# Lab Notebook

**Project:** KLS iGEM 2025
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## SUNDAY, 7/6/2025

Attendees: Alyssa, Ansh, Khushi
Summary: Started 2 5ml cultures of E. coli K12 from stab

Inoculated with loop from E. coli K12 stab into 5 ml of terrific broth.
Took values at OD 600, blank OD value is 0.118

- did so by taking a small sample out of one of the cultures, which is now deemed "contaminated"

Put in shaker 37C at 220rpm, let stay over for 2 nights

## TUESDAY, 7/8/2025

Attendees: Alyssa, Ansh, Khushi
Summary: glycerol stocks for E. coli K12

Measured OD 600 from another sample taken from "contaminated" culture -- 0.47

**Glycerol Stocks:**

- Filter sterilized glycerol (using a 0.45 um pore membrane) , diluted with dH2O to make 50% glycerol (in storage).
- 500ul culture + 500ul 50% gycerol to make glycerol stock in 2ml tube
- made 2 glycerol stocks per culture tube. Tubes labeled with red-dot were taken from "contaminated" culture, white dot taken from "uncontaminated" culture

Placed stocks in freezer -20C overnight, with plans to transfer to -80 C later this week.

## WEDNESDAY, 7/9/2025

Attendees: Alyssa, Ansh, Khushi
Summary: made glycerol stocks of electrically competent cells, made chemically competent cells, suspended DNA

**Electrically competent cells**
Following: https://www.eppendorf.com/product-media/doc/en/045157_Protocol/Eppendorf_Cell-Technology_Protocol_Escherichia-coli-K12_Protocol-No-4308-915514-Escherichia-coli-K12.pdf
Made two centrifuge tubes of 1ml of culture from each of our two culture tubes - aka, total 4 2ml centrifuge tubes

- culture from "contaminated" tubes with red dots, from "uncontaminated" tubes with white dots

Tubes were chilled on ice for 15 minutes, before being centrifuged for 20 minutes at 5000g at 4°C
Removed supernatant (aka harvested cells), before resuspending pellet in 1ml of ice cold water
Washed two more times by centrifuging & resuspending in 1ml of ice cold water, as described above
Moved to step 4b of protocol, freezing for later use.

Added 80 ul of 10% glycerol (ice-cold), moved mixture from original centrifuge snap-tubes to screw tubes.

- again placiing red dots + white dots on cap as appropriate

Rapidly froze tubes on dry ice.
Stored in -80°C

**Chemically competent cells**

Pipetted 50ul of transformation mix into 2 2ml centrifuge tube
Added 20ul of culture into tubes, pipetting gently to mix

- marked with red dot = taken from "contaminated" culture, marked with white dot = taken from "uncontaminated" culture

Stored in 4°C
(ran out of time to do transformation)

**Suspended DNA**

Opened dried Plasmids from Twist, 105.1 nanograms
Suspended DNA in 100ul of nuclease-free TE buffer - final conc 1.05 ng/ul
Aliquoted 50ul into another screw-top tube, to be used as "working" solution, while the other will sit in storage
Stored in -20°C

Potential issue: We have used isopropyl alcohol to sterilize screw cap tubes instead of autoclaving them.

Transfered E. coli glycerol stocks to 80C

**SATURDAY, 7/12/2025**

Attendees: Ansh, Alyssa, Khushi, Meilin, Sameer
Summary: Did both chemical transformation and electroporation transformation, plated transformants on LB-ampicillin

**LB broth creation:**

Used a 0.45 um filter to sterilzed 3 ml of lb broth.

**Chemical transformation**

Took out/thawed tubes of transformation mix + culture from 4C

- one from contaminated culture, one from uncontaminated culture

Pipetted 2ul of resuspended DNA (~21 ng) into each tube, incubated tubes on ice for 15 min
Heat shocked tubes at 42°C for 45 sec in a water bath
Incubated tubes on ice for 5 min
Added 500uL of LB to each tube
Incubated at 37°C for 1 hour, shaking at 200-300rpm

- ended up incubating for ~50 min

Used incoulation loops to spread culture on LB-ampicillin plate

- each half is one culture

Leftover culture was placed in 4C

**Electroporation transformation**

Took out competent cells from -80C. Thawed one uncomtaminated culture tube, let the remaining 7 in -20C.
Added 80 ul of competent cells to a prechilled 0.2 cm electroporation cuvette.
Electroporated for 6 ms at 1800v.
Added 1 ml of lb broth, mixed and transfered to a 15 ml sterile culture tube.
Added 2ul of 10.5 ng/ul dna, incubated for 45 min at 37C 220 rpm.
Plated on ampicillin selective plate utilizing inoculation loop streaking method.
leftover transormed cells culture placed at 4C.
Transfered the rest of the competent cell tubes to -80C.

## SUNDAY, 7/13/2025

Attendees: Alyssa, Ansh, Khushi, Meilin
Summary: checked plates, started S. pasteurii culture, made LB-amp plates

Looked at LB-amp plates from yesterday's chemical transformation & electroporation transformation -- no sign of transformants thus far.
Will check again tomorrow, and if still no transformants, will proceed to redo transformation protocol.

**S. pasteurii culture**

Started S. pasteurii culture using protocol from Kelsey, as seen below.
Put 15mL of BHI w/ 2% urea into a 50mL tube
Thawed 1 cryovial of S. pasteurii culture

- Put 150ul of culture into the tube
- The original cryovial of S. pasteurii culture was returned to -80C in a orange foam thing (now our working solution)

Incubated culture at 30C, with dubious rpm (was supposed to be 150 rpm...)

- probably was 300 rpm

**LB-amp plates**

Mixed 100mL of water with 2.8g nutrient agar
Autoclaving took about 1 hour  (was set for unkown aboutn of time, likely 20-25 min)
Added 100mg of ampicillin to 1ml water make 100mg/ml concentration stock
Added 100 ul of stock to 100 ml of autoclaved nutrient agar and poured plates.
Put plates in 4C, stock in -20C.

## MONDAY, 7/14/2025

Attendees: Ansh, Khushi
Summary: did S. pasteurii prep transfer culture

**Prep transfer culture (6:30 pm)**

Added 990 ul of BHI w/ 2% urea into a sterile culture flask
Transfered 10 ul of the starter culture into the new flask
Placed the flask on 30° C incubated shaker table at 150 rpm
Left flask on shaker for 20 hours

**TUESDAY, 7/15/2025**

Attendees: Alyssa, Ansh, Khushi
Summary: transferred S. pasteurii culture, did electroporation transformation

Note: Still no colonies on the set of originally transformed plates

**Resuspending DNA**

Planning on doing an electroporation transformation using the ampicillin high copy plasmid backbone from the iGEM distribution kit (plate 2, O7)
Following instructions, we resuspended the DNA with 10ul of dH2O

- an estimated 1-2ng of DNA in each well.
- so about 100-200pg/ul

**Electroporation transformation**

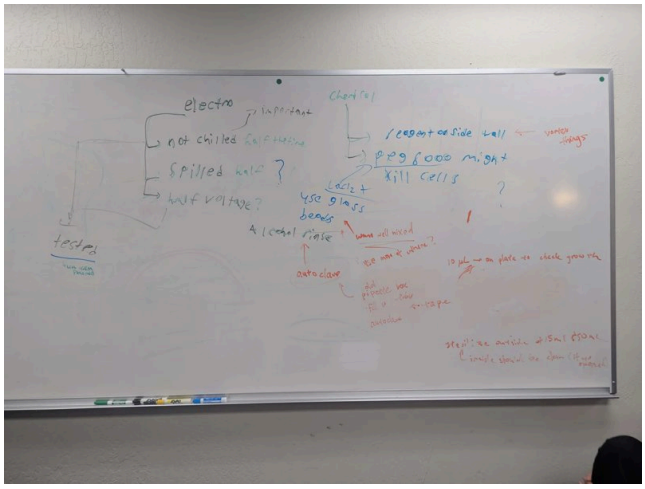
Took out competent cells from -80C. Thawed one [uncontaminated] culture tube.
Added 80 ul of competent cells to a prechilled 0.2 cm electroporation cuvette.
Electroporated for 5.8 ms at 2990V.
Added 1 ml of lb broth, mixed and transfered to a 15 ml sterile culture tube.
Added 3.3 ul of resuspended DNA (from iGEM kit), incubated for ~60 min at 37C 220 rpm.
Plated on ampicillin selective plate by pipetting 100ul of culture & spreading via plating beads
Leftover transformed cells culture placed at 4C.

**Transferring S. pasteurii culture (~7:30 pm)**

Put 990 ul of BHI w/ 2% urea + 10ul of previous S. pasteurii culture into sterile culture tube (15 ml)
Placed the flask on 30C incubated shaker at 150 rpm
Left flask on shaker for 20 hours

Troubleshooting:

**PXL_20250716_022303875 (1).png**




This diagram depicts our thought process while troubleshooting why we have no transformations with Jenny Mae (one of our mentors). On the left is the electroporation transformation, and each of the sub-bullets under it in black lists reasons why it might not be transformed. Same for the right, which is chemical transformation. The translation is written below.

Electroporation transformation (this regards to the very first electroporation transformation we did this summer)

- Our solution was not chilled the whole time (probably cause of cell death -- chilling is very very important, as Jenny Mae later told us)
- Spilled about half of our sample on accident
- Used 0.2 cm cuvettes instead of 0.1 cm at the same voltage - can't change the cuvette unless we buy new ones)

Chemical transformation

- Reagent/liquid was present on on the side of the side wall of tubes, not everything at the bottom
- When making the cells chemically competent, we left them in transformation mix overnight since there wasn't enough time to do the chemical transformation — PEG1000 probably killed the cells during the wait

We also throughout other processes did an alcohol rinses for sterilizatio. Left over alcohol residue could have killed our cultures.

Revisions/Solutions

- Plan ahead + autoclave a bunch of tubes in an empty pipette box, so that we can have sterile tubes
- Do the chemical transformation all in one go instead of waiting overnight (see: PEG1000 comment)
- Try doubling the voltage for electroporation transformation
- Keep the solution chilled the whole time (for electroporation transformation)
- When plating the theoretically transformed culture, use plating beads instead of an inoculation loop
    - Can also pipette a small amount of the transformed culture (10ul? 100ul?) on to another plate just to see if there are cells

**WEDNESDAY, 7/16/2025**

Attendees: Ansh, Khushi

Summary: transferred S. pasteurii culture

**Transferring S. pasteurii culture (12:30 pm)**

Put 20 ml of CMM- w/ 2% urea + 50 ul of previous S. pasteurii culture into sterile culture tube (15 ml)

- 1:400 dilution of S. pasteurii to CMM-

Placed the flask on 30C incubated shaker at 200-300 rpm

Left flask on shaker for 20 hours

Ran OD reading on nanodrop and got 3.0, which was concerningly high. Caveat: We later find that the nanodrop is unreliable for OD 600 measurements, likely making this reading a fluke.

## THURSDAY, 7/17/2025

Attendees: Ansh, Khushi, Alexei, Meilin, Nick

Start time: 2:00 pm, End time: 5:30 pm

Summary: Start on creating biomineralized material, clean out culture from fridge

Got two types sand from local parks. Sterilized the sand by washing with regular water, washing with bleach, and then two washes with reverse osmosis water. Dried sand over hotplate.

Put 5 ml of CMM- with 50 ul of previous S. pasteurii culture into a sterile culture tube (15 ml)

- 1:100 dilution of S. pasteurii to CMM- media

Did calculation to figure out how much calcium chloride should be added to make CMM+ media:

- (40.08 (molar mass of calcium) + 35.42 (molar mass of chloride) *2 + 18.016 * 2)/40.08 * 13.3 * 0.050 = 2.44g calcium chloride
- made 50 ml of CMM+ media, setrilzed by autoclaving, stored 250 ml tube

Added sand to two molds, added CMM-/S.pastuerii combination culture:

- 1 ml to the small mold
- 2 ml to the big mold

Let sit for hour and 30 mins at 30C. Drained both molds, added CMM+ media in similar amounts, left at 30 C overnight.

Autoclaved remaining sand.

## FRIDAY, 7/18/2025

Attendees: Alyssa, Ansh, Khushi

Summary: S. paseturii culture/biomineralization, started E. coli culture

**S. pasteurii biomineralization**

Made transfer culture of BHI + urea at ~5:30 pm

- 1:200 dilution, with 5 ul of previous day culture & 1ml of BHI
- left in shaker at 30C (for next 18ish hours)

Made CMM- + S. pasteurii culture using previous day's transfer culture

- 30ul of S. pasteurii culture for 3 ml of CMM- media

Using 80 wire mesh screen, drained previous day's precipitation media from the molds

Added in CMM- + S. pasteurii culture to each mold

- approx 1 ml
- Let sit for ~1 1/2 hours

Again drained culture via 80 wire mesh screen
Added in CMM+ precipitation media to molds (~7:30pm)

- Let sit overnight at 30C

**E. coli culture**

Put 500ul of terrific broth in 2ml tube
Took working E. coli glycerol stook & used inoculation loop to inoculate culture
37C, on shaker

Note: don't use nanodrop for measuring OD600 of bacteria culture, use cuvettes!!

## SATURDAY, 7/19/2025

Attendees: Alyssa, Sameer
Summary: S. pasteurii culture/biomineralization, chemical transformation

**S. pasteurii biomineralization**

Made CMM- + S. pasteurii culture using previous day's transfer culture

- 20ul of S. pasteurii culture for 2 ml of CMM- media

Using 80 wire mesh screen, drained previous day's precipitation media from the molds
Added in CMM- + S. pasteurii culture to each mold

- approx 1 ml
- Let sit for ~2 hours

Again drained culture via 80 wire mesh screen
Added in CMM+ precipitation media to molds (~5 pm)

- Let sit overnight at 30C

Made transfer culture of BHI + urea (~5 pm)

- 1:200 dilution, with 5 ul of previous day culture & 1ml of BHI
- left in shaker at 30C (for next 18ish hours)

**Chemical transformation**

2 microcentrifuge tubes, appropriately labelled as I & II
Pipetted 50ul of transformation mix in each + ~20ul of fresh E. coli culture
Pipetted 2ul of resuspended DNA (plasmid csg-CA-amp) into each tube
Incubated on ice for ~15 minutes
Heat shock for 45 sec at 42C
Incubated on ice for another 5 min
Added 500ul of terrific broth & incubated for 1 hour at 37C at 200rpm
Added 100ul of culture to LB-amp plate, used beads to spread.
Placed in incubator at 37C (~6:30pm)

Note: Acquired agar and placed in our storage bin (room temp).

**SUNDAY, 7/20/2025**

Attendees: Alyssa, Ansh, Khushi
Summary: S. pasteurii culture/biomineralization, making plates

**S. pasteurii biomineralization**

Made CMM- + S. pasteurii culture using previous day's transfer culture

- 3ul of S. pasteurii culture for 3 ml of CMM- media (1:100 dilution ratio)
- should make more next time -- used 2ml for black mold & 1ml for white mold

Using 80 wire mesh screen, drained previous day's precipitation media from the molds
Added in CMM- + S. pasteurii culture to each mold

- approx 1 ml
- Let sit for ~2 hours

Using 80 wire mesh screen, drained culture from the molds
Added in CMM+ to each mold, let sit overnight

Made transfer culture, in 1:400 dilution ratio (~1pm)

- 2.5ul of previous transfer culture + 1ml of BHI
- **Sidenote: when changing dilution ratios to adjust for a longer waiting period, we've assumed the culture undergoing exponential growth during the time added by the dilution.**

**LB agar plates**

Made 9 1/2 total plates, 5 without salt and 4 1/2 with salt (LB agar)

- really just 4 plates with salt

Used standard lb agar recipes, for 100 ml of media:

- 0.5 g yeast extract
- 1.0 g tryptone
- 1.0 g NaCl (skipped for salt free plates)
- 1.2 g agar

Autoclaved, allowed to cool and poured. In this particular case, waited too long during cooling, resulting in slightly lumpy plates.

Plated electrocompetent cells (contaminated) on LB agar plate
Also plated ecoli stock from yesterday to have cells the next day for transformation.

Note: Biosafety cabinet malfunctioning at the end of the day. Had two people holding up the glass pane as we did the plating.

**MONDAY, 7/21/2025**

Attendees: Alyssa, Ansh, Khushi
Summary: transfer culture of S. pasteurii

Note: Biosafety cabinet still malfuncitoning, the window will not stay up. The lab has dismantled it. Hopefully it will be back soon. At the moment, we're using the good old-fashioned alcohol lamp to do the transfer culture.

