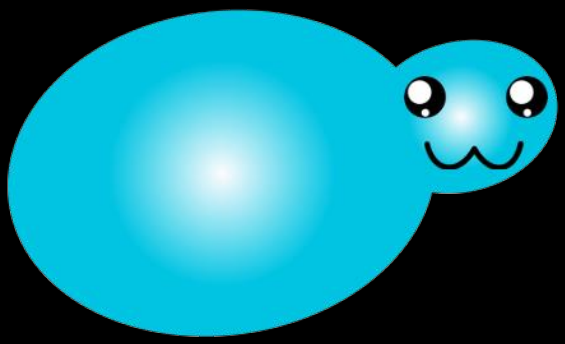
GENETICS

NOTEBOOK



iGEM CONCORDIA

iGEM CONCORDIA

ASTROYEAST 2020

Genetics Minutes

Fall/ Winter Term Tasks (08/12/2020)

- SMG: involved in the design
    - Adjustable for Moon, Mars
- Promoters: Earth application
    - Insulin
    - Fermentation
    - Other nutrient
- **Inventory: Gabe & Davindra**
- **Chris Brett...Gabe & Lancia**
- Protocols- January 1st
    - Radiation- Nislow
    - Freeze dried- Nislow
    - Natasha:
        - Oxidative H2O2
        - Heat
        - pH
        - Osmoregularity Salt
    - Nhi
        - ***Promega Water Glo - yeast compatible viability test, 20 single tests***
        - Viability Freeze/thaw
        - Yeast viability dehydrate/ rehydrate
    - Lancia
        - Histone Control
    - Brian
        - ***Wizard® SV Gel and PCR Clean-Up System, 250 preps***
        - ***Wizard® Plus SV Minipreps DNA Purification Systems (250 count)***
        - ***GoTaq® Master Mixes (+dNTPs) Colorless, 100 reactions***

        - ***Q5® High-Fidelity 2X Master Mix (NEB #M0492S)***
        - ***NEB 5-alpha Competent E. coli ** (NEB #C2987I - Not available if ordering #E5520S)***
        - Cloning
        - CRISPR insertion
            - Screening/ colony PCR
        - transfection
    - SMG
    - Evelyn
        - GFP Fluorescence Screening
    - Real-time monitoring
    - Mars conditions
- Training:
    - Courses on your own (list)
    - Mentors
- Bioreactor:
    - Media
        - Can we use a waste stream
        - What other production methods produce sugars
    - Closed loop
    - Interviews
- Schedule: Gabe in the lab May 1st
    - If SMG works
    - If it doesn't
    - How you would imagine full-time schedule 7 days a week
        - Davindra
- Ordering
    - March 1st
    - In the lab May 1st
- Back up plan: if SMG doesn't work or if yeast is a bad host
- Interview Yeast microgravity researcher
- **Ballooning or microgravity Fall 2021**

09/08/2020 Minutes

- Low rates of insertion a concern with CRISPR
    - Picking pam sites close to cut sites..?
- Sequencing → what more do we need to do? (Gabe) → guide RNAs
    - What is the deadline (Lancia)
    - For CRISPR knock-ins and plasmid insertion

- Wiki content
- Presentation for Genetics:

09/01/2020 Minutes

1. **Follow up** - please put links to documents here
    - Brian- Bioreactors [doc link]
    - Plasmids vs cripsr [doc link]
    - Natasha- What nutrients does yeast culture need and what is the rate of consumption of nutrients?
        - Away (will add more soon)
        - Carbon source for energy and building blocks
        - Nitrogen source
        - Amino acids (also act as nitrogen source)

        - Gabe- What kinds of membranes are used in bioreactors for the nutrient/ cellular compartment? How does exchange occur? [doc link]

    - Labrini- What gases are produced in yeast cultures and at what rate/ concentration? What is the viscosity of the media? [doc link]
    - Lancia- Draft emails- not yet, waiting for a full hardware team
    - Nhi- [doc link]

2. **Recruitment-** bring new team members on throughout the year and vote in a co-lead at a later date, see who is a good fit, commits to the project etc. This could be a current team member or a new recruit. Team votes for the co-lead early spring with mentor consultation.

3. New ***Genetics meeting*** time for Fall
    a. Create a when to meet
    b. Choose together now: How often? Every two weeks? Once a month?
        i. → We will still have weekly team meetings until the Giant Jamboree in November

Once every two weeks

4. **Assign for wiki, content & text writing- questions? message Lancia**
        1. Promoter selection -Done

2. [Nhi] Building the reporter- infographic protocols/ methods, short text and suggested diagrams to make/ use. Check out other wikis.
3. **[Labrini]** Inserting into yeast
    a. short text point form about process/ methods with proposed fun diagrams to make/ use
4. [Natasha] Evolutionary experiments
    a. short text with proposed diagrams to use/ make. Define how evolutionary experiments work and also how we will use SMG to do so.
5. [Gabe] Proposed implementation (genetics focus)- 1 page max
    a. How would these resistant strains be implemented in the real world? Could be in storytelling format. **Specific to Genetics details**- control strain/ resistant strains
    b. biosafety/ kill switch…
    c. Who would use it
    d. How will they know about it?
        i. Promote on AdGene (advice from Dr. Nislow)
        ii. ...
    e. How would it be used by the researchers or bioproducers? Where, how and why?
6. [Brian] Safety & Ethics - short and sweet
    a. For the whole project- Software & Genetics
    b. Short text. Consult other wikis, read RTTAs
    c. Ask Software team what the safety concerns are and ethical concerns
        i. As a start:
            1. Privacy and data handling
            2. Data curation- researchers will trust the platform, is the data inputted reliable?
            3. Accessibility, who has access to this technology we have designed? --> Open source
    d. Genetics
        i. Biosafety, kill switches
    e. " We perform Human Practices to assure our project is good and responsible for the world. We also consult science and technology ethicist throughout our project"
7. **[Lancia]** summarising the RTTA processes, whole project proposed implementation- different from above.

