Week of June 10, 2019 : Primers were designed and ordered. Cyanobacteria was received.
First attempts at PCR of pAM and PET.

<table>
<tr><td>Monday
06/10</td><td>1) Designed primers (DK & SM)</td><td>Next Steps:
Check and order primers</td></tr>
<tr><td>Tuesday
06/11</td><td>1) Received cyanobacteria(NM)

2) ordered primers (NM)</td><td>Next Steps:
Need to gather protocol for keeping bacteria alive</td></tr>
<tr><td>Thursday
06/13</td><td>1) Received primers (NM)</td><td>Next Steps:
PCR up fragments (3 fragments)</td></tr>
<tr><td>Friday
06/14</td><td>1) PCR of PET (SH)</td><td>Next Steps:
1) PCR frag of pAM
2) run a gel of PCR’d PET</td></tr>
<tr><td>Saturday
06/15</td><td>1) Prepared gel recipe (SM)
2) PCR of pAM (fragment A and B, see details in table 1 below) (SM & SH)
3) ran a gel of PCR’d PET from friday--unsuccessful :(

Notes:
PET PCR was unsuccessful

Table 1<table><tr><td>Frag A</td><td>pAM_r and pAM35k_f</td></tr><tr><td>Frag B</td><td>pAM_f and pAM35k_r</td></tr></table></td><td>Next Steps:
1) another PCR of PET
2) run a gel of PCR’d Frag A and B</td></tr>
<tr><td>Sunday
06/16</td><td>1) Attempted to make the gel fragments. We ran 2 tests.

2) This will result in us needing new primers because the current ones are not helping us run proper fragments</td><td>Next Steps:</td></tr>
</table>

Week of June 17, 2019 : Cyanobacteria subcultured. PET fragment completed and ready for Gibson assembly. New pCS primers were designed and ordered.

Monday 06/17	AE, DK, SH, SM - 3x PCR of PET Notes: AE did a 1µL Q5 Poly and DK & SH did a .5µL Q5	Next Steps: Gel of pAM frag A and B, SM&SK's practice PCR, 3x PET
Tuesday 06/18	(9:30 - 11:40 AM) SH, AE - Gel electrophoresis of PET(x3), Fragments A and B: PET successful, Fragments A and B unsuccessful (1-1:45 PM) Make a 100 microliter PCR of PET that worked for purification - DK & AE, MD observed Notes: - Most likely need to design smaller A and B fragments to get it to work (3 2200-2500 frags) - SM and EK went to lab to do subculturing, but couldn't find the autoclaved BG11	Next Steps 1) Retry Fragments A and B 2) purify PET frag (100 ul new +2*20 ug initial PCR) 3) subculture cyanobacteria with BG11 medium
Wednesday 06/19	1) Purified PET fragments and checked spectroscopy for success -SM & AE 2) made BG11 plates - EK Notes: - Our PET fragment is complete and ready for the gibson assembly as soon as we finish our pCS fragments - NM said that when he ran his own personal PCRs of pCS and PET, two worked and one did not - Checked off "purify and validate PET frag" from card "PETase into vector"	Next Steps 1) retry frags A and B 2) design new pCS primers
Friday 06/21	PCR for Frag A and B (2 samples each) - SH Notes: Samples are labeled A1, A2, B1, B2 along with SH initials	Next Steps Gel for Frag A and B 2nd try
Sunday 06/23	Gel for Frag A and B 2nd try (unsuccessful) - SH, AE, JH	Next Steps Order new primers, repeat PCR and gel for smaller size

Week of June 24, 2019: Ran PCR of fragments A-E and PET (control).

<table>
<tr><td>Monday
06/24</td><td>Turbidity reading for cyanobacteria</td><td>Next Steps:</td></tr>
<tr><td rowspan="2">Tuesday
06/25</td><td>PCR for PET (control) and Fragments A and B- SH</td><td rowspan="2">Next Steps

Gel; if does not work must order new primers -AE</td></tr>
<tr><td>Notes: 2 samples of each, labeled 1 and 2</td></tr>
<tr><td rowspan="2">Wednesday
06/26</td><td>1) gel of PET, Frag A and B - AE JH
2) PCR of Frag C, D, and E and PET -SM JH
3) Some cyano preservation - EK</td><td rowspan="2">Next Steps:

