09/20/2013 AR

- Organized for conductivity and NMR spin coupling test sample preparation
- Handed off NMR sample to NMR lab at Stanford.

09/19/2013 AR

- Prepped X-Ray sample

09/18/2013 AR

- Prepped NMR sample

09/17/2013 AR

- Received sequencing results. 1 was correct.
- Sent in SBB biobrick (BBa_K1218026) to registry

09/16/2013 AR

- Miniprepped liquid cultures
- Sequenced all 10 minipreps

09/14/2013 AR

- Made recipe for COSY-H NMR sample prep
    - Assume we were given 4umol of DNA
    - Have 2:1 ratio of silver ions to total C-C mismatches in solution
    - Recipe for sample 1 (all reagents already suspended in D_2O)
        - § 10uL 0.2M $AgNO_3$
        - § 19.5uL 1M $NaNO_3$
        - § 26uL 0.1M K_3PO_4
        - § 200uL ssDNA (half of the DNA)
        - § 0.5uL D_2O
        - § 260uL total
    - Recipe for sample 2 (all reagents already suspended in D_2O)
        - § 19.5uL 1M $NaNO_3$
        - § 26uL 0.1M K_3PO_4
        - § 200uL ssDNA (half of the DNA)
        - § 10.5uL D_2O
        - § 260uL total
- Made recipe for X-Ray sample prep
    - Have 2:1 ratio of silver ions to total C-C mismatches in solution
    - Assume we were given 2umol of DNA
    - Recipe
        - § 50uL 0.2M $AgNO_3$
        - § 19.5uL 1M $NaNO_3$
        - § 26uL 0.1M K_3PO_4
        - § 82.25uL DNA from tube 1

        - § 82.25uL DNA from tube 2
        - § 260uL total
- Made 10 liquid cultures of colonies from SBB-mut colonies on the plates

09/13/2013 AR

- Received SBB addition primers. Did addition PCR. Cleaned up result.
- Did T4 PNK Phosphorylation on addition-PCR result
    - Recipe
        - § 1uL ligase buffer
        - § 1uL T4 PNK
        - § 3uL DNA
        - § 5uL DI Water
        - § 10uL total
    - Heat cycler
        - § 37˚C – 1 hr
- Then added 1uL T4 DNA Ligase to PNK result
    - Incubated at 25˚C for 2 hrs
- Transformed and plated the result at 6 PM
- Did inventory of all Biowires DNA currently in the lab

09/12/2013 AR

- Sequencing results show 2 of the samples were 100% what we wanted to be (165/165 bp were correct).
- However, we forgot to add 2 base pairs when designing the sequence, so these must now be added through addition-PCR.
- Designed and ordered primers for addition-PCR.
    - Reverse: 5' - CGG CCG CGA ATT CCA GAA ATC - 3'
    - Forward: 5' - CTT CTA GAG CCA AGC ACG CCC - 3'
    - These primers will amplify the entire SBB-pSB1C3 plasmid and also add 2 base pairs (AG)

09/11/2013 AR

- Sent in 3 samples for sequencing
- Drying DNA for COSY-H NMR in rotovap (will resuspend in D_2O)

09/10/2013 AR

- Plates for SBB-pSB1C3 had colonies in the morning. Made liquid cultures for 6 of the colonies in the morning.
- In afternoon, did colony PCR on the 6 day-cultures.
    - Recipe
        - § 1uL from SBB-pSB1C3 liquid culture
        - § 1uL VF2 primer
        - § 1uL VR primer

        - § 10uL GoTaq mix
        - § 7uL DI Water
        - § 20uL total
    - Heat cycler
        - § 94˚C – 120 sec
        - § 30 repeats
            - ú 94˚C – 30 sec
            - ú 57˚C – 30 sec
            - ú 72˚C – 120 sec
        - § 72˚C – 5 min
    - Gel analysis of colony PCR
        - § 3 of the colonies showed correct bands. Will sequence tomorrow
- Split day-culture of SBB-pSB1C3 into 3 overnight cultures

