Nanodrop Observation (8/11) New Oligos

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-08-11

FRIDAY, 8/11/17

Table1

	A	B	C	D
1	Sample	[]	260/280	Notes
2	A	1843.4	1.69	
3	B	1795.2	0.86	
4	C	272.9	1.76	
5	D	891.9	1.79	
6	E	1911.8	1.18	
7	F	1185.5	1.93	
8	B-1	1954.5	1.26	
9	C-1	261.9	1.74	Nick says it's due to the length of the primer, but may remake the dilution

puc19 KpnI digest, Gibson assembly

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-08-20

SUNDAY, 8/20/17

From prior PCR

C sMMO2 (worked at the higher temp)
E sMMO1 (worked at the lower temp)
D puc19 (worked at both temp)

5 uL puc19
1 uL CutSmart
0.7 uL KpnI
3.3 ddH20

puc19 KpnI cut
14.9 uL 1.87

Possible Assembly
10.6 ng/uL 10.75??? Very left shifted

Current Inventory

Project: TCE Biodegredation Project
Authors: Katie Brown
Date: 2017-06-10

SATURDAY, 6/10/17

Table1

	A	B	C	D
1	Item	Count	Location	Ownership Status
2	Fisherbrand Micropippette TIps (1-200uL)	5 Boxes	Shelf 2	Donated (Forrest)
3	PetriDishes	6 Sleeves	By Oven	Donated (Forrest)
4	Aluminum Foil	1 Roll	Shelf 2	?
5	iGEM DNA Kit	1 Kit	Shelf 4	BOUGHT BOI
6	Odin Amp Medium	14 15 ml Tubes	Drawer 42	Bought
7	Odin Amp. Sodium	1 Bottle	Drawer 42	Bought
8	Odin LB Powder Media	1 Bottle	Drawer 42	Bought
9	Odin Tris Buffer 50 mM	1 Bottle	Drawer 42	Bought
10	Odin Agarose High Read	2 Bottle	Drawer 42	Bought
11	Odin Kanamyacin Monosulfate	1 Bottle	Drawer 42	Bought
12	Odin NaOH 50 mM	1 Bottle	Drawer 42	Bought
13	Odin TAE Buffer Mix	1 Bottle	Drawer 42	Bought
14	Micropippete Tips (1000 uL)	5 Boxes	Shelf 2	Donated (Forrest)
15	Filter Micropipette Tips (1-200uL)	7 Boxes	Shelf 2	Donated (Forrest)
16	500 mL Squirt-Bottles	4 Bottles	Shelf 1	?
17	1000 mL Erlenmeyer Flask	2 Bottles	Shelf 3	Borrowed (P-Chem La
18	FIsher Electrophoresis System	1 Unit	Drawer 42	Borrowed (Steed)
19	Electrophoresis Plate and Leads	2 Units	Drawer 43	Borrowed (Meigs)
20	Odin Electrophoresis System	1 Unit	Drawer 43	Bought (Nick)
21	Tabletop Centrifuge	1 System	Drawer 43	Bought (Nick)
22	Fisher Magnetic Stirrer w/Magnets	1 System	Window Bench	Borrowed (P-Chem La
23	Electrophoresis Power Supply	1 System	Window Bench	Borrewed (Meigs)
24	PCR Machine	1 System	Window Bench	Bought (Nick)
25	Campstove Burner	1 Unit	Shelf 4	Bought (Nick)
26	Camp Fuel Canisters	2 Units	Shelf 4	Bought (Nick)
27	Cotton Tipper Applicators	1 Bag	Drawer 46	Donated (Forrest)
28	Monarch Plasmid Miniprep Kit	2 Kits	Shelf 3	Donated (Steed)
29	Monarch PCR and DNA Cleanup Kit	1 Kit	Shelf 3	Donated (Steed)

29	Monarch PCR and DNA Cleanup Kit	1 Kit	Shelf 3	Donated (Steed)
30	Kimwipes	3 Boxes	Shelf 1	?
31	Mixed Colored Microcentriguge Tubes	1 Bag/500 Count	Top of Shelving Unit	Donated (Forrest)
32	Gilson P20 Pipetteman	1	Shelf 3	Donated (Meigs)
33	Gilson P200 Pipetteman	1	Shelf 3	Donated (Meigs)
34	Glison P1000 Pipetteman	1	Shelf 3	Donated (Meigs)
35	Fisherbrand 0.2-2uL Pippeteman	1	Fridge Table	Donated (Steed)
36	Fisherbrand 2-20uL Pippeteman	1	Fridge Table	Donated (Steed)
37	Fisherbrand 20-200uL Pippeteman	1	Fridge Table	Donated (Steed)
38	Odin Pipetteman 2-20 uL	1	Fridge Table	Bought (Nick)
39	Odin Pipetteman 20-200 uL	1	Fridge Table	Bought (Nick)
40	Odin Pippeteman 200-1000 uL	1	Fridge Table	Bought (Nick)
41	Antibiotic Purification Needle Thing	1	Shelf 2	?
42	Misc Sized Vials	3	Shelf 2	?
43	Falcon Tubes	18	Shelf 2	Donated (Meigs)
44	iGEM Drugdealer Scale	1	Shelf 42	Bought (Nick)
45	Latex Gloves (Medium)	1 Box	Shelf 4	Donated (P-Chem Lat
46	Latex Gloves (Large)	1 Box	Shelf 4	Donated (P-Chem Lat
47	FIsherbrand Pippettes 25 mL	1 Box	Under Fridge Table	Donated (Forrest)
48	500 mL Graduated Cylinder	2	By Oven	Donated (P-Chem Lat
49	Chloremphenacol Plates	12	Our Fridge	Made
50				
51				
52				
53				
54				

Fridge Contents

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-06-10

SATURDAY, 6/10/17

Table1

	A	B	C	D	E
1	Name	Location	Amount	Origin	Notes
2	sMMO Part 1 Ligation	F. Shelf 2	1	IDT	
3	sMMO Part 2 Ligation	F. Shelf 2	1	IDT	
4	GroEL/ES Ligation	F. Shelf 2	1	IDT	
5	dhlB Ligation	F. Shelf 2	1	IDT	
6	Monarch Plasmid Buffer 3	F. Shelf 2	1	Donated (Steed)	
7	1x TAE Buffer	F. Door	1 Bottle	Donated (Steed)	
8	Competent Cell Test Kit	F. Freezer	1 Kit	iGEM	
9	Plasmid Backbone Kit	F. Freezer	1 Kit	iGEM	
10	Cloremphenicol Concentrate	F. Freezer	1 Vial	Donated (Meigs)	
11	Ampicilin 100	F. Freezer	1 Vial	Donated (Meigs)	
12					
13					
14					
15					
16					
17					
18					
19					
20					

Upstair Fridge

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-06-21

WEDNESDAY, 6/21/17

sMMO and GRoEL/ES come in

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-07

WEDNESDAY, 6/7/17

Resuspended IDT DNA

Concentration of stock DNA

Table1

	A	B	C
1		Concentration	260/280
2	sMMO1	236 ng/uL	2.24
3	sMMO2	314 ng/uL	1.98
4	GroEL/Es	186 ng/uL	2.31

Making Chloramphenicol plates (Katie)

Restriction digest (sMMO 1, sMMO2, GroEL/ES, chloramphenacol vector **pSB1C3)**

Ran purification gel

Column Purified

Cell Transformation

4 uL instead of 1, 100 uL Matt Greene's competent cells

Ligation
6 uL Vector
2 uL buffer
1 uL T4 Lyz

If plating ligations doesn't work, attempt liquid cultures?
In: 2 am
Out 4 pm

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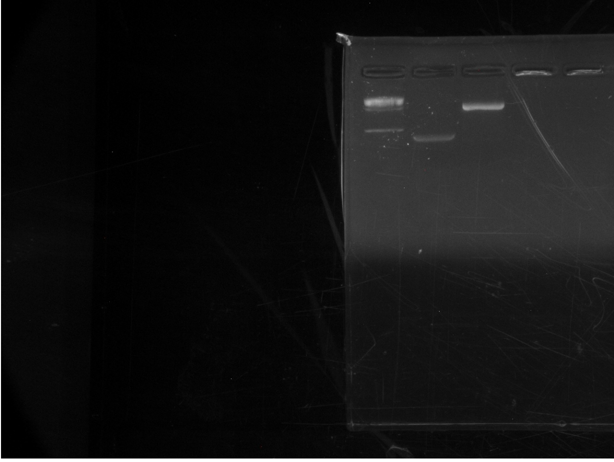

Oligo primers for dhlB, sMMO 1,2 GroEL/Es

Project: TCE Biodegredation Project

Authors: Nicholas White

Date: 2017-06-08

THURSDAY, 6/8/17

Colony Counts [By Hand]

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-06-09

FRIDAY, 6/9/17

Table1

	A	B	C	D	E
1	Plate	Colonies	Counter	Notes	
2	10A	13	Nick	All Peripherel	
3	10B	9	Nick		
4	10C	31	Nick	No presence of red	
5	50A	86	Nick		
6	50B	122	Nick		
7	100A	16	NIck		
8	100B	126	Nick		
9					
10					

FRIDAY STUFF

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-09

FRIDAY, 6/9/17

Making primers because IDT sequences are low concentration, not enough volume

sMMO 1

FWD, lac 5' gcatgaattcgcggccgcttctagagaattgtgagcgga 3'
FWD, w/ weak Anderson
FWD w. cut sites?
REV . 5' tacgctgcagcggccgctactagtactctagtatca'

dhlB liquid cultures, in 2mL w/ Amp

In: 5:10 pm
Out: 8 am

Plates in 8
Out 10 am

Minipreps, liquid cultures

Project: TCE Biodegredation Project

Authors: Nicholas White

Date: 2017-06-10

SATURDAY, 6/10/17

Checked on Liquid cultures. dhlB picks were blank meaning cells did not pick up ligation. Amp and Chloro picks also turned pink, indicating contamination from RFP bacteria or plasmid. Miniprep yields were also fairly low. Experiment scrapped and re-attempted.

Amp/Chloro plates

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-11

SUNDAY, 6/11/17

Transformed Matt Greene's JM109s with Amp and Chloro iGEM plasmids for future minipreps

100 uL cells
2 uL DNA

30 min on ice
Heat shock 30 sec at 42

1 mL of LB for ~1 hour shaking 37, 230 rpm
plate 150 uL on each plate (One chloro made by iGEM, 1 amp, made by steed)

In: midnight 12
Out: 2 pm

Making Calcium Competent Cells

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-12

MONDAY, 6/12/17

Colorimetric Asssay, expenses, etc

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-13

TUESDAY, 6/13/17

sMMO colorimetric assay

https://www.scbt.com/scbt/product/azoic-diazo-component-48-91-91-8
https://www.spectrumchemical.com/OA_HTML/chemical-products_Azoic-Diazo-Component-48_TCI-F0093.jsp?minisite=10020&respid=22372

http://www.sigmaaldrich.com/catalog/product/aldrich/185507?lang=en®ion=US
http://www.sigmaaldrich.com/catalog/product/sial/n1000?lang=en®ion=US
http://www.sigmaaldrich.com/catalog/product/aldrich/147141?lang=en®ion=US

Napthalene $31.70
1-Napthol $29.30
2-Napthol $27
tetrazotized o-dianisidine $184-$268

School discount?

Primers come In

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-14

WEDNESDAY, 6/14/17

1:20 dilution of oligos, test on nanodrop
Make 10 uM Stock solution for PCR (use excel spreadsheet)

PCR reactions

Table1

	A	B	C	D
1	**1 sMMO 1**	**2 sMMO2**	**3 GroEL/ES**	**4 dhlB**
2	10 uL MM	10 uL MM	10 uL MM	10 uL MM
3	1 uL FWD	1 uL FWD	1 uL FWD	1 uL FWD
4	1 uL REV	1 uL REV	1 uL REV	1 uL REV
5	1 uL temp	1 ul temp	1 uL temp	37 temp
6	37 uL H20	37 uL H20	37 uL H20	1 uL H2O

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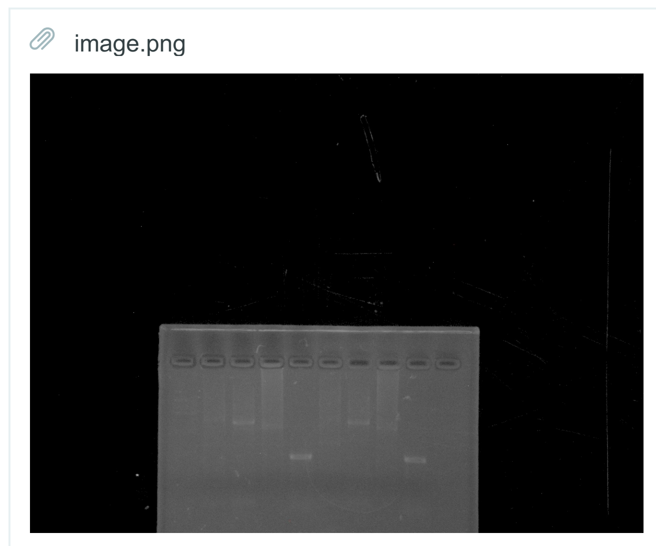


Edit 7/7 WTF is this Gel?

