Author: Simonne Guenette
Entry 123/123: Brief Update 10/15/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 15.10.2019 23:26
updated: 15.10.2019 23:28

Recap of the past two weeks.. Many assembly troubles for sty, but finally have a successful transformation, will be send out for sequencing tomorrow (10/16/19) and should have results by 10/17/19.

PHA plasmid has been sent into production and EM is being performed on samples on 10/18/19.

The two plasmid will be transformed together into already electrocompetent E. coli TG1 cells that already have our PHA plasmid in them tomorrow (10/16/19)

Author: Aarati Pokharel
Entry 122/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 15.10.2019 04:25
updated: 15.10.2019 04:29

To Do	• Transform Golden Gate Assembly Sty • Pha pre-cultures • If sty assebmly from yesterday grew, start per-cultures for dna extraction
Accomplished	• Transformed Golden Gate Assembly Sty • Pha pre-cultures : 2 started by Alec • Sty assebmly plates grew! Started 6 pre-cultures for dna extraction

Author: Simonne Guenette
Entry 121/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 05.10.2019 20:43
updated: 05.10.2019 20:45

To Do	• Electroporate PHA plasmids into TG1 E. coli so they can be cultured for competency
Accomplishment	• Electroporated PHA plasmids 1 and 4 into TG1 Cells • plated on Cam plates to grow overnight

Author: Simonne Guenette
Entry 120/123: 10/2/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 02.10.2019 17:57
updated: 03.10.2019 00:39

To Do	• Re-digest same sty parts with BsaI in the am
	• Run digested parts on a gel made form sea-plaque agarose
	  • extract gel fragments by cutting out from gel and melting
	• Perform a sequential ligation with all sty parts in the follow order and time
	  • amp backbone parts
	  • sty 1
	  • sty 2
	  • sty 3
	  • sty4
	  • all done in 15 minute intervals and then continue reaction at room temperature for 2 more hours (3 hours total of ligation reaction)
	• Transform ligated product into DH5-alpha competent cells
	  • plate on amp plates and let incubate overnight

Accomplishment	• Re-digested sty parts with BsaI for 2 hours • Ran digested parts on sea-plaque gel • then cut out bands and melted for 10 minutes at 65 degrees • Performed sequential ligation with all sty parts as specified above for a total of three hours at room temp • Transformed the ligated product into DH5-alpha competent cells

Author: Kobe Rogers
Entry 119/123: Lab 10/1/2019
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 02.10.2019 01:02
updated: 02.10.2019 01:07

To Do:	• Resuspend primers and add them to the sequencing tube with the DNA. • Ship off the PHA DNA to be sequenced. • Resassemble the sty plasmid + transform

Accomplished:	• Resuspended primers to 5 pmol/uL concentration and added 5 uL of the primers into their respective PCR tubes. These were then added with 10 uL of DNA to the tube and sent off for sequencing.
	• The DNA that was PCR'd was purified yielding concentrations:
	  • sty1: 104 ng/uL
	  • sty2: 71.9 ng/uL
	  • sty3: 209.6 ng/uL
	  • sty4: 133.4 ng/uL
	  • sty5: 95.4 ng/uL
	  • sty6: 92.1 ng/uL
	•

Author: Alec Brewer
created: 30.09.2019 23:15
updated: 30.09.2019 23:19
Entry 118/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

9/30 Prepared PHA
precultures in LB + Chlor from plates 1,2,4,5,6.

Author: Simonne Guenette
Entry 117/123: lab 9/30/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 30.09.2019 17:12
updated: 30.09.2019 17:17

To Do	• Make dilutions of new primers • Prep pha DNA for sequencing and get it sent out • Check the precultures of the potential sty plasmid

Accomplishment	• Hunted down new primers, were in the Psych department for some reason?
	• Found that we were missing extracted DNA from a few colonies, so I made precultures of all of them to extract tomorrow so we have them all to send out for sequencing
	• streaked a plate and made another preculture of the one sty preculture that did grow overnight
	  • will see what these new ones do
	  • Used an old amp plate incase there is something off with the concentration of amp on the new ones

Author: Katie Zhang
Entry 116/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 27.09.2019 17:41
updated: 27.09.2019 17:44

9/26

Accomplished	• extracted DNA from PHA VGEM plasmid • most concentrations above 100 ng/ul • ready for purification

Author: Simonne Guenette
Entry 115/123: lab 9/25/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 25.09.2019 17:04
updated: 25.09.2019 20:50

To Do	• check plates from transformation last night • transform overnight assembly via heat shock • PCR all the sty parts so we have enough, check concentration • order more sequencing primers that are specific to overlaps
Accomplishment	• Plates did not grow, will have to reassess and reassemble after transformation today (if this second transformation is a failure) • Re-transformed the sty assembly that occurred overnight • plated on amp plates

PCR tubes labs

1 - sty1

2 - sty2

3 - sty3

4 - sty4

5 - amp1

6 - amp2

Author: Jermaine Austin
Entry 114/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 24.09.2019 04:13
updated: 24.09.2019 04:15

To do:	- Autoclave Eppendorf Tubes, Glass Beads and Culture Tubes -Make Amp Plates - Preculture PHA Colonies
Accomplished:	- Autoclaved Autoclave Eppendorf Tubes, Glass Beads and Culture Tubes - Made Ampl Plates - Made a stock solution of LB + CamR - Made six precultures of PHA Colonies

Author: Evan Biedermann
Entry 113/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 22.09.2019 17:48
updated: 22.09.2019 17:50

To do:	• Digest Sty DNA with Bsa1 • Make 2 gels
Accomplished:	• Ran the digest • Made 2 gels, one is drying in the hood and the other is in the 4C fridge.

Author: Kobe Rogers
Entry 112/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 22.09.2019 00:28
updated: 22.09.2019 00:30

To do:	• Extract DNA from pieces of gel
Accomplished:	• DNA extracted for sty Part 3 and sty Part 4, revealing concentrations, respectively, of 28.2 ng/ul and 11.5 ng/ul. These will be used for PCR reactions to increase and purify the DNA.

Author: Simonne Guenette
Entry 111/123: Lab 9/21/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 22.09.2019 00:25
updated: 22.09.2019 00:29

To Do	• Dilute Primers for PCR to 10mM (do in two different dilution steps) • PCR all of the sty constructs using the NEB Q5 Master Mix • PCR purify with GenScript mini prep kit

Accomplishment	• Diluted the primers in the IDT tubes to .1nmol/uL
	  • Diluted a second set of the primers in epindorf tubes to 10mM
	• PCRd the sty constructs (25 cycles)
	  • used the NEB Q5 master mix
	• PCR purified the PCR product
	• Nanodrop results after PCR purification are below

Author: Simonne Guenette
Entry 110/123: Lab 09/20/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 22.09.2019 00:23
updated: 22.09.2019 00:25

To Do	• Prepare potential pha assembled constructs for sequencing and get them about before 12pm • Order the rest of the DNA we need more of
Accomplishment	• Prepared 3 of the potential pha constructs for sequencing, each with 5 primers, should get the results back for them on Monday • Ordered more of the pVGEMsty (3 and 4) DNA from Twist

Author: Aarati Pokharel
Entry 109/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated
created: 18.09.2019 02:24
updated: 18.09.2019 02:34

To Do	• Extract pVGEM pha plasmid DNA • Send for sequencing
Accomplished	• Extracted pVGEM pha plasmid DNA • Missed the sequencing time, do it tomorrow!

pVGEM PHA plasmid Nanodrop values

Name	**Concentration (ng/uL)**
1a	43.0
1b	42.0
2	39.6
3	65.1
4	73.8
5	49.0

Author: Kobe Rogers
Entry 108/123: Lab 09/16/2019
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 16.09.2019 23:40
updated: 16.09.2019 23:44

To Do:	• Retransform bacteria onto chloramphenicol plates
Accomplishment:	• Performed heat shock on DH5alpha competent cells for the DNA potentially containing the entire sty plasmid, pha 1 & 2, pha 3 & 4, sty 1 & 2, sty 3 & 4, and sty 5 & 6 respectively. These were 6 separate transformations and each were plated onto two plates

Author: Jermaine Austin
Entry 107/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated
created: 16.09.2019 23:34
updated: 16.09.2019 23:43

To Do:	Make precultures of the plates that succesfully grew from transformation of the day previous (9/16/2019)
Accomplished:	Five precultures where made of the VGEM PHA plasmid

Author: Aarati Pokharel
created: 15.09.2019 21:29
updated: 15.09.2019 23:45
Entry 106/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

GOLDEN GATE ASSEMBLY KEY

pVGEM pha

Name of Part Tube (tube, lid)

pha 1 & pha 2 (camR 1 & camR 2)	green, orange
pha 3 & pha 4	green, green

pVGEM sty

sty1 and sty2	clear, clear
sty 3 and sty 4	clear, orange
sty 5 and sty 6 (amp 1 & amp2)	clear, green

Author: Simonne Guenette
Entry 105/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.09.2019 22:41
updated: 11.09.2019 23:21

To Do	• Performed Golden Gate assembly on DNA that arrived yesterday to try to assembly pVGEMsty and pVGEMpha plasmids • Once DNA has been assembled transform both assembled constructs using DH5-alpha heat shock competent cells and a positive control
Accompishment	• Diluted all DNA samples in 13.33 uL to that each 1uL would be at a concentration of 150ng/uL • Performed Golden Gate Assembly on pVGEMsty and pVGEMpha dan fragments • Transformed pVGEMsty and pVGEMpha into competent DH5-alpha cells using heat shock • Also transformed a camR plasmids as a positive control for transformation

Author: Aarati Pokharel
Entry 104/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.09.2019 22:40
updated: 11.09.2019 22:42

To Do	• Make 200 mL of M9 for iGEM pha plasmid pre-culture. 48 hour culture will be ready on Friday for EM!
Accomplished	• Made 200 mL of M9 for iGEM pha plasmid pre-culture

Author: Shaalini Desai
Entry 103/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.09.2019 16:31
updated: 11.09.2019 16:32

Author: Shaalini Desai
Entry 102/123: 08/31/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.09.2019 16:19
updated: 11.09.2019 16:31

To Do	• Make pre-cultures of iGEM PHA plasmid
Accomplishment	• Make two 200mL pre-cultures of iGEM PHA plasmid

Author: Alec Brewer
Entry 101/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.09.2019 16:15
updated: 11.09.2019 16:16

Author: Simonne Guenette
Entry 100/123: 9/10/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.09.2019 16:06
updated: 11.09.2019 16:11

To Do	• 6am - Switch preculture into fernbok flask and put in shaking incubator to culture for competency, move everything into the cold room • 5 pm- perform electroporation with amp plasmid on cells prepared earlier by Aarati to test for electrocompetency
Accomplishment	• 6am - Moved the precut lure into fernbok and moved over to shaking incubator in PLSB, moved all materials needed for electrocompetency prep into the cold room • 5:30pm - Performed electroporation with amp plasmid on cells prepared earlier • plated onto cam, amp, and cam + amp plates to see which cells could grow, indicating which plasmids are retained.

Author: Aarati Pokharel
Entry 99/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 10.09.2019 23:33
updated: 10.09.2019 23:35

To Do	• Make the iGEM pha plasmid cells electrocompetent (from Simmone's 6AM pre-culture)
Accomplished	• Made the iGEM pha plasmid cells electrocompetent

Author: Simonne Guenette
Entry 98/123: 9/9/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 09.09.2019 19:49
updated: 11.09.2019 16:06

To Do	• Make preculture of cells that were transformed around 1pm so that they can be switched into the larger culture at 6am tomorrow
Accomplishment	• Made the preculture of newly transformed cells at 1:30pm, will come it at 6am tomorrow to switch into the larger culture.

Author: Simonne Guenette
Entry 97/123: 9/7/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 09.09.2019 19:44
updated: 09.09.2019 19:49

To Do	• Talk to Koz and continue with prep for electrocompetency throughout the day • First, run red nile on cultures to see if any indicator that PHBs are present • Determine where to proceed based on both of the results of those
Accomplishment	• Troubleshooted to realize that the reason that transformations were failing was because the transformed cells were being plated onto amp + cam plates, so the only colony that grew had been exposed to amp already, and cells would not be viable to prepare for electrocompetency again • Re-did transformation with iGEM PHA plasmid and TG1 cells and plated on correct, cam only plates

Author: Simonne Guenette
Entry 96/123: Lab 9/6/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 09.09.2019 19:41
updated: 09.09.2019 19:45

To Do	• Check cells to see if transformation works • if it did, begin preculture in the evening to prepare for electrocompetency again
Accomplishment	• Cells did not grow so further troubleshooting is needed • one single colony grew on each plate, make pre-cultures for them anyways and plan to talk to Koz to see if they are viable to use

Author: Simonne Guenette
Entry 95/123: 9/5/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 09.09.2019 19:38
updated: 09.09.2019 19:41

To Do	• Tranform iGEM PHA plasmid into TG1 electrcompetent cells and plate overnight to grow
Accomplisment	• Transformed IGEM PHA plasmid into cells and plated in Koz's incubator to grow overnight

Author: Simonne Guenette
Entry 94/123: 9/4/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 08.09.2019 17:54
updated: 09.09.2019 19:38

To Do	• Look at PHB pellet further, see if a dry pellet can be isolated
Accomplishment	• Spun down ~1ml of bottom PHB pellet from density differential extraction in 1ml epindorf tubes • Took some of the pellet out and put on parafilm in fume hood to dry • Took some of the pellet and dissolved in deuterated chloroform in flask, left to shake overnight to see success is dissolving • added about .4mL of wet weight of PHB to 10mL deuterated chloroform

Author: Simonne Guenette
Entry 93/123: 9/03/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 04.09.2019 00:33
updated: 04.09.2019 02:53

To Do	• Switch PHA pre-culture into M9 • Start new PHA pre-cultures (6 of them) for DNA extraction • Spin down further PHB pellet and try to isolate • Somewhat improvising to see what's going on just try to get PHBs alone

