Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 04/25/2017
Week#: 14

Objective: (in one or two sentences)
Observe Tribolium injected with dsRNA

Procedures: (bullet point the procedures)

- Observe

Conclusion:
Observations of dead adult Tribolium:
It appears that most of the beetles died during adult eclosion
Pigmentation funny on abdominal sclerites; mottled
No sensory bristles
Small eyes (half the size). Eye structure, no ommatidia

Speculation: Since they have no sensory hairs, the eclosion process is stunted due to the fact that the beetles cannot sense their surroundings

DEATH TOLL TODAY: 10
TOTAL DEAD: 12
LIVING: 0

Next step:
Complete online report

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 04/20/2017
Week#: 13

Objective: (in one or two sentences)
Observe Tribolium injected with dsRNA

Procedures: (bullet point the procedures)

- Observe

Conclusion:
Pupae
Urogomphi shortened/nearly missing in pupae
Gin-traps are disrupted, some nearly missing
Missing sensory bristles

All pupae have the above phenotype

DEATH TOLL TODAY: 0
TOTAL DEAD: 2
LIVING:
Total Larvae: 1 (but appears to be either dying or pupating)
Total Pupae: 12

Next step:
Continue Tribolium analysis and complete online report (username: BIO464, password: beetles)

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 04/18/2017
Week#: 13

Objective: (in one or two sentences)
Observe Tribolium injected with dsRNA

Procedures: (bullet point the procedures)

- Draw mutant Tribolium

Note:

Conclusion:
Larvae
Loss of sensory bristles on injected larva?

Pupae
Urogomphi shortened/nearly missing in pupae
Gin-traps are disrupted, some nearly missing
Missing sensory bristles

DEATH TOLL TODAY: 2
TOTAL DEAD: 2
LIVING:
Total Larvae: 1
Total Pupae: 12

Next step:
Continue Tribolium analysis and complete online report (username: BIO464, password: beetles)

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 04/11/2017
Week#: 12

Objective: (in one or two sentences)
Learn how to use microscopes and observe wild type Tribolium at three different stages.

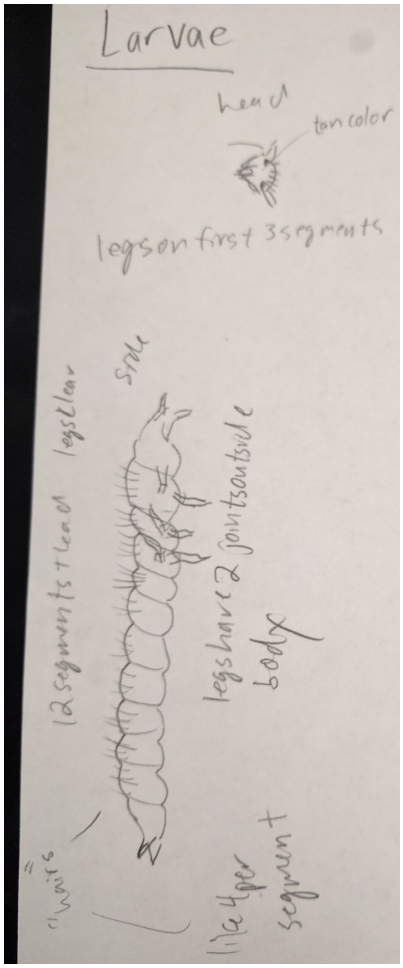
Procedures: (bullet point the procedures)

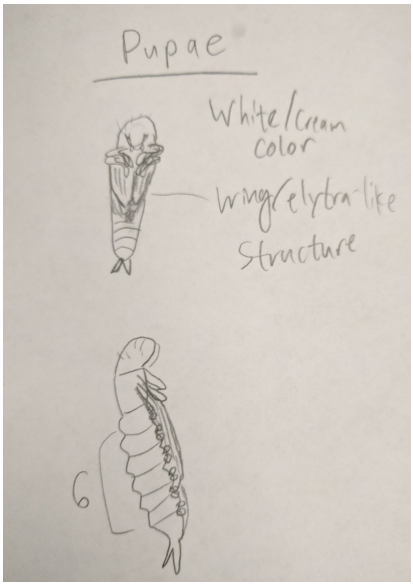
- Observe wild type Tribolium under the microscope and draw them in their three stages

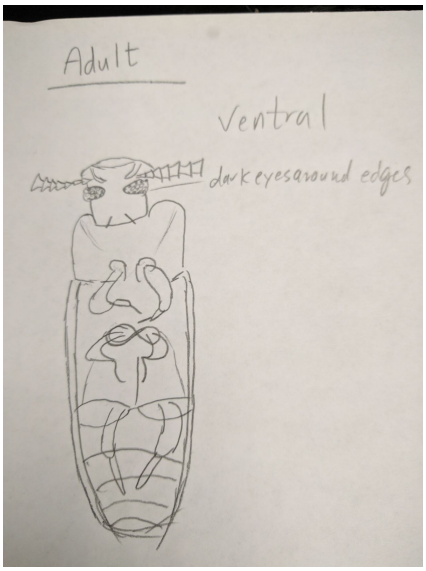
Note:
Insects have three pairs of legs. Hemimetabolous insects look the same as young and adults. Holometabolous insects look different during their different stages (like beetles, which are larvae before being adults). The pupal stage is when the insect drastically changes its form (metamorphosis)... this form is a recent evolutionary change and is very successful.