Thurday

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-15

THURSDAY, 6/15/17

Katie checked Amp plates by streaking, iGEM 2 sleeves were bad

I've made a Huge Mistake

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-17

SATURDAY, 6/17/17

The key part in the phrase "linearized plasmid backbone" is the word **linearized.**

195 uL chloro in 250 lb AGAR

Plain plate IN: 11:50
Out: 3:00 pm

Other plates: In 2:37
Out: 4:37

Digest[edit]

- Enzyme Master Mix for Plasmid Backbone (25ul total, for 5 rxns)
  - 5 ul NEB Buffer 2
  - 0.5 ul BSA
  - 0.5 ul EcoRI-HF
  - 0.5 ul PstI
  - 0.5 ul DpnI (Used to digest any template DNA from production)
  - 18 ul dH20
- Digest Plasmid Backbone
  - Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total)
  - Add 4 ul of Enzyme Master Mix
  - Digest 37C/30 min, heat kill 80C/20 min

Ligation[edit]

- Add 2ul of digested plasmid backbone (25 ng)
- Add equimolar amount of EcoRI-HF SpeI digested fragment (< 3 ul)
- Add equimolar amount of XbaI PstI digested fragment (< 3 ul)
- Add 1 ul T4 DNA ligase buffer. **Note:** Do not use quick ligase
- Add 0.5 ul T4 DNA ligase
- Add water to 10 ul
- Ligate 16C/30 min, heat kill 80C/20 min
- Transform with 1-2 ul of product

Note: For linearized plasmid backbones provided by iGEM HQ, a plasmid backbone with an insert of BBa_J04450 was used as template. As a result any red colonies that appear during your ligation may be due to the template as a background. Digesting with Dpn1 before use should reduce this occurrence.

Monday Funday

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-19

MONDAY, 6/19/17

Agenda:

Column purify dhlB and smmo2 from first PCR reaction.

Re-do PCR reaction with Jackson using Steed's taq 5x mix
    possibly tweak extension time?*

Lunch

Transfect HEK cells for Meigs

Restriction digest (need Dpn1)
Ligation
Transformation

Shopping list for Steed

5x Taq master mix
Chloramphenicol
DpnI
Napthalene
1-napthol
2-napthol
Tetrazotized o-dianisidine

Availble

Master Mix Recipe

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-20

TUESDAY, 6/20/17

Plasmid Master Mix

Table1

	A	B	C
1	2.5	uL	2.1 Buffer Mix 10x
2	0.5	uL	EcoRI
3	0.5	uL	PstI
4	0.5	uL	DpnI
5	20.75	uL	ddH20
6	24.75		
7			

Forward Master Mix

Table2

	A	B	C
1	2.5	uL	2.1 Bufer Mix 10x
2	0.5	uL	EcoRI
3	0.5	uL	SpeI
4	21.25	uL	ddH20
5	24.75	Total	
6			

Reverse Master Mix

Table3			
	A	B	C
1	2.5	uL	2.1 Buffer Mix 10x
2	0.5	uL	XbaI
3	0.5	uL	PstI
4	21.25	uL	ddH20
5	24.75	Total	
6			

Steps taken:

Restriction Digest (1 hour ~37, 20 min heat inactivation @ 80 C)

Ligation (1 hour, 20 min heat inactivation @ 80 C)

Transformations

Digest

- Digest Plasmid Backbone
  - Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total)
  - Add 4 ul of Enzyme Master Mix
  - Digest 37C/30 min, heat kill 80C/20 min
- Inserts
  - Add 4 uL of 25ng/uL insert (equimolar, make a dilution)

Ligation[edit]

- Add 2ul of digested plasmid backbone (25 ng)
- Add equimolar amount of EcoRI-HF SpeI digested fragment (< 3 ul)
- Add equimolar amount of XbaI PstI digested fragment (< 3 ul)
- Add 1 ul T4 DNA ligase buffer. **Note:** Do not use quick ligase
- Add 0.5 ul T4 DNA ligase
- Add water to 10 ul
- Ligate 16C/30 min, heat kill 80C/20 min
- Transform with 1-2 ul of product

Shopping List

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-20

TUESDAY, 6/20/17

Shopping list for Steed

Table1

	A	B	C
1	5x Taq master mix	140	ttps://www.neb.com/products/m0270-taq-2x-master-mix
2	Chloramphenicol	51.86	https://www.fishersci.com/shop/products/chloramphenicol-98-acros-organics-3/p-3734180
3	DpnI	63	https://www.neb.com/products/r0176-dpni
4	Napthalene	31.70	http://www.sigmaaldrich.com/catalog/product/aldrich/147141?lang=en®ion=US
5	Tetrazotized o-dianisidine	268	https://www.scbt.com/scbt/product/azoic-diazo-component-48-91-91-8
6			
7			
8			
9			
10			

PCR tubes
Pipette Tips
SOC
1-napthol (optional)
2-napthol (optional)

TBE - tris, boric acid, EDTA (seems like chemistry department has this) alternative acetate for boric -> TAE

Running Confirmation Gel

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-26

MONDAY, 6/26/17

Table1

	A	B	C
1	2.5	uL	2.1 Buffer Mix 10x
2	0.5	uL	EcoRI
3	0.5	uL	PstI
4	21.5	uL	ddH20
5			
6			

4 uL of each miniprep, 4 uL master mix, 37 C 30 min, run on gel

Ladder GroA sMMOB C D E F

***Needs picture of gel, everything came out at 2KB. Initially thought that there might have been undigested circular plasmid in the ligation, transfering chloramphenicol resistence. However, the backbone is linearized, theoretically meaning it shouldn't transfer resistence. ***

coding plasmid for amplification used by iGEM contains RFP, size is ~3 kB

PCR amplification

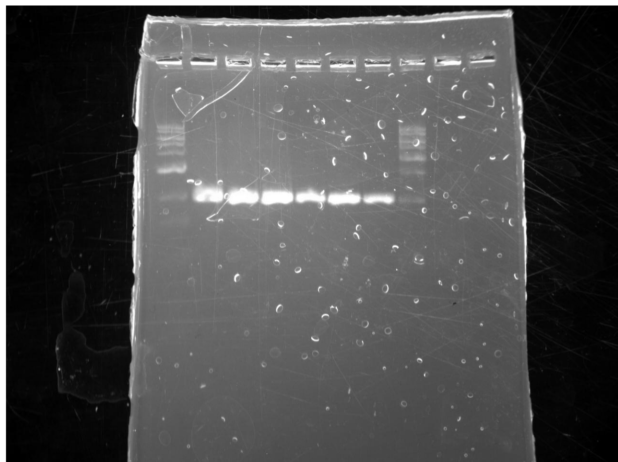
We'll use VF and VR2 to size up what's inside the plasmids.

3250 for GroEl/dhlb
5733 for sMMMO 1 &2.
314 Negative result

Gel with internal bubbles

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-06-27

TUESDAY, 6/27/17



BIG SHOT RESTRICTION DIGEST

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-29

THURSDAY, 6/29/17

Going for a 1:1 ratio of insert to vector

sMMO 1 236ng/uL
sMMO2 314 ng/uL
Gro/El 131 ng/uL (PCR reaction)
dhlB2 ~300 ng/uL

Puc19 279 ng/uL

Shooting for 300 ng each, vector needs 1200 ng

1.27 uL smo1
0.96 smo2
2.3 uL Gro
1 uL dhlB
4.3 uL puc19

1 uL of 2.1 buffer
0.5 PstI
0.5 EcoRI
    DNA?
    ddH20 to 10 uL

1 hour digest @ 37
20 min at 80 to inactivate enzymes

Ligation:

Restriction Digests for 7/1

Project: TCE Biodegredation Project
Authors: Katie Brown
Date: 2017-07-01

SATURDAY, 7/1/17

Restrictions were performed for sMMO1 A&B, sMMO1 A&B, dHLB A&B, and Gro A&B.

Master mix:
4 uL EcoRI
4 uL PstI
4 uL 2.1 NEB buffer
28 uL ddH2O

Restriction digest:
4 uL DNA sample
4 uL master mix

Run at 37 C for 30 minutes

Gel was then loaded and run for 45 minutes at 110 volts

Loading of gel:
Lane 1 - Steed's ladder (1.5 uL)
2 - sMMO1 A (8 uL)
3 - sMMO1 B
4 - sMMO2 B
5 - sMMO2 A
6 - dH B
7 - dH A
8 - Gro A
9 - Gr0 B
Lane 10 - Odin ladder (12 uL)

Back to Basics

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-06

THURSDAY, 7/6/17

Jackson is running another restriction digest -> visualization gel on our puc19 insertion attempts

I am running a viz gel on our old PCR to see if we have product of the right size. If we do, I'll re-do PCR to get more of our inserts (50 uL reaction volume). Then PCR cleanup, restriction digest, purification gel, nanodrop, test ligation etc...

1.5 ul Ladder

4 uL part +1 uL 6x dye

Ladder sMMO1 sMMO2 GroEl/Es dhlB Puc19 (undigested)

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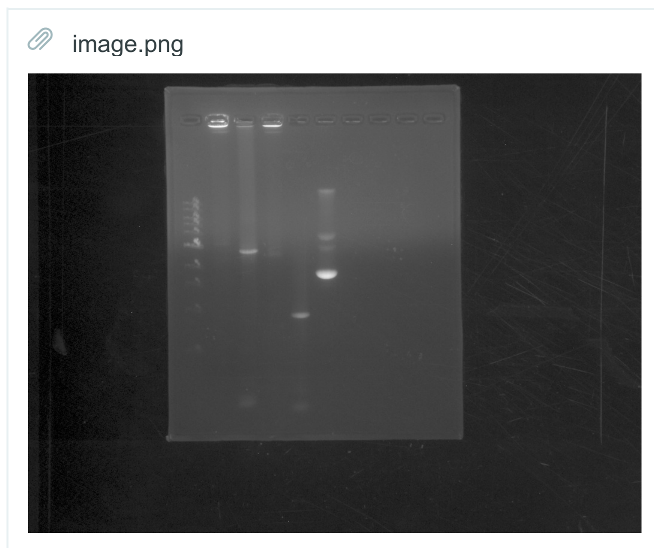

Ladder Smo1A Smo1B Smo2A SMo2B GroA GroB dhlbA dhlbB puc19

Picture????