09/09/2013 AR

- Digestion of cleaned-up SBB PCR result
    - Recipe
        - § 0.5uL EcoR1 HF
        - § 0.5uL Pst1 HF
        - § 2uL Cutsmart Buffer
        - § 17 uL DNA (~900 ng)
        - § 20uL total
    - Heat cycler
        - § 37˚C for 60 min
    - Mini-elute cleanup
- Digestion of pSB1C3
    - Recipe
        - § 0.5uL EcoR1 HF
        - § 0.5uL Pst1 HF
        - § 2uL Cutsmart Buffer
        - § 17 uL DNA
        - § 20uL total
    - Heat cycler
        - § 37˚C for 60 min
    - Mini-elute cleanup
- Ligation of digested SBB and pSB1C3
    - Recipe
        - § 1uL digested pSB1C3
        - § 1uL digested SBB
        - § 3uL T4 buffer
        - § 1uL T4 Ligase
        - § 6uL total
    - Heat cycler

        - § 25˚C – 10 min
        - § 80˚C – 20 min
    - Result saved in -30˚C freezer
- Transformation of ligated SBB-pSB1C3 product into NEB-5-alpha
    - Plated 2 plates at 4:50 pm (1 100uL plate, 1 150uL plate)
    - Put unplated, transformed cells in 4˚C
- Received BBa_K1218022 (complete brick for Biowires) from DNA 2.0
- Made glycerol stock and streaked plates of BBa_K1218022
- Prepped BBa_K1218022 for submission
    - Created page on the Registry of Standard Biological Parts for BBa_K1218022
    - Added 50uL to dry DNA, put in 50mL falcon tube, shipped to iGEM HQ

09/06/2013 AR

- Received “Star Brick Basic” (SBB) oligo
    - Concentration was 297 ng/uL
    - Did 1:5 dillution to deconcentrate. Ended up with concentration of around 60 ng/uL
- Made 1:10 dillution of primers (2 primers that will be used to amplify Star Brick Basic)
    - 200 uM à 20 uM final concentration
    - F Primer SBB: 967.25 ng/uL à 96.725 ng/uL final concentration
    - R Primer SBB: 993.88 à 99.388 ng/uL
- Did PCR amplification of SBB
    - Recipe
        - § 25uL 2x Q5 Polymerase master mix
        - § 1uL SBB (60ng/uL)
        - § 1uL Primer mix (equal parts F Primer and R Primer)
        - § 23uL deionized water (DI Water)
        - § 50uL Total
    - Heat cycler
        - § 98˚C – 30 sec
        - § 28 repeats
            - ú 98˚C – 10 sec
            - ú 57˚C – 10 sec
            - ú 72˚C – 15 sec
        - § 72˚C – 120 sec
- Ran Gel on PCR result à correct band at 200 bp rung on ladder
- Ran PCR cleanup à 50.7 ng/uL result

08/30/2013 AR

- Organized for sample preparation

08/29/2013 AR

- Outlined sample preparation recipe for COSY-H NMR tests.
- Dried buffers in rotovap to get them ready for COSY-H NMR sample preparation

ESI MS sample prep

1. Mini prep:

- Weigh 0.156 g of Ammonium Acetate. Dissolve in 1ml of water.This gives us 1M solution of Ammonium Acetate Buffer.
- Weigh 0.017 g of silver nitrate and dilute in 1ml of the solvent A. This gives us 100mM solution of Ag+. Take 10 µL of this and dilute in 990µl of solvent A so that we have 1ml of 1mM Ag+ solution

2. Actual prep:

a) Molar ratio definition:

Molar DNA: 10 times lower==> 10 µM

Molar Ag: 100 times lower ==>10

1 eq of Ag	2 eq of silver	3 eq of silver
10 µL of DNA Temp 10µL of DNA Mis 10µL of Buffer 40µL of Methanol 2µL of 1mM Ag+ 128µL of qWater	10µL of DNA Temp 10µL of DNA Mis 10µL of Buffer 40µL of Methanol 4µL of 1mM Ag+ 126µL of qWater	10µL of DNA Temp 10µL of DNA Mis 10µL of Buffer 40µL of Methanol 6µL of 1mM Ag+ 124 µL of solvent A

b) Efficiency assessment:

- 10µL of DNA Temp
- 10µL of DNA Mis
- 10µL of Buffer
- 40µL of Methanol
- 20µL of Ag+
- 120µL of solvent

3. Intercalation stimulus:

- Heat 4 samples to 90^{o}C in 2 minutes
- Take the whole heat block out and cool down in an hour
- Store in the fridge (5^{0}C) for another hour[NL1]