**Transfer culture (~6pm)**

- 2.5 ul of previous culture + 1ml of BHI

**TUESDAY, 7/22/2025**

Attendees: Ansh, Khushi
Summary: transfer culture 3:30 pm

1:400 dilution of previous culture, 2.5 ul of previous culture + 1ml of BHI, placed in 30 C.
Still done with alcohol lamp.

**WEDNESDAY, 7/23/2025**

Attendees: Alyssa
Summary: transfer culture, LB broth, chemical competency solutions

**LB broth**

approximately following https://static.igem.org/mediawiki/2015/1/1f/Exeter_LB_recipe.pdf (standard recipe)

- Tryptone powder (5g)
- Yeast extract (2.5g)
- NaCl (5g)
- MiliQ water (500 ml)

Autoclaved, kept in personal storage

**Transfer culture (~2pm)**

- 5ul of previous culture + 1ml of BHI (maybe. the pipette for the culture was mildly iffy, so it might be a lil titchy bit more)

Placed in 30C shaker

**Chemical transformation solutions**

Solution A: ~40 ml of 100 mM $CaCl_2$

- 40 ml of MilliQ water
- ~588.04 mg of $CaCl_2 * 2H_2O$

Solution B: ~40 ml of 100 mM $MgCl_2$

- 40 ml of MilliQ water
- ~800.2? mg of $MgCl_2 * 6H_2O$

Solution C: ~10ml of 100 mM $CaCl_2$ + 10% glycerol

- 8 ml of solution A
- 2 ml of 50% glycerol

Autoclaved all 3 solutions
Parafilmed them, and put them in the fridge at 4C
Note: Solution A was dropped after autoclaving. It needs to be remade.

**THURSDAY, 7/24/2025**

Attendees: Alyssa, Avery, Meilin?
Summary: S. pastuerii culture/biomineralization, chemical competency solutions, E. coli culture (for biofilm or smth)

**Transfer culture (~5 pm, 5:20ish)**

- 1:400 dilution ratio
- 2.5 ul previous culture to 1 ml BHI

**Biomineralization**

- made 4ml of CMM- + S. pastuerii
  - 4 ml of CMM-, 40ul of previous S. pasteurii culture
  - used approx 2ml per mold
- Let sit for 2 hours at 30C
- (painfully) filter sterilized the CMM+ media
- Drained culture from molds
- Added ~2ml of CMM+ media to each mold
- Let sit overnight in 30C

**Chemical competency solutions**

Made solution A of 100mM $CaCl_2$

- 588.04 mg of $CaCl_2 * 2H_2O$
- 40 ml of MilliQ water

Autoclave

**E. coli culture**

- 5ml LB broth (kinda...)
- ~1 colony (more like a streak...) from inoculation loop (from loong ago E. coli... we'll see)
  - it's only E. coli in there tho so it should be fine? lol
- No shaker at 37C -- just let sit horizontally in incubator

Made 2 E. coli cultures -- one for biofilm protocol, one for chemical transformation protocol

## FRIDAY, 7/25/2025

Attendees: Alyssa, Meilin

Summary: S. pastuerii culture/biomineralization, E. coli biofilm, starting to make chemically competent cells

Note: biosafety cabinet finally fixed.

**Transfer culture (~6 pm)**

- 1:200 dilution ratio
- 5 ul previous culture to 1 ml BHI

**Biomineralization**

- made 4ml of CMM- + S. pastuerii
  - 4 ml of CMM-, 40ul of previous S. pasteurii culture
  - used approx 2ml per mold
- Let sit for 2 hours at 30C
- Drained culture from molds

- Added ~2ml of CMM+ media to each mold
- Let sit overnight in 30C

**E. coli biofilm:**

Inoculated an LB-agar salt-free plate with 9 drops of 5 ul of bacterial suspension

- we had 2 tubes of bacterial suspension -- our OD600s were 0.2 and 1.2 or soo..... ummmm anyways

Let airdry

Put in incubator at 37C

**Chemically competent cells**

Secondary cultures from overnight cultures are prepared by making 1000x, 5000x dilutions

- 100ml of LB per tube
- 1000x dilution --> 100ul
- 5000x dilution --> 20ul

Put in incubator at 37C

## SATURDAY, 7/26/2025

Attendees: Alyssa

Summary: S pasteurii culture/biomineralization, E. coli biofilm, chemically competent cells

**Transfer culture (~3:30 pm)**

The culture started yesterday (7/25/25) looked clear... so instead I made two cultures, one using the 7/25/25 culture and one using the 7/24/25 culture.

- the culture made using the 7/25/25 culture had a 1:400 dilution ratio, with 2.5 ul of previous culture to 1 ml BHI
- the culture made using the 7/24/25 culture had a 1:200 dilution ratio, with 5 ul of previous culture to 1 ml of BHI
    - the 7/24/25 culture had been sitting in the fridge, as we didn't think we would be needing it. To compensate for this, I used a little more culture.

Put both new cultures in the incubator at 30C on the shaker.

**Biomineralization**

Got feedback from Chris, our secondary PI that perhaps we need to check out our S. pasteurii since the biomineralizations seems to be very slow.

Nonetheless, went ahead and did the biomineralization cycle for today using the 7/25/25 culture.

- made 4ml of CMM- + S. pastuerii
    - 4 ml of CMM-, 40ul of previous S. pasteurii culture
    - used approx 2ml per mold
- Let sit for 2 hours at 30C
- Drained culture from molds
- Added ~2ml of CMM+ media to each mold
- Let sit overnight in 30C

**E. coli biofilm**

Realized yesterday that I accidentally put the plate for biofilm formation in 37C instead of in 30C. so I moved it to 30C today

I also made another biofilm plate on salt-free LB agar, just in case

- 9 drops of 5ul of some of the 1000x dilution culture (which was started the previous day)
    - should be the right OD -- see below

Again, both plates are now correctly in 30C

- the technically optimal temperature is 28C, but we don't exactly have that many incubators, and Meilin reassures me that 2C just means that there will be less biofilm.

**Chemically competent cells**

Using protocol from the Cellular Computing Team at INRAE

- Each of the 100ml cultures (1000x dilution & 5000x dilution from the prev day) were split into 50ml falcon tubes
- Chilled on ice for 1 hour 30 minutes (was supposed to be 30 minutes, technical delays/difficulties)
- Centrifuged for 15 minutes at 3000rpm at 4C
    - slightly different from protocol written
- Removed supernatant & resuspended cells in 5ml of solution A (100mM $CaCl_2$) before chilling cells on ice for ~30 minutes
- Again centrifuged for 15 minutes at 3000rpm at 4C
- Removed supernatant & resuspended cells in 5ml of solution B (100mM $MgCl_2$) before chilling cells on ice for ~30 minutes
- Again centrifuged for 15 minutes at 3000rpm at 4C
- Removed supernatant & resuspended cells in 2ml of solution C (100mM $CaCl_2$ + 10% glycerol)
- Aliquoted into smaller tubes
    - protocol calls for aliquoting into 50ul portions
    - ended up with a total of 6x 1ml portions, 8x 200ul portions, and 4x 100ul portions (due to lack of time)
- Put in -80C freezer
    - 2nd shelf (top down), 2nd in the row (left right), 3rd in depth, 3rd down
    - Box:
      row 1: 1000x dilution culture, smaller volumes
      row 2: 1000x dilution culture, ~1ml
      row 3: 5000x dilution culture, smaller volumes
      row 4: 5000x dilution culture, ~1ml

Also took OD600 of the 1000x and 5000x dilution culture

- blank -- LB agar
- 1000x dilution culture had approx 0.7-0.8 val
- 5000x dilution culture had approx 0.8-0.9 val
- which doesn't entirely make sense, but it might also be bc this is the scaled up version of me using a 10x dilution... which probably made it less accurate.

## SUNDAY, 7/27/2025

Attendees: Alyssa, Ansh

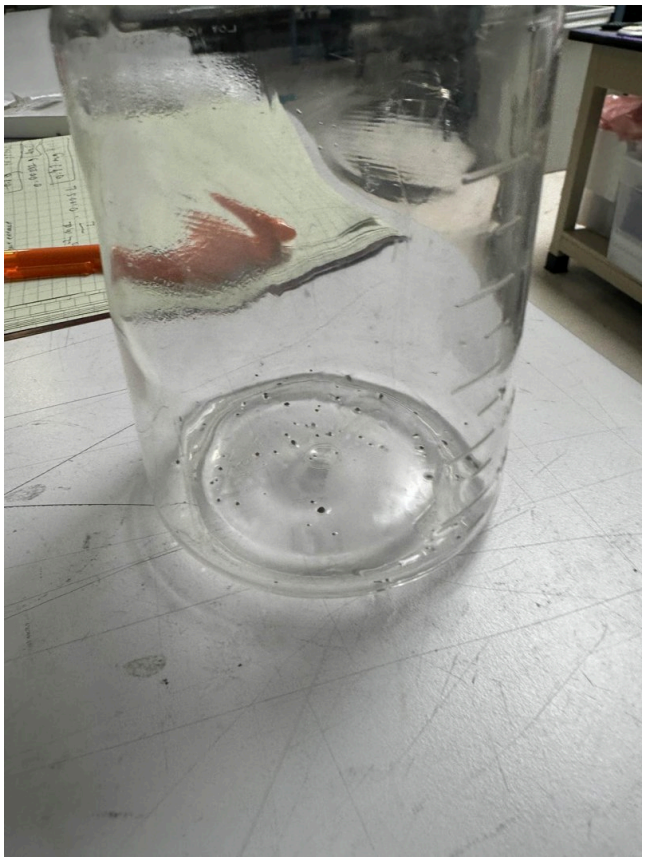
Summary: S. pasteurii culture/biomineralization, chemical transformation, started E. coli culture using Joshi plasmids

Autoclaved the BHI, CMM-, and LB broth we had in storage

- suspicious looking nature of mold/bacteria/black spots in CMM- bottle, so we autoclaved all the broths in our personal storage bin for good measure. Suspect contamination may have occured when using alcohol lamp.
- placed said broths in community cabinet, in the liquids and media section. All are labeled with our name and are sterile.

Autoclaved box of P10 pipette tips, placed in storage

**IMG_4961.jpg**



suspicious looking black spots in jar.... (empty jar as we transfered the CMM- to a seperate jar to autoclave)

**Transfer culture (~6:30 pm)**

- 1:400 dilution ratio
- 2.5 ul previous culture to 1 ml BHI

**Biomineralization**

Uncertain -- is it truly biomineralizing?? we shall see...

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.5 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**SOC media**

https://static.igem.org/mediawiki/2014/6/64/BGU14-06-SOC_medium_prep.pdf +
https://www.thermofisher.com/order/catalog/product/15544034
Recipe for 1L

- 5 g yeast extract
- 20 g tryptone
- 0.584 g NaCl
- 0.186 g KCl
- 2.4 g MgSO4
- + also 20mM of glucose

We scaled down for 500 ml

Autoclaved 50 ml, filter sterilized 5 ml.

**Chemical transformation**

Using protocol from the Cellular Computing Team at INRAE

- Let 50ul of chemically competent cells sit on ice for 30 min (2 tubes)
- Add in 10ul of resuspended pET-csg-CA-amp plasmid (100 ng)
    - Only added DNA to one without red dot
- Heat shock for 90 sec
- Let sit on ice for 10 min
- Add in 250ul of SOC media
- Put in shaking incubator at 37C for an hour
- Plate cells via plating beads ~100ul per plate
    - Put cells on both LB-amp plate (technically nutrient agar-amp) and LB plate
- Put in incubator at 37C

In summary, we have two tubes which underwent the same protocol, just that we did not add DNA to one of the tubes (marked with a red dot)
We plated our cells on plates with antibiotic and plates without antibiotic

- LB agar & nutrient agar/amp

**1000x Kanamycin stock:**

Made 1 ml of 50 mg/ml kanamycin sulfate stock from the dry pwoder that was in community use. May be expired. Stored stock at -20C.

**E. coli culture for Joshi plasmids**

Acquired transformed strain with plasmid from Neel Joshi lab

- curli-GFP plasmid: https://www.addgene.org/166858/
- already transformed into E. coli Mach 1 & put on plate

Made 2 ml culture by adding 2 ml lb broth, 2 ul of 1000x kanamycin stock, and a single colony from the plated bacteria. Incubated at 37 C, 200 rpm. Placed both agar plates at 4 C.

**MONDAY, 7/28/2025**

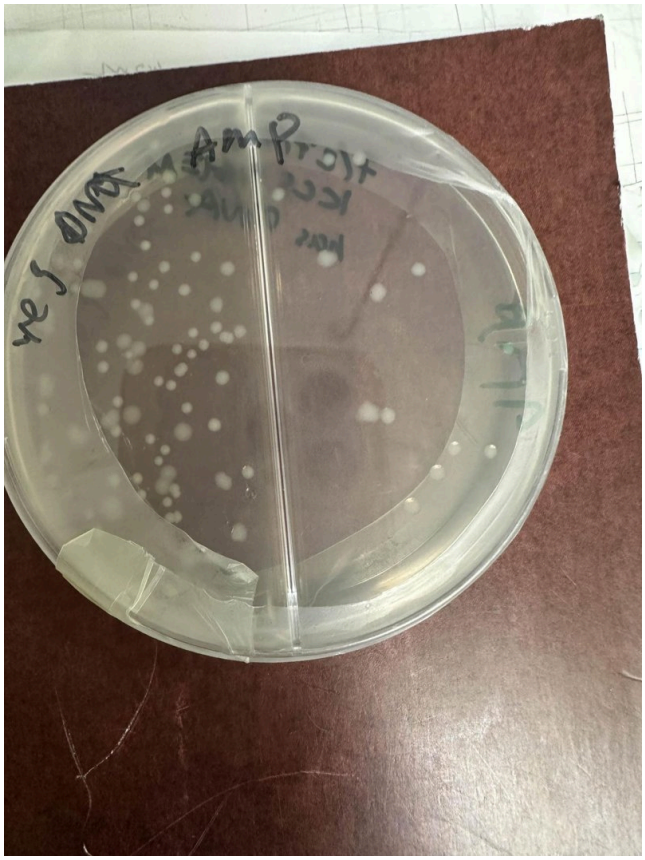
Attendees: Alyssa, Ansh, Nick
Summary: S. pasteurii culture/biomineralization, glycerol stock for culture of Joshi plasmids, transfer culture of Joshi E. coli, overnight culture of E. coli transformants (csg-CA)

We have transformants!!

**IMG_4964.jpg**

top left: nutrient agar/amp plate, cells without added DNA
top right: nutrient agar/amp plate, cells with added DNA
bottom left: LB agar plate, cells without added DNA
bottom right: LB agar plate, cells with added DNA
controls are as hypothesized!
-- no E. coli on top left plate
-- E. coli smears on LB agar plates
-- E. coli colonies on top right plate

IMG_4968.jpg



zoomed in picture of nutrient agar/amp plate with our successfully transformed colonies

**Transfer culture, S.pasteurii (~4 pm)**

- 1:400 dilution ratio
- 2.5 ul previous culture to 1 ml BHI

**Biomineralization**

Uncertain -- is it truly biomineralizing?? we shall see...

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.5 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Glycerol stock of E. coli (csg-NbGFP)**

- 500ul of overnight culture
- 500ul of 50% glycerol
- stored with electro competent cells at -80C

**Transfer culture of E. coli (csg-NbGFP)**

- 2:5000 dilution ratio
- 2 ul of previous culture to 5 ml of LB/Kan (concentration 50 ug/m)

**Overnight culture of E. coli transformants (csg-sazCA)**

- made 3 tubes with 1ml of LB and 1ul of Amp stock
  - Amp stock: 100mg/ml
  - concentration of broth: 100ug/ml
- 1 colony per tube
- in shaker in 37C incubator overnight

**TUESDAY, 7/29/2025**

## 9am

Attendees: Nick

Summary: S. pasteurii biomineralization

Did one cycle of biomineralization as per usual protocol.

## 4pm

Attendees: Alyssa, Ansh

Summary: S. pasteurii transfer culture/biomineralization, new S. pasteurii culture, plasmid miniprep of E. coli (csg-NbGFP), assays

**Transfer culture (~4 pm)**

- 1:400 dilution ratio
- 2.5 ul previous culture to 1 ml BHI
- Place in 30C

**Biomineralization**

Uncertain -- is it truly biomineralizing?? we shall see...

