Lab Notebook (Week 11.2)

Name: Saphira & Angel
Date: April 12th
Week#: 11

Objective:

- Concentrate dsRNA for injection (remove DNA with DNase, ssRNA with RNase, salts, NTP, protein)

Procedures:

- RNase + DNase digestion
- Incubation
- Purify dsRNA by filtration

Note:

- Prepare the RNase + DNase digestion on ice by adding RNase and DNase in the dsRNA solution with 10x binding buffer (page 11 Megascript RNAi)
- Incubate the digestion at 37C for 30min
- Purify dsRNA through a filter cartridge in a collection tube
    + Pipet 500uL of dsRNA binding mix into the filter cartridge. Centrifuge
    + Pipet 500uL of wash solution onto the filter cartridge. Centrifuge and discard the flow-through
    + Repeat with 500uL of wash solution. Centrifuge and discard the flow-through. Centrifuge again for 1min to remove last traces of liquid.
    + Transfer the filter cartridge to a new collection tube
    + Recover the dsRNA with 50 uL Elution buffer (EB) and incubate at 70C for 10min
    + Centrifuge for 2min at maximum speed. DO NOT DISCARD FLOW-THROUGH!!!
    + Repeat with second 50 uL of EB

Conclusion:
N/A

Next step:
Inject dsRNA into *Tribolium*

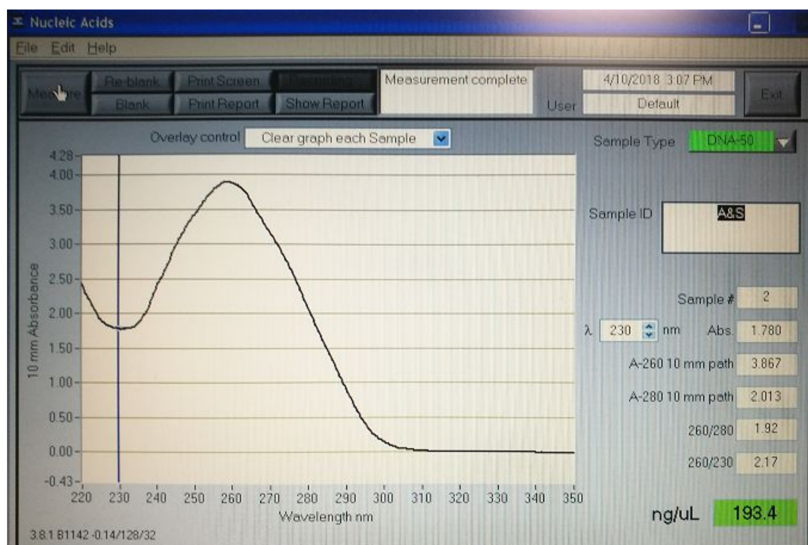
<u>Lab Notebook (Week 11.1)</u>

Name: Saphira & Angel
Date: April 10th
Week#: 11

Objective:
Purify our PCR product from last week to obtain dsDNA, then make dsRNA by *in vitro* transcription using the dsDNA as template.

Procedures:
- Purify our PCR product using QIAquick PCR Purification Kit
- Measure the concentration of our dsDNA using nanodrop
- Set up *in vitro* transcription reaction

Note:
- Purification procedure
  - In a 1.5mL microcentrifuge tube, Add 1mL of PB buffer and 200uL (8 x 25uL) of PCR mixture
  - Turn the vacuum on and attach a column to the vacuum, transfer the 1.2mL of mixture 600uL at a time into the column and let it drain
  - Add 750uL of PE buffer into the column and let it drain, then turn off the vacuum
  - Put the column into the provided 2mL tube, centrifuge it at 13000 rpm for 1 min to remove the excess PE buffer.
  - Place the column into a new 1.5mL microcentrifuge tube and add 30uL of EB buffer, then let it stand for 1 min.
  - Centrifuge the mixture at 13000 rpm for 1 min to collect the flow through
- dsDNA concentration



- In vitro transcription reaction mix:
    - $H_2 0$ µl
    - NTP mix 8 µl
    - 10 x buffer 2 µl
    - Template DNA µl
    - T7 RNA pol 2 µl
    - *Total* *20 µl*

Conclusion:
N/A

Next step:
Isolate and purify dsRNA to prepare for injection

Lab notebook(Week 10.2)