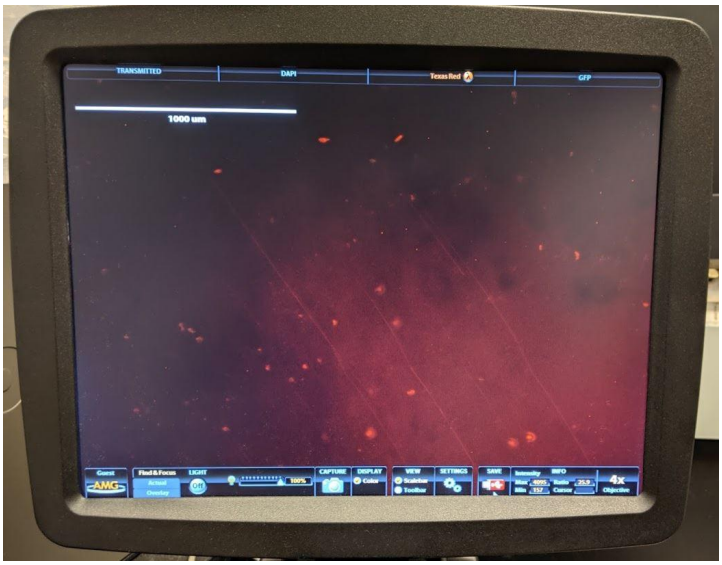
Accomplishment	• Switched pre-culture in M9 ready for 72 hour incubation
	• Started new pre-culture (6) that can be DNA extracted tomorrow
	• Spun down and further extracted PHBs, isolated them into epindorf tubes with minimal liquid in it
	  • extracted top pellet and water and sucrose leaving only the pellet
	  • resuspended in 5 ml of water, and distributed that evenly into 4, 1.5mL epindorf tubes
	  • spun down at 5000 g's for 5 minutes and extracted supernatant

Author: Aarati Pokharel
Entry 92/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

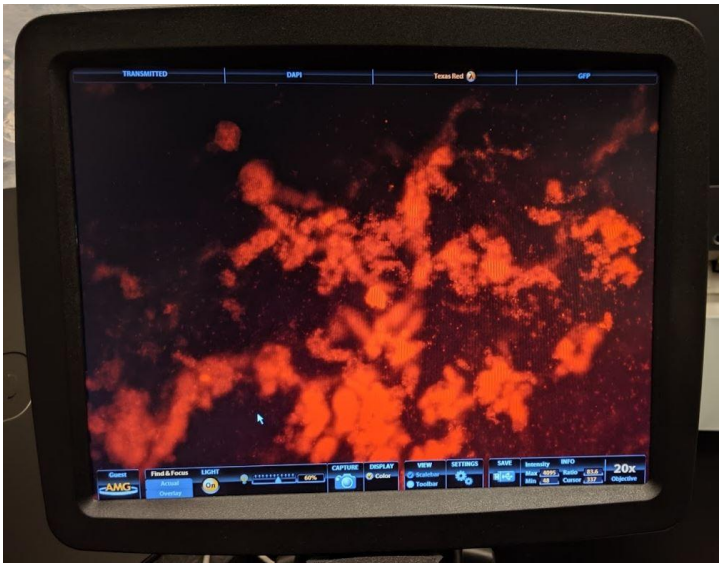
created: 03.09.2019 03:56
updated: 03.09.2019 04:06

To Do	• Red Nile Staining of iGEM pha plasmid culture • Switichover of iGEM pha plasmid from LB pre-culture into 2, 200 mL of M9 Medium
Accomplished	• Red Nile Staining of iGEM pha plasmid culture (control and pha plasmid listed below) • Switichover of iGEM pha plasmid from LB pre-culture into 2, 200 mL of M9 Medium

control_culutre.JPG



iGEM_pha_plasmid.JPG



Author: Simonne Guenette
Entry 91/123: 9/02/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 02.09.2019 22:32
updated: 03.09.2019 16:41

To Do	• Sonicate 72 hour PHA culture in M9 to lyse cells
	  • then add sucrose solution made up earlier today to create a cushion for cell debris to float on and PHB to sink in
	  • Follow procedure in google drive (general steps will be laid out below in notes)
	• Wash all centrifuge tubes

Accomplishment	• Performed sonication, left cell pellet in tube to extract tomorrow • Cleaned all bottles and left them to dry

General Procedure for Sonication PHB extraction (still in the works)

1. Place M9 media and cells in centrifuge bottles and spin at 5000rpm for 20 minutes
2. remove supernatant and resuspend in ~40 mLs of DI water
3. sonicate at 80% for 30 seconds on, 15 seconds off for 5-12 times (depends on the day and cells how long it'll take)
4. spin down sonicated material and remove as much supernatant as possible
5. using a 9' pasture pipette, add sucrose cushion to the bottom of the solution
6. Spin down for 20 minutes at 5000g's (two pellets should form as a result of this)
7. remove supernatant and top pellet, leaving only the smaller pellets (this should be your PHBs)

Author: Kobe Rogers
Entry 90/123: Lab 09/2/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 02.09.2019 18:54
updated: 02.09.2019 18:55

To Do	• Make s
Accomplishment	• Made s

Author: Simonne Guenette
Entry 89/123: 8/30/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 30.08.2019 17:13
updated: 31.08.2019 20:19

To Do	• Make more M9 • Look at the sonication under microscope and troubleshoot further • Meet with EM people at the hospital

Accomplishment	• Made more M9
	• Looked at sanitation debris
	  • Looks like the cell debris did not separate from the PHA material with the sucrose fusion
	  • We will make a more accurate sucrose solution to be 1.20-1.22 g/L so that it works to separate
	  • Also add a larger volume of sucrose to the cushion is larger so that the pellets may separate more

Author: Jainam Modh
Entry 88/123: 8/30
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 30.08.2019 15:45
updated: 30.08.2019 15:48

To Do	• Possibly extract PHA or PHA plasmid
Accomplishments	• Created 2 PHA precultures each from 3 different plates. One was the glycerol stock from 8/28 and the other two were from 8/22.

Author: Evan Biedermann
Entry 87/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 29.08.2019 21:32
updated: 29.08.2019 21:32

Author: Aarati Pokharel
Entry 86/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 29.08.2019 20:21
updated: 29.08.2019 20:21

Author: Kobe Rogers
Entry 85/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 28.08.2019 18:15
updated: 28.08.2019 23:20

To Do	• Filter Ch • Add met
Accomplishment	• Filtered solution • Added th dry • Plan to p • Started 2 • Created

Author: Kobe Rogers
Entry 84/123: Lab 8/27/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 28.08.2019 18:11
updated: 28.08.2019 18:15

To Do	• Make 5x • Move SD overnigh
Accomplishment	• Made 5x • SDS ext • Prepare

Author: Simonne Guenette
Entry 83/123: Lab 8/27/19
In Project: Transfoam (WetLab Notebook)
No tags associated
created: 23.08.2019 15:55
updated: 27.08.2019 19:18

To Do	• Perform DNA extraction on master plasmid, nanodrop, prepare for sequencing, and send off to be sequenced
	• Do PHB extraction of 72 hour with iGEM part BBa_K114905
	  • SDS in the morning and leave to dry for 5 hours
	  • Finish with adding chloroform 5 hours later and shake overnight

Accomplishment	• Performed DNA extraction of master plasmid, nano drop results listed below
	  • M1 - 136.5 ug/uL
	  • M2 - 121.4 ug/uL
	  • M3 - 122.0 ug/uL
	  • M4 - 108.0 ug/uL
	  • M5 - 89.0 ug/uL
	• Did PHB extraction with SDS in the am, left in the drying oven at 60 degrees for 5 hours

Author: Simonne Guenette
Entry 82/123: 8/23/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 23.08.2019 15:35
updated: 23.08.2019 15:53

To Do	• Start pre-cultures of plated BBa_K114905 and mystery plasmid to grow overnight
Accomplishment	• Began both pre-cultures

Author: Simonne Guenette
Entry 81/123: 8/22/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 22.08.2019 15:22
updated: 23.08.2019 15:53

To Do	• Extract DNA from mystery plasmid and BBa_K114905 for measurement and transformation • Re-transform iGEM part BBa_K114905 into competent DH5-alphas to start up growth on PHA again • Plate pre-culture of mystery plasmid to grown overnight to get larger precultures • Extract DNA from most of the pre-culture, but the concentration was not high enough to send out for sequencing to determine identity
Accomplishment	• Extract DNA from most of the pre-culture of the mystery plasmid, but the concentration was not high enough to send out for sequencing to determine identity (~40 ng/uL) • Extracted DNA from BBa_K114905 • Re-trasnformed BBa_K114905 into DH5-alpha and plated to grow overnight

Author: Simonne Guenette
Entry 80/123: Lab 8/21/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 21.08.2019 16:32
updated: 22.08.2019 15:24

Cells did not assembly from last night, there was a single colony that formed on one of the plates

We will pre-culture the single colony that grew overnight and see if it grown on the antibiotics

To Do	• Run gel extraction of the other tube of CamR backbone that we digested yesterday to try to get a higher yield • Perform gibson assembly of pVGEMsty and pVGEMpha on both plasmids and the positive control • Transform all assembled constructs and incubate overnight • Transfer single colony into pre-culture with amp to see if anything grows
Accomplishment	• Ran gel extraction of CamR backbone, got a yield of 4.5 ng /uL, which is slightly higher than yesterdays and good to use for assembly • Performed gibson assembly on all constructs, exact quantities of everything used pictured below • Transformed pVGEMsty and the positive control • pVGEMpha was not transforming properly so we let it be and will troubleshoot further • Transferred the single colony to pre-culture

IMG_0481.jpg

Author: Simonne Guenette
Entry 79/123: Lab 8/20/19
In Project: Transfoam (WetLab Notebook)
No tags associated
created: 20.08.2019 21:14
updated: 21.08.2019 22:37

To Do	• Restriction enzyme digest CamR Plasmid with HindIII-HF and EagI
	  • Do a sequential digest with HindIII first, then EagI
	• Run a gel of restriction enzyme digest
	  • Then use gel extraction kit to extract the smaller band of the digest, to be used for pVGEMpha assembly
	• Transform the assembled pVGEmsty construct from 8/19/19

Accomplishment	• Tried to transform pVGEMsty, but the electroporator was reading arc for every transformation, meaning that it was not completing its circuit correctly
	  • The DNA was found to be the problem, and it is likely that there are excess ions in the NEB High Fidelity Assembly Master Mix, so to work around this, we precipitated our DNA to elute it into DI water to rid the DNA of ions so that transformation might succeed
	  • Transformations worked after we precipitated the DNA
	• Ran restriction enzyme digest for 90 minutes (45 minutes for each enzyme respectively)
	• Made gel for extraction with .8% sea-plaque agarose to improve extraction

Pictured below: Gel run with restriction enzyme digest under UV light (lower band is the one we want for assembly)

IMG_3721.jpg

Author: Kobe Rogers
Entry 78/123: Lab 8/19/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 19.08.2019 15:45
updated: 20.08.2019 19:41

To Do	• Extract DNA of CamR plasmid backbone from overnight culture • Assemble sty plasmid • Transform TG1 competent cells with the sty plasmid and streak on amp plates

Accomplishment	• Extracted DNA of CamR plasmid yielding concentrations:
	  • C6 -
	  • C7 - 89.0 ng/ul
	• Assembled the sty plasmid and a positive control
	• Transformed once with 25 ul of competent cells and 1 ul of diluted DNA
	• The transformed cells were streaked onto 2 ampicillin plates

Author: Kobe Rogers
Entry 77/123: Lab 8/18/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 19.08.2019 15:43
updated: 19.08.2019 22:01

To Do	• Start an O/N pre-culture of the CamR backbone from streaked plate for DNA extraction • Extract amp backbone plasmid from the overnight preculture

Accomplishment	• O/N pre-culture of the CamR backbone was started
	• Amp backbone plasmid was extracted yielding concentrations:
	  • A6 - 47.7 ng/ul
	  • A7 - 29.1 ng/ul

Author: Kobe Rogers
Entry 76/123: Lab 8/17/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 19.08.2019 15:39
updated: 19.08.2019 15:43

To Do	• Start an O/N pre-culture of the ampicillin backbone from the streaked plate for DNA extraction • Streak a Chloramphenicol plate with the glycerol stock of the CamR backbone plasmid
Accomplishment	• Overnight pre-culture was started for the ampicillin backbone • CamR backbone plasmid was streaked onto a CamR plate

Author: Kobe Rogers
Entry 75/123: Lab 8/16/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 19.08.2019 15:29
updated: 19.08.2019 15:39

To Do	• Run a digest of the Amp backbone (addgene) and extract the DNA
Accomplishment	• Ran a restriction digest of the Amp backbone (addgene) with the restriction enzymes EcoRI-HF and AflII • Made a Sea-plaque gel (0.8% agarose) • Gel extracted the larger band (vector) to yield a concentration of 141.8 ng/ul • Streaked an ampicillin plate with the glycerol stock of the ampicillin backbone plasmid

Author: Simonne Guenette
Entry 74/123: Lab 8/16/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 16.08.2019 15:07
updated: 16.08.2019 15:07

Author: Simonne Guenette
Entry 73/123: Lab 8/15/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 15.08.2019 15:10
updated: 15.08.2019 21:11

To Do	• Plate Controls of the TG1 cells the grew with pVGEMsty and pVGEMpha
	  • amp only -> both pVGEMsty and pVGEMpha
	  • cam only -> both pVGEMsty and pVGEMpha
	  • amp + cam -> cells with just pVGEMsty
	  • amp + cam -> cells with just pVGEMpha
	• Pre-culture some of the cells with both plasmids as well
	  • LB with amp and cam to maintain both pre-cultures
	• Send out sequencing for pVGEMpha if the primers arrive in time
	• Extract DNA from pre-cutlures
	• Re-culture both pVGEMsty and pVGEMpha in their respective medium

Accomplishment	• Plated controls of all listed above to double check that both plasmids are being maintained within each cell • Pre-cultured cells with pVGEMsty and pVGEMpha to grow for integration • Sent out pVGEm for sequencing • 5 primers -> 5 samples sent out • Made plates with amp + cam • Extracted DNA from pre-cultures of pVGEMsty and pVGEMpha • Re-culutre cultures of pVGEMsty in styrene with IPTG to induce transcription • Re-culture cultures of pvGEMpha in M9 with IPTG to induce transcription • Made 20, .5mL IPTG stocks of 800 micro molar

IMG_0369.jpg

Author: Simonne Guenette
Entry 72/123: 8/14/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 14.08.2019 15:27
updated: 15.08.2019 21:11

To Do	• Transform the TG1 cells that were made competent again with pVGEMpha so that both plasmids are in each cell
	  • Competency prep worked for the cells with pVGEMsty, but not pVGEMpha, from 8.13.19
	  • Plate on both Kan and Amp + Cam
	• Prepare pVGEmsty for sequencing (drop box for GeneWiz in PLSB 1)
	• Setup cultures of pVGEMsty in styrene
	• Setup cultures of pVGEMpha to produce phas
	• Make pre-cultures of pVGEMsty and pVGEMpha for DNA extraction tomorrow

Accomplishment	•

Pictured below: duly competent cells that contain both pVGEMsty and CamR backbone (from AddGene) that grew on Cam + Amp plates, ensuring both plasmids are maintained within each cell.