1) Gel of Frag C, D, E and PET
2) cyano preservation</td></tr>
<tr><td>Notes:
- Could not find methanol for cyanopres.
- EK took turbidity measurements of culture
- see table 2 for frag descriptions
- 2 samples of each, labeled 1 and 2

Table 2<table><tr><td>Frag C</td><td>pAM_f and 23k_r</td></tr><tr><td>Frag D</td><td>23k_f and 48k_r</td></tr><tr><td>Frag E</td><td>48k_f and pAM_r</td></tr></table></td></tr>
<tr><td rowspan="2">Thursday
06/27</td><td>1) Gel of fragments C, D, and E with PET control - AE

2) took cyano light and spect readings -SM</td><td rowspan="2">Next Steps:
1) PCR of fragments/purification (?)
2) cryopreservation of cyanobacteria</td></tr>
<tr><td>Notes:
- see cloning document
- gave EK cyano light/spect data</td></tr>
<tr><td>Saturday
06/29</td><td>1) took cyano light and spect readings -SM/SH</td><td>Next Steps:</td></tr>
</table>

Week of July 1,2019 : Ran PCR, gel, and amplification of fragments A, D’ and E.

Monday 07/01	PCR of frags E and D' -AE	Next Steps
Tuesday 07/02	Gel of fragments A, D' and E twice -AE Notes: -Gel unsuccessful both times, retry PCR and gels	Next Steps : 1) Split cyano culture 2) frag A amplification and purification 3) PCR frag D’ and E
Wednesday 07/03	1) PCR of D’ and E + amplification of A -AE + SM	Next Steps : 1) Gel of PCR frag
Thursday 07/04	-gel of frags D', E, A - SM	Next Steps: Amplify frags A, D' and E
Saturday 07/06	1) Amplify Frag A D’ and E -SH SM	Next Steps: 1) Run gel of frag A D’ and E

Week of July 8, 2019: Successful PCR confirmed by gel for fragments A and D’. PCR unsuccessful for fragment E.

Tuesday 07/09	1) Retest frag A, amplify frag E -AE 2) Gel of frag A and E - DK SM Notes: - Its highly likely the amplification for E did not work, must be reamplified - frag A ran with program Q5_55_120 - gel of Frag A and E didn’t work - If A **DOES** works, amplify A, D' and E using the new cycle for A. if A **DOES NOT** work, amplify C, D and E -DONT DO THE ABOVE JUST YET, need to really figure out what primers we need to use for each frag to put it all together	Next Steps : 1) figure out the proper primers to use for PCR 2) redo PCR
Wednesday	1) Split liquid cyano culture - SH, EK	Next Steps:

07/10	2) New streak plates done to get individual colonies - SH, EK
	3) PCR of B and amplification of A (with high fidelity) -SM SH JH
	4) PCR of B and amp of A D' and E (with hot start high fidelity) -SM
	
	Notes:
	- new streak plates are **COLOR CODED** according to where they sit on the cabinet
	  - **RED** should stay closest to the window
	  - **BROWN** is on the edge of the yellow cabinet
	  - **PURPLE** is set up on the white block of foam in the middle of the cabinet
	- See bottom of page for primers used
		1. Make more Bg11 media (need ferric ammonium citrate for this)
		
		2. If individual colonies acquired, can use control plasmid to see if there are problems with uptake into cyanobacteria
		
		3. Gel of PCR set 1 with high fidelity and set 2 with hot start

Thursday 07/11	1) Gel of PCR of B (with hf and hs) & amplification PCR of A(hf and hs) D' E (hs) -SM 2) PCR of A D' E -SM Notes: - Gel unsuccessful, again - Redid A D' E with menhart, if doesn't work we'll need to figure out what's happening	Next Steps : 1) Gel of A D' E
Friday 07/12	1) Gel of A, D', and E - SH Notes: - NM is rerunning frag E tom am (hopefully) so we know what's going on with that - successful gel of A and D'	Next Steps : 1) amplify A D' (if not already) 2) purify frag A D' 3) rerun gel of E

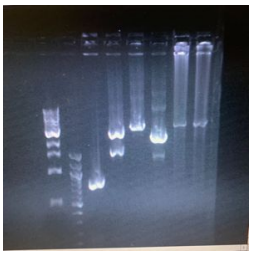
Week of July 15, 2019: Primers for fragment E redesigned and ordered. Preliminary conjugation protocol developed.

