Date: 4/20/17
Week#: 14

Objective: (in one or two sentences)
Observe changes in tribolium castaneum

Procedures: (bullet point the procedures)

Note:

10 alive all pupae same as Tuesday.
One pupae has possible clubbed foot phenotype.
It appears that there may be two distal points on the middle set of legs
Still not adults
PIC:

Incredible quality^

Conclusion:

Next step:

Date:4/18/17
Week#: 14

Objective: (in one or two sentences)
Look at how RNAi effects in pupae/ larval stages of tribolium castaneum

Procedures: (bullet point the procedures)

Note:
Larval stage: 5 dead
Pupae : 10 alive
Did not show development.

Conclusion:
Nothing exciting this week. Wait for adulthood.

Next step:
Keep updated on the development of the pupae into adults. Look for new changes and furthering development.

Date: 4/11/2017
Week#:13?

Objective:

Familiarize ourselves with the microscope, and document (draw) wild type *Tribolium castaneum.*

Procedures:

Ovserve & Draw larvae
Observe & Draw pupae
Observe & Draw Adult

All drawn to the best of our artistic ability

Note:

Conclusion:

I conclude that we drew the pupae

Next step:

Use these skills to document phenotype of RNAi beetles

Date: 4/6/17
Week#:12

Objective: (in one or two sentences)
dsRNA Purification

Procedures: (bullet point the procedures)

D. Nuclease Digestion to Remove DNA and ssRNA

This DNase/RNase treatment digests template DNA and any ssRNA that did not anneal. RNase will not degrade dsRNA when using the reaction conditions specified below.

1. Assemble RNase digestion reaction on ice

The amounts shown are for a 20 µL transcription reaction; scale up if your transcription reaction was larger.

Amount	Component
20 µL	dsRNA (from step B.4 or step C.2)
21 µL	Nuclease-free Water
5 µL	10X Digestion Buffer
2 µL	DNase I
2 µL	RNase

2. Incubate at 37°C for 1 hr

The ssRNA will be digested after 15 min but allow the incubation to proceed for 1 hr to completely digest the DNA template.

Do ***not*** continue this incubation longer than 2 hr.

E. Purification of dsRNA

This purification removes proteins, free nucleotides, and nucleic acid degradation products from the dsRNA.

NOTE

For the quickest dsRNA purification, preheat the Elution Solution to ~95°C before starting the purification procedure.

1. Assemble the dsRNA binding mix

Assemble the dsRNA binding mix by adding 10X Binding Buffer, water, and 100% ethanol to the dsRNA according to the table below.

Amount	Component
50 µL	dsRNA (from step D.2 above)
50 µL	10X Binding Buffer
150 µL	Nuclease-free Water
250 µL	100% Ethanol

Gently mix the reaction by pipetting up and down.

2. Apply binding mix to the Filter Cartridge, and draw it through

Pipet the entire 500 µL dsRNA binding mix onto the filter in the Filter Cartridge, and draw it through by centrifugation or with a vacuum manifold.

Centrifuge users:

a. For each dsRNA sample, place a Filter Cartridge in a Collection Tube. Use the Collection Tubes supplied with the kit.

b. Pipet the entire 500 µL dsRNA mixture onto the filter in the Filter Cartridge. Centrifuge at maximum speed for 2 min.

c. Discard the flow-through and replace the Filter Cartridge in the Collection Tube.

Vacuum manifold users:

a. For each dsRNA sample, place a 5 mL syringe barrel on the vacuum manifold, load it with a Filter Cartridge, and turn on the vacuum.

b. Pipet the entire 500 µL dsRNA mixture onto the filter in the Filter Cartridge. The vacuum will draw the lysate through the filter.

IMPORTANT

Verify that 12 mL of 100% ethanol was added to the 2X Wash Solution.

a. Pipet 500 µL of Wash Solution onto the filter in the Filter Cartridge. Draw the wash solution through the filter as in the previous step.

b. Repeat with a second 500 µL of Wash Solution.

c. After discarding the Wash Solution, continue centrifugation, or leave on the vacuum manifold for ~10–30 sec to remove the last traces of liquid.

a. *The Elution Solution provided with the kit is 10 mM Tris-HCl pH 7, 1 mM EDTA. It is compatible with dsRNA injection, or 2X Injection Buffer can be added to the purified dsRNA for a final concentration of 1X Injection Buffer. Alternatively, the dsRNA can be eluted into any sterile low salt solution (≤30 mM), e.g. 5 mM KCl, 0.1 mM sodium phosphate buffer as used by Rubin and Spradling (1982).*Transfer the Filter Cartridge to a fresh Collection Tube.

b. Apply 50–100 µL (hot) Elution Solution to the filter in the Filter Cartridge.
   - Apply preheated (≥95°C) Elution Solution to the filter, ***or***
   - Apply room temperature Elution Solution, close the tube lid over the Filter Cartridge, and incubate in a heat block set to 65°C or warmer for 2 min.

c. Centrifuge for 2 min at maximum speed.

d. Repeat steps b–c with a second 50–100 µL aliquot of Elution Solution collecting the RNA into the same Collection Tube. Most of the RNA will be eluted in the first elution. The second elution is included to recover any remaining RNA.

Quantitate the reaction product by measuring its absorbance at 260 nm and calculating the concentration (see section *V.A. Quantitation of RNA by Spectrophotometry* on page 19).

The dsRNA is stable when stored at –20°C in Elution Solution.

Run 1/400th of the dsRNA on a 1% agarose gel (nondenaturing) to examine the integrity and efficiency of duplex formation.