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.5 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Glycerol stocks of E. coli transformants (csg-CA)**

- 3 tubes of ~1ml overnight culture
- 500ul of culture + 500ul of 50% glycerol
- Placed in -80C

**CMM+**

We were running low on CMM+ in our storage, so we made more:

- ~2.44g of $CaCl_2$ * $2H_2O$
- 50ml of CMM-
- Autoclave & store at room temperature

**New S. pastuerii culture**

There are suspicions that we are unable to biomineralize because we have contaminated our S. pastuerii, so we decided to make a new one. As the S. pasteurii cultures had previously been placed in a 30C incubator with yeast, we hypothesize that the yeast may have outgrown and contamined our cultures. As such, we plan to place them in a different, larger incubator and set it to 30C.

- following protocol, 1:100 dilution
- 10ul of S. pasteurii glycerol stock + 1ml of BHI
- Placed in (big) 30C incubator, shaking at 180 rpm

**Transfer cultures of E. coli (csg-CA)**

- 5ml of LB + 5ul of ampicillin stock
    - ampicillin stock: 100 mg/ml
- put 5ul of old E. coli culture in (aka, 1:1000 dilution)
- all three cultures from the previous day were transfered.
- placed in 37C shaker

**Transfer cultures of E. coli (csg-NbGFP, Joshi)**

- 5ml of LB + 5ul of kanamycin stock
    - kanamycin stock: 50 mg/ml
- put 2ul of old E. coli culture in (aka, 1:5000 dilution)
- placed in 37C shaker

**Congo Red dye 5000x stock**

- Resuspended 100 mg in 1 ml of water
- Filter sterilized
- **We need to still make a 5x stock via dilution**

## WEDNESDAY, 7/30/2025

Attendees: Alyssa, Ansh, Khushi

Summary: S. pastuerii culture/biomineralization, LB broth, prepping media for induction, plasmid miniprep

Originally, we set aside 5 hours and intended to use IPTG and arabinose to induce expression in both of our E. coli bacteria

- IPTG for our transformants, the E. coli with csg-CA plasmid
- arabinose for the E. coli from the Joshi lab, that have the csg-NbGFP plasmid

But we ended up taking too much time and decided to do it on Friday instead.

**Diluted Congo Red dye**

- first made 50x stock by adding 10ul of the 5000x stock to 990ul of MilliQ water.
- then. made 5x stock by adding 100ul of the 50x stock to 900 ul of MIlliQ water.

During this process we realized that our 5000x congo red stock was super viscous, making pippeting accurately very difficult.

**100x IPTG stock**

- Added 15 mg of solid IPTG to 1 ml of sterile water.
- Filter sterilized (using 0.45 um pore filter)

**1000x arabinose stock**

- Added 23.8 mg of L-arabinose to 1 ml of sterile water.
- Filter sterilized (using 0.45 um pore filter).

**Media made**

- 3 of LB/amp
  - 5ml of LB, 5ul of 1000x ampicillin stock
- 3 of LB/amp/IPTG
  - 5ml of LB, 5ul of 1000x ampicillin stock, 50ul 100x IPTG stock
- 1 of LB/kan
  - 5ml of LB, 5ul of 1000x kanamycin stock
- 1 of LB/kan/ara
  - 5ml of LB, 5ul of 1000x kanamycin stock, 5ul of 1000x arabinose stock

Stored in 4C

**(Old) transfer culture (~7:30 pm)**

- 1:200 dilution ratio
- 5 ul previous culture to 1 ml BHI
  - the culture in this case was the possibly yeast-contaminated one, as the new S. pastuerii culture is still in the process of being grown
- Placed in shaker in 30C incubator (yeast one)
  - we placed this old culture in the yeast incubator, as we do not want to potentially contaminate our new culture with yeast.

**(New) transfer cutlure (~7:30 pm)**

- 1:200 dilution ratio
- 5ul of previous culture to 1ml BHI
  - this culture is the new batch currently being cultivated.
- Placed in shaker in 30C incubator (the big one, no yeast)

**Biomineralization**

Again, used the possibly yeast-contaminated culture as the new S. pasteurii culture is still in the process of being grown.

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold

- Let sit for 2 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**LB broth**

Approximately following https://static.igem.org/mediawiki/2015/1/1f/Exeter_LB_recipe.pdf (standard recipe)
Our current LB broth has been contanminated. We centrifuged a small sample of it, and there was clearly cell pellet at the bottom. As such, we are making a new batch of LB broth.
Made ~100ml of LB broth:

- Tryptone powder (1g)
- Yeast extract (0.5g)
- NaCl (1g)
- MiliQ water (100 ml)

Ran out of time to autoclave -- left in storage, to be autoclaved tomorrow.

**Plasmid miniprep : Followed**
**https://files.zymoresearch.com/protocols/_d4208t_d4209_d4210_d4211_d4212_zymopure_plasmid_miniprep.pdf**

1. Followed above protocol exactly using 1.5 ml of Overnight LB culture of Joshi lab E-coli K-12 strain.

**THURSDAY, 7/31/2025**

Attendees: Ansh, Alyssa
Summary: S. pasteurii culture/biomineralization, autoclave LB broth, check on biofilm, E. coli transfer cultures, bleach bath

LB broth was autoclaved & placed in community storage.

**(New) S. pasteurii transfer culture (~3:30 pm)**

- 1:400 dilution ratio
- 2.5ul of previous culture to 1ml BHI
  - this culture is the new batch currently being cultivated.
- Placed in shaker in 30C incubator (the big one, no yeast)

**Biomineralization (ended 6:00)**

Uncertain -- is it truly biomineralizing?? we shall see...

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**E. coli transfer culture**

- 1ml tubes, transferred all 4 of them
- 1:1000? dilution ratio
- added antibiotics as appropriate

Bleached bag of old culture tubes.

**FRIDAY, 8/1/2025**

Attendees: Ansh, Alyssa, Khushi,
Summary: S. pasteurii culture/biomineralization, induction of E. coli cultures, Congo Red assays

**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5ul of previous culture to 1ml CMM-
  - BHI is contaminated
- Placed in shaker in 30C incubator

**Biomineralization**

Uncertain -- is it truly biomineralizing?? we shall see...

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

Autoclaved old 15 ml eppendorf conicals for potential future use.
Autoclaved CMM-
Made aliquots/stocks of our CMM- and LB broth (just in case)

- We noted that our BHI broth was contaminated, and after further discussion wtih Jenny Mae, we concluded that it was best to aliquot out our remaining stocks of CMM- and LB broth into smaller portions considering the small amount we use every day. This way, we will not need to toss out a lot of our stock due to accidental contamination.
- 2 50ml tubes of LB broth in storage, 2 50ml fube of CMM- in storage

**Induction protocol, started (4:15):**

Using media made on 7/30/25

- Added 1:30 dilution of all non induced cultures
- Added 1:100 dilution of all induced cultures

5ml cultures

OD 600 of select cultures by removing 1 ml into transfer cuvettes:

**Table1**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | J (joshi lab strain, no inducer) | J_i (joshi lab strain, yes arabinose) | K1 (Our transformants, colony one, no inducer) | K1_i (Our transformants, colony one, yes iptg) |
| 2 | | | | |

At 6:15, added induction media to K1, K3, and J cultures, saved K2 as control. Below is an OD 600 of all cultures.

**Table2**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | J (joshi lab strain, no inducer) | J_i (joshi lab strain, yes arabinose) | K1 (Our transformants, colony one, no inducer) | K1_i (Our transformants, colony one, yes iptg) | K2 (Our transformants, colony two, no inducer) | K2_i (Our transformants, colony two, yes iptg) | K3 (Our transformants, colony three, no inducer) | K3_i (Our transformants, colony three, yes iptg) |
| 2 | 0.6528 | 0.2570 | 1.2850 | 0.6598 | 1.3575 | 0.6217 | 1.1781 | 0.4655 |

Followed measuring Curli with flourscence and absorbance (see below) protocol on E.coli K-12 biofilm grown at 30 C, started 7/25/2025. Used our uninduced transformants, as well as regular E.coli K-12 and uninduced Mach1 and controls.

**Table3**

| | A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|
| 1 | | Biofilm, (theoretically) | Joshi lab un induced strain | Our transformants, un induced, 4C | Regular plated ecoli K-12 left at 37 C for 4 days. | | | |
| 2 | Florscence Emmision:625nm Excitation: 525nm | 3.5 | 3.075 | 3.6, 2.89 | 6.8 | | | |
| 3 | ABS480 | 8.2 | | | 7.4 | | | |

Added in inducer to the "uninduced cultures" except K2
Waited 2 hours 30 minutes, then repeated the measuring Culri with flourscence and absorbance protocols.
Flourescence measured using flourometer, absorbance measured using UV-Vis

**Table4**

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | J (joshi lab strain, no inducer) | J_i (joshi lab strain, yes arabinose) | K1 (Our transformants, colony one, late inducer) | K1_i (Our transformants, colony one, yes iptg) | K2 (Our transformants, colony two, no inducer) | K2_i (Our transformants, colony two, yes iptg) | K3 (Our transformants ,colony three, late inducer) | K3_i (Our transformants, colony three, yes iptg) |
| 2 | Florscence Emmision:625nm Excitation: 525nm | 53.360 | 55.346 | 64.992 | 69.687 | 65.406 | 64.609 | 64.006 | 70.833 |
| 3 | ABS480 (blanked with LB) | 0.5919 | 0.4356 | 0.55 | 0.533 | 0.6559 | 0ish?? | 0.61ish? | 0.4576 |

**Measuring Curli with flourscence:**

- Take one colony from plate of choice
- Mix it in 500 ul of water
- Add 160 ul of 100 ug/ml congo red
- Titerate up and down or vortex until colony is dissolved
- Measure flourscence at 625 nm with input of 525 nm

**Measuring Curli with absorbance:**

- Start with tube from Measuring curli with flourscence
- Centrifuge for 5 min, 14,000 rpm
- Check color of cell pellet fromed - more red = more curli binding
- Check ABS480 of supertanant - The tube with lower ABS480 has more curli in the sample

## SATURDAY, 8/2/2025

Attendees: Alyssa, Ansh, Khushi

Summary: S. pasteurii culture/biomineralization, congo red serial dilution (calibration curve)

**S. pasteurii transfer culture**

- the current S. pasteurii culture looks clear... so we ended up making two transfer cultures: one using the 8/1/25 culture, and one using the 7/31/25 culture
- 1:100 dilution ratio
- 10 ul of previous culture to 1ml CMM-
  - BHI is contaminated, more BHI broth is being ordered
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold

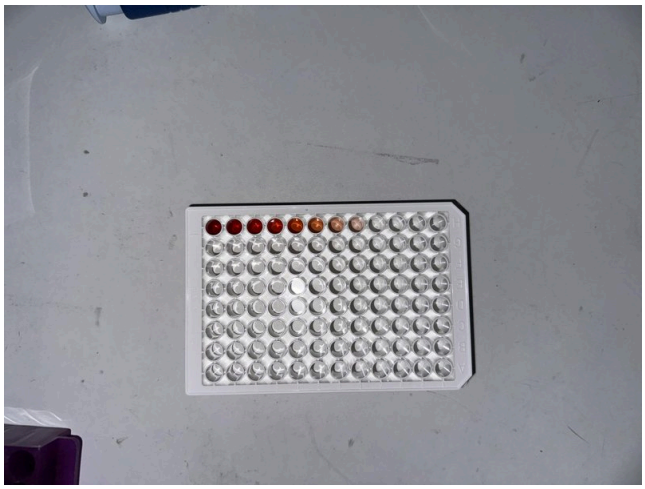
- Let sit overnight in 30C

**Congo Red calibration curve**

Made serial dilutions of congo red solution

- did twofold serial dilution
- started with 20x congo red solution that was in 4C fridge
    - 250ul of MiliQ water, 250ul of prev dilution etc.
- $20*2^{0}$, ..., $20*2^{-11}$
- ended with a total of 12 solutions (filling up one row of the white 96 well plate)
- measured using plate reader -- reading flourescence intensity, using filter wheel.
    - limited filters available, according to the plate reader.

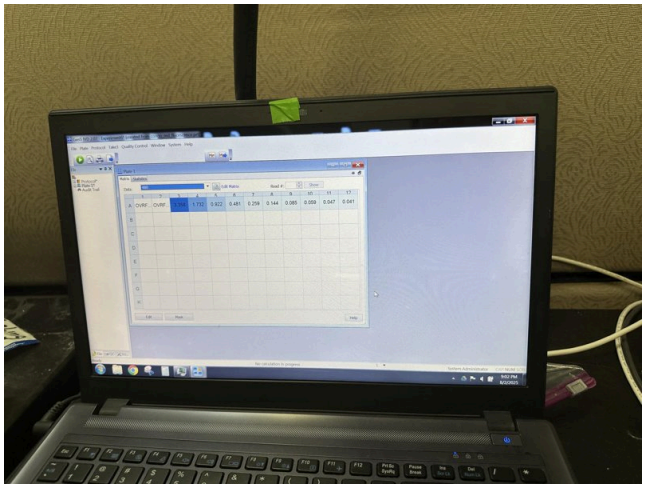
**IMG_4980.jpg**



20x congo red solution on right, all the way down to $20*2^{-11}$ dilution
dilutions take up all 12 wells

Measured absorbance at 480nm -- got expected results

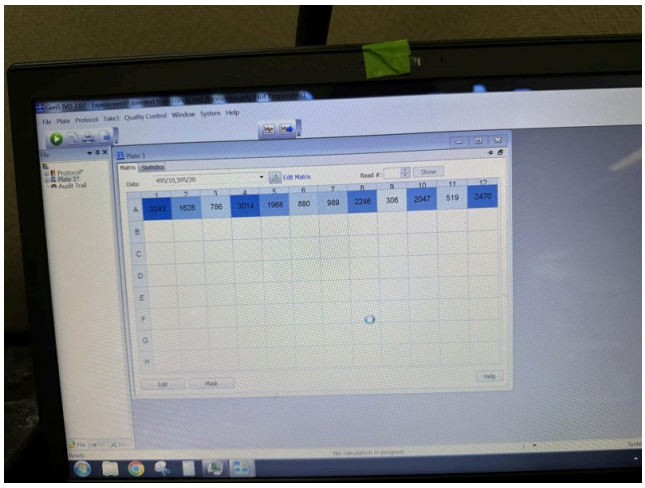
**IMG_4988 (1).jpg**



two rightmost cells were overflowed, but the other results decreased about twofold each dilution, which is as expected.

Measured flourescence, with varying excitation & emission wavelengths

**IMG_4984.jpg**



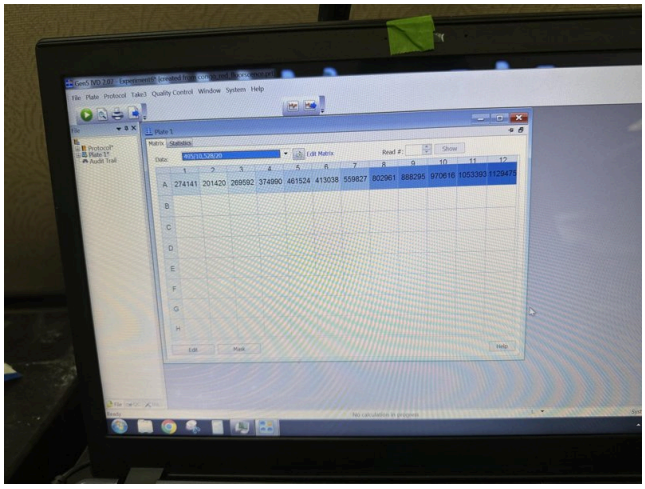
excitation: 495/10, emission: 595/20
darker in color = higher value
results not as expected, looking more like random values than a linear line

^^ results/images using the same emission filter but varying excitation filters look similarly scrambled

- excitation: 495/10, emission: 595/20
- excitation: 485/20, emission: 595/20
- excitation: 360/40, emission: 595/20

all other images/results taken with a different emission filter were opposite of what we expected

**IMG_4989.jpg**



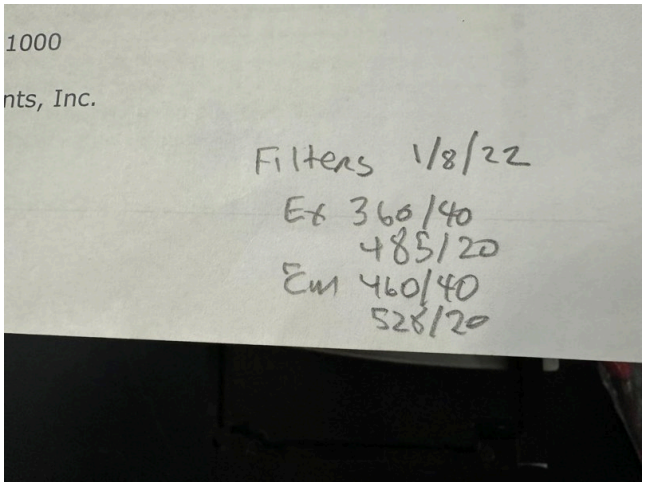
excitation: 495/10, emission: 528/20
darker in color = higher value
results oppostie of what expected -- expecting to see higher values for more concentrated samples

tested for:

- excitation: 495/10, emission: 528/20
- excitation: 485/10, emission: 528/20
- excitation: 360/40, emission: 528/20
- excitation: 360/40, emission: 460/40

Later found in the manual that it seems like the only filters available are the 360/40 & 485/20 for excitation filters and the 460/40 & 525/20 filters
However, this may be different, as this note was from 2022...