PCR 50 uL reaction, w/ 25 ng/uL dilution of parts:

25 uL 2x Q5 Master mix
4 uL part (100ng)
2.5 uL Prefix F (10 uM)
2.5 uL Suffix R (10 uM)
16 uL ddH2O

FRIDAY IN THE SKY

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-07

FRIDAY, 7/7/17

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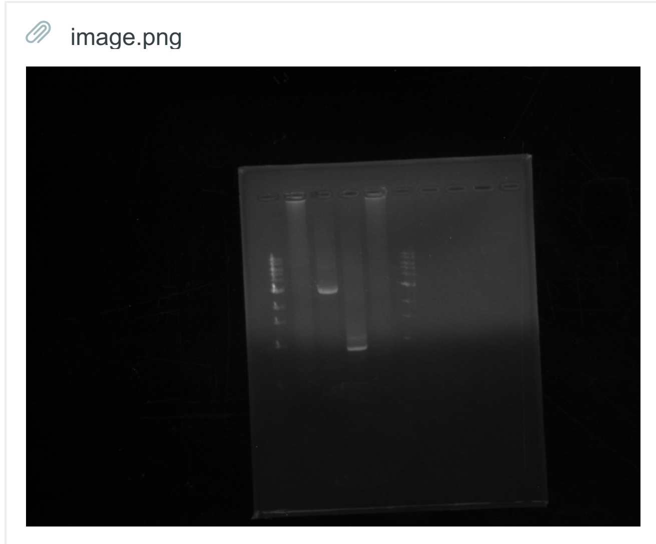


Ladder Smo1 Smo2 dhlB GroEl/ES

Will re-try PRC with all parts using a temperature gradient on the block

Smo1 Smo2 GroEl dhlb
A B C D 60 C
E F G H 55

CURRENT DNA INVENTORY AND PLAN
1 uL Original sMMO 1 (dilute to 25 ng/uL, attempt PCR)
<1 ul Orignal sMMO2 (dilute to 25 ng/uL, attempt PCR)
0 GroEL/ES (add 5 uL ddH2O, attempt PCR)
2 uL dhlB (make some more 25 ng/uL, attempt PCR)

25ng/uL dilutions of parts (less than 3 uL each, close to 0)

PCR products

7/7
sMMO2: gel confirmed 82.5 ng/uL
dhlB: gel confirmed 141 ng/uL HOWEVER 260/280 ratio low, 1.58

Unknown Date products, no confirmation gel or nanodrop results
sMMO1 111 ng/uL
GroEl/ES 131ng/uL

sMMO part 2 117 ng/uL
dhlB 107 ng/uL

PLAN:

PCR Using:

2x Q5 Master Mix, temperature variable, reduce to 25 cycles

sMMO 1 remade 25 ng/uL
sMMO2 remade 25 ng/uL
GroEL/Es prayer shot
dhlB 25ng/uL

sMMO2 7/7 PCR product
dhlB 7/7 PCR product

Unknown PCR products?
sMMO1 111 ng/uL
sMMO2 117 ng/uL (6/19)
GroEl/Es 131 ng/uL
dhlB 107 ng/uL (6/19)

Tuesday PCR

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-11

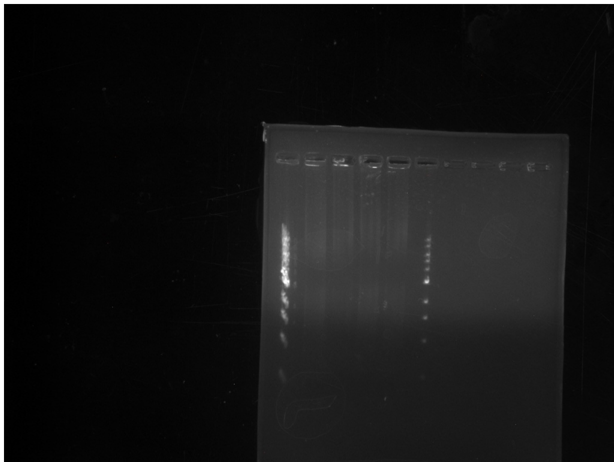
TUESDAY, 7/11/17

Q5 PCR reaction conditions
100 ng DNA
25 uL 2x Q5 Master mix
2.5 uL Prefix F (10 uM)
2.5 uL Suffix R (10 uM)
19-18 uL ddH2O

30 cycles @ 66

L M N O = SMo1 sMo2 Gro dhlb

1 uL smo1 @ 111
2 uL Sm02 @ 45.9
1 uL GroEl @ 131
2 uL dhlb @ 42.7



Will try again with 1-10ng DNA, 30 cycles

ALSO, may need to consider Gibson assembly to speed up process, need to get on the mass spec ASAP.

RE-doing puc19 digest

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-19

WEDNESDAY, 7/19/17

Digest with EcoRI
9 uL puc19
2 uL CutSmart
1 uL EcoRi
8 uL ddH2O

Run on Low melt agarose gel (0.7g low melt + 50 mL TAE + 5 uL)
ladder cut puc19 plasmid puc19

middle band = 38.1 ng/uL

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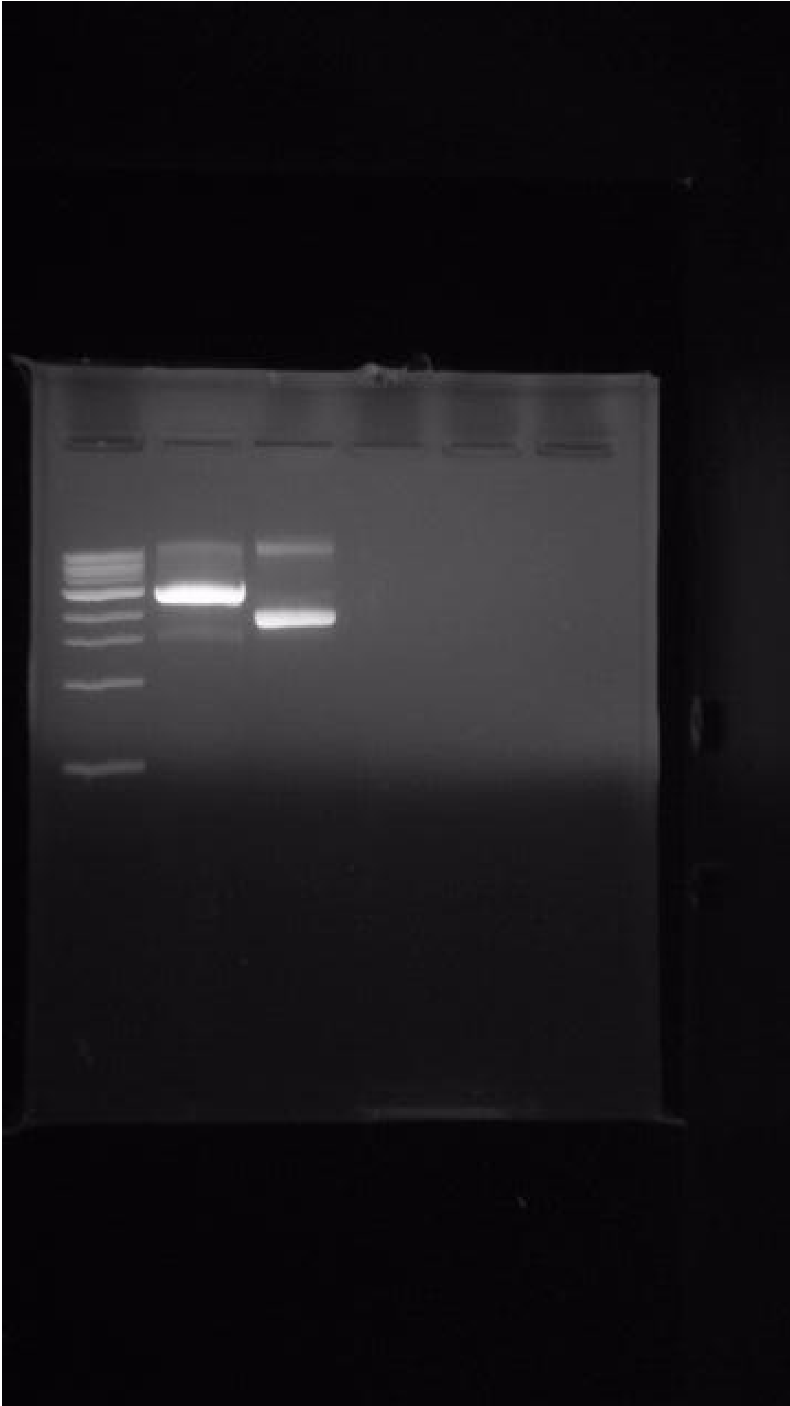

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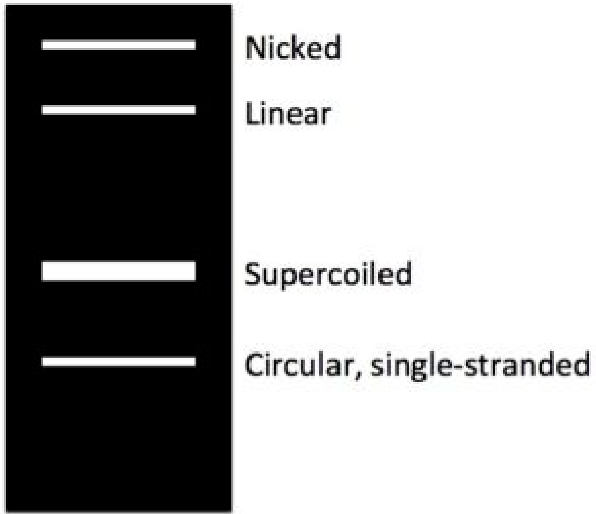


GIBSON PCR

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-20

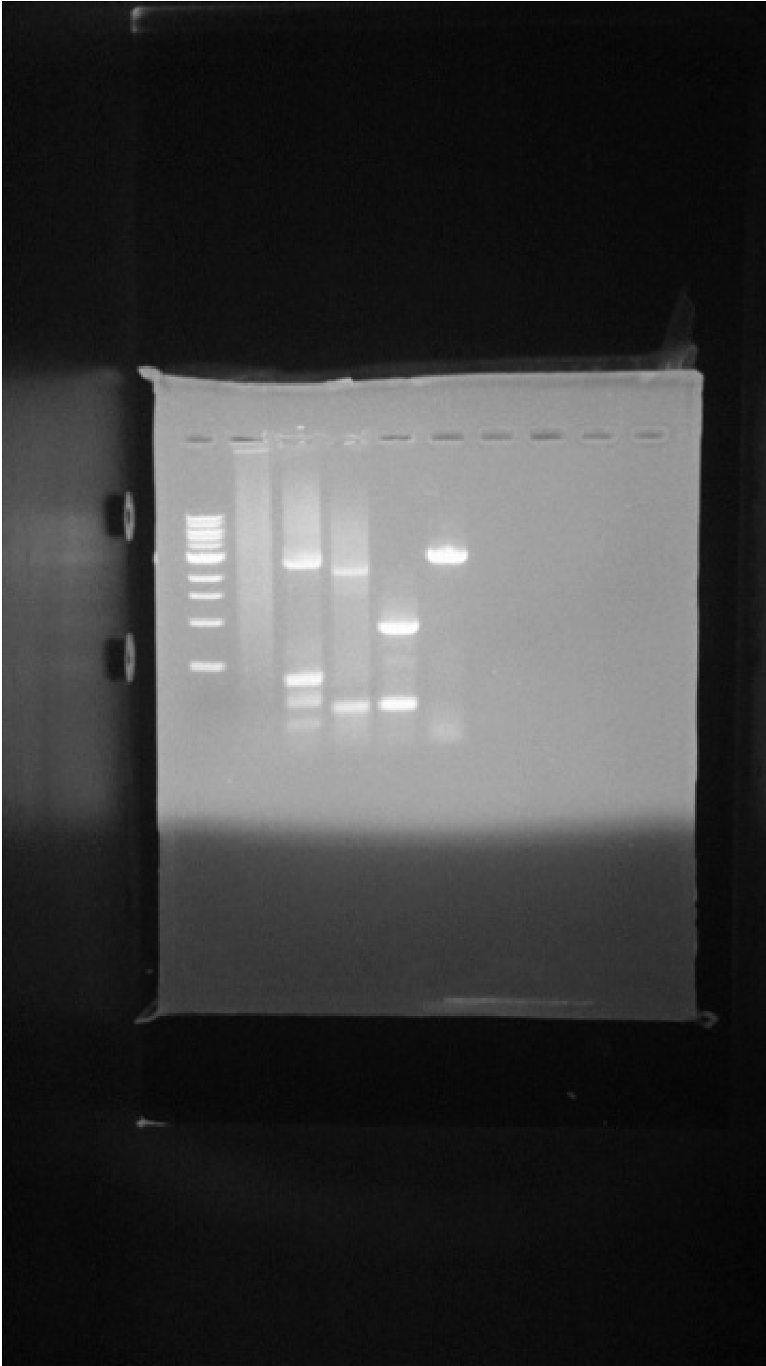
THURSDAY, 7/20/17

Oligos came in, resuspended in 25 uL, 1:20 dilution, nanodrop, 10 uM concentration

PCR 25 uL (I think annealing was at 66?)
12.5 uL 2x Q5 mix
1.25 uL 10uM FWD
1.25 uL 10uM REV
1 uL 1ng/uL PCR product
9 uL dd H_2O

1 sMMO1
2 sMMO2
3 GRoEl/ES
4 dhlb
5 puc19

image.png



sMMO1: Need to lower annealing temperature
sMMO2: up annealing temp
GroEL/ES: up annealing temp
dhlB: up annealing temp
puc19: looks perfect

Puc19 FWD 1 PCR

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-24

MONDAY, 7/24/17

Resuspend PUC19 FWD, Nanodrop

PCR Puc19 with FWD 1, dhlB with REV 1, and sMMO1 with re-done primer suspensions, at low annealing temp.