08/18/2020 Minutes

- Read: https://onlinelibrary.wiley.com/doi/full/10.1002/yea.1705
- Primers: polish your assigned promoters for next week (video in Genetics Slack)
- Let's do CRISPR

Assignments:

1- Primers
2- Bioreactor research

i. Connect with UCalgary about bioreactors. Flow rate in yeast bioreactors [Brian]
ii. What nutrients does yeast culture need and what is the rate of consumption of nutrients? [Natasha]
iii. What kinds of membranes are used in bioreactors for the nutrient/ cellular compartment? How does exchange occur? [Gabe]
iv. What gases are produced in yeast cultures and at what rate/ concentration? What is the viscosity of the media? [Labrini]
v. Write draft emails to interview [Lancia]
    1. Tufenkji, Nathalie, McGill https://www.mcgill.ca/study/2017-2018/courses/chee-474
    2. Kamen, Amine https://www.mcgill.ca/study/2019-2020/courses/bien-590

For Thursday's meeting:

- Vitamin A or...Vitamin D….
- Two-three enzyme system, native in yeast
- 3 degrees dif in primers, but some ppl have up to 10degrees. Ideally 3C
- Nomenclature in Benchling

08/18/2020 Minutes

- Bioreactor:
    - Calgary (Mackenzie ?)
    - Determine: Flow rate & rate of consumption of nutrients, what nutrients, what kind of membrane, what gases, growth rate of yeast, viscosity of media
    - Rate of consumption by yeast [Genetics]
    - Nutrient pack/ modularity → weight, ug, rate of consumption, how does exchange occur,

- Tufenkji, Nathalie, McGill [Lancia→ set up interview, concept first] https://www.mcgill.ca/study/2017-2018/courses/chee-474
- Kamen, Amine https://www.mcgill.ca/study/2019-2020/courses/bien-590

Space Concordia

Wiki checklist [doc link]

- Lancia
  - List of Microgravity Journals
  - Bonn criteria
    -
  - Microgravity events (annual meetings, seminars, what else?…)
- Gabe
  - How will we visualize our experiments in space:
    Think about chromoproteins/camera vs fluorometer. Explain briefly how it would be used in space.
    - Fluorescence best way to go- sensitivity
    - Chromoprotein has a **baseline expression**
    - Gal/X system
    - We want a system that adapts and changes, can't get a chromoprotein system
- Khash
  - Probably done for the year
- Natasha
  - Methods used for microgravity studies (not hardware)
    Gene deletion/ microarray, RNA-seq, etc… with one or two sentences describing the technique and with references
  - APA
- Nhi: [doc link]
-
  - Which vitamins/ nutrients degrade with long-term space travel?
    Which vitamins do astronauts need supplemented & why? [doc link]
  - How would the astronauts eat our yeast or the nutrients it provides?
    (yeast for bioproduction of nutrients examples, look at food deserts here on Earth, Lallemand is working on enriching yeast)
- Brian
  - Integrate into the genome:

        - Integrated plasmids or CRISPR
        - Price range how does crispr compare to plasmids? Is it around the same?
        - 2010/2012 - papers comparing CRISPR to normal integrated plasmids (in yeast)
- Labrini maybe
    - Send google doc for last week's work

- Other:
    - Defend SMG changes in yeast, not just in space conditions [Nhi]
    - How would our yeast be cultured in space? (ie look at bioreactors in space for yeast & look at nutrient production in yeast here on Earth)

08/13/2020 Minutes

Here are some quick and fun things to complete this week &
Design Primers *wooooooooohooooooooooo*
@Gabe & @Natasha Letourneau: send protocol to ***Toulouse*** and follow up to confirm they have everything they need from us.
@Gabe One more ***promoter*** for Dr.Kachroo (actin, myosin, tubulin)

@Natasha Letourneau: List of ***Genome-wide analysis papers in microgravity*** (SMG & Spaceflown). 10 papers [doc link]

@Labrini Vlassopoulos: Can you make a point form document with one or two sentences for each point about why ***yeast is a good choice for microgravity studies*** please (with references) Min 10pts

- Genome is sequenced
- Generalise the medicine part up to microbiology (medecine, nutrients, polymers)
- Molecular tools are figured out, not all of the organisms have that
- Non-lab yeasts, don't always have tools to engineer them vs standard baker's yeast which is a standard

@Brian: ***Upload*** Bronze medal doc, then look at plasmid vs CRISPR, what are the pros and cons for inserting ***GFP into yeast*** [doc link]

@Nhi: A list of how microgravity affects ***yeast as a cell*** (ie random budding, cytoskeleton alterations, what else?) in point form with references. Minimum 10 other pts. [doc link]

(11). Sebastiaan E. Van Mulders (2011). The Influence of Microgravity on Invasive Growth in Saccharomyces cerevisiae

Lancia: Microgravity doc

08/11/2020 Minutes

- Put up bronze medal criteria- Brian
- Yeast strains (Labrini)
- Clinostat→ HARV?

Primer design

- Restriction sites
- Sequence
- Which tag are we adding
- Which method of cloning? Directional? Gateway? Gibson?
- Know our vectors

Finalize RPM/HARV
Defend our project (wiki)
Finalize our promoters

- Everyone- read the paper Mo posted directly above
- Gabe & Natasha: Experimental protocol (notes in the minutes) Measure the activity of the promoters expressing GFP, put it in a 96 well plate and measure it with dif stressors. Gabe- size of things (96 well, agar dish, falcon tube rack and tubes)
- @Brian: rpm of 3D Clinostats in yeast papers, question for Nislow, can you do the mini video?
- @Labrini Vlassopoulos @Khash: Look at and list what strains of yeast are used in microgravity experiments, including unconventional strains of yeast.
- Nhi- one week off
- Lancia: Defend the use of a 3D Clinostat

- Actin, myosin, tubulins
- Mo- Dr. Kachroo: GFP collection in 96 well plate in SMG

Feedback from Dr.Kachroo

•Actin, myosin, tubulins

•GFP collection strains, throw on a 96 well plate and see what happens

•Translational fusion, very close to ORF, protein is made and then protein is expressed with GFP tag

•Upregulation of mRNA relate to upregulated of protein, ie stability of proteins

•Looking at protein data

•Saba biofluorescence reporter CFP (degraded) YFP (internal control). Cfp/yfp

•RAD52 (very conserved protein in yeast- up expressed) radiation studies in yeast. DNA damage, double stranded breakage.

