plasmid purification from E.coli Retained 10 PCR tubes digested but not purified on door
    - Sample 250uL 277 ng/uL of purified plasmid, 100 of TANGO buffer, 12.5uL NotI, 12.5 uL BsaI, 625 uL autoclaved H2O
    - [37C, 2hr] [72C, 30min] [12C, infinity]
    - 12 samples of digested plasmid obtained (in falcon tube on door).
- Autoclaved glassware and pipette boxes
- We also dropped a lot of things
- Used all of the NotI Anza.
- Need autoclaved water next autoclave run
- Ran Cas9 plasmid restriction enzyme digest on gel. Loaded 6 microL mixed with loading dye. Loaded gel: ladder, undigested plasmid, digested plasmid. To verify proper cutting of plasmid.

- 
  - Digested plasmid lane 3 didn't travel as far which confirms it was digested as linear DNA does not travel as quickly as circular DNA
- Performed DNA purification protocol for digested cas9 plasmid In box 1
- measured purified cas9 plasmid DNA concentration to ensure usable yield
  - Tube 1 = 112 ng/microL, 1.85 ratio
  - Tube 2 = 141.9 ng/microL, 1.83 ratio
  - Tube 3 = 160.1 ng/microL, 1.81 ratio
- Discarded yeast cells with perfect OD because its 6pm, Thursday we can transform finally
- Cultured new yeast cells for mañana

PEG- binding of DNA to yeast cells
LiOAc- enhances binding of DNA to yeast cells and lithium ions increase permeability of the yeast cell wall
Salmon Sperm increases the chance of our plasmid passing nucleases and not binding to the cell wall because the salmon sperm is. Bulking up DNA.

Tuesday June 8th
OD blank = 0.071
OD value = 0.1655

OD Difference = 0.0945 with 10x dilution so 0.945
→ achieved after 3.5 hours

Saturday June 6th
Natasha and Gabe

Prepared yeast for next day's transformation

Saturday June 5th
No one

Friday June 4th
Gabe and L

- Made YPD
- **Made 50ml, 50% peg**
- CRISPR fragment purified and quantified - 47.6 ng/uL
- CPS1 terminator purified and quantified - 76.6 ng/uL

Autoclave training:

- Polypropylene bin
- What setting? Your judgment- small bottle 20, even 3-4 large bottles 30
- Fill only to ½ volume
- Loose lids and/ or foil
- No volatile components aka bleach, ethanol, flammable…
- If you combine solids and liquids use liquid setting. But will get condensation on tips for example. Better to separate.
- Biohazardous
    - EHS bag
    - Fill ⅔
    - Close with autoclave tape, leave a hole for steam
    - Liquid30
    - Wait until completely cooled down and double bag in black garbage bags
    - Place garbage bag outside of the door
    - Wash the bin

Thursday, June 3rd

Afternoon Gabe and Nhi

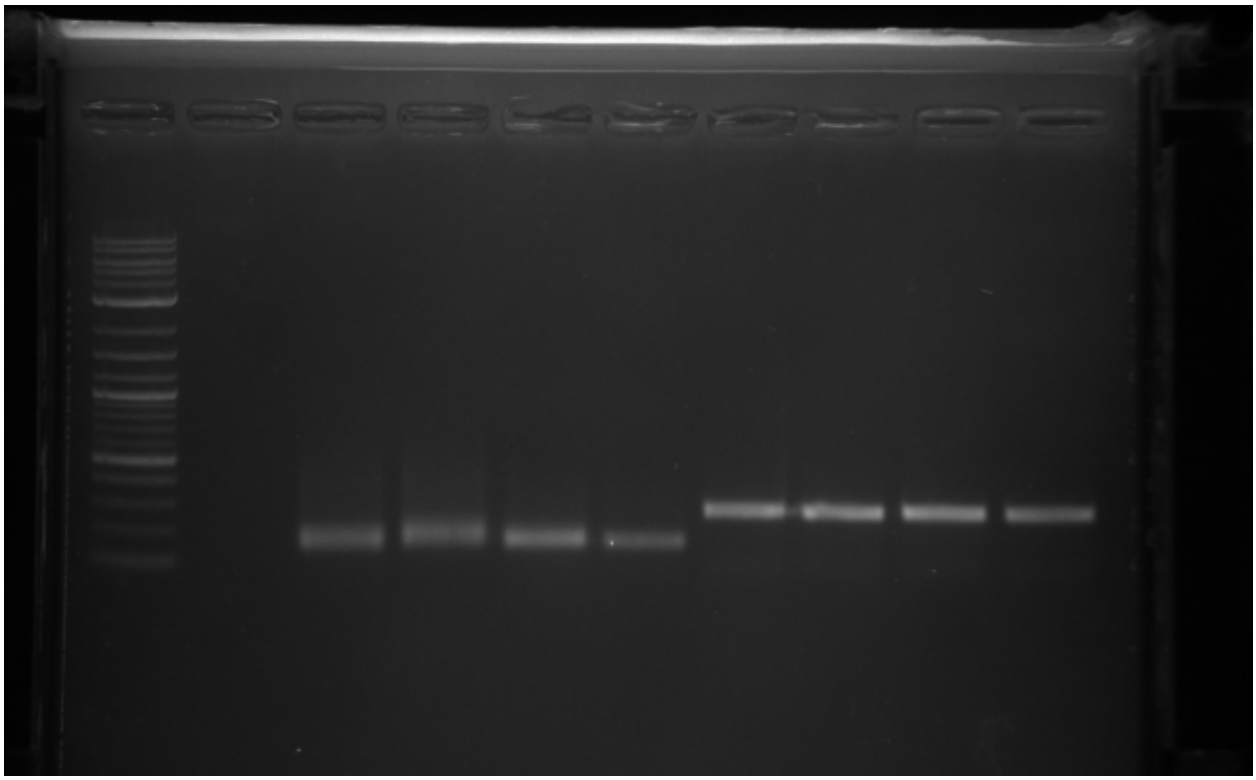
- check gel (below). I think we used the wrong ladder?