IMG_0361.jpg

IMG_0369.png

Author: Simonne Guenette
Entry 71/123: 8/13/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 13.08.2019 23:29
updated: 13.08.2019 23:38

To Do	• Prepare both cultures of TG1 cells with pVGEMsty and pVGEMpha for electrocompetency
	  • Pre-culture transferred to large culture at 6am
	  • Grow until OD is between .5-.7 then prep for competency
	  • Do for each culture
	  • Do competency check with electroporation after prep is finished and cells have been frozen in the -80
	• Make more glycerol stock
	• Prepare precultures of pVGEMsty and pVGEMpha in case competency prep fails so we can redo tomorrow
	• DNA extraction of pVGEMsty and pVGEMpha to prepare samples for sequencing
	• Transfer pre-cutlures of pVGEMpha to M9 to grow to produce PHB

Accomplishment	• Prepared cultures for competency, once OD was ~0.5 for each culture
	  • All "competent" cells are stored in the -80
	  • Transformed ampR backbone into pVGEMpha for competency check
	  • Transformed camR backbone into pVGEMsty for competency check
	• Made 10% glycerol stock
	• Began pre-cutlures of pVGEMpha and pVGEMsty incase competency prep failed, will know in the am tomorrow
	• DNA extraction >80ng/uL for both plasmids so we can prep to send out for sequencing
	• Trasnfered pVGEMpha culture into M9 to culture to produce PHB

Author: Aarati Pokharel
Entry 70/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 13.08.2019 15:23
updated: 13.08.2019 15:23

Author: Simonne Guenette
Entry 69/123: 8/8/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 08.08.2019 15:10
updated: 13.08.2019 15:27

To Do	• Reassemble the pVGEMsty plasmid again, with necessary changes to prevent the ampR backbone from reassembling
	  • Re-do restriction enzyme digest for plasmid backbone, then run gel extraction on the piece that we want for our linearized backbone
	• Prepare pVGEMpha plasmid for sequencing if primers arrive, setup service with GeneWiz
	• Work on styrene integration, setup apparatus in shake incubator and allow to shake for 24 hours
	  • Perform GC on it tomorrow
	• Begin pre-culture of pVGEMpha plasmid and E. coli
	  • Start pre-culture at 6pm for
	• Begin pre-culture of ampR and camR

Accomplishment	• Re-did restriction enzyme digest from plasmid backbone, then ran gel extraction but bands were weak and low yield • Going to re-do tomorrow • Worked on styrene integration, set up aparatus to run Gas Chromatography • Began all precultures

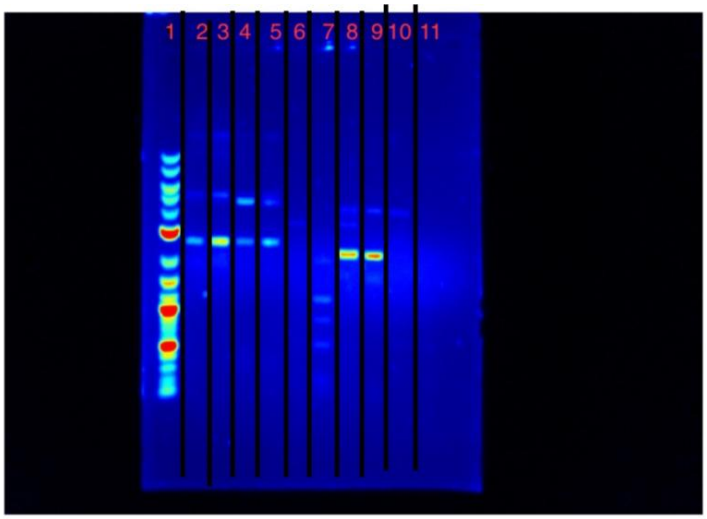
Author: Simonne Guenette
Entry 68/123: 8/7/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 07.08.2019 15:08
updated: 08.08.2019 19:52

To Do	• Run Gel one more time to confirm identity of pVGEMpha and pVGEMsty
	  • Also run with an un-cut plasmid to see if bands show up for this one
	• Continue PHB extraction with chloroform and see if samples can be prepared for NMR
	  • Filter out chloroform from precipitate
	• If Gels confirm that plasmids are what they appear to be, then continue one with sequencing and preparing pre-culture for competency, and if gel does not confirm or deny anything, then re-do assembly and transformation
	• Start pVGEMpha pre-culture to transfer over into M9 tomorrow

Accomplishment	• Ran restriction digest and gel on pVGEMsty and pVGEMpha along with camR and ampR Addgene plasmids to troubleshoot • Results of gel displayed below • Attempted to complete chloroform extraction with sample we had, did not work because of low yield so we scrapped the sample • Planned out action to take from here on out in regards to setting up plasmid and extraction and moving forward with styrene integration

BANDZ.jpg



Pictured above: gel run with enzyme digests from pVGEMsty, pVGEMpha, ampR (Addgene), camR (addGene)

1 - 1kb ladder

2 - pVGEMsty (EagI)

3 - pVGEMsty (MluI + EagI)

4 - ampR (EagI)

5 - ampR (MluI + EagI)

6 - pVGEMpha (MluI + BglII)

7 - pVGEMpha (AluI)

8 - camR (MluI + BglII)

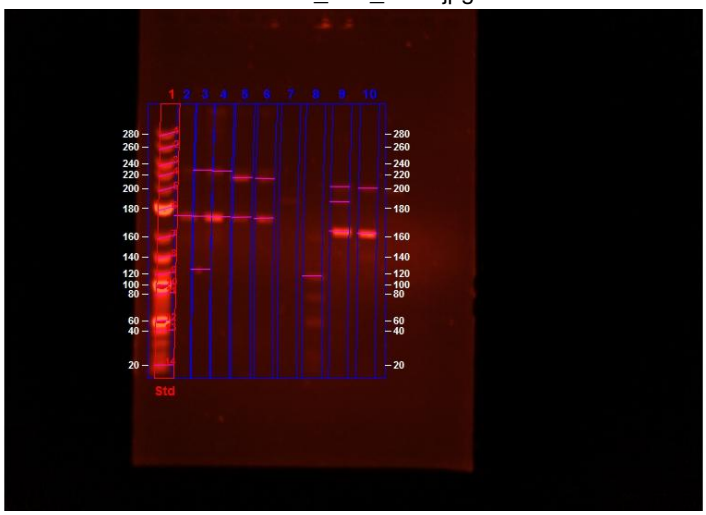
9 - camR (AluI)

10 - pVGEMsty (uncut)

11 - pVGEMpha (uncut)

Gel with more analysis pictured below

BANDZ_with_LINZ.jpg



What we can conclude from the results and how to move forward

Based on the bands from the gel, it looks like the pVGEMpha plasmid has correctly assembled, so we will proceed with preparing those cultures for competency and sequencing

The pVGEMsty plasmid looks like the digest of the ampR backbone, so it looks like the backbone re-annealed and did not assemble to our

Author: Simonne Guenette
Entry 67/123: 8/6/18
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 06.08.2019 02:21
updated: 06.08.2019 21:28

To Do	• Re-run gel with pVGEMsty and pVGEMpha to get thicker bands
	  • Can use extracted DNA from pm of 8/5 to re-do restriction enzyme digest to have higher concentrations
	• Transfer pre-cultures of pVGEMsty and pVGEMpha into fernbok flasks to culture for competency
	  • Check OD of cultures every hour, and more frequently once they reach a close OD
	  • Once these cultures have reached an OD of .5-.7 continue with competency check
	• Prepare cells to be sent for sequencing, order primers if needed (try to confirm of deny identity with gels to some extent first)
	• Extract PHBs from cells culturing with iGEM plasmid
	  • Figure out which methods to use in am

Accomplishment	• Re-ran gel with pVGEMsty and pVGEMpha
	  • Re-did digest with higher conentrations of DNA (100 ng minimum)
	  • Also digested CamR and AmpR Addgene plasmids as a comparison to ensure they are not the plasmids that we are claiming to be pVGEMsty and pVGEMpha
	  • Get results below
	• Extracted DNA from pre-culture of pVGEMsty and pVGEMpha from yesterday because we were not ready to begin preparation for competency until we can confirm the plasmids are their true identity
	• Looked into sequencing, not going to prepare of send out until gel gives us an indication of the plasmid identity
	• Extracted PHBs from cultures using SDS procedure

IMG_0316.jpg

Results from DNA extraction from 8/5/19 in pm (units of ng/uL)

pVGEMsty1 - 47.5

pVGEMsty2 - 40.6

pVGEMsty3 - 56.4

pVGEMsty4 - 67.5

pVGEMsty5 - 41.1

pVGEMpha1 - 49.9

pVGEMpha2 - 41.8

pVGEMpha3 - 64.7

pVGEMpha4 - 40.6

pVGEMpha5 - 44.3

Pictured Below: Setup of the gel that was run and the results of the gel after it was run

Author: Aarati Pokharel
created: 05.08.2019 16:02
updated: 05.08.2019 16:04
Entry 66/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

Author: Aarati Pokharel
created: 05.08.2019 15:59
updated: 05.08.2019 16:02
Entry 65/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

Weekend (8/3 and 8/4): Pre-cultures of both plasmids for glycerol stocks/ DNA extraction

Author: Simonne Guenette
Entry 64/123: 8/5/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 05.08.2019 15:57
updated: 06.08.2019 02:21

To Do	• Run DNA extraction of pVGEMsty and pVGEMpha
	  • Measure DNA concentration on Nanodrop
	  • Prepare samples of both plasmids for sequencing
	  • Run restriction digest on each plasmid and run gel for preliminary confirmation of plasmid identity
	• Start pVGEMpha pre-culture for glycerol stocks
	• Move cell cultures with pVGEMpha from pre-culture into M9
	• Begin extraction of PHAs from cells with BBa_K114905

Accomplishment	• Extracted DNA from pVGEMsty and pVGEMpha
	  • extracted some in am for gel confirmation, and more in the pm from remaining pre-cutlure
	  • Ran restriction enzyme digest and gels to confirm identity of both plasmids
	• Made glycerol stocks of both plasmids
	• Re-made M9 and glucose stocks because they were found to be contaminated
	• Re-plated and Re-precultured pVGEMpha and pVGEMsty

Author: Aarati Pokharel
Entry 63/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 05.08.2019 15:18
updated: 05.08.2019 15:59

Accomplished	• Re-doing PHA restriction digest, assembly, and electroporation transformation • Create 10% glycerol for making electrocompetent cells • Transferring iGEM pha plasmid into M9 medium • Made new M9 Medium

Author: Simonne Guenette
Entry 62/123: 8/2/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 01.08.2019 20:47
updated: 05.08.2019 15:18

Author: Simonne Guenette
Entry 61/123: Lab 8/1/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 01.08.2019 15:22
updated: 05.08.2019 15:15

To Do	• Make plates of Cam + Amp and then plates of Kan for when both plasmids are transformed into
	• Restriction Enzyme digest the AddGene plasmids that were extracted yesterday
	  • For AmpR Plasmid, digest with EcoRI-HF, and AflII
	  • For CamR Plasmid, digest with HindIII, and EagI
	  • Must do this digest sequentially, adding HindIII first and then EagI second with a different buffer
	• Perform Gibson assembly for sty plasmid and pha plasmid
	  • Trying new incubation time of 45 minutes
	• Electroporate TG1 E. coli with assembled plasmids
	  • Plate varying amounts (2-50 uL) once transformed
	  • 4 main transformations will be performed
	  • positive control
	  • sty
	  • pha
	  • sty + pha (plate on both amp + cam, and kan)

Accomplishment	• Made plates • Performed digest • Performed Gibson assembly • Performed electroporation

Pictured Below: Notes and Calculation from experiments today

IMG_0220.jpg

IMG_0221.jpg

Author: Simonne Guenette
Entry 60/123: Lab 7/31/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 30.07.2019 21:10
updated: 01.08.2019 19:16

To Do	• Troubleshoot further what is wrong with assembly /transformation • Re-assemble and re-transform (if restriction enzyme site comes)
Accomplishment	• Talked to Koz to try to determine what is going wrong with assembly • Began design of DNA synthesized constructs for Golden gate assembly • Extracted DNA from AddGene plasmids and checked concentrations in the NanoDrop

Troubleshooting with koz:

- Run Gibson assembly reaction for longer
- Heat block our suspended synthesized DNA at 60 degrees for 10 minutes
- Golden Gate is probably not necessary, but good to have as a backup plan since we can order from Twist for free and need to do so soon

Results from the NanoDrop:

- C1: 41.1 ng/uL
- C2: 41.8 ng/uL
- C3: 73.8 ng/uL
- C4: 42.6 ng/uL

- A1: 38.3 ng/uL
- A2: 48.7 ng/uL
- A3: 124.2 ng/uL
- A4: 77.8 ng/uL

Author: Simonne Guenette
Entry 59/123: Lab 7/30/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 30.07.2019 15:18
updated: 05.08.2019 16:05

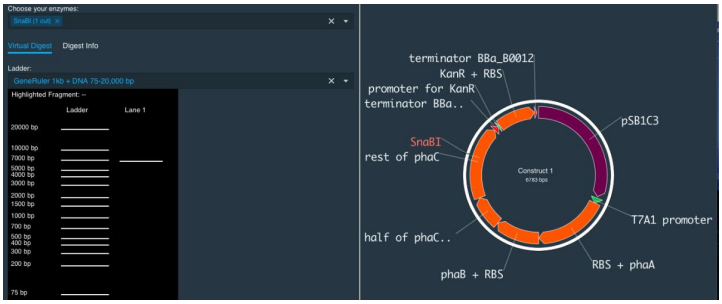
To Do	• Transfer pre-culture of pha (part BBa_K114905) cells into M9
	• Transfer control plasmid (part BBa_B1616) culture into M9 with styrene as well
	• Culture cells with AddGene plasmid backbones so that we can extract DNA tomorrow
	• Trouble shoot why assembly of PHA plasmid did not work
	  ◦ Restriction enzyme digest of plasmid to confirm what it looks like
	  ◦ Talk to Koz and see what's wrong
	• Assembly sty plasmid and reassemble pha plasmid (only if we feel comfortable with our troubleshooting)

Accomplishment	• Transferred Pre-Culture of pha cells and control cells into M9 • Cultured Addgene plasmid cells • Ran restriction enzyme digest to troubleshoot why PHA plasmid assembly did not work • Going to hold off on further assembly until we talk to Koz further to troubleshoot what we may have done wrong and how we can fix it ◦ Also discuss with him how to optimize transformation of two plasmids • Began pre-culture of AddGene plasmid backbones so that they can be extracted tomorrow

Results from the restriction enzyme digest of the plasmid and running the gel

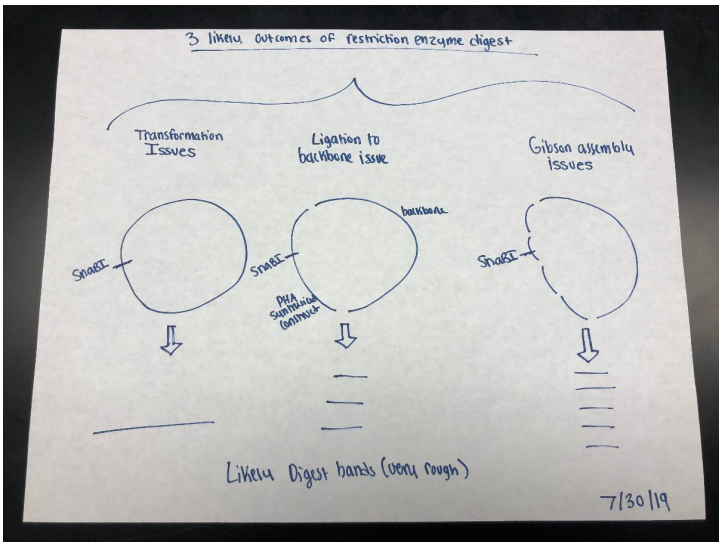
Using Teselagen we simulated a restriction enzyme digest for what our synthesized pha dna would look like clone into iGEM part pSB1C3, and then cut with restriction enzyme SnaBI. A screenshot of the expected results are below.

Screen_Shot_2019-07-30_at_3.21.50_PM.png



By cutting in one place we can try to trouble shoot where in our assembly and transformation we are failing. If the gel runs as expected then the construct should be correctly assembled and our problem is likely in the transformation. If the gel runs and shows 3 distinct medium sized bands then the issue arises form the ligation of the backbone to our synthesized construct, since the SnaBI will be cutting the synthesized construct into two. If there are many smaller bands that appear in the gel then there was no success in the assembly of the dna constructs.

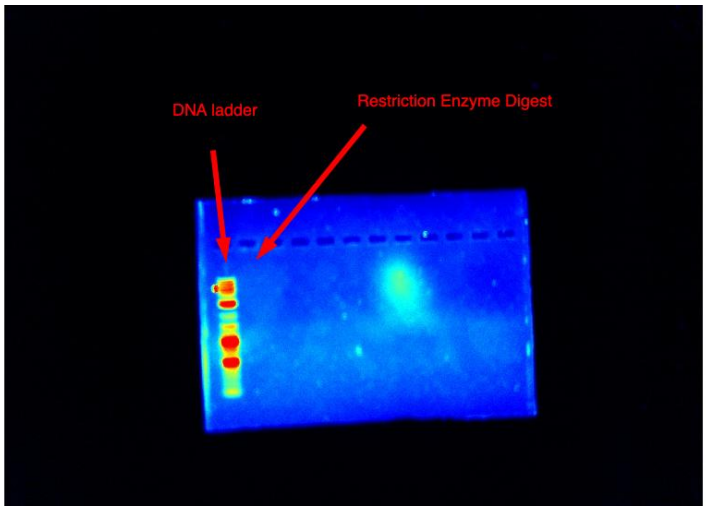
PlasmidTroubleshooting_.jpg



After performing the restriction enzyme digest and running the gel, we imaged the gel and no band appeared in well 2 where our digest was. This thoroughly confused us and we will now have to troubleshoot further :(

The Image of the gel is pictured below.

PHA_plasmid_Test_digestion_colorful.jpg



Author: Simonne Guenette
Entry 58/123: Lab 7/29/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 29.07.2019 15:11
updated: 30.07.2019 18:07

To Do	• Restriction Enzyme Digest of chloramphenicol plasmid from iGEM distribution kit • Gibson Assembly of PHA plasmid • restriction enzyme digest for ligation • BioBrick Assembly for PHA plasmid and pBC13 backbone • Troubleshoot why cultures aren't growing

Accomplishment	• For cultures not growing in Cam (troubleshooting what is going wrong)
	  • Re-made stock anti-biotites for relaxed plasmid
	  • Re-made plates with new stocks
	  • Re-plated cells on new plates
	  • re-started pre-cultures of CamR plasmid cultures with new stocks
	• Restriction enzyme digested pSB31C backbone
	  • Restriction enzyme digest with EcoRI and PstI
	  • Nano Drop to get concentration
	• Gibson assembly on PHA coding sequence
	  • Restriction enzyme digest with EcoRI and PstI
	  • Nanodrop to get DNA concentration
	• Ligate backbone and PHA coding sequence
	• Transform plasmid into electrocompetent TG1 strains of bacteria
	  • positive control with ampR and two pha plates with CamR

Results from nano drop:

- pBC13 = 29.5 ng/uL

- PHA synthesized = 329.5 ng/uL

Ratio of synthesized.... 11 : 1 of PHA : backbone

Author: Simonne Guenette
Entry 57/123: Lab 7/26/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 26.07.2019 15:10
updated: 30.07.2019 20:50

To Do	• Switch pre-cultures of BBa_K1149051 and BBa_1616 from LB to larger culture of M9 • DNA extraction of Amp and Cam plasmid backbones • Gibson Assembly of PHA plasmid • Develop specifics of protocol for styrene integration • Create protocol for PHA extraction with density differentials
Accomplishment	• Created protocol for PHA extraction with density differentials • Got the specific numbers for styrene integration • Began practice styrene integration

Author: Simonne Guenette
Entry 56/123: Lab 7/25/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 25.07.2019 16:38
updated: 26.07.2019 22:03

To Do	• Begin Pre-Cultures of all cell strains with varying amounts of anti-biotics
Accomplishment	• Re-did all pre-cultres for incubation overnight

Author: Simonne Guenette
Entry 55/123: Lab 7/24/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 24.07.2019 15:10
updated: 26.07.2019 15:22

To Do	• Take pre-cultures form BBa_K1149051 and BBa_1616 cells and begin 72 hour culture in M9 (jk none of them grew except for the ampR plasmid) • Make new chloramphenicol stocks to troubleshoot why nothing is growing • Re-start pre-cultures of all Cam cultures • Figure out why none of the chloramphenicol plates are growing.
Accomplishment	• Made pre-culture of all cells • Make new stocks of all antibiotics • Ran practice gel electrophoresis to get an understanding of the process

Author: Simonne Guenette
Entry 54/123: Lab 7/23/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 23.07.2019 15:08
updated: 26.07.2019 15:13

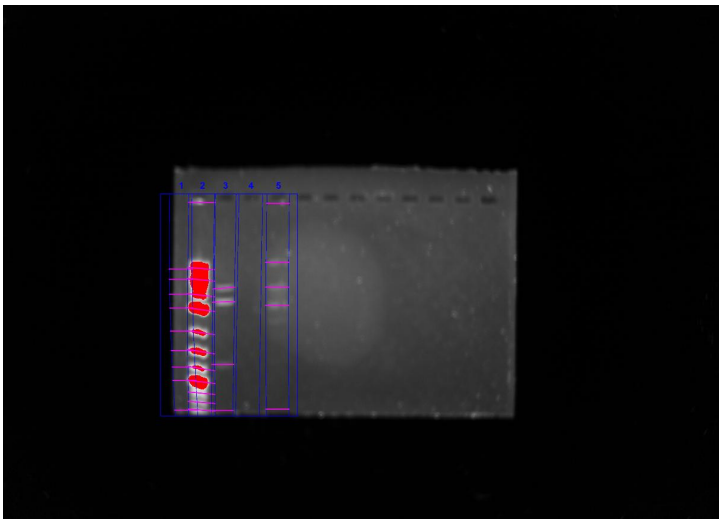
To Do	• Pre-culture all plated bacteria (both stab cultures and BBa_K1149051 and BBa_1616) • culture in LB and chloramphenicol • Setup Styrene integration shaking • Talk to Koz about Gibson Assembly procedure and when we are going to carry everything out • Make plates with gradient of chloramphenicol to test effectiveness
Accomplishment	• Began all precultures • Make plates with a gradient of chloramphenicol to test effect of concentration

Author: Simonne Guenette
Entry 53/123: Lab 7/22/19
In Project: Transfoam (WetLab Notebook)
No tags associated

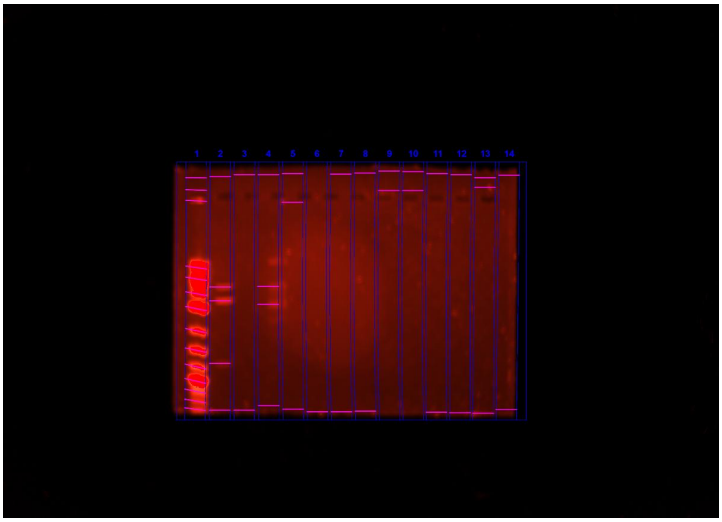
created: 18.07.2019 23:25
updated: 26.07.2019 15:13

To Do	• Gibson Assembly of pha plasmid • Re-make amp and cam plates in am • Re-preculture cells with BBa_K1149051 and BBa_1616 in chloramphenicol • also replace bacterial stab cultures • Go over EM protocol with Koz to see if any changes need to be made • Figure out styrene integration, get back to chemistry • Update chloroform extraction protocol to include details about chemical control • Check on M9 and make more if necessary • Run gel of restriction enzyme digest from 7/19/19
Accomplishment	• Made cam plates • Fixed extraction protocols to include chemical controls • Streaked plates with all bacteria necessary for re-pre-culturing • Ran gel of restriction enzyme digest of Amp plasmid • Updated extraction protocol for chloroform and SDS to include chemical controls

AmpPlasmidCheckAdddGeneGrey.jpg



Bio-Gel_Dock_Center_2019-07-22_11hr_22min.jpg



Pictured Above: Imaging from gel electrophoresis restriction enzyme digest of Amp AddGene plasmid

Author: Simonne Guenette
Entry 52/123: Lab 7/19/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 18.07.2019 15:26
updated: 19.07.2019 15:37

To Do	• Extract DNA of cultures with AddGene plasmids
	  • Restriction Enzyme digest DNA extracted from plasmid backbone cultures
	  • Run gel electrophoresis
	• Meet with Koz to talk about EM protocol to reform it
	• Get everyone acquainted with Gibson Assembly Protocol for Monday

Accomplishment	

Author: Simonne Guenette
Entry 51/123: Lab 7/18/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 17.07.2019 20:58
updated: 18.07.2019 22:31

To Do	• Continue Chloroform and SDS extractions • Attempt to dissolve PHBs in deuterated chloroform • Finish developing EM protocol • Culture overnight the bacterial stab in 3 mL liquid cultures • Replate culture of DH5-alpha with BBa_K1149051
Accomoplishment	• Finished EM protocol, will meet with koz soon so discuss it • re-plated and re-cultured DH5-alpha with BBa_K1149051 • Began overnight culture of bacterial stab again

Author: Simonne Guenette
Entry 50/123: 7/17/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 16.07.2019 20:06
updated: 17.07.2019 21:00

To Do	• Perform DNA extraction of plasmid backbones
	  • Restriction enzyme digest DNA extracted from plasmid backbone cultures
	  • Run gel electrophoresis of digested DNA
	• Continue chloroform and SDS extractions
	• Meet with Chem grad students at 10:30am to discuss gas chromatography
	• Create EM protocol for E. coli with PHA plasmid based on literature and EM protocols from Koz
	• Create glycerol stocks with plasmids from AddGene