- 1/400th of 100 µL elution volume is 2.5 µL of a 1:10 dilution
- 1/400th of 200 µL elution volume is 5 µL of a 1:10 dilution
- Dilute the gel samples in TE (10 mM Tris, 1 mM EDTA) or in gel loading buffer

(Instructions for running the gel are in section V.B on page 19). The dsRNA will migrate slightly slower than DNA markers of the same length. See Figure 4 on page 15 for an example of how the dsRNA reaction products will look on a gel.

Note:
For part D the solution was made in the same PCR tube that was used to make dsRNA(to remove ssRNA and DNA).

Ran through centrifuge again to confirm filter is dry. Before adding elution buffer

After Elution(60uL) - Take 1uL mix with 19uL H20 20X dilution
Nano-Drop
Concentration dsRNA 33.5 ng/uL

Conclusion:

Next step:

Date: 4/4/2016
Week#: 12

Objective: (in one or two sentences)

dsRNA Synthesis

Procedures: (bullet point the procedures)

NTP Mix: 8ul
10x Buffer: 2ul
Enzyme Mix: 2ul
Template: x ul (1-1.5 Ng)
Add nuclease free water to: 20 ul
1.06ul nuclease free H_2O
Calculate volume template as follows:

x ul template * (0.216 ug/ul) = 20 ul (1.5 ug/20 ul)

x ul template = 6.94ul
Therefore 1.06 ul nuclease free water.

Note:

We made the reaction solution as calculated above. This was allowed to incubate for 5-6 hr. We will purify this on Friday.

Conclusion:

We conclude that we correctly mixed our dsRNA Synthesis solution.

Next step:

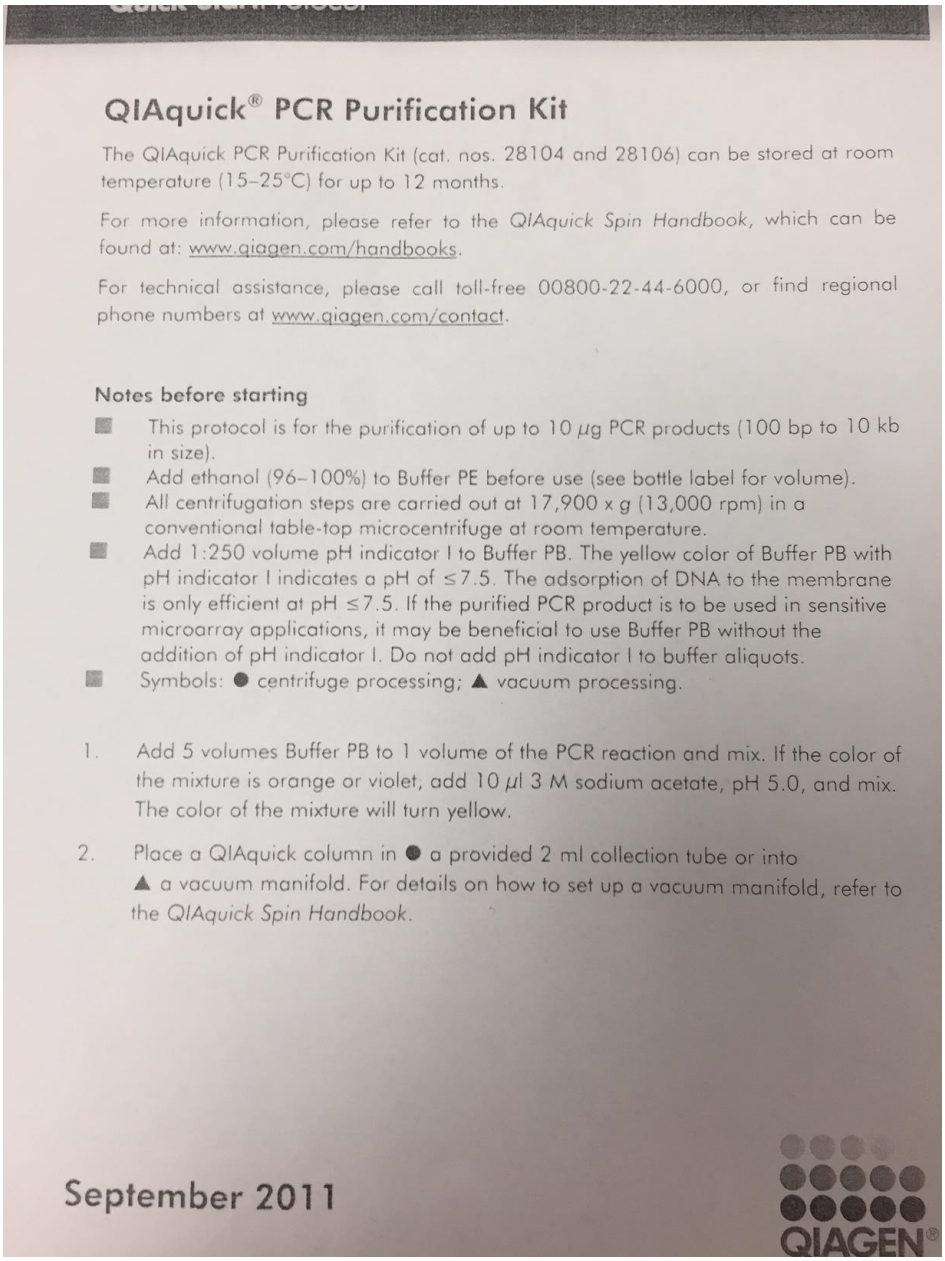
Purify the dsRNA.

Date: 3/30
Week#:11

Objective: (in one or two sentences)
The objective of the day was to isolate the template DNA to prepare for dsRNA

Procedures: (bullet point the procedures)

We made 40ul of a 1.5% agarose gel for the class.