BCD

puc19 (anneal at 65, but I'll do 66)
12.5 uL Q5
1 uL puc19 (1 ng/uL)
1.25 uL puc19 REV (10 uM)
10.25 uL puc19 FWD 1 (0.2 uM)

dhlB (anneal at 66)
12.5 Q5
1 uL dhlB
1.25 FWD 1
1.25 REV 1
9 uL ddH2O

sMMO1 (anneal at 58, extra extension time)
12.5 Q5
2 uL sMMO1
1.25 FWD 1
1.25 REV 1
8 uL ddH2O

PCR and Gel cleanup

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-26

WEDNESDAY, 7/26/17

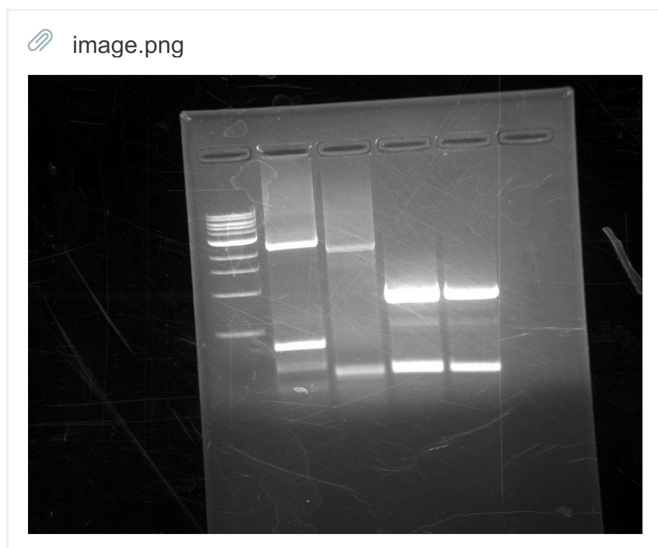
I've combined all past working PCR reactions into one tube. Some of the PCR had multiple bands, I'm attempting to isolate the correct ones via gel purification. I will then re-attempt PCR, which should bump up the annealing temp of the oligos since more bp will anneal. I'll try 2 step pcr and at 69 with various concentrations of DMSO, which hopefully will peel off the extraneous binding sites. Note that this round of PCR I used the set of primers that has dhlB ending with the Biobrick suffix, NOT the terminators + Biobrick ending which required so many basepairs.

sMMO1 still isn't cooperating in PCR. I will order the sMMO1 REV 2 later today.
puc19 looked great and was very concentrated. Still needs PCR cleanup. May PCR up more just have in the back pocket.

If we assemble the way things are going now, we'll need to add in terminators and possibly promoter after the pathway is assembled. I should look through the biobrick catalog to see if there is a backbone which comes with those, or if we'll have to add them in with ligation, or PCR etc...

Ladder sMMO2 GroEl/ES dhlB more dhlB

image.png



sMMO2: 37.1 ng/uL
GroEL/Es 19.3 ng/uL
dhlB 175.6 ng/uL

GroA 50 uL (x2 @ 64 anneal) (GroA, B)
25 uL Q5 MM
2.5 uL FWD
2.5 uL REV
1 uL GroA
19 uL ddH20

sMMO1 (62x10,66x10,72x10) (C)
12.5 uL Q5 MM
1.25 sMMO1 FWD
1.25 sMMO1 REV
1 uL sMMO1
9 uL ddH20

sMMO1 (D) (5% DMSO, 62 annealing)
12.5 uL Q5 MM
1.25 FWD
1.25 REV
1 uL sMMO1
1.25 uL DMSO
7.75 ddH2O

sMMO1 (5% DMSO, stepped) E
12.5 uL Q5 MM
1.25 FWD
1.25 REV
1 uL sMMO1
1.25 uL DMSO
7.75 ddH2O

sMMO2 (66 annealing) F,G
25 uL Q5
2.5 FWD
2.5 FWD
1 uL sMMO2
19 uL ddH2O

G
2.5 uL DMSO
16.5 uL ddH2O

Ladder C, E, D, Gro, B, F, G

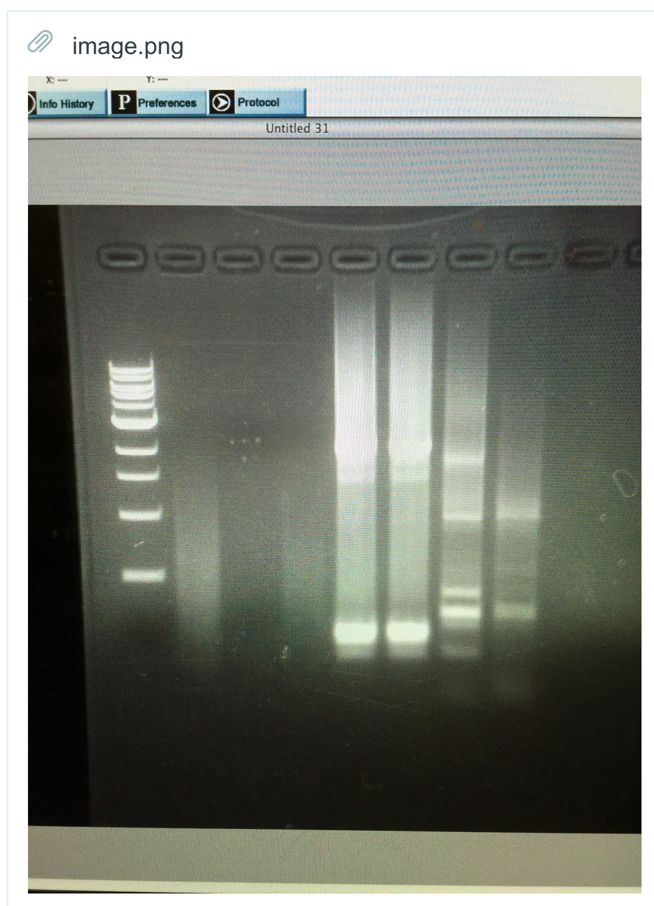


image.png

Attempt to Gel purify Gro + B and also F

GIBSON PCR

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-08-11

FRIDAY, 8/11/17

A-F oligos came in, resuspended

Made 10 uM stock

Table1

	A	B
1		25 uL
2	Template (1ng/uL)	1 uL
3	Forward primer (10 uM)	1.25 uL
4	Reverse Primer (10 uM)	1.25 uL
5	2x Q5 Master Mix	12.5 uL
6	ddH2O	9 uL

Annealing Temps

Table2

	A	B	C
1	A	puc19	69
2	B	sMMO1	70
3	C	sMMO2	70

3 uL + 1uL dye

Ladder A B C

Crap

RE-Nanodrop
A 2351.3 1.76

Table3

	A	B	C	D	E
1	A 2351.3	2351.3	1.76		
2	B	2776.4	1.65		New
3	C	339.6	1.68	946	1.82
4	D	1433.3	1.78		
5	E	2995	1.68		
6	F	1991.5	2.0		

ABC puc, smo1, smo2 69,70,70

DEF puc, smo1, smo2 67, 68, 68

Table4

	A	B
1	50 uL	
2	2 uL	DNA
3	2.5 uL 10uM	FWD
4	2.5 uL 10uM	REV
5	25 uL	Q5
6	18 uL	

Thursday

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-08-23

WEDNESDAY, 8/23/17

Table1

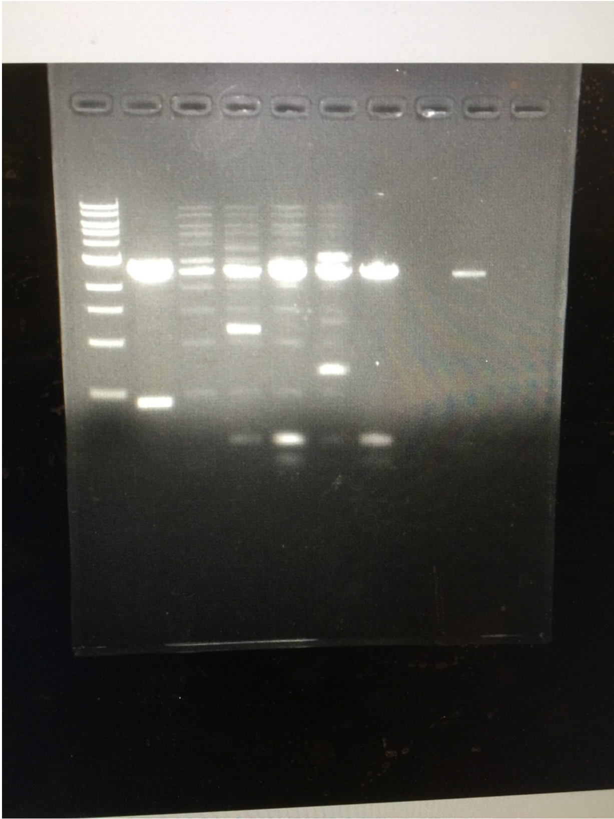
	A	B	C	D	E	F	G
1	Label	ng/uL	Ratio				
2	1-1	129	1.91				
3	2-1	85.5	1.9				
4	3-1	30	1.79				
5	4-1	39	1.85				
6							
7	1-2	156.9	1.91				
8	2-2	113	1.97				
9	3-2	131.6	1.73				

image.png

thumbnail

Master mix (7x)
3.5 EcoRI
3.5 PstI
7 uL 2.1 Buffer

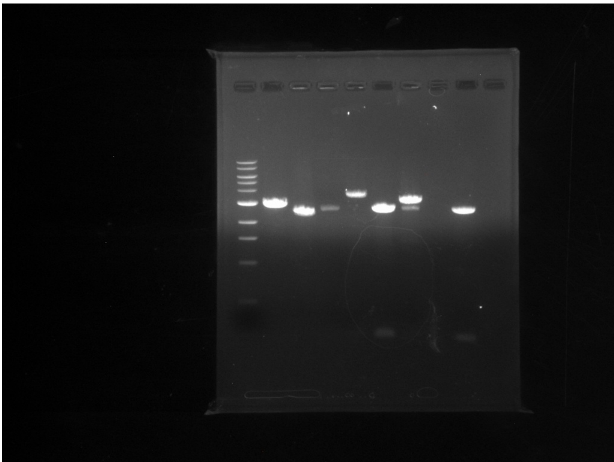
image.png



New Gel that doesn't suck:

Ladder 1-1 2-1 3-1 **4-1** . 1-2 2-2 3-2

image.png



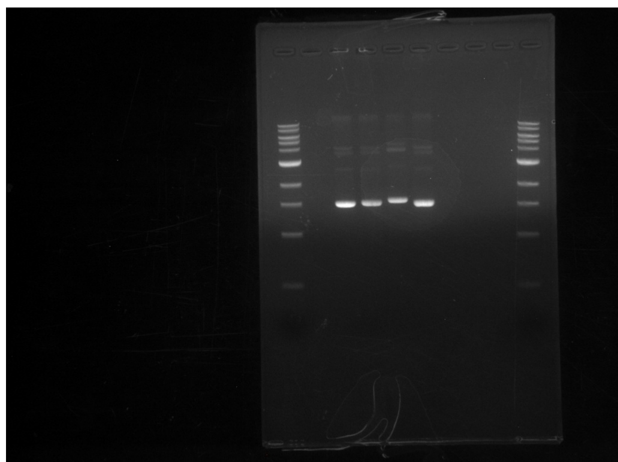
Friday

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-08-25

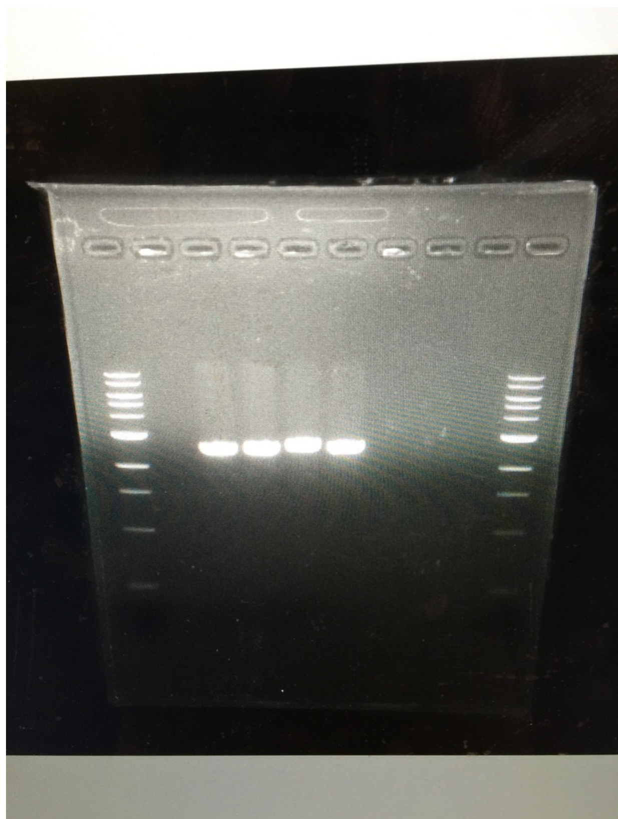
FRIDAY, 8/25/17

Miniprepped liquid cultures,
Ran digested and un-digested gels

undigested



Digested with EcoRi



dhlB PCR + others

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-08-28

MONDAY, 8/28/17

Table1

	A	B	C
1		**25 uL**	
2	Template (1 ng/uL)	**1 uL**	
3	Forward primer	**1.25 uL 10uM**	
4	Reverse Primer	**1.25 uL 10uM**	
5	2x Master mix	**12.5 uL**	
6	ddH2O	**9 uL**	