- Wizard SV Gel and PCR clean-up for sample 13 & 15

    Quantify DNA

- Do Sample 1 sgr assembly PCR (missing), protocol from Mo below and if gel isn't good for sample 13 or 15 repeat as well (only ~2uL of 13/SAF1 left)

- Filtered Lithium acetate prepared (3 M and 0.1 M)
- PCR run for 13 and 15
    - CPS1 terminator was successful (200 uL prepared)
    - Saf1 unsuccessful (200 uL prepared)
    - Run 2% gel tomorrow for better separation at low end and run with 100 bp ladder



1: 1 kb ladder, 2 blank, 3-6 Saf1, 7-10 Cps1 Terminator

Sample1- sgr assembly CRISPR FF PCR

Protocol from Mo

- Q5 Mastermix 80uL
- 10uL of 10uM Primer 1
- 10uL of 10uM Primer 2
- No need to check on a gel
- Use 2uL per transformation

- 100uL single tube PCR
- PCR settings:
    - 98, 30s
    - 6 times
        - 98, 10s
        - 57, 20s
        - 72, 20s
    - 72, 5min

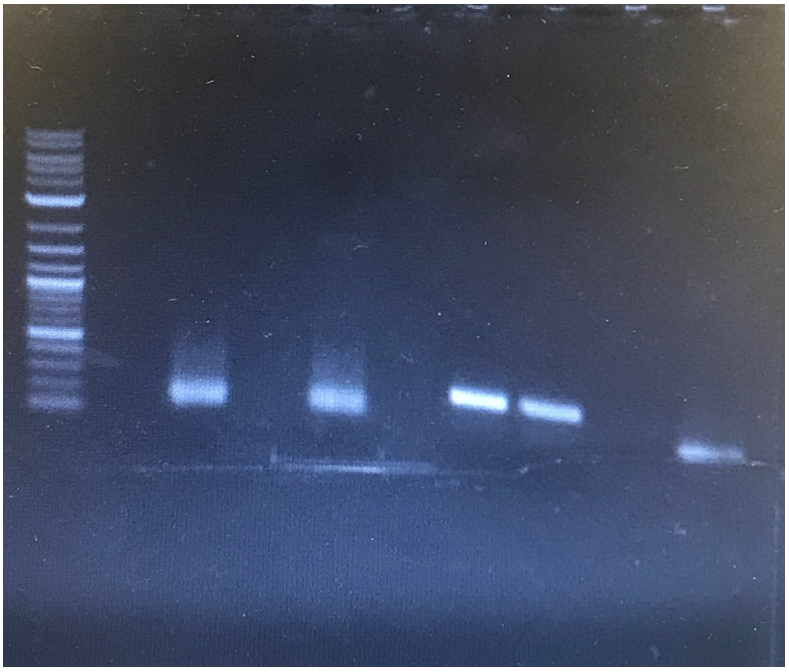
L & Brian

Notes:

- Right fridge leaking water, cleaned it up, hoping it's transient. We let Brandon (Dr. Kharma's lab know). Please check temp with handheld thermometer during your lab time.
- Microgravity simulator parts are on the above left shelf. Please treat with love.
- DNA concentrations/ Tecan→ use 2.5ul

Morning Objectives:

1. Run a gel with terminator CSPIT and SAF1
    a. PCR was run at two different temperatures to get better results (Nhi & Brian)
    b. Used non-autoclaved tips
    c. SAF1 ran 2uL promoter with 3 uL water and 1uL loading dye (not a lot of sample left)
    d. Gel:
        i. Lane 1- 1kb purple ladder ← ? wrong ladder maybe
        ii. Lane 2- 13A (SAF1)
        iii. Lane 3- 13B (SAF1)
        iv. Lane 4- 15A (CPSIT)
        v. Lane 5- 15B (CPSIT)
        vi. Lane 6- 13 unknown



2. Measure DNA concentrations from yesterday's purifications (blanked with air, loaded 2.5uL)

Samples	Concentrations (ng/microL)	Ratio
2	161.4	1.77
3	187.6	1.86
4	266.9	1.85
5	190.4	1.76
6	145.8	1.81
7	293.1	1.82
8	427	1.84
9	185.3	1.81
10	199	1.79
11	73	1.77
12	294.9	1.81
13	186.7	1.82
14	95.8	1.81

15 76.6 ng/uL

→ **Results are good because they are promoters**

3. Perform DNA purification with these as well if good gel results are obtained.

4. Measure DNA concentrations for these remaining samples.

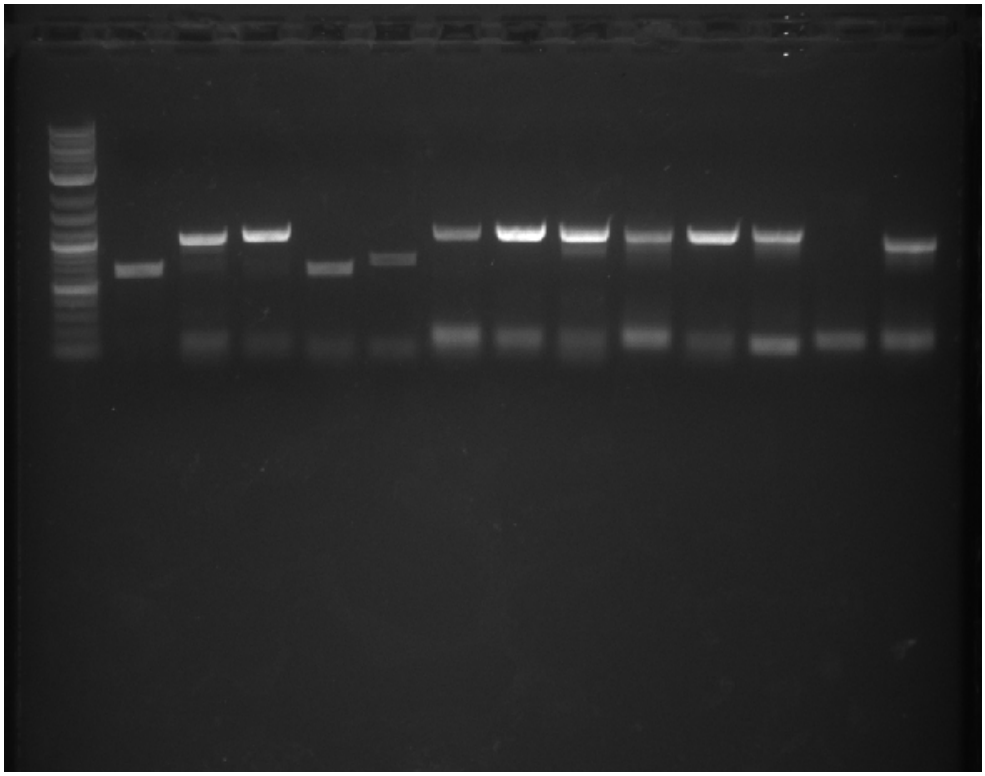
Samples	Concentrations (ng/microL)
13a	
13b	
15a	
15b	