Monday 07/15	Redo gel of fragments A, D', and E samples from 07/11 - SH, AE, SE, DK (E still unsuccessful) Notes: If other sample of E works and bp length checks out then we are on our way with fragments	Next Steps : Try a gel previous samples of E that worked Amplification of A and D'
Tuesday	-Gel of E samples from date - SH -Amplification of A, D' with primers pAM35k_r and pAM_f for	Next Steps : -gel for A and

07/16	frag A and pAM35k_r and pAM48k_f for D' but ran on the wrong cycle. -AE+PC -Gel for frags A, D' and E - AE -amplification of A and D' with the same primers and used the _Q5_57_120 cycle -AE	D' -Redesign primers for frag E
	Notes: All fragments unsuccessful for the first PCR	
Wednesday 07/17	Gel for fragment and of A and D' samples.-PC	Next Steps: Test about the template.
	Notes: Fragments of A and D' still unsuccessful for the PCR.	
Thursday 07/18	-Made a new template DNA and redo the Gels for D',but no result.-PC -Made a new blank gel and test it with standard, only a weak result. -PC	Next Steps -Try a new gel
	Notes: New template with no result, and weak result for standard.	
Sunday 07/21	- started a protocol for conjugation & made new primers for E -SM, SH	Next Steps: - order new primers - confirm protocol
	Notes: - need e. Coli to try conjugation (where's it at?)	

Week of July 22, 2019: First successful PCR for fragments A and B. Amplification was run with taq and Q5 for both fragments.

Monday 07/22	PCR of frag B -SM SH PC	Next Steps: gel of frag B
Tuesday 07/23	1) ran gel of frag b -PC	Next Steps:
	Notes: - Frag B= unsuccessful - rerun with NM	
Wednesday 07/24	1) PCR of multiple Frag -PC NM 2) started 15 ml culture of pet-PET -SM AE PC NM 3) Gel of PCR -SM AE PC	Next Steps: 1) Purify pet-PET culture 2) NM need to review gel to

<table>
<tr>
<td></td>
<td>

<table>
<tr><th>lane</th><th>P_f</th><th>P_r</th><th>expected (approx)</th><th>class name??</th><th>result</th></tr>
<tr><td>1</td><td>1kb</td><td>-</td><td></td><td></td><td>-</td></tr>
<tr><td>2</td><td>100bp</td><td>-</td><td></td><td></td><td>-</td></tr>
<tr><td>3</td><td>63</td><td>pAM_r</td><td>?</td><td>E?</td><td>ok</td></tr>
<tr><td>4</td><td>35</td><td>63</td><td>?</td><td>E?</td><td>no good, two bands</td></tr>
<tr><td>5</td><td>F2</td><td>35</td><td>3500</td><td>A</td><td>ok</td></tr>
<tr><td>6</td><td>F2</td><td>23</td><td>2300</td><td>C</td><td>maybe ok, but has two bands</td></tr>
<tr><td>7?</td><td>35</td><td>pAM_r</td><td>3500</td><td>B</td><td>ok</td></tr>
<tr><td>8?</td><td>48</td><td>pAM_r</td><td>2300</td><td>E?</td><td>ok</td></tr>
</table>

Notes:
- FINALLY HAVE SUCCESSFUL FRAGS!!!! (A & B)
- For some PCR used Q5, for others did taq
- Still working on whether to use taq or Q5
- Transforming pET-PET into competent E. Coli to asses PETase
</td>
<td>determine next steps
a) Large scale PCR of working frag== FRAG A & B
b) Check on gel
c) Purify
3) transform pET-PET into competent T7 cell</td>
</tr>
<tr>
<td>Thursday
07/25</td>
<td>1) Amplification for Frag A & B with different enzyme both(Q5 and taq) -AE PC
<table>
<tr><td>Fragment :</td><td>w/taq</td><td>w/Q5</td></tr>
<tr><td>A</td><td>pAM_F2 + 35k_r</td><td>pAM_F2 + 35k_r</td></tr>
<tr><td>B</td><td>35k_f + pAM_r</td><td>35k_f + pAM_r</td></tr>
</table>
2) Purified pet-PET culture -AE PC