•Clinostat add detector

••Histone Control:

•Global response, how to counter the argument.

•Histone , GPD, tubulin, showing a transcriptional level change in proteins

•Evolution:

•LTE (long term evolution)

Can use for yeast as well, write to him: https://en.wikipedia.org/wiki/E._coli_long-term_evolution_experiment

•Yeast growth defect?

•Colony size, long time does colony size increase?

•Know the phenotype.

•Directed- knock out/ overexpressed, wild type internal control and see which provides a growth advantage. Can pick 10/20/100 genes. May not necessarily provide an advantage.

•Reporter that gives a clear readout right away and can be visualized.

•GFP would be great or a chromoprotein

•Successful sequencing,, Sanger, Genome 700-1000$

•GFP collection for growth rate assay- someone else done it?

•Wild type in GFP collection

•Protocols plan

•Dr.Kachroo collection promoter is regulated, can up down regulate with the collection

•Talk to Saba

•Specificity****

•Expression Changes

•Diploidy/ Haploid

Meeting

- Clinostat
    - Shaking speed: yeast settles quickly
    - Modular (Gabe dimensions)
        - Liquid culture : 50ml falcon tube rack 9 tubes (rack & tube dimension)
        - Agar plate (dimensions of standard plates & product) : does this make sense?
        - 96 well plate (dimension)
    - Clinostat 3D defend the choice for yeast (defense) Lancia
    - RPM - repetitions per minute. Materials and Methods/ Supplementary (Brian)

08/05/2020 Minutes

- NO LOGIC GATE
- Genetics experiment **Toulouse-** GAL3, GAL10 promoters express GFP
  (Gabe & Natasha)
    - Activity of the promoters
    - GFP
    - 96 well plate
    - Salt (Osmoregularity), Ethanol, pH decrease, H2O2
    - Triplicates and dif concentrations

- Dif second meeting time? Thursdays after team meeting
- Cell will forgo protein synthesis to protect ER and mitochondria in ug
- Considering half-life degradation will be affected in

- Enhanced fluorescent proteins in yeast yEGFP

- **Non-conventional strains**
    - Iain from Lallemand
    - Papers we have: which ones do they use?
    - BioNutrients (NASAs bionutrient program with VitA)

- Clinostat
    - Shaking speed: yeast settles quickly
    - Modular (Gabe dimensions)

        - Liquid culture : 50ml falcon tube rack 9 tubes (rack & tube dimension)
        - Agar plate (dimensions of standard plates & product) : does this make sense?
        - 96 well plate (dimension)
    - Clinostat 3D defend the choice for yeast (defense) Lancia
    - RPM - repetitions per minute. Materials and Methods/ Supplementary (Brian)

08/04/2020 Minutes

Tues: Meeting new tasks and goals, Gabe sends out tasks individually after the meeting
Thurs: Lancia team checkin in the morning (can cover big issues at the meeting)
Friday: Have two papers posted with a brief summary

- Review tasks
- Bronze medal- let's wrap it up :)
- Logic gate:

Answer

1. What makes a good reporter in yeast?
2. What are the specifications for our reporter? What does it need to do?
    - Give a clear signal when there is a change in ug
    - Quantifiable
    - Be consistent
    - Be bright
3. What is the main design used in research papers?
4. Why do they use this design?
5. What are the pros and cons of the methods found in relation to our project?
6. Are there any criteria we haven't considered?

    - Does the reporter we choose influence the design? eGFP...
        - Nislow for the reporter:
            - brightest one possible
            - Half-life
            - Gut: bright and short-lived, but depends on experiment
        - Can't analyze until splashdown two weeks later
        - "You might consider keeping it simple to start, but building in the ability to reuse the pieces. Take out the classic synthetic layout modular synthetic biology approach where things you build first might not be what you want, ultimately, but at least you can reuse those parts."

    - Adaptive evolution as a better choice, letting the cell tell us what it needs to survive in that condition. Moving to directed once you have good candidates.
    - Work with diploids or strains with duplicated genes of interest

- WIKI
    - How the cell is affected in microgravity
        - Space yeast with popup?
    - Promoter selection process (software, criteria, HP, validation by Dr. Nislow)
        - Infographic
    - Why we chose yeast
    - Proposed methods & experimental design
        - Infographic
    - Implementation
        - Bioreactors for space applications
        - AddGene

Promoters:

GAL 3 - Vit A Toulouse
GAL 10 - Vit A Toulouse
HSP 30 - heat shock
OXR 1 - oxidative
OPI 10 - DNA replication stress
SAF 1 - Nutrient Signaling
RAV 2 - ATP-ase RAVE complex
BTN 2 - snare
Add (Nislow)
-SOD1
SOD2

Maybes:
TDH1 - Vit A Toulouse
COS8
DSC2

07/29/2020

Task & 1-2 slides next week + post 1 paper to the channel with a short summary

- Does yeast make H2O2 in microgravity? (Gabe)
- Oxidative conditions of yeast in microgravity. (Natasha)
- Do beta-carotenoids & Vitamin A cause stress in yeast when produced? (Khash)
    - BioNutrients program producing carotenoids
- Logic gate in Yeast (Labrini & Nhi)
    - Note researchers
- Bronze medal criteria finish next week, 1 week to add if needed, take for review Aug 10th (Brian)
- Promoters (Lancia & Gabe)
- Microgravity document (Lancia)

- Slides due every tuesday. 1-2 summarising what you have been working on
- During the week, post min 1 paper/ review/ video to genetics with a summary. Let's learn together!