ORDER OF ENTRIES BELOW NEEDS TO BE FLIPPED

Tuesday, June 2nd
Nhi & Brian

Objectives:

1. Prepare a medium sized gel, load the ladder and PCR samples 2-14 in the following wells, analyze to ensure they are there and the right size and not if some are showing multiple bands



Piece and length:

2 - FF16D - 673bp - Success
3 - OXR1 - 967bp- Success
4- HSP30 - 1000bp- Success
5 - FF16U - 627bp - Success
6 - GFP ENVY - 770bp - Success
7 - BTN2 - 1000bp - Success
8 - SOD1 - 1000bp - Success
9 - SOD2 - 1000bp - Success
10 - SLT2 - 1000bp - Success
11 - ARC19 - 1000bp - Success
12 - RAV2 - 1056 - Success
13 - SAF1 - 1920 - Unsuccessful
14 - GAL10 - 889bp - Success

2. Run PCR for the CISPIT with gotaq as well as SAF1 with the traditional Q5 (failed last run).
3. Performed processing of PCR amplifications without gel extraction on samples 2-14 (except 13). Moe mentioned not to do gel extraction for any of the samples as we will be

able to tell if we have good results by running the colony PCR. As well in an attempt to get higher DNA concentration we diluted into 21 microL rather than 50 microL.

Samples stored in freezer for measurement tomorrow

Tuesday, June 1st
Brian and Paula

Summary:

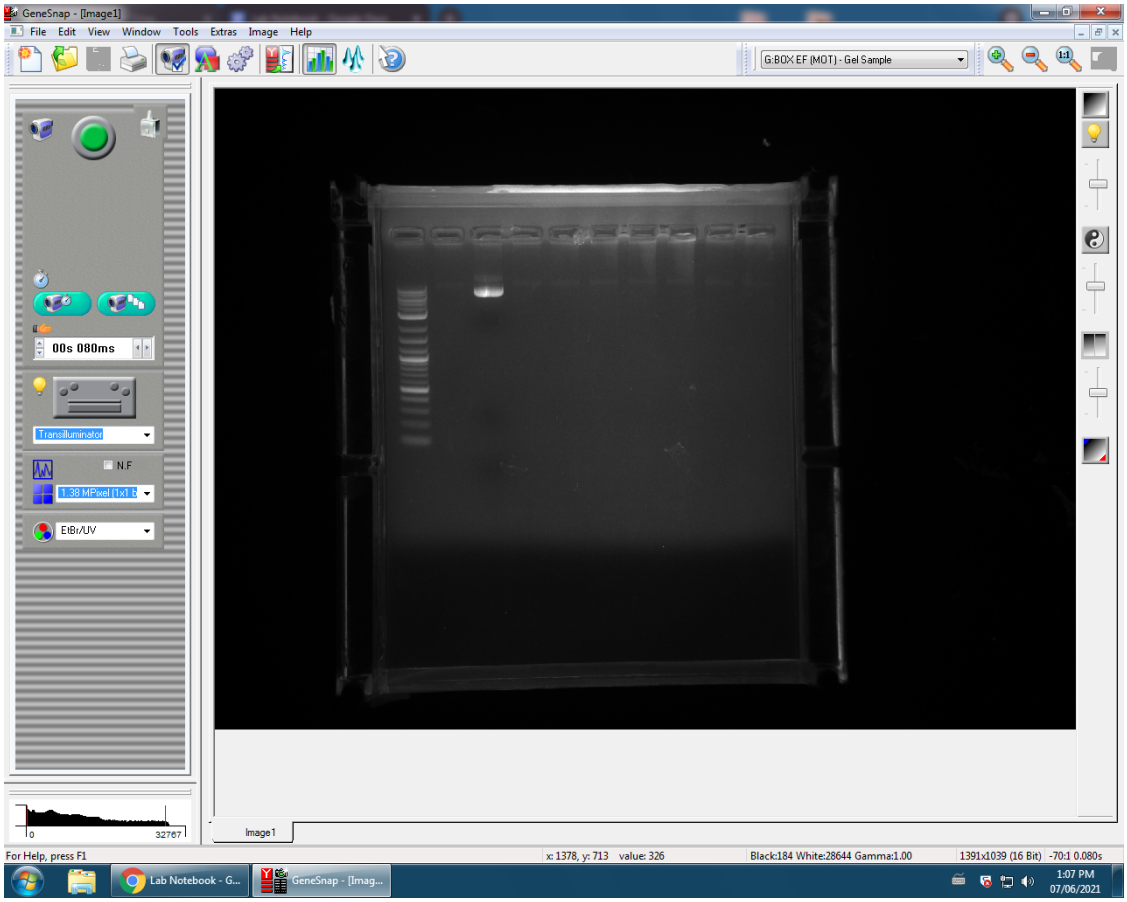
DNA concentrations were far too low (the goal should be 100ng/microL) and so we are starting to reprepare the PCR amplifications. This time we are doubling the amounts of everything on the samples which had a DNA concentration under 20ng/microL marked by a blue dot on the cap of their tubes, and as precaution will PCR purify where possible rather than Gel purify and extract.

Due to the way we began a PCR Q5 master mix was not prepared but rather all was added just as described in the PCR document with Q5 (polymerase, ligase etc) added to genomic DNA, water, and primer.

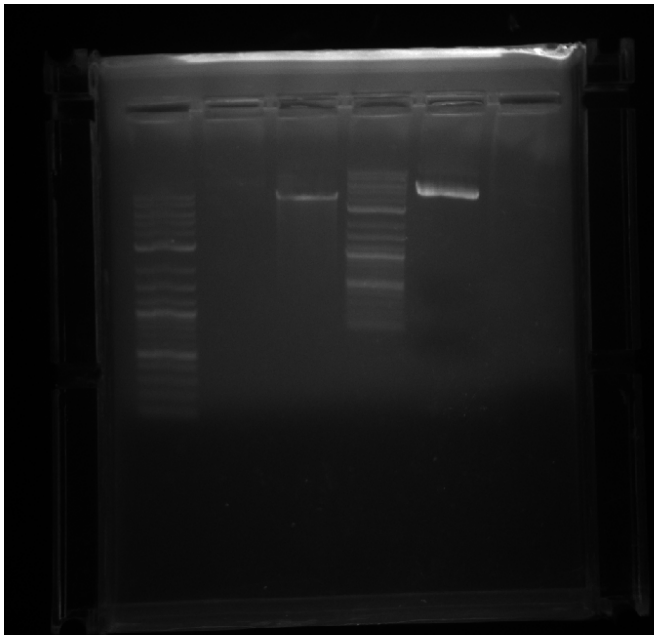
Objectives

1. Analyze what needs to be re-done, add adjustments to our procedure and re start preparing PCR
2. Perform PCR for all samples except GFP and CPSIT which will be done tomorrow. SOD2 is being attempted at 61.5 C which was the ideal temperature.

