21/6/2017 (Wednesday)

Preparation of Buffers

2X TM buffer (80 mM Tris-HCl, 200mM $MgCl_2$, pH 7)

- Volume: 300mL
- 24mL 1M Tris-HCl
- $MgCl_2 \cdot 6H_2O$: 12.19816g

Procedures:

1. Prepare a 2X TM buffer by adding 24mL 1M Tris solution & 276mL ddH_2O.
2. Dissolve 12.19816g $MgCl_2 \cdot 6H_2O$ into the solution.
3. Calibrate pH meter from highest pH buffer to lowest pH buffer available, washing the electrode with ddH_2O and wiping with lint-free tissue (Kimwipes) between each measurement.
4. Use a pH meter to measure the pH of TM buffer and adjust to pH 7 by adding 1M HCl drop by drop.
5. Autoclave.
6. Dilute 2X TM buffer to 1X in a 50mL Falcon tube.

1X TBE buffer (1M Tris, 1M boric acid, 0.03M EDTA)

1. Dilute 10X TBE buffer available to 1X by adding 100mL of 10X TBE buffer to 900mL ddH_2O.

2X Triton X-100 Buffer (100mM Tris-HCl, 300mM NH_4Cl, 40mM KCl, 0.03% Triton X-100, pH 7.5)

- Volume to prepare: 300mL
- 30mL 1M Tris-HCl
- NH_4Cl: 4.814g
- KCl: 0.8946g
- Triton X-100: 90µL

Procedures:

1. Add 30mL 1M Tris & 220mL ddH2O (save 50mL for the addition of HCl to adjust pH and Triton).
2. Dissolve 4.814g NH_4Cl and 0.8946g KCl.
3. Use a pH meter to measure the pH and adjust to pH 7.5 by adding 1M HCl drop by drop.
4. Add 90ul Triton X-100 and shake vigorously.
5. Dilute to 1X.

22/6/2017 (Thursday)

Preparation of Working Oligo Solutions

1. Wipe the bench and your gloves with 70% ethanol.
2. Briefly vortex and centrifuge the oligo stock solutions before dilution.
3. Dilute the stock solutions with 1X TM buffer as follows:

Eppendorf/PCR tube label	Initial conc. of stock solutions	Final conc.	Volume of stock solution	Volume of 1X TM buffer	Final volume
O1 (5μM)	100μM	5μM	15μL	285μL	300μL
O2 (5μM)		5μM	15μL	285μL	300μL
O3 (5μM)		5μM	15μM	285μL	300μL
O4 (5μM)		5μM	15μM	285μL	300μL
O5 (5μM)		5μM	15μM	285μL	300μL
O1 (25μM)		25μM	5.5μL	16.5μL	22μL
O2 (25μM)		25μM	5.5μL	16.5μL	22μL
O3 (25μM)		25μM	5.5μL	16.5μL	22μL
O4 (25μM)		25μM	5.5μL	16.5μL	22μL
O5 (25μM)		25μM	5.5μL	16.5μL	22μL
Input (5μM)		5μM	15μL	285μL	300μL

4. Mix well by tapping and briefly centrifuge the tubes.
5. Store at -20ºC.

Tetrahedron Assembly

1. Tap and centrifuge the prepared working oligo solutions.
2. Prepare the following PCR tubes:

PCR tube label	O1 (5μM)	O2 (5μM)	O3 (5μM)	O4 (5μM)	O5 (5μM)	1X TM buffer	Final volume	Final oligo conc.
A1	10μL	10μL	/	/	/	30μL	50μL	1μM
A2	10μL	/	10μL	/	/			
A3	10μL	/	/	10μL	/			
A4	10μL	/	/	/	10μL			
A5	/	10μL	10μL	/	/		50μL	1μM
A6	/	10μL	/	10μL	/			
A7	/	10μL	/	/	10μL			
A8	/	/	10μL	10μL	/			
A9	/	/	10μL	/	10μL			
A10	/	/	/	10μL	10μL			
B1	10μL	10μL	10μL	/	/	20μL	50μL	1μM
B2	10μL	10μL	/	10μL	/			
B3	10μL	10μL	/	/	10μL			

B4	10µL	/	10µL	10µL	/			
B5	10µL	/	10µL	/	10µL			
B6	10µL	/	/	10µL	10µL			
B7	/	10µL	10µL	10µL	/			
B8	/	10µL	10µL	/	10µL			
B9	/	10µL	/	10µL	10µL			
B10	/	/	10µL	10µL	10µL			
C1	10µL	10µL	10µL	10µL	/	10µL	50µL	1µM
C2	10µL	10µL	10µL	/	10µL			
C3	10µL	10µL	/	10µL	10µL			
C4	10µL	/	10µL	10µL	10µL			
C5	/	10µL	10µL	10µL	10µL			

PCR tube label	**O5 (5µM)**	**Input (5µM)**	**1X TM buffer**	**Final volume**	**Final oligo conc.**
Output	10µL	10µL	30µL	50µL	1µM

PCR tube label	**O1 (25µM)**	**O2 (25µM)**	**O3 (25µM)**	**O4 (25µM)**	**O5 (25µM)**	**1X TM buffer**	**Final volume**	**Final oligo conc.**
Tetra	10µL	10µL	10µL	10µL	10µL	/	50µL	5µM

3. Tap to mix and briefly centrifuge the PCR tubes.
4. Incubate the PCR tubes at 95°C for 5 min and cool them down to 20°C with a 0.5°C drop every 30 seconds and then drop to 10°C using a thermocycler (program called "igem annealing" already saved in thermocycler).
5. Store at 4°C.

****It is good practice to return all pipettes to maximum volume after using at the end of the day so as to allow them to last longer.**

23/6/2017 (Friday)

Strand Displacement Reaction

1. Prepare the following mixture:

Eppendorf/PCR tube label	Tetra (5μM)	ddH_2O	Input (5μM)	Final volume	Final oligo conc.
Tetra XO5	4μL	12μL	4μL	20μL	1μM

2. Place the tubes in a shaker and incubate them at room temperature for 30 minutes for strand displacement reaction.

Making Native PAGE

For 1 gel:

%	30% Acrylamide (29:1)	ddH_2O	10X TBE	10% APS	TEMED
12%	4800 μL	6000 μL	1200 μL	200 μL	10 μL

For 2 gels:

%	30% Acrylamide (29:1)	ddH_2O	10X TBE	10% APS	TEMED
12%	9600 μL	12000 μL	2400 μL	400 μL	20 μL

Procedures:

1. Get the gel caster ready.
2. Pipette into 2 clean 50mL Falcon tubes the chemicals needed to make 4 gels (mixture for 2 gels in each tube) according to the percentage required (12%), ensuring that APS and TEMED are added at the end.
3. Vortex quickly to mix.
4. Pour mixture into the gel caster and quickly insert the comb. For each gel caster, pour mixture for one and pipette mixture quickly for the other so as to not disturb the first gel.
5. Let it polymerize for about 15 minutes. You can check the polymerisation of the unused mixture left behind in the Falcon tube to estimate the time of polymerisation.
6. If you do not use the gels, wrap in wet tissue paper and plastic bag and put in fridge at 4^oC.

26/6/2017 (Monday)

Preparation of Solutions for PAGE

1. Dilute all 1μM solutions to be loaded, namely A1-A10, B1-B10, C1-C5, Tetra XO5 and Output, as follows:

Oligo (1μM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
1.8μL	8.2μL	2μL	12μL	150nM

2. Dilute all 5μM solutions to be loaded, namely O1, O2, O3, O4, O5, Input and Tetra as follows:

Oligo (5μM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
0.36μL	9.64μL	2μL	12μL	150nM

3. Dilute the Quick-Load® Purple 2-Log DNA ladder as follows: 1μL DNA ladder + 11μL 1X TM buffer.

Running PAGE

1	2	3	4	5	6	7	8	9	10
Ladder	O1	O2	O3	O4	O5	Input	A1	A2	A3

1. Put the gel in the electrophoresis tank, add 1X TBE buffer and remove the comb.
2. Load 12μL of each of the DNA samples as follows:
3. Run the gel at a constant voltage of 100V for around 60 minutes or until the bands of the dye reach 3/4 of the length of the gel.
4. Use GelRed to post-stain the gel for 15-20 minutes on a shaker and observe the gel under UV light.

27/6/2017 (Tuesday)

PAGE (Continued)

1. Run 3 gels in total.
2. Load 12μL of each of the DNA samples as follows:

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Ladder	A4	A5	A6	A7	A8	A9	A10	B1	B2
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Ladder	B3	B4	B5	B6	B7	B8	B9	B10	C1
21	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Ladder	C2	C3	C4	C5	Tetra	Tetra XO5	Output	O5	Input

3. Run the gel at a constant voltage of 100V for around 60 minutes or until the bands of the dye reach 3/4 of the length of the gel.
4. Use GelRed to post-stain the gel for 15-20 minutes on a shaker and observe the gel under UV light.

28/6/2017 (Wednesday)

ABTS Assay

Procedures:
1. Prepare the following working concentrations of reagents:

Reagents	Stock conc.	Working conc.	Volume of stock reagent	Volume of diluent	Final volume	Remarks
DNA nanostructure	/	5μM	/	/	/	/
Input (Target DNA)	100μM	5μM	5μL	95μL	100μL	Or RNA, prepared during preparation of working oligo solutions
Hemin	113μM	10μM	8.8496μL	91.1504μL	100μL	Light sensitive, diluted in DMSO, stored in -20°C
ABTS	20mM	20mM	/	/	/	Diluted in DMSO, stored in -20°C
H_2O_2	9.79M	120mM	1.226μL	98.774μL	100μL	Freshly diluted with ddH_2O. stored in 4°C
Triton X-100 Buffer	/	/	/	/	/	Prepared previously

1. Prepare the reaction mixture as follows, following the subsequent steps:

Reagent	Working conc.	Volume in reaction mixture	Final conc.
DNA nanostructure	5μM	2μL	100nM
Target DNA	5μM	2μL	100nM
Hemin	10μM	4μL	0.4μM
ABTS	20mM	10μL	2mM

H_2O_2	120mM	10µL	12mM
Triton X-100 Buffer	/	72µL	/
Total volume		100µL	/

6. Add 72µL buffer, 2µL DNA nanostructure, 2µL target DNA and 4µL hemin and briefly vortex the solution.
7. Incubate at room temperature on shaker for 20-30 minutes, using a tissue paper to cover the tube holder (as hemin is light-sensitive).
8. Add 10µL ABTS and 10µL H_2O_2 and briefly vortex the solution.
9. Prepare the following controls as well by adjusting the volume of buffer used so that the final volume is 100µL:
    a) Positive control (2µL EAD2 instead of DNA nanostructure & target DNA)
    b) Negative control (blank with no DNA)
    c) With DNA nanostructure only
    d) With target DNA only
    e) DNA nanostructure + random RNA/RNA mutant
10. Transfer the reaction mixtures to a 96-well plate in the following order: Tetra+Input, Tetra only, Input only, RNA mutant, Positive control, Negative control.
11. Ensure that you do not introduce bubbles.
12. Measure the absorbance at 420nm by a spectrophotometer at 30-second intervals for 20 minutes.

❼ RNA mutant displays a similar absorbance to that of the positive control. There is a possibility that the RNA mutant may have been mixed up with the positive control.

30/6/2017 (Friday)

ABTS Assay (Continued)

1. Make a new working concentration of the RNA mutant by diluting 100μM stock solution to 5μM working solution.
2. Repeat the ABTS assay in the same way as on 28/6/2017, except with the addition of the new RNA mutant solution.
3. Transfer the reaction mixtures to a 96-well plate in the following order: Tetra+Input, Tetra only, Input only, New RNA mutant, Old RNA mutant, Positive control, Negative control.
4. Measure the absorbance at 420nm by a spectrophotometer at 30-second intervals for 20 minutes.