Team progress:

- Benefits and usefulness of a logic gate (using AND vs OR): Labrini, Nhi
    - Labrini working on it today
    - Nhi learning what a logic gate is.
    - And gate both open to know there is a signal, OR gate if have a signal don't know which one was open
- Other possible ways of doing it: Natasha, Gabriel
    - 2 micron plasmid replicates autonomously, con 60 copies
    - Yeast integrating plasmid
    - Yeast artificial chromosome
        - Large, telomeres and centromere (degradation)
        - Low copy number
    - Idea: Insert

- Bronze medal Brian (finish by next week)
- What kind of stressors tend to be predominant in microgravity (and specific to microgravity) Khash
    - Does yeast make H2O2 in microgravity?
    - DNA replication & recombination, chromatin remodeling
        - Give drugs to emulate effects

- Gabe and Lancia (promoter review & Eppendorf)
    - Eppendorf submitted
    - Promoter review still underway. Tomorrow.
    - Check radiation

This week's tasks, be ready to present with one or two slides
Tuesday, July 27th at 5pm:

July 21- July 27th :

- Benefits and usefulness of a logic gate (using AND vs OR): Labrini, Nhi
- Other possible ways of doing it: Natasha, Gabriel
- Bronze medal Brian (finish by next week)
- What kind of stressors tend to be predominant in microgravity (and specific to microgravity) Khash, Sam
- Gabe and Lancia (promoter review & Eppendorf)

July 21st 2020 Meeting

Gabe, Brian, Labrini, Natasha, Mo, Lancia

- NEB:
    - Q5® High-Fidelity 2X Master Mix (NEB #M0492S)
    - NEB 5-alpha Competent E. coli ** (NEB #C2987I - Not available if ordering #E5520S)

- Brian Bronze medal
    - Biobricks in independent research * incorporate their data
    - Doing research and presenting

- Feedback from interviews:
    - Macauley
        - Study with SMG in *E. coli.* Developed 8 dif antibiotic resistances, back on Earth lost 6 of them. Some stay, a lot of them are going, which is why it's a **tolerance** not a **resistance**.
        -
    - Dr. Potvin-Trottier
        - READ and gain all that insight
        - Look at master regulators.
        - OR gates
          Remember feedback. If you have an AND gate what you are seeing might be due to another promoter being upregulated that is part of a feedback loop causing a

change in expression of your candidate. False positive. Better to have one reporter per promoter.
    - Up or down regulation shouldn't be an issue
    - Generalised (MSN 2/4) and also not generalised
    - GFPs, no luciferases
    - Drs, Whiteway, Martin, Kachroo when we have a better plan

- Is this stress response even a problem in space, in yeast? Find literature to back it up.
- What does SBOL stand for? https://sbolstandard.org/applications/
- Why is median abundance/cell relevant?
- Why are genetic interactions relevant?
- What is a physical interaction vs a genetic interaction?
- **Why are we developing microgravity-induced stress resistant strains?**
- **Why are we working in yeast?**
    - Nutrients productivity in yeast vs *E.coli.* And other model organisms
- Accessing papers online

CLARITY:
Project Goal: To develop space compatible yeast for bioproduction of *nutrients* in space.
Genetics Goal: Want a signal that is specific to microgravity which can be used to engineer more tolerant or resistant organisms to microgravity resistant stress.

- Why need a logic gate
    - Selected X promoter candidates. Start without logic gates; one promoter, one GFP per strain. Evaluate single reporters/ efficiency.

- RHO1- good for generalised, actin & cytoskeleton master regulator
- TDA10- not a lot of regulation from other stressors (~15 individual stressors)
- GYP7- Very abundant, good FC, 64 interactions, 11 regulators
- BTN2- v-SNARE, weird choice, SNARE imp to budding endosomes, great FC, lots of interactions and regulators, lots of stressors, lot of literature
- OXR1- oxidative and heat damage. Low abundance, FC1.59, lots of interactions, mostly genetics, only 3 regulators, lots of stress response
- DSC2- ubiquitin ligase
- IMA3- cleaves signaling sugars, important for recycling and degradation of proteins and membranes. Not super abundant. VERY LOW abundance (166). VERY low interactions. Control?
- COS8- plasma membrane, v abundant. Low genetic interactions 14
- CMK2- calmodulin-dependant protein kinase, neg feedback Ca pathway, lots of interactions.
- SAF1 - F-Box protein involved in proteasome-dependent degradation of Aah1p, not very abundant; 24 genetic interactions.

- PLN1 - Stability of lipid droplets (respiratory growth, possible ATP/ADP exchange), very abundance
- HEM3 - Heme biosynthesis (cytoplasm and nucleus), very abundant and highly regulated, only one known regulator (for transcription of genes in hypoxia)
- YSC84 - Actin binding protein, actin binding protein, stabilises binding between actin globules and actual actin fibers, ok abundance,
- OPI10 - Possible role in phospholipid biosynthesis, ok abundance and regulation
- RAB2 - Part of RAV complex, associates with vacuolar membrane atpase, low change, ok abundance, a lot of interactions

Tasks: (No more promoter research :( )
Benefits and usefulness of a logic gate (using AND vs OR): Labrini, Nhi
Other possible ways of doing it: Natasha, Gabriel
Bronze medal criteria: Brian
What kind of stressors tend to be predominant in microgravity (and specific to microgravity): Kash, Sam

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July 15th 2020

Attendance: Labrini, Gabriel, Natasha, Nhi, Khash, Lancia, Brian

Tasks For Tuesday the 21st :

- Do in Benchling, create a new entry using 'Promoter Selection/ Round 2'
- Are the interactions protein/protein, or genetic interactions, and how do they interact?
- Yeast Stress Expression database: http://www.ystrexdb.com/search.php
- Yeastract:
  Pattern matching → Find tf binding site, gray our DNA motifs (clikc on it), add fasta promoter sequence and search
  Fasta format:
  >nameit
  Pastesequence
- Designing logic gates in yeast. promoter→ signal→ terminator
  - See papers Mo is going to post (Nielsen, Voigt 'Genetic Circuits' in *E.coli*, Fussenegger 'Genetic Circuit review' in mammalian cells)
  - Find other papers, what kind of architecture is good for us? Do people build logic gates in yeast as reporters? Who, can we contact them? What did they do?