Note: Please run a bit of 6 and 8 on the gels to double check that they are labelled properly



Res june 7th



Restriction enzyme digest - june 8th

Monday, May 17th
Gabe and Brian

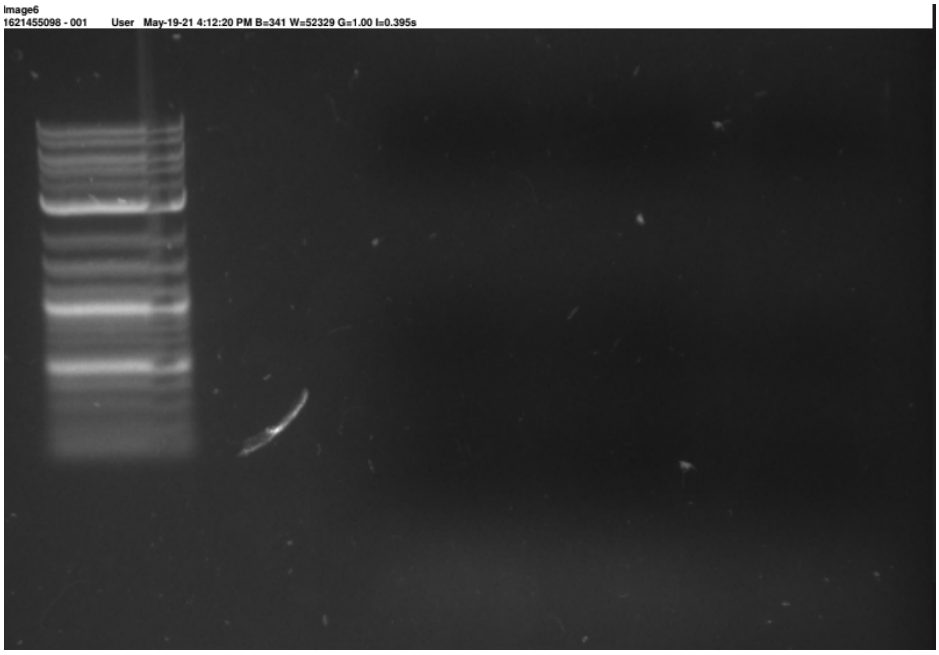
1. Preparation of 1000x Kan stock solutions (-20 degrees)
2. Inoculation of 8 mL of LB for miniprep
3. Autoclaved stock solutions and water
4. Cultured Cas9 containing e.coli (37 degrees) and yeast for stocks (30 degrees)

Tuesday, May 18th
Paula and Brian

1. Made 1L fresh YPD (1x). and 60% glycerol and autoclaved them
2. Made 3 cultures of yeast, one of e.coli with the plasmid.
3. Two extra cas9 plasmid cultures to be stored - Mini-prepped the bacteria to isolate the cas9 plasmid
    - 3 large mini-preps with the 3 cultures
        - sample 1 = 287.8 ng/microL
        - sample 2 = 231 ng/microL
        - sample 3 = 200.2 ng/microL
4. Found concentration of 3 preps of plasmid DNA (concentrations recorded)
5. Prepared a gel
6. Prepared the 2 hour restriction enzyme digest (NotI, BsaI), stored at 4C to be run tomorrow.
7. Yeast growth unsuccessful

Wednesday, May 19th
Paula and Brian

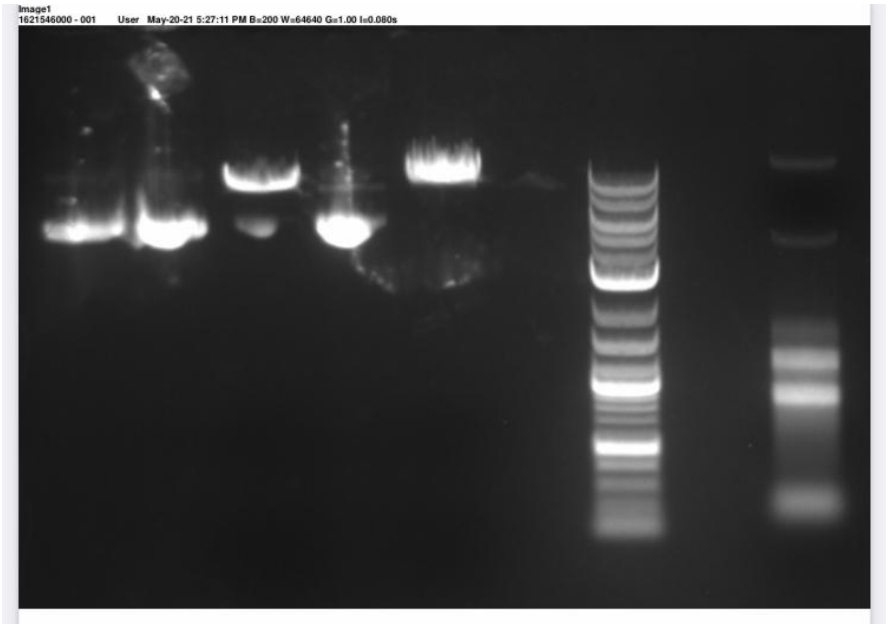
1. Transferred 60% glycerol to a falcon tube where some is stored
2. Combined plasmid and yeast cultures with glycerol and stored in -80 C
3. Run the Gel to verify successful results unsuccessful, No bands were displayed in the gel. Most likely was lost in the mini-prep



4. Perform Genomic DNA extraction on Yeast cultures. This is to be used for PCR.
5. Make 50mL of 10% SDS stock
6. Made culture of e.coli with plasmid, mini-prep was unsuccessful unfortunately and needs to be re-done

Thursday, May 20th
L and Brian

1. Re-did the mini prep
    - DNA concentrations of 228.8 ng/microL (1.84 ratio) and 213.6 ng/microL (1.82 ratio)
2. Re did the genomic extraction to be extra sure. Quickly measure DNA concentration tmr.
3. Ran gel with mini-prep and genomic DNA extraction to make sure everything is fine.