Conclusion:

Micah Drawings:

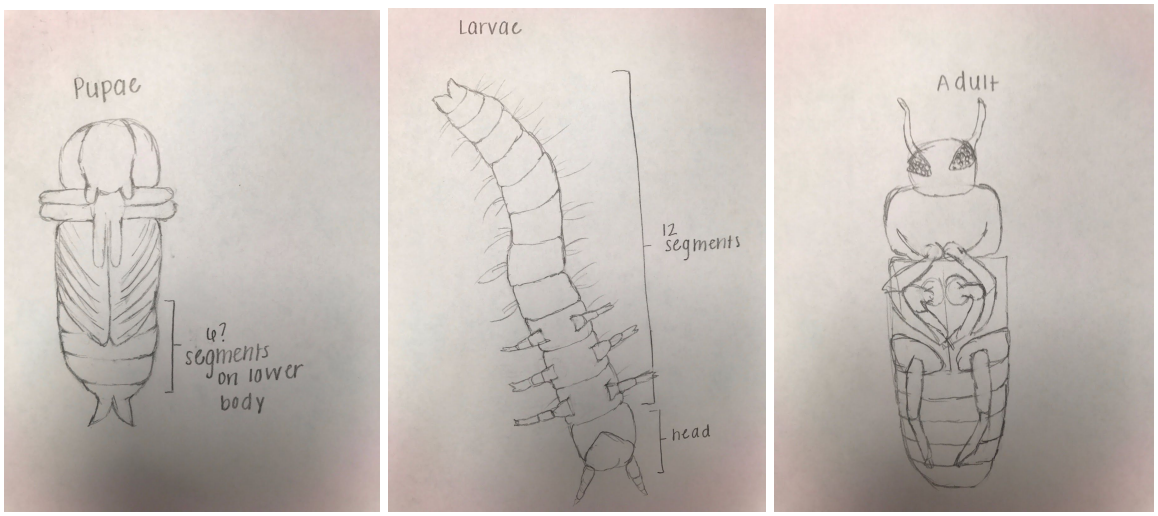






Lab notebook
Megan Lytle, Micah Morris
BIO464W

Megan Drawings:



Next step:
Exam Thursday, observe Tribolium after inserting dsRNA.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 04/06/2017
Week#: 11

Objective: (in one or two sentences)
DNase treatment and dsRNA purification.

Procedures: (bullet point the procedures)

- Set up RNase Digestion Reaction, incubate for 1 hr at 37˚C:
    - 20 µL dsRNA from previous step
    - 21 µL nuclease free water
    - 5 µL 10x digestion buffer
    - 2 µL DNase I
    - 2µL RNase
- Procedure for dsRNA purification
    - Ingredients:
        - 50 µL dsRNA from previous step
        - 50 µL 10x binding buffer
        - 150 µL nuclease free water
        - 250 µL 100% ethanol
    - Put the entire 500 µL into the filter tube and draw through with a centrifuge at maximum speed for 2 minutes
    - Pipet 500 µL of wash solution onto the filter and draw the wash solution through the filter at maximum speed for 2 minutes
    - Repeat with another 500 µL wash buffer
    - Centrifuge again for 2 min at max speed to ensure all liquid was removed
    - Elute with 60 µL (hot) elution buffer only 1x by centrifuging 2 min at max speed
        - If the concentration is high enough, stop at this point
        - If not, elute again and proceed to concentration step
    - Nanodrop

Note:
Now, we should have dsRNA but the mixture also contains some dsDNA (the template we used to transcribe the RNA which annealed to become dsRNA) and leftover ssRNA that we want to get rid of. We do a DNase treatment to get rid of the dsDNA and do dsRNA purification to get rid of the ssRNA.