QIAquick® PCR Purification Kit

The QIAquick PCR Purification Kit (cat. nos. 28104 and 28106) can be stored at room temperature (15–25°C) for up to 12 months.

For more information, please refer to the *QIAquick Spin Handbook*, which can be found at: www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

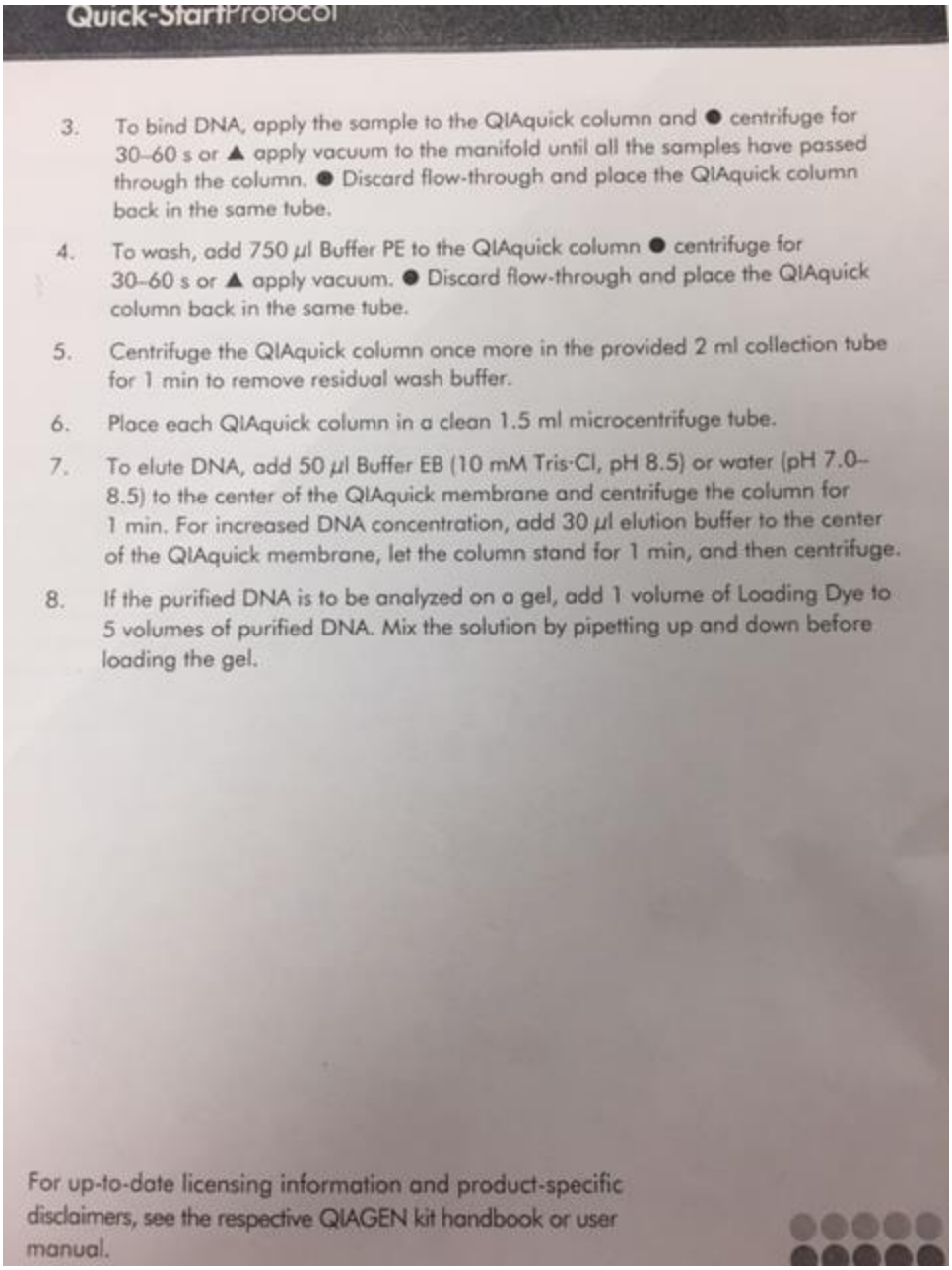
Notes before starting

- This protocol is for the purification of up to 10 µg PCR products (100 bp to 10 kb in size).
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x *g* (13,000 rpm) in a conventional table-top microcentrifuge at room temperature.
- Add 1:250 volume pH indicator I to Buffer PB. The yellow color of Buffer PB with pH indicator I indicates a pH of ≤7.5. The adsorption of DNA to the membrane is only efficient at pH ≤7.5. If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH indicator I. Do not add pH indicator I to buffer aliquots.
- Symbols: ● centrifuge processing; ▲ vacuum processing.

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
2. Place a QIAquick column in ● a provided 2 ml collection tube or into ▲ a vacuum manifold. For details on how to set up a vacuum manifold, refer to the *QIAquick Spin Handbook*.