a. 

   b. Lane 1,2,4 Plasmid control
   c. Lane 3,5 Plasmid Miniprep RE digest (NotI/BsaI)
   d. Lane 7 1kb ladder
   e. Lane 9 yeast genomic prep
4. Froze two yeast cultures with glycerol in -80C
5. Prepared a e.coli liquid culture to make a guaranteed good frozen version with glycerol tomorrow

Friday, May 21st
L and Gabe

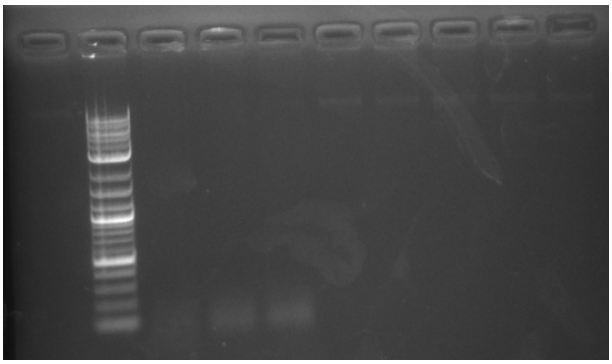
1. Measure DNA concentration of yeast genomic DNA extraction
2. Make frozen e.coli stock
3. Prepare primer stocks at 100 uM per tube

Saturday May 22nd
Gabe and Natasha

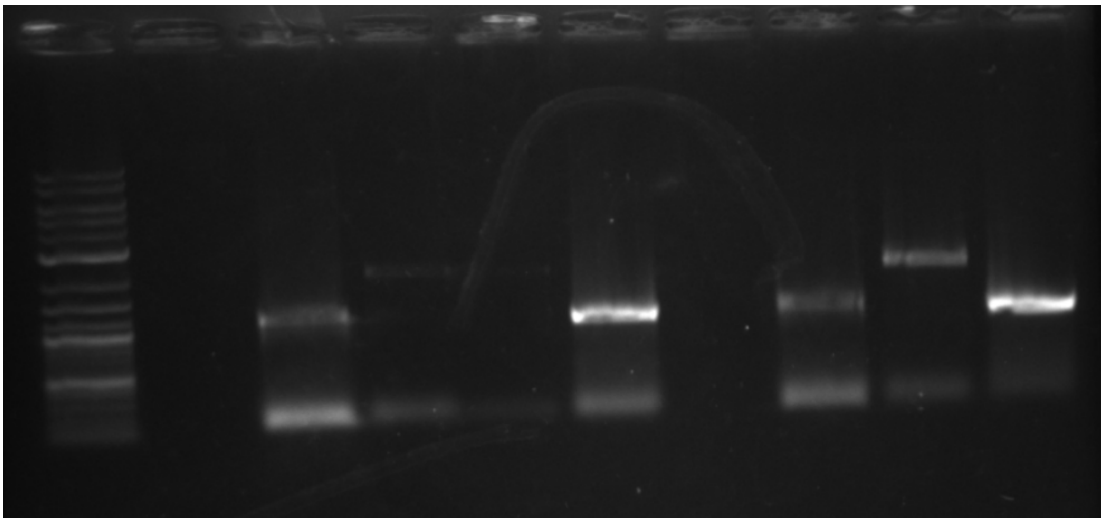
1. Genome extraction

Sample	Ratio	Average ratio	Concentration (ng/uL)	Average concentration (ng/uL
1	1.96	1.96	109.9	101.9
	1.96		93.9	
2	1.94	1.95	54	53.85
	1.96		53.7	
3	1.77	1.785	36.3	37.05
	1.8		37.6	

Sunday May 23rd
Gabe, Natasha, and Nhi

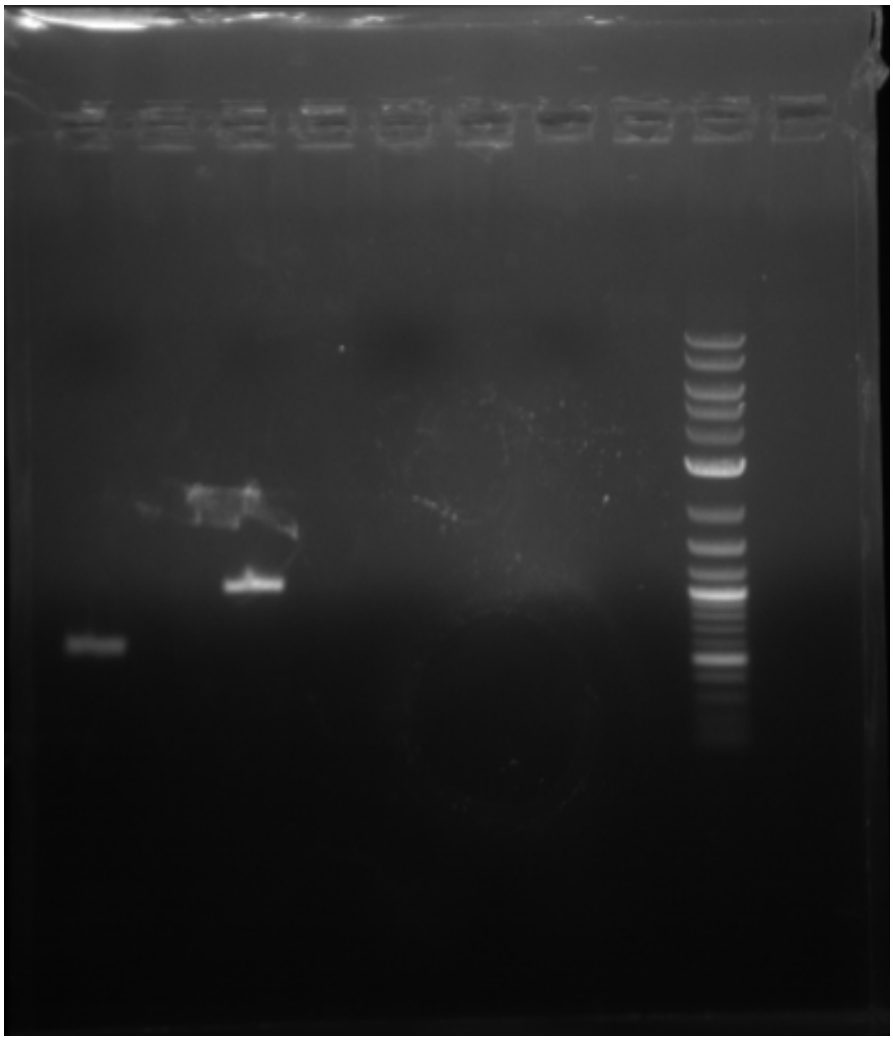
1. PCR amplification
    a. Sample: FF16D, HSP30, OXR1
        i. Protocol: “Plasmid QC”
    b. Gel:
        i. Lane 2 = ladder
        ii. Lane 3 = FF16D
        iii. Lane 4 = OXR1
        iv. Lane 5 = HSP30
        v. 
    c. Check “PCR fragments” google sheets
    d. PCR purification unsuccessful