Conclusion:
Nanodrop results: 55.2 ng/µL = 1104 ng/µL*58µL

Lab notebook

Megan Lytle, Micah Morris

BIO464W

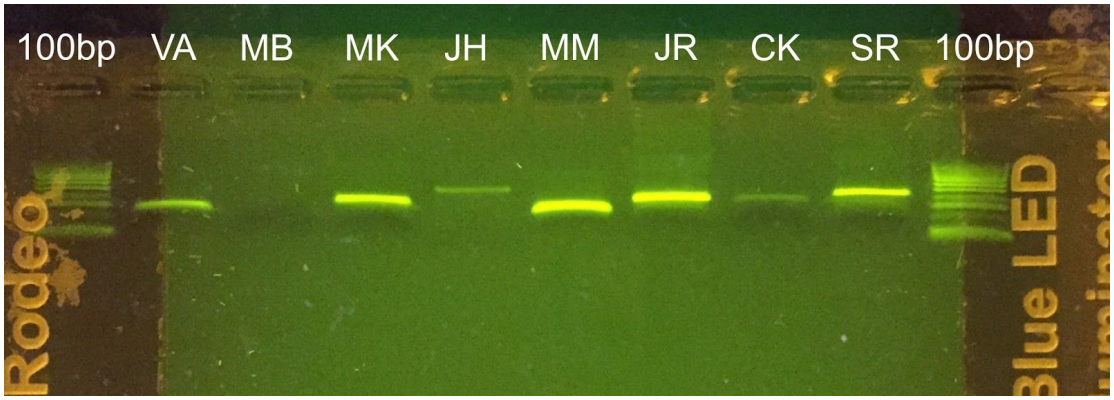


Figure 1. Gel electrophoresis results after RNA isolation.

Next step:

Concentrate the results.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 04/04/2017
Week#: 11

Objective: (in one or two sentences)
Complete *in vitro* transcription to produce double stranded RNA with the same sequence as our target gene.

Procedures: (bullet point the procedures)

- Ingredients for *in vitro* transcription
    - 8 µL NTP mix
    - 2 µL buffer
    - 2 µL enzyme
    - 8 µL template (138.11ng/µL)

Note:
At the start of this lab, we have a linear PCR product containing our gene fragment and the T7 promoter on both ends. We will do *in vitro* transcription to get double stranded RNA with the same sequence as our target gene.

Conclusion:
Conclusions to be drawn next lab session.

Next step:
DNase treatment and dsRNA purification.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 03/30/2017
Week#: 10

Objective: (in one or two sentences)
Get linear PCR product of our gene with T7 promoters on the ends.

Procedures: (bullet point the procedures)

- Make and run gel (1.5%, 40 ml TBE, 0.6 g agar)
    - Refer to 02/28/2017 entry for instructions on making and running a gel
    - Run 2μL sample
    - Lanes
        - 1 MM
        - 2 RJ
        - 3 CK
        - 4 SR
        - 5 100bp ladder
- With the remaining sample (98μL), purify the PCR product
    - Refer to 03/14/2017 entry for instructions, disregarding the portion where cells were killed
    - Change to procedure: elute with 30μL to make sure concentration is as high as possible

Note:

The expected length of the PCR product should be our fragment + 40bp (coming from the primers on either end) = 246 + 40 = 286bp.

Figure 1. PCR result. Lane 1 contains our plasmid.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Conclusion:
We now have a linear PCR product containing our gene fragment and the T7 promoter on both ends.
Concentration: 138.11ng/μL

Next step:
In vitro transcription.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 03/28/2017
Week#: 10

Objective: (in one or two sentences)
Analyze our sequence to make sure it is correct. Make sure it matches with the sequence we used to design the primer. Set up the PCR for the next step.

Procedures: (bullet point the procedures)

- For sequence analysis procedures, see "2017/03/28 Sequence Analysis" doc
- Set up 100µL PCR reaction (which will add a promoter that is attached to the T7 primer)
    - Refer to 02/28/2017 entry for instructions on PCR
    - "Ingredients" list for rxn
        - 46 µL H_2O
        - 20 µL 5x Green Buffer
        - 8 µL dNTP mix
        - 15 µL PCR4_Topo Primer
        - 1 µL Taq
        - 10 µL of 10ng/µL Plasmid
    - Plasmid must be diluted to make 30 µL of 10ng/µL
        - (539ng/µL)x=(10ng/µL)(30µL) → x= 0.56µL

Note:
Sanger sequencing works using a mixture containing the template DNA, a short primer (T7), DNA pol, and dideoxy NTPs. ddNTPs inserted into the growing chain will terminate strand synthesis. The contents of the reactions are transferred to a gel, which can tell at what point each nucleotide was placed in the strand.

Conclusion:
With the exception of the deletion, the amino acid sequences matched.

Next step:
Run gel and purify the fragment.