Labrini- COS8, CMK2, SAF1
Gabe- IMA3, TDA10, GYP7
Natasha- YFC84, OPI10, RAV2
Nhi- DCS2, BTN2, OXR1
Khash- TRX3, RGI1, EMP46
Lancia- RHO1, promega/ NEB order, description
Brian- HEM3, PET10, bronze medal

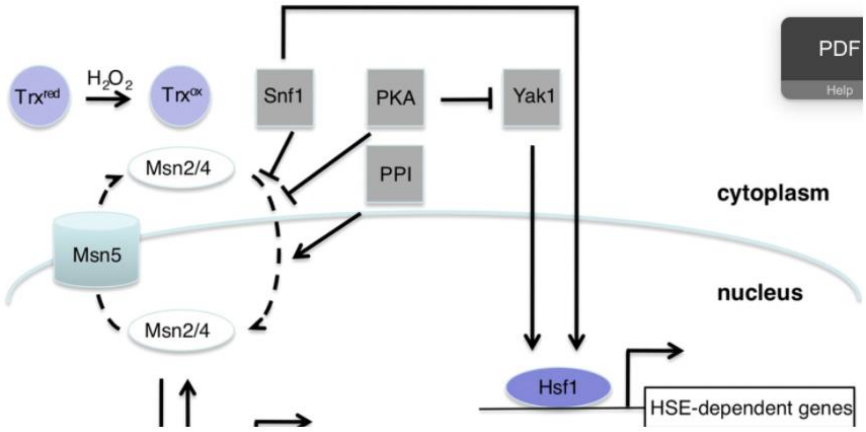
Primary.

Add your research

1. **IMA3**
    a. Low abundance (166), but upregulated almost 100 times
    b. Alpha-glucosidase, cleaves alpha-1,3 linkage of nigerose and turanose (signalling sugars rather than for energy)
    c. Only 5 interactions (know the interactions)
2. **RHO1**
    a. Up 1.7, 5 generations, 281 interactions
    b. Establishment of cell polarity and cell wall synthesis, role in the actin cytoskeleton, focal adhesion, cell wall expansion
    c. Considered the 'master regulator of CWI signalling' because it receives major input from the cell surface and also regulates a variety of outputs in cell wall biogenesis, actin organization and polarized secretion
    d. Homolog of RhoA mammalian (but downregulated in microg
    e. In microgravity: Undesirable changes in microtubule, actin stress fiber and intermediate filament organization. Perinuclear clustering in microtubular network.
    f. GTPase, essential gene
    g. Papers: see Benchling
    h. Positioning in pathway- see Benchling

3. **TRX3**
    a. Up, 1.42, 5 generations,
    b. 68 interactions, 5 regulators, med abundance 7223 +/- 4845 (yeastgenome.org)
    c. Oxidative Stress Response. Cell redox homeostasis.
    Disulfide oxidoreductase, acting on a sulfur group of donors
    d. Regulates Msn 2/4 (stress-response transcriptional activator which then migrates to nucleus and activates transcription of STRE genes; stress responsive elements)

e. Mitochondrial



Secondary

- CMK2
- TDA10
- OPI10
- BTN2
- RGI1
- SAF1
- GYP7
- RAV2
- OXR1
  OXR1 - OXidation Resistance - Unknown molecular function
  1265 +/- 238
  1 pathway
  88 interactions
  Midrochonria

- EMP46
- PET10

Questions for tomorrow:

- If the function of the protein is unknown can we use it? Should be ok, we're just using the promoter
- Mitochondrial, ER… For tagging -> soluble cytoplasm, degradation is an issue too, tagging not helpful for the actual reporter

- Do they have to be near each other?
- promoter→ signal→ terminator
- Localization important for tagging, vs promoters
- Yeastract can say what transcription factors affect the promoters
- Clarify what the interactions are. Is it in the promoter or the protein?
- Yeast Stress database

07/14/2020 Minutes

Updates:

- **Brian:** Bronze medal criteria
    - List of parts on Benchling. See 'Part List'
    - RFP in microgravity *E.coli*
    - *Cell, cycle degradation tag*
    - To join office hours Friday and see what they mean by 'add literature'
- **Promega Grant**
- **Spreadsheet from Sheenan: [doc link]**

2 days Promoter selection

These will be long meetings heads up.
When the zoom session ends, we take a 5 minute break, then join back in.

Day 1

1. Explain project Goal: Resistant strains
2. Each team member presents what they have on their promoters. Good candidates:
    a. Gabe:
        i. **IMA3- only 6 interactions!, metabolic**
        ii. CMK2- abundant, protein kinase, central to other pathways, Ca2+ tolerance, lots of interactions
        iii. **YFC84 (cytoskeleton)**
        iv. **DCS2, 48 interactions, very abundant**
    b. Labrini
        i. TDA10- ATP binding unknown function
        ii. OPI10- upregulated against industrial anti-foam against, phospholipid biosynthesis
        iii. BTN2- v-snare (might be tricky)
        iv. RGI1- cell membrane, periphery
        v. SAF1- same study as RGI1

vi. **COS8 - membrane protein**

c. Nhi
   i. GYP7 Gtpase-activating protein for Ypt7 Protein
   ii. RAV2 (unknown function, lots of interactions with other genes)
   iii. OXR1

d. Brian
   i. HEM3

e. Lancia
   i. RHO1
   ii. TRX3
   iii. EMP46
   iv. PET10/ PLN 1 (lipid droplets)

