PLEASE STAY TRUE TO THESE CONVENTIONS WHEN WRITING IN THIS NOTEBOOK:

Before each new date, page break.(ctrl + Enter)
Each date has at least 3 portions: Summary, Procedures, What to do next.
Always include time at lab (all after Day 4)

Please take note of any changes to the lab/working space (what was used, where things were put).
Some such places include:

- **4C Fridge**
- **37C Incubator**
- **At lab space**
- **(other precise description)**

Some tools include:

- **milli-Q H2O**
- **TAE Buffer**
- **Plates**
- **Gels**
- **Gloves**
- **Goggles**
- **Pipets (take care of these please)**
- **(anything else)**

PORTIONS OF EACH DATE:

Summary:

- **What happened; what you did**

Procedures:

- **Include all steps if you can in detail (how long to wait, what you used)**
- **Always make note of where you place things; be SPECIFIC.**

What to do next:

- **Only a possibility (not always needed) but points others in the direction where we are headed. Should be a description worthy of being the summary of the next day**
- **ie. Run SDS page gels tomorrow**

Optional Portions:
Results, Observations, Measurements/Data, etc.

Day 1 afternoon: August 3, 2015: (Nathan, Aidan, Abhi)

SUMMARY:

- Transformation of e. Coli Dh5-alpha with ordered plasmid.

PROCEDURE:

1. Thaw competent cells on ice (plasmid and Dh5-alpha)
2. Dilute pure plasmid (4ug) with 40ul of milli-Q water for ~100ng/uL ratio
3. Add 1uL diluted plasmid to e.Coli
4. Incubate in ice for 15 mins.
5. Heat shock at 42deg C for 45sec
6. Ice for 2 mins to complete heat shock
7. Add 900uL of LB
8. Let sit for ~35 mins at 37deg C
9. Plate 50 - 100 uL on LB+Amp plate
10. Let it grow overnight

WHAT TO DO NEXT:

- Culture GOOD bacterial colonies
- Replate colonies on LB+Amp Agar plates and incubate at 37deg C
- Create 2-3 new plates of transformed e. coli, following the same procedure as above - aliquot 5uL of the plasmid to prevent contamination of the "master broth"

Day 2 morning: August 4, 2015: (Caroline, Patricia, Dane, Abhi)

WHAT WE FOUND:

- no colonies on either plate-> put both plates back in the incubator

SUMMARY:

- Transformation of e. Coli Dh5-alpha with ordered plasmid. (we used 2.5 ul diluted plasmid as opposed to the 1 ul used yesterday)
- Made changes to transformation protocol-> centrifuged to concentrate bacteria

PROCEDURE:

1. Thaw competent cells on ice (Dh5-alpha)
2. Add 2.5 uL diluted plasmid to e.Coli
3. Incubate in ice for 15 mins.
4. Heat shock at 42deg C for 45sec
5. Ice for 2 mins to complete heat shock
6. Add 900uL of LB
7. Let sit for ~40 mins at 37deg C
8. Centrifuge for 2 min. to collect bacteria
9. Vacuum so that ~100 ul is left
10. Pipet to resuspend bacteria
11. Plate 50 - 100 uL on LB+Amp plate
12. Let it grow overnight.

WHAT TO DO NEXT:

- hope we have colonies :)

Day 2 afternoon: August 4, 2015: (Nathan)

SUMMARY:

- checked on plates

Day 3 afternoon: August 5, 2015: (Nathan, Anthony, Catherine, Madeline)

WHAT WE FOUND:

- Colonies grew on both plates from August 4; plates from August 3 still had no colonies

SUMMARY:

- Streak plated colonies from the more populated of the two original plates onto two more
- Created 6 LB+Amp plates

PROCEDURE:

Streaking:

1. Using a sterilized transfer loop, identify and sample a “good” colony
2. Streak this colony across the first sector
3. Repeat steps 1-2 four more times, with each streak performed perpendicular to the previous streak so that 5 sectors are created
4. Repeat steps 1-3 on a second LB+Amp plate
5. Place in 37 degC incubator and let them sit O/N

LB+Amp Plates:

1. Place 10g of LB, 6g of Agar, and 400mL of dH2O in an autoclave beaker
2. Autoclave for 1 hour
3. Remove flask and let cool for 20 min
4. Add 400 uL of ampicillin; swirl gently to avoid making bubbles
5. Pour into plates
6. Let plates sit for 20 min to solidify
7. Turn upside down, label, and store in fridge

WHAT TO DO NEXT:

- Nathan - autoclave remaining LB+Agar to create additional plates because LB+Amp agar solidified; make more plates
- IPTG induction and SDS Page

Day 4 morning: August 6, 2015: (Caroline, Patricia)

SUMMARY:

- Made part of the solutions for the page gel
- Recipes for gels on instruction paper in iGEM box or this helpful website below http://www.assay-protocol.com/molecular-biology/electrophoresis/denaturing-page

PROCEDURE:

- Calculated Protein Size (LbCHI31) by entering amino acid sequence in http://web.expasy.org/compute_pi/ : 33 kDa
- Made a Stacking Gel solution and Separating Gel solution for 12% Gel (we added everything except AP & TEMED which should be in the 4C fridge)

WHAT TO DO NEXT:

- Finish making gels (instruction sheet in iGEM box): solutions are in 15mL falcon tubes on the iGEM 4C fridge rack
- Do SDS Page
- Make Stock solutions (if you have time) for 10% SDS, 1.5 M Tris(ph=8.8), and 0.5M Tris-HCl(ph=6.8)

Day 4 afternoon: August 6, 2015: (Aidan, Catherine, Patricia, Anthony, Jonathan) Time: 15:00 - 17:40

SUMMARY:

- We made 2 12% page gels

PROCEDURE:

For 10% Separating Gel: (10.11mL) - Enough for 2 gels

ddH2O	4.1mL
30% Acrylamide	3.3mL
1.5M Tris pH **8.8**	2.5mL
10% SDS	0.1mL = 100uL
10% APS	100uL
TEMED	10uL

NOTE: TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

For 4% Stacking Gel: (4.088mL) - Enough for 2 gels

ddH2O	2.93mL
30% Acrylamide	0.53mL
1.0M Tris pH **6.8**	0.5mL
10% SDS	0.04mL = 40uL
10% APS	80uL
TEMED	8uL

NOTE: Again, TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

1. Make Separating and Stacking Gel (all except APS and TEMED)
2. Get gel frames and clean with ethanol
3. Line up gel plates (should not be able to feel the crease at bottom easily)
4. Test if plates are aligned with ddH2O (pipet in as you would the gel solution)
5. Pour out ddH2O
6. Add TEMED to separating gel in fume hood.
7. Add APS to separating gel and immediately load onto gel plates. (Load about ¾ way [separating])
8. Add ddH2O or isopropanol to make sure that it is level with no bubbles
9. Let it sit for 20 mins
10. Repeat step 6 and 7 for stacking gel.
11. When you load the stacking gel, fill to the top of the plates and place combs
12. Make sure there are no bubbles
13. Let sit for 15 mins or so and either run or store in fridge

TO STORE IN FRIDGE:

Keep gels with combs and plates and wrap in damp paper towel.
Wrap again with cling wrap and label with the DATE and iGEM and % gel (ie 10% or 12%)

10% APS STOCK: Not labeled with date but made on 08/06/15 (In 4C fridge)

WHAT TO DO NEXT:

- Make more stock solutions for future use (maybe 1.5M Tris ph 8.8, 1.0M Tris ph 6.8, 10% SDS, TAE buffer)
- http://openwetware.org/wiki/Qiagen_Buffers We should make sure we have tools necessary to conduct minipreps. (don't think we'll create an overnight saturated culture over the weekend)
- Basically stock up on things we will be needing in the future to make more gels, run said gels, conduct miniprep, western blots
- Next week Monday we will likely make an overnight saturated culture of our E.Coli and run a miniprep (if we can) on Tuesday [this is all hypothetical]

Day 5 morning: August 7, 2015: (Patricia, Caroline, Catherine) Time: 09:30 - 12:00

SUMMARY:

- We made 10% SDS Solution, 1.0M Tris pH 6.8, and 1.5m Tris pH 8.8
- All three solutions are stored at room temperature.

PROCEDURE:

For 10% SDS

ddH2O	25mL
20% SDS solution	25mL

NOTE: Since we used 20% SDS solution, we only had to dilute it.