➐ Done twice but did not work both times (including the positive control), possibly due to degradation of ABTS, hemin or H_2O_2?

5/7/2017 (Wednesday)

ABTS assay (re-do)

Procedures:

1. Prepare an ice box with ice for the Hemin, ABTS, and H_2O_2.

2. Prepare the following working concentrations of reagents:

Reagents	Stock conc.	Working conc.	Volume of stock reagent	Volume of diluent	Final volume	Remarks
DNA nanostructure	/	5μM	/	/	/	/
Input (Target DNA)	100μM	5μM	5μL	95μL	100μL	Or RNA, prepared during preparation of working oligo solutions
Hemin	113μM	10μM	8.85μL	91.15μL	100μL	Light sensitive, diluted in DMSO, stored in -20°C (a new batch of diluted hemin is made)
ABTS	20mM	20mM	/	/	/	Diluted in DMSO, stored in -20°C
H_2O_2	9.79M	120mM	1.23μL	98.77μL	100μL	Freshly diluted with ddH_2O. stored in 4°C
Triton X-100 Buffer	/	/	/	/	/	Prepared previously

3. Prepare the reaction mixture as follows, following the subsequent steps:

Reagent	Working conc.	Volume in reaction mixture	Final conc.
DNA nanostructure	5μM	2μL	100nM
Target DNA	5μM	2μL	100nM
Hemin	10μM	4μL	0.4μM

ABTS	20mM	10µL	2mM
H_2O_2	120mM	10µL	12mM
Triton X-100 Buffer	/	72µL	/
Total volume		100µL	/

4. Add 72µL buffer, 2µL DNA nanostructure, 2µL target DNA and 4µL hemin and briefly vortex the solution.
5. Prepare the following controls as well by adjusting the volume of buffer used so that the final volume is 80µL:

    a. Positive control (2µL EAD2 instead of DNA nanostructure & target DNA)
    = 74µL buffer, 2µL EAD2, and 4µL hemin
    b. Negative control (blank with no DNA)
    = 76µL buffer, and 4µL hemin
    c. With DNA nanostructure only
    = 74µL buffer, 2µL DNA nanostructure, and 4µL hemin
    d. With target DNA only
    = 74µL buffer, 2µL target DNA, and 4µL hemin
    e. DNA nanostructure + random RNA/RNA mutant
    = 72µL buffer, 2µL DNA nanostructure, 2µL random RNA, and 4µL hemin

6. Incubate at room temperature on shaker for 20-30 minutes, using a piece of aluminium foil to cover the tube holder (as hemin is light-sensitive).

7. Starting with the last control (DNA nanostructure + random DNA/RNA mutant), add 10µL ABTS and 10µL H_2O_2 and briefly vortex the solution.
8. Transfer the reaction mixtures to a 96-well plate. Ensure that you do not introduce bubbles.
9. Measure the absorbance at 420nm by a spectrophotometer at 30-second intervals for 20 minutes.

→ None of the wells changed colour at all, so the assay was redone for just the positive control (just EAD2 + buffer + hemin + ABTS + H_2O_2)

→ the positive control also did not change colour
→ Simon will be giving us a new batch of ABTS soon

<u>DNA Dilution</u>

We prepared the stock solution for Tim's Design according to the following concentrations:

Oligos	Initial nmoles	Final stock conc.	Volume of ddH_2O
Tim1 (200µM)	80.4	200µM	402µL
Tim2 (200µM)	75.2	200µM	376µL
Tim3 (200µM)	77.2	200µM	386µL
Tim4 (100µM)	20.8	100µM	208µL
Tim5 (100µM)	25.8	100µM	258µL
TimTetraTarget (100µM)	19.3	100µM	193µL
TimPaperProbeA1 (100µM)	23	100µM	230µL
TimPaperProbeB3 (100µM)	27.3	100µM	273µL
TimPaperTemplate5 (100µM)	23.3	100µM	233µL

Afterwhich, the DNA stock solution were made into working solutions using the following volumes . This eppendorfs of working solutions were stored in -20^{o}C.

Eppendorf/PCR tube label	Initial conc. of stock solutions	Final conc.	Volume of stock solution	Volume of 1X TM buffer	Final volume
Tim1 (5µM)	200µM	5µM	7.5µL	292.5µL	300µL
Tim2 (5µM)		5µM	7.5µL	292.5µL	300µL
Tim3 (5µM)		5µM	7.5µL	292.5µL	300µL
Tim4 (5µM)	100µM	5µM	15µL	285µL	300µL
Tim5 (5µM)		5µM	15µL	285µL	300µL
TimPaperProbeA1 (5µM)	100µM	5µM	15µL	285µL	300µL
TimPaperProbeB3 (5µM)		5µM	15µL	285µL	300µL
TimPaperTemplate5 (5µM)		5µM	15µL	285µL	300µL
Tim1 (25µM)	200µM	25µM	2.75µL	19.25µL	22µL
Tim2 (25µM)		25µM	2.75µL	19.25µL	22µL
Tim3 (25µM)		25µM	2.75µL	19.25µL	22µL
Tim4 (25µM)	100µM	25µM	5.5µL	16.5µL	22µL
Tim5 (25µM)		25µM	5.5µL	16.5µL	22µL
TimPaperProbeA1 (25µM)	100µM	25µM	5.5µL	16.5µL	22µL

TimPaperProbeB3 (25µM)		25µM	5.5µL	16.5µL	22µL
TimPaperTemplate5 (25µM)		25µM	5.5µL	16.5µL	22µL
TimTetraTarget (5µM)	100µM	5µM	15µL	285µL	300µL

Four PAGE Gels were made which are to be used tomorrow.

6/7/2017 (Thursday)

Errors occurred in the protocol

1. We did not account for the changes in Tim's Design into the protocol .
2. The dilution of each of the nucleotide solutions did not lead to a 1X TM buffer.
3. Amendment of the DNA oligo working solutions from 25uM to 24uM.

7/7/2017 (Friday)

Finalized protocol for Native PAGE

- Began labelling PCR tubes and making the tetrahedron assembly solutions for thermocycling

Changes made to the Native PAGE protocol

1. Cut down from 72 wells to 9 wells (not all combinations are required to be tested)
2. Storage methods of gels to be used later are amended
    a. Wet tissue paper with buffer instead of water
    b. Store gels in plastic bags/cling wrap instead of aluminium foil

Met with Hei Wai and Simon

Integrated Human Practices Suggestions

Interviews with:

- Professors (theory)
- Doctors (application)
- Technicians (biosafety)

→ modify experiments' procedures, targets and structures based on the interview insights

JSSE and high school collaborations:

- Nanostructures
  → draw on Tiamat
- Targets
  → test them out on structures

Mathematical Modelling Suggestions

ABTS assay (need to consult Dr Tanner)

- Find rate constant and order of reaction
- Find duration of optimal reaction

^ETH's modelling is recommended for reading up

Experiment

Reasons for using E.Coli (for pitching to judges):

- Mass production and screening both done inside live E.Coli
    - Screening using fluorescence and addition of target

Genetic circuit?

Cell transformation (talk to Jacky)

- We only have 1 plasmid aka 1 go
    - Will need to email MIT again, and shipment of plasmid will take around 3 weeks (assuming they send us new plasmids)
- E.Coli is with Jacky
- Need to ask Jacky for flasks, alcohol lamp, agarose, etc

InterLab (talk to Jacky)

- Pending reply from iGEM HQ
- Upon arrival of the kit, store it properly AT ROOM TEMP, then contact Jacky
- Ask Jacky to book plate-reader on 3/F for 1-2 hours
- Make sure Jacky is also available during that time to take the measurements together

10/7/2017 (Monday)

Tetrahedron Assembly

1. Tap and centrifuge the prepared working oligo solutions.
2. Prepare the following PCR tubes:

PCR tube label	T1 (5µM)	T2 (5µM)	T3 (5µM)	T4 (5µM)	T5 (5µM)	TTT (5µM)	2X TM buffer	ddH_2O	Final volume	Final oligo conc.
A6	-	10uL	10uL	-	-	-	15.25uL	14.75uL	50uL	1uM
A13	-	-	-	10uL	10uL	-	15.5uL	14.5uL		
B1	10uL	10uL	10uL	-	-	-	10.375uL	9.625uL	50µL	1µM
B8	10uL	-	-	10uL	10uL	-	10.6uL	9.4uL		
B11	-	10uL	10uL	10uL	-	-	10.5uL	9.5uL		
B12	-	10uL	10uL	-	10uL	-	10.5uL	9.5uL		
B13	-	10uL	10uL	-	-	10uL	10.5uL	9.5uL		
C10	10uL	-	-	10uL	10uL	10uL	5.8uL	4.2uL	50µL	1µM
D1(x2)	11uL	11uL	11uL	11uL	11uL	-	5uL	-	60uL	1uM

PCR tube label	T1 (24µM)	T2 (24µM)	T3 (24µM)	T4 (24µM)	T5 (24µM)	TTT (24uM)	1X TM buffer	Final volume	Final oligo conc.
Tetra	10µL	10µL	10µL	10µL	10µL	10uL	/	60µL	4µM

3. Tap to mix and briefly centrifuge the PCR tubes.
4. Incubate the PCR tubes at 95°C for 5 min and cool them down to 20°C with a 0.5°C drop every 30 seconds and then drop to 10°C using a thermocycler (program called "igem annealing" already saved in thermocycler).
5. Store at 4°C.

INCUBATE THE TARGET DNA

1. Prepare the following mixture:

Eppendorf Label	D1(1uM)	ddH_2O	2X TM buffer	TTT (5uM)	Final Volume	Final oligo conc.
D1 + TTT	5ul	6.975uL	7.025uL	1uL	20uL	250nM

2. Place the tubes in a shaker and incubate them at room temperature for 30 minutes for the tetrahedron to form.

Native PAGE - made 3 gels

Volumes of reagents for making 1 gel:

%	30% Acrylamide (29:1)	ddH$_2$O	10X TBE	10% APS	TEMED
8%	3200 µL	7600 µL	1200 µL	200 µL	10 µL
12%	4800 µL	6000 µL			
15%	6000 µL	4800 µL			

GelRed (20mL): 200µL 100X GelRed + 19.8mL 1X TBE

- It can be reused many times to stain multiple gels.

Procedures:

1. Get the gel caster ready.
2. Pipette into a clean 50mL Falcon tube the chemicals needed according to the percentage required (12%), ensuring that APS and TEMED are added at the end.
3. Vortex quickly to mix.
4. Pour mixture into the gel caster and quickly insert the comb.
5. Let it polymerize for about 15 minutes.
6. Dilute all 1µM solutions to be loaded, namely A6-D1, as follows:

Oligo (1µM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
1.8µL	8.2µL	2µL	12µL	150nM

7. Dilute all 5µM solutions to be loaded, namely T1, T2, T3, T4, T5, TTT, Tetra and D1+TTT as follows:

Oligo (5µM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
0.36µL	9.64µL	2µL	12µL	150nM

Tetra (4µM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
0.45µL	9.64µL	2µL	12µL	150nM

highlighted = protocol calculation amended

D1+TTT (250nM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
9µL	3.5µL	2.5µL	15µL	150nM

8. Dilute the DNA ladder as follows:

1µL DNA ladder + 11µL 1X TM buffer.