Accomplishment	• DNA extraction with GenScript MiniPrep Kit (only the amp plasmid)
	  • one DNA sample was 27.5ng/mL and one was 33.5 ng /mL
	  • Since samples were cultured at 30 degrees and will likely get a higher DNA yield if cultured at 37, so we will start a new culture
	• Ran restriction Enzyme digest on extracted DNA (one sample cut with HaeIII, one cut with ScaI and KpnI)
	  • Digest occurring for 4 hours, will run gel tonight at 6:22pm
	• Developed rough draft for EM, will give to Koz for review to make sure everything looks good
	• Greated glycerol stocks of the plasmids and cells from AddGene, stored in the -80 freezer
	• Met with Chemistry grad students to learn more about gas chromatography, will figure out how we are going to prepare our samples for them in the next few coming days
	• Re-plated stab bacteria with plasmid backbones from AddGene

Author: Simonne Guenette
Entry 49/123: 7/16/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 16.07.2019 15:09
updated: 17.07.2019 15:24

To Do	• Begin SDS extraction with 72 hour culture at 10:30 • Check on chloroform extraction and continue it PHB looks completely dissolved • Transfer plasmid backbone cultures to liquid culture overnight
Accomplishment	• SDS extraction with 72 hour culture • Transfered plasmid cultures to liquid culture to incubate overnight • Began Pre-culture of DH5-alpha cells (pha plasmid and control)

Author: Simonne Guenette
Entry 48/123: 7/15/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 15.07.2019 15:16
updated: 30.07.2019 21:08

To Do	• Perform SDS extraction with 72 hour culture at 12:00pm • Begin chloroform extraction on cells that just completed SDS • Talk to Koz to work through plasmid extraction and confirmation protocol for AddGene plasmids • Make and pour more amp plates • Begin culture of stab bacteria
Accomplishment	• Performed SDS extraction • began chloroform extraction following completion of SDS extraction • made more amp plates • Began plate culture of stab bacteria (amp and cam backbones)

Author: Simonne Guenette
Entry 47/123: Lab 7/13/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 15.07.2019 15:12
updated: 15.07.2019 15:16

To Do	• Transfer pre-cultures form LB to M9
Accomplishment	• Transfered pre-cultures form LB to M9

Author: Simonne Guenette
Entry 46/123: Lab 7/12/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 11.07.2019 21:51
updated: 26.07.2019 15:13

To Do	• Begin another pre-sulture of DH5-alpha, one with BBa_1616, one with BBa_K1149051 • Perform SDS extraction on culture from Tuesday during the mid day • If dried during the day, begin chloroform extraction • If chloroform filters arrive, finish chloroform extraction from 7/10/19 • In any free time, everyone acquaint themselves well with gas chromatography • Work on rolling tasks, finish data management plan
Accomplishment	• Began pre-culture of both DH5-alpha strains • Performed SDS extraction on cells cultured on Tuesday • Finished data management plan for wet lab

Author: Simonne Guenette
Entry 45/123: Lab 7/11/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 10.07.2019 21:52
updated: 11.07.2019 21:58

To Do	• Begin pre-culture of DH5-alpha, one with BBa_K1149051, one with BBa_I616
Accomplishment	• Began pre-cultures • Worked on developing gas chromatography protocol with Professor Gunnoe and Earl • Begun Data Management Layout and Plan for wetlab

Author: Simonne Guenette
Entry 44/123: Lab 7/10/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 09.07.2019 21:16
updated: 11.07.2019 19:42

To Do	• Continue SDS extraction, collect PHB from solution • perform chloroform extraction on top of SDS to purify PHBs • Determine what filters should ordered for chloroform extraction • Perform another transformation into DH5-alpha for control PHB cells	
Accomplishment	• Finished SDS extraction, began with chloroform extraction and purification, will collect in 48 hours • Ordered filter for chloroform filtration system • Transformed BBa_I616 in DH5-alpha cells again	

Author: Simonne Guenette
Entry 43/123: Lab 7/9/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 08.07.2019 16:26
updated: 11.07.2019 21:54

To Do	• Prepare PHB standard and experimental sample for NMR • Transform DH5-alpha cells with empty chloramphenicol plasmid to use to grow as a control for out PHB plasmid cells • Begin culture of this strain • Order primers for Gibson • Perform SDS extraction with new cell cultures
Accomplishment	• Ordered primers for gibson assembly of plasmids • Tranformed BBa_I616 into competent DH5-alpha to grow as a control alongside of the BBa_K1149051 for cell comparison • Performed SDS extraction, solution left to evaporate overnight

Author: Simonne Guenette
Entry 42/123: Lab 7/8/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 08.07.2019 16:00
updated: 30.07.2019 21:08

To Do	• Determine protocol for NMR for 7/9/19 • PHB solubility in Chloroform • Begin new pre culture in am • Meet with Koz to discuss protocols/project updates /improvements that can be made
Accomplishment	• Figured out kinks in Extraction protocols, improvements to be made in this weeks extractions • Preculture began

Notes from meeting with Koz:

- Core facility at the Med school, we can look at the cells under an EM microscope. More cost effective if you do some of it on your own. process the samples in our lab and then just hand it to them to analyze. Have them microtone the block to cut the sections once they are in resins. they na do this really quickly and prepare the grids, then ask for a refresher to show you how to run it. Koz can help with processing the samples so they are ready.
- Look at cells with PHB - and PHB + under the microscope to check cultures. PHB - needs to be a strain with an empty plasmid but with resistance of chloramphicol
- Could do a double chloroform extraction, do calculations based on the yield of how much chloroform we actually extract
- Filtration system could be improved, look into one that can work with chloroform 0.2 micro filter -> millipore
- 75 degrees should be fine for SDS extraction, doesn't need to come to a boil, do that for 10 minutes.
- SDS in conjunction with chloroform, look into how the would work together to dissolve both of them
  - Minimum of three washes to get rid of SDS
- SDS is really variable, make sure we have enough for the experiment. See what kind they use in the literature. Write everything down on the label, including the lot number, percentage of purity
- Dont transfer from plate to culture to culture more than twice

Author: Simonne Guenette
Entry 41/123: Lab 7/6/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 08.07.2019 15:57
updated: 08.07.2019 16:26

To Do	• Transfer Pre-culture from 7/5/19 to 200 mL culture in M9
Accomplishment	• Transferred Pre-culture from 7/5/19 to 200 mL culture in M9 (will culture for 72 hours)

Author: Simonne Guenette
Entry 40/123: Lab 7/5/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 08.07.2019 15:17
updated: 08.07.2019 15:59

To Do	• Pre-culture new strain of DH5-alpha in LB
Accomplishment	• Pre-culture new strain of DH5-alpha in LB (12:00pm)

Author: Simonne Guenette
Entry 39/123: Lab 7/4/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 04.07.2019 15:15
updated: 08.07.2019 15:17

To Do	• Perform SDS extraction • Finish Chloroform extraction • Submit Plasmid Order • Send NMR info to Jeff Ellena
Accomplishment	• Began SDS extraction • Finished chloroform extraction • Plasmid Order submitted

SDS extraction notes:

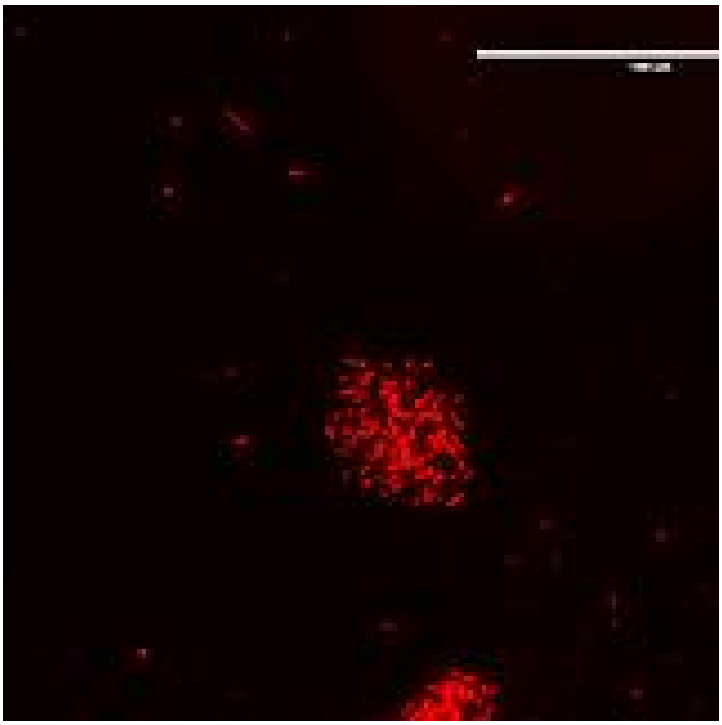
- 12.32 mg SDS added to 200mL culture, cell dry weight of 17.6 mg
- 0.3 g SDS added to 200 mL culture cell dry weight

Author: Simonne Guenette
Entry 38/123: Lab 7/3/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 03.07.2019 15:04
updated: 04.07.2019 15:16

To Do	• Complete plasmid design and put order into IDT/Twist • Continue chloroform extraction
Accomplishment	• Chloroform extraction, let dry to extract dry weight of PHB

phb_s1_07-03-2019_160.png



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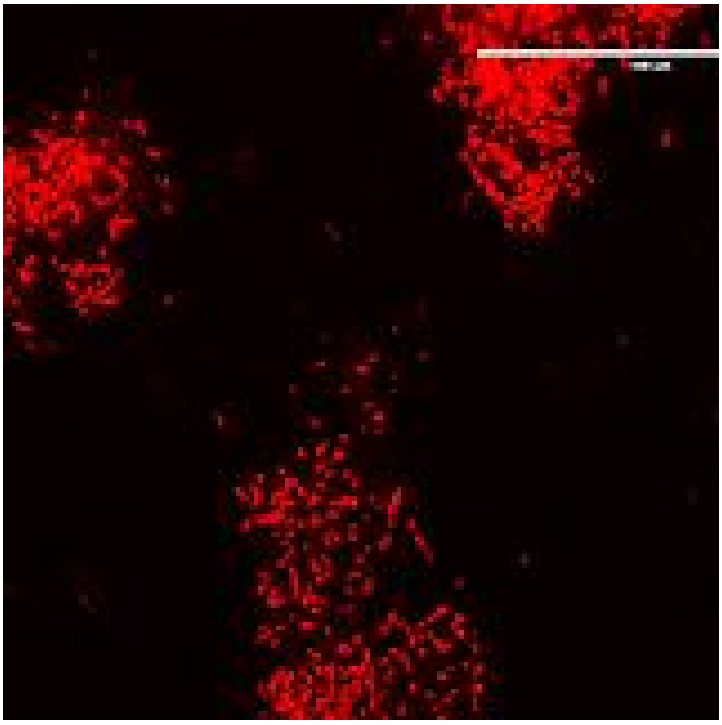

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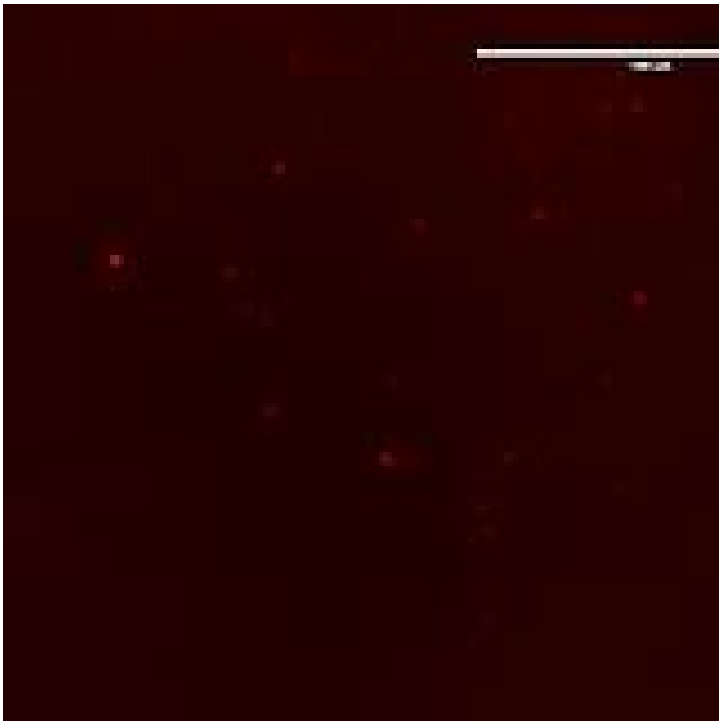


Author: Simonne Guenette
Entry 37/123: Lab 7/2/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 01.07.2019 21:56
updated: 03.07.2019 15:15

To Do	• Finish Chloroform extraction • Meet at 10am to discuss NMR with Jeff Ellena • Finish Plasmid and Submit by the end of the day • Perform Red Nile tests
Accomplishment	• Finished chloroform extraction • Red Nile Preliminary tests

Lab Notes: cell dry weight to figure out protocol for SDS extraction method

- cell dry weight : 17.6 mg -> will use to determine amount of SDS added to standard solutions

SDS protocol (subject to changes)

- Acquire 20ml of culture broth and note the concentration
- Add SDS to liquid culture in a weight by weight ratio (range from 0.1-0.7)
- Let the culture/SDS solution sit for one hour (react for one hour)
- Heat treat the solution at 121 degrees Celsius for 15 min
- Centrifuge at 13,000g for 10 minutes
- Harvest solid sample and was P3HB with distilled water
- Dry recovered P(3HB) at 60 degrees celsius for 5 hours.