**IMG_4999.jpg**



**SUNDAY, 8/3/2025**

Attendees: Nick, Ansh, Khushi

Summary: S. pasteurii culture/biomineralization, Microscope images

**Biomineralization (6:00 am)**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**S. pasteurii transfer cutlure (4:00 pm)**

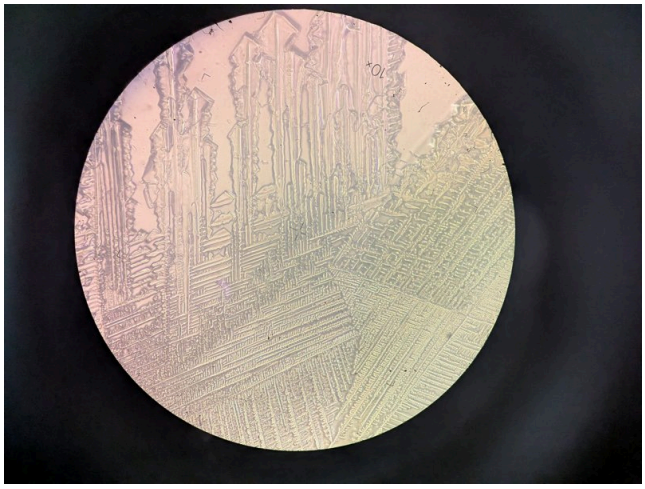
- 1:400 dilution ratio
- 2.5ul of previous culture to 1ml CMM-
  - BHI is contaminated
- Placed in shaker in 30C incubator

**Biomineralization (4:00 pm)**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

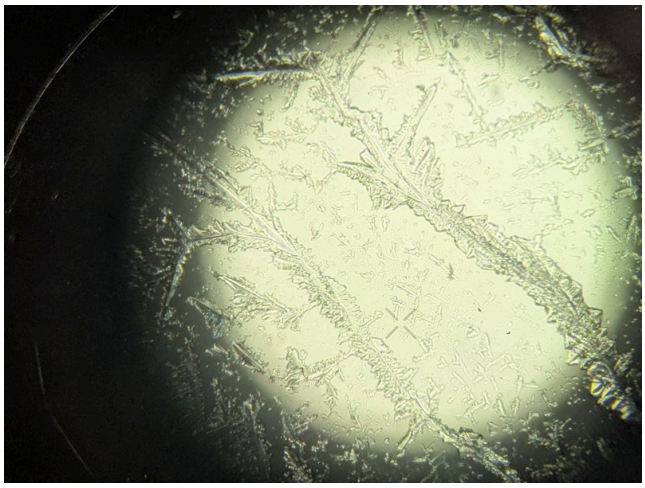
Plated both of 8/2/2025 transfer cultures on microscope slides to check viability. Also plated CMM- and CMM- that was incubated at 37C for a few days in a quasi-sterile tube. Both transfer cultures had significant crystalization under a microscope.

**PXL_20250803_214029954.jpg**



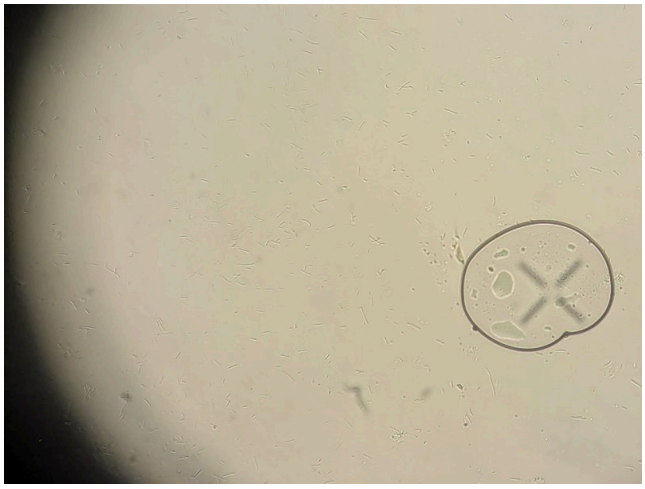
CMM- s. pastuerii transfer culture x100

**PXL_20250803_214356736.jpg**



CMM- s. pastuerii transfer culture x400

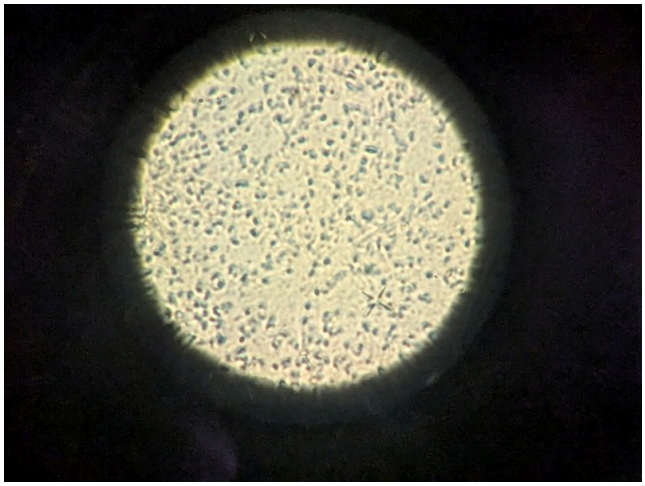
**PXL_20250803_214801256.jpg**



CMM-, relatively clear, no crystals

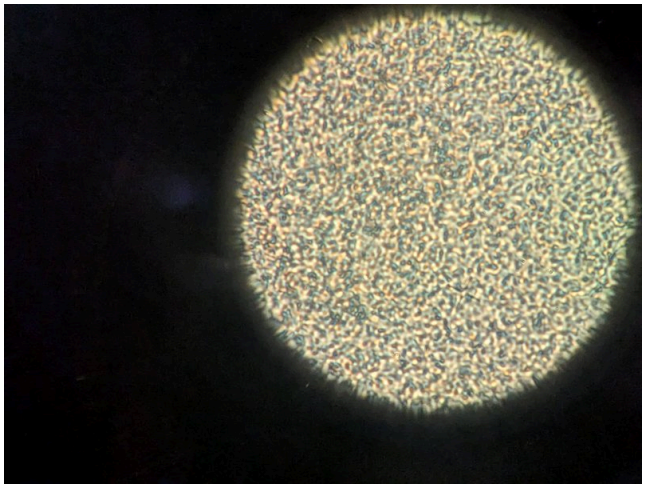
Further atttempted to congo red sticking to cell pellets. Placed induced joshi lab and transformant three samples on a slide, along with 1.25x congo red stock to act as a control. The results were not very conclusive due to only being able to use brightfield microscopy:

**PXL_20250803_222248512.jpg**



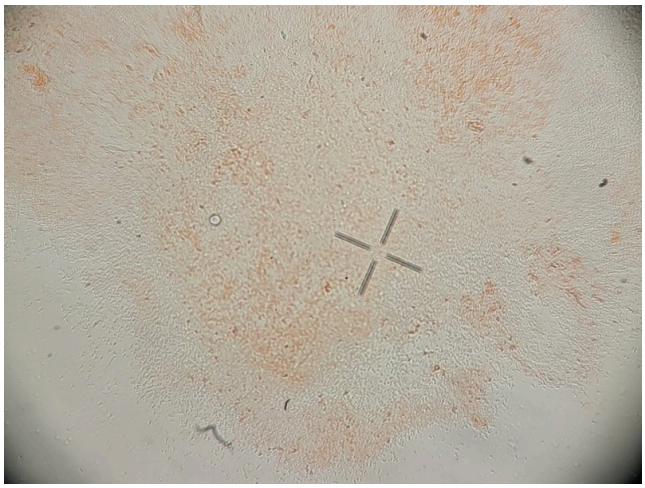
Our transformants, x1000 magnification

**PXL_20250803_222605504.jpg**



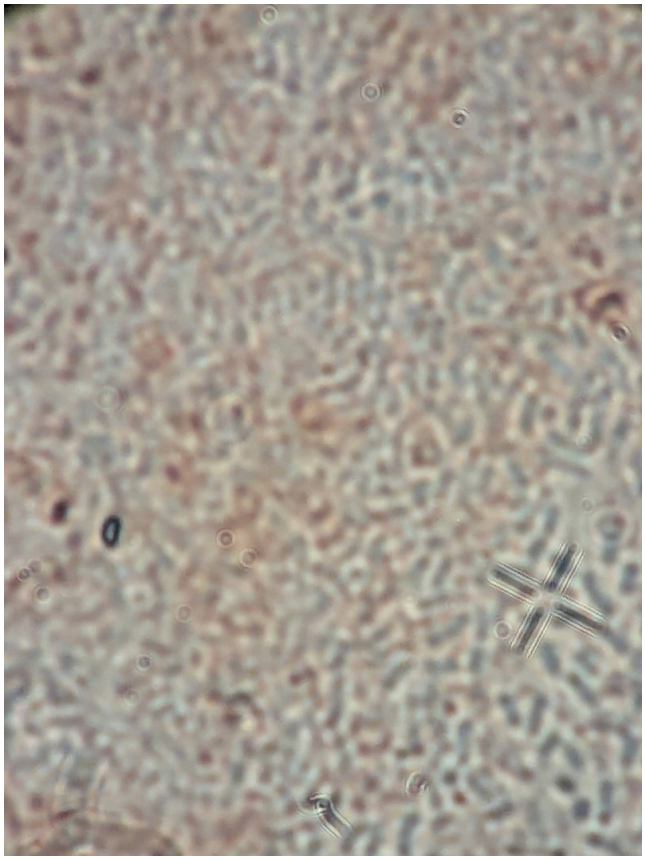
Our transformants, x1000 magnification

**PXL_20250803_223203705.jpg**



Joshi lab 400x

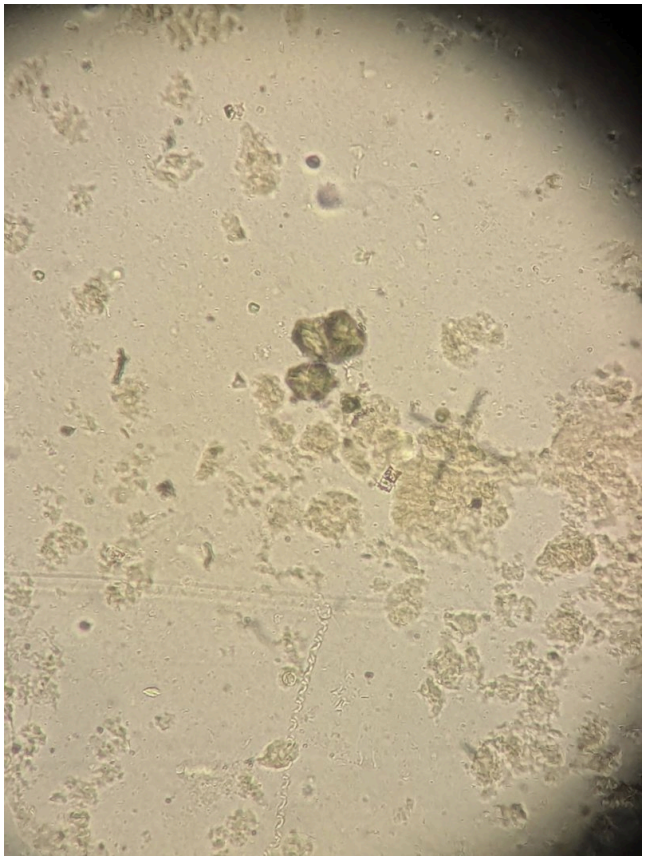
PXL_20250803_223432911.jpg



Joshi lab x1000

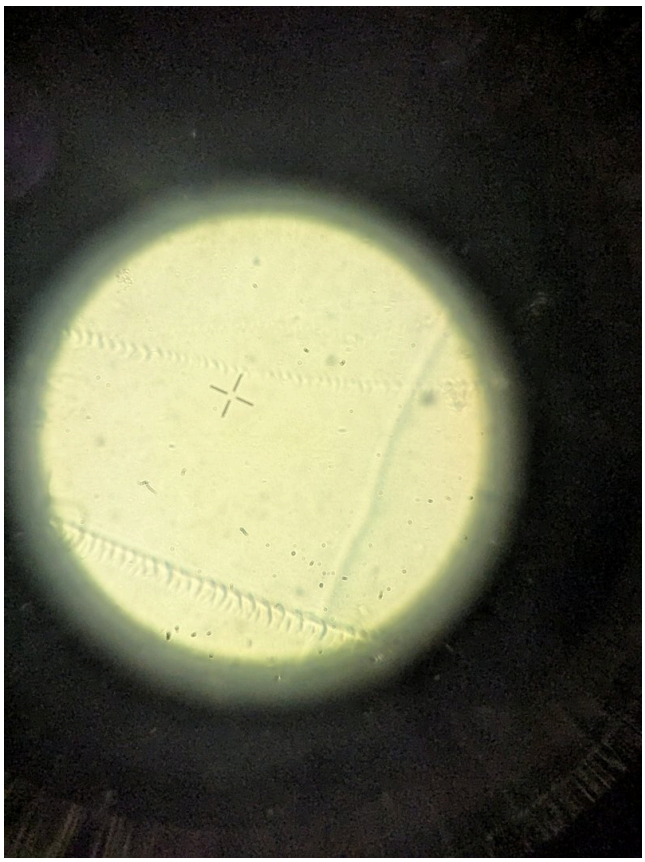
At this point we attempted to check the purity of CMM+ and CMM- stocks we had. We found the CMM+ to be certainly contaminated, and the CMM- to be potentially fine or potentially contaminated.

**PXL_20250803_231118197.jpg**



CMM+, very clear bacterial colonies present at 400x magnification.

PXL_20250803_232555765.jpg



CMM -, mostly clear except for these odd line formations. Unsure what those are.

CMM+ was then centrifuged resulting in a cell pellet, supporting that it was contaminated.

To reduce contamination related issues, CMM+ and CMM- stocks were moved to 4C for storage instead of room temp.

**MONDAY, 8/4/2025**

Attendees: Alyssa, Ansh, Khushi
Summary: S. pasteurii culture/biomineralization, congo red calibration curve

**S. pasteurii transfer cutlure (6:00 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml CMM-
  - BHI is contaminated
- Placed in shaker in 30C incubator

**Biomineralization (started 4:30? pm)**

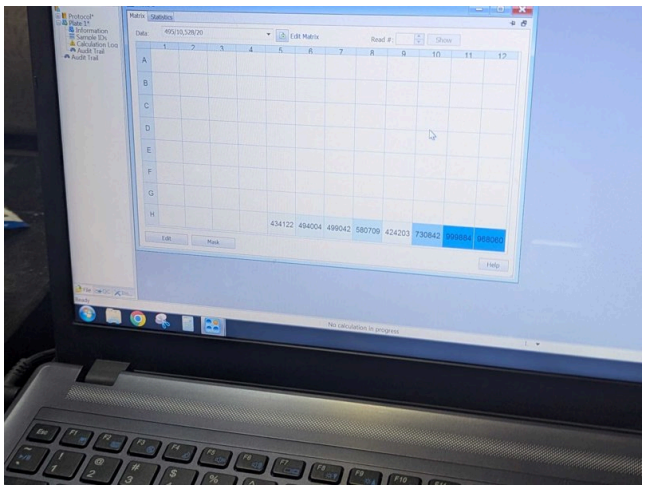
- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture

    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Congo Red standards in plate reader**

- Discovered that specifically the 595/20 emmision filter is missing, resulting in any data generated using that filter being random numbers
- Managed to get flourscence readings as expected using a black 96 well plate with opaque bottom and Excitation: 495/10, Emmision: 528/20.
- Put standards 1 through 7 (prepared prior day) in wells H12 though H5 of the plate, starting with standard one (highest conc) in H12

**PXL_20250805_024210834.jpg**



**TUESDAY, 8/5/2025**

Attendees: Alyssa, Ansh,
Summary; S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (6:00 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml CMM-
    - BHI is contaminated
- Placed in shaker in 30C incubator

**Biomineralization (started 4:30? pm)**

- made 3ml of CMM- + S. pastuerii

    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Congo red standards:**

Realized that only concentrations between standards 5, 6, 7, 8, and 9 are actually linear on the flourometer and plate reader.