For 1M Tris pH 6.8

Tris Base	12.1g
ddH2O	70+mL
HCl	as necessary

1. Dissolve 12.1g Tris base in 70mL ddH2O.
2. Adjust pH by adding HCl while using a pH meter.
3. Add ddH2O until the solution reaches 100mL.

For 1.5M Tris pH 8.8

Tris Base	27.23g
ddH2O	80+mL
HCl	as necessary

1. Dissolve 27.23g Tris base in 80mL ddH2O.
2. Adjust pH by adding HCl while using a pH meter.
3. Add ddH2O until solution reaches 150mL.

WHAT TO DO NEXT:
-Create TAE Buffer.

Day 5 afternoon: August 7, 2015: (Aidan, Madeline, Priya) 2:35-3:30

SUMMARY:

- Made 200mL LB broth for future use and saturated culture.

PROCEDURE:

LB broth:
25g LB mix (found in tub on shelf directly above scales) per 1L of water.
autoclave 15 mins at ~125deg

WHAT TO DO NEXT:

- Probably remake a saturated overnight culture (LB + e.Coli + incubate overnight)
- Make some TAE buffer (if we need to)
- Gather materials needed for miniprep....

Day 6 morning: August 10, 2015: (Nathan, Caroline, Abhi, Patricia): 10:00-12:30

SUMMARY:

- Made 500 mL TAE buffer for SDS Page (on iGEM shelf)
- Created an overnight (4-6 hour) culture for SDS Page (in top 37 deg.C shaker)

PROCEDURE:

TAE buffer:

1. 100 mL of 50x TAE buffer diluted with 400 mL ddH2O
2. Stored 500 mL of TAE on iGEM shelf

Overnight Culture:

1. Make LB+Amp solution (3 mL LB & 3 uL Amp for each tube)
2. Pick one “good” colony and place in a shaker-compatible tube
3. Repeat steps 1+2 for two more samples
4. Make a control using DH5 Alpha instead of colonies from transformed plates
5. Place in 37 deg.C shaker

WHAT TO DO NEXT:

- Perform SDS Page (Save analysis for tomorrow) - Store in fridge after de-dyeing
    - Store by submerging in ddH2O (use gel container - ask Aaron/Brian/Reta)

Day 6 afternoon: August 10, 2015: (Anthony, Liana, Nathan, Dane, Edric)

SUMMARY:

- Ran a gel for SDS Page analysis (Ladder, control, and three copies of the samples)
- Staining gel in Coomassie Blue overnight

PROCEDURE:

DNA Sample Prep

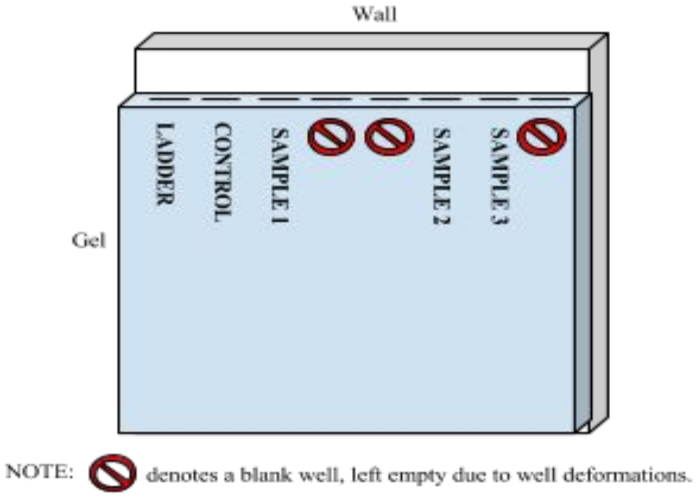
1. Pipet 20 microliters of each sample into Eppendorf tubes (Control, Sample 1, Sample 2, Sample 3)
2. Centrifuge for 1min (to get the solutions at the bottom of the tube).
3. Place in dry hot bath (90-95C) for 10min. This allows the cells to open up and release materials.
4. Centrifuge for 1min to allow proteins to separate from other cellular masses
5. Separate the supernatant from pellet (if pellet is nearly invisible, pipet supernatant into another Eppendorf tube anyway).
6. Add 5 microliters of 5x SDS Buffer dye to each sample.

SDS Page setup

1. Load small gel and dummy gel plate in gel box. Lock into place
2. Remove comb carefully from gel
3. Fill inner chamber to the brim (or until slightly overflowing) with 1x SDS Buffer
4. Fill outer chamber so that the bottom of the gel is submerged - this allows for the samples to reach the opposite end of the gel

SDS Page gel run

1. Clean each well with SDS Buffer using a well-pipet tip set at 80 microliters. Take care not to break well walls. (Place end of tip on the gel wall and slide down into the well).
2. Load wells with a ladder, Control, Sample 1, Sample 2, and Sample 3. Use a pipette set to 18 uL. See diagram below for setup used today.



NOTE: ⊘ denotes a blank well, left empty due to well deformations.

3. Cover gel box with cover - match red to red and black to black
4. Hook the gel box up to the volt generator
5. Set the electrical current to 110 V (Milliamps should be between 20-50; Watts should be around 2 (~5 max)) for about 30 minutes or until the loaded samples surpass the separating gels
6. Increase the voltage to 200V for about 15 min (check after 10 and wait around gel box in case the samples already reached the bottom)

Storing SDS Page gel

1. Dump out SDS buffer from the inner chamber
2. Remove the dummy gel (clean it off) and the gel that was run
3. Submerge gel in Coomassie Blue and place on gel rocker O/N

WHAT TO DO NEXT: (Just making notes here for the rest of this week- Nathan)

- Analyze SDS page results and see if procedure worked well (Monday afternoon = test run) - Tuesday morning + afternoon
- Make one set of stacking gels for an SDS run to save time on Wednesday (if possible) - Tuesday afternoon
- Two sets of stacking gels for two more runs of SDS page - Wednesday afternoon
- Prepare LB+Amp+colony vs LB+e.coli for SDS page runs - Thursday morning
- Run both gels at the same time in SDS gel box - Thursday afternoon
- Analyze gel results (expression) - Friday morning and afternoon

Day 7 morning: August 11, 2015 9:00-10:30 (Patricia, Priya, Gita)

SUMMARY:

- Prepared solutions for 2 12% stacking gels

PROCEDURE:

For 10% Separating Gel: (10.11mL) - Enough for 2 gels

ddH2O	4.1mL
30% Acrylamide	3.3mL
1.5M Tris pH **8.8**	2.5mL
10% SDS	0.1mL = 100uL
10% APS	100uL
TEMED	10uL

NOTE: TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

For 4% Stacking Gel: (4.088mL) - Enough for 2 gels

ddH2O	2.93mL
30% Acrylamide	0.53mL
1.0M Tris pH **6.8**	0.5mL
10% SDS	0.04mL
10% APS	80uL
TEMED	8uL

NOTE: Again, TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

1. Make Separating and Stacking Gel (all except APS and TEMED)

WHAT TO DO NEXT:

- Finish making Gels

Day 7 morning: August 11, 2015 11:30-1:30 (Caroline)

SUMMARY:
Staining and De-staining (Gel plates were not taken off, so staining from Monday night did not take effect)

PROCEDURE:
*Please take off gel plates before staining

Staining and De-staining

1. Put gel into a gel box and cover it with Coomassie Blue
2. Put box on shaker in darkroom for ~20 min.
3. Use thumb to hold gel gently against box as you pour out Coomassie Blue into used bottle
4. Pour Destaining Buffer in gel box
5. Crumple up Kimwipe and put in gel box too (helps absorb stain)
6. Put box on shaker in darkroom for ~10 min.
7. Use thumb to hold gel gently against box as you pour out Destaining Buffer into waste storage bottle under the table
8. Repeat steps 4,5, and 7 two more times (you can put gel box on the light to see how bands are developing)
9. Put deionized water(or MilliQ H2O) in gel box (this helps the gel grow again because it shrinks from de-staining)
10. Put box on shaker for 5-10 min.