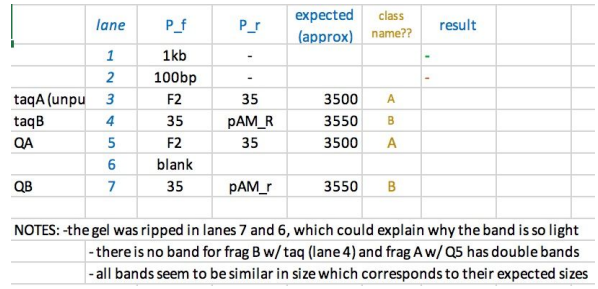
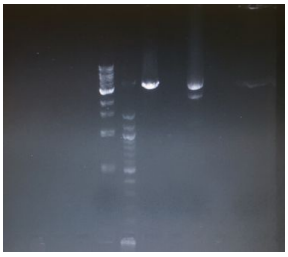
Notes:

- Documented the “PCR Purification” on google drive
- did amplification of BOTH frags with taq AND Q5</td>
<td>Next Steps :

-Gels for the frags
-Replicas plate NEB3013</td>
</tr>
<tr>
<td>Friday
07/26</td>
<td>1) Gels of Frag A , B, PET(taq)
<table>
<tr><th></th><th>lane</th><th>P_f</th><th>P_r</th><th>expected (approx)</th><th>class name??</th><th>result</th></tr>
<tr><td></td><td>1</td><td>-</td><td>-</td><td></td><td></td><td>-</td></tr>
<tr><td></td><td>2</td><td>100bp</td><td>-</td><td></td><td></td><td>-</td></tr>
<tr><td></td><td>3</td><td>pAM_f</td><td>pAM_r</td><td>1034</td><td>PET</td><td>ok</td></tr>
<tr><td>taq</td><td>4</td><td>F2</td><td>35</td><td>3521</td><td>A</td><td>maybe ok, the size might be too big</td></tr>
<tr><td>taq</td><td>5</td><td>35</td><td>pAM_R</td><td>3554</td><td>B</td><td>no</td></tr>
<tr><td>Q5</td><td>6</td><td>F2</td><td>35</td><td>3521</td><td>A</td><td>maybe ok, but has two bands</td></tr>
<tr><td>Q5</td><td>7?</td><td>35</td><td>pAM_r</td><td>3554</td><td>B</td><td>ok</td></tr>
</table>
Notes:</td>
<td>Next Steps:
-Replicas plate NEB3013
-Purify the B(taq) and both A,B(Q5) - NM DECISION
-Rerun the gel of A(pAM_f2 + 35k_r, taq) (1 ul) with 1kb and 1ul of purified samples. - NM</td>
</tr>
</table>

	-Most of the gels are good, but A(pAM_f2 + 35k_r, Q5) shows 2 bands B(taq) no band A(pAM_f2 + 35k_r, taq) may be too big -No colonies shows off on the plate NEB3013, need wait more 24 hours and see.	

Week of July 29, 2019: Purification of fragments QA, QB and taq B. Gibson reaction of the primer A, B , pet-PET documented. Started liquid culture of pet_PET/3013.

Tuesday 07/30	1) Reran the gel of A(pAM_f2 + 35k_r, taq) (1 ul) with 1kb and 1ul of purified samples -SM AE PC  	To Do: - need info/assistance with NEB3013 - need info/assistance on concentrations for GFP conjugation media - go over gel from 0730
Thursday 08/01	1) spec of purified frags (QB QA and taq B) 2) pcr of taqB -purify taq A and Q5B -PC Notes: - using taq A and Q5B with pet-PET for plasmid	Next Steps : 1) gel of purified fragments 2) redo competency for pet_PETase 3) try conjugation with e. Coli 4) rewrite “intro to project” for JH - Powerpoint of stuff 5) gather docs/pics for website

	lane	P_f	P_r	expected (approx)	class name??	result
	1	1kb	-			-
	2	100bp	-			-
taqA (unpu	3	F2	35	3500	A	
taqB	4	35	pAM_R	3550	B	
QA	5	F2	35	3500	A	
	6	blank				
QB	7	35	pAM_r	3550	B	

NOTES: -the gel was ripped in lanes 7 and 6, which could explain why the band is so light
- there is no band for frag B w/ taq (lane 4) and frag A w/ Q5 has double bands
- all bands seem to be similar in size which corresponds to their expected sizes