9. Put the gel in the electrophoresis tank, add 1X TBE buffer, and remove the comb.
10. At least 2 gels are needed. Load 12µL of each of the DNA samples as follows:

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Ladder	T1	T2	T3	T4	T5	TTT	A6	A13	B1
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Ladder	B10	B11	B12	B13	C10	D1	Tetra	D1+TTT	1X Loading buffer

									(left empty this time)

11. Run the gel at a constant voltage of 100V until the bands of the dye reach 3/4 of the length of the gel. **(used constant voltage of 150V instead of 100V)**
12. Use GelRed to post-stain the gel and observe the gel under UV light.

→ The GelRed (re-used) is light blue in colour instead of purple when used. Gel images are very dark, may be a result of the GelRed losing its function.

→ Store GelRed in containers covered with aluminium foil at all times. When post-staining gels, cover the container (with the GelRed and gels) with aluminium foil.

11/7/2017 (Tuesday)

Native PAGE

Volumes of reagents for making 1 gel:

%	30% Acrylamide (29:1)	ddH$_2$O	10X TBE	10% APS	TEMED
8%	3200 µL	7600 µL	1200 µL	200 µL	10 µL
12%	4800 µL	6000 µL			
15%	6000 µL	4800 µL			

GelRed - A new batch has been made by Jess and placed next to the gel doc.

Procedures:

7. Get the gel caster ready.
8. Pipette into a clean 50mL Falcon tube the chemicals needed according to the percentage required (12%), ensuring that APS and TEMED are added at the end.
9. Vortex quickly to mix.
10. Pour mixture into the gel caster and quickly insert the comb.
11. Let it polymerize for about 15 minutes.
12. Dilute all 1µM solutions to be loaded, namely A6-D1, as follows:

Oligo (1µM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
1.8µL	8.2µL	2µL	12µL	150nM

7. Dilute all 5µM solutions to be loaded, namely T1, T2, T3, T4, T5, TTT, Tetra and D1+TTT as follows:

Oligo (5µM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
0.36µL	9.64µL	2µL	12µL	150nM

Tetra (4µM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
0.45µL	9.64µL	2µL	12µL	150nM

D1+TTT (250nM)	1X TM buffer	6X Loading dye	Final volume	Final conc.
9µL	3.5µL	2.5µL	15µL	150nM

8. Dilute the DNA ladder as follows:

1µL DNA ladder + 11µL 1X TM buffer.

9. Put the gel in the electrophoresis tank, add 1X TBE buffer, and remove the comb.
10. At least 2 gels are needed. Load 12µL of each of the DNA samples as follows:

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Ladder	T1	T2	T3	T4	T5	TTT	A6	A13	B1
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
B10^	B11	B12	B13	C10	D1	Tetra	D1+TTT	1X Loading	**Ladder^**

								buffer **(left empty this time)**	

red: 2uL of 1X loading buffer was used instead of 2uL of 6X loading buffer, as we used up all the 6X loading buffer.
^forgot to put ladder in first, hence the ladder is placed in the last well instead.

11. Run the gel at a constant voltage of 100V until the bands of the dye reach 3/4 of the length of the gel.
12. Use GelRed to post-stain the gel and observe the gel under UV light.

Results:
Wells that didn't match our hypotheses:

Well (Combination)	**Hypothesis**	**Observed result**
B1 (T1+T2+T3)	All 3 oligos should bind together, the subsequent band should be formed closer towards the top of the lane.	No clear band formed towards the top of the lane (light smearing only), a band is seen at the same position as the individual T1, T2, and T3 bands.
B11 (T2+T3+T4)	T3 and T4 should bind together to form one band towards the top of the lane, T2 should remain separate, forming an individual band.	The expected band of T3+T4 is nonexistent. Only a band is seen at the same position as the individual T1, T2, and T3 bands. Observed results does not match with B12 (T2+T3+T5), B12's results match the hypothesis, which is the same as B11's.
B13 (T2+T3+TTT)	All 3 oligos should bind together, the subsequent band should be formed closer towards the top of the lane.	Only one band is observed, which is at the same position as the individual T1, T2, and T3 bands.
D1 (T1+T2+T3+T4+T5)	A flat structure should be formed due to the binding of all 5 oligos, the subsequent band should be formed closer towards the top of the	Band is nonexistent.

	lane, if not stuck in the well.	
Tetra	A 3D tetrahedron should have been formed during the thermocycling, the subsequent band should be formed closer towards the top of the lane, if not stuck in the well.	Band is nonexistent.
D1+TTT	The flat structure should bind to the target, resulting in a 3D nanostructure, the subsequent band should be formed closer towards the top of the lane, if not stuck in the well.	Band is nonexistent.

Modifications for PAGE next time:
→ Remove well B8 next time.
→ Make wells T1+T2, and T1+T3.
→ Make 12uL per PCR tube, but put only 10uL in each well.
→ No touching gel with hands, except the corners.
→ No need to put cling wrap on the reader. Clean with water, then alcohol, then water, and put some water on the reader before putting gel (to remove the dust particles).

→ Try higher % of PAGE.
→ Mix 1X TM buffer (used for incubation) with 1X TBE buffer (the running buffer), redo calculations to ensure that we have the same concentration of Magnesium ions (avoid diffusing out and breaking the structures).

→ Re-run PAGE again.

12/7/2017 (Wednesday)

Native PAGE

Running buffer (with the inclusion of Mg ions to assist the formation of nanostructures):
1X TBE, 12.8 mM Mg2+:

- 500 mL 1X TBE + 1.30112g $MgCl_2 \cdot 6H_2O$

Got 10X loading dye from Jess, hence the changes in calculations as follows:

Oligo (1µM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
1.8µL	9µL	1.2µL	12µL	150nM

Dilute all 5µM solutions to be loaded, namely T1, T2, T3, T4, T5, TTT, Tetra and D1+TTT as follows:

Oligo (5µM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
0.36µL	10.44µL	1.2µL	12µL	150nM

Tetra (4µM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
0.45µL	10.35µL	1.2µL	12µL	150nM

D1+TTT (250nM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
9µL	4.5µL	1.5µL	15µL	150nM

Use 120V max, not 150V → DNA nanostructures may be broken due to the high heat, and smearing is more likely to occur

ABTS Assay

Procedures:

1. Prepare an ice box with ice for the Hemin, ABTS, and H_2O_2.
2. Prepare the following working concentrations of reagents:

Reagents	Stock conc.	Working conc.	Volume of stock reagent	Volume of diluent	Final volume	Remarks
D1 (nanostructure only)	/	1µM	/	/	/	/
TTT (target)	100µM	5µM	5µL	95µL	100µL	Or RNA, prepared during preparation of working oligo solutions
Hemin	113µM	10µM	8.85µL	91.15µL	100µL	Light sensitive, diluted in DMSO, stored in -20°C

ABTS	123.3mM	20mM	16.22uL	83.78uL	10uL	Diluted in DMSO, stored in -20°C
H_2O_2	9.79M	120mM	1.23µL	98.77µL	100µL	Freshly diluted with ddH_2O. stored in 4°C
Triton X-100 Buffer	/	/	/	/	/	Prepared previously

3. Prepare the reaction mixture as follows, following the subsequent steps:

Reagent	**Working conc.**	**Volume in reaction mixture**	**Final conc.**
DNA nanostructure	1µM	10µL	100nM
Target DNA	5µM	2µL	100nM
Hemin	10µM	4µL	0.4µM
ABTS	20mM	10µL	2mM
H_2O_2	120mM	10µL	12mM
Triton X-100 Buffer	/	64µL	/
Total volume		100µL	/

4. Add 64µL buffer, 10µL DNA nanostructure, 2µL target DNA and 4µL hemin and briefly vortex the solution.
5. Prepare the following controls as well by adjusting the volume of buffer used so that the final volume is 80µL:
    a. Positive control 1
        2µL EAD2 (5uM) instead of DNA nanostructure & target DNA,+72uL buffer, +4uL of 10uM Hemin
    b. Positive control 2
        2µL of 5µM T4 + 2µL of 5µM T5 + 72uL buffer + 4uL of 10uM Hemin
    c. Positive control 3
        2uL of 5uM TimPaperProbeA1
        + 2uL of 5uM TimPaperProbeB3, 72uL buffer
    d. Negative control
        blank with no DNA + 76uL buffer +4uL of 10uM Hemin

e. With DNA nanostructure only
D1 only (10µL) + 66uL buffer +4uL of 10uM Hemin
f. With target DNA only
TTT only + 74uL buffer + 4uL of 10uM Hemin
g. DNA nanostructure + random RNA/RNA mutant
D1 + random DNA + 64uL buffer + 4uL of 10uM Hemin

6. Incubate at room temperature on shaker for 20-30 minutes, using a piece of aluminium foil to cover the tube holder (as hemin is light-sensitive).
7. Add 10µL ABTS and 10µL H_2O_2 to each tube very quickly and briefly vortex the solution.
8. Transfer the reaction mixtures to a 96-well plate. Ensure that you do not introduce bubbles.
9. Measure the absorbance at 420nm by a spectrophotometer at 30-second intervals for 20 minutes. Hold a stopwatch near the spectrophotometer's screen & take a picture of the absorbance & time every 30s.

No colour change observed for the ABTS assay.
→ Assay is done with new ABTS stock, meaning ABTS isn't the problem
→ either the hemin or H_2O_2 isn't working
→ the -20ºC fridge sometimes can't be shut tightly and the things inside would melt, the hemin stored in there may have degraded in the process
→ might re-do the assay with new hemin

Storage:
- DNA stock solution: -20ºC
- DNA working solution: 4ºC
- Hemin: wrap with aluminium foil, store at -20ºC
- ABTS: wrap with aluminium foil, store at -20ºC
- H_2O_2: store at 4ºC

13/7/2017 (Thursday)

1. We have received a new stock of Hemin which 86.63mM in concentration
2. The hemin was diluted to a 100uM solution for easy pipetting.

NATIVE PAGE

1. We ran the page at 80 volts and additionally provided it with some cooling using water.
2. The voltage is too low. The contents of the well diffused into the running buffer. The gels had no bands.
3. We used yesterday's protocol.