September 2011

QIAGEN®



Quick-Start Protocol

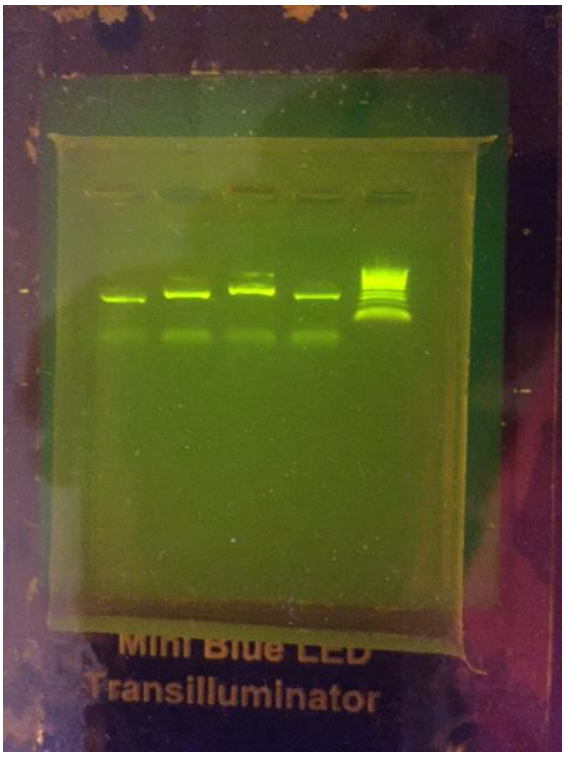
3. To bind DNA, apply the sample to the QIAquick column and ● centrifuge for 30–60 s or ▲ apply vacuum to the manifold until all the samples have passed through the column. ● Discard flow-through and place the QIAquick column back in the same tube.
4. To wash, add 750 µl Buffer PE to the QIAquick column ● centrifuge for 30–60 s or ▲ apply vacuum. ● Discard flow-through and place the QIAquick column back in the same tube.
5. Centrifuge the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
6. Place each QIAquick column in a clean 1.5 ml microcentrifuge tube.
7. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. For increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.
8. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

Loaded 2 ul of sample into gel. Run at 100v for 30 mins.

Note:

Our sample is in well 8. Not 9.
We loaded 2ul of our sample into the third from the left spot on the rightmost gel.
T7 is 20bp multiply that by two for each side - our fragment in the gel should be 469 bp
Elute in 30 uL EB not 50ul



Our sample was in well 3

Results from nanodrop are:
216 ng/uL DNA

Conclusion:

We isolated and concentrated our template DNA.

Next step:

Synthesize dsRNA.

Date: 3/28/2017
Week#: 11

Objective: (in one or two sentences)

Confirm the sequence that we received from the company is correct through some sequence comparison. Then set up next week's PCR.

Procedures: (bullet point the procedures)

Fill out the sequence analysis form.

Set up PCR reaction as described in weeks below.

Recipe		
ddH_2O	46uL	
5x GoTaq buffer w/ Mg		20uL
dNTP		8uL
PCR-TOPO		15uL
Plasmid DNA		10uL
GoTaq		1 uL (10ng/uL)
Total		100uL

Note:

We filled out the sequence analysis form. We found that the amplicon is sense, correct length, and contains two mutations from predicted, however looking at the protein sequence showed that both were silent.

Conclusion:

Next step:

Date: 3/14 (PIE DAY)
Week#: 10

Objective:

Mini Prep To isolate GOI plasmid from our E. coli cultures.

Procedures:

Make sure to write down all the details about the procedures.

1) Pellet E.coli (max speed for 5 min)
    a) Obtain 2 mL tube
    b) Add 2 ml of 5 ml sample to tube
    c) Spin down
    d) Repeat b and c
    e) Retain 1 ml stock as backup
2) Remove LB
3) Add P1 250uL
4) Vortex
5) Add P2 250uL gently
    a) P2- not too long or will dissolve DNA (P2 used to get rid of RNA and genomic DNA "interference" on agarose gel)
6) Mix and let dissolve Ecoli (3 minutes)
7) Add N3 350uL
    a) Make sure to mix thoroughly
8) Put on ice 5 minutes
9) Spin at 12krpm for 10 min
10) Add 750 uL supernantant to column
    a) don't get pellet in new tube
11) Vacuum down
12) Add 500 ul PE
13) Vacuum down
14) Column to clean 1.5 ul tube
15) Add 30 ul Elution Buffer
16) Let sit 1 min, then spin down @ 12000rpm for 1 min

Note:

Pelleted E. coli as above. Worked great. Remaining LB+E.coli stock kept in the large tube as a backup culture. Procedure followed as above (N3 had salt in it. Made pipetting kinda funny. Didn't quite mix enough directly after adding N3, so it may not have neutralized the soln. In time). BIG problem, Yoshi thought it was PB to start, it was actually P1… so that's not good.

Followed the rest of the procedure, eluting what we hope is our GOI plasmid. In order to confirm, we used the CBFG's nanodrop to determine concentration of whatever we eluted. It was bad. 91.6 ng/ul (should have been ~500 ng/ul).

Conclusion:

We did not correctly do this, however we gained experience in miniprep and nanodrop procedures. We are just going to send out the E. coli sample out to a company to miniprep and sequence.

Next step:

Get the sequence back, annotate it, and use this plasmid as a template to make dsRNA for our gene.

Date: 3/9/2017
Week#: 9

Objective:

Run a gel to confirm the colony PCR amplicon length match our designed GOI fragment

Procedures:

Make a 1.5% agarose gel (Procedure below)

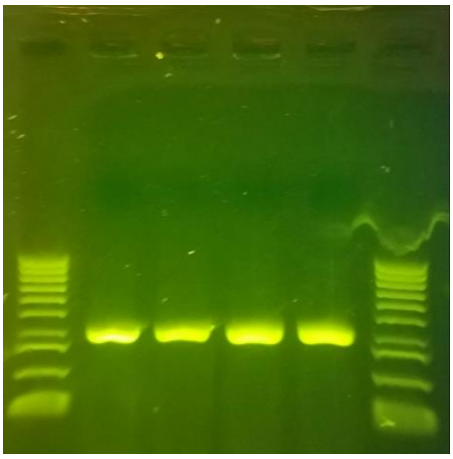
Load 2 ul of each of 4 samples, and run for 30 mins @ 100 V

Use the 100 bp ladder to estimate the length of the samples we ran

Note:

The gel was prepared for us, we loaded 20 ul on accident in of each of our 4 samples we used for PCR last week.