Name: Saphira & Angel
Date: April 5th
Week#: 10

Objective:

- Make dsDNA of our gene fragment, using T7 containing primers (TOPO_RNAi primer) in order to introduce T7 promoters on the 5' ends of both strands

Procedures:

- Make 40uL of 10ng/uL plasmid DNA solution using our stock from last week
- Use PCR to construct and amplify linear dsDNA with T7 promoters

Note:

- Make 40uL of 10ng/uL of plasmid DNA in a 1.5ml tube by adding 0.67uL of original plasmid DNA (596.2ng/uL) in 39.3 uL of water
- Make a 200uL PCR master mix and split into 8 PCR tubes (25uL each)
- Run PCR

PCR form:

Date: 4-5-18
Aim: Construct and amplify dsDNA with T7 promoters on the 5' end of both strands

Reaction

	Maxter mix	*Split (8)*
H_20	102 µl	12.75µl
5 X PCR buffer	40 µl	5 µl
dNTP mix (2.5mM stock)	16 µl	2 µl
primer (10µM)	30 µl	3.75 µl
Go Taq (5U/ µl)	2 µl	0.25 µl
Plasmid DNA	10 µl	1.25 µl
Total	200 µl	25 µl

*No $MgCl_2$ if using TAKARA Ex Taq or Promega GoTaq

Reaction Cycle

Program name:
Denaturation: 94 ℃ X 2 min
↓
Denaturation: 94 ℃ X 30 sec _
Annealing: 57 ℃ X 30 sec | 30 cycles
Extension: 72 ℃ X 30 sec _|
↓

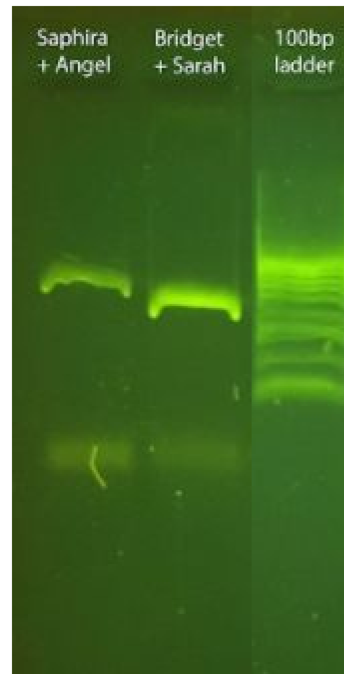
Extension: 72 ℃ X 5 min
↓
Hold: 4 ℃

*2: Primer Tm + 1-2℃
*3: 500bp/30sec, 1,000bp/1min

Conclusion:
N/A

Next step:
Purify the PCR products and set up in vitro transcription reaction

Result



Lab notebook(Week 10.1)

Name: Saphira & Angel
Date: April 3rd
Week#: 10

Objective:
Analyze the DNA sequencing data of our plasmid.

Procedures:
- Install the sequence viewer software Snapgene Viewer
- Download the 4 sequence files from our class Google Drive
- Analyze the sequencing results by completing the Sequence Analysis form

Note:
- See details in the Sequence Analysis form

Conclusion:
- The sequenced portion of the plasmid contains the complete sequence of our cloned DNA fragment, with one synonymous change.

Next step:
- Construct linear dsDNA with T7 promoters added to the 5'-end on both strand

Lab notebook(Week 9.2)

Name: Saphira & Angel
Date: March 29th
Week#: 9

Objective:
Harvest the plasmids from *E. coli* and purify them for sequencing.

Procedures:
- In a 2mL tube, add 2mL of E. coli suspension (4)
- Centrifuge the E. coli suspension and remove the supernatant.
- Add another 2mL into the tube, centrifuge again, and remove the supernatant.
- Resuspend the E. coli cells in P1 buffer with added RNase A
- Increase the pH for 1 minute by adding P2 buffer to break the cell and destroy unwanted macromolecules
- Neutralize the solution by adding N3 buffer
- Centrifuge the solution again and transfer the supernatant to the column

Note:
Centrifugation of *E. coli* suspension
- Add 2mL of E. coli suspension into a 2mL tube
- Centrifuge at 12000 rmp for 3 min.
- Remove supernatant

Resuspension
- Add 250uL of P1 RNase buffer into the 2mL tube
- Mix vigorously until no cell clumps remain

Lysing the cells and destroying other macromolecules
- Add 250uL of P2 buffer
- Invert tube gently then let it sit
- This step should last for exactly 1 minute

Neutralizing the suspension
- Add 350uL of N3 buffer
- Invert tube, slowly at first, then progressively faster
- Let it sit for 10 min.