- optimal concentration is looking to be 0.3x that of what we originally thought we would need (as we originally based it off of the paper)

Also realized that LB media has significant autofloursence. The flourometer is thankfully not too dependent on volume put into the reader.

Need to transfer details/measurements from the physical lab notebook

**Transformant culture prep for phenol red assay:**

- Made a 5ml lb culture with 1 mM iptg, and 1x ampicillin with transformant 3
- Made 5 ml lb culture with 1x ampicillin with transformant 3
- Made 5 ml lb culture with ecoil k-12

**PDMS mold:**

- measured out 1.35 g of base and 0.15 g of curing agent
- stirred for 3 min, then degassed in a vacuum chamber for 2 min
- poured into petg mold what was coated with easy release
    - further removed bubbles by poking it with a toothpick
- placed in oven at 60 C for 72 h.

PXL_20250806_031708589.jpg

Mold inside lab oven.

**WEDNESDAY, 8/6/2025**

Attendees: Ansh, Khushi
Summary: S. pasteurii biomineralization/culture, making tris buffers

**S. pasteurii transfer cutlure (6:00 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml CMM-
  - BHI is contaminated
- Placed in shaker in 30C incubator

**Biomineralization (6:00 am, 4:30 pm)**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold

- Let sit overnight in 30C

**Tris buffers:**

- Made 20 mM tris buffer at pHs 6.4, 7.4, 8.4
    - Dissloved 0.242 g tris-HCL into 100 ml water
    - Split solution across 3 tubes
    - Added 1 N HCL and 0.5 N NaOH until each tube was at the appropriate pH

**Transformant culture transfers for phenol red assay:**

- Made a 1ml lb culture with 1 mM iptg, and 1x ampicillin with transformant 3
- Made 1 ml lb culture with 1x ampicillin with transformant 3
- Made 1 ml lb culture with ecoil k-12

## THURSDAY, 8/7/2025

Attendees: Ansh, Khushi, Nick
Summary: S. pasteurii biomineralization/culture, Carbonic anhydrase assay v1

Recieved and made aliquots of new BHI broth.

**S. pasteurii transfer cutlure (6:00 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI-
- Placed in shaker in 30C incubator

**Biomineralization (6:00 am, 4:30 pm)**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

A $CO_2$ (aq) solution was prepared by adding dry ice to water and stiring for 30 minutes, replenishing the dry ice as it dissolves.

**Carbonic Anhydrase assay standards:**

- Pipetted 200 ul of phenol red and 800 ul of each of the three tris buffers (6.4, 7.4, 8.4) into three seperate tubes
- Split the total volume of each tube into three sepereate well on a 96 well plate on a plate reader and measured A560 and full spectrum data.

**Carbonic anhydrase assay v1:**

- 177 ul of phenol red and 700 ul of ph 8.4 20 mm tris buffer was added a set of tubes.
- Tubes were prepared with 10 ul, 30 ul, and 50 ul of induced transformants, as well as 30 ul of uninduced transformants, 30 ul of ecoli k 12 and 30 ul of LB.
- Finally, 200 ul of co2 (aq) was added to each tube.
- At this point, each tube's volume was split three ways and placed into wells in a 96 well plate. The intention was to use a plate reader to check the rate of pH change. Howver by the time the pipeting finished, the reaction was over. Thus no data was collected.

**FRIDAY, 8/8/2025**

Attendees: Ansh, Khushi, Nick
Summary: S. pasteurii biomineralization/culture, Carbonic anhydrase assay v2, v3

**S. pasteurii transfer cutlure (6:00 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

**Biomineralization (6:00 am, 4:30 pm)**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Carbonic anhydrase assay v2:**

- 177 ul of phenol red and 823 ul of ph 8.4 20 mm tris buffer was added a set of tubes.
- Tubes were prepared with 30 ul of induced transformants, uninduced transformants, ecoli k 12. One tube was left as a control with no additions.
- All tubes were chilled on ice.
- 200 ul of co2 (aq) was added to each tube.

The result was the tube with no addition finishing first.

**Table5**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Tube contents: | Induced transformants | Un induced transformants | ecoli-k12 wild type | No addition |
| 2 | Time | 559 | 600 | 600 | 500 |

**Carbonic anhydrase assay v3:**
Tubes were prepared as in Carbonic anhydrase assay v2, but were prepared simultaneously using a repeater pippet to reduce the time between the input of co2 to each tube. Additionally, the reactiosn were conducted at room temperature. It is also suspected that by this point, the co2 solution had been sitting out for a minute and lost some concentration.

**Table6**

| | A | B | C | D | E |
|---|---|---|---|---|---|
| 1 | Tube contents: | Induced transformants | Un induced transformants | ecoli-k12 wild type | No addition |
| 2 | Time | 900 | 750 | 900 | 690 |

Finally, we took out the PDMS mold that was sitting in the oven for the last three days ay 60C (see 8/5/25 lab notes for details).

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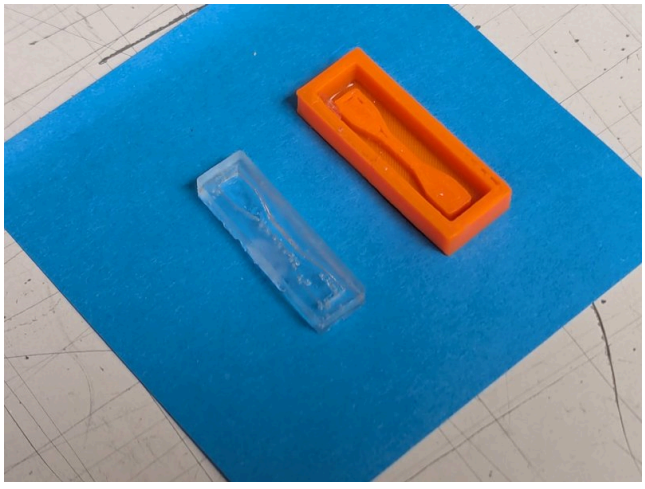

PDMS mold taken out from oven. Note there are minor bumps on the mold due to imperfections in the 3d print.

## SATURDAY, 8/9/2025

Attendees: Ansh, Khushi,
Summary: S. pasteurii transfer culture

**S. pasteurii transfer cutlure (8:00 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

## SUNDAY, 8/10/2025

Attendees: Ansh, Khushi, Alyssa
Summary: S. pasteurii transfer culture + biomineralization, DNA gel

**S. pasteurii transfer cutlure (1 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

**Biomineralization (1:30 pm)**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**DNA gel**

Made 1.5% agarose gel with ritcher green (similar to gelgreen)

- 375 mg of agarose, 22.5 ml water, 2.5 ml 10x TAE, 2.5 ul ritcher green

Let gel solidfy & placed in gel box with 1x TAE buffer
Pipette DNA samples into wells:

- Ran extracted Joshi lab plasmid (pBbB8k-csg-NbGFP, 7.5 kb), as well our synthetic plasmid ordered from twist, (??? kb)
- per DNA sample: 10ul of sample + 2ul of 6x loading dye

Ran at 90 volts for 15 mins, viewed under blue light with and orange filter.

(Prior to this we attempted making a 1% agarose gel, but it melted while running)

**PXL_20250811_203120588.NIGHT-EDIT.jpg**


On the left is the extracted joshi lab plasmid, on the right our synthetic plasmid. At this point we did not have a DNA ladder, and hence making conclusions about size is hard.

## MONDAY, 8/11/2025

Attendees: Ansh, Alyssa
Summary: Induction of E. coli, Curli assay, Biomineralization

**S. pasteurii transfer cutlure (7:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~9pm)**
- Let sit overnight in 30C

**white plate, induction**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Induced at start, Transformant 1 | Induced at start , Transformant 2 | Induced at start, Transformant 3 | Induced at start, Joshi | Induced at start, K-12 | Induced at start, LB | Induced at start, Transformant 1 | Induced at start , Transformant 2 | Induced at start, Transformant 3 | Induced at start, Joshi | Induced at start, K-12 | Induced at start, LB |
| B | Induced late, Transformant 1 | Induced late, Transformant 2 | Induced late, Transformant 3 | Induced late, Joshi | Induced late, K-12 | Induced late, LB | Induced late, Transformant 1 | Induced late, Transformant 2 | Induced late, Transformant 3 | Induced late, Joshi | Induced late, K-12 | Induced late, LB |
| C | No induction, Transformant 1 | No induction, Transformant 2 | No induction, Transformant 3 | No induction, Joshi | No induction, K-12 | No induction, LB | No induction, Transformant 1 | No induction, Transformant 2 | No induction, Transformant 3 | No induction, Joshi | No induction, K-12 | No induction, LB |
| D | | | | | | | | | | | | |
| E | Induced at start, Transformant 1 | Induced at start , Transformant 2 | Induced at start, Transformant 3 | Induced at start, Joshi | Induced at start, K-12 | Induced at start, LB | Induced at start, Transformant 1 | Induced at start , Transformant 2 | Induced at start, Transformant 3 | Induced at start, Joshi | Induced at start, K-12 | Induced at start, LB |
| F | Induced late, Transformant 1 | Induced late, Transformant 2 | Induced late, Transformant 3 | Induced late, Joshi | Induced late, K-12 | Induced late, LB | Induced late, Transformant 1 | Induced late, Transformant 2 | Induced late, Transformant 3 | Induced late, Joshi | Induced late, K-12 | Induced late, LB |
| G | No induction, Transformant 1 | No induction, Transformant 2 | No induction, Transformant 3 | No induction, Joshi | No induction, K-12 | No induction, LB | No induction, Transformant 1 | No induction, Transformant 2 | No induction, Transformant 3 | No induction, Joshi | No induction, K-12 | No induction, LB |
| H | | | | | | | | | | | | |

First set for curli fiber absorbance assay, second set for curli fiber flourescence assay duplicates.

**Induction**

- following pET system manual's example induction protocol -- https://research.fredhutch.org/content/dam/stripe/hahn/methods/biochem/pet.pdf
  - accordingly used 1mM of iPTG for our solutions
- also following other article that said 5 hours -- [insert here]
- pipetted in LB + antibiotics as needed

**Curli absorbance assay**

- took rows A-C, pipetted in congo red, measured absorbance
- transfered to small PCR tubes, centrifuged at 14000 for 1 min
- transfered supernatant  (~150 ul) back into wells, used plate reader to measure absorbance
- per well, also did 9-point hting

## TUESDAY, 8/12/2025

Attendees: Ansh, Alyssa, Khushi
Summary: S. pasteurii biomineralization/culture,  curli assay

**S. pasteurii transfer cutlure (~2:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold

- Let sit overnight in 30C

**Curli flourescence assay**

- Finished PBS wash, took reads of flourescence in between against PBS to see when the LB had been mostly washed
  - ended up taking ~3 washes
- Then added in congo red, gently mixed with a pipette, and took flourescence measurements on DeNovix
- See data in drive for more info. [insert graphs here for analysis?]

## WEDNESDAY, 8/13/2025

Attendees: Alyssa, Ansh,
Summary: S. pasteurii biomineralization/culture, curli assay, transfer cultures, trouble shooting, colony PCR or so

**S. pasteurii transfer cutlure (4 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~6:30pm)**
- Let sit overnight in 30C

300ul per well

**white plate, induction #2**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB |
| B | Not induced, Transformant 1 | Not induced, Transformant 2 | Not induced, Transformant 3 | Not induced, Joshi | Not induced, E. coli K12 | Not induced, LB | Not induced, Transformant 1 | Not induced, Transformant 2 | Not induced, Transformant 3 | Not induced, Joshi | Not induced, E. coli K12 | Not induced, LB |
| C | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB |
| D | Not induced, Transformant 1 | Not induced, Transformant 2 | Not induced, Transformant 3 | Not induced, Joshi | Not induced, E. coli K12 | Not induced, LB | Not induced, Transformant 1 | Not induced, Transformant 2 | Not induced, Transformant 3 | Not induced, Joshi | Not induced, E. coli K12 | Not induced, LB |
| E | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB |
| F | Not induced, Transformant 1 | Not induced, Transformant 2 | Not induced, Transformant 3 | Not induced, Joshi | Not induced, E. coli K12 | Not induced, LB | Not induced, Transformant 1 | Not induced, Transformant 2 | Not induced, Transformant 3 | Not induced, Joshi | Not induced, E. coli K12 | Not induced, LB |
| G | | | | | | | | | | | | |
| H | Induced, Transformant 1 | Induced, Transformant 2 | Induced, Transformant 3 | Induced, Joshi | Induced, E. coli K12 | Induced, LB | | | | | | |

left side (A-F, 1-6) -- curli fiber absorbance assay
right side (A-F, 7-12) -- curli fiber flourescence assay
bottom-most row (H1-6) -- reserved for CA assay

- since CA assay only needs 30ul of culture or so

**Induction**

Added E. coli cultures to the plate

- 300ul per well
- antibiotics + LB
- 1:100 dilution ratio of glycerol stock culture to media

Put plate in the plate reader ~5pm

- incubated at 37C, 500 rpm
- takes a read every 10 minutes (absorbance 600nm)

Popped out at ~9:30pm, at which point we added in iPTG and arabinose as apporpriate

Put back in the plate reader again

- incubated at 37C, 500rpm
- takes a read every 20 minutes (absorbance 600nm)

Will see results and take assays ~12pm tomorrow

Note: also made 50x iPTG stock & 50x arabinose stock, for convenience, since the wells have 300ul of culture in them

Sterilized sand, will use to start biomineralization on PDMS mold tomorrow.

Aliquoted PBS & CMM+ into tubes, now stored in fridge at 4C

streaked out transformants 1, 2, 3, onto LB/agar/amp plates

## THURSDAY, 8/14/2025

Attendees: Alyssa, Ansh, Khushi

Summary: S. pasteurii biomineralization/culture, colony PCR, curli assays, carbonic anhydrase assays

**S. pasteurii transfer cutlure (1:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

**Biomineralization**

Note: We have now started biomineralization of the small PDMS mold that we made at Biocurious. The black mold and the white mold also continue to undergo biomineralization.

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~3 pm)**
- Let sit overnight in 30C

**Resuspending DNA**

Urease gene fragments came in a powder form, 1000ng.

- we resuspended it in 100ul of TE buffer to a concentration of 10ng/ul

Primers also came in a powder form, ~25 nmol

- They were resuspended to a 10x stock, with ~250ul of TE buffer

- 10ul of this 10x was then added to 90ul of TE buffer, to create a 100ul 1x stock of the primers

**Colony PCR**

We took a colony from the previously streaked plate, from transformant 1,2, and 3

Added to 12.5 ul NEB hotstart mastermix 2x

Added 1.25ul each of appropriate 1x primers

- Primers target T7 promoter + terminator

Added 10 uL of water

**PCR settings:**

- 21 cycles
- 98 C - 10 sec
- 60 C - 30 sec
- 72 C - 2 min

**Carbonic anhydrase assay:**

- 100 ul of phenol red (0.01%)
- 700 ul of 15 mM pH 8.4 tris buffer
- 30 ul of test culture
- 200 ul of CO2 (aq)

Reagents are all chilled on ice and added in the order above. A spectrophotometer is used to measure A435 and A560 every 5 seconds. Transformant 2,3 and LB are tested.