Table2

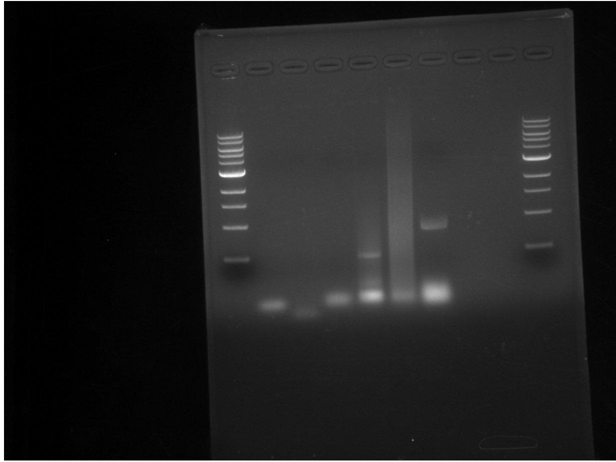
	A	B	C	D	E
1		**Biobrick Part**	**FWD**	**REV**	**Anneal Temp**
2	**1**	dhlB	K	Real Suffix	72
3	**2**	Biobrick J04450	B-prep 1	SB prep	65****
4	**3**	sMMO1	Real Pre	Real Suffix	72
5	**4**	sMMO2	Real Pre	Real Suffix	72
6	**5**	GroEL/Es	Real Pre	Real Suffix	72
7	**6**	dhlB	Real Pre	Real Suffix	72

****** Nick relearns his lesson that iGEM is not to be trusted ****** TM Bprep = 74, TM SB 65

Biobrick 11.9 ng/uL 1.67 ratio

ladder 1-7 ladder

image.png



#6 dhlB concentration was 863?! on nanodrop downstairs.

Table3

	A	B	C	D	E	F	G
1		**Biobrick Part**	**FWD**	**REV**	**Anneal Temp**		
2	**1**	dhlB	K	Real Suffix	72	69	
3	**2**	sMMO1	Real Pre	Real Suffix	72	69	
4	**3**	sMMO2	Real Pre	Real Suffix	72	69	
5	**4**	GroEL/Es	Real Pre	Real Suffix	72	69	
6	**5**	dhlB	Real Pre	Real Suffix	72	69	
7	**6**	Gibson	C 8.32	F 2	72	69	

1:5 dilution of dhlB pcr

30.3 ng/uL 1.81

10 226.7 1.85
11 380 1.65
12 13.1 1.45

9 221 1.90

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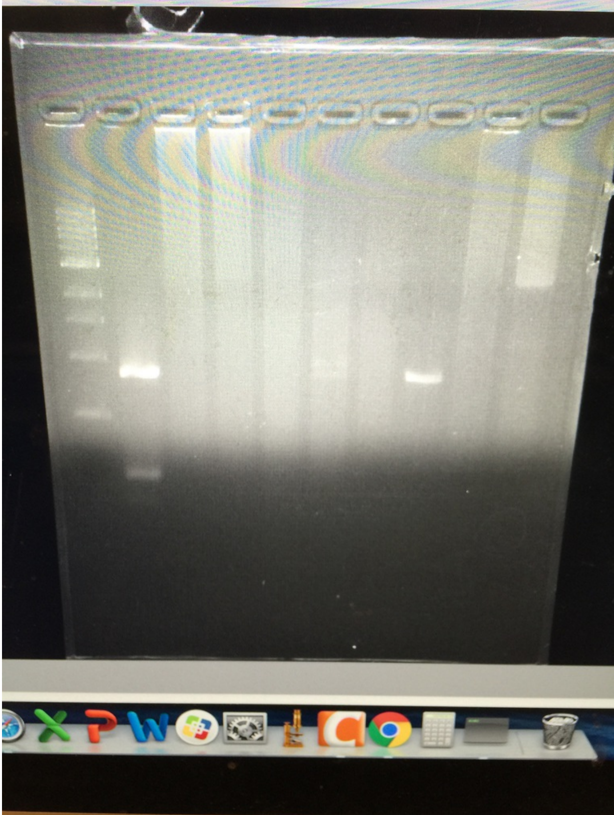

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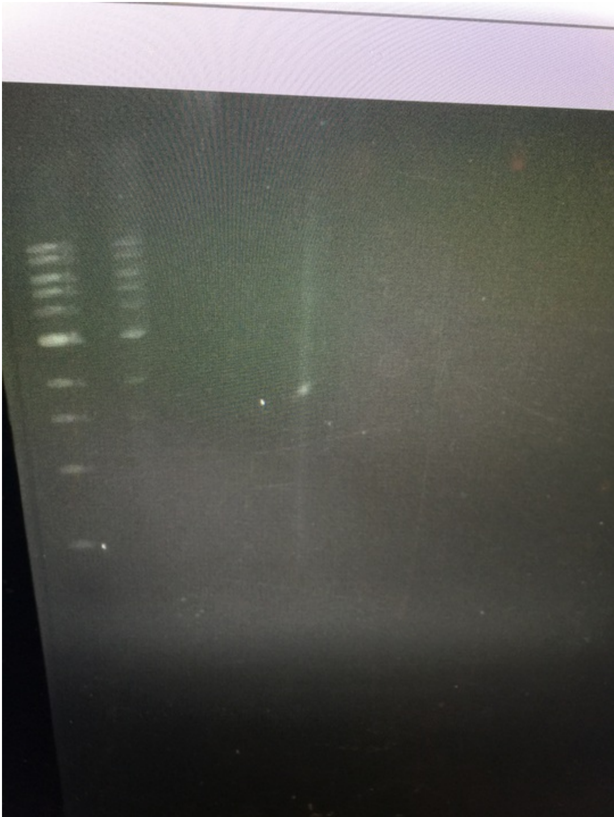

Table4

	A	B	C	D
1	Gibson assembly	1430	2.55	
2	Real Prefix	1060	1.79	
3	Real Suffix	1849.4	2.0	
4	B-prep	407.6	1.96	
5	SB-prep	1417	1.93	
6	K	128.1	1.97	
7	C	9.1	1.44	14.7
8	F	43.8	1.66	

Wed Gib Grodhlb

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-08-30

WEDNESDAY, 8/30/17

1:5 dilution of dhlB pcr

30.3 ng/uL 1.81

10 226.7 1.85
11 380 1.65
12 13.1 1.45

9 221 1.90

image.png

10-16

image.png

1-9

ladder 4 EcoRI/PstI   7Eco/P 10 11 (repeats)

Labor Day

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-09-04

MONDAY, 9/4/17

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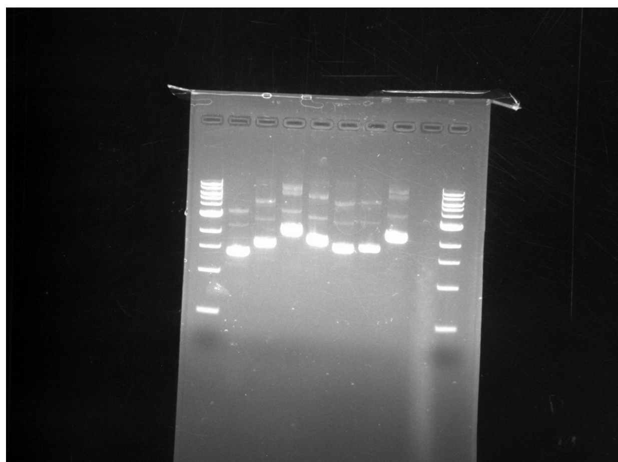
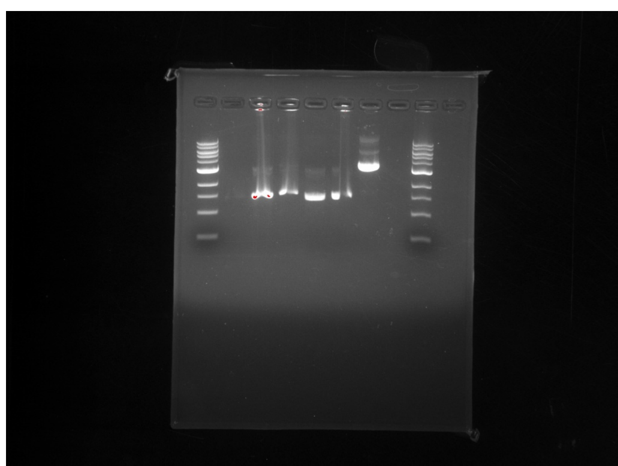


1-7 Gro lux Gro Red sMMO SMMO Gro

image.png



8-16 sMMO Gro Lux sMMO sMMO Gro Red

Restriction of Xbai 1 and SPE

Project: TCE Biodegredation Project
Authors: Katie Brown
Date: 2017-09-05

TUESDAY, 9/5/17

Restrictions were performed for Xbai1 and SPE

Restriction digest:
6 uL DNA
1 uL 2.1 buffer
1 uL of restriction enzyme
Water to fill to 30 uL total

Run at 37 C for 30 minutes

Gel was then loaded and run for 45 minutes at 115 volts

Loading of gel:
Ladder
3s
3s+x
7s
7s+x

Untitled

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-09-21

THURSDAY, 9/21/17

Table1

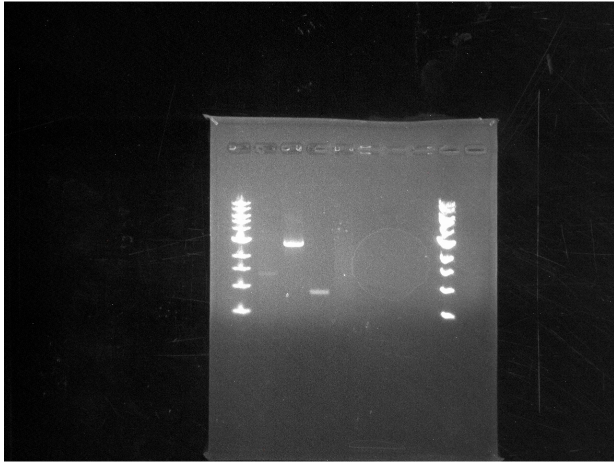
	A	B
1		
2		
3		
4		
5		
6		
7		

PCR Reaction Product 8/07/2017

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-07-10

MONDAY, 7/10/17

image.png



1. 1 kB Ladder
2. sMMO1 (Hotter Temp)
3. sMMO2 (Hotter Temp)
4. dhlb (Hotter Temp)
5. GroEL/ES (Hotter Temp)
6. sMMO1 (Colder Temp)
7. sMMO2 (Colder Temp)
8. dhlb (Colder Temp)
9. GroEL/ES (Hotter Temp)
10. 1 kb Ladder

Unknown PCR Product of sMMO1/GroEL/ES (7/10)