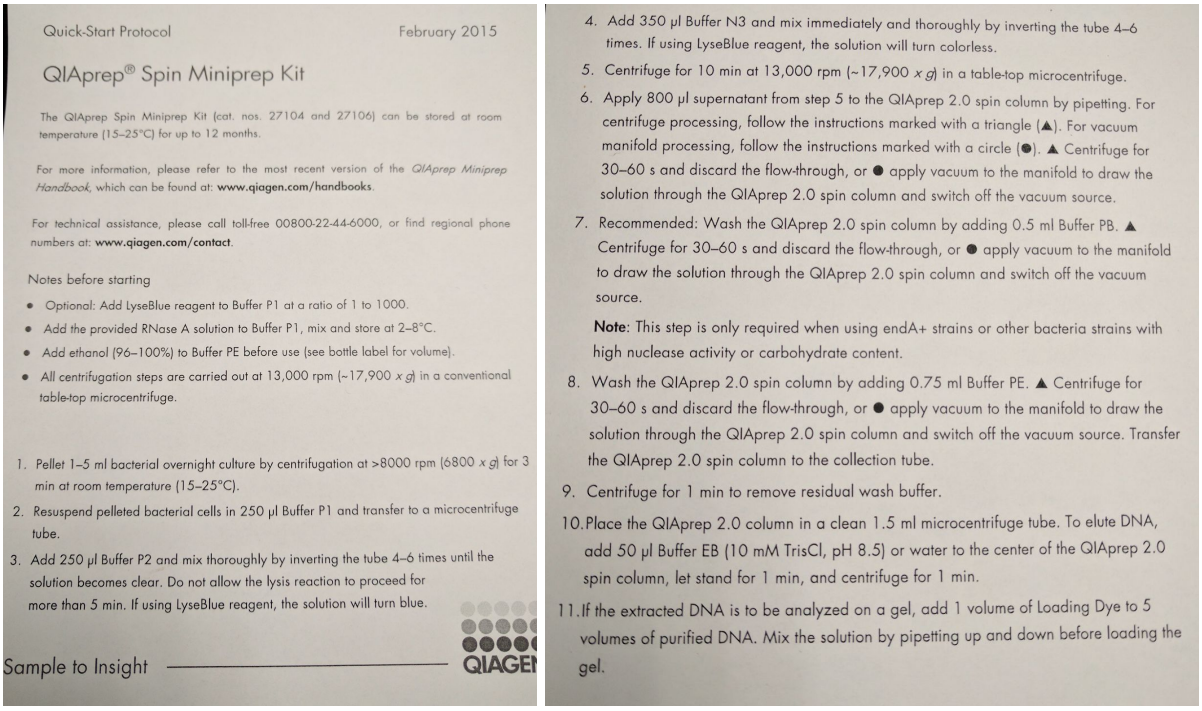
Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 03/14/2017
Week#: 8

Objective: (in one or two sentences)
Use the Miniprep procedure to isolate and purify the plasmids containing our GOI.

Procedures: (bullet point the procedures)

- Miniprep procedure:
    - Centrifuge 2ml *E. coli* culture solution at 12,000 rpm for 2 minutes (x2)
    - Remove supernatant
    - Add 250µL P1 (a buffer) and vortex
    - Gently add 250µL P2 (highly alkaline) and invert; leave for 3 minutes
    - Add N3 to neutralize pH and invert
    - Leave on ice for 5 minutes
    - Centrifuge at 12,000 rpm for 10 minutes
    - Pipette 750µL of solution (all of it) into Miniprep column
    - Place blue piece of column onto vacuum so that as solutions are added, they are automatically pulled through the column
    - Add 750µL PE
    - Transfer column to clean 1.5ml tube
    - Add 30µL EB
    - Let sit 1 minute then spin 1 minute
- Sequence the fragment to make sure that it is the gene that you meant to clone
- Minikit Procedures



Quick-Start Protocol February 2015

QIAprep® Spin Miniprep Kit

The QIAprep Spin Miniprep Kit (cat. nos. 27104 and 27106) can be stored at room temperature (15–25°C) for up to 12 months.

For more information, please refer to the most recent version of the *QIAprep Miniprep 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

- Optional: Add LyseBlue reagent to Buffer P1 at a ratio of 1 to 1000.
- Add the provided RNase A solution to Buffer P1, mix and store at 2–8°C.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 13,000 rpm (~17,900 *x g*) in a conventional table-top microcentrifuge.

1. Pellet 1–5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 *x g*) for 3 min at room temperature (15–25°C).
2. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
3. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.

Sample to Insight QIAGEN

4. Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times. If using LyseBlue reagent, the solution will turn colorless.
5. Centrifuge for 10 min at 13,000 rpm (~17,900 *x g*) in a table-top microcentrifuge.
6. Apply 800 µl supernatant from step 5 to the QIAprep 2.0 spin column by pipetting. For centrifuge processing, follow the instructions marked with a triangle (▲). For vacuum manifold processing, follow the instructions marked with a circle (●). ▲ Centrifuge for 30–60 s and discard the flow-through, or ● apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.
7. Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB. ▲ Centrifuge for 30–60 s and discard the flow-through, or ● apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source.

   Note: This step is only required when using endA+ strains or other bacteria strains with high nuclease activity or carbohydrate content.
8. Wash the QIAprep 2.0 spin column by adding 0.75 ml Buffer PE. ▲ Centrifuge for 30–60 s and discard the flow-through, or ● apply vacuum to the manifold to draw the solution through the QIAprep 2.0 spin column and switch off the vacuum source. Transfer the QIAprep 2.0 spin column to the collection tube.
9. Centrifuge for 1 min to remove residual wash buffer.
10. Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM TrisCl, pH 8.5) or water to the center of the QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.
11. If the extracted 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.