Centrifugation of the E. coli in buffer
- 13000 rpm for 10 min.

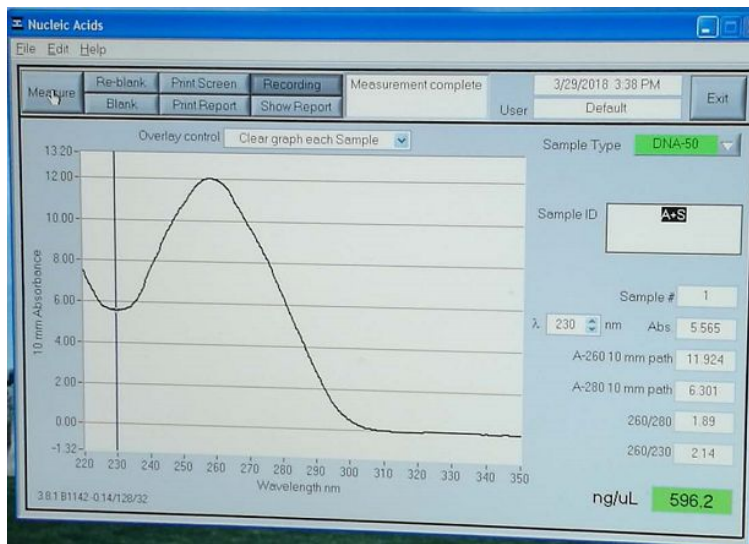
purifying plasmid DNA
- Put a column on a vacuum, turn on the vacuum, and…

- Transfer 750uL of the supernatant into the spin column and allow all the liquid to pull through
    - Add 500uL of PB buffer and allow the liquid to pull through
    - Add 750uL of PE buffer and allow the liquid to pull through
- Take the column off the vacuum
- Centrifuge the column at 13000 rpm for 1 min.
- Put the column on a new microcentrifuge tube
- Add 50uL of EB buffer and let it sit for 1 min.
- Centrifuge the column + tube at 12000 rpm for 1 min, then discard the column

Measuring DNA concentration

- 2uL of DNA was added to a NanoDrop
- The concentration was measured using 260nm light

Result



The concentration of our plasmid DNA is 596.2 ng/uL
The single peak suggests that our DNA is pure

Conclusion:

The extraction and purification of our plasmid DNA was successful because we have a pure DNA solution with a very high concentration.

Next step:

Our plasmid DNA will be sequenced as the final and definitive confirmation that it was cloned successfully

Lab notebook(Week 9.1)

Name: Saphira & Angel
Date: March 27th
Week#: 9

Objective:
Run DNA gel electrophoresis of our colony PCR product from last lab to confirm that our gene fragment was successfully cloned.

Procedures:

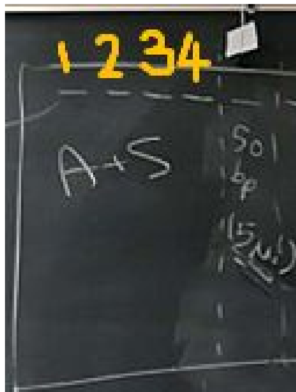
- Make 1.5% agarose gel
- Load and run the gel at 100V for 30 minutes
- Visualize the gel and pick the best two *E. coli* colonies for the next steps

Note:
Making the gel:

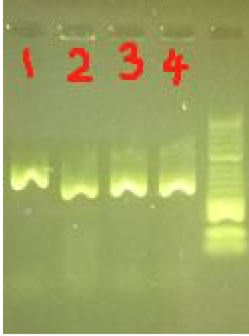
- 1.2g of agarose was added into 80mL of 0.5x gel green TB buffer
- The solution was then microwaved for all the agarose to dissolve
  *after microwaving, make sure not to pick up by the beaker, otherwise, the flask would fall and break.
- The agarose solution was poured into three gel trays and solidified

Loading the gel:

- The gel was loaded with 2uL of each sample and 5 uL of ladder according to the following scheme



Gel result



- The bands were distorted, probably due to the gel being too thin
- The expected size of the products is 485 bps
- Samples 2, 3, and 4 fit the expected size
- Samples 3 and 4 were marked for future experiments

Conclusion:
It was confirmed that samples 2, 3, and 4 contained our DNA fragment

Next step:
Purify the plasmids from samples 3 and 4, then sequence the plasmids.

Lab notebook(Week 7.2)

Name: Saphira & Angel
Date: March 15th
Week#: 7

Objective:

The goal of today's lab is to conduct colony PCR to confirm the cloning from last lab was successful.

Procedures:

- Pick and prepare 4 E. coli colonies
  - Pick 4 colonies from plate into 15 uL LB +amp
  - Inoculate into 4 different tubes at 37C for 1 hour with mixing (by tapping) every 15 min
- Set up 4 colony PCR reactions according to the recipe (detailed in next section)

Note:

The image of agar plate from previous lab is shown below:

- Circled colonies were picked for colony PCR

Details for the PCR setup are shown below

PCR form:

Date: 3-15-18
Aim: amplify the region in our plasmid to confirm that our DNA fragment was successfully inserted
Reaction

	Maxter mix	*Split*

H_2O	32.6 µl	8.15 µl
5 X PCR buffer	16 µl	4 µl
dNTP mix (2.5mM stock)	6.4 µl	1.6 µl
T3 primer (10µM)	8 µl	2 µl (__µM)
T7 primer (10µM)	8 µl	2 µl (__µM)
Go Taq (5U/ µl)	0.5 µl	0.25 µl
Colony		2 µl
Total	72 µl	20 µl

*No $MgCl_2$ if using TAKARA Ex Taq or Promega GoTaq

Reaction Cycle **Result**

Program name:

Denaturation: 95 ℃ X 5 min

↓

Denaturation: 94 ℃ X 30 sec _

Annealing: _55_℃ X 30 sec | _30_ cycles

Extension: 72 ℃ X _30_sec _|

↓

Extension: 72 ℃ X 2 min

↓

Hold: 4 ℃

*2: Primer Tm + 1-2℃

*3: 500bp/30sec, 1,000bp/1min

Conclusion:

E. coli colonies successfully grew on the selective LB-agar plate. Confirming that the E. coli cells carry plasmids with inserted DNA fragments.

Next step:

Run DNA gel electrophoresis of today's PCR products.

Lab notebook(Week 7.1)

Name: Saphira & Angel
Date: March 13th
Week#: 7

Objective:
The goal of today's lab is to clone the amplified region into TOPO plasmids and transform the plasmid into *E. coli*.

Procedures:

- Ligate our DNA fragment into TOPO plasmids
- Transform the plasmids into E. coli cells
- Grow transformed E. coli cells on an agar plate overnight in a 37 degree celsius incubator

Note:
The TOPO ligation reaction mixture was made in a 1.5mL tube using the following volumes of each components:

- Purified DNA: 4uL
- Salt solution: 1uL
- Topo vector: 1uL

It was then left at room temperature for 30 min for the reaction to complete

The plasmids was then transformed into E. coli cells according to the following procedure

- Thaw a tube of frozen E. coli on ice
- Pipette 3uL of TOPO reaction mixture into the *E. coli* tube
- Put the *E. coli* tube on ice for 5 minutes
- Heat shock the E. coli at 42 degrees Celsius for 30 sec
- Put the *E. coli* tube back on ice for another 5 minutes
- Pipette 250 uL of SOC into the E.coli cell tube
- Incubate the tube at 37 degree Celsius for 45 min-1 hour

120 uL of *E. coli* cell suspension was then pipetted onto one agar plate and spread across the plate. The plate was left in 37 degree Celsius overnight for the *E. coli* to grow.

Conclusion:
N/A

Next step:
Pick the E. coli colony and confirm that our gene has been successfully cloned

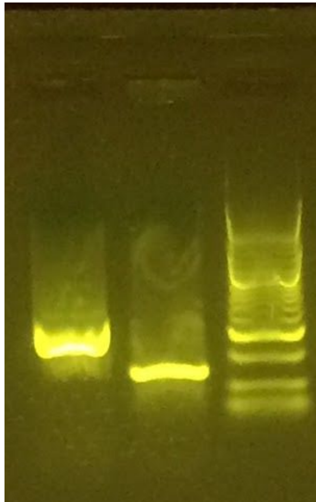
Lab notebook(Week 6.2)