All went well, and the gel showed all bands were exactly in the 400-430 bp range, right where the predicted F2R2 sequence should be. The gel is shown below (100 bp ladder):



Conclusion:

We conclude that the colonies we isolated contained our GOI fragment.

Next step:

Prepare the DNA fragments we isolated to use in dsRNA synthesis.

Date: 3/7/2017
Week#: 8

Objective:

Make sure that our GOI plasmid transformation into *E. coli* was successful. In order to do this we will do colony PCR using our gene specific primers, and run a gel to confirm that a fragment the size of our GOI fragment was amplified.

Procedures: (bullet point the procedures)

Pick Some Colonies:

Pipette a 100 ul aliquot of LB+Ampr from the stock tube into a 1.5 ml tube. Next, prepare 4 small PCR tubes by pipetting 15 ul LB+Ampr into each. Next, use a pipette tip to touch each of the 4 desired colonies (do not select any satellite colonies around large colonies that may have survived the antibiotics) and transfer each to their own PCR tube by touching the contaminated pipette tip to the LB+Ampr solution in the tube. Label each tube, take these, and culture them @ 37C for ~1 hr.

Colony PCR:

Now we will use these cultured samples to prepare 4 PCR solutions from a master mix (master volumes indicated on the right []), each as follows:

● H20	-	8.2 ul	[41 ul]
● 5X Green Buffer	-	4 ul	[20 ul]
● dNTP mix	-	1.6 ul	[8 ul]
● Primer 1 (F2)	-	2 ul	[10 ul]
● Primer 2 (R2)	-	2ul	[10 ul]
● Taq	-	0.2 ul	[1 ul]
● Culture	-	2 ul	2 ul per cult.
● Total	-	20 ul	

*Make sure to use F2R2 primer pair

We will then run these 4 PCR in order to determine which of these colonies contained our GOI fragment.

Note:
(describe what you actually did, along with all data you obtained (gel pictures, DNA quantification results, etc)

Make sure to write down all the details about the procedures.

We obtained a 100 ul aliquot of LB, and transferred 15 ul of this into 4 separate PCR tubes. We then used the 2 ul pipette tips to select 4 promising (large and isolated) colonies on our plate from last week, and transferred them into the LB tubes. We then cultured these @ 37C for ~ 1 hr.

While the samples were culturing, we mixed a master mix without the taq enzyme and samples. Once the culture was finished, the enzyme and a sample from each were added to an individual PCR tube along with ¼ of the master mix. This was given to Yoshi and David to run a PCR.

Conclusion:

We conclude that we selected 4 colonies that may contain our GOI fragment plasmid, and will run a PCR using gene specific primers in order to assess which (if any) contain our GOI fragment.

Next step:

Run a gel of each of the PCR fragments, and compare the dominant band to the size of the F2R2 TcBar fragment we designed to confirm that we successfully cloned and amplified our GOI.

Date: 3/2/2017
Week#: 7

Objective:

1. Make + run gels of last week's PCR product
2. Cut out DNA from Gel
3. Purify DNA
4. Clone the DNA fragment into plasmid
5. Transform into E. coli

Procedures:

Again, our PCR buffer had loading dye included, so no need to add any before running the gel.

- Load 20 ul of each fragment
- Load 5 ul of 100 bp ladder
- Run @ 100 V for 30 mins

In order to preserve, and be able to easily amplify our gene of interest, we will put it into a plasmid, and transform it into E. coli. Once the plasmid is in E. coli, we can culture the E. coli, effectively amplifying the plasmid, then miniprep the plasmid to isolate it from the culture, and use this as a template, then run a PCR to make DNA fragment with T7 on both ends (later).

<u>TA cloning</u>
In order to clone our fragment into a plasmid (because we have taqPOL which adds a hanging A on the 3' sides, we can TA clone):

- Use a specific 'opened' plasmid that has hanging T on the 5' ends, and a pre conjugated topoisomerase.
- Simply mix your GOI DNA fragment and this plasmid, and it will combine into a plasmid containing your GOI DNA fragment as below:
    - PCR product - 4 ul
    - Salt soln - 1 ul
    - Topo plasmid - 1 ul

    - Allow to react for 15 min @ Room Temp

This will give us a mixture of plasmids with our GOI inside (BarH1), as well as many copies of empty plasmid that were closed anyway.

Transformation

In order to transform, we use a chemical to damage the cell membrane to allow some plasmid into the E. coli cells, then do a voodoo heat shock. These damaged cells are then incubated, then plated for isolation. Do this as follows:

- Add 3 ul rxn from 'Cloning' into a new tube with E. coli cell solution

- Heat shock at 42C for 30 sec
- Cool for 5 mins on Ice

- Add 250 ul of SOC (super optimal culture)
- Incubate at 37C for 1 hour

- Shake down, and plate 120 ul on agar plate described below
- Use a spreader to completely spread the sample
- Flip the plate upside down, and incubate @ 37C overnight