PAGE gel protocol 7.30.13 - ET

- 8 mL Long Ranger (50%)
- 4 mL 10X TBE Buffer
- 28 mL DW

(*note: skip mix filtration step because no urea used)

Obtain 5mL of this solution and add:
- 22uL of 10% APS (0.1g APS/1mL DW)
- 2.2 uL TEMED

Prerun (100V): run gel with 1X TBE Buffer after washing each well with injection needle (1hr)
Run (150 V): 1hr

Ligation reaction 7.26.13 - ET

Set one:
- 5uL dsDNA (10uM)
- 1 uL 10X T4 ligase buffer
- 1uL PNK
- 3 uL DW

incubate at 37°C for 1 hr

- 1 uL T4 ligase
- set at RT for 2 hrs

- 5uL ssDNA
- 1uL 10X T4 ligase buffer
- 1 uL PNK
- 3 uL DW

incubate at 37°C for 1 hr

- 3.5 uL PEG 8000
- 0.5 uL T4 ligase buffer
- 1 uL ssRNA ligase
- set at 16°C overnight

Set two:
- repeat entire procedure for ssDNA
- for dsDNA, use mastermix for ligation step instead

7% Gel 7.10.13 - ET

motivation: 15-25 bp DNA is very small, so a dense lattice is required
hinge: no ladder small enough to use
preparation: 8.4g agarose in 120 mL of TAE buffer
microwave at power 60, removing every 15-30 seconds and gently swirling
precaution: use a large beaker/flask (500mL) to avoid overflow
add 2 aliquots of GelRed

MOPS buffer recipe 7.10.13 -ET

get 400 mL qH2O, add:
1.048 g MOPS
4.25 g NaNO3

adjust pH to 7 with NaOH
add qH2O to get final volume of 0.5 L

autoclave
*note: buffer is light sensitive, so wrap in aluminum foil

R8.2 Protocol

SYBR Gold
MOPS/NaNO3

Dye: SYBR Gold
Buffer: MOPS/NaNO3
Loading Dye?

Duplicate?
Mixed?

0: 3 comp
1: Ono
3: 3 mis
6: 6 mis
10: 10 mis
28: lol

6CC set:

ATAT C A C TAA C AA C ATA C AT C TTAT
ATAA C AT C TAT C TT C TTA C T C ATAT

R8: Protocol **7.9.13 SV**

Objective: to determine if 4% agarose can resolve strands at 15bp and 25bp

Pitfall: Electroplating of gel box in presence of silver

Test case: duplexed strands of length 15 and 25, perfect compliments, in absence of silver

Further: can SYBR Gold, a non-intercolating dye, accurately stain fragments of this length in gel?

Procedure

-Pour 4% agarose gel

-Anneal 2 duplexes: **CTT ATG AGT TGA AAC**
GTT TCA ACT CAT AAG

ATA TAA ATC TAA CTT AAT ACA TTA T
A TAA TGT ATT AAG TTA GAT TTA TAT

annealing done with 5uL each strand, 90uL MOPS/NaNO3 buffer

-Run wells (no ladder) of duplex 1, duplex 2, and duplexes 1 and 2 in same well

-Determine appropriate running time

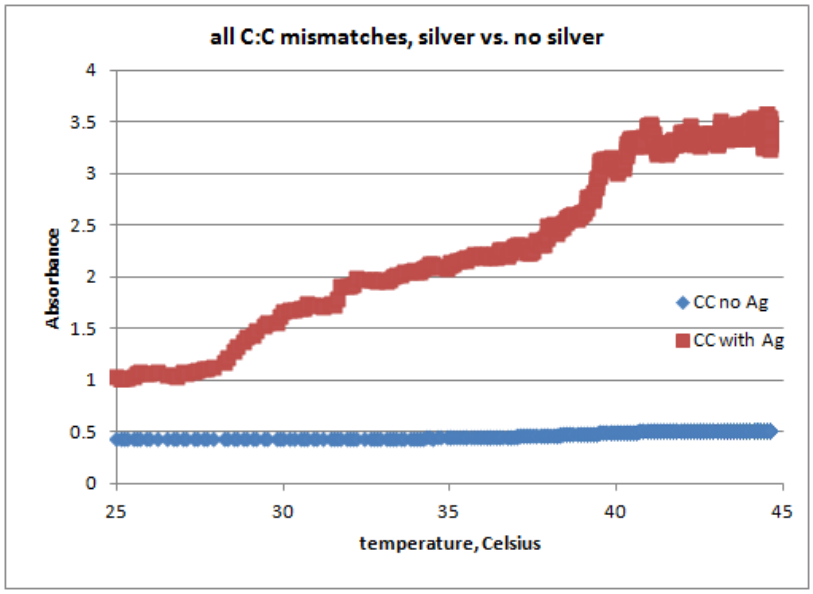
-Try with gel red

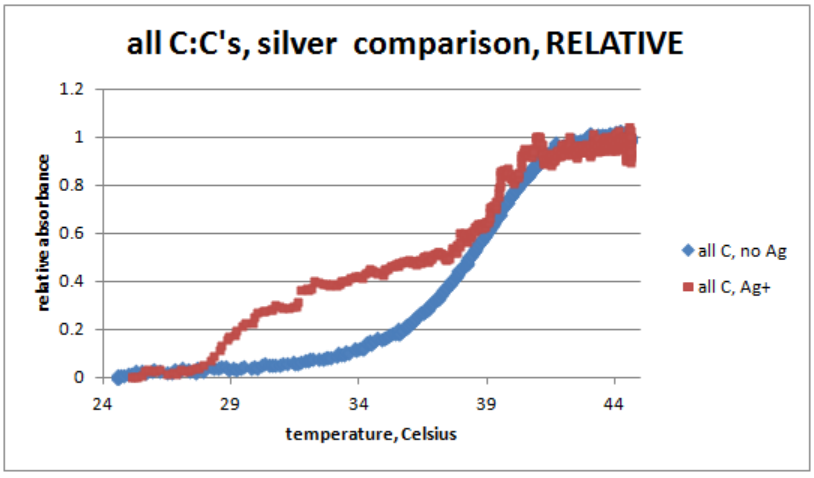
-Perform in duplicate (6 wells total)

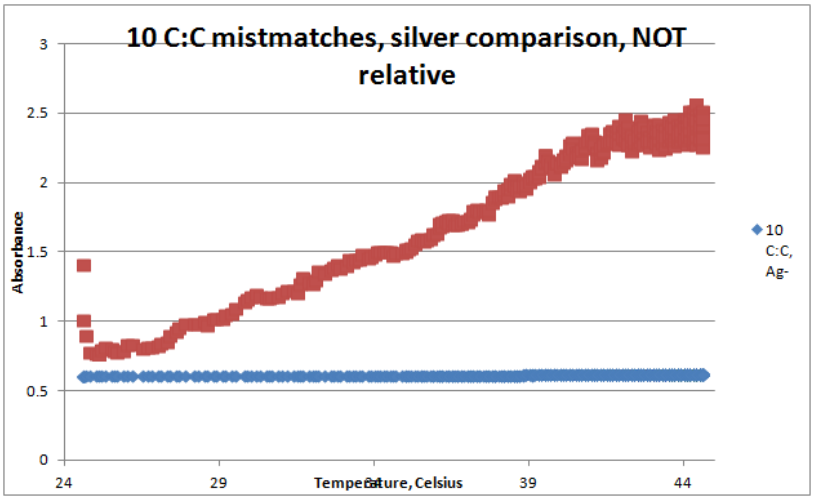
Result: Separation visible, but not enough to see difference between 1-2 bp. Max resolution about 5bp.

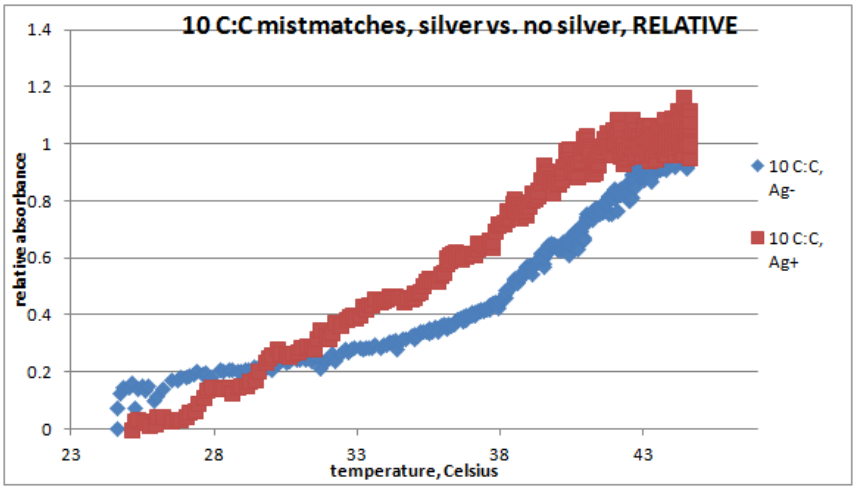
Next steps: PAGE gel, 7% agarose TAE

R7 Results: 7.8.13 ET









(A_{rel} = (A,given temp - A, start temp)/(A, end temp - A, start temp)

R7: 7.2.13-7.3.13

Objective: to determine the melting temperature of various oligonucleotide mixes via UV spectroscopy

Ono.1	GTG ACC ACT GCA GTG	x
Ono.2	CAC TGG TCA CGT CAC	Ag+, Ag-, MOPS, PBS
Torigoe.1	CTC AGA TCC TGC CCT TCA AAA ACA A	x
Torigoe.2	T TGT TTT TGA AGC GCA GGA TCT GAG	Ag+, Ag-, MOPS, PBS
15.1.1	CTT ATG ACT TGA AAC	x
15.1.2	GTT TCA ACT CAT AAG	Ag+, Ag-, MOPS, PBS
15.1.3	GTT TCA AGT CAT AAG	Ag+, Ag-, MOPS, PBS
15.2e.1	CTT ATG AGT TGA AAC	x
15.2e.2	CTT TCA ACT CAT AAC	Ag+, Ag-, MOPS, PBS
15.2e.3	GTT TCA ACT CAT AAG	Ag+, Ag-, MOPS, PBS

3.1	ATA TAA ATC TAA CTT AAT ACA TTA T	x
3.2	A TAA TCT ATT AAC TTA CAT TTA TAT	Ag+, Ag-, MOPS, PBS
3.3	A TAA TGT ATT AAG TTA GAT TTA TAT	Ag+, Ag-, MOPS, PBS
10.1	TTA TAT TTA CC A CC T CC T CC A CC TTT TAA ATT	x
10.2	AAT TTA AAA CC T CC A CC A CC T CC TAA ATA TAA	Ag+, Ag-, MOPS, PBS
28	CCC CCC CCC CCC CCC CCC CCC CCC CCC C	Ag+, Ag-, MOPS, PBS
25A	GAT AAA TAT AAT ATC	x
25B	GAT ATT ATA TTT ATC	NaNO3
30A	GAT AAA TAT GAA TAT C	x
30B	G ATA TTC ATA TTT ATC	NaNO3
35A	GAT ACC ATG AAT ATC	x
35B	GAT ATT CAT GGT ATC	NaNO3
40A	GAT CCG ATT CAT AAT C	x
40B	G ATT ATG AAT CGG ATC	NaNO3

Tube numbers

1-MOPS, Ono Ag-*
2- MOPS, Ono, Ag+*
3- PBS, Ono, Ag-*
4- PBS, Ono, Ag+*
5-8- repeat, Torigoe*
9-12- 15.1.2
13-16- 15.1.3
17-20- 15.2e.2
21-24- 15.2e.3 (control)
25-28- 3.2 (3 mismatches)+
29-32- 3.3 (control)
33-36- 10 C:C
37-40- 28 C:C
41- 25A&B
42- 30 A&B
43- 35 A&B
44- 40 A&B

Bring each tube to a final volume of 100uL. In solutions without silver, 90uL of the appropriate buffer, and in silver solutions, 85uL. DNA oligos are added in 5uL of 200mM stock solution. Silver is added in 5uL in a 1:1 molarity with the C-C mismatches, from the appropriate stock solutions: 200mM, 600mM, 2M, 5.6M.

Annealing is done by heating in a thermal cycler to 90C, letting the solutions cook for 5min, and then letting them cool to room temperature. After an hour, they are refrigerated at 4C for another hour. At this point they are added to the plates.
Not all DNA came on 7.2, thus the experiment was split into two days.

Plate 1: 7.2.13

1	1	1	1	17	17	17	17	25	25	25	25
2	2	2	2	18	18	18	18	26	26	26	26
3	3	3	3	19	19	19	19	27	27	27	27
4	4	4	4	20	20	20	20	28	28	28	28
5	5	5	5	21	21	21	21	29	29	29	29
6	6	6	6	22	22	22	22	30	30	30	30
7	7	7	7	23	23	23	23	31	31	31	31
8	8	8	8	24	24	24	24	32	32	32	32

Plate 2

33	33	33	33	-	-	-	-	-	-	-	-
34	34	34	34	-	-	-	-	-	-	-	-
37	37	37	37	-	-	-	-	-	-	-	-
38	38	38	38	-	-	-	-	-	-	-	-
41	41	41	41	-	-	-	-	-	-	-	-
42	42	42	42	-	-	-	-	-	-	-	-
43	43	43	43	-	-	-	-	-	-	-	-
44	44	44	44	-	-	-	-	-	-	-	-

Added 75uL to each well to accommodate 4x dilution from tubes, bringing total volume to 100uL.

Note: PBS precipitated silver in two highest concentrations. MOPS appeared to be nearing saturation (reddish color) but showed no precipitation. PBS will be discarded as a potential buffer.

6/27 - 7/2

Updates of Phen Green testing

‘

	DNA with mismatch	DNA without mismatches
Solution preparation	We will mix these reagents in PCR tube and centrifuge: - 10µlof 200µM DNA * 2 check - 200µl of 30µM Ag+ check - 2µL of 1M of K3PO4 check - Add water : 178µl check Thermocyclic both: - Heat 2 tubes to 90C in 5 minutes 1 - Cool down to room temp in 45 minutes.	Same
	Expectation: DNA would take up silver	Expectation: DNA would not take up silver
Precipitate DNA	We will try precipitating DNA with 3 methods: isopropyl Alcohol, ethyl alcohol and column. - Extract 200µl of DNA mixture into two Eppendorf tubes - Add 22µl of 3M CH3COONa to each tube (10% of final volume) (made by 0.0414g in 100µl solution) - Add 467 µl of Isopropanol to each tube (70% of final volume) - Extract 200µl of the supernatant. Let them both dry in vacuum centrifuge. Mix with 200µl of DMSO. Then centrifuge. * Correction: After realizing that sample should not dissolve in DMSO, we dried DMSO by vacuum centrifuge and **refilled with q water**. This is because DMSO only	Same

	serves as Phen Green SK stock protection, but in order for the “green” color to fluoresce, PGSK has to gone through hydrolysis.	
	Expectation: DNA has taken up silver, so there should be lower silver concentration in the supernatant.	Expectation: DNA has NOT taken up silver, therefore the concentration of silver in the supernatant should be higher.

Phen Green dilution	- Dilute 1mg of Phen Green in 1ml of DMSO - Extract 1µl and dilute to 140µl to attain 100µM solution Extract 50µl and dilute to 500µl to attain 10µM solution

Standard curve setting

- Prepare 7 solutions of silver in q water with following concentration: 500µM, 200µM, 100µM, 60µM, 20µM, 2µM, 200nM. Pipet 100µL of each into column of 96-wells plate. The excitation wavelength is set on 507nm and emission varies from 565nm (wavelength of maximum intensity of just PGSK and water after running spectrum test), 527 (wavelength of maximum intensity of PGSK, Ag and water after running spectrum test), and 532 (suggested by manual guide of invitrogen). The following chart illustrates the result :

Well	Conc	Emission at 565	At 527	At 532
1A	500µM	-2010	-2856	-2164
B	200µM	-3658	-3513	-2925
C	100µM	-3724	-4095	-4013
D	60µM	-3723	-4095	-4013
E	20µM	-3514	-3822	-3531

<table>
<tr><td></td><td>
<table>
<tr><td>F</td><td>2μM</td><td>-479.6</td><td>-557</td><td>103.04</td></tr>
<tr><td>G</td><td>200nM</td><td>3.673</td><td>43.452</td><td>600.06</td></tr>
<tr><td>H</td><td>0</td><td>Blank</td><td>Blank</td><td>Blank</td></tr>
</table>
· There has been little certainty in the interaction between silver and PGSK. In fact, silver is not commonly seen to quench the fluorescence of PGSK. This experiment weakly shows the ability to quench by silver. The reverse relationship : more silver, less fluorescence is only illustrated in the range of concentration from 200nM to 60μM.
</td></tr>
<tr><td>Comparison of sample</td><td>
- As the concentration of Ag left in the supernatant is expected to fall between 15μM (the original concentration of silver in the solution with DNA, assuming that no silver reacts at all with DNA) and 0μM (when all silver is taken up by DNA, which in this case is via C-C mismatch), the range of concentration for standard curve is narrowed down to 200nM – 60μM. The following chart presents the result of sample prepared with isolation method by ethyl acohol :
<table>
<tr><td>Well</td><td>Conc</td><td>Emission at 532</td><td>565</td></tr>
<tr><td>3A</td><td>60μM</td><td>-18737</td><td>-18865</td></tr>
<tr><td>B</td><td>20μM</td><td>-4827</td><td>-3565</td></tr>
<tr><td>C</td><td>2μM</td><td>637.44</td><td>3782.3</td></tr>
<tr><td>D</td><td>200nM</td><td>1379.4</td><td>4757.7</td></tr>
<tr><td>E</td><td>100nM</td><td>1176.1</td><td>4568.4</td></tr>
<tr><td>F</td><td>Supernatant from DNA without C-C</td><td>14941</td><td>15130</td></tr>
<tr><td>G</td><td>With C-C</td><td>15285</td><td>15343</td></tr>
<tr><td>H</td><td>blank</td><td>Blank (0)</td><td>0</td></tr>
</table>
- The test was run once again. This time, we also tested the sample that was prepared by column isolation method. *** This test does not have blank
</td></tr>
</table>

and hence there is no negative value.

Well	Conc	Emission at 532
4A	60µM	683.77
B	20µM	722.10
C	2µM	1513.2
D	200nM	1665.0
E	-CC by ethanol	22380
F	+C-C by ethanol	21949
G	-CC by column	22793
H	+CC by column	22928

After 25 minutes, the test is run again. *** This test is based on the blank at 3H.

Well	Emission at 532	Well	Emission at 532
3A	-24405	**4A**	-26580
B	-5789	B	-26811
C	234.2	C	-25854
D	1514.6	D	-25701
E	1113.2	E	-1656
F	21409	F	-2563
G	22235	G	-1143

	H	Blank (0)	H	-1095

6/28 ET

Updates since last lab entry:

- performed UV spec on 25 bp oligos in various combinations (mostly 3 C:C mismatches and 1 C:C mismatch with either silver or no silver) and tested melting temperature changes for evaluation of silver stabilization. **result:** tests were largely inconclusive due to the limited maximum temperature of the machine (44.8 °C). When comparing mean change in absorbance, the solutions with 3 C:C mismatches showed a larger result for the sample containing silver than the one without silver. However, no such difference was seen in solutions for one mismatch.
- due to lack of success with UV spec, experiments performed with qPCR. Were able to show stabilization for one trial of samples, looking to repeat the results in both qH_2O and MOPS buffer.
- started work with PhenGreen for alternative method of Ag+ binding confirmation
- ordered strand with 10 C:C mismatches, as well as control strands from the 2011 and 2012 Ono papers. Testing them on qPCR today.

6/10

- First orders were made; supplies should be in tomorrow
- Drafted ideas for preliminary testing:

1. gel electrophoresis on linear pieces of DNA with varying silver arrangements to test if the pattern of ion distribution will affect the conductivity. However, there's a potential risk that the silver ions will "jump" out of the DNA towards the negative side of the gel, so we need to cut out the resultant bands and run an analysis on them to see if the silver is still bound to the structure (Joe: how would you analyze to see if silver is present?) ← Joe, I think we would test the melting temp of the sample, since it would be lower if silver was no longer there.
2. Nanodrop the products, and use the equation A=CIe (Beer's law) to get e (the molar absorptivity) with a known concentration and path length. We can also perform a thermal titration on the Nanodrop, and compare the melting temperature of the altered DNA with that of normal DNA. We would expect DNA containing silver ions to have a higher melting temperature than normal DNA.
3. check to see if we can get circularized DNA by using ligase to seal the plasmids. Then, if we apply exonuclease, the enzyme should be unable to break apart circularized DNA, so we can check the lack of linearity by rating the “success” of the exonuclease. (Joe: Could the silver interfere with ligation?) (SV: possibly, but we’d ideally place the break away from a CC bond. It’d be an interesting test to do though)
4. we might also be able to use X-ray crystallography to get a better view of the structure, but that's definitely a "might"...