Friday 08/02	-gel of purified frags(Q5B taqA) -PC -Gibson reaction of the primer A,B,pet-PET -PC -Documented Gibson reaction setup in the lab manual.	Next Steps :
Saturday 08/03	1) replicata plated pet_PET/3013 -SM Notes: - strong colonies chosen for replication; placed replica plate in incubator - gibson plate put back into incubator; no colonies at 1100a, had strong colonies on the control plate	Next Steps : 1) need to grow pet_PET/3013 culture in liquid media 2) may need to make 100/200 ul of Frag
Sunday 08/04	1) started liquid culture of pet_PET/3013 -SM Notes: - strong colonies of pet_PET/3013 on LBa plate - liq culture of pet_PET/3013 in shaker in 311 - LBa plate w/ pet_PET/3013 in fridge with LBc plate - one small, questionable colony on gibson plate (pAM_PET/NEB5a on LBc) - pAM_PET/NEB5a in incubator, control on lab bench	Next Steps : 1) need 200ul rxn of frag A and B

Week of August 5, 2019: PCR and purification of fragments A and B with Q5. Gibson reaction ran and transformed.

Monday 08/05	1) prepped gel -AE 2) PCR: 150ul taqA, 100ul Q5B 3) plated pAM4788 Notes: - didn't have enough enough template for 20 ul rxn, made some new plates today (0805), mini prep tomorrow (08	Next Steps : 1) 20 ul of Q5A (gave funny two bands, refer to pAM primer test doc) with mastermix 2) make mini preps of pet_PET/3013
Tuesday	1) Ran gel for amplified fragments A and B -AE 2) Purified fragments A and B and took a spec	Next Steps :

08/06	reading both -AE 3) Prepared new template for PCR - AE and NM 4) Set up and incubated Gibson reaction -AE SM Notes: - start trying to do conjugation between e. Coli and cyano -- make media -stored the remaining gibson in the -20 freezer	1) Mini prep plasmids from over the weekend
Wednesday 08/07	Notes: - no growth on plates AE SM did with gibson	
Thursday 08/08	1) replated gibson rxn -NM 2) PCR of frag A and B with Q5 mastermix Notes: - SM AE didn't transfer bacteria onto plate correctly, NM took the stored gibson bacteria and replated (who knows if the bacteria will grow after freezing)	Next Steps: - mini prep plasmids from weekend (pet_PET/3013)
Friday 08/09	1) PCR of frag A and B with Q5 -NM 2) Purification of PCR -SM 3) Gibson -SM Notes: - Plate with gibson from tues 06 didn't take	Next Steps : 1) transformation from gibson
Saturday 08/10	1) transformation from gibson -SM	

Week of August 12, 2019: PCR of Gibson reaction (also cultured and plated colonies). No successful Gibson reaction with the correct plasmid.

Monday 08/12	1) PCR of Gibson reaction -AE 2) Cultured colonies from Gibson plate -AE 3) Plated the colonies from the Gibson -AE 4) 2 gels for the PCR -AE Notes: - no successful gibson with CORRECT plasmid	Next Steps : - mini prep plasmids (pet_PET/3013) - website - redo gibson ???

Week of August 19, 2019: Did mini preps of pet_PET and Gibson transformation.

Saturday 08/24	- mini preps of pet_PET(red minicent tubes) and gibson transformation (blue minicent. tubes) -SM JT WJB	Next Steps : - screen pET-PET

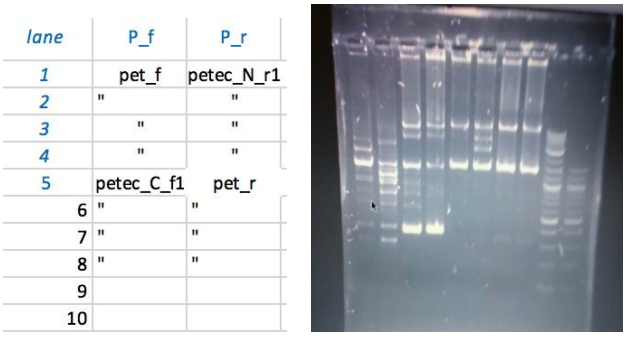
		and pAM_PET - redo gibson ???
	Notes: - refer back to gel doc located ****** for replica plate colonies for gibson	

Week of August 26, 2019: Re-ran gel of pET_PET to ensure colony growth. Made culture of pET_PET colonies grown from replica plate.