Name: Saphira & Angel
Date: March 8th
Week#: 6

Objective: (in one or two sentences)
The goal of lab today is to run gel electrophoresis to purify our amplified gene after PCR

Procedures:
- Make 1.2% Agarose gel (50mL/gel) using 0.5X TBE
- Run the gel using 20uL of each sample and obtain an image of the bands
- Extract the DNA from the chosen pair of primers from the gel

Note:
We added 5uL of ladder in each gel and on both sides of the ladder, each group injected 20uL of PCR products of primer 1 and primer 2 in 2 wells. Gel was run for 25 min in 100V (see image of bands below). We chose to extract the PCR product of primer pair 1 from the gel and used the freeze-and-squeeze kit to get the gene out.



Left: primer 1
Middle: primer 2
Right: ladder

Conclusion:
Both primer pairs worked. Primer 1 was chosen because it has a longer amplicon (~385bp).

Next step:
Clone the gene into a plasmid vector and transform the plasmid into *E. coli*

Lab notebook(Week 6.1)

Name: Saphira & Angel
Date: Match 6th, 2018
Week#: 6

Objective:

The goal of today's lab is to use PCR to select and amplify the chosen regions in our gene

Procedures:

- Make 100uM primer stock solutions by adding calculated amounts of ddH2O
- Make 10uM primer working solutions by adding calculated amounts of ddH2O
- Set up and run PCR according to the PCR form

Note:

We dissolved the primers in ddH20 to make 100uM stock solutions for each primer.

	TC008204_F1	TC008204_R1	TC008204_F2	TC008204_R2
Initial amount	28.2 nmol	25.4 nmol	26.5 nmol	32.8 nmol
Volume of water added	282 uL	254 uL	265 uL	328 uL

The stock solutions were vortexed and centrifuged for mixing. Then 50uL of 10uM working solutions were prepared from the stock solutions by adding 5uL of stock solution into 45uL of ddH2O for each primer.

PCR form:

Date: 3-6-18
Aim: select and amplify regions in our chosen gene using pre-designed primers
Gene name: Dopamine N-acetyltransferase (DAT)

Primer Set 1

Forward Primer(TC008204_F1): Tm= 59.97℃:
sequence 5'--CCGGAGCTGGAGTCGTATTC--3'
Reverse Primer(TC008204_R1): Tm= 59.96℃:
sequence 5'--TCAGAGCCCTTGCTGTGAAG -3'

Primer Set 2

Forward Primer(TC008204_F2): Tm=59.82℃
sequence 5'--GTCGATTCAGTCTCCACCCC -3'
Reverse Primer(TC008204_R2): Tm=60.11℃

sequence 5'--GTGGTTCAGGGGCTCATCTC -3'

Reaction

	Maxter mix	*Split*
H_20	30.5 µl	15.25 µl
5 X PCR buffer	10 µl	5 µl
dNTP mix (2.5mM stock)	4 µl	2 µl
Primer1 (10µM)		1 µl (_µM)
Primer2 (10µM)		1 µl (_µM)
Sample DNA (cDNA)	1 µl	0.5 µl
Taq polymerase (5U/ µl)	0.5 µl	0.25 µl
Total	50 µl	25 µl

*No $MgCl_2$ if using TAKARA Ex Taq or Promega GoTaq

Reaction Cycle(primer set 1)
Program name:
Denaturation: 95 ℃ X 5 min
↓
Denaturation: 94 ℃ X 30 sec
Annealing: 61 ℃ X 30 sec | 35 cycles
Extension: 72 ℃ X 30 m / s
↓
Extension: 72 ℃ X 5 min
↓
Hold: 4 ℃

Reaction Cycle(primer set 2)
Program name:
Denaturation: 95 ℃ X 5 min
↓
Denaturation: 94 ℃ X 30 sec
Annealing: 61 ℃ X 30 sec
Extension: 72 ℃ X 30 m / s | 35 cycles
↓
Extension: 72 ℃ X 5 min
↓
Hold: 4 ℃

*2: Primer Tm + 1-2℃
*3: 500bp/30sec, 1,000bp/1min

Result
See entry 3-8-18

Conclusion:
N/A

Next step:
Next lab, we will be running gel electrophoresis to determine which set of primers worked the best

Lab notebook(Week 5)

Name: Saphira & Angel
Date: March 1st, 2018
Week#: 5

Objective:

In today's lab, we will practice to get familiar with DNA gel electrophoresis and identify the size of three unknown DNA samples A, B, and C.