ABTS ASSAY

1. The assay did not give any signal. The experiment to the controls , no colouration observed.

14/7/2017 (Friday)

Tetrahedron Assembly

1. Tap and centrifuge the prepared working oligo solutions.
2. Prepare the following PCR tubes:

PCR tube label	T1 (5µM)	T2 (5µM)	T3 (5µM)	T4 (5µM)	T5 (5µM)	TTT (5µM)	2X TM buffer	ddH_2O	Final volume	Final oligo conc.
A6	-	10uL	10uL	-	-	-	15.25uL	14.75uL	50uL	1uM
A13	-	-	-	10uL	10uL	-	15.5uL	14.5uL		
B1	10uL	10uL	10uL	-	-	-	10.375uL	9.625uL	50µL	1µM
B8	10uL	-	-	10uL	10uL	-	10.6uL	9.4uL		
B11	-	10uL	10uL	10uL	-	-	10.5uL	9.5uL		
B12	-	10uL	10uL	-	10uL	-	10.5uL	9.5uL		
B13	-	10uL	10uL	-	-	10uL	10.5uL	9.5uL		
C10	10uL	-	-	10uL	10uL	10uL	5.8uL	4.2uL	50µL	1µM
D1(x2)	11uL	11uL	11uL	11uL	11uL	-	5uL	-	60uL	1uM

PCR tube label	T1 (24µM)	T2 (24µM)	T3 (24µM)	T4 (24µM)	T5 (24µM)	TTT (24uM)	1X TM buffer	Final volume	Final oligo conc.
Tetra	10µL	10µL	10µL	10µL	10µL	10uL	/	60µL	4µM

PCR tube label	A1 (5uM)	B3 (5uM)	Template (5uM)	2X TM buffer	ddH_2O	Final volume	Final oligo conc.
+ve control	10µL	10µL	10µL	10.375uL	9.625uL	50µL	1µM

Native PAGE

Oligo (1µM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
1.8µL	9µL	1.2µL	12µL	150nM

Dilute all 5µM solutions to be loaded, namely T1, T2, T3, T4, T5, TTT, Tetra and D1+TTT:

Oligo (5µM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
0.36µL	10.44µL	1.2µL	12µL	150nM

Tetra (4µM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
0.45µL	10.35µL	1.2µL	12µL	150nM

D1+TTT (250nM)	1X TM buffer	10X Loading dye	Final volume	Final conc.
9µL	4.5µL	1.5µL	15µL	150nM

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Ladder	T1	T2	T3	T4	T5	TTT	A6	A13	B1
11	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
Ladder	B8	B11	B12	B13	C10	D1	Tetra	D1+TTT	+ve control (PaperProbeA1 and PaperProbeB3)

B1 (T1+T2+T3),
B11 (T2+T3+T4),
B13 (T2+T3+TTT),
D1,
Tetra, and
D1+TTT do not have clear bands at the expected positions.
(see doc in the ‘gel images folder’)

18/7/2017 (Tuesday)

ABTS Assay

- made blank, 100nm EAD2, 200nm EAD2
- 100nm EAD2 showed colour change, but not for 200nm EAD2

Native PAGE

- ran gel with continuous water flow at 100V
- made 2 gels

To-do:
MAKE 10% APS
Run gel at 80V and run it longer
ABTS: stick to 100nm

19/7/2017 (Wednesday)

1. The gels were Run in the 12.8mM MgCl2 +1xTBE buffer in the -20^{o}C Cold room at 80V
2. The bands appeared to be smeared and distorted. Furthermore, the loading buffer Bromophenol blue turned to a yellow color half way thorough the run.
3. The ABTS assay functioned well as the positive control (EAD2) did show a blue output. There was a greater signal observed for the DNA nanostructure with its target than without it. But, the DNA nanostructure did show a significant signal with the random mRNA

20/7/2017 (Thursday)

1. Made new 1X TBE and 12.8mM MgCl2 +1X TBE buffer, as well as new gels
2. The gels were ran in the 12.8mM MgCl2 +1X TBE buffer in the -20^{o}C Cold room at 80V
3. The bands appeared to be even more smeared and distorted than Wednesday's. The loading buffer Bromophenol blue also turned yellow halfway through the run again.
4. The ABTS assay did not function, minimal colour change was observed
    - a test was done by running the assay for just the EAD2 setup → worked properly → previous failure should be due to human errors

21/7/2017 (Friday)

1. Measured pH of the gel running buffer: alkaline
2. Assembled tetrahedron using the thermocycler
    - made 6 PCR tubes of D1s
3. Run 12% gel at 100V using plain 1X TBE buffer
    - contents of the well floated out, gel could not be run
4. Made 2 8% gels and 2 12% gels
5. ABTS assay

Protocol used:

1. Prepare the reaction mixture as follows, following the subsequent steps:

Reagent	**Working conc.**	**Volume in reaction mixture**	**Final conc.**
DNA nanostructure (D1)	1μM	15μL	100nM
Target DNA(TTT)	5μM	3μL	100nM
Hemin	10μM	6μL	0.4μM

ABTS	21.02mM	14.28µL	2mM
H_2O_2	120mM	15µL	12mM
(2X) ABTS Assay Buffer	/	75µL	/
ddH_2O	/	21.72uL	/
Total volume		150µL	/

2. Add 75µL buffer, 15µL DNA nanostructure, 3µL target DNA and 6µL hemin and briefly vortex the solution.
3. Prepare the following controls as well by adjusting the volume of buffer used so that the final volume is:
    a. Positive control 1

        3 µL EAD2 (5uM) (instead of DNA nanostructure & target DNA) + 75uL buffer, + 6 uL of 10uM Hemin, +36.69uL ddH_2O

    b. Positive control 2

        3µL of 5µM T4 + 3 µL of 5µM T5 + 75uL buffer(2x) + 6uL of 10uM Hemin +33.69uL ddH_2O
    c. Positive control 3

        15 uL of thermocycled +ve + 75 uL buffer +6 uL of 10uM Hemin+ 24.69 uL of ddH_2
    d. Negative control

        blank with no DNA + 75 uL buffer +6 uL of 10uM Hemin+39.69uL of ddH_2O
    e. With DNA nanostructure (D1) only

        15 uL of D1 + 75 uL buffer +3 uL of 10uM Hemin+ 24.69 uL of ddH_2O
    f. With target DNA (TTT)only

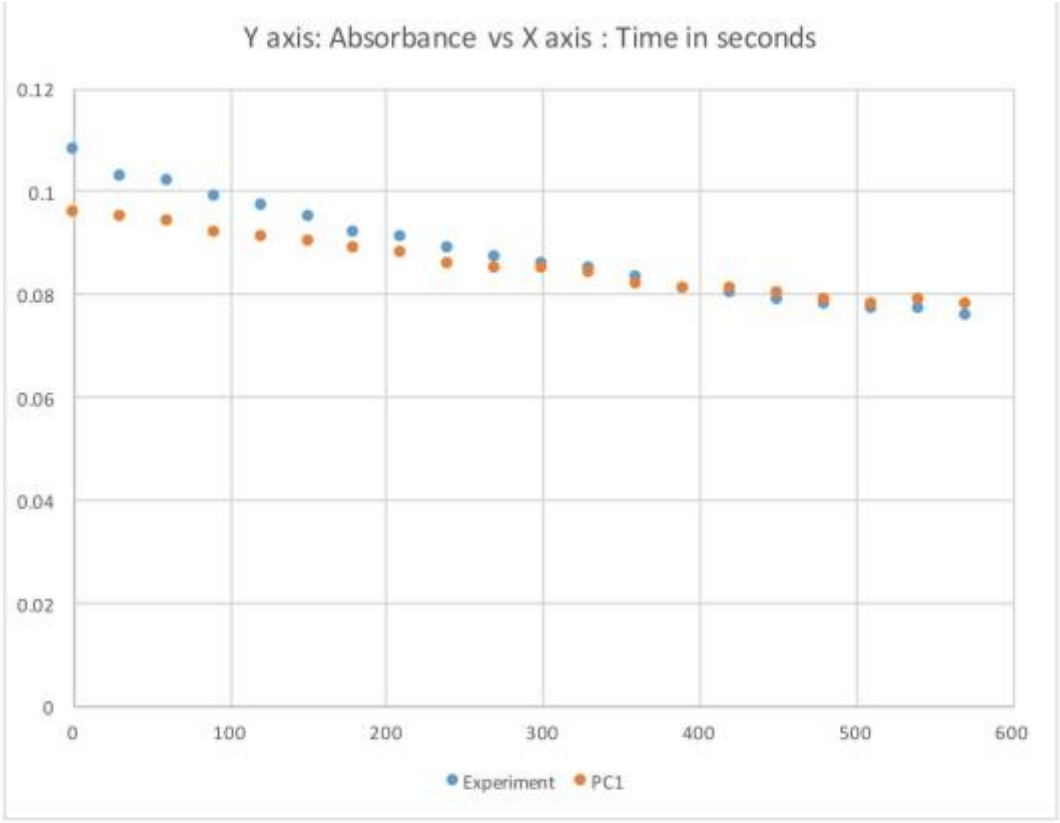
        3 uL of TTT + 75 uL buffer + 6 uL of 10uM Hemin +35.69 uL of ddH_2O
    g. DNA nanostructure + random RNA/RNA mutant

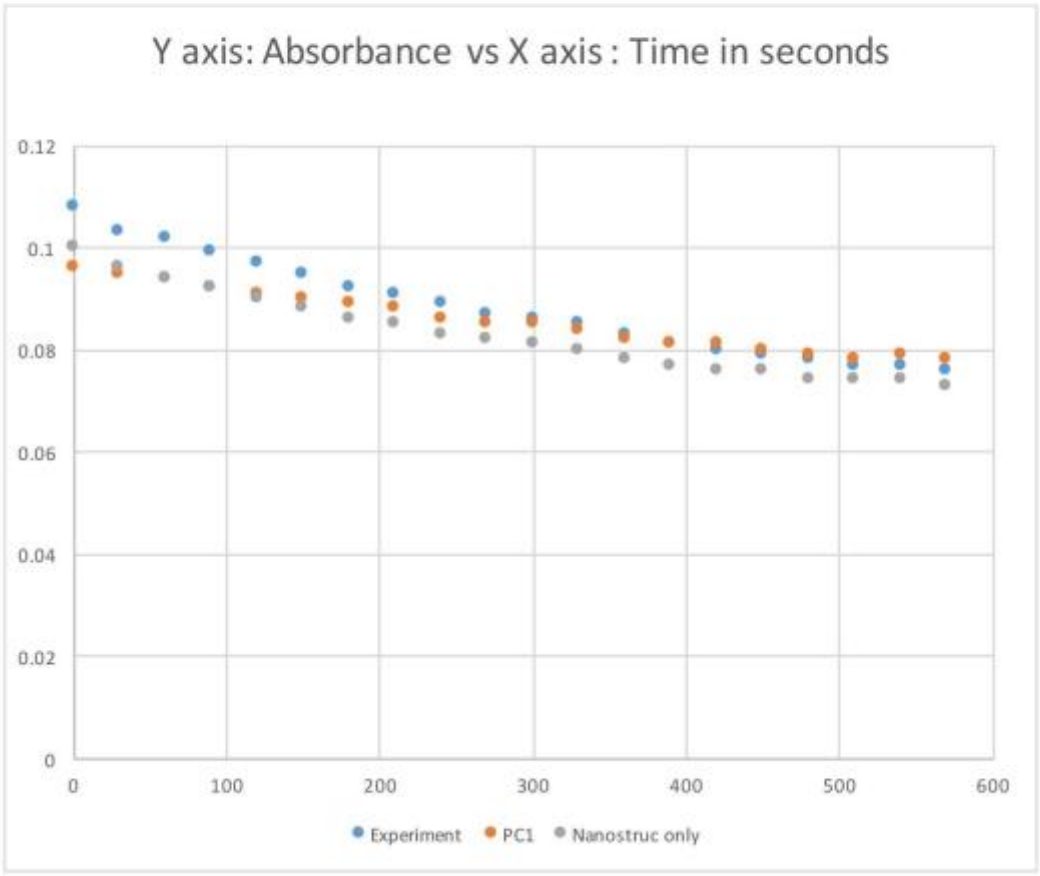
        15 uL of D1 +6 uL random DNA + 75 uL buffer + 6uL of 10uM Hemin + 21.69 uL of ddH_2O
4. Incubate at room temperature on shaker for 20-30 minutes, using a piece of aluminium foil to cover the tube holder (as hemin is light-sensitive).
5. Add **4.76µL ABTS and 5µL H_2O_2** to each tube very quickly and briefly vortex the solution.
6. Transfer the reaction mixtures to a 96-well plate. Ensure that you do not introduce