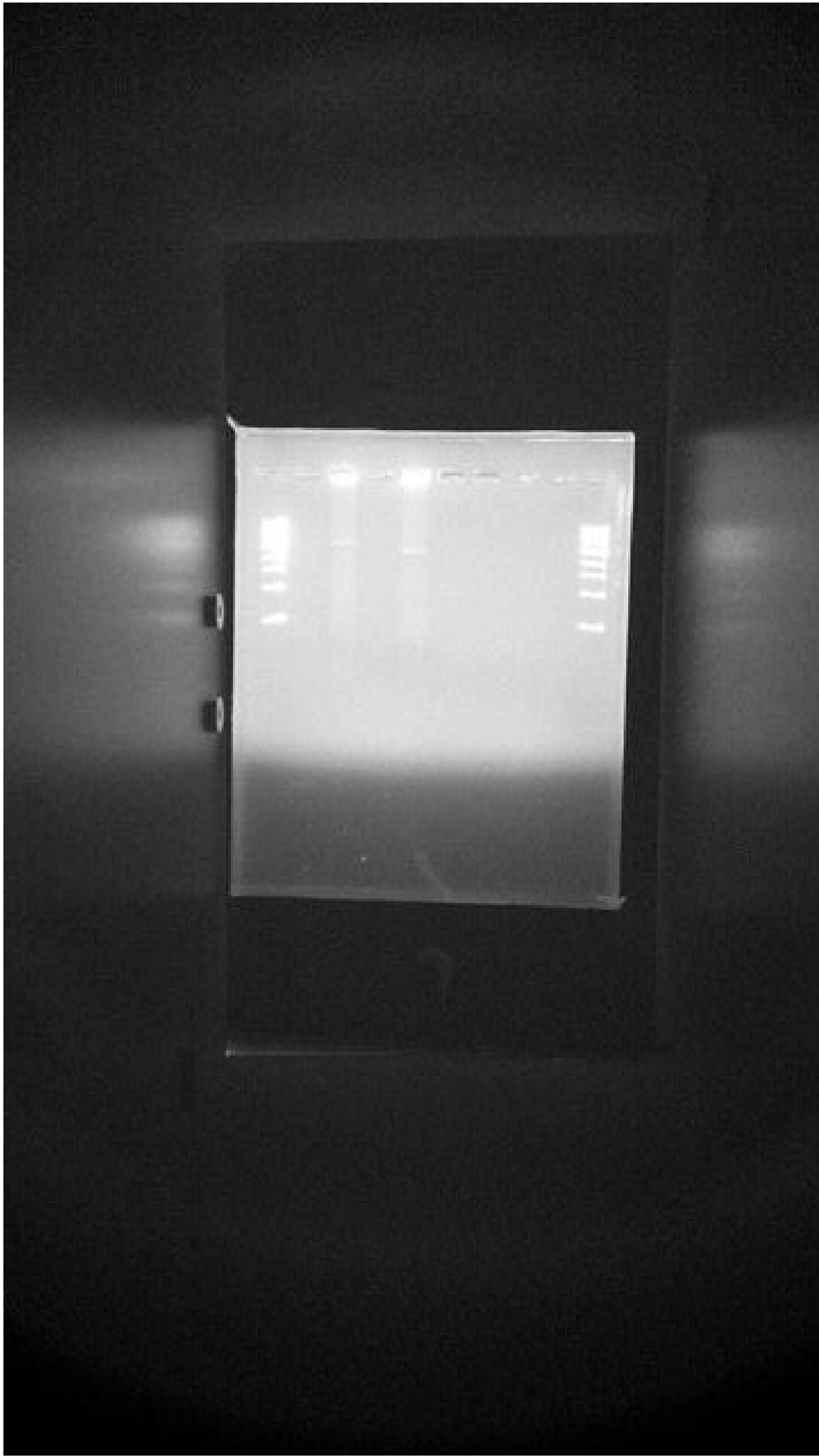
Project: TCE Biodegredation Project

Authors: William Jackson

Date: 2017-07-10

MONDAY, 7/10/17

2017-07-10 17-17-49.760.jpg



1. 1 kb ladder

3. sMMO-1

5. GroEL/ES

10. 1 kb ladder

PCR Reaction Product 7/14/2017 (Jackson's First PCR)

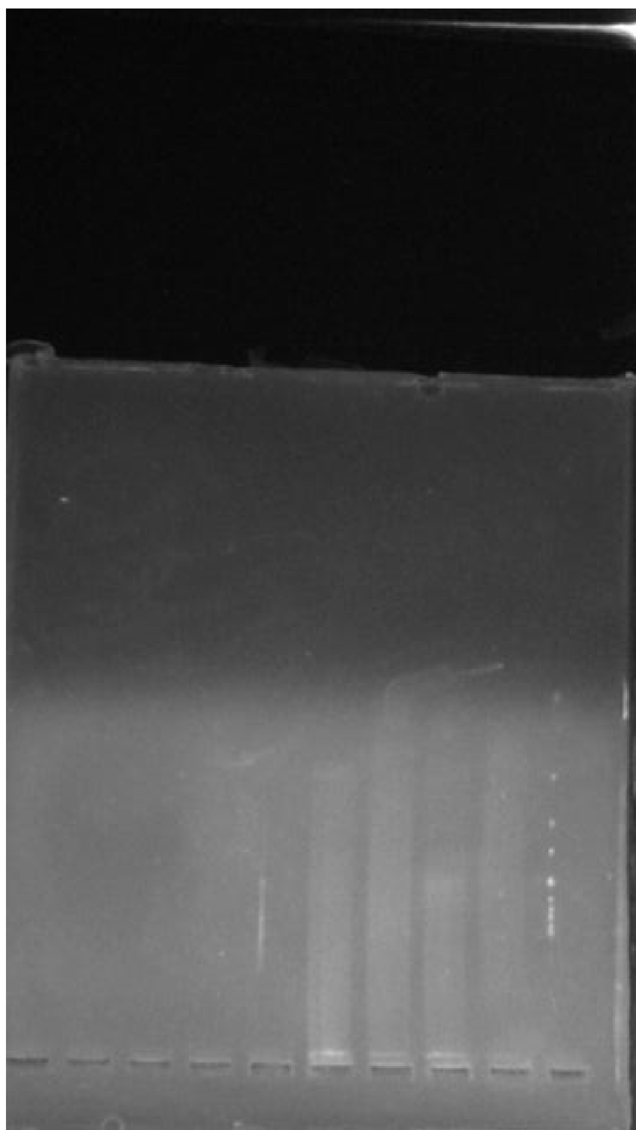
Project: TCE Biodegredation Project

Authors: William Jackson

Date: 2017-07-14

FRIDAY, 7/14/17

2017-07-14 11-26-11.159.jpg



Gel ran on 7/14. PCR Reaction done by Jackson, looks like it didn't work.

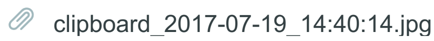
Puc19 Restriction Digest (7/19/2017) Prep for Gibson

Project: TCE Biodegredation Project

Authors: Nicholas White

Date: 2017-07-19

WEDNESDAY, 7/19/17



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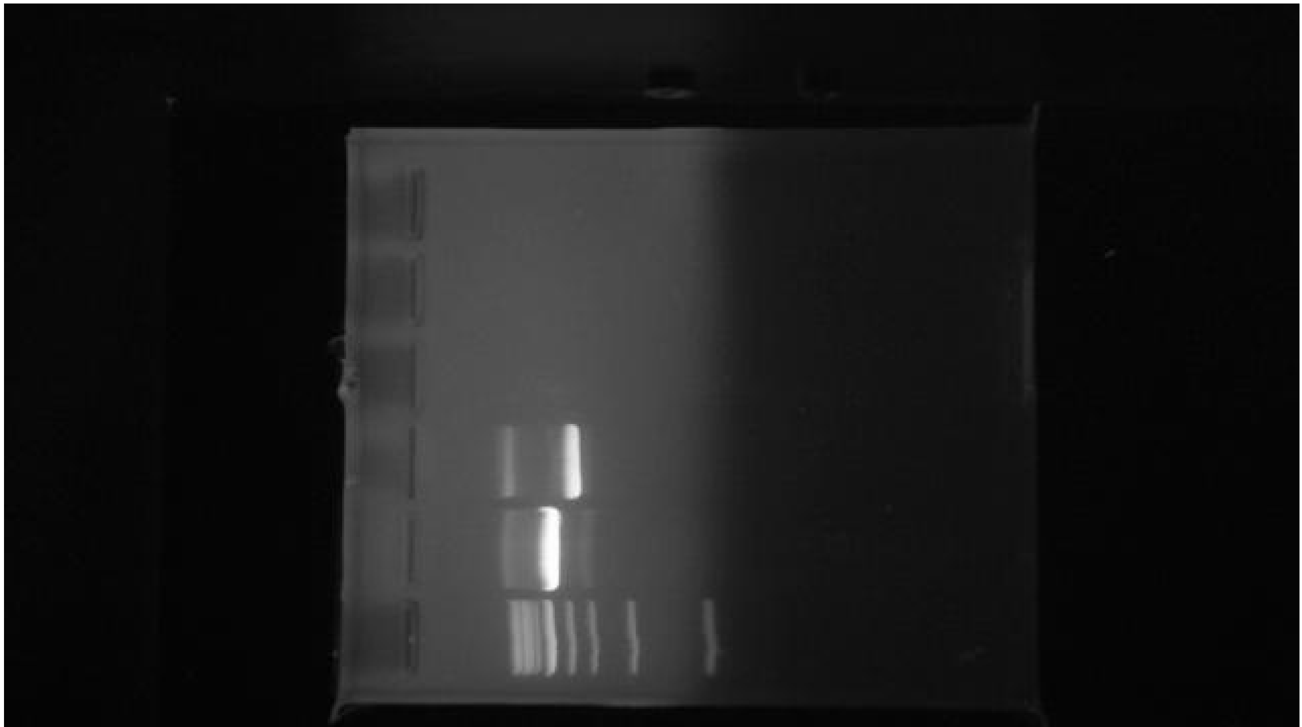
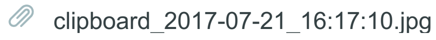


PCR Product (7/21) Q5 Two-Step for Gibson Assembly

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-07-21

FRIDAY, 7/21/17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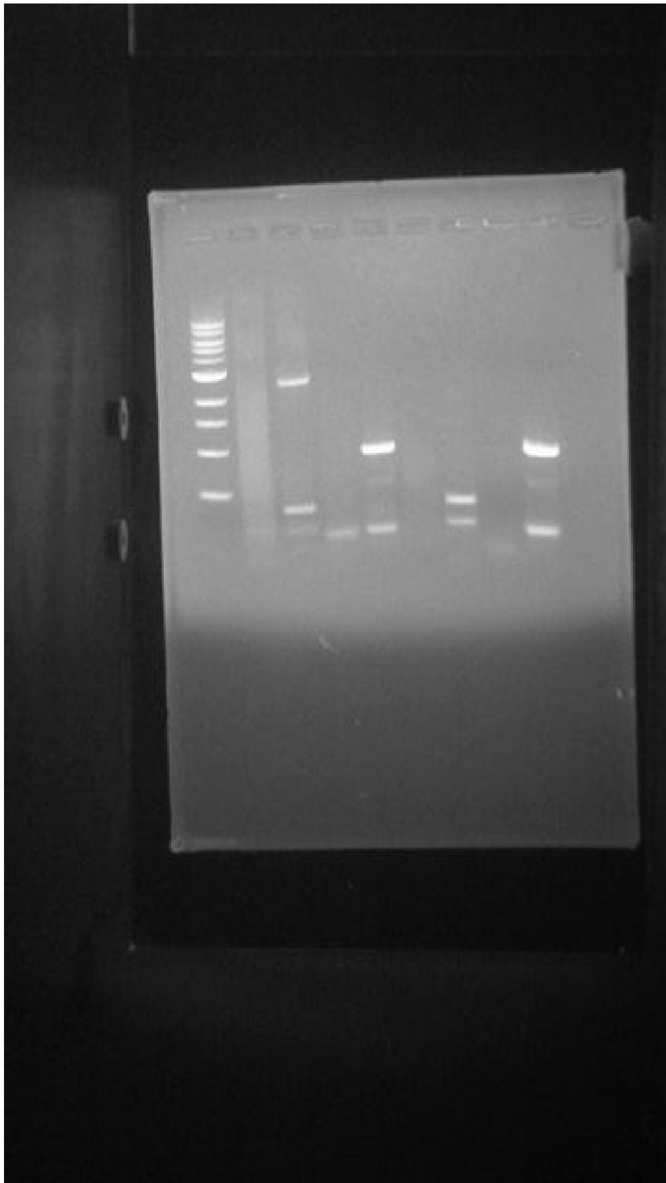

PCR Reaction Product (8/12) New Oligos

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-08-12

SATURDAY, 8/12/17

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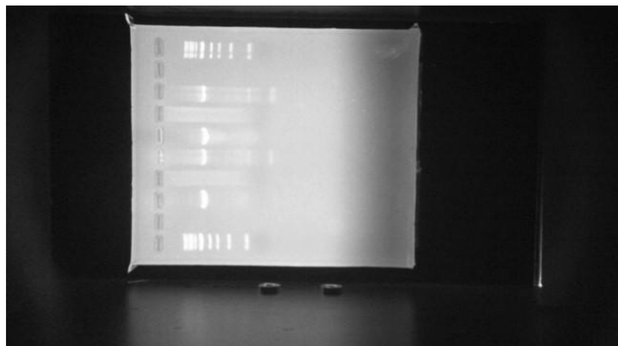

Table1

	A	B
1	Lane	Product
2	1	Ladder
3	2	
4	3	F
5	4	E
6	5	D
7	6	C
8	7	B
9	8	A
10	9	
11	10	Ladder

Nanodrop Results (6/22/2017)

Project: TCE Biodegredation Project

Authors: Nicholas White

Date: 2017-06-22

THURSDAY, 6/22/17

Table3

	A	B	C	D
1	Gro A	105.1	1.85	
2	Smo B	116		
3	Smo C	148	1.87	
4	Smo D	137	1.9	
5	Smo E	167	1.85	
6	Smo F	125	1.89	1
7				

Final Observation of the day at Time 6:36 PM.