Monday May 24th
Gabe and Brian

1. 1x primer stocks prepared (use this for PCR) (use 45 uL of master mix and 2.5 uL of each primer)
2. Diluted primers and are being kept in a 50mL plastic falcon tube.
3. 12 (RAV2), 13 (SAF1), 14 (GAL10) PCR
    a. PCR successful
4. Compared against mentor's template DNA
5. Gel
    a. 
        i. Lane 1 = ladder
        ii. Lane 3 = RAV2 - SUCCESS
        iii. Lane 4 = SAF1 - SUCCESS
        iv. Lane 6 = GAL10 - SUCCESS
        v. Lane 8 = RAV2 (with Mo's genomic DNA)
        vi. Lane 9 = SAF1 (with Mo's genomic DNA)
        vii. Lane 10 = GAL10 (with Mo's genomic DNA)

Tuesday May 25th
Brian

1. Prepared PCR run for samples. Used numbers from PCR fragment checklist
    a. 5 - FF16U - melt = 59C, anneal = 62C - SUCCESSFUL
    b. 8 - SOD1 - melt = 59C, anneal = 62 C - SUCCESSFUL
    c. 9 - SOD2 - melt = 57C, anneal = 60C - UNSUCCESSFUL
    d. 10 - SLT2 - melt = 60C, anneal = 63C - UNSUCCESSFUL



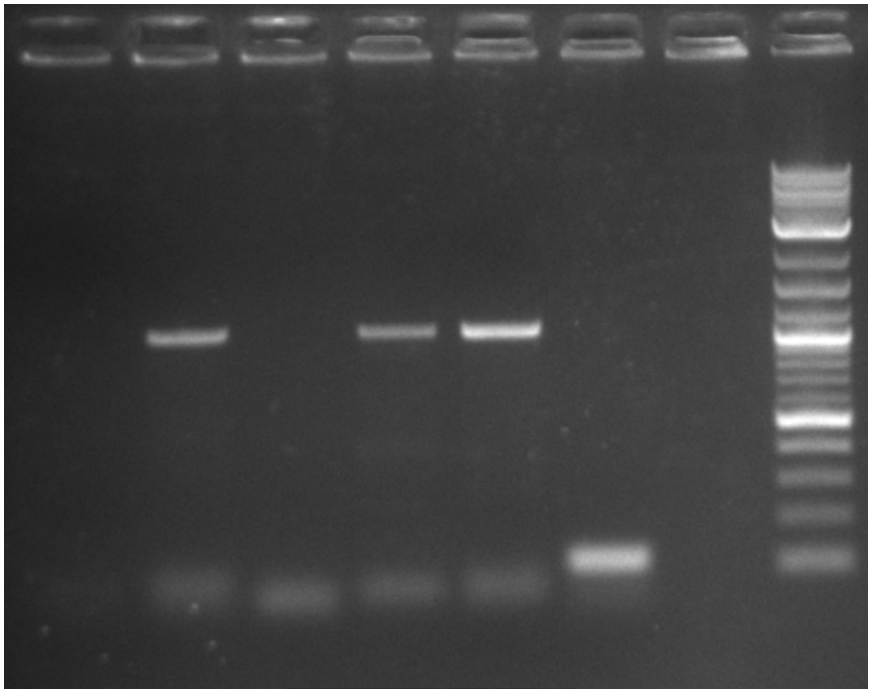
2. Prepped a gel

Wednesday May 26th
Paula & Brian

1. Ran the gel, used 15 microlitres of PCR products however looking back should have used 5 microlitres. Why give up the juice? PCRs were loaded in numerical order.

2. Prepared 6 more samples today:
    - Arc19, #11
    - BTN2, #7 - Success
    - GFP, #6 - Not same template
    - CPSIT, #15 (to verify difficulty)
    - SOD2, #9 - Success
    - SLT2, #10 -success

3. These were loaded into the gel in numerical order

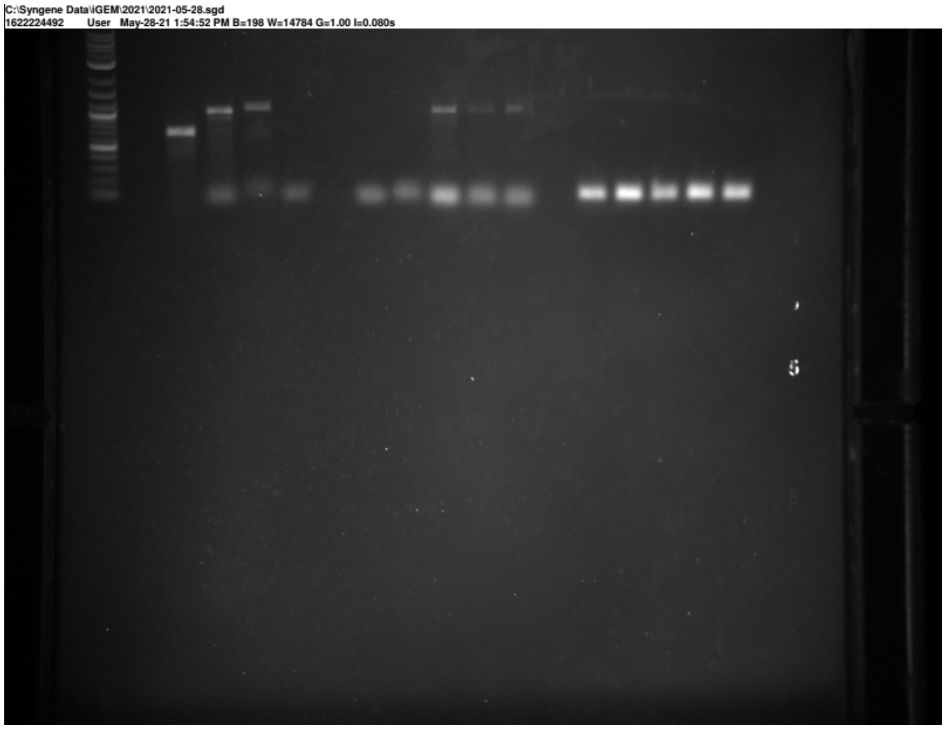


Thursday May 27th
Paula & Brian

1. Verified that all PCR bands were of an appropriate length in order to confirm correct production. All bands which appeared were an acceptable length.
2. Continued with the final 5 (3 unsuccessful from the sunday gel, 2 unsuccessful one from tuesday and one from both tuesday and wednesday)
3. Samples were prepared and designed an experiment to find a better annealing temperature of SOD1 and CPS1T (check yellow notebook for more details , in the office drawer)
4. Gel was not run