Figure 1. Minikit procedure.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Note:
Miniprep allows us to purify ONLY the plasmid with our GOI and not the genome, lipids, proteins, etc. The P2 buffer is highly alkaline and will destroy the membrane. Therefore, the time it is used must be measured very precisely

In the Miniprep column, the DNA is retained in the membrane

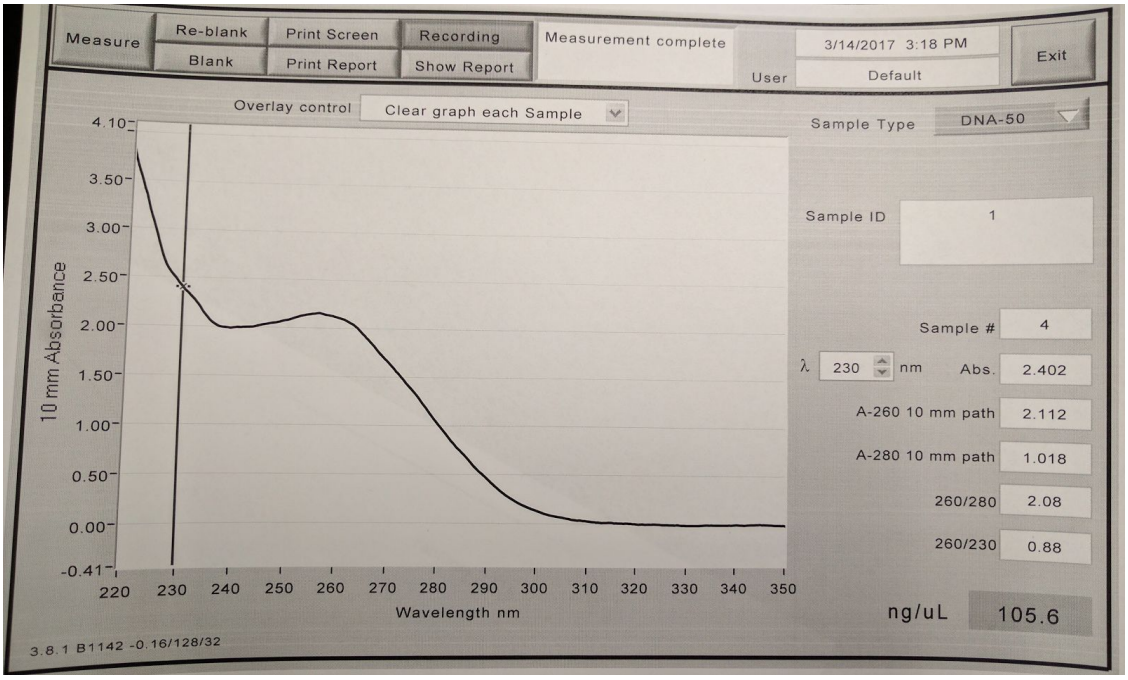


Figure 2. Nanodrop results. The low 260/240 ratio and low concentration indicated the the buffer mistake significantly affected the plasmid extraction and isolation process.

Conclusion:
Unfortunately, we messed up some of the buffers and so our samples did not work.

Next step:
Send plasmids out for sequencing and annotate the sequence.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 03/09/2017
Week#: 7

Objective: (in one or two sentences)
Run our PCR samples from last week on a gel

Procedures: (bullet point the procedures)

- Make and run gel (1.5%, 75ml TBE, 1.125g agar)
    - Refer to 02/28/2017 entry for instructions on making and running a gel
    - Run 2µL sample
    - Lanes
        - 1 CK
        - 2 CK
        - 3 CK
        - 4 CK
        - 5 100bp ladder
        - 6 MMML Colony #1
        - 7 MMML Colony #2
        - 8 MMML Colony #3
        - 9 MMML Colony #4

Note:
Prediction for PCR amplified fragments from colonies digested with F2R2: 267bp

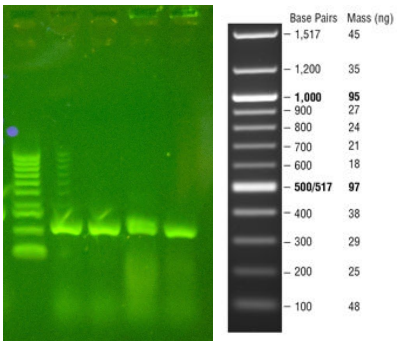


Figure 1. PCR products from isolated *E. coli* colonies selected with F2R2 Primers. Size is approximately 200-250bp, expected fragment size

Conclusion: Colony 2 will be utilized for overexpression of dsRNA

Next step:

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 03/07/2017
Week#: 7

Objective: (in one or two sentences)
Today we will be picking colonies and setting up PCR.