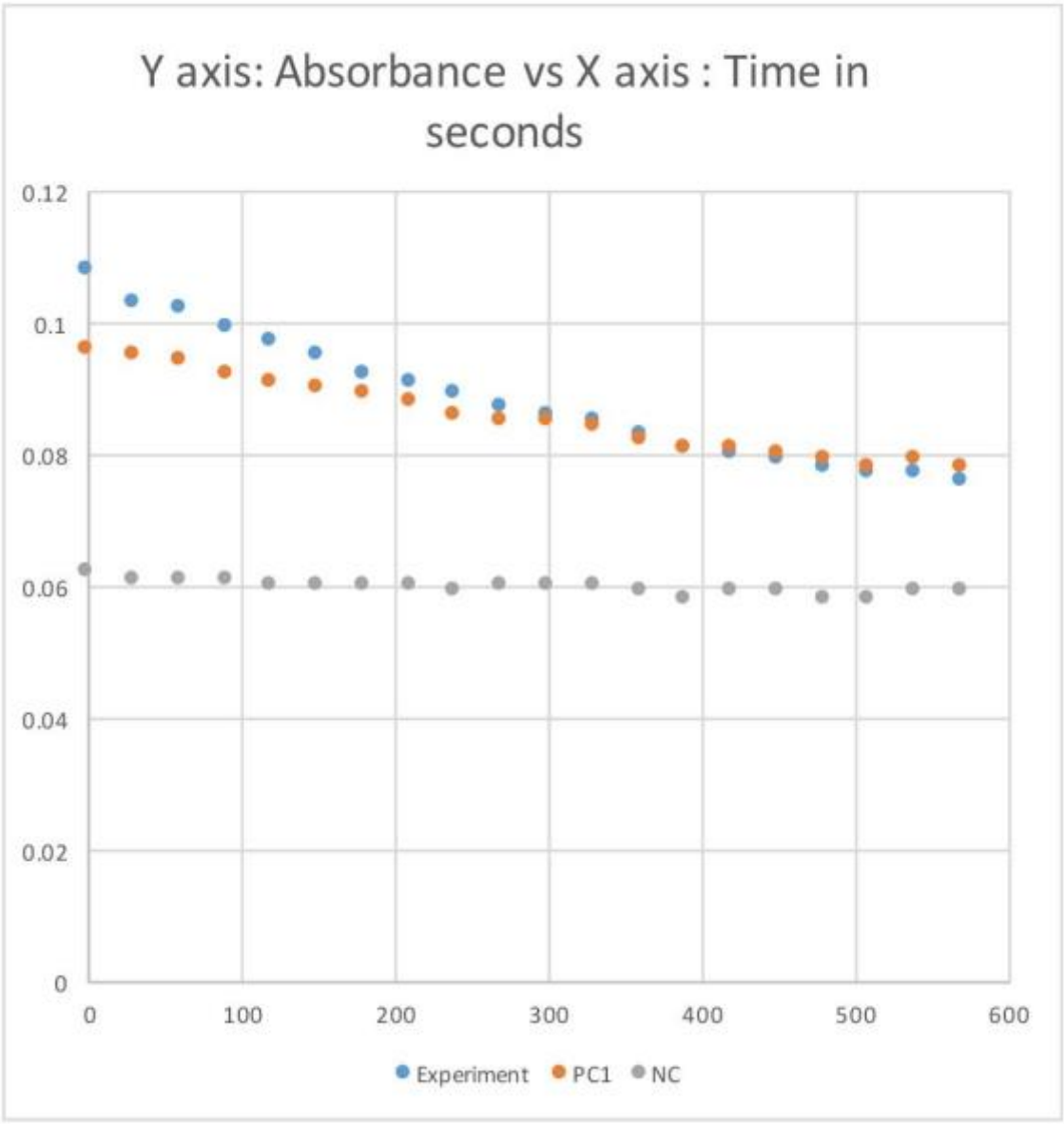
bubbles.

7. Measure the absorbance at 420nm by a spectrophotometer at 30-second intervals for 20 minutes. Hold a stopwatch near the spectrophotometer's screen & take a picture of the absorbance & time every 30s.

The Signal output is high for the first 30 seconds after which it progressively decreases. From the graphs plotted here:


Y axis: Absorbance vs X axis : Time in seconds
0.12
0.1
0.08
0.06
0.04
0.02
0
0
100
200
300
400
500
600
Experiment
PC1


Y axis: Absorbance vs X axis : Time in seconds
0.12
0.1
0.08
0.06
0.04
0.02
0
0
100
200
300
400
500
600
Experiment
PC1
Nanostruc only



24/7/2017 (Monday)

Native PAGE

- Ran 8% gel at 100V in cold room
  → distortion and yellowing occurred towards the end
- Redo Native PAGE and run agarose gel tmr

ABTS Assay

- very weak signal observed
- redid the EAD2 setup, weak colour change observed as well.
- The ABTS assay was done by Rachel. She observed a few mistakes in the volume and the concentration in the previous protocols. Corrections were made.

25/7/2017 (Tuesday)

Native PAGE

- ran 12% gel at 80V in cold room for 1hr 30mins
- distortion and yellowing is minimal
- and does not affect reading of the gel (band qualities were better)

Agarose gel

- 1% gel at 100V at room temp for 2hrs 15mins
- 130mL of agarose (small one is 60mL)
- post-stained (pre-stain will affect mobility according to jess)
- dust accumulated within the gel, smearing occurs, suspected diffusion

ABTS assay

- The ABTS assay was ran twice
- In the first run, the only change made to the protocol was the incubation time, from 30 mins to 15 mins
- In the second run the pcr tray was put on ice and the H_2O_2 was added at the end next to the spectrometer.

Mini-prep for DH5alpha

- 5mL of LB + one colony of DH5alpha
- incubating overnight at 37C 100rpm

26/7/2017 (Wednesday)

Stock solutions

- made 5uM stock solution for Tim's oligos, and 5uM + 25uM stock solution for Rachel's oligos from stock
    - For Rachel's Oligo 2: a mistake was made with the addition of 321uL ddH2O instead of 291uL ddH2O, hence the stock for oligo 2 is 90.7uM instead of 100uM

Reagents

- made 5X TM buffer, checked for pH (7.0) and autoclaved

Competent cell

- failed → no growth was observed in the 5mL LB Broth
  → negative absorbance
- redid mini-prep for DH5alpha
    - 5mL of LB + one colony of DH5alpha
    - incubating overnight at 37C 100rpm

Native PAGE

- Ran gel with all wells containing 10uL of 1X loading buffer in cold room at 100V for around 2 hours
    - buffer: Mg + 1X TBE
    - minimal distortion and no yellowing was observed

27/7/2017 (Thursday)

Stock and Working solutions

- Made stock solutions for Yash's oligos
- Made working solutions for Tim's, Rachel's, and Yash's oligos
  labels: plain black marker - Tim's
  blue dot - Rachel's
  red dot - Yash's

→ For Tim's Oligos:
TM buffer concentration has been changed to 5X TM buffer in working solutions, hence the calculation changes in the tetrahedron assembly as well (see below)

Preparation of Working Oligo Solutions (Tim's)

1. Wipe the bench and your gloves with 70% ethanol.
2. Briefly vortex the oligo stock solutions before dilution.
3. Dilute the stock solutions with 1X TM buffer as follows:

Eppendorf/PCR tube label	**Initial conc. of stock solutions**	**Final conc.**	**Volume of stock solution**	**Volume of 5X TM buffer**	**Volume of ddH_2O**	**Final volume (1X TM)**
Tim1 (5µM)	200µM	5µM	7.5µL	60µL	232.5µL	300µL
Tim2 (5µM)		5µM	7.5µL	60µL		300µL
Tim3 (5µM)		5µM	7.5µL	60µL		300µL
Tim4 (5µM)	100µM	5µM	15µL	60µL	165µL	300µL
Tim5 (5µM)		5µM	15µL	60µL		300µL
TimTetraTarget (5µM)		5µM	15µL	60µL		300µL

4. Mix well by tapping and briefly centrifuge the tubes.
5. Store at -20°C

Tetrahedron Assembly (Tim's)

1. Tap and centrifuge the prepared working oligo solutions.
2. Prepare the following PCR tubes:

PCR tube label	**T1 (5µM)**	**T2 (5µM)**	**T3 (5µM)**	**T4 (5µM)**	**T5 (5µM)**	**TTT (5µM)**	**1X TM buffer**	**Final volume**	**Final oligo conc. (1X TM)**
A6	-	10uL	10uL	-	-	-	30uL	50uL	1uM
A13	-	-	-	10uL	10uL	-			
B1	10uL	10uL	10uL	-	-	-	20uL	50µL	1µM
B8	10uL	-	-	10uL	10uL	-			

B11	-	10uL	10uL	10uL	-	-			
B12	-	10uL	10uL	-	10uL	-			
B13	-	10uL	10uL	-	-	10uL			
C10	10uL	-	-	10uL	10uL	10uL	10uL	50µL	1µM
D1(x4)	10uL	10uL	10uL	10uL	10uL	-	-	50uL	1uM

3. Tap to mix and briefly centrifuge the PCR tubes.
4. Incubate the PCR tubes at 95°C for 5 min and cool them down to 20°C with a 0.5°C drop every 30 seconds and then drop to 10°C using a thermocycler (program called "igem annealing" already saved in thermocycler).
5. Store at 4°C.

DNA nanostructure assembling

- assembled Tim's, Rachel's and Yash's DNA nanostructures

Native PAGE

- obtained a Bio Rad gel setup
- made 2 12% gels

Agarose gel

- made 1.2% agar gel, ran at room temperature for 1 hour, post-stained with GelRed
- gel image showed very faint and thick bands
- gel thickness is suspected to be affecting the auto-exposure of the gel doc
  → consider reducing thickness of the gel in future runs

ABTS Assay

- done twice, both times the assay was incubated for 15mins, and triplicated after the addition of ABTS and H2O2
- first assay:
  - D1+TTT, EAD2, T4+T5, blank, D1 only, TTT only, D1+mutant RNA
- second assay:
  - D1+TTT, T4+T5, blank, D1 only, TTT only

- for both assays, ABTS was added at the bench, H2O2 was added next to the spectrophotometer, with one person adding 15uL H2O2 to each of the PCR tube (starting with the controls with H2O2 added last to the D1+TTT tube), and another person pipetting 45uL from the PCR tube to each well
  - absorbance is then measured at 1-minute intervals for 10 minutes

- for the first assay: H2O2 was added to the PCR tubes only when the person triplicating is pipetting into the last well for the tube
  → this may have lead to higher discrepancies of absorbance levels due to the reactions at a later time than the previous tube

- for the second assay: H2O2 was added immediately without waiting for the person triplicating to be done with the pipetting, the tubes are kept in the ice box in the meantime

Competent cell

- minimal growth was observed again, with the LB broth remaining clear
- as the LB broth was from last year, new LB broth was made from pure LB agar powder and autoclaved
- LB agar was also made from pure LB agar powder and put onto the petri dish
- a colony is taken from the DH5alpha stock and streaked onto a petri dish, this process is done twice
- the two petri dishes are then sealed with parafilm until it was deposited in the warm room (37^0C) , where they are kept aerated overnight for bacterial growth

TMR:

Native PAGE

- make 2 8% gels for Rachel's PAGE
- run Yash's page using the 2 12% gels made previously
  → all 4 gels run in the Bio Rad tank at 80V in cold room

ABTS assay by Yash

- D1+TTT, EAD2, T4+T5, blank, D1 only, TTT only
  (EAD2 to make sure the assay is working)

checked on the two petri dishes in the warm room

MONDAY:

ask for more 10X TBE

Changes to be made to the agarose Gel electrophoresis

1. Run a 3%- 4% Agar gel. The recommended percentage for a 25- 1000 Bp DNA is 3%.
2. Calculate the voltage using the following equation = (5-10 V) x the distance between the two electrodes
3. Run the gel until bromophenol blue (purple, lower band) is 2/3rd of the way of teh gel.