Across the board a final concentration of 0.5x congo red was used (20 ug/ml)

**Curli flourescence assay**

- put culture in transparent PCR tubes
  - lost culture due to evaporation, at most ~200 ul per well or so
- wash 3x w PBS
  - centrifuge at 12000 rpm for 2 min, remove supernatant, add PBS, repeat
- after last centrifuge, remove supernatant, add PBS, and also add in congo red dye
  - this time, 0.5x concentration or so
- shake for 10 min at room temperature (25C)
- use DeNovix flouremeter to measure flourescence
  - excite at green (~525 nm), emit at red (~625 nm)

**Curli absorbance assay**

- put in 0.5x congo red (20 ug/ml)
- let shake at room temperature (~25C) for 10 min
- transfer culture from wells to centrifuge tubes, centrifuge for 2 min at 12000 rpm
- put supernatant into wells & read absorbance at 480nm

**FRIDAY, 8/15/2025**

Attendees: Alyssa, Ansh, Khushi

Summary: S. pasteurii culture/biomineralization, plasmidsaurus plasmid prep, gel electrophoresis

**S. pasteurii transfer cutlure (5 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

Note: Also realized that our first batch of BHI, which Kelsey had graciously provided, was BHI + 2% urea. Our current BHI broth is just BHI, with no urea. We will be getting urea shortly.

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~7:30 pm)**
- Let sit overnight in 30C

Note: we looked at our stocks of CMM+, and the calcium chloride has precipitated out to a certain degree.

Yesterday we had inconclusive results from the gel, meaning that our transformants may or may not have our plasmid. So today we decided to have plasmidsaurus sequence our plasmids, so that we could move things along.

**Plasmid prep**

One of the options is to send in 100ul of culture.

We used leftover culture that we had from the induction process yesterday

- we diluted it down with some LB/amp media because we didn't have enough culture
- 20ul old culture, 80ul media

3 samples

- two from our transformants (T1 & T2)
- one from the joshi culture, as a positive control

**Gel electrophoresis**

made 1.5% agarose gel with cybersafe green dye

wells

- ladder (NEB 1kb+ ladder)
- ladder (NEB fastload 2)
- PCR product from colony PCR of transformant 1
- PCR product from colony PCR of transformant 2
- PCR product from colony PCR of transformant 3
- PCR product of Ure1 fragment (first half of our urease construct)
- PCR product of Ure2 fragment (second half of our urease construct)
- Joshi lab plasmid
- Our twist plasmid

2ul of loading dye per sample

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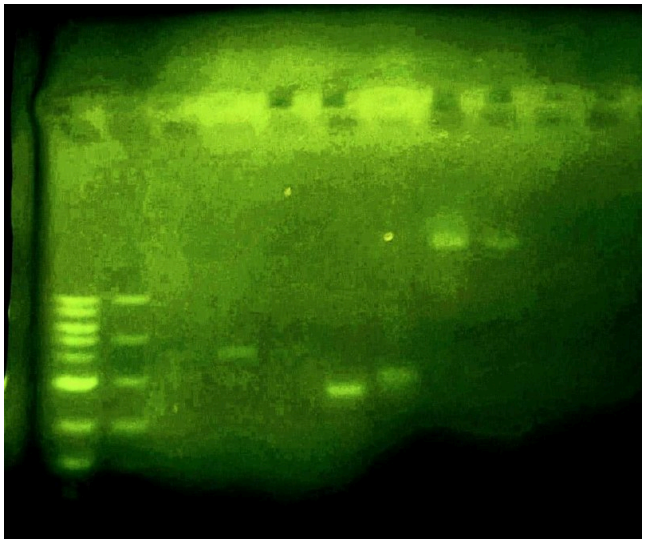

Neb 1kb+ ladder for safe stain, Neb fast load 2 ladder, Transformant 1, Transformant 2, transformant 3, Ure (a), Ure (b), Joshi lab plasmid, Twist plasmid.

**Tris buffer:**

Made 20 mM pH 8.4 tris Buffer.

- Weighed out 0.31 g of tris-hcl
- Added to 75 ml of water
- Adjusted pH to 8.4 using 0.5 N NaOH
- added water to 100 ml

**CMM+ concerns:**

We noted precipitation of CaCl2 in our cmm+ stocks, even ones autoclaved very recently.

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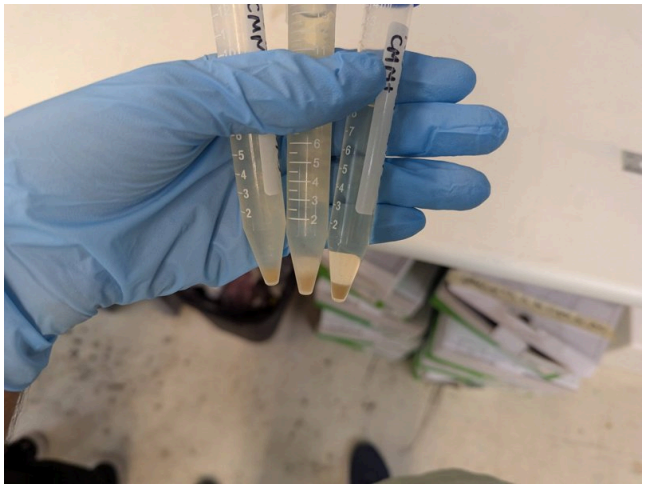


**SATURDAY, 8/16/2025**

Attendees: Alyssa, Ansh, Khushi, Uday,

Summary: S. pasteurii culture/biomineralization, DNA assembly, gel electrophoresis, Phosphate buffers, Induces cultures

**S. pasteurii transfer cutlure (12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

Note: Also realized that our first batch of BHI, which Kelsey had graciously provided, was BHI + 2% urea. Our current BHI broth is just BHI, with no urea. We will be getting urea shortly.

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~3:00 pm)**
- Let sit overnight in 30C

**DNA assembly:**

Used: https://www.neb.com/en-us/protocols/2014/11/26/nebuilder-hifi-dna-assembly-reaction-protocol?srsltid=AfmBOors4YavaxcNW984fxxXwiofKXL4VQQFO14SLwgGczWboWkxmaNU protocol.

- 10 µl NEB hifi dna assembly mix
- 2 ul of 40 ng/ul fragment ure(a)

- 2 ul of 40 ng/ul fragment ure(b)
- 6ul of pcr grade water

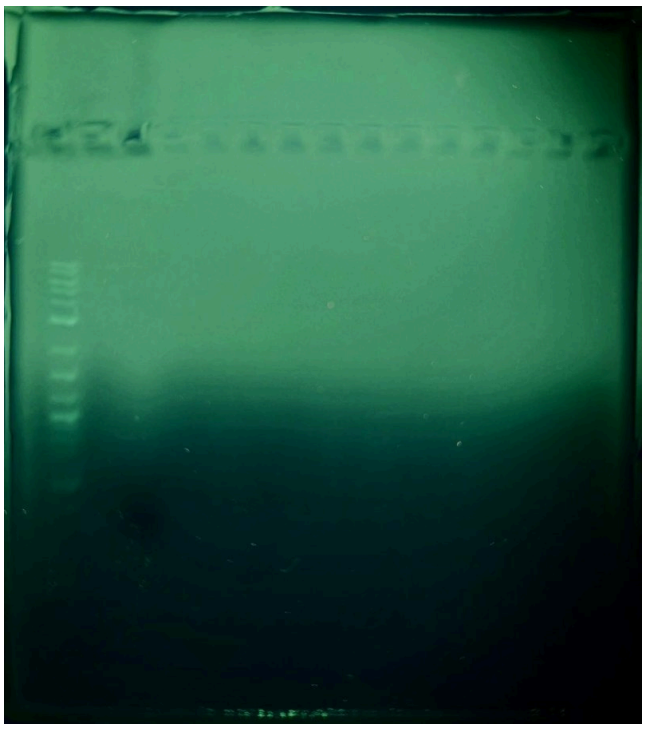
**Gel electrophoreis**

Made 1.5% agarose gel

- 375 mg agarose
- 22.5 ml of water
- 2.5 ml 10x TAE buffer

Ran ladder + PCR'd up urease construct

**PXL_20250817_030510425.NIGHT~3.jpg**



No visible assembled fragments, only an NEB 1 kb+ ladder.

**Induction**

made 3ml cultures w antibiotics as needed (~3pm)

- transformant 1, 2, 3, Joshi, E. coli
- fresh colonies for our transformants, fridge cultures (30ul, 1:100 dilution) for joshi + e. coli

added in IPTG (~8:30 pm)

also did varying IPTG concentrations for transformant 2 culture

- aliquoted ~600ul into 5ml tubes
- 0.1x, 0.3x, 1x, 3x, 10x

**SUNDAY, 8/17/2025**

Attendees: Alyssa, Ansh, Khushi, Nick

Summary: S. pasteurii culture/biomineralization, induction assay, urease assay

**Biomineralization (6am)**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~4 pm)**
- Let sit overnight in 30C

**S. pasteurii transfer cutlure (~2 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI
- Placed in shaker in 30C incubator

Note: Also realized that our first batch of BHI, which Kelsey had graciously provided, was BHI + 2% urea. Our current BHI broth is just BHI, with no urea. We will be getting urea shortly.

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~4 pm)**
- Let sit overnight in 30C

**Induction assay:**

Followed the below protocosl for each sample gneerated for the induction assay yesterday.

**Measuring Curli with fluorscence:**

- Take 200 ul of culture from tube
- Wash with sterile PBS 3 times, using 300 ul each (sufficient washing can be confirmed by measuring the flouresence of the tube after wash. It should have a similar profile to plain pbs).
- Measure flourscence at 625 nm with input of 525 nm.

**Measuring Curli with absorbance:**

- Take 200 ul of culture from the tube
- Centrifuge for 2 min, 14,000 rpm
- Check color of cell pellet fromed - more red = more curli binding
- Check ABS480 of supertanant using plate reader - The tube with lower ABS480 has more curli in the sample

**MONDAY, 8/18/2025**

Attendees: Alyssa, Ansh, Khushi

Summary: S. pasteurii culture/biomineralization, RNA extraction

**S. pasteurii transfer cutlure (~4 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~4 pm)**
- Let sit overnight in 30C

**RNA extraction:**

Utilized Bioneer maglisto 5M RNA extraction kit: https://s3-us-west-1.amazonaws.com/resource.bioneer.us/juan2/pagecat1/accuprep/MagListo-5M-Tissue-Total-RNA-Extraction-Kit/UG-MagListo_Tissue_Total_RNA_Kit_V2(EN)_201703.pdf

On 300 ul samples of our transformant culture (colonies 1,2,3) as well as culture from joshi lab. In the process, we forgot about the recomended addition of betamercaptaethanol in the protocol, potentially resulting in RNA degradation.

The resulting samples had between 10 to 25 ng/ul concentrations on RNA on nanodrop, though with mediocre purity ratios. Accordingly we plan to redo this expirement.

## TUESDAY, 8/19/2025

Attendees: Ansh, Khushi, Uday

Summary: S. pasteurii culture/biomineralization, Overlap extension PCR

**S. pasteurii transfer cutlure (~2 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~4 pm)**

- Let sit overnight in 30C

**Overlap extension PCR part one:**

Prepapred 4 tubes with the following reagents. Ran overlap cycle with 15 iterations, with denaturation at 98C (0:10), annealing at 60 C (0:30), and extension at 72C (1:30). Stored results in the freezer to run extension cycles tommorow.

**Table7**

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | Tube Name | 2x q5 hot start master mx NEB | Forward primer (1x stock, 10 uM) | Reverse primer (1x stock 10 uM) | PCRd urease gene fragement one | PCRd urease gene fragment 2 |
| 2 | fn | 12.5 ul | 1.25 ul (added during extension cycle) | 1.25 ul (added during extensio n cycle) | 1 ul | 1 ul |
| 3 | fp | 12.5 ul | 1.25 ul (added during overlap cycle) | 1.25 ul (added during overlap cycle) | 1 ul | 1 ul |
| 4 | 40n | 12.5 ul | 1.25 ul (added during extension cycle) | 1.25 ul (added during extensio n cycle) | 1 ul 1/10x stock, 40ng | 1 ul 1/10x stock, 40ng |
| 5 | 40p | 12.5 ul | 1.25 ul (added during overlap cycle) | 1.25 ul (added during overlap cycle) | 1 ul 1/10x stock, 40 ng | 1 ul 1/10x stock, 40 ng |

**Urea stock:**

- Made 20x urea stock by disolving 1g of urea in 4 ml of water and filter sterilizing.
- Added appropriate concentration of Urea stock to reach a final conc of 20g/L in all BHI stocks in fridge.

**WEDNESDAY, 8/20/2025**

Attendees: Ansh, Khushi, Uday

Summary: S. pasteurii culture/biomineralization, Gel

**S. pasteurii transfer cutlure (~2 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~4 pm)**
- Let sit overnight in 30C

**Overlap extension PCR part two:**

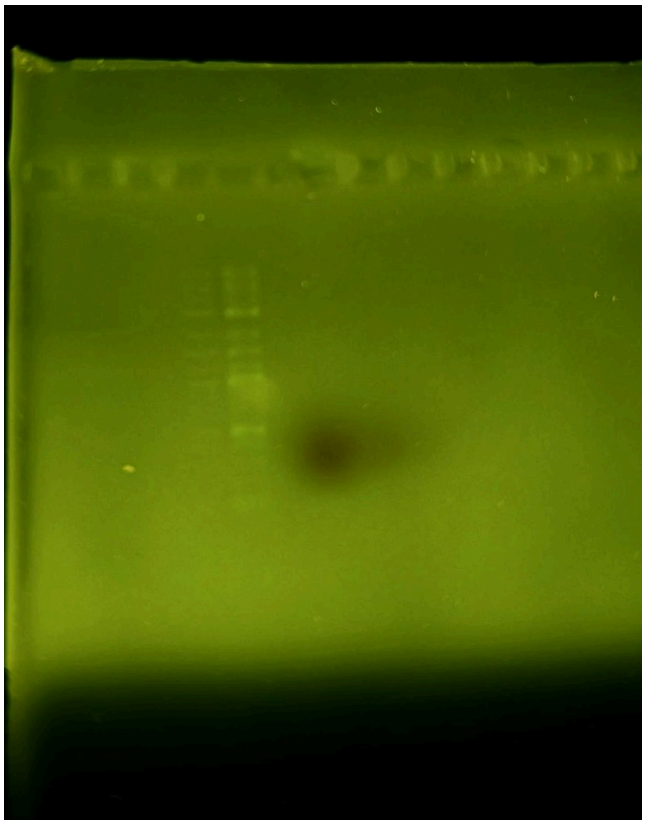
Ran extension cyles on yesterdays sample, after adding primers.

Settings: 20 iterations, with denaturation at 98C (0:10), annealing at 60 C (0:30), and extension at 72C (1:00). Note that this was on a different thermocycler than prior due ot availability.

**Gel of Urease assembly:**

We ran a 1.5% agarose gel with syber safe to view our overlap extension PCR products. We seem to have no product.

PXL_20250821_031809451.NIGHT~3.jpg



2 NEB 1kb+ ladders, followed by 4 samples of overlap extension PCR.

**THURSDAY, 8/21/2025**

Attendees: Uday
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (~6 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~8 pm)**
- Let sit overnight in 30C

## FRIDAY, 8/22/2025

Attendees: Ansh, Khushi, Uday
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (~6 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~8 pm)**
- Let sit overnight in 30C

**Culture prep:**

- Used glycerol stocks to make fresh overnight culture of transformant 1,2,3 as will ask joshi lab strain and E.coli K12

**S.pastuerii testing:**

- Plated S.pastuerii active stock along side glycerol stock (on BHI plate) to check for differences in colony morphology
- Made 1 ml 1:400 dilution S.pastuerii culture in CMM+ to compare to the culture in BHI +2 % urea tommorow under microscope to check for crystalization.

## SATURDAY, 8/23/2025

Attendees: Alyssa, Ansh, Khushi, Uday
Summary: S. pasteurii culture/biomineralization, induction, LB broth, microscope examination of S. pasteurii

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C

- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

**Induction/Curli assay prep:**

(Using induction protocol provided by Shreyas)

- Created transfer cultures of all ecoli cultures from yesterday: (transformant 1,2,3, joshi lab, k-12) as well as an LB control) with 1:20 dilution. (1ml cultures)
    - Created transfers into both LB+antibiotic, as well as LB+antibiotic+inducer
- Waited 3 hours
- Move in quadruplet to a 96 well plate in a 1:20 dilution, keepeing seperate samples for induced and not induced.

**Induction Assay (Shreyas protocol)**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB |
| B | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB |
| C | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB |
| D | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB |
| E | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB |
| F | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB |
| G | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB | induced #1 | induced #2 | induced #3 | induced Joshi | E. coli K12 | LB |
| H | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB | not induced #1 | not induced #2 | not induced #3 | not induced Joshi | E. coli K12 | LB |

most of column 12 used filter-sterilized LB instead of autoclaved LB
columns 1-6 to be used for flourescence assay, whereas columns 7-12 to be used for absorbance assay (quantifying curli fiber)

We also needed induced culture for the carbonic anhydrase assay & for RNA extraction. The originally induced 1ml cultures still had plenty of culture left in them.