Isolating E. coli With our GOI plasmid

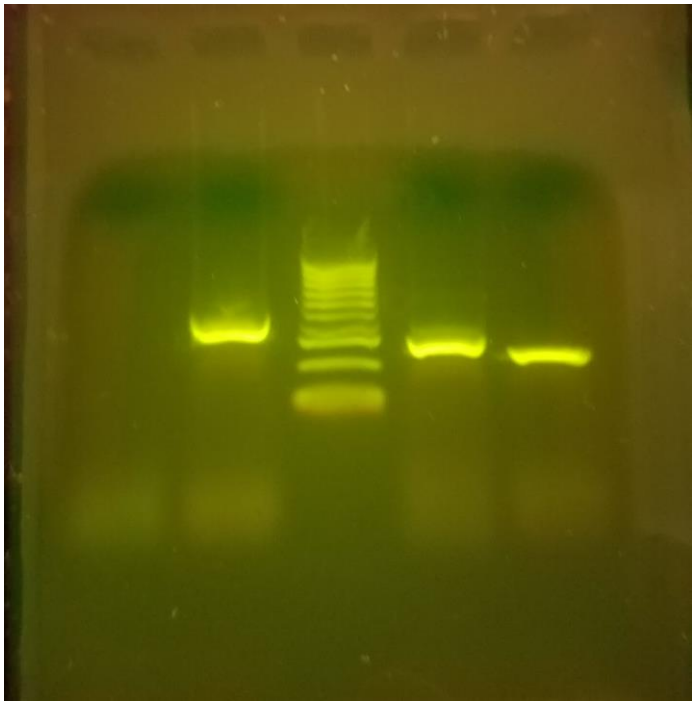
The plasmid we used above also has an antibiotic resistance gene included, which allows for simple screening with antibiotics (as those without our plasmid will die) to isolate E. coli with our GOI plasmid. However, many plasmids are simply closed without inserting our GOI DNA fragment. In order to get rid of these, the plasmid has a ccdB gene split by the insertion site of our DNA, which when completed by plasmid closing w/o our GOI causes lethality. This combined with the antibiotic agar, kills all E. coli that do not have the plasmid with our GOI DNA fragment. Then we will isolate a colony with a freeze and squeeze column, and spin down to isolate our GOI.

Note:

Jess and clayton made a 1.5% agarose gel for all of us to run our PCR products on.

We loaded 20 ul of both of our samples in the two leftmost wells on the gel on the right side of the gel box, with 5 ul of 100 bp ladder directly to the right of our sample. Ran @ 100 v for ~30 minutes.

Below is the gel we ran with our two PCR products, in positions 1 and 2. Note that there is nothing in position 1, meaning that something went wrong (user error, primer issues, etc). However we wanted the longer fragment anyways, which is F2R2 in position 2, so we are all good.



Using the Freeze and squeeze kit, we cut out our Bar F2R2 fragment, put it into the double column, froze it for ~5 min, the centrifuged for 5 min @ 14,000 rpm, then discarded the filter portion of the column.

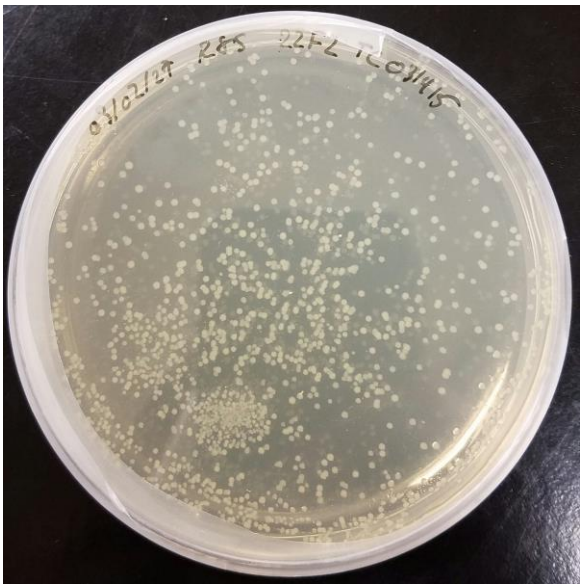
We then put 4 ul of our reaction into a new tube and added 1 ul salt soln and 1 ul of the topo plasmid. We then pipetted this up and down and let it sit at room temperature for 15 mins.

After the rxn, we pipetted 3 ul of this soln into a pre prepared tube of E. coli, and heat shocked at 42C for 30 seconds on a heat block. After heat shocking, we rested them on ice for 5 mins.

Once rested, we added 250 ul of SOC into the same tube, and incubated this for only ~40 mins @ 37C on a shaker plate, as we were running out of class time and could not fit in a full hour.

We pipetted 120 ul of the solution onto a bacteria plate, spread using the spreader, flipped it, labeled it, and gave it to yoshi to put it in the incubator to stay overnight.

The following is the resulting colonies hopefully containing our GOI fragment plasmid:



Conclusion:

We conclude that we successfully cloned and transformed our Bar F2R2 gene fragment into E. coli, and cultured this E. coli in order to preserve our GOI DNA fragment.

Next step:

Pick colonies, and confirm that they have our GOI fragment plasmid. Then prep the DNA fragment we obtained in order to make dsRNA to do RNAi for our GOI.

Date: 2/28/2017
Week#: 6

Objective:

First dilute the primers, then mix PCR reaction.

Procedures:

Important parts to PCR Mix

- Taq DNA pol
- Primer F
- Primer R
- dNTPs
- (MgCl) 10X Buffer
- cDNA Template/genomic DNA
- H_2O

When we want to amplify a GOI, we have a few starting places for template

- Genomic DNA (contains Introns, GOI hard to locate)
- **cDNA (DNA sequence of all mRNA) [USUALLY BETTER]**
    - We will be using whole body mixture of larvae, pupae and adults to source cDNA.

We have dehydrated Primers that need to be made into 100uM stock. Need then to dilute to 100 ul of 10uM solution. <= Working solution

- F1 - 6114.0 g/mol - 34.5 nmol - 0.0345 umol
- R1 - 6010.9 g/mol - 25.9 nmol - 0.0259 umol
- F2 - 6151.0 g/mol - 30.6 nmol - 0.0306 umol
- R2 - 6028.0 g/mol - 30.8 nmol - 0.0308 umol

Use the following equation to give you the necessary volume water to dilute:

umol stock / x L = 100umol final / 1 L final

This gives us the following necessary water to carry out the dilutions to 100uM stock soln:

- F1 - 345 ul ddH20
- R1 - 259 ul ddH20
- F2 - 306 ul ddH20

- R2 - 308 ul ddH20

Once we have our desired 10uM soln of each primer, we are ready to move on to Mixing or reaction soln for PCR.