Author: Simonne Guenette
Entry 36/123: Lab 7/1/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 01.07.2019 15:39
updated: 02.07.2019 15:02

To Do	• Make Kanamycin plates • Pre culture into M9 • Take out most recently lyophilized cells • Finish plasmid design and call George to discuss • Perform Chloroform extraction with lyophilized cells
Accomplishment	• Began culture of cells in M9 for 72 hours • Began another Chloroform extraction protocol • Talked to George about plasmid design and ordering in parts

Author: Aarati Pokharel
Entry 35/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 01.07.2019 15:21
updated: 01.07.2019 15:21

Author: Aarati Pokharel
Entry 34/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 28.06.2019 15:14
updated: 01.07.2019 15:21

To Do	- Learn how to use the red nile dye and write up protocol - Take out culture from freeze drier - Talk to Gunnoe about responding to our E-mail - Finialize SDS protocol
What We Did	- Made Nile Red Protocol - Plated a DH5 Alpha Culture for a control - Made 1000x Kanamycin Stock - Meet with McArthur to discuss plasmid design - Made Kanamycin Plates

Author: Aarati Pokharel
Entry 33/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 27.06.2019 15:15
updated: 28.06.2019 15:14

To Do	-Tranferr LB pre-culture to M9 medium -Freeze-Dry culture in M9 -Styrene came in! design a couple apparatuses/brainstorm ideas -Are we doing SDS??
What We Did	-Moved LB pre-culture to M9 medium -Freeze dried culture for extraction, learned how to use lyopholizer -Red Nile Came In!

Author: Aarati Pokharel
Entry 32/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 26.06.2019 15:06
updated: 27.06.2019 15:40

To Do	• Check Plate, start 24 LB preculture • Figure out all the information for Gas Chromatography and email Professor Gunnoe • Liquid Nitrogen location • Custom RBS sites for iGem PHA plasmid
What We Did	• Emailed Professor Gunnoe about Gas Chromotography • Custom RBS sites for iGem PHA plasmid: DONE • LB preculture started at 11:25 AM

Author: Simonne Guenette
Entry 31/123: Lab 6/25/19
In Project: Transfoam (WetLab Notebook)
No tags associated

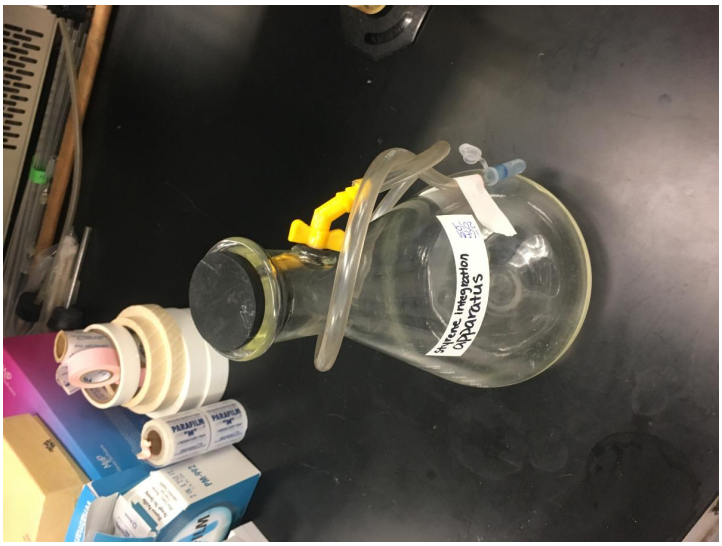
created: 25.06.2019 15:17
updated: 25.06.2019 20:10

To Do	• Finish basic styrene apparatus

Accomplishment	• Designed and setup preliminary styrene integration apparatus
	  • this apparatus will utilize vapor pressure to integrate styrene into the growth media
	  • testing with ethanol to determine how well it generally works

IMG_6510.JPG

IMG_3402.JPG



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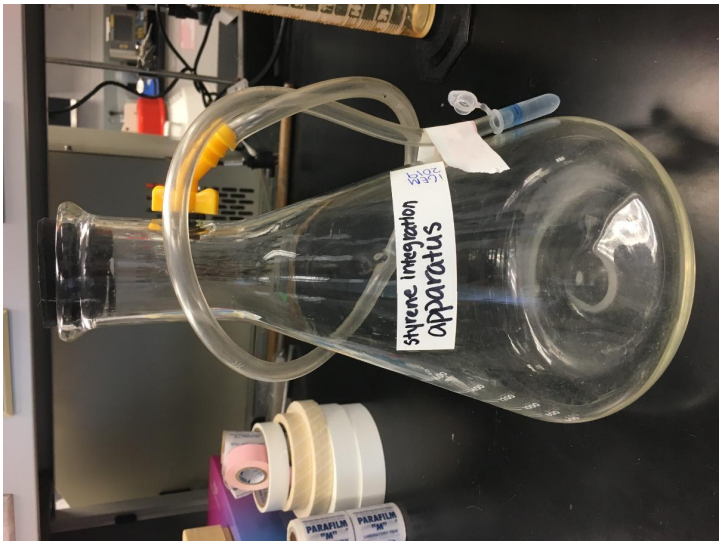


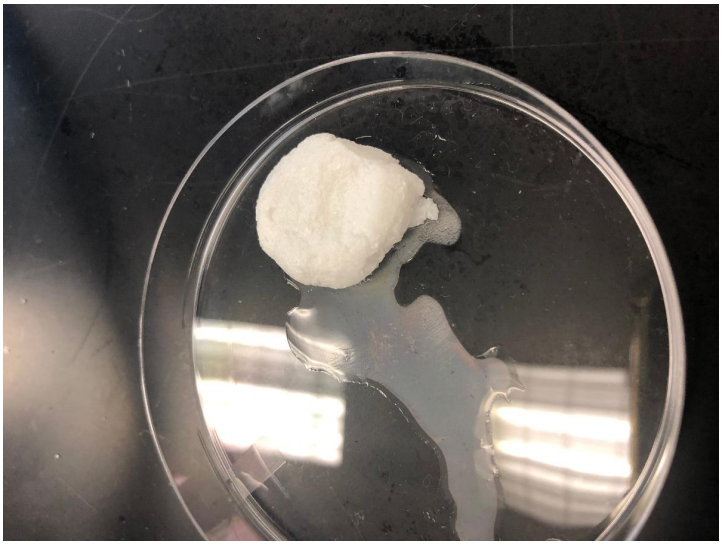
Pictured Above: Preliminary Styrene apparatus

Author: Simonne Guenette
Entry 30/123: Lab 6/24/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 24.06.2019 15:13
updated: 25.06.2019 19:56

To Do	• Complete chloroform extraction procedure • Begin new culture of cells in M9 media (72 hours)
Accomplishment	• Chloroform extraction completed, PHB granules left to dry overnight • Began new culture of cells in M9

IMG_3553.jpeg



Pictured above: PHA lump extracted from cultures using the chloroform protocol

Author: Simonne Guenette
created: 21.06.2019 21:06
updated: 25.06.2019 15:16
Entry 29/123: Lab Weekly Goals: 6/24-30
In Project: Transfoam (WetLab Notebook)
No tags associated

Goals for the week 6/24-6/28

- Continue PHA extraction and determine the exact protocol to be used for entire protocol (needs to be done by the end of the week)
- Once styrene is received begin styrene integration apparatus design and protocol (get close to having that done by the end of the week)
- Order plasmid in beginning of the week (by Tuesday)

Author: Simonne Guenette
Entry 28/123: 6/22/19 Lab
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 21.06.2019 17:42
updated: 25.06.2019 15:16

To Do	• Come in and check OD fo cells culturing at 12:10 • Begin Chloroform extraction
Accomplishment	• Setup Chlorform extraction procedure

Author: Simonne Guenette
Entry 27/123: Lab 6/21/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 21.06.2019 15:22
updated: 23.06.2019 19:19

To Do	• Finish Description page writing, talk to Ben about layout • Chloroform extraction continued • Finish Plasmid design, send to George for review, have lab review • Use teselegen to create primers and determine assembly setup • Take OD of cell culture at 12:10
Accomplishment	• Finished description page • Took OD for cultures • Continued plasmid design (get in contact with george over the weekend to finalize last things)

Cell Culture OD (taken at 1:30) -- 0.265 & 0.715 are the ODs of the two cultures (24 hours)

Cell Culture OD (taken at 1:30) -- 1.929, 1.8884 (48 hours)

Author: Simonne Guenette
Entry 26/123: Lab 6/20/19
In Project: Transfoam (WetLab Notebook)
No tags associated
created: 20.06.2019 15:25
updated: 21.06.2019 17:44

To Do	• Continue culture of cells on M9 and PHA extraction techniques • Plasmid Design on Teselagen
Accomplishment	• Started 72 hour culture of DH5-alpha and BBa_K1149051 for revision to PHA culture protocol in M9 media (12:10 pm) • Performed another trial of chloroform extraction technique

Lab Notes:

- Decided to use a T7A1 promoter system for our plasmid design for both plasmids
- Going to test out hypochlorite PHA extraction method as well, ordered necessary chemicals

Author: Simonne Guenette
Entry 25/123: Lab 6/19/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 18.06.2019 15:45
updated: 20.06.2019 15:27

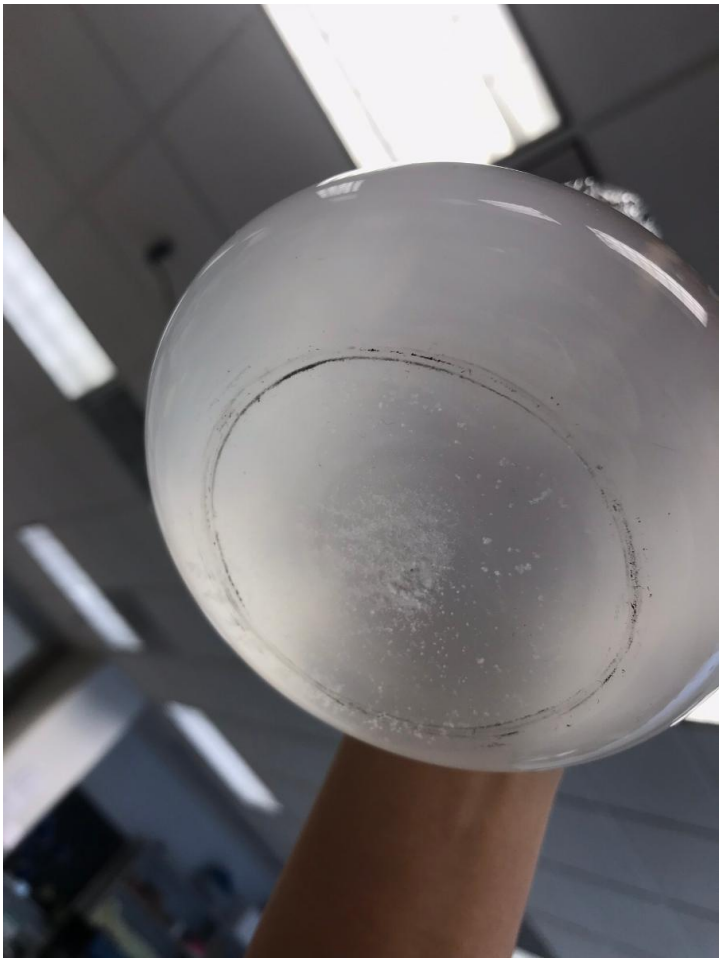
To Do	• Email Berger about styrene integration
	• PHA extraction and quantification procedures
	  • 24 hour preculture and 24 hour culture before extraction
	  • grown in 200 mL of M9 media with chloramphenicol
	  • OD of cultures at extraction 0.215

Accomplishment	• Continued PHA extraction technique • Worked on plasmid design with Teselagen • Talked to George McArthur about use of Teselegen and De Novo

Lab Notes from today:

- PHAs form outside of the cell without need for extraction from culture, did they kill the cells and lyse them?
- PHAs are not separating form the cell way, how can we optimize this?

IMG_0049.JPG



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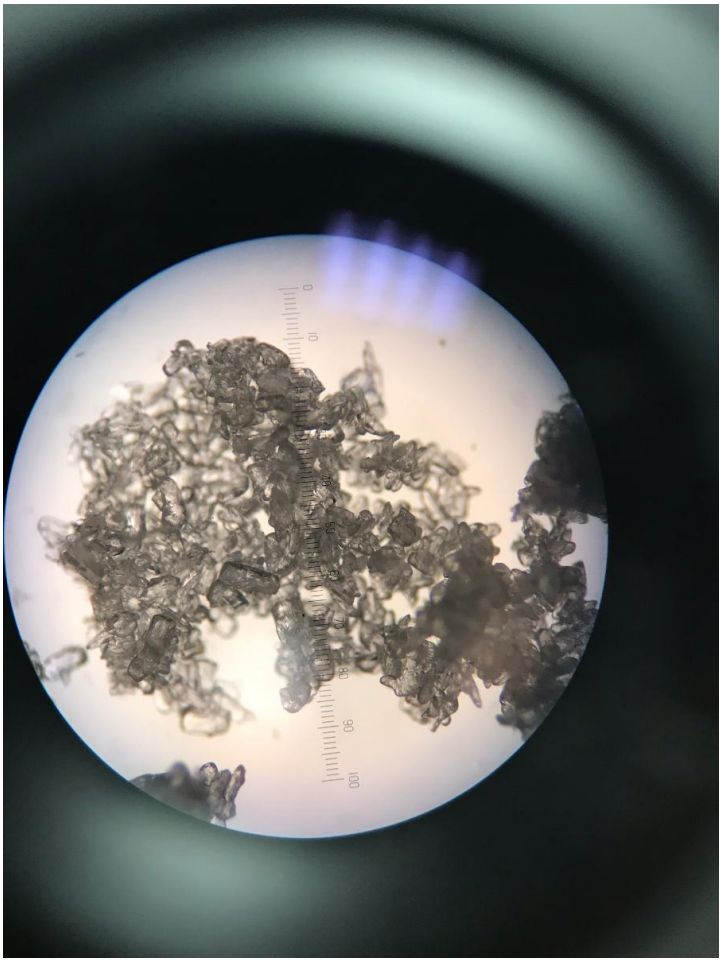