28/7/2017 (Friday)

- ABTS assay for Tim's structure
-

31/7/2017 (Monday)

- Agarose gel of Tim's structure → error with electricity/power source
- PAGE of Rachel's structure (8%) → error with buffer leakage so unclear/wavy bands.
- ABTS assay → pipetting error with addition of H2O2

1/8/2017 (Tuesday)

- Tested the ABTS assay using EAD2 → 2 samples, one with and one without time lag, readout was low
- Prepared petri dish/cell culture for DH5alpha
- PAGE for Rachel's and Yash's structure → error with PBS, no pre-running, wells not washed.

2/8/2017 (Wednesday)

- ABTS assay for Rachel & Tim (with plate reader 3/F)
- Agarose gel for Tim's
- Mini-prep for competent cells (at 11am)

3/8/2017 (Thursday)

4/8/2017 (Friday)

- ABTS assay for all structures using 3/F plate reader (following Simon's protocol)
  → Ivan's structure and structure + target showed observable increase in absorbance, but not the other structures (inc. EAD2)

→ plan: change all TM buffer to PBS buffer

- remake working solutions and thermocycle new products next week

7/8/2017 (Monday)

- made 1X PBS and 2X PBS buffer
- tested assay with just EAD2
  → dilution of 2.5uM EAD2 to 1uM using various buffers
  - one setup: 1X PBS buffer
  - one setup: Ivan's 1X TM buffer
  - very weak colour change, obtained new 2X ABTS buffer from Simon → colour change becomes more observable, but both setups had similar observed colour change intensity

8/8/2017 (Tuesday)

- made working solutions using PBS buffer and thermocycled

- tested ABTS assay with different concentrations of EAD2: blank, 1uM, and 2.5uM
  → 2.5uM EAD2 generated more observable absorbance level increases
  → protocol changed from 1uM ND to 2.5uM ND
  → final concentration of ND in assay = 250nM

9/8/2017 (Wednesday)

- ran 12% PAGE gel for Tim's structures
  - gel images showed significant smearing: suspected due to well-washing
- Yash did ABTS assay for all 4 structures in one-go (without triplicating) using the new protocol of 250nM ND
  - the assay is incubated for 3 minutes after the addition of ABTS and H2O2

Results:

	ND*	**ND+T***	**Ratio (|ND+T/ND|)**
Tim	-0.003	0.114	38
Rachel	0.004	0	0
Yash	-0.005	0.039	7.8
Ivan	0.007	0.292	41.71

*Noise has been deducted

- Yash did ABTS assay again with just Tim's and Ivan's structures (with duplicating)
  → all NDs showed higher absorbance levels than their corresponding ND+Ts
- thermocycled new assembled structures

10/8/2017 (Thursday)

- Yash redid the ABTS assay with just Tim's and Ivan's structures (with duplicating)
  → all NDs showed higher absorbance levels than their corresponding ND+Ts
- Ravneet redid the ABTS assay with newly thermocycled solutions
  → all NDs showed higher absorbance levels than their corresponding ND+Ts
- Rachel redid the ABTS assay with Tim's, Yash's, and Ivan's structures (with duplicating)
  → pipetting error: added 15uL (instead of 10uL) of H2O2 to blank and hemin-only
  → Tim's and Ivan's NDs showed higher absorbance levels than their corresponding ND+Ts
  → Yash's duplicates were inconclusive
  1: ND showed slightly higher absorbance levels than their corresponding ND+Ts
  1: ND showed slightly lower absorbance levels than their corresponding ND+Ts

11/8/2017 (Friday)

- ABTS Assay by Yash
- The ABTS assay was erroneous and hence Simon rana fluorescent dye test to check out results. The following volumes were used -

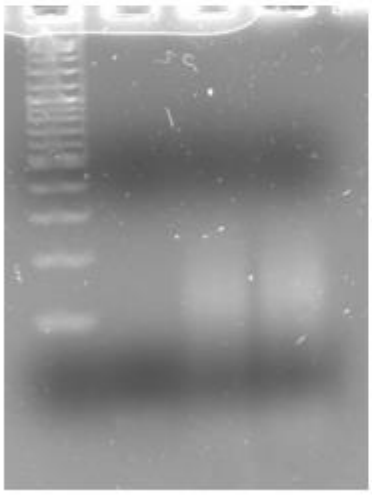
Nanostructure (Labeled as Y/T/R/I)	2 µM	22 µl
Target (Labeled as Y-T1/ Y-T2/T-T/R-T/I-T)	2 µM	12 µl

- The results showed that the nanostructure turns on without target.
- The competent cell work failed multiple times due incorrect sterilization techniques, causing contamination. The previous year's LB broth contained antibiotics, which we were unaware of.
- We ran an agarose gel shown below

Agarose Gel

25th August 2017

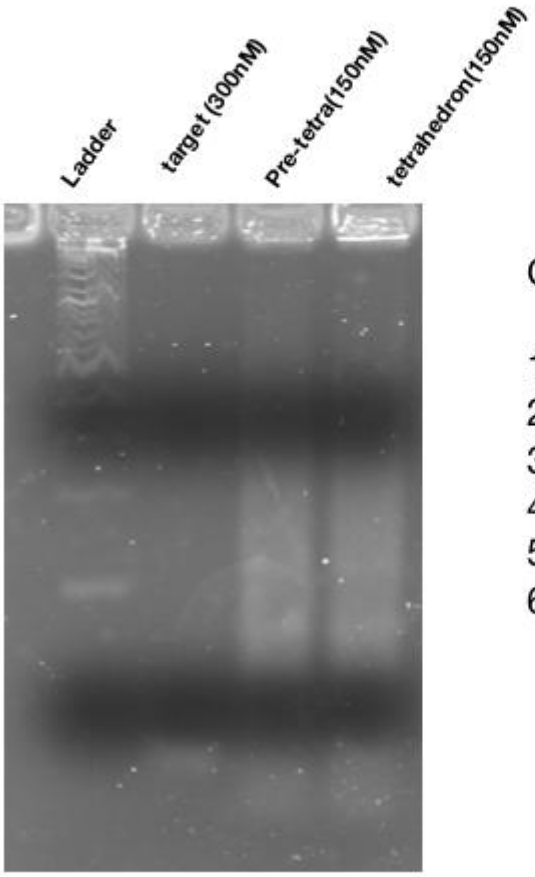
22nd August 2017



Conditions:

1. 100 Volts
2. no Pre-run gels
3. 2% agarose
4. Run at room temperature
5. Stain 30 mins
6. Quality of Gel: bubbles

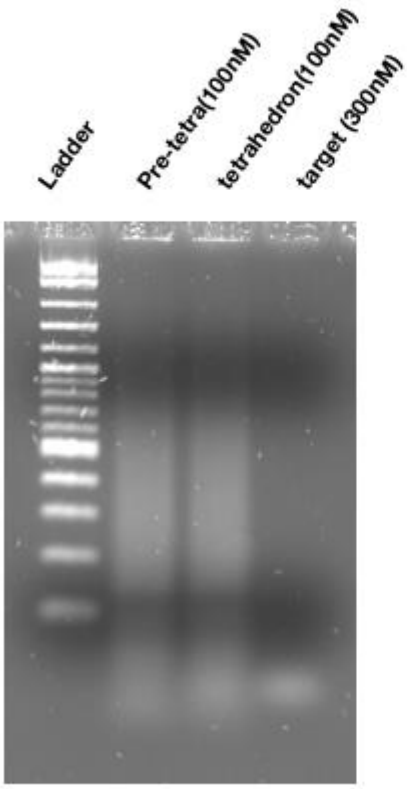
24th August 2017



Conditions:

1. 100 Volts
2. no Pre-run gels
3. 2% agarose
4. Run at 4C Cold room
5. Stain 15mins
6. Quality of Gel: few bubbles

25th August 2017



Conditions:

1. 80Volts
2. Pre-run gels for 10 mins
3. 2% agarose
4. Run at 4C Cold room
5. Quality of Gel setting better
6. Stain for 45mins

25/8/2017 (Friday)

1. Competent cell work:

    1. Currently working on DH10beta. The cell worked failed so we will get a batch of competent cells from Jess. Which means we can start working on the plasmid part

    2. Gels : Agarose

    1. Having tried different combinations of Target and nanostructure. And furthermore, having tried running it in the cold room, we are currently seeing only smears in the form of a normal distribution on the gel. This means that the individual oligos are not in a 1:1:1:1 ratio. Therefore, page gel for each individual oligo has to be run and analysed using imageJ to check if the stock concentration is correct. Upon assessing that we can make correction and redo the therm-cycling again.

    https://drive.google.com/a/connect.hku.hk/file/d/0B8D80gO4HPq2eHNmVWp0c3pSSVU/view?usp=sharing

    3. The Green fluorescent test: The signal on is very minimal in our current structure. In order to make it better we ordered the complementary sequence.

    4. Current goal: To produce the plasmid with our inserts and prove that Tim's structure works.

4 Sep 2017

- ran a 2% agarose gel at 80V

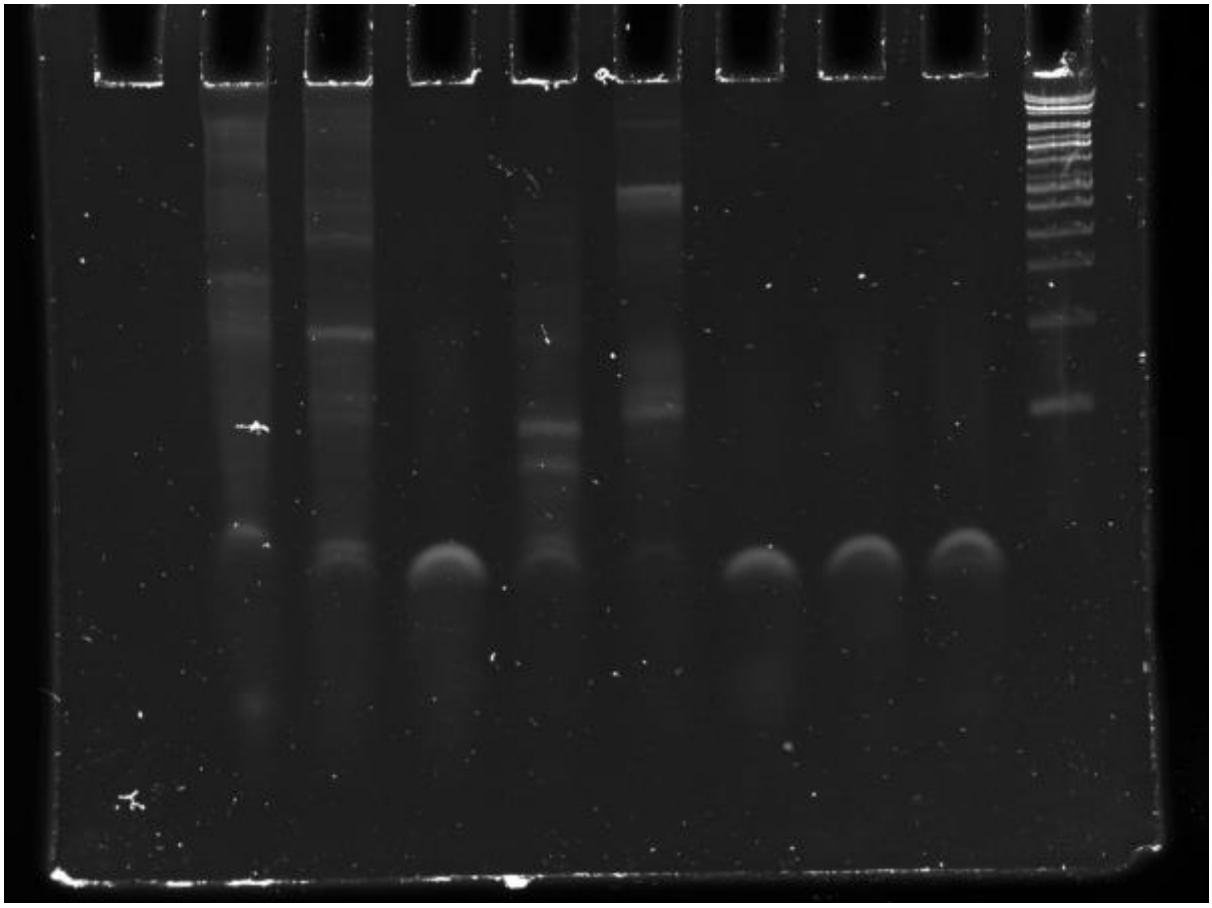
Reagents	T1 (5uM)	T2(5uM)	T3(5uM)	T1+T2 (2.5uM)	T2+T3 (2.5uM)	T1+T3 (2.5uM)	T1+T2+ T3(2.5uM)	T1+T2+ T3+TTT (2.5uM)
Oligos	0.36uL			0.72uL				
Loading (6x)	2uL							
1x PBS	9.64uL			9.28uL				
Final Conc	100nM							
Final Vol	12uL							