Procedures: (bullet point the procedures)

- Pick colonies from plate and culture them
    - Add 15µL LB/Ampicillin mix to each of the four small tubes
    - Use pipette tip and grab colony from plate then transfer to a tube (4x)
    - Incubate at 37˚C for 1 hour
- Set up Colony PCR
    - Refer to 02/28/2017 entry for instructions on PCR
    - "Ingredients" list for rxn
        - 8.2 µL H_2O
        - 4 µL 5x Green Buffer
        - 1.6 µL dNTP mix
        - 2 µL Tc_3570 Forward Primer #2
        - 2 µL Tc_3570 Reverse Primer #2
        - 0.2 µL Taq
        - 2 µL Culture
    - "Master mix" consists of everything except the culture; multiply ingredients by factor of 5 to make the master mix
    - Add 18µL of master mix to each tube then add the corresponding culture

Note:

- What is the main goal/final product of what we want from these experiments? dsRNA
- What kind of techniques have we been using?
    - PCR, gel electrophoresis
    - primer design/blast
    - Transformation
    - T-A cloning with topoisomerase
- How did we select for *E. coli* that have the GOI?
    - Ampicillin resistance gene in plasmid (beta lactamase) and antibiotic in growth medium selects against cells with no plasmid uptake.
    - CcdB gene in plasmid kills cells that uptake the plasmid that did not insert the gene of interest (which disrupts the ccdB gene).
- How can we make sure that our colony has the correct GOI?
    - We will do PCR directly on the colony (Colony PCR)
    - Other methods
        - Plasmid isolation and sequencing

Conclusion:

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Next step:
Thursday we will actually be running the PCR.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 03/02/2017
Week#: 6

Objective: (in one or two sentences)
The objective for today is to make gels, run gels, cut out DNA from gel, purify DNA, clone the DNA fragment into a plasmid, and transform *E. coli* with the plasmid.

Procedures: (bullet point the procedures)

- Make and run gel (150 ml, 1.5%)
    - Refer to 02/23/2017 entry for instructions
    - Lanes
        - 1 MMML Tc003570 #1
        - 2 MMML Tc003570 #2
        - 3 100bp Ladder
        - 4 MK
        - 5 MK
- Cut out DNA from gel using the extractor tool
    - Freeze the sample
- Purify DNA
    - Centrifuge the sample to separate the DNA from the agarose
- Clone the DNA fragment into plasmid (topo-reaction)
    - Mix 4µL PCR product, 1µL salt, 1µL Topo plasmid
    - Leave at room temperature for 15 min.
- Transform *E. coli* with the plasmid
    - Add 3µl of the reaction and pipette into *E. coli* cell solution
    - Heat shock at 42˚C for 30 sec.
    - Cool at 4˚C for 5 min. (recovery)
    - Add 200µl super optimal culture (SOC) (more recovery)
    - Shake at 37˚C for 40 minutes
- Plate 120µl bacteria

Note:
Due to the length of our amplicon, the gel that we will be running our DNA on is 1.5% agarose. We will chose the longer fragment to clone. For us, this corresponds to primers F2R2.

The plasmid that we use already has topoisomerase conjugated to it and therefore the reaction does not require ligase. We mix the plasmid (that has T overhangs at a cut portion of the plasmid) with our PCR product (which has A overhangs as a result of PCR using Taq polymerase) and the topoisomerase will cause these to join (T-A cloning). Sometimes, the plasmid will self-anneal without the gene of interest (GOI) in it and the *E. coli* will still grow. We get past this using a lac operon gene which, if the gene is intact (which will only happen in the *E. coli* that have the empty plasmid), the colonies will turn blue (blue-white selection). Those that picked up the GOI will stay white because the lac operon gene was interrupted with the GOI

insertion. Also, the plasmids have ampicillin resistance so that the *E. coli* will die if they do not receive the plasmid. Therefore, we use two methods to select for *E. coli* that have picked up the plasmid containing the GOI.

In this lab, we do not do the blue-white selection but rather do a similar method where instead of the lacZ gene, there is a disrupted ccdB gene that will automatically kill any bacteria that have the gene intact (those that have not picked up the GOI).

Transformation is done by heat shocking the *E. coli* and changing the concentration of its media to allow for the plasmid to be picked up by the bacteria.

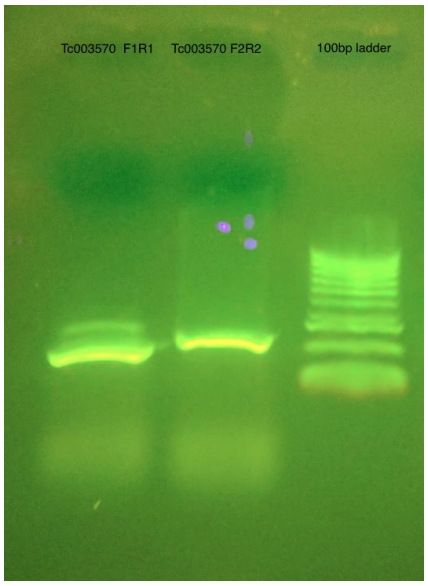


Figure 1. PCR products of Tc003570 with F1R1 (lane 1) and F2R2 (lane 2) primers. F1R1 contained unspecific bands and therefore the brighter band in lane two corresponding to primers F2R2 was chosen for further experimentation

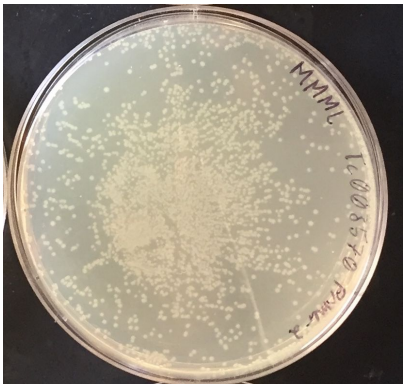


Figure 2. Results from plating of *E. coli* transformed with GOI

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Conclusion:
Growth of *E. coli* was observed indicating that the correct plasmid containing our GOI was transformed into the bacteria.