- Created transfer cultures, 1:400 into 2ml of appropriate media -- LB/antibiotic, LB/antibiotic/inducer
- red dots mark induced cultures

**LB**

Made ~200ml of LB agar, autoclaved + aliquoted into ~50ml portions sitting in the fridge

- 2g tryptone
- 1g yeast extract
- 2g NaCl
- 200 ml MilliQ water

**Microscope examination:**

**S.pastuerii stock testing:**

We plated out current S.pastuerri culture against a S.pastuerii glycerol stock to ensure that it looked morphologically similar. We wil keep track of the plate over the next few days in 30C.

**SUNDAY, 8/24/2025**

Attendees: Alyssa, Ansh, Khushi
Summary: S. pasteurii culture/biomineralization, curli fiber assay, RNA extraction?

**S. pasteurii transfer cutlure (~2 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~4:30 pm)**
- Let sit overnight in 30C

**PBS**

made 200ml of PBS

- NaCl: ~1601 mg
- KCl: ~
- $Na_2HPO_4$: ~
- $KH_2PO_4$: ~

**Curli fiber assay**

Previously let 96-well plate incubate/shake overnight in the plate reader

- saw evaporation -- wells went from ~300ul to <200ul

again, columns 1-6 for flourescence assay, columns 7-12 for absorbance assay
Columns 1-6 moved to 0.65 ml clear tubes

- washed with PBS 3x (aka centrifuge 3x)
  - centrifuge at 12000 rpm for 2 min, remove supernatant, add in 200ul of PBS
- after last wash, add in 5ul of 20x congo red (effectively 0.5x)
-

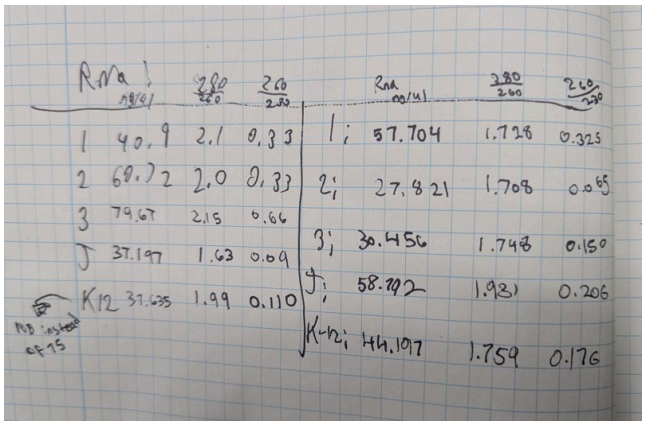
**RNA extraction v2:**

Utilized Bioneer maglisto 5M RNA extraction kit: https://s3-us-west-1.amazonaws.com/resource.bioneer.us/juan2/pagecat1/accuprep/MagListo-5M-Tissue-Total-RNA-Extraction-Kit/UG-MagListo_Tissue_Total_RNA_Kit_V2(EN)_201703.pdf

On 1 ml samples of our transformant culture (colonies 1,2,3), cultures from joshi lab, and plain E.coli k12. All cultures were extracted using both induced and uninduced samples.

The resulting samples had between 30 to ng/ul concentrations on RNA on nanodrop, with good 260/280 purity but bad 260/230 ratios. We decide to later use this for RT-PCR.

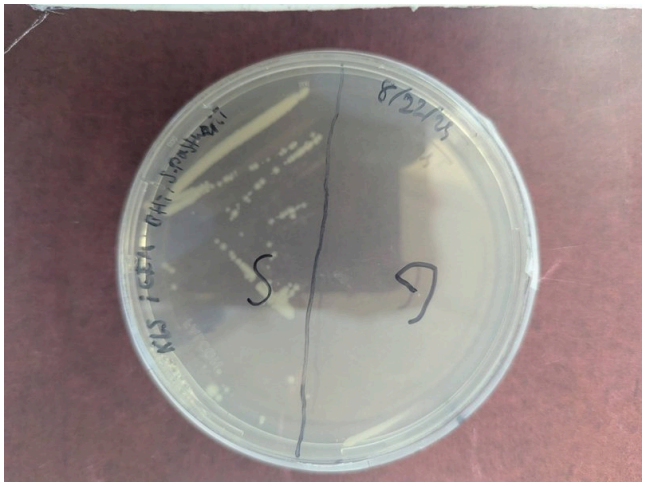
**PXL_20250911_020619016.jpg**




Here are results of a testing all samples on a nanodrop. Note that most 260/280 ratios are above 1.8, but our 260/230 is consistently around or below 0.3.

**S.pastuerii culture growth on plates:**

**PXL_20250823_214328211.jpg**




It seems that the culture is growing much faster than the glycerol stock, but at this point it is hard to make conclusions about culture similarity.

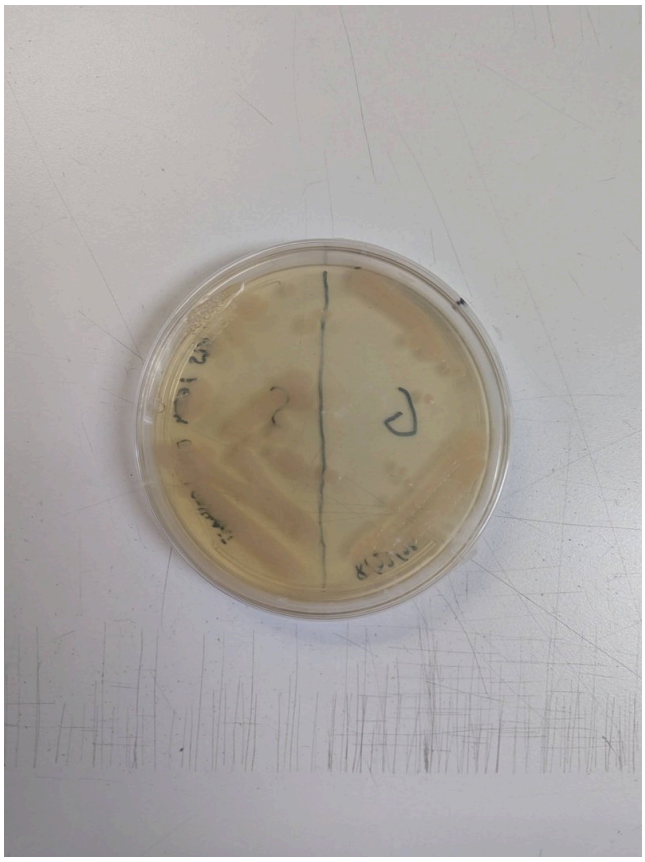
**MONDAY, 8/25/2025**

Attendees: Ansh,

Summary: Checked on plates

**S.pastuerii culture growth on plates:**

PXL_20250826_012416260 (1).jpg



Now that both sides are grown out, some differences of colony morphology are visible, but it is hard to conclude much.

**SATURDAY, 8/30/2025**

Attendees: Alyssa, Ansh,
Summary: S. pasteurii culture/biomineralization, testing of biomineralization

We paused on biomineralization today and instead measured the pH values of our S.pastuerii culture. What concenred us was that instead of the expect 8.5, it had dropped to 6.5, indicating the presence of another microbe. Accordingly, we made a fresh transfer culture from glycerol stock. We also placed some of the glycerol stock into mineralizing media to see if we could see any precipitate form.

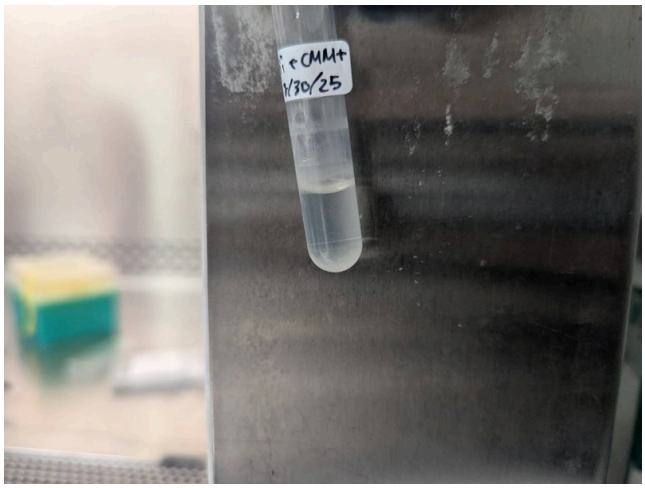
**SUNDAY, 8/31/2025**

Attendees: Ansh

Summary: S. pasteurii culture/biomineralization, testing of biomineralization

We tested the pH of our new s.pastuerii transfer culture, which was thankfully now 8.6. The pH of our s.pastuerii in mineralizing media was 8.2. The tube with mineralizing media did arguably have a little bit of precipitate that had settled to the bottom of the tube.

**PXL_20250831_192718920.jpg**




Tube of S.pastuerii in CMM+ media incubated at 30C shaking 200 rpm overnight.

**MONDAY, 9/1/2025**

Attendees: Ansh
Summary: S. pasteurii culture/biomineralization

Done with fresh culture

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

**PXL_20250901_194454246.jpg**

This is a comparision of out old s.pastuerii culture and our glycerol stock plated on BHI. There is a clear difference in strain.

## TUESDAY, 9/2/2025

Attendees: Ansh, Uday, Khushi
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

## WEDNESDAY, 9/3/2025

Attendees: Ansh, Uday, Khushi
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio

- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

## THURSDAY, 9/4/2025

Attendees: Ansh, Uday, Khushi
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

## FRIDAY, 9/5/2025

Attendees: Ansh, Uday, Khushi
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold

- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

**SATURDAY, 9/6/2025**

Attendees: Ansh, Alyssa, Uday, Khushi
Summary: S. pasteurii culture/biomineralization, RT PCR, Transfromation with CA and curli control plasmids.

**S. pasteurii transfer cutlure (~12:30 pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~2:30 pm)**
- Let sit overnight in 30C

**Transformation: u**sed Shreyas' protocol

- 3 tubes -- curli, sazCA, control
  - 50ul of competent cells + 100ng of DNA
  - control has no DNA
- incubate on ice for 30 min
- heat shock for 90 sec at 42C (in water bath)
- incubate on ice for 10 min
- add 250ul of LB to each tube, let recover at 37C
- for each tube, plate onto LB & LB/amp plates
  - ~100ul of mixture, plating beads
- incubate overnight at 37C

**RT PCR:**

Used bioneer rocketscript RT-PCR premix.
Pepared 8 tubes labeled: Transformants 1,2, 1_i, 2_i, 3_i, joshi lab, joshi lab induced, and wild type E.coli k-12.
To each tube added:

- 1 ul of RNA extract from cell culture matching the tube label
- 2 ul of csg_for primer

- 2 ul of csg_rev primer
- 2 ul of rss_for primer
- 2 ul of rss_rev primer
- 11 ul of PCR grade water

As per protocol placed tubes in a thermocyler

**SUNDAY, 9/7/2025**

Attendees: Ansh, Khushi, Alyssa
Summary: S. pasteurii culture/biomineralization, gel electrophoresis on RT PCR results, prepped cultures

**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

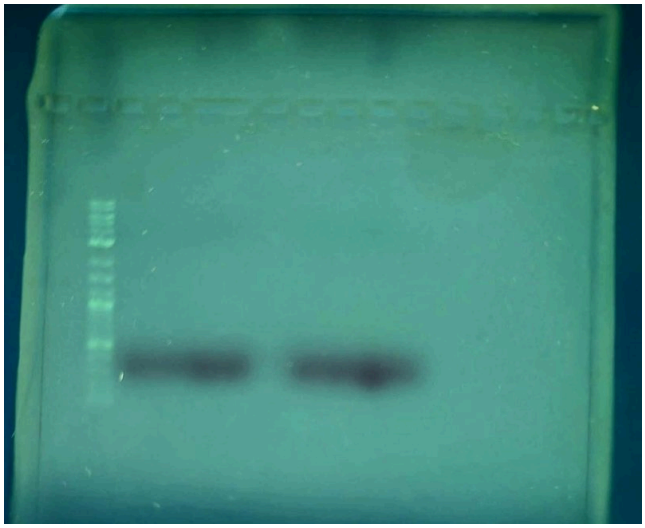
**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Gel**

Ran gel on RT PCR results -- got no visible bands other than ladder:

PXL_20250907_233437726.NIGHT~2.jpg



Also nanodropped RT PCR results -- exceptionally high ng/ul concentration, which we later found to be due to the RT PCR mix
Jenny Mae recommends we redo RT PCR with the newer kit.

**Cultures**
added transformants to media + inducer, let grow overnight at 37C

## TUESDAY, 9/9/2025

Attendees: Ansh, Khushi, Uday
Summary: S. pasteurii culture/biomineralization, curli fluorescence assay, carbonic anhydrase assay

Done with fresh culture
**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Curli flourescence assay with controls v1:**

- put 300 ul culture in transparent PCR tubes
- wash 3x w PBS
    - centrifuge at 12000 rpm for 2 min, remove supernatant, add PBS, repeat
- after last centrifuge, remove supernatant, add PBS, and also add in congo red dye
    - 7 ul of 20x stock
- Vortex
- shake for 10 min at room temperature (25C)
- use DeNovix flouremeter to measure flourescence
    - excite at green (~525 nm), emit at red (~625 nm)

**Carbonic anhdyrase (CA) assay:**

- 100 ul of phenol red (0.01%)
- 700 ul of 15 mM pH 8.4 tris buffer
- 30 ul of test culture
- 200 ul of CO2 (aq)

Reagents are all chilled on ice and added in the order above to a chilled cuvette. A spectrophotometer is used to measure A435 and A560 every 5 seconds. We noted in the results that every time the CO2 soluti
on is opened it gets weaker, skewing results.

## WEDNESDAY, 9/10/2025

Attendees: Ansh, Khushi, Uday

Summary: S. pasteurii culture/biomineralization, prepping culture

**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Culture preperation**

**THURSDAY, 9/11/2025**

Attendees: Ansh, Khushi, Uday

Summary: S. pasteurii culture/biomineralization, sterilization of biomineralized product, glycerol stocks

**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
  - 3 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**Sterilization:**

Moved the largest of our minerlized pieces (the black mold) into the oven at 75C to dry it.

Then removed the mineralized solids from the mold into a tinfoil crucible to bake at 160C overnight to sterilize.

This process did break the mineralized solids up into several pieces.

**PXL_20250912_032531159.jpg**

Photo taken after drying in ther process of removeing the solids from the mold.

**Glycerol stocks:**

We added 500 ul of sterile glycerol stock to a sterile screw cap tube along with 500 ul of appropriate culture (Curli 1, Curli2, CA1, CA2). The tubes were then mixed using a pipete and then put in -80C.

**FRIDAY, 9/12/2025**

Attendees: Ansh,
Summary: S. pasteurii culture/biomineralization

**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**SATURDAY, 9/13/2025**

Attendees: Ansh, Uday
Summary: S. pasteurii culture/biomineralization, RNA extraction,

**S. pasteurii transfer cutlure**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 3ml of CMM- + S. pastuerii
    - 3 ml of CMM-, 30ul of previous S. pasteurii culture
    - used approx 1ml per mold
- Let sit for 2.0 hours at 30C
- Drained culture from molds
- Added ~1ml of CMM+ media to each mold
- Let sit overnight in 30C

**RNA extraction v3:**

**Table8**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | **K12** | 6.17 | 0.013 | 0.4 |
| 2 | **Joshi** | 7.96 | 0.036 | 0.8 |
| 3 | **Curli 1** | 9 | 0.028 | 0.774 |
| 4 | **Curli 2** | 29.35 | 0.128 | 2.2 |
| 5 | **CSGCA** | 3.87 | 0.021 | 0.48 |

Utilized Bioneer maglisto 5M RNA extraction kit: https://s3-us-west-1.amazonaws.com/resource.bioneer.us/juan2/pagecat1/accuprep/MagListo-5M-Tissue-Total-RNA-Extraction-Kit/UG-MagListo_Tissue_Total_RNA_Kit_V2(EN)_201703.pdf

On 1 ml samples of our transformant culture (colonies 1,2,3), cultures from joshi lab, and plain E.coli k12. All cultures were extracted using both induced and uninduced samples.