Experiment BioRFID 1 (R1): started **6.11.13**

Four DNA strands ordered. Strand 1 serves as the template. Strand 2 is the mismatched compliment (1' with C-C). Strand 3 is the perfect compliment (1'). Strand 4 is a similar sequence, but as a hiarpin. It includes strands 1 and 2 in its sequence.

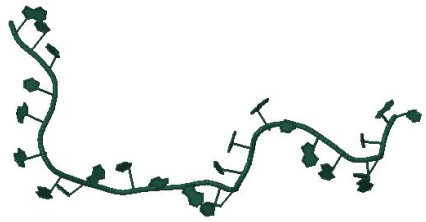
#1: 5' 3' Tm= 46.1C

#2: 5' **ataatttattaaCttatatttatat** 3'

#3: 5' **ataatttattaaGttatatttatat** 3'

#4: 5' **atataaatataacttaataaattat ggggggg ataatttattaacttatatttatat** 3'

Strand 1 models beautifully with no self-dimers:



(I modeled it with RNA composer. Yes it's actually DNA in real life. It's ok. It was visualized after all the hairpins and self dimers were smoothed out with 2D DNA modeling programs)

DNA strands 1 and 3 in conjunction form a perfect dsDNA, and strands 1 and 2 form a dsDNA with one C-C mismatch.

6.11.13: Nanodrop and plate setup for thermal denaturation

Duplex Production

Reagents and buffers were modeled to check chemical speciation. Ag(I) comprises 99.9999 of Ag atoms at pH 7 with 5nM AgNO3 and 5mM K2PO4 in solution.

Dilution of AgNO3**:** 0.0845g was added to 1mL qH2O to form a 0.5M solution. A serial dilution took place. 1uL of this solution was added to 1mL qH2O to produce a 0.5mM product. 10uL of this subsequent solution was added to 1mL qH2O, producing a 5uM solution. 5uL of this solution was added to a final 1mL aliquot of qH2O to produce a 25nM solution of AgNO3, matching that of the DNA. Volumes were not adjusted to reach a final vol of 1mL, so error was introduced into the system. As it is only buffer and ions, we accepted this error.

Dilution of K3PO4: 0.876g was added to 1mL qH2O to produce a 0.5M solution. 50uL of this solution was added to 1mL qH2O, producing a 25mM solution of K3PO4.

Solutions: 7 solutions were made at a volume of 25uL each. Various control solutions were made.

Tube	qH2O	K2PO4	AgNO3	DNA 1	DNA 2	DNA 3
A	25uL	-	-	-	-	-
B	15uL	5uL	5uL	-	-	-
C	15uL	5uL	-	-	-	5uL
D	10uL	5uL	-	5uL	-	5uL
E	10uL	5uL	-	5uL	5uL	-
F	5uL	5uL	5uL	5uL	-	5uL
G	5uL	5uL	5uL	5uL	5uL	-

Nanodrop: Each solution was read on the nanodrop machine (smaller spectrophotometer). Readings were taken at 260nm. We knew the concentration, and were testing for the duplexing of DNA, per Beale's law:

A = CeI absorbance = concentration*constant*path length

We know the concentration (5nM), and the path length (1cm). Given the absorbance, we are able to calculate the constant e. For ssDNA, e is 33ug/mL, and for dsDNA it is 50ug/mL.We can with these data determine if duplexes are present, and what % are producing a duplex. We can also see if the absorbance of silver bound DNA is abnormally high or low.

Data

	B: ions	C: ssDNA	D: Ag-CC-	E: Ag-CC+	F: Ag+CC-	G: Ag+CC+
A260	-6.333	13.806	25.223	27.696	27.725	33.588
e ???	-12.6	27.6	50.4	55.4	55.5	67.2

Thermal Denaturation

Thermal denaturation is done using a larger spectrophotometer with temperature control. Per Beer's law, as DNA melts, it changes its constant from 50 to 33, allowing for a precise determination of Tm to be obtained. Unfortunately the machine in the lab only reaches 45C, and this may pose an issue if the melting temp is higher than that.

Plate Prep

A UV-transparent plate was used to allow for UV melting. 10uL of each of the previous solutions was diluted in 190uL qH2O in a 96 well plate. This brings the concentration of DNA down to 0.25nM. Plate was parafilmed and left at room temp overnight. They were added first to row A, but the volume was too small (100uL), so new wells were poured in column 12 in order of tube number.