WHAT TO DO NEXT:
Gel Analysis:

- Take out sheet protector from drawer in darkroom
- put gel in between sheet protector (BE CAREFUL & wet ur hands first -> gel can break easily)
- Make sure there are no bubbles
- put gel on scanner(in the office room where we store our backpacks)
- On the computer-> Adobe Photoshop CS2 -> email picture to yourself and put on lab ntbk

Create 2 more gels:

- Solutions w/o AP and TEMED are on the rack in 15 ml tubes
- Gel rig is on bench with only one set of gels
- Follow procedure from before (Day 4 afternoon pg.6) and finish gels

Day 7 afternoon: August 11, 2015 (Nathan, Caroline, Liana, Dane, Abhi)

SUMMARY:

- Made 2 12% stacking gels (remade gel solutions from morning)
- Stained and destained gel from Monday (used new Coomassie instead of used one)
- Performed Gel Analysis -> no results

PROCEDURE:

Finish Making Stacking Gels from Morning:

1. Get gel frames and clean with ethanol
2. Line up gel plates (should not be able to feel the crease at bottom easily)
3. Test if plates are aligned with ddH2O (pipet in as you would the gel solution)
4. Pour out ddH2O
5. Add TEMED to separating gel in fume hood.
6. Add APS to separating gel and immediately load onto gel plates. (Load about ¾ way [separating])
7. Add ddH2O to make sure that it is level with no bubbles
8. Let it sit for 20 mins
9. Checked on gels - no polymerization
10. Cleaned out gel frames to rerun

Stacking Gel:

For 10% Separating Gel: (10.11mL) - Enough for 2 gels

ddH2O	4.1mL
30% Acrylamide	3.3mL
1.5M Tris pH **8.8**	2.5mL
10% SDS	0.1mL = 100uL
10% APS	100uL
TEMED	10uL

NOTE: TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

For 4% Stacking Gel: (4.088mL) - Enough for 2 gels

ddH2O	2.93mL
30% Acrylamide	0.53mL
1.0M Tris pH **6.8**	0.5mL
10% SDS	0.04mL
10% APS	80uL
TEMED	8uL

NOTE: Again, TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

1. Make Separating and Stacking Gel (all except APS and TEMED)
2. Get gel frames and clean with ethanol
3. Line up gel plates (should not be able to feel the crease at bottom easily)
4. Test if plates are aligned with ddH2O (pipet in as you would the gel solution)
5. Pour out ddH2O
6. Add TEMED to separating gel in fume hood.
7. Add APS to separating gel and immediately load onto gel plates. (Load about ¾ way [separating])
8. Add isopropanol to make sure that it is level with no bubbles
9. Let it sit for 20 mins
10. Repeat step 6 and 7 for stacking gel.
11. When you load the stacking gel, fill to the top of the plates and place combs
12. Make sure there are no bubbles. If there are bubbles, remove comb, add more of the 4% stacking gel and resubmerge comb from corner so you can remove bubbles as you lower it into the solution
13. Let sit for 20 mins or until polymerized and store in fridge

TO STORE IN FRIDGE:
Keep gels with combs and plates and wrap in damp paper towel.
Wrap again with cling wrap and label with the DATE and iGEM and % gel (ie 10% or 12%)

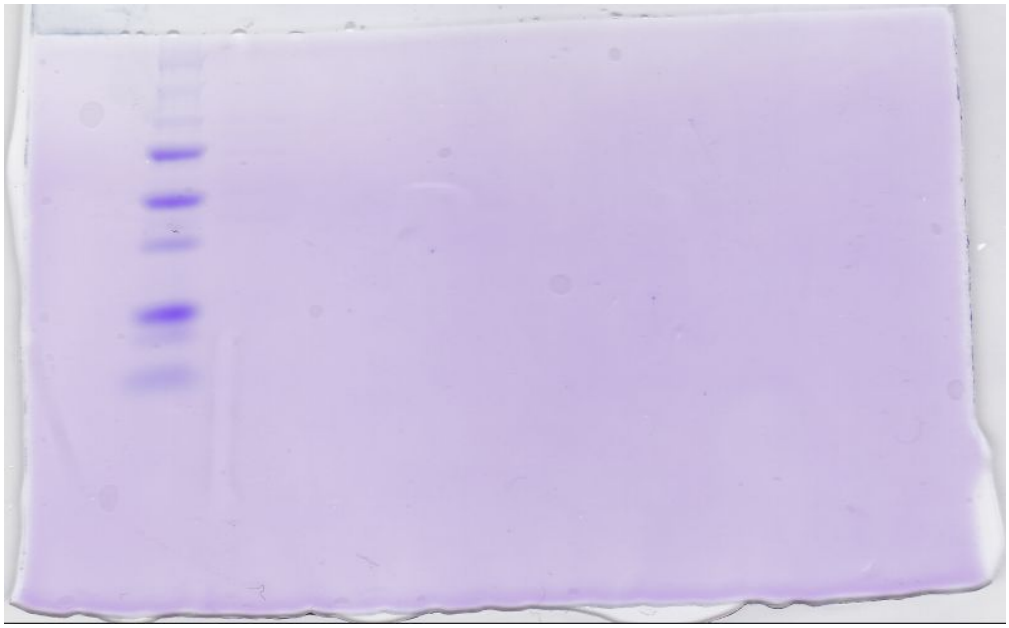
Staining and destaining:

1. Put gel into a gel box and cover it with Coomassie Blue
2. Put box on shaker in darkroom for ~20 min.
3. Use thumb to hold gel gently against box as you pour out Coomassie Blue into used bottle
4. Pour Destaining Buffer in gel box
5. Crumple up Kimwipe and put in gel box too (helps absorb stain)
6. Put box on shaker in darkroom for ~10 min.
7. Use thumb to hold gel gently against box as you pour out Destaining Buffer into waste storage bottle under the table
8. Repeat steps 4,5, and 7 two more times (you can put gel box on the light to see how bands are developing)
9. Put deionized water(or MilliQ H2O) in gel box (this helps the gel grow again because it shrinks from de-staining)
10. Put box on shaker for 5-10 min.

Gel Analysis:

1. Take out sheet protector from drawer in darkroom
2. Wet fingers and gently take out gel and put in sheet protector
3. Make sure there are no bubbles
4. Put gel in scanner (in office room where we keep backpacks)

5. On the computer: open Adobe Photoshop CS2 -> import from Canon scanner -> press Preview -> re-size the frame to focus on gel ->Scan
6. Save picture to iGEM folder (under Users) and e-mail it to yourself too
7. Throw away gel and dab sheet protector dry



WHAT TO DO NEXT:

Morning:

- Do Transformation and grow on 2 new LB+Amp plates (Label) → use **Day 2 morning** procedure for this
- Create new culture of LB+sample -> 3 samples
- **important: Rehydrate old gel**

Afternoon:

- Run two new gels for SDS page (Do not centrifuge this time, or if you do, don't just pipet the supernatant because then you're probably not getting protein → Ask Reta for her tips on running a gel) --> **Important:** Load the ladder, the 3 samples that Caroline and Nathan made, and the 3 samples that Liana and Nathan made
- Stain and destain gels; scan; save → we can analyze through discussions later

Notes:

- Run old SDS gel → use old plates as positive control for cells on new plates

Day 8 morning: August 12, 2015 (Nathan, Liana)

SUMMARY:

- Made O/N (4-6 hour) LB+Amp+culture for SDS page

PROCEDURE:

1. Make LB+Amp solution (3 mL LB & 3 uL Amp for each tube)
2. Pick one “good” colony and place in a shaker-compatible tube
3. Repeat steps 1+2 for two more samples
4. Place in 37 deg.C shaker

WHAT TO DO NEXT:

- Check old gel for dehydration - if not dehydrated, leave in wrap… if dehydrated, rehydrate
- Run the two gels made on 8/11/15… ask Reta for her tips on loading for SDS page
    - Refer to 8/11/15 on notes regarding loading the gel (remember to keep the two end lanes open)
- Aidan and Anthony run the gels
- Madeline, Priya, Gita create two new plates (in 4 degC fridge)

Day 8 afternoon: August 12, 2015 (Aidan, Madeline, Dane, Priya, Gita)

SUMMARY:

- Made 3 O/N saturated cultures and 1 control (IN 37deg C INCUBATOR)
- Threw away junk

PROCEDURE:

- TURN ON THE BUNSEN BURNER
- SANITIZE LB
- make LB + amp + culture (basically take one good colony from a culture and put it into your LB + amp mix)
- incubate in 37deg C incubator O/N
- Should look dark and cloudy if it is saturated after incubation.