Next step:

Lab notebook
Megan Lytle, Micah Morris
BIO464W

Date: 02/28/2017
Week#: 6

Objective: (in one or two sentences)
Learn about the basics of polymerase chain reaction (PCR); replicate our primers using PCR

Procedures: (bullet point the procedures)

- Materials required for PCR:
  - Taq DNA polymerase
  - Forward and reverse primers
  - dNTP mix
  - 5x buffer
  - Template (*cDNA*, genomic DNA, plasmid)
  - Magnesium chloride (depending on if it is already added into the buffer)
  - Water (to adjust concentrations)
  - PCR Cycler
  - 50bp ladder
- Add water to the dry primer mixtures to make the concentration 100μM
  - See calculations below (we used Yoshi's way)
  - Vortex then spin down
- Then, make 100μL of 10μM of each primer to use for PCR
  - Mix 10μL primer and 90μL water
- Set up PCR samples (starting with the biggest volume)
  - H20: 59.5 μL
  - 5x buffer: 20 μL
  - dNTP: 8 μL
  - cDNA: 4 μL
  - Taq polymerase: 0.5 μL
    - Before you add the enzyme, vortex the mixture well and spin down. Once enzyme is added, briefly vortex again and spin down again
- Split into two 46μL samples
  - Add 2μL Forward Primer
  - Add 2μL reverse Primer
- Run PCR under the following reaction parameters:
  - Denaturation: 95˚C x 5 min.
  - Cycle 35x times
    - Denaturation 94˚C x 30 sec.
    - Annealing: 57˚C x 30 sec.
    - Extension: 72˚C x 40 sec.
  - Extension: 72˚C x 5 min. [this step adds extra nucleotides (A or T?)]
  - Hold: 4˚C

Lab notebook
Megan Lytle, Micah Morris
BIO464W

- Calculations for 100μM
    - primer (mass on tube):

	Mass (μg)	Mw (g/mol)	Moles	Volume H_2O (L)	Volume (μL)
R1:	150	6238.1	2.405E-8	2.405E-4	240.5
R2:	220	6226.1	3.534E-8	3.534E-4	353.4
F1:	180	6071.0	2.965E-8	2.965E-4	296.5
F2:	160	6198.1	2.581E-8	2.581E-4	258.1

- Moles on tube

	nMoles	Volume H_2O (μL)
R1:	24.1	241
R2:	35.2	352
F1:	29.9	299
F2:	25	250

Note:
cDNA is a good alternative to genomic DNA because cDNA magnifies the gene of interest and has correct splicing. In this class, we will be using cDNA.

Above are the primer calculations to make the final concentration 100μM.

T_m should be the annealing temperature to make more specific binding (at the cost of less binding)

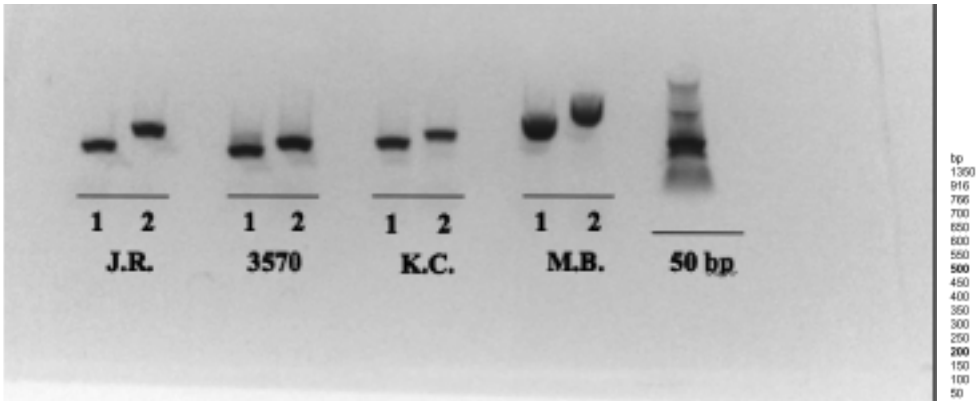


Figure 1. Amplification success of 3570 with both F1R1 and F2R2 primers demonstrated by highly concentrated gel bands at appropriate mass (~250bp between the two bold bands of 200 and 500bp)

Conclusion:
This procedure will be completed on Thursday.

Lab notebook

Megan Lytle, Micah Morris

BIO464W

Next step:

Run our primers a gel after completing polymerase chain reaction. Be able to complete a PCR by ourselves. Clone the DNA fragment into a plasmid and transform it into *E. coli*.