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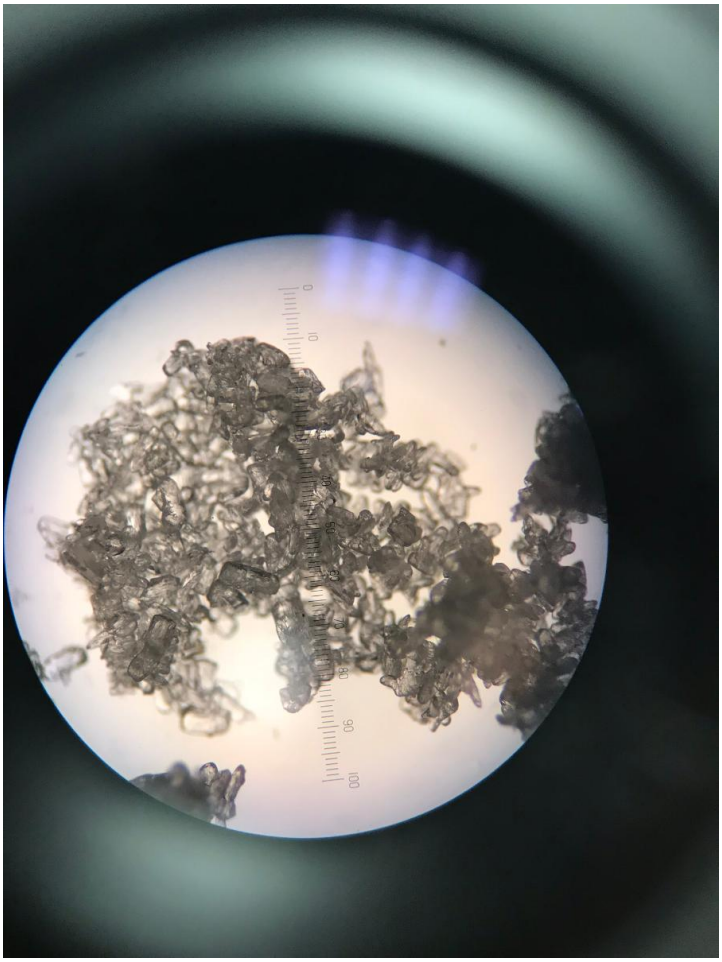

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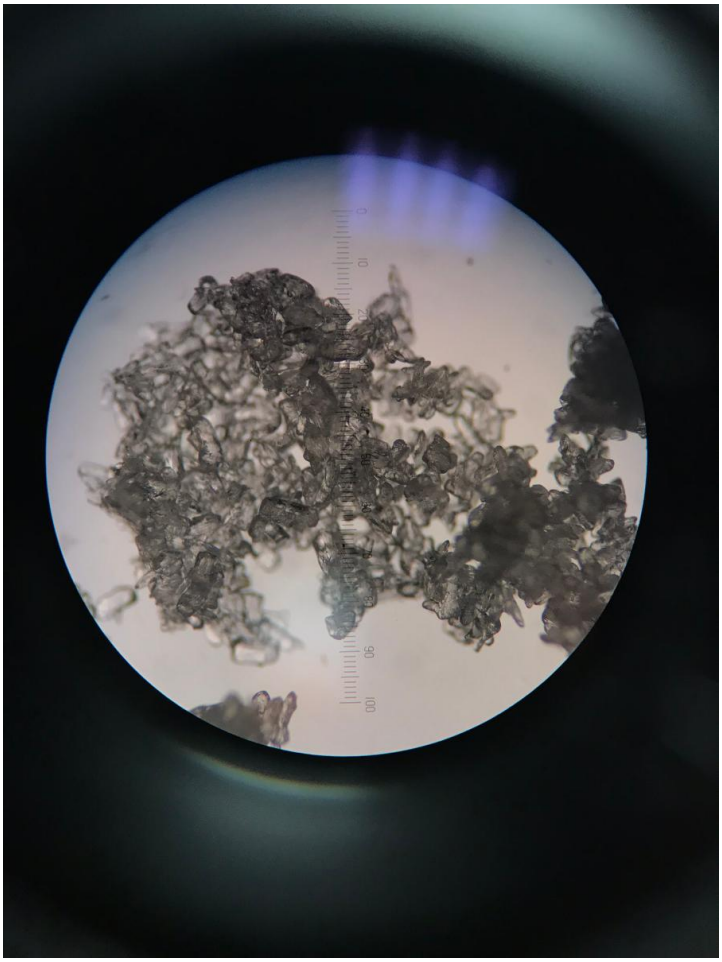

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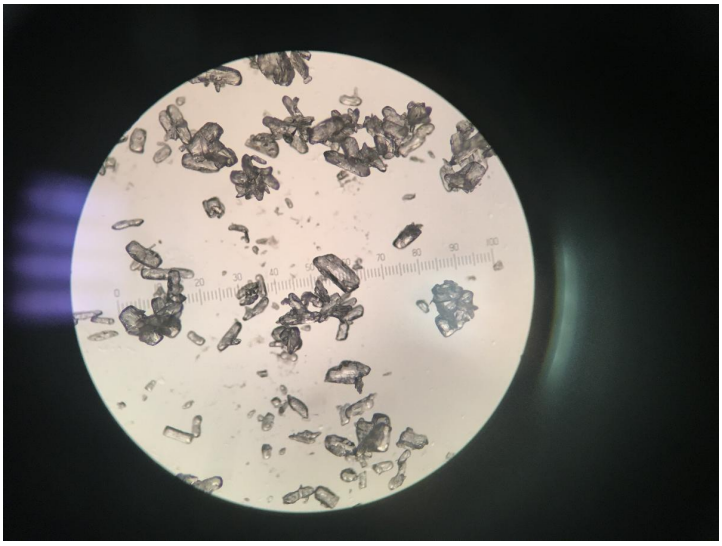


Author: Simonne Guenette
Entry 24/123: Lab 6/18/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 17.06.2019 15:21
updated: 09.07.2019 21:14

To Do	• Make LB plates + chloramphenicol • Chloroform extraction procedure • Innoculate (2) 200 mL culture of M9 + .2 mL of 1000x chloramphenicol • Streak Chloramphenicol plate with E. coli DH5-alpha and BBa_K1149051 • Call George at 11:30 to discuss plasmid/RBS design
Accomplishment	• made plates • Both extraction methods performed, little success seen • Innoculated another culture for 24 hours of E. coli DH5-alpha and BBa_K1149051 for PHA production

Author: Simonne Guenette
Entry 23/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 16.06.2019 16:33
updated: 17.06.2019 15:21

Lab Goals: Week 6/17-6/22

- Determine protocol for PHA cells growth
- Choose optimal PHA extraction protocol
- Obtain styrene and practice partitioning and distillation
- Design RBS sites for plasmids
- Order plasmids and primers

Author: Simonne Guenette
Entry 22/123: Lab 6/16/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 16.06.2019 16:28
updated: 16.06.2019 16:34

To Do	• Extract PHAs from DH5-alpha with BBa_K1149051 using the following methods below
Accomplishment	

PHB recovery by using chloroform. https://aem.asm.org/content/aem/65/6/2762.full.pdf

- Cells were collected by centrifugation at 4,000 x g for 20 min at 25°C and were washed with hot acetone for 20 min.
- After being dried, the cells were mixed with 50 volumes of chloroform for 48 h at 30°C. A clear PHB solution was recovered by centrifugation; this was followed by polishing filtration.
- Finally, pure PHB was obtained by non-solvent precipitation (five times the volume of chloroform) and filtration. Methanol in water (7:3)

PHB recovery using NaOH digestion

- Wash cell broth with distilled water and centrifuge to pellet cells (~5000 rpm for 10 min)
- Resuspend cells in distilled water, and add 0.1 M NaOH solution and allow solution to digest at 30 degrees celsius for 5 h for maximum purity and lowest endotoxins
- Separate PHB granules from the aqueous fraction containing cell debris by centrifugation at 2,500 × g for 20 min.
- Gently rinse the PHB granules with distilled water to recover, recentrifuge, and air dry.

PHB recovery using sodium hypochlorite

- Sodium hypochlorite solution was diluted with distilled water.
- The hypochlorite concentration was 30% (v/v).
- After mixing PHB-containing biomass with the hypochlorite solution, PHB was separated from the aqueous fraction rinsed with water, centrifuged again, and then rinsed with acetone.
- The biomass concentration in the suspension was 4% (w/v) and the treatment time was 150 min at 30°C.

Author: Simonne Guenette
Entry 21/123: 6/14/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 14.06.2019 16:02
updated: 16.06.2019 16:28

To Do	• Culture DH5-alpha with BBa_K1149051 in M9 media for 48 hours • Prepare competent cells of XL 1-Blue once OD of 0.12 is reached
Accomplishment	• Cultured DH5-alpha with BBa_K1149051

Author: Simonne Guenette
Entry 20/123: Lab 6/13/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 13.06.2019 17:18
updated: 21.06.2019 15:22

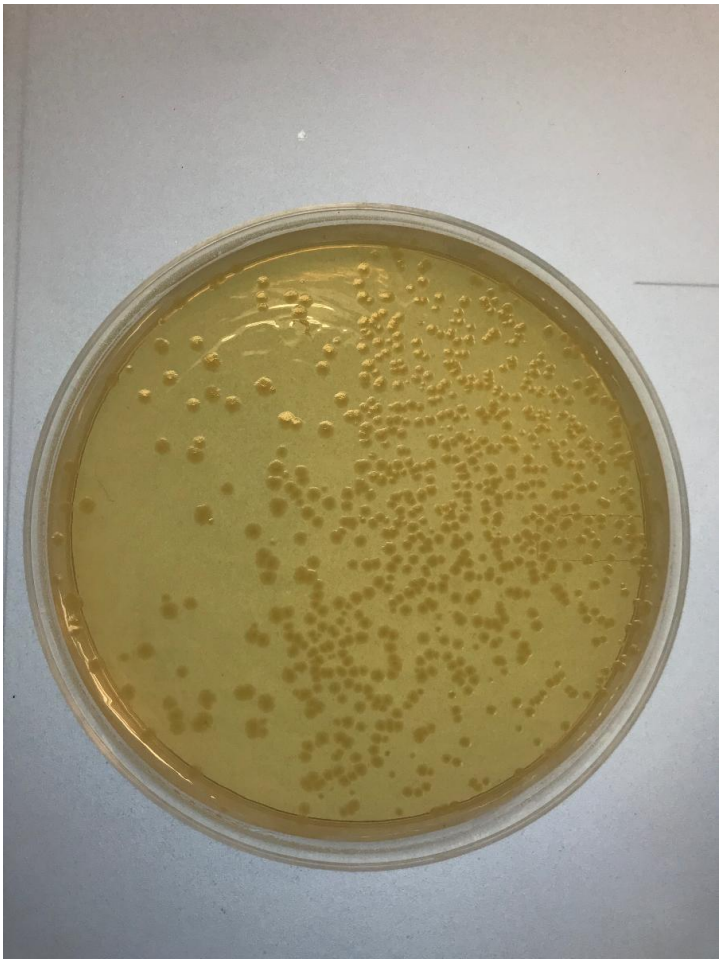
To Do	• Begin Pre-culture of DH5-alpha with BBa_K1149051 • Prepare transformation buffer and supplies for XL 1-Blue cells
Accomplishment	• Began Pre-culture of DH5-alpha with BBa_K1149051 • Prepared transformation buffer and supplies for XL 1-Blue cells

Author: Simonne Guenette
Entry 19/123: Lab 6/12/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 12.06.2019 15:15
updated: 16.06.2019 16:36

To Do	• Tranform BBa_K1149051 into competent DH5-alpha cells • Check Optical Density of XL 1-Blue strains
Accomplishment	• Performed transformation of BBa_K1149051 into DH5-alpha

IMG_2886.jpeg



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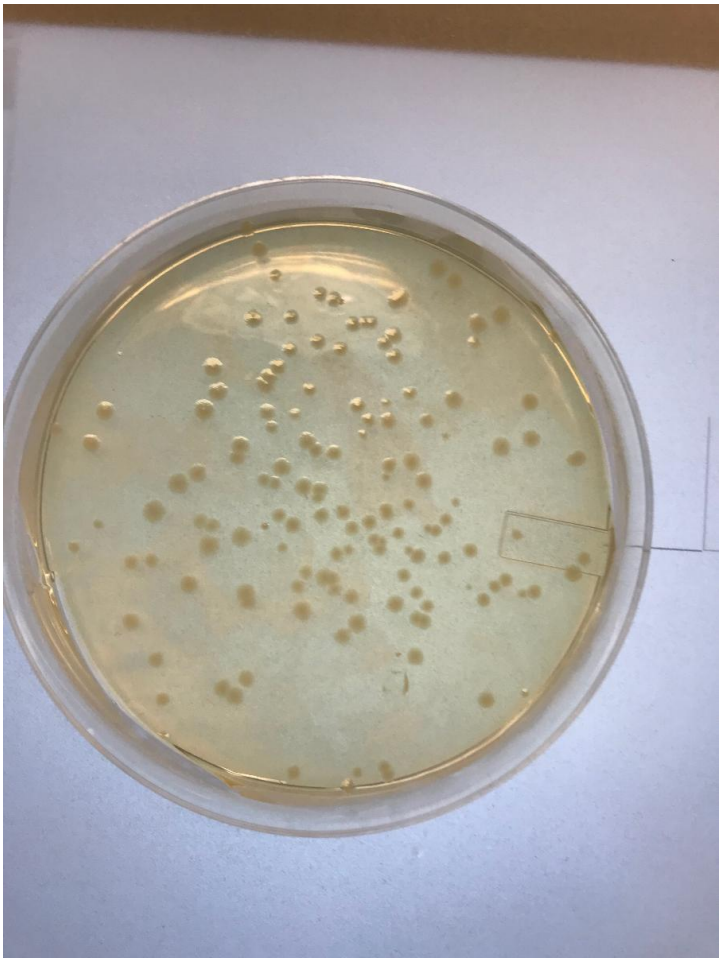

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Pictured Above: DH5-alpha cells transformed with BBa_K1149051