BEFORE WE CAN RUN SDS PAGE GELS:

1. **We need to have grown saturated O/N cultures (> 16 hours)**
2. **We need to make sure that our cultures express the plasmid of interest (Is there a specific inducer? If so, what is it? If not, how do we make sure that it is expressed?)**
3. **We need to purify the proteins from our saturated culture and use a spectrophotometer to keep concentrations relatively consistent (~ .25 OD)**
4. **Then we can run gels**

Day 9 morning: August 13, 2015 (Nathan, Caroline, Madeline, Catherine)

SUMMARY:

- Ran an SDS Page gel (1 ladder, 3 samples, 3 buffer+SDS running dye (ensures that everything runs straight))
- Made another saturated batch of LB+Amp+colony
    - The reason we made another batch is because our plasmid, pUCIDT, is a custom plasmid off IDT that was selected by IDT to meet our lab requirements. Unfortunately, pUCIDT does not contain a lac operon (so no IPTG induction), is a DNA specific plasmid (so it doesn't over express proteins, but it'll still express just at a lower concentration, which is why we created a new batch with 6 mL of LB for greater growth. The logic behind this is, because we can't use IPTG induction, we will detect the expression of our plasmid at 33 kDa via the GFP tag we linked to our protein), and does not have common restriction enzyme cut sites (eg. ECOR1, XBal, etc).

PROCEDURE:

DNA Sample Prep

1. Pipette 2 mL of saturated LB+Amp+Sample into cuvettes
2. Find absorption of LB+Amp+Sample by blanking the machine with LB and then inserting your LB+Amp+Sample cuvette
3. Repeat steps 1-2 for the next 5 samples
4. Pipet 2 mL of control into cuvette and find absorption
5. Record absorption of samples
6. Pipette 250 uL of Sample 1 into an Eppendorf tube
7. Repeat step 6 for remaining samples and control
8. Centrifuge at 10,000 rpm for 1 minute
9. Vacuum out supernatant with pipette
10. Remove samples 4-6 due to the failure of pellet formation
11. Pipette 16 uL of buffer (to break down/loosen up the bonds in our cells) to Samples 1-3
12. Pipette 4 uL of SDS running dye to Samples 1-3
13. Place Samples 1-3 (in Eppendorf tubes) in water bath for 10-15 minutes

SDS Page setup

1. Lock gel in place on one side and dummy slide on other side of gel box
    a. **Important: Load gel with short side first. Loading tall side first could damage the gel and causes the gel box to not run properly**
2. Remove comb carefully so as to not puncture/deform the wells
3. Overflow inner chamber with 1x SDS Buffer
4. Fill outer chamber with 1x SDS Buffer until the bottom of the inner box is slightly submerged

SDS Page gel run

1. Clean out each well of the gel with 1x SDS buffer with a pipette set to 18 uL (MAKE SURE TO USE WELL-COMPATIBLE-TIPS)
2. Load 7 uL of ladder into second well
3. Load 18 uL of samples 1-3 into wells 2-4, respectively
4. Create a “master mix” of buffer and SDS running dye (40 uL of buffer and 10 uL of SDS running dye)
5. Load 18 uL of buffer+SDS running dye into wells 5-7
6. Cover gel box with cover - match red to red and black to black
7. Hook the gel box up to the volt generator
8. Set the electrical current to 110 V (Milliamps should be between 20-50; Watts should be around 2 (~5 max)) for about 30 minutes or until the loaded samples surpass the separating gels
9. Increase the voltage to 200V for about 15 min (check after 10 and wait around gel box in case the samples already reached the bottom)

Storing SDS Page gel

1. Dump out SDS buffer from the inner chamber
2. Remove the dummy gel (clean it off) and the gel that was run
3. Submerge gel in Coomassie Blue and place in fridge for the afternoon team

Making LB+Amp+Sample O/N culture

1. Turn on the Bunsen burner - we do this because LB is a growth medium for bacteria, so performing such experiments near the flame helps to sanitize the LB
    a. Sanitize the opening of the LB jar
2. Make an LB+Amp mix with 6 mL of LB and 6 uL of Amp in 4 shaker-compatible-tubes
3. Pick up one good colony from plates with loop and place in mixture. Repeat for remaining tubes.
4. Incubate in 37deg C incubator O/N (at least 16 hours)
5. In order to figure out if mixture is saturated the next day, tubes should look milky

WHAT TO DO NEXT:

- Stain and destain gel
- Scan gel and upload with the light box
- Use fluorescence to look for our GFP tag around 33 kDA (size of LbCHI31)

Day 9 evening: August 13, 2015 (Aidan, Anthony, Patricia)

SUMMARY:

- Destained SDS Page gel from earlier today; left overnight on shaker in diH2O (step 11)
- Set up saturated cultures for SDS Page tomorrow evening (morning crew -- DO NOT TOUCH)

PROCEDURE:

Destaining Gel

1. Remove SDS Page from glass into gel box with lid and rinse with ddH2O
2. Add Coomassie Blue to cover gel completely (~.5-1cm over)
3. If you can (we didn't) microwave on high for 40 seconds
4. Incubate Coomassie Blue stain for 5-10 mins or >1hour if you didn't microwave.
5. Pour off stain into proper disposal container
6. Rinse with ddH2O 1-2 times
7. Add destain solution to cover gel completely (~ 1-1.5cm over)
8. Incubate destain until destain turns dark blue
9. pour out destain solution into proper disposal container
10. Repeat steps 7-9 until gel is almost clear and bands are EASILY seen.
11. Incubate in ddH2O to allow gel to 'grow'. This makes it easier to handle and scan. You can incubate for 1 hour or O/N.
12. Your destain is complete at this point.
13. The next step is to transfer your gel into a plastic laminate sheet.
14. Scan your gel
15. Send an email of the scan to yourself and post picture in LAB NTBK.

Setup saturated cultures

1. Pour 4 mL of LB media into three plastic tubes (with the nonscrew caps). Label as "Control," "Sample 1," and "Sample 2."
2. Add 4 microliters of ampicillin (taken from stock of 100 milligrams per milliliter concentration) into Samples 1 and 2. **Ampicillin is in 4C; it can only be stored in the 4C for 2-3 more days before it has to be returned to -20C (but it's our stock, so do not place it in that fridge).**
3. In control tube, you should have only LB media (no amp). Transfer a loop of wild type DH5alpha from glycerol stock.
4. In Sample 1, innoculate one colony from plate 1 and place in tube. Similarly, transfer one colony from plate 2 into Sample 2.
5. Place the the samples and control in the 37C shaking incubator.

MEASUREMENTS:

Spectrophotometry measurements
Afternoon 8/12:
SAMPLE1 → 2.016A = 0.304 OD (taken from plate 1)
SAMPLE2 → 2.033A = 0.308 OD (taken from plate 2)
SAMPLE3 → 0.963A = -0.02 OD (taken from plate 3)

Morning 8/12:
SAMPLE4 → 0.021A = very low (taken from plate **???**)
SAMPLE5 → 0.037A = very low (taken from plate **???**)
SAMPLE6 → 0.041A = very low (taken from plate **???**)
note: OD ~0.25 recommended

OBSERVATIONS:

- The SDS Page gel that was run earlier seemed to have no visible ladder. This means that we can't really use it to determine if our protein is expressed.
- If you worked with cultures (ie. putting LB + anything into eppendorf, falcon tubes, or cuvettes) please bleach it when you are done and leave it in the sink to die for at least 10 minutes before dumping and placing used containers into biohazard waste (should be a covered container under the sink. Otherwise, before and after using any equipment, such as plates, please wipe with ethanol and kimwipes in order to sanitize as well as rinse in sink if necessary before putting said equipment back.

WHAT TO DO NEXT:

- Gel from today's SDS Page was left overnight on the dark room shaker in diH2O. Laminate the gel (use the binder plastic page covers in the drawers of the dark room in front of the shaker) and analyze.