Monday 08/26	Made cyano medium, checked progress of subculture - EK MD	Next Steps :
Tuesday 08/27	- screened mini preps from aug 24 -SM AE	Next Steps : - run a gel of PCR - finalize a protocol for GFP transformation between e.coli and cyano
	Notes - ran: TAQ_57_120 to screen	
Wednesday 08/28	-Assay team touched base with Matt from summer	Next Steps :
Friday 08/30	- re-ran gel of pET_PET to make sure there was a colony -SM AE JT ML	Next Steps : - grow culture of pET_PET colonies from replica plate
	Notes: - may have some colonies with pET_PET, confusion on which so growing up two	
Saturday 08/31	- plated colonies 6,7, and control for pET_PET -SM	Next Steps : - Move pET_PT plates to fridge
	Notes: - plates in incubator, need to be moved to fridge Sun.	
Sunday 09/01	- pET_PET plates in fridge -SM	Next Steps :

Week of September 2, 2019: Made a liquid culture of pET_PET and did mini preps of four cultures (-6, -7, C, and C). Another Gibson reaction was set up and transformation was run. By the end of the week, no transformed colonies were observed.

	Assay Team: - liq culture of pET_PET -SM SS WB RB	Next Steps :

<table>
<tr><td>Tuesday
09/03</td><td></td><td>- minipreps of 4 cultures</td></tr>
<tr><td rowspan="2">Wednesday
09/04</td><td>Assay : Miniprep of four cultures -SS,CJ,LG</td><td rowspan="2">Next Steps :
Cloning: Gibson
Assay: PCR screen, Protein mini prep</td></tr>
<tr><td>Notes: minipreps left in incubator overnight,suggested by NM, labeled -6,-7,C,C.</td></tr>
<tr><td rowspan="2">Thursday
09/05</td><td>Cloning:
- set up gibson rxn -SM JT RB
- transformation -SM AE
- made LBsm plates (~24)
Assay:
- made liq cultures of -6, -7, -NOs -SM WB AE</td><td rowspan="2">Next Steps :
Cloning:
Assay:
- mini preps of liq cultures (protein screen)
- PCR screen</td></tr>
<tr><td>Notes:
Gibson:
- “NEBulider DNA assembly master mix”
- “NEBuilder positive control”
- 2 control tubes (labeled: +)(one SM one RB)
- 1 pAM_PET tube (labeled: G)
Assay:
- unable to do PCR because water wasn’t clean enough</td></tr>
<tr><td rowspan="2">Friday
09/06</td><td>Cloning:
- no observed transformed colonies on plate @ 1410pm-AE
Assay:
- screen PCR -SM WB
- screen PCR gel of EN and EC - ML
- Protein mini preps -JC
 
</td><td rowspan="2">Next Steps :
Cloning:
- check for growth later in the day
- if growth take out and replica plate. Replica plate goes in incubator and old plate goes in fridge
- if no growth, figure out what is wrong with gibson (consult NM)</td></tr>
<tr><td>Notes:
Assay:
- questionable mini prep of -7 and -C2</td></tr>
</table>

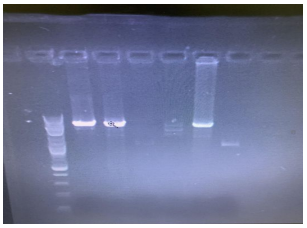
	- PCR tubes under “EE” (with pET_f/r) had drastic volume differences didn’t run a gel - used primer pET_f rather than pET_f2	

Week of September 9, 2019: Redid Gibson reaction with LBamp plates for control and performed transformation procedure. No growth was observed on Gibson plates once again. Made cultures of pCS_PET and pAM4788. Ran PCR for PET, A, and B. Also ran PCR for EE screening of -6, -7, C1, C2 from pET_PET and did protein purification.

Monday 09/09	Cloning: - took out neg gibson plates -SM Notes: Cloning: - Had “positive” colonies last time we ran gibson, why no colonies this time - Used LBsm plates for control, needs to be LBamp	Next Steps: Cloning: - redo gibson one more time
Tuesday 09/10	Cloning: - redid gibson. one experimental tube and one positive control -WB MD - Transforming gibson -SM ML - Made gel for frag a b pet check -SM ML Notes: - NEED TO ORDER MORE C2987 CELLS FROM NEB - PCR used flat lids instead of rounded, so tube became malformed. Took a lot of work to get product ou	Next Steps: - Ran a gel of fragments A, B and PET - Check plates in incubator
Wednesday 09/11	Cloning: - no growth on gibson plates -SM - conformation gel of A B and PET (see gel below) -SM Notes: Cloning: - no band for PET, may be the cause of failed gibson reactions - control gibson grew, no rxn for pAM_PET	Next Steps: Cloning: - make frag A and B and PET via PCR - talk to NM tomorrow about what to do with no gibson growth
Thursday	Cloning:	Next Steps:

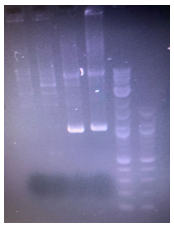
09/12	- replated pCS_PET and pAM4788 -SM Notes: Cloning: - might need to make new template b/c it's getting old, hence why replated	Cloning: - PCR of frag A B and PET - if no growth, figure out what is wrong with gibson (consult NM)
Friday 09/13	Cloning: - Ran PCR for PET, A, and B -SS, AE, MD - started pAM4788 and pSC_PET cultures -SM CJ Assay: - PCR for EE screening of -6, -7, C1, C2 from pET_PET -SM - started liq cultures of -6, -7, NOS control, cindel control (for protein prep) -JC Notes: Cloning: Q5MM with _Q5_57_120 Assay: See below for "EE" PCR parts	Next Steps: Cloning: - gel of frag A B PET - do PCR purification with carts Assay: - gel of EE screening
Saturday 09/14	Assay: Ran Protein Purification from Friday's overnight cultures- LG JCT	Next Steps: Run the gel to determine expression
Sunday 09/15	Assay: Vlad set up + ran gel to verify results	

Week of September 16, 2019: Purified fragments A, B and PET. Received a very low A260 value for PET frag, but OK A260 values for fragments A and B. Started new PCR of PET fragment to redo. Transformation of pAM4788 and Gibson into E. coli. Did high quality mini preps of pET_PET for assay.

<table>
<tr>
<td>Monday
09/16</td>
<td>Cloning:
- ran gel of Frag A B and PET -SM
Assay:
Read gel from 15 sept; empty
 
<table>
<tr><th></th><th>lane</th><th>P_f</th><th>P_r</th><th>expected</th><th>class</th><th>result</th></tr>
<tr><td></td><td>1</td><td>1kb</td><td>-</td><td></td><td></td><td>good</td></tr>
<tr><td>A</td><td>2</td><td>f2</td><td>35r</td><td>3500</td><td>A</td><td>good</td></tr>
<tr><td>B</td><td>3</td><td>35f</td><td>pAM_R</td><td>3500</td><td>B</td><td>good</td></tr>
<tr><td>PET</td><td>4</td><td>pam_petf</td><td>pam_petr</td><td>1034</td><td>PET</td><td>very light, not so great</td></tr>
<tr><td>A</td><td>5</td><td>f2</td><td>35r</td><td>3500</td><td>A</td><td>not so great, appears to have 3 bands</td></tr>
<tr><td>B</td><td>6</td><td>35f</td><td>pamr</td><td>3500</td><td>B</td><td>good</td></tr>
<tr><td>PET</td><td>7</td><td>pam_petf</td><td>pam_petr</td><td>1034</td><td>PET</td><td>light band, but present</td></tr>
</table>

Notes:
Cloning:
- positive bands for A B PET</td>
<td>Next Steps:
Cloning:
- PCR purification
- gibson/ transformation

Assay:
- gel of PET “EE” screen</td>
</tr>
<tr>
<td>Thursday
09/19</td>
<td>Cloning:
- purified frag A B PET -SM

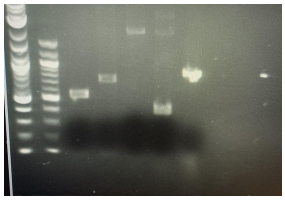
Assay:
-- gel of pET_PET screen of “EE” (pet_f and pet_r) -SM
<table>
<tr><th>Lane</th><th>P_f</th><th>P_r</th><th>size exp</th><th>results</th></tr>
<tr><td>1</td><td>pet_f</td><td>pet_r</td><td>1034</td><td>poor</td></tr>
<tr><td>2</td><td>"</td><td>"</td><td></td><td>poor</td></tr>
<tr><td>3</td><td>"</td><td>"</td><td></td><td>well defined band</td></tr>
<tr><td>4</td><td>"</td><td>"</td><td></td><td>well defined band</td></tr>
<tr><td>5</td><td></td><td></td><td></td><td>-</td></tr>
<tr><td>6</td><td></td><td></td><td></td><td>-</td></tr>
</table>