- We were provided ***5x*** *Green GoTaq Reaction Buffer* (contains loading dye, so no need to add this when running a gel of the sample at the end).

PCR Primerless Master Reaction Solution (This contains everything shared by the two reactions, i.e. everything except for primers. You need to calculated volume of water to bring to final 100 ul volume, so factor in the volume of primers needed *).

H20	-	59.5 ul
5x PCR buffer	-	20 ul
dNTP	-	8 ul
Primer F	-	*4 ul (2 ul F1, 2 ul F2)
Primer R	-	*4 ul (2 ul R1, 2 ul R2)
cDNA	-	4 ul
TaqPol	-	0.5 ul
Total	**-**	**92 ul**
		***100 ul**

Individual (F1R1 and F2R2) PCR Solutions (Now the above solution gets split into two reactions. Follow these instructions for each. *Add the above accounted for volumes of Primers into their respective tubes).

Primerless Soln. (above)	-	46 ul
Forward Primer	-	2 ul
Reverse Primer	-	2 ul
Total	**-**	**50 ul**

The PCR will follow the following steps:

Denaturation:	95C X 5 min
Denaturation:	94C X 30 sec --
Annealing:	*57C X 20 sec | - cycles
Extension:	72C X **30 sec --
Extension:	72C X 5 min
Hold:	4C

: Primer tm + 1-2C* *: 500bp/s, 1,000bp/1min*

Note:

We followed the above procedure to yield 4 stock tubes of 100uM Primer Stock soln. In the shipping tubes.

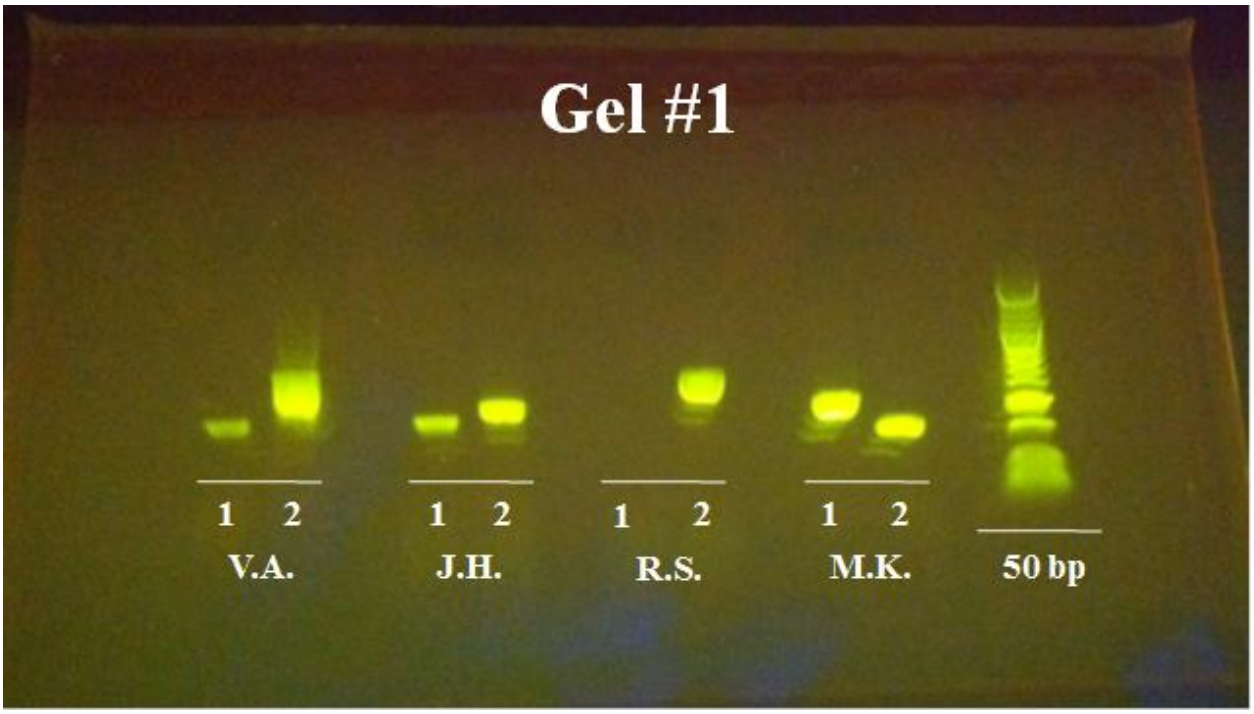
We then took 10 ul of each of these, put them in appropriately labeled tubes, and diluted them to 100 ul of 10uM with 90 ul of ddH20.

All of these were then stored in our sweet hot pink tube box, which we labeled 'R+S BIO 464'.

We then used the 10uM solutions we prepared as the forward and reverse primers referenced in the procedures above to calculate the volume of the Primerless Master Reaction Solution. We then mixed this, and split it into two reaction tubes following the individual PCR soln procedure. After this was split we then added F1 and R1 into the F1R1 PCR solution, and F2 and R2 into the F2R2 PCR solution following the Individual PCR Solution procedure above.

Once these were made, vortexed (careful after enzyme is added), and spun down, we transferred them into the small PCR tubes, labeled RS 1 and RS 2.

These were then given to Yoshi and David to run (as it takes a few hours).



RS 1 did not show amplification
RS 2 did show amplification <= continue experimentation with this primer set

Conclusion:

We conclude that our Primer dilutions were successful, and that we correctly mixed our PCR solutions.
RS 1 did not show amplification, most likely due to mis representation and not binding to cDNA. Supported because all other PCR products showed favorable results.
Continue experimentation on BarH1 using **RS 2**

Next step:

Run a gel with each of the amplicons of these reactions, and check the lengths found against the intended lengths determined when we designed the fragments.