Day 10 morning: 8/14/15 (Caroline, Madeline, Jonathan, Catherine)

SUMMARY:

- Checked on gel
- Spectrophotometry: measured Concentrations

PROCEDURE:

MEASUREMENTS:

Spectrophotometry measurements:

AFTERNOON: (saturated solutions made 8/13 afternoon)

control = 1.716A (wt rH5-alpha)

1 = 0.101A (taken from plate 1)

2 = 1.37 A (taken from plate 2)

MORNING: (saturated solutions made 8/13 morning)

1 = 0.020 A (taken from plate **???**)

2 = 2.758 A (taken from plate **???**)

3 = 0.017 A (taken from plate **???**)

4 = 0.041 A (taken from plate **???**)

WHAT TO DO NEXT:

- See afternoon

Day 10 afternoon: 8/14/15 (Aidan Catherine, Anthony)

SUMMARY:

- We ran SDS Page with a ladder, control (wt dH5-alpha e. coli), and 2 samples (sample 1 had no visible pellet [not enough growth]) in that order
- Left said gel in coomassie blue stain O/N

PROCEDURE:

- See above for SDS page procedures
- See above for staining/destaining procedures

WHAT TO DO NEXT:

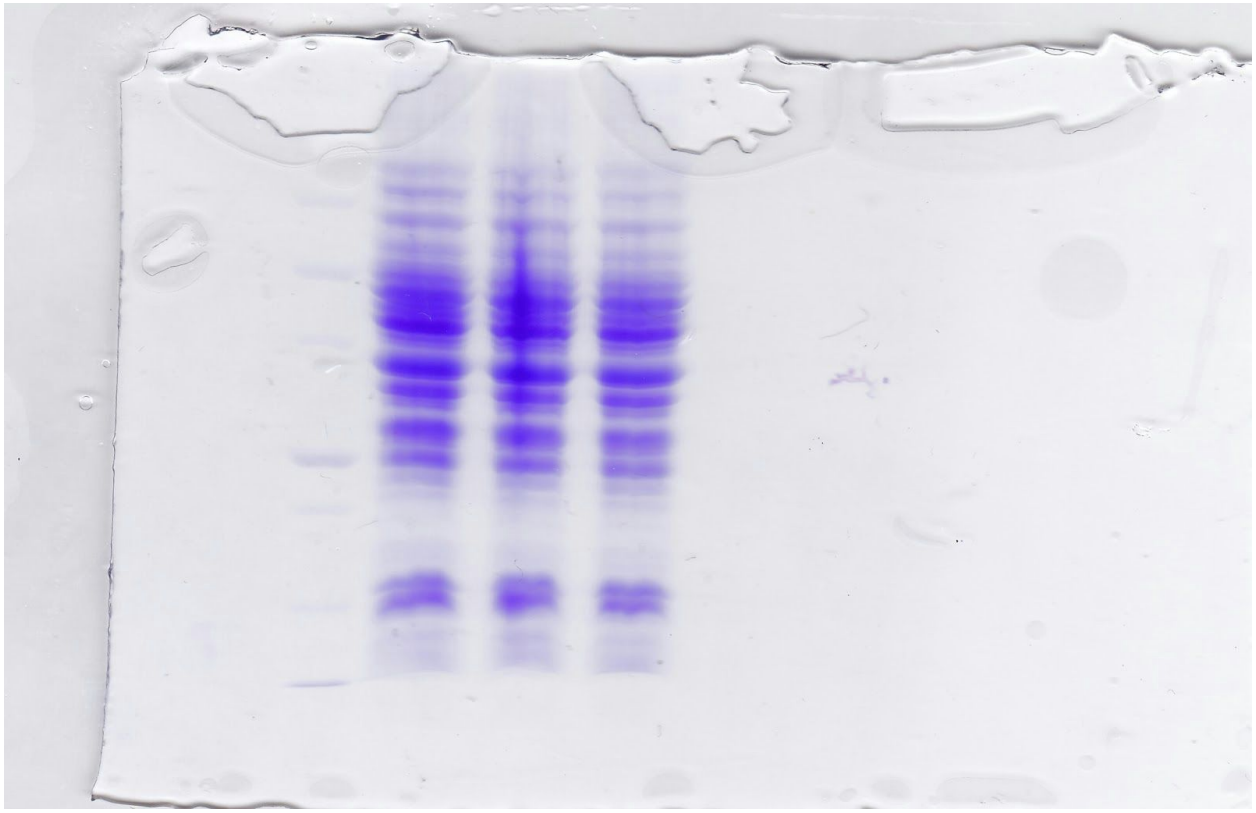
- Destain gel and scan both gels (other one was from Thursday) (both on shaker)
- Analyze gels
- Look at LB + Amp plates under UV to see GFP + take pictures

Day 11 morning: 8/17/15 (Nathan, Caroline)

SUMMARY:

- Destained gel from 8/14/15
- Scanned gel from 8/13/15

Gel from 8/13/15:



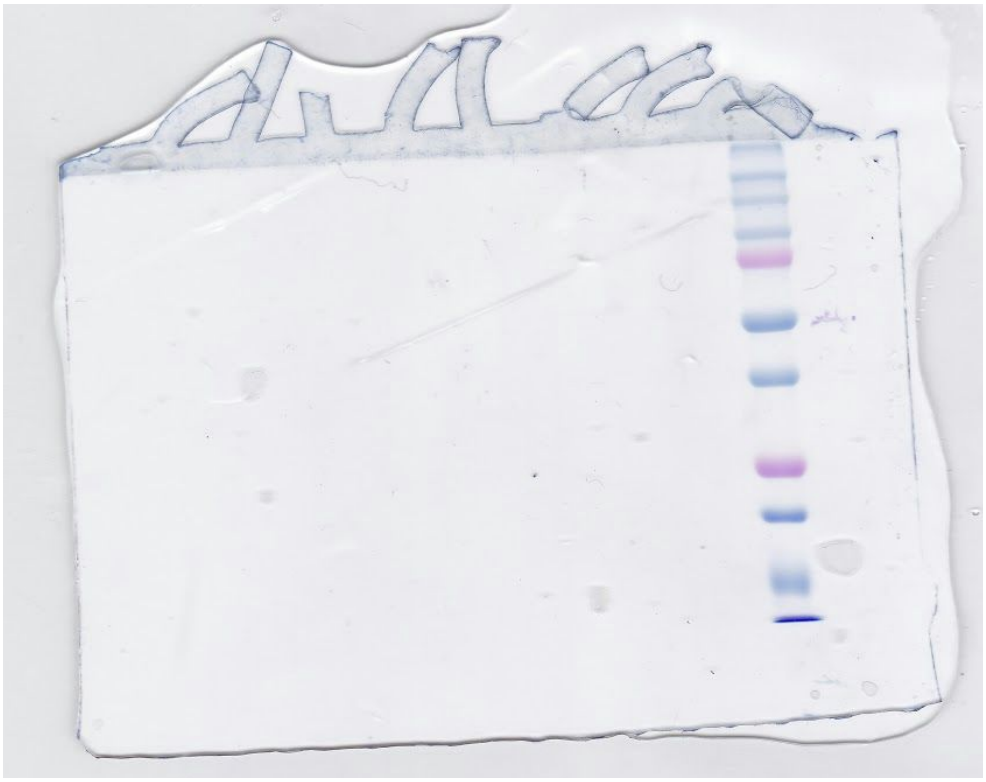
WHAT TO DO NEXT:

- Leaving the gel in Coomassie Blue stained gel really heavily, so continue to destain (if needed)
- Look at LB + Amp plates and check for GFP
- Store gels in box (submerge in diH2O)

Day 11 Evening: 8/17/15 (Anthony)

SUMMARY:

- Finished destaining 8/14/15 gel and scanned gel
- Setup for saturated samples for SDS Page tomorrow (redo)



8/14/15 Gel (inverted left to right)

PROCEDURE:

Destaining Gel

- *See procedure on destaining*
- Upon arrival in lab, gel was clear, and only bands from the ladder were showing (nothing from the wells containing control and samples).
- Washed out the Destain Solution and incubated on the rocker in diH2O for 30 min.

Scanning Gel

- *See procedure on scanning*
- Note that the Canon gel scanner is connected to the computer running Windows, not MacOS

Setting up Saturated Samples for SDS Page tomorrow

1. Pour 4 mL of LB media into three plastic tubes (with the nonscrew caps). Label as “Control,” “Sample 1,” and “Sample 2.”