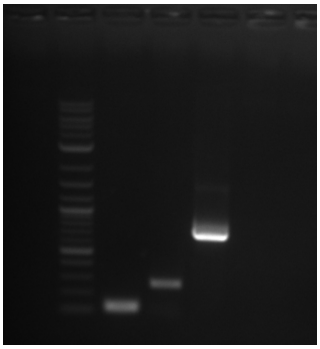
Friday May 28th
Gabe and Paula

1. QC Gel of remaining fragments (shown below)
    a. FF16D, OXR1, HSP30 amplified successfully
    b. GFP negative control passed, ensuring that the primers are not non-specific to potions of the yeast genome
    c. Optimal temperature for SOD2 was determined to be 62.1 °C
    d. No temperatures tested allowed for the successful amplification of the CPS1 terminator.
        i. This will be re-attempted with new temperatures and polymerases
2. Gel Picture



1 Ladder
2 Blank
3 FF16D
4 OXR1
5 HSP30
6 GFP
7 Blank
8 SOD2 59.9 ºC
9 SOD2 59.4 ºC
10 SOD2 62.1 ºC
11 SOD2 65 ºC
12 SOD 50 ºC
13 Blank
14 CPS1T 59.4 ºC
15 CPS1T 65 ºC
16 CPS1T 50.0 ºC
17 CPS1T 53.4 ºC
18 CPS1T 55.9 ºC
19 Blank
20 Blank

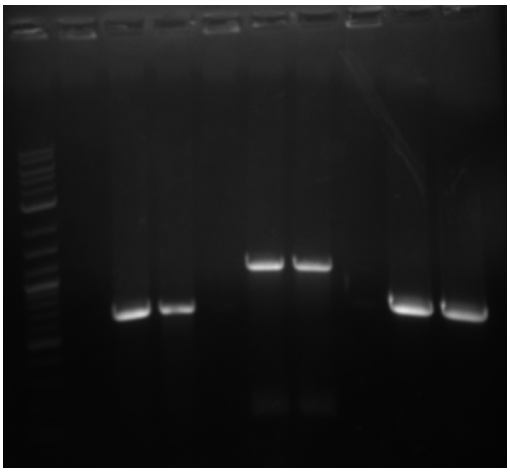
Saturday May 29th
Gabe, Natasha, Nhi

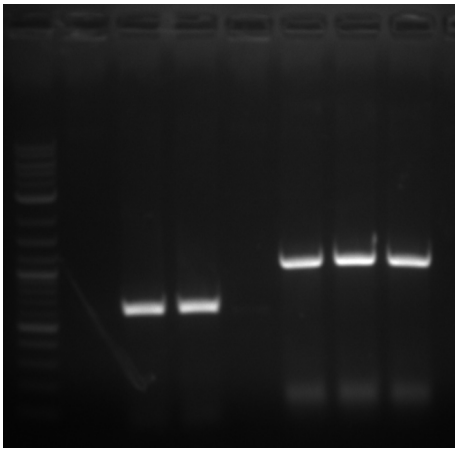
- The CPS1T terminator sequence was amplified using Q5 high fidelity polymerase along with Gotaq polymerase (same concentrations of all components and run at 58 ºC).
    - The Q5 polymerase did not amplify CPS1T
    - GoTaq was successful in amplifying CPS1T
    - GFP was amplified to ensure that the primer was designed properly, and was amplified as well.
        - 5 uL of a 10 ng/uL stock of template DNA was used
- Gel
    - 
    - 1. Ladder
    - 2. 15 (Cps1T) using Q5 polymerase
    - 3. 15 (Cps1T) using gotaq polymerase (SUCCESSFUL)

- 4. GFP (SUCCESSFUL)
-

Sunday May 30th

Gabe and Natasha

- Made CRISPR E. coli stock (9 tubes in -80ºC freezer)
  - 1 mL per tube
- Made G418 antibiotic (10 tubes, in -20ºC freezer in box #2)
  - 2g of G418 + 10mL of water
  - 1 mL per tube
- PCR to amplify CPS1T (15), HSP30 (4), OXR1 (3) and RAV2 (12) in order to have more PCR product for the gel extraction
  - PCR mix
    - 2.5 uL of each primer (F+R)
    - 19 uL nuclease free water
    - 1 uL of yeast genome #2
    - 25 uL of enzyme master mix (CPS1T = GoTaq, other 3 were Q5)
  - PCR program
    - 98 ºC 30s
    - 98 ºC 10s
    - 59.2-52.9 ºC (gradient)
    - 72 ºC 30s
    - 35X (number of cycles)
    - 72 ºC 5min
    - Hold at 20 ºC
- Gel extraction to purify the PCR products
  - Pooled all PCR products of the same promoter/gene
  - Gel #1 (loading order: ladder, FF16D (2), SOD1 (8), GFP (6))
    - Each sample was loaded into two wells
    - 
  - Gel #2 (loading order: ladder, FF16U (5), BTN2 (7))
    - FF16U loaded into two wells (lane 3+4), BTN2 loaded into three wells (lane 6,7+8)

    - 
  - For gel extraction, followed this protocol: https://www.promega.ca/-/media/files/resources/protcards/wizard-sv-gel-and-pcr-clean-up-system-quick-protocol.pdf?la=en
    - For the “Gel Slice and PCR Product Preparation” step, follow part A (Dissolving the Gel Slice)
  - Gel weight
    -

	Empty tube (g)	Tube + gel (g)	Weight of gel (g)
2	0.8888	1.2364	0.3476
5	0.8711	1.3073	0.4362
6	0.8840	1.2093	0.3253
7	0.8804	1.7155	0.8351
8	0.8892	1.3412	0.4520

- Quantification of purified DNA
  -

Sample	Concentration (ng/uL)
FF16D (2)	10.3
FF16U (5)	14.2
GFP (6)	13.1
BTN2 (7)	17.3
SOD1 (8)	5.7

  - All these samples are stored in box #1 in the -20 C freezer

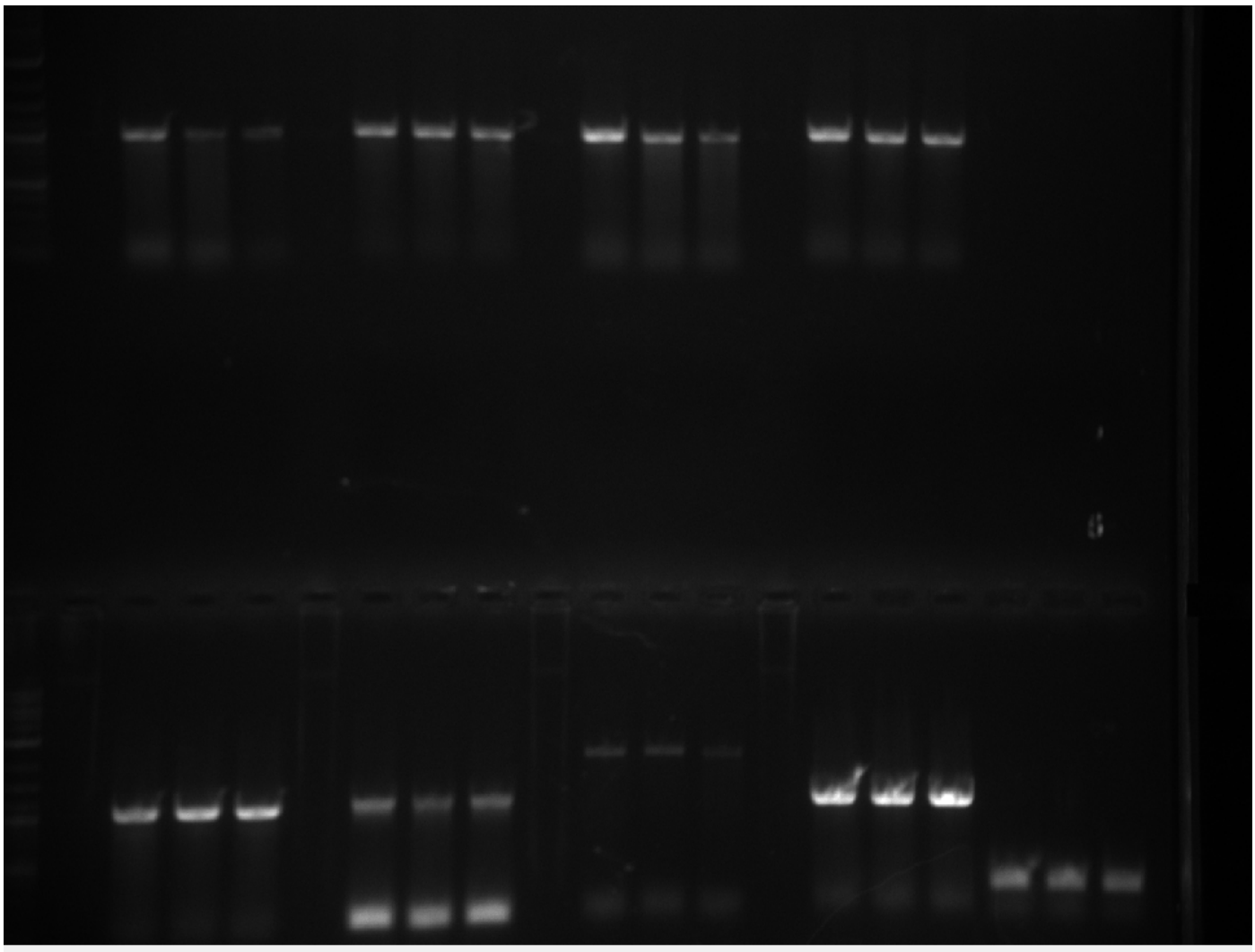
Monday, May 31st
Brian and Paula

Objectives

1. To prepare a gel and perform a DNA extraction
2. Perform DNA purification
3. To prepare 50 Agar plates with new YPD
4. To measure the YDP pH for the hardware team

What did we do ?

- Ran a gel with the pre-prepared PCR products on a medium size gel. Three wells loaded per sample. Each well was loaded with 35uL of sample.



First row

- 1Kb Ladder
- Blank

- 3
- 3
- 3
- Blank
- 4
- 4
- 4
- Blank
- 9
- 9
- 9
- Blank
- 10
- 10
- 10
- Blank
- Blank
- Blank

Second Row

- 1 Kb Ladder
- blank
- 11
- 11
- 11
- Blank
- 12
- 12
- 12
- Blank
- 13
- 13
- 13
- Blank
- 14
- 14
- 14
- 15
- 15
- 15

- A 100ml Agar solution was prepared to make about 50 agar plates. The protocol used to make the Agar Plate was this one :
    - Agar plate protocol

Note : one of the YDP bottles was emptied into the other YDP bottle because we were missing a bottle in order to autoclave our Liquid media at 30 degrees.

- The pH of the YDP solution was found to be : 6.34
- The regular pH of YDP solution is : 6.8

Gel extraction

Sample	Vial Weight (g)	Vial Weight + gel (g)	Weight of gel (g)
3	0.92	1.12	0.2 = 200mg
4	0.90	1.07	0.17 = 170mg
9	0.91	1.16	0.25 = 250mg
10	0.89	1.09	0.2 = 200mg
11	0.88	1.14	0.26 = 260mg
12	0.89	1.04	0.15 = 150mg
13	0.88	1.08	0.25 = 250mg
14	0.91	1.41	0.5 = 500 mg
15	0.90	1.18	0.28 = 280 mg

** All three samples were added together in a vial

Quantification of purified DNA

-

Sample	Concentration (ng/uL)
OXR1 (3)	7.8
HSP30 (4)	13.0
SOD2 (9)	23.8
SLT2 (10)	30.8
ARC19 (11)	20.1
RAV2 (12)	10.9
SAF1 (13)	3.5

GAL10 (14)	24.8
CPSIT (15)	21.0

- All these samples are stored in box #1 in the -20 C freezer

\