Lab notebook
Megan Lytle, Micah Morris
BIO464W

10/10
Very good note taking! So, what is the length of your DNA? --Yoshi

Date: 02/23/2017
Week#: 5

Objective: (in one or two sentences)
Determine size of unknown DNA fragments via agarose gel electrophoresis

Procedures: (bullet point the procedures)

- Make agarose gel (1.27% w/v or 1.27 g agarose/100 ml TBE)
    - Materials: Tris/Borate/EDTA (TBE) buffer (both with and without Gel Green Dye), Agarose, DNA Ladder (100bp), Gel Green Dye
    - Combine TBE w/ Gel Green and agarose
    - Microwave 2 minutes at 30% power then remove and swirl
    - Microwave again for 1 minute at 30% power
    - Pour gel, add comb
    - Wait 30 min to polymerize
- Put gel in electrophoresis cell, add TBE buffer without Gel Green Dye (enough to have the entire gel submerged)
    - Be sure to wipe any excess gel off the bottom of the cast
    - The side with the DNA should be facing the black electrode
- Prep DNA samples
    - 5x loading dye + DNA
        - 8µL DNA + 2µL Dye
- Load gel lanes with DNA, ladder
    - Lanes
        - 1 Megan & Micah Unknown A (10µL)
        - 2 -Empty-
        - 3 100 bp ladder (5 µL)
        - 4 Brooklynn & Maddie Unknown C (10 µL)
        - 5 -Empty-
        - 6 Valerine & Anna Unknown A (10 µL)
- Add more running buffer, run electrophoresis at 100 V for 20-30 min.
- Visualize gel

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

Smaller portions of DNA will go faster through the gel and thus move farther. Using a ladder, you can determine the size of the DNA you have run by comparing the bands. Typically, we will use a 100 bp ladder. If the gel is too concentrated (too high of a concentration of agarose), then

the bands won't separate as much since the DNA will be unable to move through as easily. On the other hand, if you use a gel that is not concentrated enough, then the DNA runs very far and separates much more. This can make it harder to actually get a result/tell the difference between the bands. The concentration should be adjusted based on the size of DNA you expect to see. For example, you could use 0.7% for DNA that's between 1kb-10kb, 1% for DNA that's between 300bp-1kb, and 1.5% for DNA that's between 50bp-300bp.

EtBr is a gel stain for DNA used to visualize the DNA. EtBr is an intercalating agent and gets between the DNA strands. Here, we will be using TBE with staining chemical already in it. For this class, we are using Gel Green rather than EtBr since EtBr can be dangerous to work with. Unlike EtBr, Gel Green cannot penetrate the lipid bilayer and this will not penetrate our own cells. Also, Gel Green allows us to visualize the bands without UV but rather 480 nm light (blue) which excites the chemical and makes it appear green in the gel.

Today, we made 1.2% gel or 1.2 g agarose/100 ml TBE.

DNA is mixed with 5x DNA loading dye (sugar) and this makes it more dense so that it is not lost in the buffer. We were given 8 µL DNA and therefore needed 2 µL of DNA loading dye to make the total volume 10 µL.

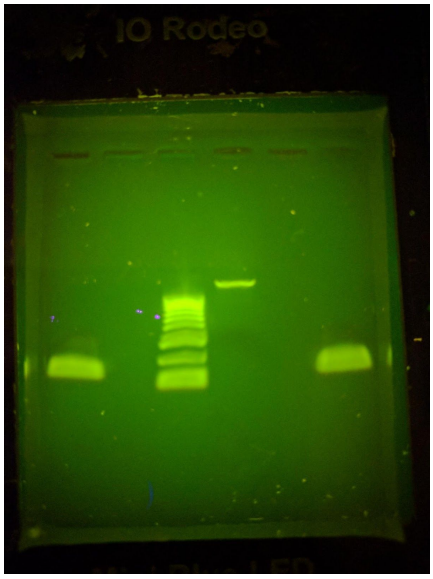


Figure 1. DNA samples run with the above layout scheme

Lab notebook
Megan Lytle, Micah Morris
BIO464W

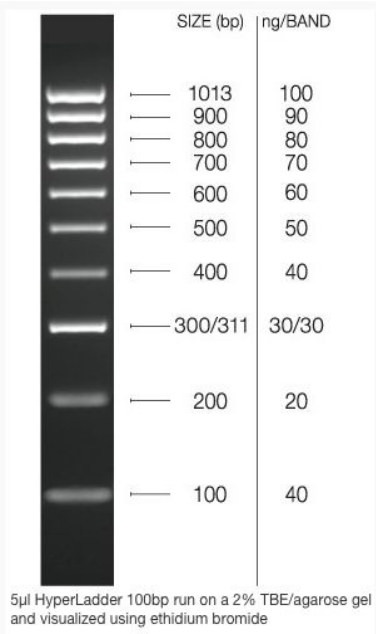


Figure 2. Ladder

Conclusion:
Upon visualization, unknown A appears to be around 150 bp DNA.

Next step:
The next steps are to be able to do this procedure with no help and then do it to our own samples.