DNA Ladder: 1ul DNA + 2uL Loading +9uL 1xPBS

6 Sep 2017

- ran a 8% agarose gel

Reagents	DNA Ladder	T1 (5uM)	T2(5uM)	T3(5uM)	T1+T2 (2.5uM)	T1+T3 (2.5uM)	T2+T3 (2.5uM)	T1+T2+ T3(2.5uM)	T1+T2+ T3+TTT (2.5uM)
Oligos	0.5uL	0.36uL			0.72uL				
Loading (6x)	2uL								
1x PBS	9.5uL	9.28uL			9.64uL				
Final Conc	-	100nM							
Final Vol	12uL								



we noticed a multimer structure of about 600bp ,when T1 and T2 are put together, being formed.

8 Sep 2017

- **In order to assess the formation of this multimer rather than a error in pipetting error we are remaking the T1-T2 complex in different ratios and observing the change in formation of the DNA bands. The T1:T2 ratio we are using are 1:1, 1:2,2:1.**
- **We prepared the following solutions for Thermocycling**

PCR tube label	T1 (20µM)	T2 (20µM)	1X PBS	Final volume	Final oligo conc.
T12 A	6.25uL	6.25uL	37.5uL	50 uL	2.5uM

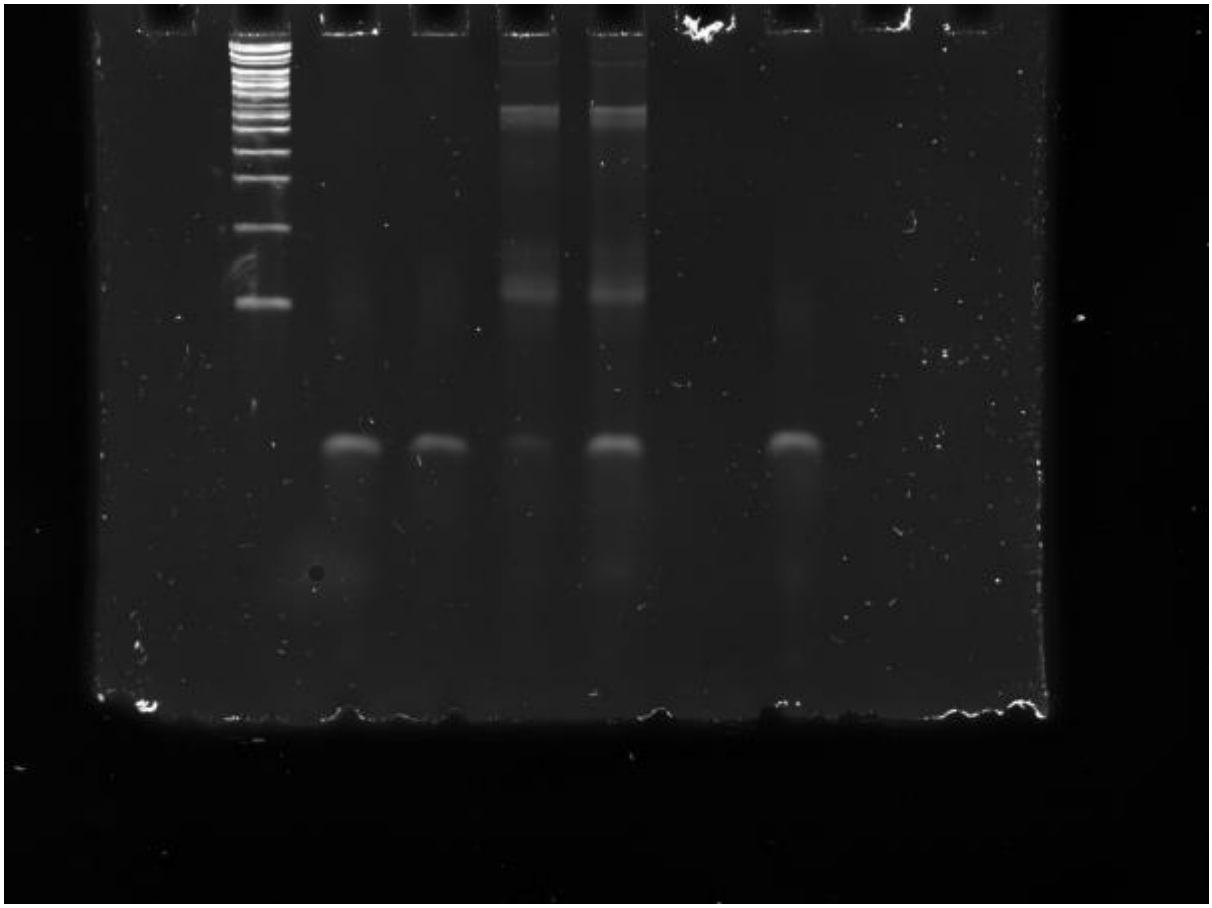
PCR tube label	T1 (20µM)	T2 (20µM)	1X PBS	Final volume	Final oligo conc.
T12 B	12.5uL	6.25uL	31.25uL	50uL	5um:2.5uM

PCR tube label	T1 (20µM)	T2 (20µM)	1X PBS	Final volume	Final oligo

					conc.
T12 D	6.25uL	12.5uL	31.25uL	50uL	2.5uM:5uM

- **We ran a 8% PAGE gel**

Reagents	Ladder	T1 (5uM)	T2(5uM)	T12A (2.5uM)	T12B (2.5uM)	T12D (2.5uM)
Oligos	0.5uL	0.36uL		0.72uL		
Loading (6x)	2uL					
1x PBS	9.5	9.64uL		9.28uL		
Final Conc	N/A	150nM		150nM		
Final Vol	12uL					



11 Sep 2017

- We prepared the following **solutions for Thermocycling**

PCR tube label	T1 (20µM)	T2 (20µM)	T6 (20uM	1X PBS	Final volume	Final oligo conc.
T12 A	6.25uL	6.25uL	6.25	31.5uL	50 uL	2.5uM

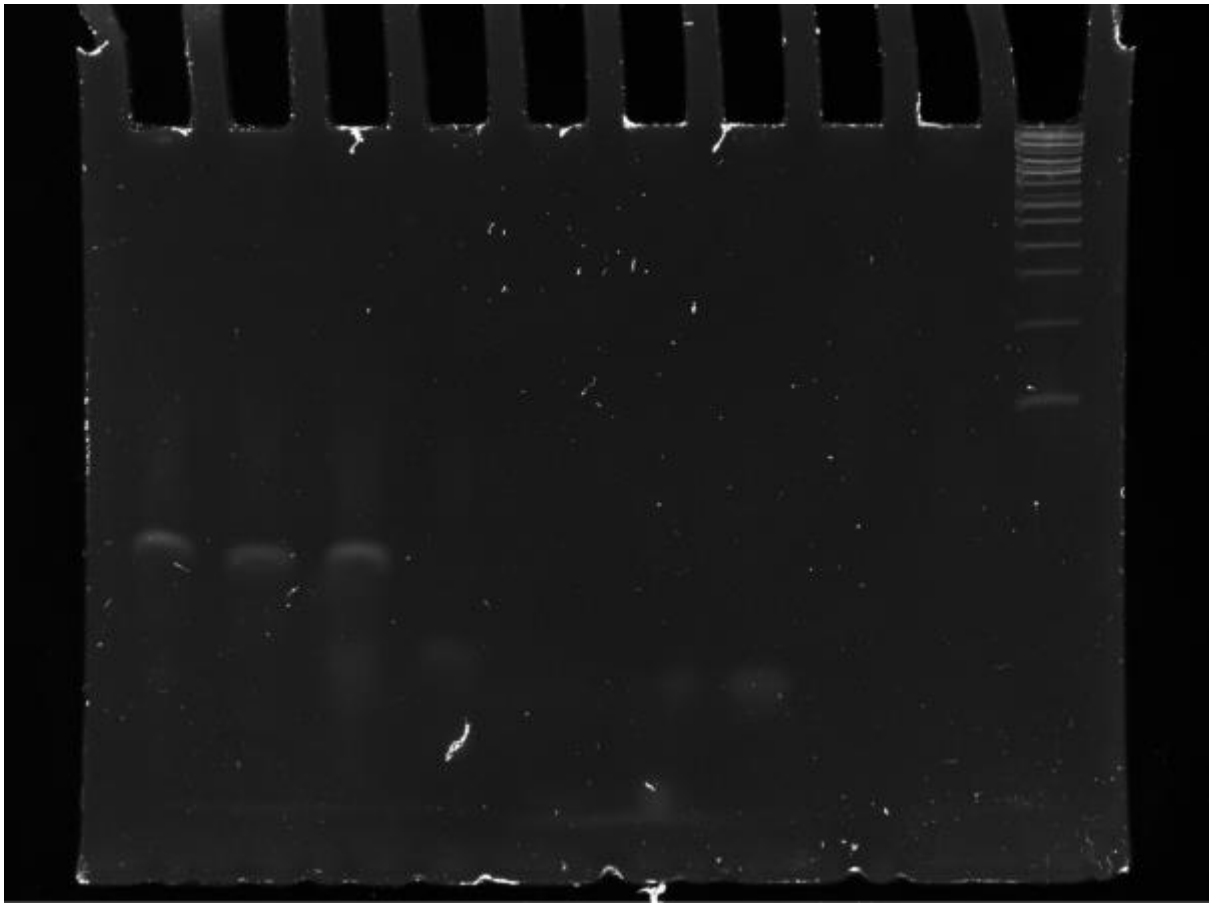
PCR tube label	T1 (20µM)	T3 (20µM)	T6 (20uM)	1X PBS	Final volume	Final oligo conc.
T12 B	6.2.5uL	6.25uL	6.23uL	31.25uL	50uL	2.5uM

PCR tube label	T1 (20µM)	T2 (20µM)	1X PBS	Final volume	Final oligo conc.
T12 D	6.25uL	12.5uL	31.25uL	50uL	2.5uM:5uM