Procedures:

- Make 1.2% agarose gel using 0.5x gel green TBE buffer.
- Make 10 ul of 10 ng/ul DNA samples
- Add calculated amounts of 5x dye to the samples
- Load the samples and a ladder into the gel
- Obtain an image of the resolved bands in the gel
- Determine the size of the unknown DNA samples

Note:

To make a small 1.2% gel for electrophoresis using 0.5x TBE buffer, we mixed 0.6g of Agarose in 50mL of gel green mixed TBE buffer and microwaved it for 3 minutes with 30% power, took it out to mix then continued to microwave for 1 minute. After it was ready, we poured the solution onto the gel tray and let it sit until solidified. The DNA samples were loaded into the 8-well gel in the following order: A, B, C, 50bp ladder, A, B, C.

We calculated the amount of DNA needed to make 10ul of 10 ng/ul of each sample using initial concentrations and equation c1v1=c2v2.

Sample	Initial concentration	Volume of sample	Volume of ddH2O
A	82 ng/ul	1.2 uL	8.8 uL
B	118 ng/ul	0.85 uL	9.15 uL
C	115 ng/ul	0.87 uL	9.13 uL

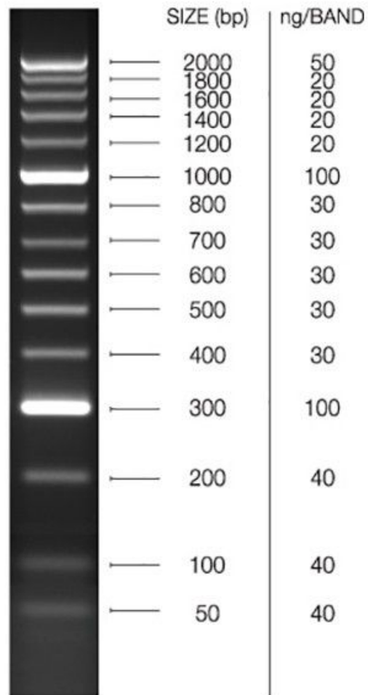
We calculated the amount of 5x loading dye to add to each DNA samples:

$$\frac{1}{5} = x / (10 + x)$$
$$4x = 10$$
$$x = 2.5$$

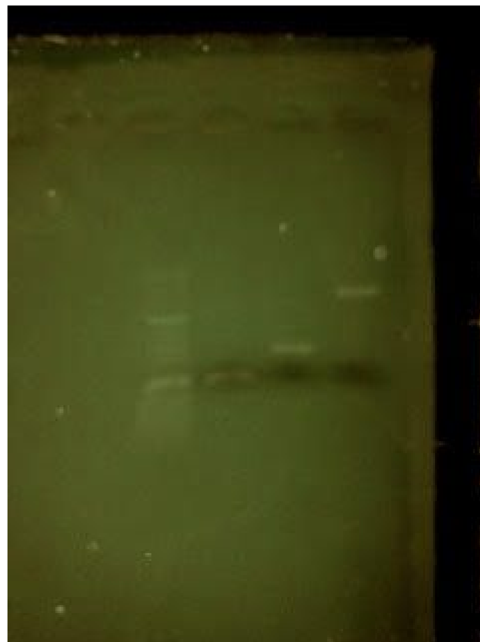
We then loaded our samples and a ladder into the gel (left 4 wells):

- 10 uL of each sample

- 5 uL of ladder (HyperLadder 50 bp)



We ran the gel for ~25 minutes using 135V, then tried to visualize the gel using a blue light, but no bands were visible for the unknown samples or ladder. Probably due to outdated gel green, only one out of nine groups (Connor & Hunter) had visibly separated DNA bands on their gel. We then borrowed their result to determine the sizes of DNA in the unknown samples.



Conclusion:

- The size of DNA in unknown A is approximately 300 bp
- The size of DNA in unknown B is approximately 600 bp
- The size of DNA in unknown C is approximately 1200 bp

Next step:

Next week, we will be purifying *Tribolium* cDNA using gel electrophoresis to obtain the cDNA of the correct size. Then using PCR and our previously designed primers, wel will amplify the regions that'll ultimately be our dsRNA.