3. Select 10 strong candidates. If we don't have them, we will find them, in-meeting, together from this extended list:
   a. *REG1- glc starvation- Lancia
   b. RTS3 - Lancia
   c. KIN82-Lancia
   d. TPK1 - Gabe
   e. SDH8- Lancia
   f. DCS2 - Gabe
   g. BDH2 - Gabe
   h. YKL091C- Lancia
   i. EMP46-Lancia
   j. SAF1 - Labrini
   k. VHS3 - Gabe
   l. CBP4 - Labrini
   m. OXR1
   n. PET10- Lancia
   o. YMR087W
   p. BLI1 - Gabe
   q. ACH1 - Labrini
   r. YGR237C - Gabe
   s. COS8- Labrini
   t. GLC8 -Labrini
   u. PTP1- Lancia
4. From the ten good candidates we have, validate our findings with other papers- are the results consistent?
5. Prepare for tomorrow's meeting, Wednesday at 4pm with Mo:
   a. Familiarize yourself with the ten promoters we have selected
   b. Think about how these could make a good project

Day 2

- Present promoter findings and discuss them with Mo

07_06_2020

Genes and tasks for July 14th 5pm meeting

Labrini	Nhi	Gabe	Khash	Brian- Bronze medal criteria.	Lancia
RGI1 TDA10 MPC3 BTN2 OPI10 YMR084W	AFR1 ARG82 RNY1 GYP7 RAV2 TDA1	STP4 IMA3 OSW2 CMK2 VID30 THI4	SDS22 YPI1 RRT8 MDH1 YHR016C PRM8	#1 PRIORITY List of 10 parts we could characterize/ add information to. Start with teams that have worked with microgravity. Go to the parts they submitted in the registry and find ones that are poorly characterized, aka have little information. Another option is EGFP codon-optimized for yeast. #2 If you come across parts we could maybe use in our project make a list, don't go into detail	Project Description Promega Submission IGO2 RPN4 PCL8 TRX3

1. Housekeeping
2. Promega Product List
3. 10 promoters and their gene sequences
4. Project Description
5. Tasks, Goals & Deadlines

---

1. Housekeeping
    a. Yeastract
    b. Take initiative, collaboration to execute our subteam goals.
    c. Successful if you are all critical of one another 'Dr.Kachroo' Promega Product List
    d. Two meetings?
2. Promega Product List
3. 10 promoters and their gene sequences
    a. Choose best candidates from different pathways
    b. Top 20-30 genes that are upregulated/down regulated
    c. Don't overthink interactions
    d. Next tuesday's meeting:
        i. Be thorough, choose top 3 as per gene selection criteria
        ii. Upregulation preferred- production better
        iii. New list of genes
    e. Software team
    f. Do people build genetic circuits with downregulation?
4. Slides- visuals
    a. Split GFPs two promoters producing the same product
    b. GFP/YFP overlap & crosstalk
5. When to Meet
6. Project Description: [doc link]
7. Tasks, Goals & Deadlines
    a. Wednesday, July 8th, 10am
        i. iGEM Toulouse, France collaboration meeting
    b. Friday, July 10th
        i. Confirm & submit Promega list
            1. Promega luciferases
            2. Kits Mo
        ii. List of genes in the registry we could use for our project and for the Bronze medal criteria
            1. EGFP codon-optimized for yeast or luciferases?
            2. Microgravity yeast parts?
    c. Tuesday, July 15th
        i. Promoters and Gene sequences:

Upcoming tasks August 1st

ii. Building the reporter circuit
iii. Develop a plan for presenting Genetics work on the wiki
    1. What will we showcase?
        a. Description
        b. Logic gate

c. Protocols?
d. Collaboration with Software

07_03_2020

to HiBit or not to HiBit! That is the question

Gabe, Khash, Lancia, Hajar, Brian

1. HiBit or GFP or luciferase?
    a. Transcription factors
    b. and/or gate
    c. Dynamic expression testing - when affected, but not to what degree, says when microgravity is on. HiBiT
        i. Can have micro spectro
    d. Grouping genes by physiological function
    e. Applying the reporter (on/off)- other ie GFP
    f. Sending it to space…
    g. Sequencing genes after experiment
2. Experiments/ methods
    a. Slides for CRISPR protocol (overexpression) RNP purified Cas-9
    b. Plasmids- specific copy numbers, plasmids for insertion- cheaper but longer
        i. Restriction enzymes
        ii. Mini-prep
        iii. Polymerase
    c. iGEM kit
3. Products
4. Lancia send list to mentors Sunday pm
    a. Ask Vin to check if we already have lots of something from last year
    b. Send outline of methods we plan to use
    c. Example protocols
5. Remember
    a. Goal is control strain to develop microgravity strains.
    b. Real-time monitoring is cool

    Draft/ Working doc: [doc link]

Promega 2020 Catalog: [doc link]

Notes:

- Conflicting studies
- Want to look at regulation/ expression levels ourselves
- Mo- promoter strength

Gene/ Promoter Selection Criteria for Control Strain

- Use data obtained by software team.
  Look at FC and p-values <0.5
- Looking for well- characterized OR consistent and predictable expression
- Is the gene/promoter part of many pathways or relatively isolated? Prefer genes that don't have too much cross-talk; helps isolate the response, prevents false positives
- Is the gene affected by other stressors such as salt/ion concentration, pH, or changes in osmoregularity? We prefer it is only affected by microgravity (as much as possible) to prevent false positives.
- Gene/ Promoter does not have to be significantly affected by microgravity but we do need consistent and predictable expression.

In Benchling, using the template, answer:

1. Gene ID and strain for ID (GenBank)
2. Gene name
3. Function of gene- what does it do?
4. In microgravity- how is the function altered?
5. Methods use in publication (HARV, microarray...)
6. Median Abundance in molecules/cell (yeastgenome.org)
7. Regulation (up or down)
8. Amount Fold Change in regulation (FC) ie +/- 1.8
9. Units of fold change (ie 5 generations FC vs control)
10. Position on Chromosome
11. Sequences (NCBI)
12. Promoter Expression levels (Use Yeastract)
13. Brief effects of stresses such as osmoregulation, salt or pH stress in normal gravity
14. Adequate concentrations in the cell- Yeast Genome
15. Paper title and link (assure is on Zotero)

- Gene/promoter selection
- What makes a good reporter in yeast?
- Hibit vs GFP….
- Transcriptional vs translational fusion
- Logic gates….

06_29_2020

This week: FOCUS on GENE/ PROMOTER selection

SLIDES: overview [doc link]

- Hi-bit advantages in detection, high sensitivity, 7OrdersMagnitude larger linear curve than GFP
- Approach: Real-time Hibit or transcriptomics; proteomics- large number of proteins, not ideal
- CRISPR - overexpression artifacts vs Plasmids- promoter for gene with tag inserted
- Looking for accuracy, therefore suggest tagging the gene itself, won't have more copies than there would be, more promoters, etc… or underexpression. Using CRISPR is more reflective of what is really happening in the cell. Plasmids are cheaper.
- GFP- higher standard of error

Tasks:

1. **Brian:**
    a. Browse the registry. Make a point-form list of any parts you come across that could be useful to our project. We will also investigate these for Bronze medal criteria
    b. Complete questions below for promoter/ gene research you've already done

2. **Gabe/ Lancia:**
    a. What is required to make a good reporter in yeast? Is fold-change the only factor we should consider?
    b. Complete questions below for promoter/ gene research you've already done

3. **Software:**
    - T-test: control vs experimental results
    - P value is confidence taking into account everytime we run a t-test
        $P < 0.05$
        Adjusted p-value is stricter
    - GEO2R
    - Transformed to logFC
    - Coexpression analysis: microgravity vs other stressors coming these weeks to help narrow down

4. **Nhi, Labrini, Brian, Gabe, Lancia**
    Gene research for reporter strain (assigned genes that met the p-value criteria from the software team)
    This can be informal and in point form (remember in yeast!)

For each gene input results in Benchling using the Gene/ Promoter template

i. Promoter- *Use Yeastract*
   What info? Expression levels….
ii. Median Abundance (molecules/cell)- *yeastgenome.org*
iii. Sequences- *Use NCBI*
iv. Function of gene- what does it do?
v. Well- characterized OR Consistent and predictable expression
vi. Brief effect of stresses such as osmoregulation, salt or pH stress in normal gravity
vii. Adequate concentrations in the cell- Yeast Genome
viii. Add references
   1. Which papers did you use?
   2. What methods did they use?
      SMG/ real space...microarray…

Genes with low p-value and high change in regulation.

[doc link]

Gabe	Brian	Lancia	Labrini	Nhi	Khash
AAH1	ARX1	PWP1	CSI2	DSE1	MCD1
PHO3	RNH201	PHO84	INA1	PCL2	RNR1
ALB1	TOS4	SDA1	UTP4	PLB2	YOR338W
NSR1	YTM1	RPA34	BMT5	RSA4	LEU9
GCD10	PXR1	HAS1	FAL1	RRB1	YGR079W
ADE17	DHR2	JJJ3	TMA46	REI1	DBP2
GCV1	HIS1	ENP2	FCY2	SPB1	CLB6
NOC4	NIP7	PRP43	NRM1	GFD2	UTP21
IMP4	HMT1	UTR2	MAE1	PHO5	WSC4
			NOG1		

****If you need more genes let us know*

Mo's feedback:

Hey team, I've looked into your minutes and I have a few comments. Please make sure to read them, and let me know what you think. It's a long post (Sorry!), and it contains the word reporter at least 50 times.

1- I'm not very clear about why you still want to use HiBit. You've all agreed you want to build a reporter strain. From what I've seen in the literature, scientists with many years of experience say that transcriptional fusions (using promoters) to create these reporter/control strains are simple and very effective. HiBiT is a method for creating translational fusions (tagging genes) to study gene expression levels. Also, you can't build logic gates using translational fusions (or at least not nearly as complex as the

ones you can build using promoters). So why not use promoters? I understand the usefulness of HiBiT since it doesn't require excitation and so is easier in space, but you could also use Luciferase, or any other non-excitable reporter gene for this purpose. If the goal is just CRISPRing something into the genome you could still do that with transcriptional fusions using promoters.

2- Regarding the GFP higher standard of error comment: GFP has been a useful tool for years for scientists everywhere. Why would it not be good enough for your reporter/control strain (apart from it not working in space)? Do you really need 7 orders of magnitude for a reporter strain? You are not studying levels of gene expression fluctuation, or quantifying protein abundance, which this type of tag is used for. You most likely will not need that level of dynamic range for a reporter strain. You just want a strain that lights up in a certain condition.

3- As I've mentioned before, discussions about what type of reporter gene are very premature at the moment, and I wouldn't waste my time on them for now if I were you. You can choose fluorescence, luminscence, chromoproteins, etc, and you can choose when the time comes (In particular for example if you're actually building genetic circuits with split reporters then you might be stuck with splittable ones like GFP and Luciferase). For now, you should decide on the strategy of the reporter strain, either transcriptional or translational, with sound justifications for whoever is judging your work. And as I've mentioned before, you're not going to build the one ultimate reporter strain. You can always use multiple reporter genes of different types, which last year's team has done btw. For now, because we have a deadline for Promega, we need to sort out HiBiT once and for all. In order to do this you need to answer the following questions, to convince your fellow team members, mentors and yourselves that it's worth spending a significant chunk of our Promega winnings on this system:

A- Are translational fusions better than transcriptional ones? (i.e. is tagging genes with a reporter better than using promoters to express a reporter gene). If so, what are the advantages of using translational fusions as reporters? Please provide examples from the literature (Not examples of using HiBiT, just translational fusions).
B- Do you need that massive 7 order of magnitude dynamic range for a reporter strain? If so why?
C- Create an experimental outline for how you envision the experiment to be done in both simulated and actual microgravity, with a HiBiT reporter and with a Luciferase-based reporter (4 very brief, rough, experimental outlines in total, even a small table should do. Just briefly cover details like cell lysis, measurement, incubation etc).

Finally, and that's a piece of advice for y'all, overly complicated and convoluted solutions don't always work in biology. Just keep in mind you're engineering a living biological system, not some inanimate material, and there's a lot of unforeseen results and outcomes to experiments.

IMPORTANT PAPER TO READ: Schwinn, M.K. et al. (2018) CRISPR-mediated tagging of endogenous proteins with a luminescent peptide. ACS Chem. Biol. 13(2), 467-474.

New members resource document: [doc link]

Next week:

Registry research

b. Bronze medal criteria **#4 Contribution by adding literature**
   https://2020.igem.org/Judging/Medals
   i. Search for an iGEM part that has little to no information
   ii. Find out what information is usually present in part pages. We are making a contribution. What form could this take?
   iii. Try to find info from literature to contribute to a lacking iGEM part

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06_25_2020

What we are doing:
[doc link]

Current action item (Due by July 7th): List of 10 promoters for your reporter strain. Please read reviews and papers about building reporters in various systems. If fold upregulation is really the only factor taken into account, then this is really just a one day job (by collaborating with software). We can always modify and add to the list later on. Please prepare a google doc with the promoter and gene sequences, from databases like yeastract and ncbi, respectively. Once looked over by everyone we can then upload our first promoter parts to Benchling!!
Upcoming action item: Design genetic circuits using these promoters. Please look at this review for preliminary reading into designing genetic circuits (https://www.nature.com/articles/s41580-018-0024-z). There's nothing that comprehensive for yeast unfortunately as far as I know, but a lot of principles are shared with mammalian cells. Particularly, check out **two-hybrid and split expression systems** for building logic gates. We can discuss this more when you start. One question we will need to answer is: Does modelling circuits actually help? Genetic circuits are usually designed without modelling, as the principles for building them are already known. Modelling is applied sometimes for later circuit streamlining and tuning, but may not be necessary for our purposes (https://www.nature.com/articles/nmeth.2926) (This paper is only for the basics of designing circuits, the details are specific for bacteria, and don't apply for eukaryotes).

06_22_2020

- Team plan & tasks including timeline
- Goals for next week
    - All papers on Zotero
    - Benchling tutorial
- recruitment….

06_09_2020

A. Assign Tasks to other team members:

Name	**Task**	**Deadline**
Someone who knows modelling well	Bronze Medal: Contribution Add to an existing part- model in Matlab?	July 30th
	Silver: Engineering Success	Aug 30th
Lancia	What kind of collaboration would we need and can we propose? Make a List	June 15th
	Timeline	Next week?
	Genetics tasks assigned weekly Thursdays. - Khash Ptp2 & Sdp1	Wednesdays

NOTES:

- Hibit lifetime ~6min

a. MEDAL CRITERIA
[doc link]

i. BRONZE: Contribution:

a. Make RTTA accessible to other teams

b. ***or*** **add documentation to an existing part on the Registry-** we could model something in Matlab even- something simple and clean

c. *or* Build upon existing software or hardware tool

ii. SILVER: Engineering Success:
Demonstrate engineering success in at least one aspect of your project. This achievement should be distinct from your Contribution for Bronze.
   a. Model Reporter Strain
   b. Software & Database (ask iGEM)

iii. GOLD: Use modeling to gain insight into how your project works or should be implemented. Explain your model's assumptions, data, parameters, and results in a way that anyone could understand.
Has to be different than Silver
   1. Model Control Strain

b. Collaboration Proposals
   i. Standford-Brown-Princeton *Astropharmacy* (2019) in bacteria…
      1. SMG for them
      2. Model something together
      3. Express their medicine in yeast
      4. Resistant strain
   ii. Copenhagen- *Ovulaid* (2019) and *PharMARSy* (2018) both in yeast
      1. Express their medicine in yeast
      2. Resistant
   iii. FYI Queens Chronic Kidney Disease
   iv. ULaval down for collaboration but working with maple syrup production- more details to come

B. Interview questions- insert below
   a. Do you use control strains in your studies
   b. What databases do you access for your research or what are your go-tos for research?
   c. What other disciplines do you collaborate with? What are the benefits or challenges?
   d. What methods would you use?

06_02_2020

Attendance: Gabe, Paula, Lancia, Maher, Brian

- What have we found?
  - Gabe: Zotero posted Chapter on yeast autoregulation
- Input Database

- Common approach to research, all on the same page
    - Refer to project description and mentor's notes
- **End Goal of** :
    - Database: to display data
    - Reporters: to reveal mechanisms of microgravity
    - Resistant Strains: to mitigate the effects of microgravity on yeast to contribute to the end goal of yeast cells resistant to yeast.
        - Control will compensate for radiation/ UV…
        - Don't look at studies in space solely, follow up with LSMMG or Low Shear, if findings are
        - Location of the gene:
            - Cell wall signaling proteins are difficult, cytoplasm, nucleus ok- we are lysing the cell.
            - Proteins under normal conditions are expressed normally, don't need specific condition to express
            - Instead of tagging, use same promoter and express luminescent protein
            - Why not make our cell wall protein the reporter? More specific. Caveat- if cell wall protein need to assure its expressed at convenient levels. Want the gene to be on relatively consistently so we can monitor the changes.
            - Could induce gene to be on regularly by ie putting the cells in salt if they are turned on in salt.

05_26_2020 Genetics

Attendance: Gabe, Hajar, Lancia, Brian, Khash

- Genetics → Sponsorship, what do we need?
- Software → Database any other criteria needed?
    - Use **Zotero** for papers- tool to make it easier for the software team.
    - Enter information here:
      [doc link]
    - Input to **Benchling**:
      Gene name/ GenBank ID/ Yeast Genome/ Description of Gene and Function/ Explain Relevance/ start in Benchling.
- Presentation Slides
- Goal 3-4 pathways, 20-30 genes
- For next week: 3 genes each
- Notes while researching: Not cell membrane proteins (harder for tagging), constitutively active, concentrations protein secretes, expressed differently under microgravity

[Beginning of Genetics Subteam Meetings]

iGEM CONCORDIA

ASTROYEAST 2020