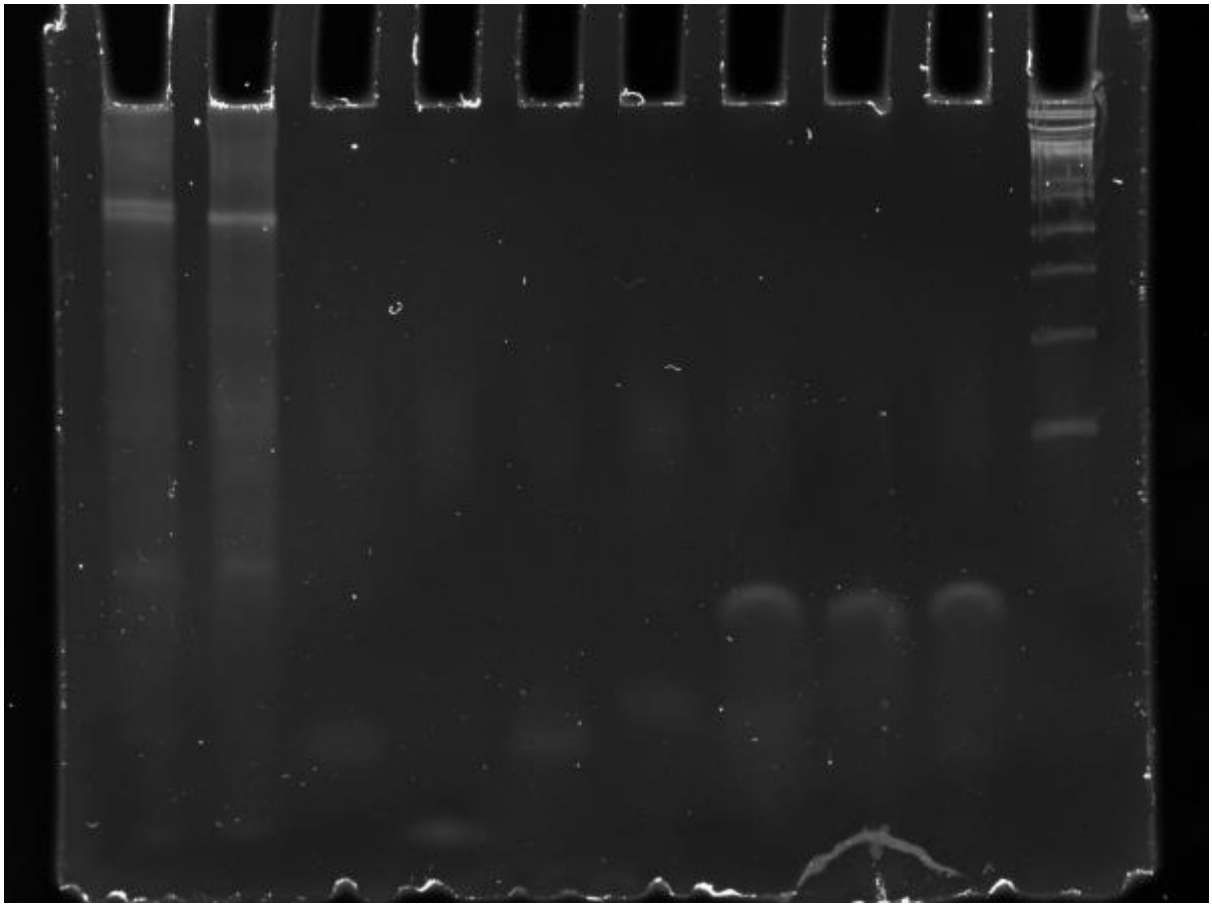
- We ran a 8% gel at 70V

Reagents	Ladder	T1 (5uM)	T2(5uM)	T12A (2.5uM)	T12B (2.5uM)	T12D (2.5uM)
Oligos	0.5uL	0.36uL		0.72uL		
Loading (6x)	2uL					
1x PBS	9.5	9.64uL		9.28uL		
Final Conc	N/A	150nM		150nM		
Final Vol	12uL					

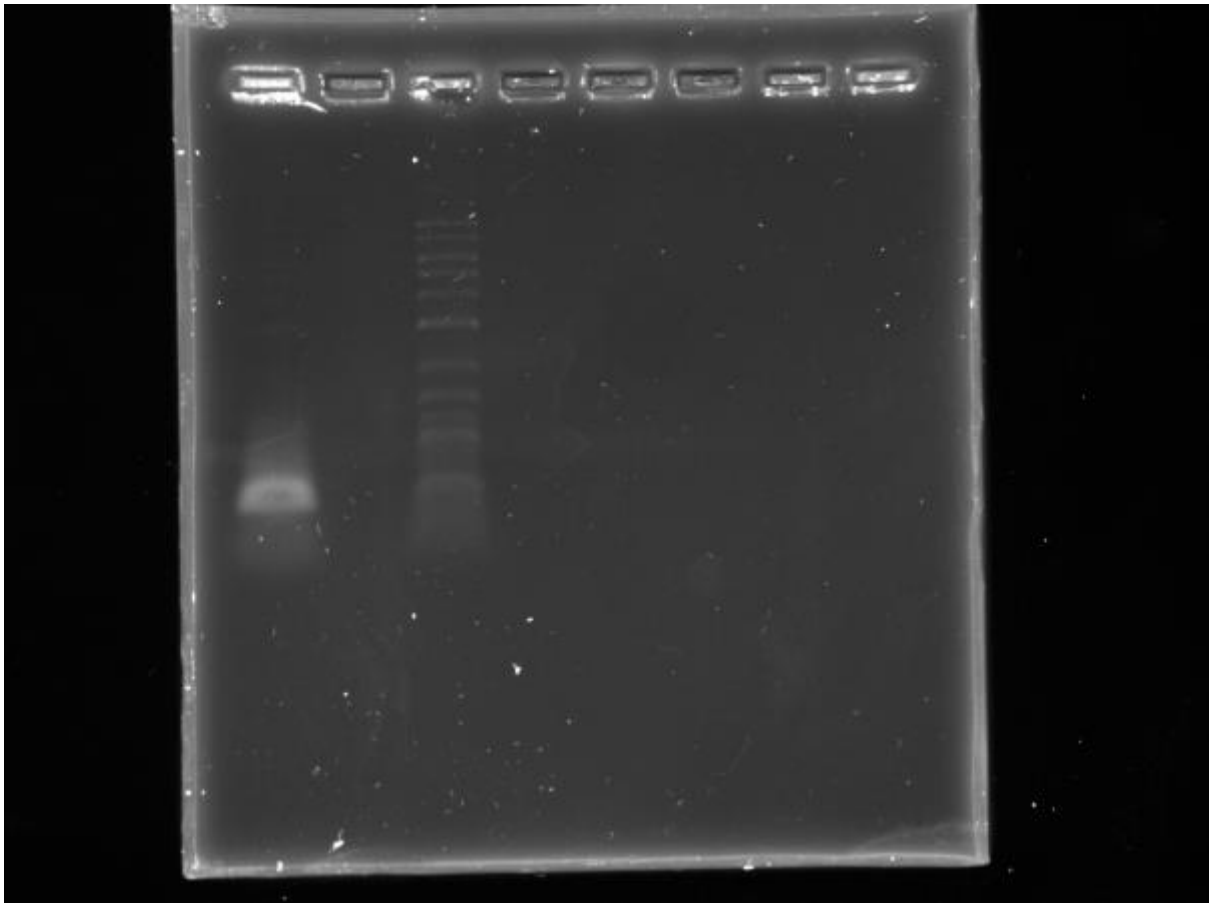
12 Sep 2017



13 Sep 2017



18 Sep 2017

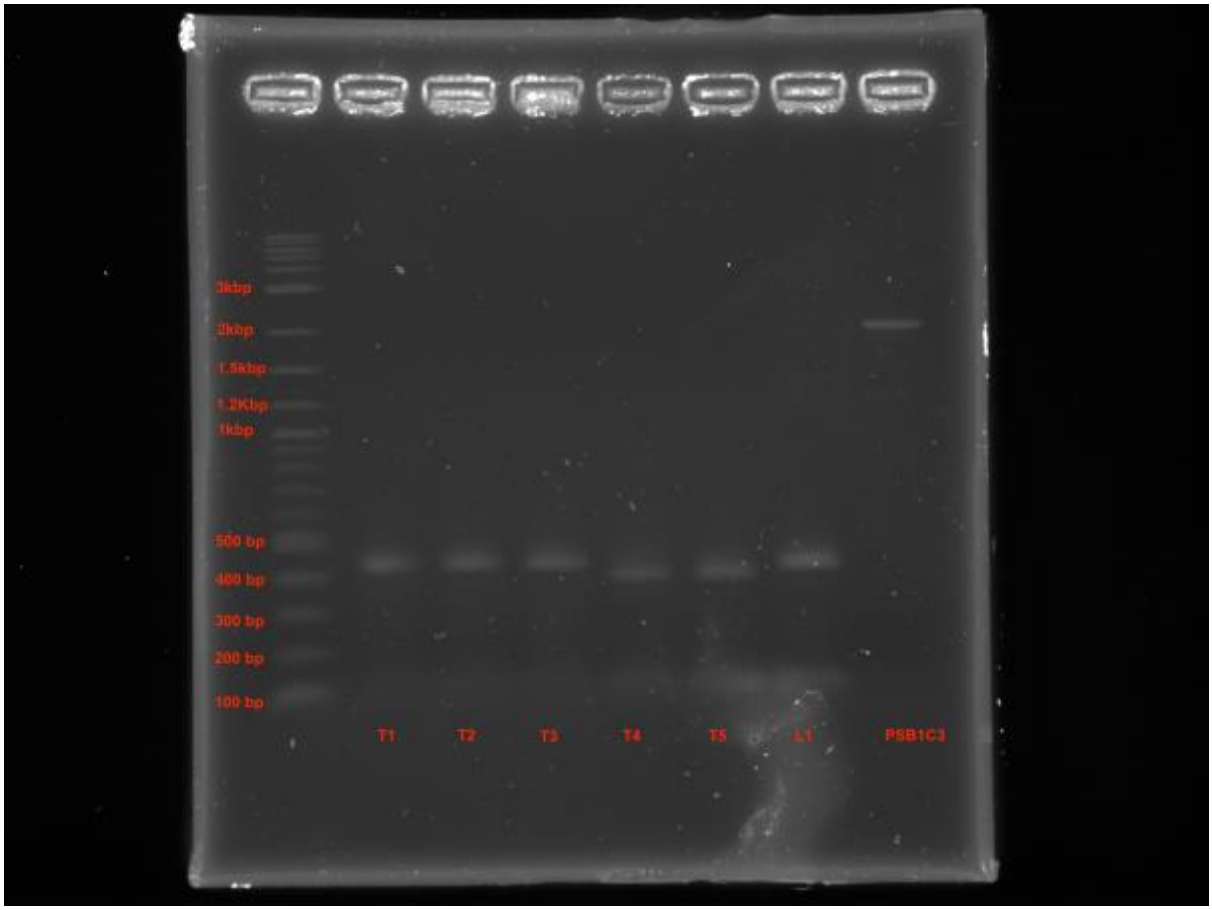


9 Oct 2017

- We assessed the formation of our insert PCR product in an agarose gel
- We assessed the concentration of our insert PCR product using nanodrop.

12 - 16 Oct 2017

- We digested our insert & iGEM backbone pSB1C3 using restriction enzymes



- We ligated our insert to iGEM backbone pSB1C3 & transformed the plasmid into competent *E. coli*

13/10
working 2.5um for ead2,p1,p2,t
template 100um to 20 um working, 2.5um final, **4**ul DNA, H20 **124**, 5X PBS **32**, TT 160
p1+p2 15, 15 6hem, 75, 9.72h20 (29.28)
p1+p2+t, 15,15,15,6hem, 75 xh202
\+ 15, 6 hem,75 24.72h20

ABTS, 60+40dmso
h2o2, 1 1.23+98.77
2 1.85+98.15