Author: Simonne Guenette
Entry 18/123: 6/6/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 05.06.2019 21:48
updated: 12.06.2019 15:15

To Do	• Start pre-culture of XL1-blue E. coli strain • Re-perform the competency check for DH5-alpha cells
Accomplishment	• Pre-cultured XL 1-blue strains and placed in 18 degree incubator • Performed competency check for DH5-alpha strain

Author: Simonne Guenette
Entry 17/123: Lab 6/4/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 04.06.2019 21:31
updated: 05.06.2019 21:48

To Do	• Finish Plasmid Design (decide if Berkley 2008 sequence will get integrated) • Focus on styrene setup mechanism • Talk to Ms. Christopher about TG1 cell strain order • Check DH5-alpha for competency • Find Hilinsky
Accomplishment	• Performed Competency Tests of DH5-alpha strains • Talked to Hilinski, contacted Professor Zhang about styrene advice • Finished Plasmid Design

Author: Aarati Pokharel
Entry 16/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated
created: 03.06.2019 22:04
updated: 04.06.2019 16:07

Protocols for Electrocompetent Tg1 Cells

*2nd one came with the order but doesn't say where to add in the DNA

200123.pdf

MA053-Phage-Display(1).pdf

Author: Simonne Guenette
Entry 15/123: Lab 6/03/19-6/04/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 03.06.2019 15:41
updated: 05.06.2019 14:54

To Do	• 24 hour incubation of DH5-alpha (checking every 30 minutes-2 hours) until OD is 0.12 • Finish Plasmid Design (codon optimization and check restriction sites)
Accomplishment	• Checked Codon Optimization and restriction sites of plasmid design • Ordered E. coli K12-TG1 cell strains • Created layout flowchart of protocols and materials that need to get • Prepared competent cells of DH5-alpha

Author: Simonne Guenette
Entry 14/123: Lab 5/31/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 30.05.2019 21:58
updated: 14.06.2019 16:17

To Do	• Prepare competent DH5-alpha cell cultures (after 24 hours of incubation) • Make ampicillin + chloramphenicol plates
Accomplishment	• Prepared Ampicillin + chloramphenicol plates

Author: Simonne Guenette
Entry 13/123: Lab 5/30/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 29.05.2019 20:36
updated: 30.05.2019 21:58

To Do	• Culture competent DH5-alpha cells • Locate liquid nitrogen stock solution • Make ampicillin plates (+chloramphenicol) • Prepare sterile tranformation buffer
Accomplishment	• cultured competent DH5-alpha cells • located liquid nitrogen • made ampicillin plates • Prepared sterile transformation buffer • Prepared stock of PIPES

Author: Simonne Guenette
Entry 12/123: Lab 5/29/19
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 28.05.2019 22:15
updated: 29.05.2019 20:42

To Do	• Locate Liquid nitrogen stock • Find dewar • Bring autoclaved epindorphin tubes, sterile tips, p1000 to Koz's lab • Contact chemistry about styrene setup
Accomplishment	• Autoclaved epindorphin tubes, sterile tips • Found dewar • Finished Plasmid design for sty and pha plasmids

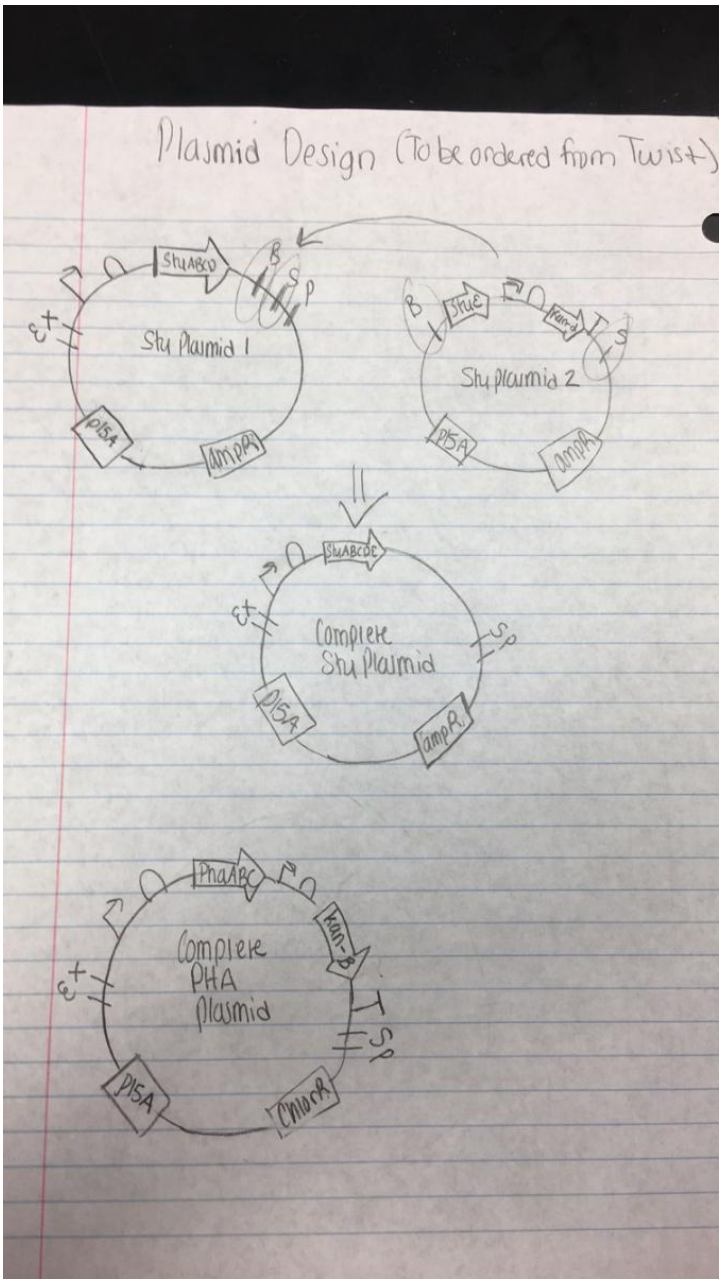
Author: Simonne Guenette
Entry 11/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
No tags associated

created: 28.05.2019 22:14
updated: 29.05.2019 21:09

Plasmid Design for BioBrick Construct

- Sty Plasmid will encode for enzymes associated with the degradation of styrene to phenylacetyl-coa
- Pha Plasmid will encode for enzymes associated with the proaction of PHBs from acetyl-coa

IMG_1499.JPG



Author: Jermaine Austin
created: 28.05.2019 20:28
updated: 18.06.2019 20:42
Entry 10/123: PROTOCOLS
In Project: Transfoam (WetLab Notebook)
No tags associated

LB Medium (Luria-Bertani Medium)

Per liter:

To 950ml of DI water (H_2O) add:

tryptone	10g
yeast extract	5g
NaCl	10g

***Add 15g/liter of agarose to liquid media before autoclaving**

Antibiotics

Ampicillin (50 micrograms/milliliter)

Chloramphenicol (25 micrograms/milliliter)

For 1000x concentration:

3.4mL of chloramphenicol per liter

For 50x concentration:

0.5mL of ampicillin per liter

Competent Cell Preparation

Starting culture from:

- 500 mL LB
- 2.5mL of 2 Morality $MgCl_2$
- 1mL 9hrs-o/n preculture on DH5a, SCS1, XL1-blue etc. in 2L flask
- Culture cells to OD = 0.12 (18 degrees Celsius)

==========On ice from now on==========

1. Transfer culture to 500mL bottle chilled on ice and incubate for 10 minute
2. Centrifuge at 5000 rpm 10 min at -4 degree Celsius
3. Resuspended cells in 150mL transformation buffer
4. Centrifuge at 5000 rpm 10 min at -4 degrees Celsius
5. Resuspend cells in 40mL transformation buffer
6. Add 3mL DMSO, and mix well
7. Make 500 microliter aliquots of cells and promptly freeze in liquid-N_2
8. Transfer tubes to storage racks in -80 degree Celsius

Competency Check

Mix 1 microliters of 1 ng/ml pRS315 and 300 microliters Competent Cells (1 picogram DNA)

Incubate on ice for 10 min

Heat Shock 2min at 42 degrees Celsius

Spread 30 microliters cells on LB + Ampicillin plate

If you can see 100 colonies, then competency is 1 x 10^9/micrograms of DNA for 300 microliters E.coli

Usually you can get at least 0.1 x 10^8/micrograms competency

Transformation Buffer

*Never adjust pH after mixing the solution!! (If you do, then the solution will turn into brown.)

*Never autoclave the final solution!! (Filter it!)

*Use PIPES-disodium salt for 500mM PIPES [pH 7.0]

15mM $CaCl_2/2H_2O$

55mM $MnCl_2/4H_2O$

250mM KCl

10mM PIPES (Disodium Salt)

pH is around 6.7

for 500mL:

1M $CaCl_2/2H_2O$ (7.5mL)

0.5M $MnCl_2/4H_2O$ (55mL)

2.5M KCl (50mL)

0.5M PIPES [pH7.0] (10mL) **or** 0.25M 1.5 Sodium PIPES

water (377.5mL)

final water pH should be around 6.7

(2) 2M $MgCl_2$

Liquid N_2

DMSO

500 mL LB in 2L flask

Others:

Autoclaved centrifuge bottles

Before starting:

Chill

Author: Jermaine Austin
Entry 9/123: LAB NOTEBOOK ENTRY: 5/28/2019
In Project: Transfoam (WetLab Notebook)
With tags: notebook, organization
created: 28.05.2019 20:12
updated: 28.05.2019 22:14

To Do	• Culture competent cells in Koz's lab • Finish Plasmid design
Accomplishment	• Prepared/Autoclaved LB broth for competent cultures • Finished Plasmid Design

Author: Jermaine Austin
Entry 8/123: LAB NOTEBOOK ENTRY: 5/27/2019
In Project: Transfoam (WetLab Notebook)
With tags: notebook, organization

created: 28.05.2019 20:08
updated: 28.05.2019 20:10

To Do	• Prepare competent cell culture with incubated DH5-alpha cells • Prepare transformation buffer (need pH) • Autoclave LB medium and flask for culture
Accomplishment	• Autoclaved LB medium and flask • Re-Incubated cell culture in preparation for competency

Author: Jermaine Austin
Entry 7/123: LAB NOTEBOOK ENTRY: 5/26/2019
In Project: Transfoam (WetLab Notebook)
With tags: notebook, organization
created: 28.05.2019 20:06
updated: 28.05.2019 20:08

To Do	• Incubate 1 mL DH5-alpha for 9 hours (see competent cell preparation protocol)
Accomplishment	• DH5-alpha preculture was inoculated and incubated for 9 hours

Author: Jermaine Austin
Entry 6/123: LAB NOTEBOOK ENTRY: 5/24/2019
In Project: Transfoam (WetLab Notebook)
With tags: notebook, organization
created: 28.05.2019 19:38
updated: 28.05.2019 19:45

To Do	• Contact professionals to confirm correct project pathway • Order Restriction enzymes /go through enzyme bucket and dispose of expired enzymes • Create LB Medium (At least 1 L) • Develop protocol for growth of culture with Styrene • Double check with Ms. Christopher about filters • Talk to Koz in the morning about asking for E. coli B strains and if we can figure out if paa will still be expressed (Strain number?) • Removed liquid culture, addition of glycerol, placed in -20 and -80 freezers (3rd row down in -80) • Refined plasmid design
Accomplishment	• Created LB Medium • Contacted Professionals • Planned 2 plasmid design

Author: Jermaine Austin
Entry 5/123: LAB NOTEBOOK ENTRY: 5/23/2019
In Project: Transfoam (WetLab Notebook)
With tags: organization, notebook
created: 28.05.2019 19:35
updated: 28.05.2019 19:40

To Do	• Filters for sterilization needed (Ms. Christopher) • Remove liquid culture and prepare glycerol freezing solution • Finalize and order Plasmid synthesis • Contact Chemistry about PS to styrene conversion
Accomplishment	• Removed liquid culture, addition of glycerol, placed in -20 and -80 freezers (3rd row down in -80) • Refined plasmid design

Author: Jermaine Austin
Entry 4/123: LAB NOTEBOOK ENTRY: 5/22/2019
In Project: Transfoam (WetLab Notebook)
With tags: organization, notebook

created: 28.05.2019 19:34
updated: 28.05.2019 19:40

my date: 22.05.2019

To Do	• Chloramphenicol + Ampicillin stock solution prepared • Autoclaving plates, glycerol, LB medium, and ampicillin materials • Get more filters for sterilization (ask Ms. Christopher) • Inoculate E. coli in water in shake incubate (do in morning) • Finish Plasmid design and order part from Twist (blast sequence to ensure to restriction sites are present in sequence)
Accomplishment	• 10 mL Chloramphenicol (1000x) and 5 mL Ampicillin (1000x) prepared • LB + agar medium, glycerol, and LB medium autoclaved • Agar/LB plates and Agar/LB + Chloramphenicol plates poured and wrapped • Bacterial cultures XL1 Blue, JAM 109, and DH5-alpha inoculated in 2 mL of LB medium and placed in shaking incubator at 37 degrees celsius • Plasmid fully designed and final checks needed before ordering

Author: Jermaine Austin
Entry 3/123: LAB NOTEBOOK ENTRY: 5/21/2019
In Project: Transfoam (WetLab Notebook)
With tags: organization, notebook

created: 28.05.2019 18:30
updated: 28.05.2019 19:40
my date: 21.05.2019

To Do	• Inoculate E.coli • Modifications of project design -- make sure everyone is on board and understands how the project is going to be changed /Confirming design of plasmid
Accomplishment	• Inoculated E.coli cultures • Begun LB stock solution creation • Begun Plasmid design

Author: Jermaine Austin
Entry 2/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
With tags: notebook

created: 28.05.2019 18:08
updated: 28.05.2019 18:30

To Do	
Accomplishment	

Author: Simonne Guenette
Entry 1/123: No entry title yet
In Project: Transfoam (WetLab Notebook)
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