Notes:
Cloning:
- SUPER low a260 for PET
- not great a260 for A and B, but workable</td>
<td>Next Steps:
Cloning:
-- autoclave tips !!!!
- purify and make new template of pCS_PET for PET frag
- redo PCR of frag PET</td>
</tr>
<tr>
<td>Friday
09/20</td>
<td>Cloning:
- PCR of PET frag -SS WB
- Gel of PET frag -SM
- started liq culture of pAM4788 (for control transformation into S.e) -SM
- Gibson -SM</td>
<td>Next Steps:

Cloning:
- HQ minipreps of pAM4788
→ transformation</td>
</tr>
</table>

	 lane | P_f | P_r | expected (approx) | class name?? | result 100 | 1 | - | - | | | good PET frag | 2 | pam_p | pam_PETf | 1034 | | good!! Assay: - plated pET_PET in DH5a (og) -SM - liq cultures of pET_PET (for PET screening) -SM	into TURBO → test conjugation into S.e Assay: - HQ mini preps of pET_PET → PCR screen once we have new ec_C_
	Notes: - need to autoclave pipette tips and make more LBa liq broth	
Saturday 09/21	Cloning: - HQ minipreps of pAM4788 - transformation of pAM4788 into turbo - transformation of gibson into turbo Assay: - HQ minipreps of pET_PET	Next Steps: Cloning: - check transformation plates → if pos: replica plate and screen pAM4788 and pAM_PET → if neg: redo transformation of pAM4788 and redo gibson of pAM_PET Assay: - PCR screen once new C primers come in

Week of September 23, 2019: Made mini preps of four successful Gibson colonies and ran PCR screen.

Monday 09/23	Cloning: - four isolated colonies on gibson plate (control growth=good)	Next Steps : Cloning: - make minipreps

	- growth of pAM4788 on plate	of gibson 4 colonies → run a screening PCR - screen turbo cells for pAM4788 Assay: - order pET C primers - PCR screen
Tuesday 09/24	- mini preps of gibson 4 colonies -SM AE - PCR screen -ML Notes: - no more taq MM-- order more	Next Steps : Cloning: - run gel of PCR screen - order GFP primers to screen pAM4788 - made just need to order Assay: - order pET C primers - made just need to order - PCR screen
Wednesday 09/25	- PCR screen gel -SM	Next Steps: - redo PCR screen
Thursday 09/26	- PCR screen of -1,2,3,4,+ pAM-PET (+pAM4788) -SM *lane* / P_f / P_r / expected (approx) / class name?? / result 1 / pAMse_f 2 / " / pAMse_r 3 / " / " / 1200 / / no 4 / " / " / 1200 / / no 5 / " / " / 1200 / / no 6 / " / " / 1200 / / no 7 / " / " / 900 / / YES 	

Week of October 7,2019: Redid Gibson and PCR of fragments A, B, and PET. Did Gibson extraction PCR using magic peg and had 11 colonies grow on the plate.

Wednesday	Redid gibson	Next Steps :

10/09	PCR of frag A Notes: - Need 35k_f and pAM_PET_r - Frag A= pAM_f2 and 35k_r	PCR of frag B and PET
Thursday 10/10	- PCR of B and PET -Transformation -Made gel Notes Frag B= 35k_f and pAM_r Frag PET= pAM_PET_f and PET_pAM_r	Next Steps : - Run a gel of A B PET -Purify frag
Friday 10/11	- Gibson extraction PCR (magic peg) -Started 3ml liq cultures/ replica plate of 11 gibson colonies -Started 15ml liq culture/plate of #9 colony Notes: - Had 11 colonies grow on gibson plate !	Next Steps : - Run a gel of extraction (magic peg) PCR and of A B PET frag
Saturday 10/12	- Ran gel of magic peg PCR -HQ purification of #9 -PCR screen of #9	Next Steps : - Run a gel of A B PET frag Run a gel of PCR screen

Week of October 14, 2019: TFA assay was developed to prove that the degradation of PET nanoparticles tagged with fluorescent molecules could be tracked through fluorimetry.

Monday 10/14	Ran a gel of PCR screen. Ran a gel of Re-PCR screen. Gel of restriction enzyme screen. Restriction enzyme screen.	Next Steps : -Re-PCR screen one with Q5 another with taq
Wednesday 10/16	Developed TFA assay with Dr Menhart.	Next Steps : - Calculate moles of TFA, find appropriate buffer
Thursday 10/17	Calculations done, chose tris. Set up trials with 0/20/45/90% TFA, left in incubator	Next Steps : - Add buffer & check in fluorimeter