2. Add 4 microliters of ampicillin (taken from stock of 100 milligrams per milliliter concentration) into Samples 1 and 2. Ampicillin is in 4C; it can only be stored in the 4C for 2-3 more days before it has to be returned to -20C (but it's our stock, so do not place it in that fridge).
3. In control tube, you should have only LB media (no amp). Transfer a loop of wild type DH5alpha from glycerol stock.
4. Remove plates from the 4C fridge and let plates thaw. In Sample 1, innoculate one colony from plate 1 and place in tube. Similarly, transfer one colony from plate 2 into Sample 2.
5. Place the the samples and control in the 37C shaking incubator.

WHAT TO DO NEXT

- MORNING CREW → Make an SDS Page gel for SDS Page tonight.
- Autoclave a new stock of LB media (debatable, since we will be moving to a different lab soon…)
- AFTERNOON CREW → Run SDS Page with new saturated samples (cultured in sterile LB media from Brian)
- Reanalysis and storage for Western Blot → Ask Brian about drying gel before scanning so that gel can be restored afterwards for further tests.

IMPORTANT NOTES FROM TODAY → Please read

- **The iGEM stock of LB media has been contaminated.** As such, some of our previous data may be invalid. Please use sterile techniques when handling stock solutions and cultures.
    - When opening LB media bottles from now on, adhere to the following steps. After opening the bottle, sterilize cap and bottle opening by passing it through the flame.
    - Once you are done using the media, similarly sterilize the bottle opening and cap with flame exposure.
- In continuation of the previous point, we will need a new LB stock. **Somebody tomorrow will need to autoclave a new batch of LB media for iGEM to use.** This person should be well-versed in sterile technique.
- **For future gels, it may be a good idea to create more transformed DH5alpha plates.** The more times you freeze and thaw a culture of bacteria, the more fragile the cells become, leading to cells that are either dead or nowhere as active as before.
- Gel did not show any bands in the wells other than the ladder. When filling wells with samples, **add around 20 microliters for safety to ensure the visibility of distinct bands.**
    - Because the 8/14/15 gel used 5 microliters of samples rather than the 20 microliter amount used previously, this may have resulted in fainter bands.
    - Similarly, the plate samples from the 8/14/15 gel run were exposed to a higher concentration of antibiotic in media, so note the working concentrations specific to the antibiotic in use (as well as the initial dilution of the antibiotic stock).

Day 12 Morning: August 18, 2015 9:00-11:00 (Patricia, Priya)

SUMMARY:

- Prepared solutions for 2 12% stacking gels

PROCEDURE:

For 10% Separating Gel: (10.11mL) - Enough for 2 gels

ddH2O	4.1mL
30% Acrylamide	3.3mL
1.5M Tris pH **8.8**	2.5mL
10% SDS	0.1mL = 100uL
10% APS	100uL
TEMED	10uL

NOTE: TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

For 4% Stacking Gel: (4.088mL) - Enough for 2 gels

ddH2O	2.93mL
30% Acrylamide	0.53mL
1.0M Tris pH **6.8**	0.5mL
10% SDS	0.04mL
10% APS	80uL
TEMED	8uL

NOTE: Again, TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

1. Make Separating and Stacking Gel (all except APS and TEMED)

WHAT TO DO NEXT:

- finish making gels

Day 13 afternoon: August 19, 2015 3:00 - (Aidan, Madeline)

SUMMARY:

- Finished making 12% SDS gels (1 gel was given to Norman as he needed one)
- Measured, or tried to measure concentration of growth using spectrophotometer
- Ran SDS Page Gel with Ladder, Control, Sample 1, and Sample 2

PROCEDURE:

See above for procedure on making SDS page gels (10% and 12%)

Spectrophotometry:

1. Gather materials (samples, ddH2O, cuvettes [found in foam container above spectrophotometer])
2. Blank out spectrophotometer with ddH2O in a cuvette
3. Load ~1.5mL of sample into a cuvette
4. Run sample
5. Record absorbance of sample
6. Repeat steps 3-5 until all samples are tested
7. Bleach cuvettes and leave in sink for >10 mins to kill anything in them

SDS PAGE:

Protein Purification/Getting samples ready for SDS:

1. Put 40uL of each sample in a eppendorf tubes
2. Place on dry heat bath for ~5 minutes so cells release proteins (note: cells have to be alive [reason for why we can't reuse growth, or incubate things for too long])
3. Centrifuge for 1 minute at ~10K RPM
4. You should find pellets in all of your samples at this point
5. Use a pipet set at slightly over 40uL to pipet out the supernatant (make sure you don't touch the pellet)
6. add about 8uL of 5x SDS buffer dye to each sample. (approx ⅛ of sample you centrifuged)

Running SDS Page:

1. Set up box with dummy plate and your own. Make sure no leakage.
2. Fill space between plates with 1x SDS buffer and enough overflow to touch the bottom of the plate (needed to conduct electricity through the gel)
3. Load ladder (if normal not dyed load ~10uL, if expensive dyed load ~5uL)
4. Load Control and your Samples with pipet set at 10uL

5. When loading, if you are not using special gel tips (don't fit on 10uL pipet) be careful, but don't be too shy when pipetting samples or ladder into the wells (you don't want anything to go into other wells)
6. Once loaded, run at 100V for between 15-20 mins (until you're sure the samples are in the separating gel)
7. Run at 200V for another ~30mins (until the samples hit the very bottom of the gel). You should be checking on it periodically to make sure you don't run it far too long.
8. You have finished running SDS Page.

Next Step:

1. Get a gel box (can be found in darkroom) and pour enough coomassie blue to cover the bottom about .5cm NOTE: coomassie blue is hazardous so only pour over a covered surface (in the darkroom)
2. Pour off buffer from in between plates into the box you ran it in.
3. Take gel plate out.
4. Carefully separate the plates around your gel (don't slide them or your gel will break) by digging your fingernails under the area with your wells
5. There are many different ways of doing this, but carefully remove gel from plate into gel box with coomassie blue. One method is to peel the gel off from the top (you do not want to touch the separating gel too much) upside down and use the surface tension of the coomassie blue to make sure the gel doesn't fold. If you need help with this, ask someone.

See above for procedures on staining/destaining.

WHAT TO DO NEXT:

- Destain Gels and take already destained gels out of their gel boxes.

Day 14 Morning: August 20, 2015 (Nathan, Caroline)

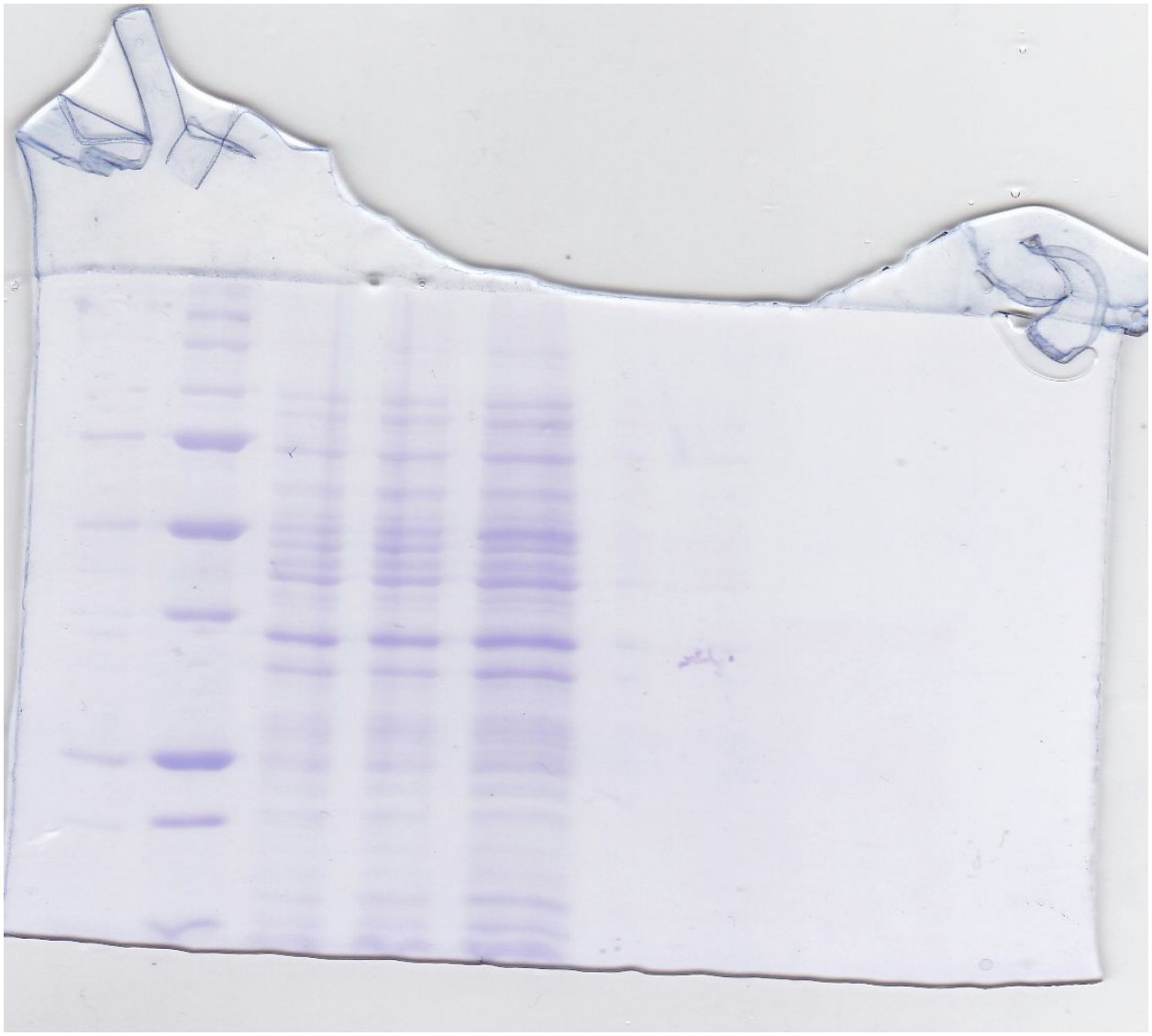
SUMMARY:

- Destained gel

WHAT TO DO NEXT:

- Scan gel

Day 14 Afternoon: August 20, 2015 5:00 - Patricia
Gel Scan:



Ignore the first well (it has part of the ladder)
sadly, there's not much visible difference between the control (well 3) and our samples (wells 4 and 5)

Day 15 Evening: August 27th, 2015 (Anthony and Patricia)

SUMMARY:
-PCR of the chitinase gene

PROCEDURE:
Primers prep

1. Dilute primers (Forward primer was originally 22 nmol, Reverse was originally 27.1 nmol) to achieve100 µM concentration (25 nmol/250 µL).
2. Suspend primers in 250 µL MilliQ H2O for dilution by centrifuging primer+H2O mix for 1 min. at Lvl 4. Keep each primer in separate tubes (i.e. keep forward primers separate from reverse).
3. Take 1 µL of the 100 µM primer mix and suspend in 9 µL MilliQ H2O to achieve 10 µL of 10 µM primers.

DNA Template prep

1. Dilute original pUCIDT plasmid 0.1 µg/µL. For this batch prep, 4 µg of plasmid + 40 µL of MilliQ were prepared together.

PCR Setup

1. Place the following into PCR tubes:
    a. 1 µL of Forward Primer (10µM)
    b. 1 µL of Reverse Primer (10µM)
    c. 1 µL of DNA Template (0.1 µg/µM)
    d. 1 µL of Phusion polymerase + 10 µL of 5x HF Buffer + 1 µL of dNTPs (25 mM)
        i. NOTE: For this lab, Part D's components were all in a single mix stored in the -20C fridge.
    e. 35 µL of MilliQ H2O
2. Cycle in PCR:
    a. 98 C: 30 sec
    b. 98 C: 10 sec
    c. 55 C: 15 sec
    d. 72 C: 2 min (1700bp region, 1min/kb)
    e. Repeat Steps 2A-2D 29 more times
    f. 72 C: 5 min
    g. Hold at 4 C overnight (~ 15 hr)

******PCR products were taken out of 4 C in machine on Aug. 28th, 2015 by Reeta to be stored in the -20 C fridge.

Day 16 Evening: September 1, 2015 (Nathan and Madeline)

SUMMARY:

- Made LB+Spectinomycin Agar Plates
- PCR of chitinase -> Re-did this because PCR product was not in 4 degC or -20 degC

PROCEDURE:

Making plates:

1. Place 5g of LB, 3g of Agar, and 200mL of dH2O in an autoclave beaker
2. Autoclave for 1 hour
3. Remove flask and let cool for 20 min
4. Add 200 uL of 50 mg/mL spectinomycin; swirl gently to avoid making bubbles
5. Pour into plates
6. Let plates sit for 20 min to solidify
7. Turn upside down, label, and store in fridge

PCR of Chitinase:

Primers prep

4. Dilute primers (Forward primer was originally 22 nmol, Reverse was originally 27.1 nmol) to achieve100 µM concentration (25 nmol/250 µL).
5. Suspend primers in 250 µL MilliQ H2O for dilution by centrifuging primer+H2O mix for 1 min. at Lvl 4. Keep each primer in separate tubes (i.e. keep forward primers separate from reverse).
6. Take 1 µL of the 100 µM primer mix and suspend in 9 µL MilliQ H2O to achieve 10 µL of 10 µM primers.

DNA Template prep

2. Dilute original pUCIDT plasmid 0.1 µg/µL. For this batch prep, 4 µg of plasmid + 40 µL of MilliQ were prepared together.

PCR Setup

3. Place the following into PCR tubes:
    a. 1 µL of Forward Primer (10µM)
    b. 1 µL of Reverse Primer (10µM)
    c. 1 µL of DNA Template (0.1 µg/µM)
    d. 1 µL of Phusion polymerase + 10 µL of 5x HF Buffer + 1 µL of dNTPs (25 mM)
        i. NOTE: For this lab, Part D's components were all in a single mix stored in the -20C fridge.
    e. 35 µL of MilliQ H2O
4. Cycle in PCR:
    a. 98 C: 30 sec
    b. 98 C: 10 sec
    c. 55 C: 15 sec
    d. 72 C: 2 min (1700bp region, 1min/kb)

e. Repeat Steps 2A-2D 29 more times
f. 72 C: 5 min
g. Hold at 4 C overnight (~ 15 hr)

*****PCR products were taken out by Bryan to be stored in -20 degC iGEM Solutions Box

Day 17 Evening: September 3rd, 2015 (Caroline)

SUMMARY:
-purified PCR products by using QIAquick PCR Purification Kit Protocol
http://2012.igem.org/wiki/images/a/a3/QIAquick_PCR-purification.pdf
-measured DNA concentrations with spectrophotometer and Nanodrop
Tube #1: 45.58 ng/uL Tube #2: 46 ng/uL

PROCEDURE:
PCR Purification with a microcentrifuge:
*All centrifuge steps carried out at 13000 rpm

1) Add 5 volumes of Buffer PB to 1 volume of PCR sample and mix.
2) To bind DNA, apply sample to QIAquick column and centrifuge for 60 s.
3) Discard flow-through. Place QIAquick column back into same tube.
4) To wash, add 0.75 mL Buffer PE to the QIAquick column and centrifuge for 60s.
5) Discard flow-through. Place QIAquick column back into same tube.
6) Centrifuge for an additional 1 min. Place QIAquick column in clean 1.5 mL tube.
7) To elute DNA, add 50 uL Buffer EB to center of QIAquick membrane and centrifuge the column for 1 min.

Measuring DNA concentration on spectrophotometer:

1) Get the quartz cuvette from box next to spectrophotometer. BE VERY CAREFUL WITH IT, COSTS LIKE $500. KEEP AWAY FROM TABLE EDGE.
2) Clean the cuvette by rinsing with MilliQ H2O and drying with vacuum.
3) Add 100 uL of water to cuvette using pipette. Wipe black sides of cuvette with kimwipe to help with spectrophotometer reading.
4) On spectrophotometer: nucleic acid, dsDNA concentration. Blank with water sample.
5) Clean cuvette.
6) Make 1:100 dilution of DNA sample and pipet into cuvette. Wipe black sides of cuvette, and measure. Type in the dilution as 100 and record concentration(ng/uL) and absorbance.
7) Clean cuvette.
8) Repeat steps 6 and 7 for any other DNA samples you may have.
9) Put cuvette back into box.

Measuring DNA concentration with Nanodrop.
* Involves going to next-door lab. ASK FOR PERMISSION.