Table2

	A	B	C	D
1	Name	Observation	260/280	Notes
2	Gro A	72.7	1.454	
3	sMMO B	70.1	1.87	
4	sMMO C	102.4	1.86	
5	sMMO D	94.4	1.72	
6	sMMO E	96.8	1.84	
7	smmO F	102.4	1.84	
8				
9				
10				
11				
12				
13				
14				

Table1

	A	B	C	D
1				Abs at 260/280
2	GroA	32.9		
3	smmoB	42.8		
4	smmoc	33.5		
5	smmoD	31.9	System gave Error	
6	smmoE	112.8	Holy Shit	
7	smmoF	97.2		
8				
9	Second Round			
10	smmoD	41		1.92
11	smmoE	40.3		1.89
12	smmoF	37.2		
13				
14	After Vacufuge			
15	smmoC	62.6		1.92

Nanodrop Observation 6/25

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-06-25

SUNDAY, 6/25/17

PUC19 Observations

Table1

	A	B	C	D
1	Sample	Ng/Ul	260/280	Notes
2	Puc19-A	279.5	1.76	Prepared by Nick
3	Puc19-B	148.3	1.95	Prepared by Jackson
4				
5				
6				

Puc19

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-06-28

WEDNESDAY, 6/28/17

Run PCR gel. Email Maria Bartolini.

Restriction digest of puc19 and parts

Ligation

Transformation

Nanodrop Results (7/1) (puc19 Single Insertion)

Project: TCE Biodegredation Project

Authors: William Jackson

Date: 2017-07-01

SATURDAY, 7/1/17

Table1

	A	B	C	D
1	Sample	ng/ul	260/280	notes
2	smmo1-A	81.1	1.92	
3	smmo1-B	185.1	1.83	Concerned about not reblanking between samples as per Nicks instruction and contamination given high variance. (Using Steed/Kaur Nanodrop)
4	smmo2-A	184.9	1.84	
5	smmo2-B	203.0	1.65	
6	dhlb-A	144.0	1.85	
7	dhlb-B	145.2	1.88	
8	gro-A	195.1	1.72	
9	gro-B	189.2	1.77	
10				
11	smmo1-A #2	323.4	1.75	I don't even fucking know.

Nanodrop Results (7/7)

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-07-07

FRIDAY, 7/7/17

Before Purification Kit

Table1

	A	B	C
1	Name	ng/ul	260/280
2	sMMO-1	426.4	1.77
3	sMMO-2	530.2	1.77
4	Gro	588.0	1.79
5	dhlb	490.8	1.81

After Purification

Table2

	A	B	C
1	Name	ng/ul	260/280
2	sMMO-1	132	1.80
3	sMMO-2	82.5	1.84
4	Gro	141	1.78
5	dhlb	114.2	1.58

Nanodrop Observation 7/10

Project: TCE Biodegredation Project

Authors: Nicholas White

Date: 2017-07-10

MONDAY, 7/10/17

Table1

	A	B	C
1	Sample	ng/uL	260/280
2	sMMO2	45.9	1.77
3	dhlB	42.7	1.67
4			

Nanodrop Observation [puc19] 7/18/2017

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-18

TUESDAY, 7/18/17

Table1

	A	B	C
1		ng/ul	260/280
2	puc19	202	1.82
3			
4			

puc19 Gel Purification (7/19)

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-07-19

WEDNESDAY, 7/19/17

Table1

	A	B	C
1	Name	[]	260/280
2	Top	2.9	0.058
3	Middle	38.1	1.95
4	Bottom	1.5	1.95

PCR troubleshooting

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-21

FRIDAY, 7/21/17

sMMO1 @ 62 & with Prefix F primer

sMMO2 @ 69 and with 2 step

GroEL/ES @ 69, 2 step

dhlB @ 69 and 2 step

50 uL reactions

25 Q5 Master Mix
2.5 uL FWD
2.5 uL REV
2 uL template
18 uL ddH2O

PCR 25 uL

12.5 uL 2x Q5 mix
1.25 uL 10uM FWD
1.25 uL 10uM REV
1 uL 1ng/uL PCR product
9 uL dd H2O

Table1

	A	B	C	D	E	F	G
1	1	2	3	4	5	6	
2	SMMO1 @ 62	sMMO2 @69	GroEL/ES@69	dhlB@69	SMMO1 @62, prefix FWD	sMMO2 2step	GroEL/ES

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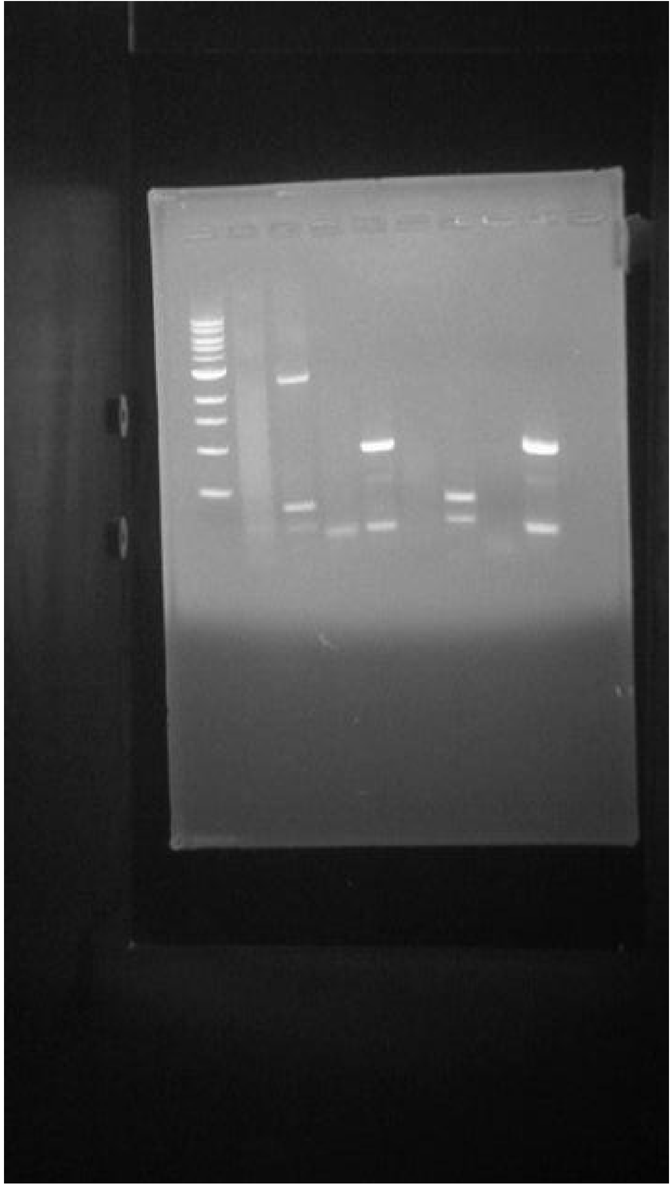


Nanodrop (8/12) New Oligos

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-08-12

SATURDAY, 8/12/17

Table1

	A	B	C	D
1	Sample	[]	260/280	Notes
2	A	403.1	1.79	PUC19
3	B	399.7	1.80	SMMO1
4	C	407.0	1.83	SMMO2
5	D	407.9	1.81	PUC19
6	E	430.1	1.78	SMMO1
7	F	418.8	1.78	SMMO2

Nanodrop Observation (8/15) LQ Gibson SMMO

Project: TCE Biodegredation Project

Authors: William Jackson

Date: 2017-08-14

MONDAY, 8/14/17

Table1

	A	B	C	D
1	Sample	[]	260/280	Notes
2	1	134.9	1.82	
3	2	241.6	1.72	
4	3	95.1	1.85	
5	4	258.8	1.63	

Nanodrop (8/18) dhlB Primer Concentrations

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-08-18

FRIDAY, 8/18/17

Table1

	A	B	C	D
1	Primer	[]	260/280	Notes
2	k primer	2989.6	1.99	
3	j primer	278.8	1.99	
4				

PCR Saturday

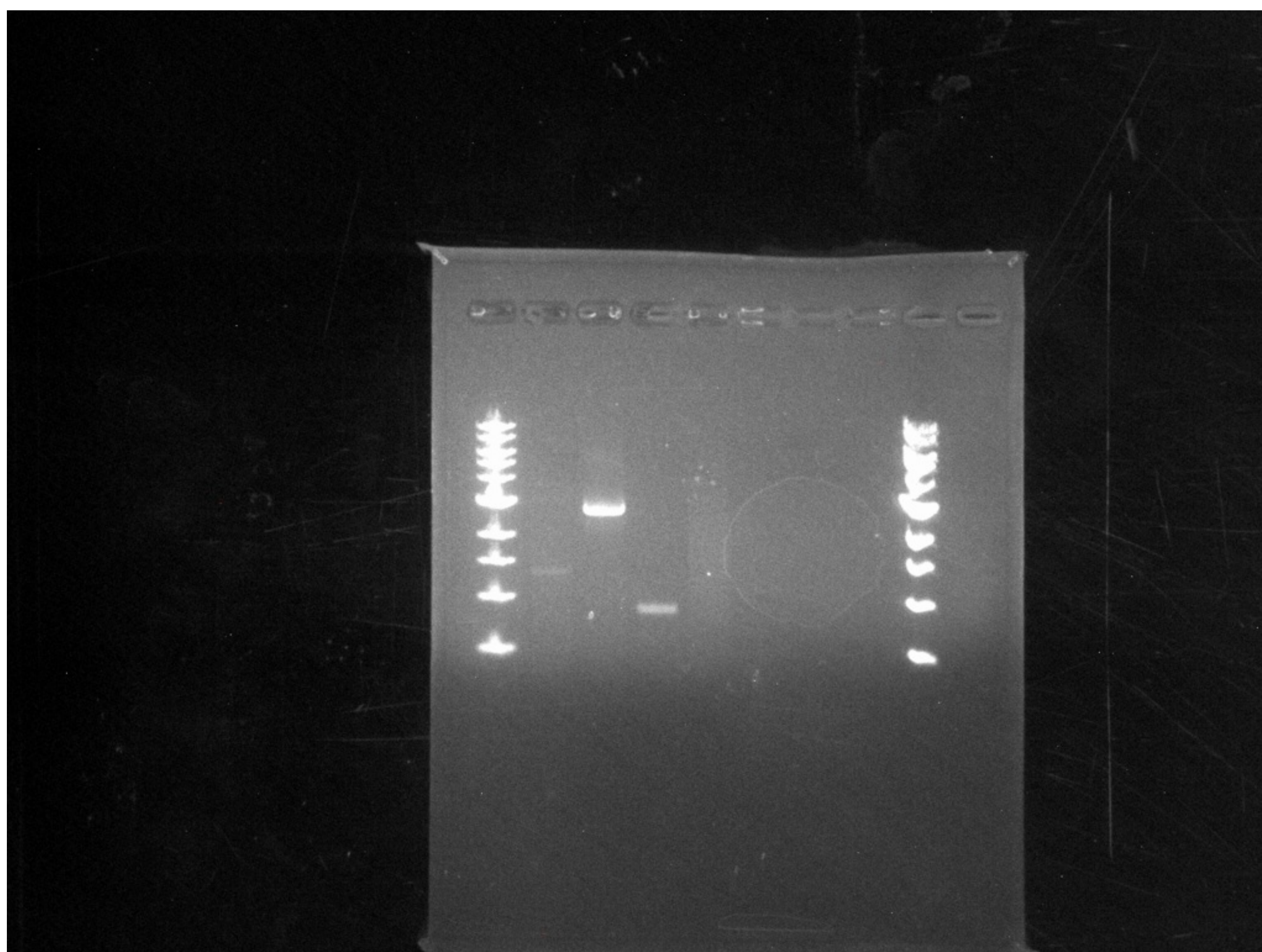
Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-08

SATURDAY, 7/8/17

25 uL 2x Q5 Master mix
1 uL part (1ng)
2.5 uL Prefix F (10 uM)
2.5 uL Suffix R (10 uM)
19 uL ddH2O

25 cycles, @ 66 and 72

image.png



Ladder smmo1 smmo2 dhlb GroEl (66C) Smmo1 sMMO2 dhlb ladder groEl/Es (72C)

Table1

	A	B	C
1	Sample	ng/uL	260/280
2	sMMO2	45.9	1.77
3	dhlB	42.7	1.67
4			

sMMO2 7/10 PCR 45.9 ng/uL
dhlB 7/10 PCR 42.7 ng/uL

Tuesday...