The resulting samples had very low ng/ul concentrations of RNA on the nanodrop, with bad 260/280 purity and bad 260/230 ratios. We decided to redo this extraction.

**Sterility test:**
We took out our baked sample from the oven (which had been in the oven for 24 h at 160C). We then plated two BHI plates, one just innoculated with a sterile innoculation loop as a control, and the other with an innoculation loop dipped in our sample.

We will view the results of this test in 3 days.

## SUNDAY, 9/14/2025

Attendees: Alyssa
Summary: S. pasteurii biomineralization/culture, induction of cultures

**S. pasteurii transfer cutlure (~3:30pm)**

- 1:400 dilution ratio
- 2.5 ul of previous culture to 1ml BHI + 2% urea
- Placed in shaker in 30C incubator

**Biomineralization**

- made 2ml of CMM- + S. pastuerii
  - 2 ml of CMM-, 30ul of previous S. pasteurii culture
  - used approx 1ml per mold
- Let sit for 2.0 hours at 30C

- Drained culture from molds
- Added ~1ml of CMM+ media to each mold **(~5:30 pm)**
- Let sit overnight in 30C

Attempted RNA extraction on the most recent batch of previously induced fridge culture -- not enough culture = not enough cells, called it quits
(Also scrounged through for RNA extraction kits and found the best one was the one we had been using, the Bioneer one)

**Induction**
Made 2ml cultures of LB w antibiotic as appropriate

- K12, Joshi, curli1, curli2, csgCA1, csgCA2
- 1:100 dilution of glycerol stock to cultures
    - everything used glycerol stock except for K12 -- K12 used old fridge cultures, still 1:100 dilution

Incubate shaking at 37C for 3 hours

- OD600 of K12 at 2 hours was 0.4, OD600 of K12 at 3 hours was 0.6

Per culture, split into two tubes w 1ml

- red dot = uninduced, no dot = induced
- added 10ul of 100x IPTG to curli 1, curli 2, csgCA1, csgCA2 (marked w/o dot)
- & added arabinose as indicated -- 1ul of 1000x ara to joshi

placed in 37C shaking incubator overnight

**MONDAY, 9/15/2025**

Attendees: Ansh, Uday, Khushi
Summary: RNA Extraction

**RNA extraction v4:**
Utilized Bioneer maglisto 5M RNA extraction kit: https://s3-us-west-1.amazonaws.com/resource.bioneer.us/juan2/pagecat1/accuprep/MagListo-5M-Tissue-Total-RNA-Extraction-Kit/UG-MagListo_Tissue_Total_RNA_Kit_V2(EN)_201703.pdf

On 1 ml samples of our transformant culture (colonies 1,2,3), cultures from joshi lab, and plain E.coli k12. All cultures were extracted using both induced and uninduced samples.

The resulting samples had between 6 to 80 ng/ul concentrations on RNA on nanodrop, with good 260/280 purity but bad 260/230 ratios. Interestingly, the induced samples seem to have higher RNA concentrations.

**Table9**

| | A | B | C | D |
|---|---|---|---|---|
| 1 | Sample Name | Concentration | A260/230 | A260/280 |
| 2 | K12 | 8.199 | 0.078 | 1.502 |
| 3 | Joshi | 9.543 | 0.045 | 1.292 |
| 4 | Curli 1 | 10.969 | 0.036 | 1.54 |
| 5 | Curli 2 | 26.643 | 0.075 | 1.831 |
| 6 | Curli CA 1 | 6.781 | 0.009 | 0.559 |
| 7 | Curli CA 2 | 9.378 | 0.021 | 1.39 |
| 8 | K12 i | 23.472 | 0.178 | 1.932 |
| 9 | Joshi i | 80.843 | 0.74 | 2.141 |
| 10 | Curli 1 i | 36.542 | 0.228 | 2.001 |
| 11 | Curli 2 i | 27.218 | 0.103 | 1.75 |
| 12 | Curli CA 1 i | 24.768 | 0.059 | 1.179 |
| 13 | Curli CA 2 i | 46.172 | 0.27 | 1.95 |

**Sterilization part 2:**

We moved our remaining two molds (the white and PDMS molds) into the oven at 75C to dry them.
Then removed the mineralized solids from the molds into a tinfoil crucible to bake at 160C overnight to sterilize.

**Media prep:**

We prepared 13 culture tubes with media: 8 lb amp, 2 lb Kan, 2 lb

## TUESDAY, 9/16/2025

Attendees: Ansh, Uday, Khushi
Summary: RNA Extraction

**Sterilization tests results:**

Our plates after 3 days at 30 C have no colonies, indicating that out samples were successfully sterilized.

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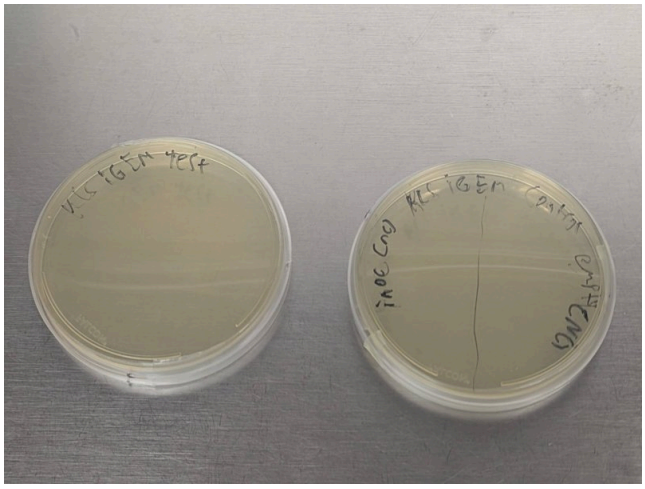

Test plate on left, control on the right. The control is split into two sections, one empty and the other swabbed with a sterile inoculation loop.

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Our samples after being transfered into a sterile bottle for storage. THe sample from the PDMS mold is pretty intact.

**FRIDAY, 9/19/2025**

Attendees: Ansh, Uday, Khushi
Summary: Culture preperation

**Culture prep:**

Made 7 5 ml cultures: Curli 2 induced, Curli 2, Curli-csg 2, Curli-csg 2 induced, Joshi, Joshi induced, and E.coli k-12.
All cultures were inoculated from glycerol stock, grown for 1.5 hr and then select cultures were induced.
The Cultures were not very cloudy on induction, likely duee to limited time given for growth.

Induction was done with 1.0 mM iptg, except for joshi lab, which used arabinose.

## SATURDAY, 9/20/2025

Attendees: Ansh, Uday, Alisha, Alyssa, Khushi
Summary: RNA Extraction

**RNA extraction v4:**
Utilized Bioneer maglisto 5M RNA extraction kit: https://s3-us-west-1.amazonaws.com/resource.bioneer.us/juan2/pagecat1/accuprep/MagListo-5M-Tissue-Total-RNA-Extraction-Kit/UG-MagListo_Tissue_Total_RNA_Kit_V2(EN)_201703.pdf

On 5 ml samples of our transformant culture (colonies 1,2,3), cultures from joshi lab, and plain E.coli k12. All cultures were extracted using both induced and uninduced samples.

The resulting samples had between 200 to 1000 ng/ul concentrations on RNA on nanodrop, with mediocre 260/280 purity and 260/230 ratios.

**RT:**
We utilized bioneer's RT premix with dn6 primers to create cDNA copies of our RNA.

- 2 ul of RNA, 18 ul of water, and the RT-pellet. Dissolve the pellet by vortexing & centrifuging.
- Followed the instructions on the protocol for the thermocycler.

## SUNDAY, 9/21/2025

Attendees: Alyssa, Ansh, Khushi, Uday
Summary: dsDNA fluorescence assay, RT, PCR cleanup, PCR, gel electrophoresis, overnight cultures

**dsDNA fluorescence assay:**
We used the DeNovix dsDNA broad range fluorescence assay, following the online protocol

- As there were insufficient amounts of enhancer, we ended up having half the appropriate concentration of both dye and enhancer. Hence, the concentration displayed was likely? half the noted concentration.
- For all of our samples, in comparison to the standards, we had abysmally low concentrations -- 0.4-1.5 ng/ul

We then discovered that the RT premix only included the RNA-binding DNA polymerase, and hence would only produce single-stranded cDNA. We then cleaned up our RT product and performed PCR with our specific primers for curli and rssA (a housekeeping gene).

**PCR**

- 1ul of specific primer for each reaction
- did 20ul reaction using NEB Taq polymerase
- specific primers: csg.FOR, csg.REV, rssA.FOR, rssA.REV -- supposed to PCR up the whole curli operon & housekeeping gene rssA

Ran gel electrophoresis on 1.5% gel, got no results. After some reflection, we realized that we had used MilliQ water for our original RT reaction. Suspecting possible contamination, we decided to re-run the pre-mix with PCR-nuclease free water.

**RT:**

We utilized bioneer's RT premix with dn6 primers to create cDNA copies of our RNA.

- 2 ul of RNA, 18 ul of water, and the RT-pellet. Dissolve the pellet by vortexing & centrifuging.
- Followed the instructions on the protocol for the thermocycler.

We than ran a gel and got no results. We suspected this had to do with the kit expiring in 2016.

**Prepared overnight cultures**

- Scraped frozen cultures from glycerol stocks to create overnight cultures
- curli, CA, csgCA

## MONDAY, 9/22/2025

Attendees: Ansh, Khushi, Uday
Summary: Passage Cultures, Induced

Passaged all cultures from 9/21 in a 1:20 ratio, induced after 2 hours of growth with variable amounts of IPTG, including 0.5 mM, 1 mM, and 2 mM.

## TUESDAY, 9/23/2025

Attendees: Ansh, Khushi, Uday
Summary: Passage Cultures, Induced

Performed Curli assay as per standard protocol (see experiment page). Got no expression across the board. Sent off glycerol stocks (Curli -1, CA 2) for sequencing and realized that both our glycerol stocks, even Curli-1, had a carbonic anhydrase only plasmid (aka, pET_sazCA_Amp).

## WEDNESDAY, 9/24/2025

Attendees: Ansh, Khushi, Uday
Summary: Tried RT using various methods

Induro RT enzyme, ordered from NEB, arrived.

**Reverse Transcription:**

Prepared RT PCR in two batches, each with the same 7 RNA samples from 9/20 as well as one negative control.

**RT one:**

1. Bioneer RT kit
2. 1 ul of induro RT enzyme (NEB)
3. 1ul RNA
4. 18 ul of nuclease free water.

This was done under the theory the random primers and DNTPs in the kit were probably okay, and the REverse transcriptase might have denatured as it aged.

**RT two** - based on NEB protocol (https://www.neb.com/en-us/protocols/2022/09/28/induro-reverse-transcriptase-protocol):

1. 1 ul RNA
2. 4 ul specific primers
3. 1 ul 10mM DNTPs
4. 4 ul nuclease free water

In RT two, we did not have and RNAse inibitor in lab, and so had to skip adding it. We also did not have randomized primers that did not already have beta mercaptaethanol added, and so instead used specific primers.

**PCR one:**

1. Added:
    a. 2 ul of sample from RT one
    b. 12.5 ul of NEB q5 hot start 2x PCR master mix
    c. 4 ul of mixed primers (1ul of each of the 4 primers at 1x conc)
    d. 6.5 ul nuclease free water
2. Ran PCR using NEB q5 hotstart master mix PCR protocol, with 25 cycles and 60C melting temp

Note: we did not run PCR cleanup after running RT, leaving a risk of presense of out dn6 rimers in this sample.

## FRIDAY, 9/26/2025

Attendees: Ansh, Khushi, Uday
Summary: PCR of RT results and gel electrophoresis

**Gel one (RT two, not PCRd):**

We ran gel electrophoresis using a 25 ml 1.5% agarose gel with sybersafe on all samples from RT two prior to running PCR.

1. 6 ul of ladder was run
2. Every other sample had 3 ul of sample, 2 ul of loading dye, and 7 ul of water

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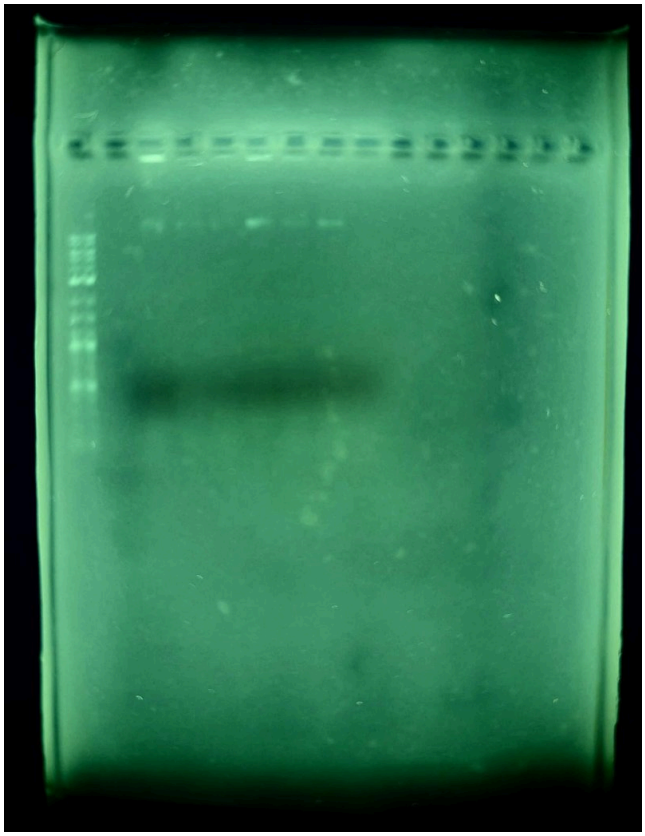


From left to right: Ladder, skipped well, K-12, Joshi, Joshi induced, Curli, Curli induced, Csg-CA, Csg-CA induced, Negative control.

This was quite promising, as it showed the presence of cDNA.

**Gel two (RT one, PCRd):**

We ran gel electrophoresis using a 25 ml 1.5% agarose gel with sybersafe on all samples from RT two prior to running PCR.

1. 6 ul of ladder was run
2. Every other sample had 3 ul of sample, 2 ul of loading dye, and 7 ul of water

This gel was completely blank out side of the ladder, indicating that this RT likely did not function.

**PCR two (for RT two):**
Followed bioneer PCR premix protocol to PCR all samples of RT two.
Added:

1. 4 ul of primers (1 ul each 1x stock)
2. 16 ul of water

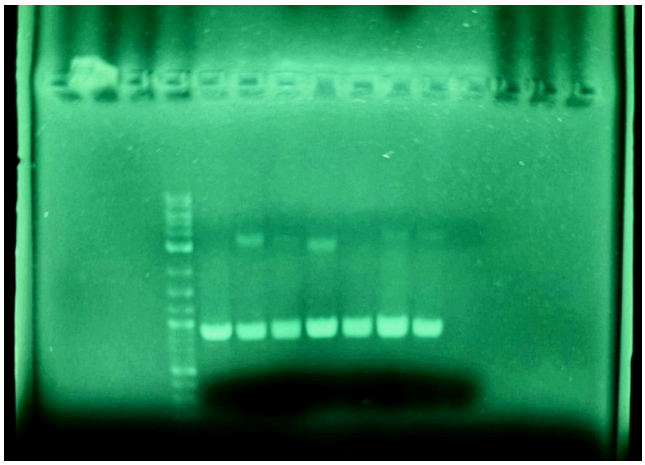
Vortexed and then briefly centrifuged tubes. Ran 25 cycles of PCR, with a metling temp of 60 C.

**Gel three (RT two, PCRd):**

We ran gel electrophoresis using a 25 ml 1.5% agarose gel with sybersafe on all samples from RT two prior to running PCR.

1. 6 ul of ladder was run
2. Every other sample had 3 ul of sample, 2 ul of loading dye, and 7 ul of water

**PXL_20250927_055910289.NIGHT4.jpg**



From left to right: K-12, Joshi, Joshi induced, Curli-2, Curli-2 induced, Csg-CA, Csg-CA induced, Negative control. Note the positive control genes show up for every sample except for negative control indicating good assay viability.

**SUNDAY, 9/28/2025**

Attendees: Ansh, Khushi, Uday
Summary: Sent samples for sequancing

Sent off inoculated LB/amp cultures of Curli-2 and CA-1 for sequencing, to see it they had our expected plasmids.
*Update, 9/30: These plasmids actually look right -- the curli has pET_csg_Amp, and the CA has pET_sazCA_Amp.

Note: All analysis of diagrams/results documented in this notebook are in Results/Documentation.