1) Bring all your materials with you (blank solution - MilliQ H2O, pens, pipet tips, pipet, sample)
2) Wipe down Nanodrop machine with a kimwipe wet with MilliQ H2O. Clean the part that measures concentration.
3) Blank with 2 uL MilliQ H2O. Wipe down machine with kimwipe.
4) Pipet 2 uL of sample onto machine. Measure. Record concentration(ng/uL) and absorbance ratio(260/280). Wipe down machine with kimwipe.
5) Repeat step 4 for any other DNA samples you may have.

WHAT TO DO NEXT:

- Gibson Assembly (~50 min.)
- Transformation

Day 18 Evening: September 11th, 2015 (Caroline)

SUMMARY:
-Gibson Assembly
https://www.neb.com/protocols/2012/12/11/gibson-assembly-protocol-e5510
-red pcr tube in -20C on igem rack contains product

PROCEDURE:
Calculations:
*Diluted vector(TCR-Ndel) from 139 ng/uL to 100 ng/uL(separate 1.5 mL tube in -20C)

vector	plasmid	
8000 bp	60 bp	
100 ng/uL	46 ng/uL	
.0192 pmol	1.179 pmol	pmols = (weight in ng) x 1,000 / (base pairs x 650 daltons)
1 uL	.424 uL	

Gibson Assembly:
*SET UP REACTION ON ICE

1. 2-3 Fragment Assembly (0.02-0.5 pmols):
   1uL vector, .43 uL insert, 10 uL Master Mix, 8.57 uL H2O
2. Incubate samples in thermocycler at 50 deg. C for 60 min.
3. Store in -20C

WHAT TO DO NEXT:

- Transformation
- Make Gels

Day 19 Morning: September 14, 2015 (Nathan, Anthony, Abhi)

SUMMARY:

- Stock solution of LB
- Transformed 2 BL21 cells and 1 Dh5-Alpha cell to make 2 plates of transformed BL21, 2 plates of transformed Dh5-Alpha, and 1 plate of BL21 negative control
- Made 12% SDS Page Gels

PROCEDURE:

Stock solution of LB:

1. Add 5 g LB mix to 200 mL of diH2O -> Use the following proportion: 25g LB mix per 1L of diH2O
2. Autoclave 15 mins at ~125deg

Transformation:

1. Thaw competent cells on ice (3 samples of competent BL21 cells and 1 sample of competent Dh5-Alpha)
2. Add 2.5 uL post-Gibson-Assembly plasmid to each sample of e.Coli
3. Incubate in ice for 15 mins
4. Heat shock at 42deg C for 45sec
5. Ice for 2 mins to complete heat shock
6. Add 900uL of LB
7. Let sit for ~40 mins at 37deg C
8. Centrifuge for 2 min. to collect bacteria
9. Vacuum out 800 uL so that ~100 ul is left
10. Pipet to resuspend bacteria
11. Plate 50 - 100 uL on LB+Amp plate
12. Let it grow overnight

SDS Page Gel:

For 10% Separating Gel: (10.11mL) - Enough for 2 gels

ddH2O	4.1mL
30% Acrylamide	3.3mL
1.5M Tris pH **8.8**	2.5mL
10% SDS	0.1mL = 100uL
10% APS	100uL
TEMED	10uL

NOTE: TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

For 4% Stacking Gel: (4.088mL) - Enough for 2 gels

ddH2O	2.93mL

30% Acrylamide	0.53mL
1.0M Tris pH **6.8**	0.5mL
10% SDS	0.04mL
10% APS	80uL
TEMED	8uL

NOTE: Again, TEMED is toxic so only add in fume hood. Add TEMED and APS **last.** APS is necessary for the gel to solidify so only add right before adding solution to plate.

1. Combine all solutions into a 50 mL Falcon tube, making sure to add TEMED and APS last
2. Pipette 10% Separating Gel to about 3/4 of the way up from the casting frames
3. Immediately add Isopropanol to get rid of bubbles
4. Let polymerize for about 15 min
5. Remove Isopropanol with Kim Wipes
6. Add TEMED and APS to 4% Stacking Gel
7. Pipette to the brim of the casting frames
8. Place combs in, making sure to avoid bubbles
9. Let sit for about 15 minutes

Storing gels for SDS:

1. Wrap gels, with the comb still in, in paper towels
2. Moisten the paper towels so as to keep the gels hydrated
3. Wrap gels in Saran wrap, label, store in 4 degC

Setting up Casting Frame and Casting Stand

1. Set up the tall plate and short plate in the casting frame, making sure that the tall plate is in the back
2. Lock plates in place and clip to casting stand
3. Pipette water between plates to make sure that there are no leaks
4. Remove water from the plates if there are no leaks; if there are leaks, reposition the casting plates

WHAT TO DO NEXT:

- Create O/N cultures (2 BL21, 2 Dh5Alpha, 1 negative control, 1 positive control)
- Perform colony PCR

Procedures to follow:

Making saturated cultures

1. Pour 4 mL of LB media into six plastic tubes (with the nonscrew caps). Label as “Control + BL21”, “Control + Dh5Alpha”, “Control -”, “BL21 1”, “BL21 2”, “Dh5Alpha 1”, and “Dh5Alpha 2”
2. Add 4 uL of Spectinomycin (taken from stock of 50 mg/mL concentration, which can be found in the iGEM box in -20 degC) into all tubes except for the controls.

3. For positive controls, only use LB media (no Spectinomycin). Transfer a loop of wild type BL21 and wildtype DH5alpha from glycerol stock (in -80 degC in right-most column)
4. Place the the samples and controls in the 37C shaking incubator.

Colony PCR

- Refer to an online protocol. The only difference between colony PCR and standard PCR is that the template DNA is now a colony and not a plasmid
- Instead of Taq, we are using Phusion, which can be found in the iGEM box in -20 degC

As a note: Run SDS, possibly send for sequencing… bring plates to JCVI for fluorometer testing… Give Caroline SDS gels for Western (make sure that any anti-GFP antibody will work for fast-folding GFP) and then order... Centrifuge cells down to obtain pellets and then resuspend them in LB media to use on varying concentrations of chitin so as to generate a graph of chitin degradation vs chitin concentration (and other variations)

Day 20: September 15th, 2015 (Caroline)

SUMMARY:
-Checked Plates(7) for Colonies -> left them in 37 deg. incubator
-Colony PCR
-labeled 7 plastic tubes (with the nonscrew caps) -> they are on a rack on the bench -> please use these for your saturated cultures so that we don't waste them

PROCEDURE:
Colony PCR:

1. Filled 4 PCR tubes with 37 uL MilliQ H20. Tubes are labeled D1, D2, B1, B2 (D = Dhalpha, B = BL21)
2. Picked 4 colonies, one from each plasmid plate and put them in tubes. Swished around to get bacteria off.
3. Put the 4 tubes in thermocycler -> program exp001 (99 deg. for 5 min) -> kill the bacteria
4. Add the following into the 4 PCR tubes:
    a. 1 µL of Forward Primer (10µM)
    b. 1 µL of Reverse Primer (10µM)
    c. 10 µL of 5x HF Buffer (includes Phusion polymerase)
    d. 1 µL of dNTPs (25 mM)
5. Cycle in PCR (program exp000):
    a. 98 C: 30 sec
    b. 98 C: 10 sec
    c. 55 C: 15 sec
    d. 72 C: 2 min (1700bp region, 1min/kb)
    e. Repeat Steps 2A-2D 29 more times
    f. 72 C: 5 min
    g. Hold at 4 C overnight (~ 15 hr)

*****PCR products will be taken out by T? to be stored in -20 degC iGEM orange PCR tube rack

WHAT TO DO NEXT:

- Run an agarose gel with the PCR products to see if the bacteria have successfully taken up the ligated plasmid
- Create saturated colonies (use the tubes already labeled)

PROPER PIPETTING TECHNIQUES

NEVER:

- Tilt a pipet to anything close to horizontal (Always keep tip pointing downwards)
- Go to second stop UNLESS you're removing everything
- Reuse pipette tips
- Touch tips to possibly unsanitary things.
- Leave the pipette tip container lid open; only open the case to take a pipette tip and close the lid whenever possible
- Let the pipetted fluid reach the end of the actual pipette (beyond the tip)
-

ALWAYS:

- Be careful
- Go to first stop before submerging tip
- Go 'up' slowly to ensure no bubbles and maximize accuracy
- Throw away tips immediately into the right disposal containers (Hazardous, Nonhazardous)

http://www.pipettecalibration.net/pipette-calibration-files/guide-to-pipetting-2.pdf