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-07-18

TUESDAY, 7/18/17

Restriction Digest

15 uL Puc19 202
2 uL Buffer
1 uL EcoRi
2 uL ddH2o

1 uL Ladder
1 uL 6x
4 water

20 uL cut DNA
4 uL 6x

6 uL uncut puc19
1 uL 6x

Digest Gibson Confirmation

Project: TCE Biodegredation Project

Authors: Nicholas White

Date: 2017-08-15

TUESDAY, 8/15/17

Master Mix

2.5 uL EcoRI

2.5 uL PstI

2 uL NEB 2.1 Buffer

14 uL ddH2O (I know this doesn't add up to 20, but it's nice to have a bit extra)

5 uL Miniprepped DNA

5 uL Master mix

Digest at 37 C for 30 min - 60 min

Run on 10 well gel. For each digest, run next to 5 uL of uncut DNA

Digested samples should have TWO bands. puc19 backbone at 2.7 KB, sMMO at 5.2 kb.

9/7 PCR Gibson

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-09-07

THURSDAY, 9/7/17

Restriction digests are throwing strange answers, decided to attempt to PCR product out of both Gibson mixes and 1 miniprep plasmid.

FWD Real Prefix
REV Real Suffix

70
1 GroGibson
2 sMMO Gibson
3 Gro Assembly (#3 from gel)

68

Blue-White Screen plates

Project: TCE Biodegredation Project
Authors: Nicholas White
Date: 2017-09-13

WEDNESDAY, 9/13/17

https://msu.edu/course/css/451/LabProtocols/IPTG%20and%20Xgal%20for%20blue%20or%20white%20selection%20_LacZ_.pdf

IPTG and X-gal for blue/white selection
CSS451-2009 Stock Solutions

IPTG Isopropyl thiogalactoside, or isopropyl beta-D-thiogalactopyranoside. Sigma stock number I5502. 0.1 M solution.
The formula weight is 238.3, so this is **0.238 g in 10 ml of water**. Sterilize by filtration, then store in the freezer.

X-gal 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside. Sigma stock number B4252. **20 mg/ml solution.** It must be dissolved in DMSO (dimethyl sulfoxide) or dimethyl formamide, not water! It must be wrapped in foil to protect it from the light, sterilize by filtration, and then stored in the freezer.

Using IPTG and X-gal for blue/white selection on Petri plates There are three basic methods: spread the chemicals on top of the plates before you use them, pour the plates with IPTG and X-gal in them, or incorporate the chemicals into top agar.

• Putting IPTG and X-gal on top of pre-made agar plates. Spread 40 ul of IPTG and 40 ul of X-gal on top of the plate with a hockey stick spreader. Then, let the plates dry before you use them. This should take 30 minutes or so if the plate is dry (i.e. a day or two old), but up to several hours for freshly made plates. I definitely prefer this method for bacteria. •

• Incorporating IPTG and X-gal into the plates before pouring. After auotclaving the media and cooling it to 65o C or less, add IPTG to a final concentration of 0.1 mM IPTG (1 ul IPTG stock solution per ml of media) and X-gal to a final concentration of 40 ug/ml (2 ul of X-gal stock solution per ml of media). Also be sure to add the selection antibiotics at this time: usually ampicillin to a final concentration of 100 ug/ml.

• Putting IPTG and X-gal into top agar. This method is generally used for bacteriophage, but also works for bacterial colonies. Use 3 ml of 0.7% agar (or agarose if you want DNA that can be cut with restriction enzymes) kept at 50o C. Add 10 ul IPTG stock and 40 ul of X-gal stock. Then add the bacteria and phage mixture, mix quickly by rolling the tube between your palms, and pour it onto the plate.

TCE Whiteboard [06/01/2017]

Project: TCE Biodegredation Project

Authors: William Jackson

Date: 2017-05-31

WEDNESDAY, 5/31/17

IMG_20170531_142739.jpg

IMG_20170531_142702.jpg

Proteins to Test

- Native E. coli adh?
- Human adh1B

TCE

sMMO

kinetics

toluene-2-monooxygenase

toluene dioxygenase

chloral hydrate

dehydrogenase

A?

?

spontaneous

CO

dhlB?

dichloroacetate halidohydrase

haloacid dehalogenase

EZ

CO_2

Oxalate

Glyoxalate

Glyoxalate

Formate

formate dehydrogenase

CO_2

Carbon cycle?

Double check dhlB works on dichloroacetate

Glyoxalate Cycle in E. Coli

dhlB might only work on di-chlorinated compounds...

Side Products?

Carbon cycle / energy

Jackson Notes

Project: TCE Biodegredation Project

Authors: William Jackson

Date: 2017-05-20

SATURDAY, 5/20/17

Wiki Notation

Project: TCE Biodegredation Project

Authors: William Jackson

Dates: 2017-05-20 to 2017-05-31

SATURDAY, 5/20/17

Wiki Notation

Project: TCE Biodegredation Project
Authors: William Jackson
Dates: 2017-05-20 to 2017-05-31

WEDNESDAY, 5/31/17

Overview of Succesful wiki designs:

- http://2016.igem.org/Team:Imperial_College
    - Deserved the win, it's pretty clean and elegant. Pretty much gonna be emulated on my end.

Pallete Choices:
https://www.awwwards.com/trendy-web-color-palettes-and-material-design-color-schemes-tools.html

- I very much like the well-storied one.
    - #262216
    - #49412c
    - #97743a
    - #b0a18e
- Nick will probably hate it, but I think it might be a good idea to move toward a more rustic (?) design. The idea to push for the school colors is a natural one, but their is a classical association with the sciences and the color blue with netural accents. It's fucking everywhere in those wikis.

Javascript Framework choices:

-

sMMO colormetric assay - notes

Project: TCE Biodegredation Project
Authors: Katie Brown
Date: 2017-06-12

MONDAY, 6/12/17

Organism used - *Methylosinus trichosporium:* obligate aerobe, methane-oxidizing bacterium. Biosynthesizes sMMO

Reagents used -
naphthalene
2-Naphthol
1-Napththol
tetrazotized o-dianisidine

Culture conditions -
Pretty typical. Except they grew that shit in media that contained copper under methane-air.

Methods -
"Naphthalene was oxidized by purified soluble methane monooxygenase or by cells grown in copper-deficient media to a mixture of 1-naphthol and 2-naphthol. The naphthols were detected by reaction with tetrazotized o-dianisidine to form purple diazo dyes with large molar absorptivities. The rate of color formation with the rapid assay correlated with the velocity of TCE oxidation that was determined by gas chromatography. Both assays were used to optimize conditions for TCE oxidation by M. trichosporium OB3b and to test several methanotrophic bacteria for the ability to oxidize TCE and naphthalene."
"The protocol is based on the ability of sMMO to oxidize the bicyclic aromatic hydrocarbon naphthalene to 1-naphthol and 2-naphthol which react spontaneously with tetrazo- tized o-dianisidine to form intense purple-colored products. The colorimetric and gas chromatogra- phy assays were used to determine which metha- notrophs synthesize sMMO and degrade TCE at rapid rates."

Colormetric assay -
"The reaction of tetrazo-tized o-dianisidine with an aqueous solution of 1- or 2-naphthol produces a violet adduct (Wackett & Gibson 1983). Each culture sample was diluted to an A6o0of 0.20 with prewarmed medium containing the same amount of copper sulfate as the original sample in a 120 ml (total volume) glass serum bottle sealed with a 20 mm Teflon-lined rubber septum (Baxter/American Scientific Products, Plymouth, MN). The resulting cell suspension was degassed to remove residual methane (Tsien et al. 1989). The culture was transferred with disposable serological pipettes (Falcon; Becton Dickinson Labware, Ox- nard, CA) in 1ml aliquots to 10ml (total volume) glass serum bottles containing crystalline naphtha- lene. The naphthalene was provided in amounts sufficient to give a saturated aqueous solution. The bottles were sealed with 20mm Teflon-lined rub- ber septa. Samples were inverted and incubated at 30° C on a platform shaker (200rpm, 2.5 cm stroke length). Samples were sacrificed at time intervals by adding 100/zl of freshly hydrated tetrazotized o-dianisi- dine (4.21 mM). Heat-killed and sterile media con- trois were also tested. If formed, the colored prod- uct was clearly visible to the naked eye or readily monitored by recording the absorption spectrum over the range of 430 to 650 mm with a Beckman DU-70 spectrophotometer (Beckman Instru- ments, Inc., Fullerton, CA). This adduct, as well as azo dyes formed from synthetic 1-naphthol and 2-naphthol, proved to be unstable in the mineral salts media, and phosphate and organic buffers used in these studies. However, the intensity of color formation immediately following the addition of tetrazotized o-dianisidine was proportional to the naphthol concentration."

Potential papers to look at - Tsein et al. (1989)

MATLAB SimBiology/Modeling Notes

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-05-19

FRIDAY, 5/19/17

MATLAB Startup Notes

1. Open a Terminal via the launch screen or press CTRL+ALT+T
2. On the command line, enter "matlab"
3. On the top of the screen, click on "APPS"
4. Click on SimBiology

MATLAB Tutorial Documentation:

- https://www.mathworks.com/videos/modeling-biology-with-simbiology-an-introduction-for-igem-teams-81817.html?elqsid=1495209483607&potential_use=Education
  - Fairly long basic overview of use of graphical interface and how to show relationships and define non-visual "rules" that define how different products interact within the model.

Previous iGEM Project Models:

- http://2016.igem.org/Team:Manchester/Model#model1
  - Generation of own code to perform Ensemble Modeling, a method in which different probable but unknown relationships are modeled and coerced into one overarching framework.
    - https://github.com/Manchester-iGem-2016/UoMiGem2016
    - MATLAB code doesn't really seem that much different from R Code in most respects.
  - Ask Nick about prevalence of Horseradish biopart. Seems prevalent in many projects.
  - This seems to be a good resource, but I get the vibe that it is only applicable to a small subgroup of projects.
- http://2016.igem.org/Team:TU_Delft/Model
  - Cool Idea. (Turning cells into lenses for lasers. It doesn't work)
  - The e.coli has to generate a certain concentration of a compound called fluorophore, so they dealt with rate constants.
  - They used some sort of physics modeling suite, but I see nary a thing about MATLAB models.
- http://2014.igem.org/Team:ETH_Zurich/modeling/overview
  - This is what I want.
  - Learn more about "standard mass action kinetics" in systems modeling.
  - Looks like there project was essentially creating a microfluidic chip that emulates the game of life in the sense of propogating living colonies across wells.
  - How do people get these rate constants. Is it derived from known information, or is it more of a representation of an experimental relationship.
- http://2014.igem.org/Team:Waterloo/Math_Book
  - Also a good resource.
  - How are these gene regulatory networks defined? Is it an understood relationship, or is it something that comes with sticking stuff together?
  - I've yet to see anyone say anything about including biobricks in their model.

Further Reading:
http://www.math.tamu.edu/~phoward/m442/modode.pdf

MAWS

Project: TCE Biodegredation Project
Authors: William Jackson
Date: 2017-05-19

FRIDAY, 5/19/17

MAWS stands for Make Aptamers without SELEX. It gives Nick boners.

It's supposed to be easy to set up, it is not.

Following instructions (https://github.com/igemsoftware/Heidelberg_15/blob/master/README_MAWS.md)

- Noticed ambermini python library. AMBER looks like it's my sticking point, so I'm gonna read up on that.
- error while installing 'defaults::dbus-1.10.10-0'.
    - Ultimately corrected due to incorrect sudo permissions, however re installation of partitioned system required due to bullshit driver bullshit.