10/10
Super nice note taking! Let's paste the gel picture and annotate the band size. --Yoshi

Date: 2/23/2017
Week#: 5

Objective: (in one or two sentences)
Run a gel, and observe injection procedure.

Procedures: (bullet point the procedures)

Gel Prep Procedure:

- Prepare a y% Agarose in TBE + GelGreen (buffer) gel
    - Choose volume of gel (~20 ml for small comb and 30 for large)
    - Choose desired % agarose in TBE (Usually around 1% weight/volume)
        - 1kb - 10kb = 0.7% agarose
        - 300bp - 1kb = 1% agarose
        - 50bp - 300bp = 1.5% agarose
    - Y % agarose (w/v) = (X g Agarose) / (Z ml Buffer)
    - Microwave for ~30sec-1min per 30mL (make sure starting mass= mass final (add H20 to equilibriate))
    - Pour into mold, and allow to cool for ~20 mins
- Observe injection methods while gel sets up
- Run 2 of 3 unknown DNA Samples for mins at power level
- Compare to ladder, and decide which samples you have

Injection Procedure:

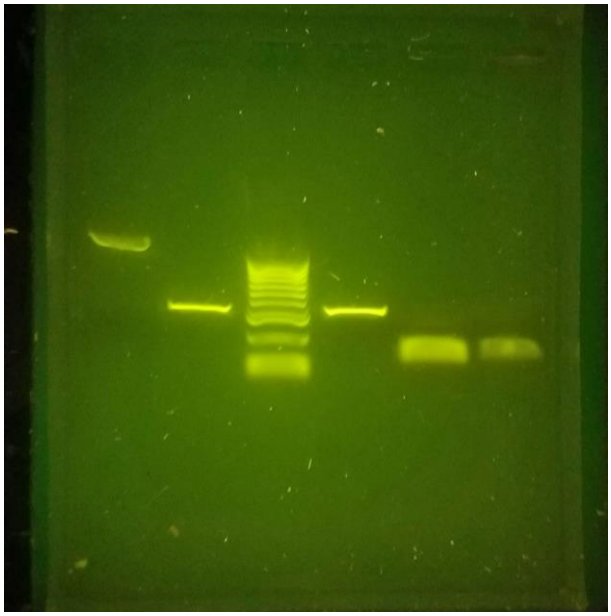
- Pick enough 3.5 day old T. castaneum larvae of desired strain (usually 15 - 20 pu11).
- Determine desired dsRNA concentration (usually 1ug/ul)
- Mix together injection soln (~0.8 ul / larvae) with ½ inj buffer, and required amount of dsRNA, coming to final volume with ddH20. Use equation below for dsRNA volume:
  (Xul stock dsRNA)*(conc stock dsRNA ug/ul) = (final dsRNA conc ug/ul)(final ul volume)
- Gently mix soln by pipetting, then vortex and briefly spin down.
- Break needle under microscope to provide sharp tip. Back load and load on holder.
- Etherize the larvae you picked earlier for ~1.5 to 2 mins.
- Quickly arrange the larvae on a sticky slide (dorsal to the top left) in however many columns of 5.
- Use the microscope (usually 16x works) to assist in injecting ~ 0.8 ul soln to each larvae. This is more of an art, so look to see color intensity, and how puffed up the larvae are. Try not to pop them.
- Once finished, dispose of needle, remove the larvae under a microscope and allow them to rest before returning them to an appropriately labeled bottle with flower in the incubator.

- Check for phenotype at desired stage later on.

Notes:

Gel Prep Done:

- Choose volume of gel **(~20 ml** for small comb and 30 for large)
- Choose desired % agarose in TBE (Usually around 1% weight/volume) = **1.2% gel**
- 1.2 % agarose (w/v) = (**X** g Agarose) / (100 ml Buffer)
  Gives us 1.27 g agarose, and 100 ml of TBE w/ GelGreen for the whole class (running 4 small combs with a little extra).
- Microwave for 2 mins on 30% power if possible, then remove and mix, put back for another ~1min on 30% power (to remove extra bubbles) in an E-Flask, with a beaker as a cap. (make sure starting mass= mass final (add H20 to equilibriate))
- Pour into mold ⅔ of the way up the well, be careful about bubbles in the well (use pipet tip to get rid of any), put in the comb, and allow to cool for ~30 mins.
- **IMMEDIATELY CLEAN THE BEAKER BY MASS DILUTION BEFORE IT SOLIDIFIES**
- Fill the gel box with enough TBE to cover the gel, make sure everything works, and connect (red positive black negative)
- Mix DNA sample B with (usually) 5x Loading dye (heavy sugar), to keep the sample in the well. We used **8 ul sample** with **2 ul loading die**. (amount loading die should be ⅕ final volume).
- load the gel and Run our unknown DNA sample B (wells on the negative/black side) and 5ul of ladder for x mins @ 100 volts
- When finished running, expose to blue light to illuminate GelGreen, and take picture with your cell phone, and upload to lab notes below:



Our unknown B is to the left of the ladder. Wells are on top.

Conclusion:

We conclude that our unknown DNA sample B, seen above as directly to the left of the ladder, is about 400bp.

Next step:

Use these procedures for our own wet experiments in the coming weeks.

Date:
Week#:

Objective: (in one or two sentences)

Procedures: (bullet point the procedures)

Note:
(describe what you actually did, along with all data you obtained (gel pictures, DNA quantification results, etc)

Make sure to write down all the details about the procedures.

Conclusion:

Next step: