BioHYDRA Lab Notebook

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Thai
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Tina

06.22.15

- Emailed Sahin lab about spores as a mechanism for folding (Kirsten)
- Grew *Bacillus subtilis* cells from spores in LB Chlor media (3 ml) (Erica, Thai, Charles)
- Ordered materials (Dan K)
    - 8-µm polyimide sheets
    - 0.8 µl poly-L-lysine solution (0.1 w/v ratio)
    - Elmer's multipurpose glue

06.23.15

- Emailed director of *Bacillus* Genetic Stock Center about different strains (Dan K)
- Washed *Bacillus subtilis* cells and incubated in DSM starvation medium (Dan K, Thai, Charles)

06.26.15

- Called Dan Zeigler, director of *Bacillus* Genetic Stock Center, who will send us six *Bacillus* strains next week (Dan K)
    - *B. megaterium* (7A16, 7A242)
    - *B. thuringiensis* (4D22, 4Q7)
    - *B. subtilis* (BKE17030, BKE28410)
        - Resistant to erythromycin (1 ug/ml)

06.29.15

- *Bacillus* strains arrived

07.06.15

- Plated *B. megaterium* (7A16, 7A242) and *B. thuringiensis* (4D22, 4Q7) onto LB plates (Charles)
    - 37°C overnight
- Picked colonies from *B. subtilis* plates (BKE17030, BKE28410) and cultured in liquid LB (Charles)
    - 37°C overnight

07.16.15

- Created 3 ml liquid cultures of each Bacillus strain (Dan K, Thai)
    - 37°C overnight

07.17.15

- Washed *Bacillus subtilis* cells and incubated in DSM starvation medium (Dan K, Thai)
    - 37°C for weekend

07.20.15

- *Bacillus* strains did not sporulate (with microscopy check). It seems like sporulation medium is strain dependent. Steps to take are:
    - Remake sporulation medium and research strain specific media
    - re-inoculate bacillus strains
    - Transform wasp waterproof protein biobricks into e.coli

07.23.15

- *Bacillus* strains re-inoculated and growing for sporulation (Tina)
- decided on using CRISPRi for knockouts
- knockout of cwID
- ideas to produce fully biological HYDRAs with cellulose binding sites & cellulose
- change color depending on state of bacteria

07.24.15

- Inoculated sporulation medium with *B. subtilis* strains with *cotE* / *gerE* mutations. (Tina)
    - *cotE* mutation - 2 tubes
    - *gerE* mutation - 2 tubes
- Incubating over the weekend in the 37 C shaker (Tina)

Plans

Next week (7/25 - 07/29)

- Make HYDRAs
- Make hella spores

07.27.15

- Check to see if spores have formed
- Make HYDRA?

07.29.15 (All Charles **from here on out)**

- Spores have not formed well. cotE seems more prone to sporulation than gerE
- media may be old, so an order was placed for newer
- Finishing up the design of cellulose binding brick
- Make HYDRA?

08.06.15

- Re-inoculated colonies, and plated on new plates with erythromycin
- Finished the design of our construct, it is a 2kB cotz-aeBLUE-CBD construct and we ordered the part from IDT

08.06.15

- Nothing grew, we need to restart getting fresh bacillus
- Will try with old growth and ask Griffin if he has some bacillus we can use

08.11.15

- Growing strains of B subt from Ryan and germinating all strains from D Ziegler except for b subt. mutation
- Found integration vector to insert cellulose binding domain w/ X and P http://www.clontech.com/US/Products/Molecular_Biology_Tools/Nucleic_Acids/DNA/ibcGetAttachment.jsp?cItemId=10858&fileId=5859575&sitex=10020:22372:US

08.18.15

- Put in backbone and KinA for miniprep, digestion and ligation tomorrow
- Can use kpnI for linearization
- some shiny spores in 100mL, will keep growing

08.19.15

- Some sporulation
- Transformation of cbd and blue, need to order primers for sporulation trigger & restriction enzymes to cut out promoter accIII et BalI

08.21.15

- Gel shows no amplification, will run through CRATER and pick more colonies
- For colony PCR, use white colonies, one red and one original backbone. The original backbone has RFP

- Gel shows that none of my white colonies have the required insert, will keep on picking from the old plate, PCR and run the gel with the CRATER colonies.

08.24.15

- CRATER worked! No red colonies in plate
- Gel run for **08.21.15** colonies and today's as well to see if there is a colony with our insert

08.25.15

- Vf and Vr primers are not compatible with the bacillus plasmid, so we ordered new custom ones that flank our insert
- will overnight grow for miniprep

08.26.15

- miniprep of the colonies
- using colony PCR to determine which colonies contain the insert

08.27.15

- Colony 1 has our 8kb plasmid! All the other colonies seem to have a very small band, perhaps a small amplification of unsuccessful ligations?
- Primers came in, and we sent in the part for sequencing

08.28.15

- Sequencing looks good. The ends of the 2kB cotz-aeBLUE-CBD construct are very well characterized, but the middle is not very well matching. The middle is 1kB away from each end, which explains the poor sequencing results in that area. We ordered primers that amplify from the center of the construct to verify it.

08.31.15

- Middle primers came in, have sent in for sequencing.

09.1.15

- Middle primer sequencing is successful! I am confident my construct is good!
- New xylose induced competent bacillus strain has arrived, will try this new method to see if we can use this to transform the cells
- While trying to make a HYDRA, nothing contracted. I have emailed Xi Chen from Columbia questions about their protocol, to make HYDRAs

09.2.15

- Xylose method did not work, so I will be trying for electroporation soon.
- Xi Chen emailed back. Will be attempting to make HYDRAs again very soon

09.4.15

- Our lab is at 45-50% humidity. I need to get down to 10% to make HYDRA work. I have decided to use the desiccator in our lab.

09.5.15

- I constructed our HYDRAs and put them in the desiccator. Let's see what happens over the weekend!
- Electroporation went without too much trouble, the cells have been plated and we will see if there are any transformants on tuesday.

09.8.15

- No transformants from electroporation. Need to troubleshoot why electroporation didn't work and will try again with a fresh new strain of bacillus that we just received today
- HYDRAs kinda worked! (?) Took a prelim video, will construct a longer hydra today and put in the desiccator

09.9.15

- Took pictures with SEM (scanning electron microscope). It looks good!
- Undertaking transformation using the LMU iGEM 2012 protocol

09.10.15

- HYDRA worked! We have a wavy form that expand and contracts 30 % from 50% to ~0% humidity

09.11.15

- Realized that we are using bacillus that is already chlor resistant, so our plates from the LMU iGEM 2012 MNGE transformation protocol are overgrown. However, using colony PCR, we found some colonies that contain our band (~2.2 kb). We are sporulating those and seeing what happens on monday.

09.14.15

- Using microscopy, we see that our bacillus has sporulated, but the spores are not blue. We are sequencing to check for the correct insert. It is possible that the linkers are preventing the aeBLUE from folding properly.