Name: Jessica and Rebekah
Date: April 25th
Week#: 13

Objective: (In one or two sentences.)
Observe adults for any mutant morphology.

Procedures: (Bullet point the procedures.)

Notes:
(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:
Larva: (in transition to pupal stage)
Pupa:
Adult: 12
Total Deceased: 3

The results were rather inconclusive. Without clear images with which we could count the number of bristles, it is hard to quantify our results. It is possible that our RNAi was not very successful, or that LRRK takes effect before the stage at which we performed injections.

Next step:

Name:Jessica and Rebekah
Date: 4/20/2017
Week#:Week 12

Objective: Observe any differences in Tribolium and quantify life stages

Procedures:

Notes:
(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.

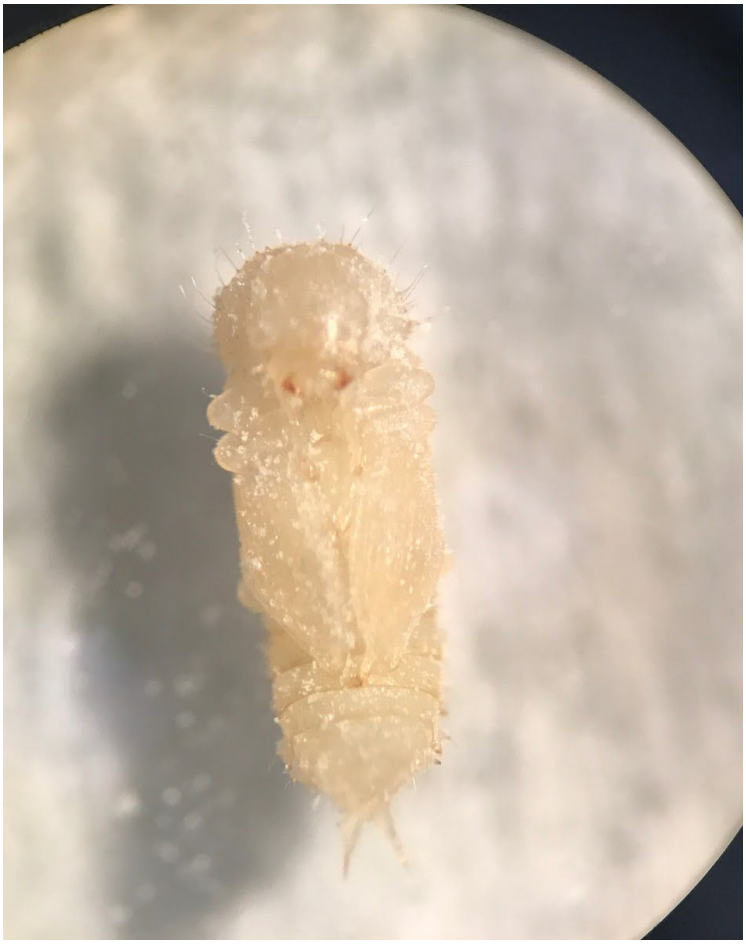


Figure 1. Image of pupae for documentation purposes (taken with iPhone).

Conclusion:

Larva: (in transition to pupal stage)
Pupa: 13
Total Deceased: 2

Next step:
Observe mutant beetles at adult stage.

Lab Notebook Template

Name:Jessica and Rebekah
Date: 4/18/2017
Week#: Week 12

Objective: To observe the injected larva and pupae

Procedures:
No difference observed

Notes:
Life Stages:

Larva: 3 (in transition to pupal stage)
Pupa: 11
Deceased: 1 black larva

Conclusion:

Next step:

Name: Jessica and Rebekah
Date: 4/6/2017
Week#: 10

Objective: Our objective today is to purify our dsRNA sample, removing any DNA or ssRNA.

Procedures:

1. **Nuclease Digestion to Remove DNA and ssRNA**
    a. Assemble RNase digestion reaction

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

    b. Incubate at 37 degrees C for 1 hr

2. **Purification of dsRNA**
    a. 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.

    b. Apply binding mix to the filter cartridge and draw it through
        i. Pipet the entire 500 microliter dsRNA binding mix onto the filter in the filter cartridge, and draw it through by centrifugation.
    c. Wash the Filter Cartridge with 2 X 500 microliter Wash Solution

i. Pipet 500 microliters of Wash Solution onto the filter in the Filter Cartridge. Draw the wash solution through the filter as in the previous step.
ii. Repeat with a second 500 microliters of Wash Solution
iii. After discarding the Wash Solution, continue centrifugation for about 10-30 seconds to remove the last traces of liquid.

d. Recover the dsRNA 2 X 60 microliters Elution Solution
    i. Mix 1 microliter of the eluted solution with 19 microliters H20 (for 20x dilution)
e. Quantitate using NanoDrop
    i. 62.4 ng/microliter x 20 = 1248 ng/microliter

Notes:

The DNase/RNase treatment digests template DNA and any ssRNA that did not anneal. dsRNA will not be degraded when using the specified reaction conditions. (ssRNA will be digested after 15 min, but it takes longer to completely digest the DNA template.)

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

The NanoDrop test gave us a concentration of 62.4 ng/microliter. However, other students didn't have usable concentrations, so everyone eluted once again.

Conclusion:

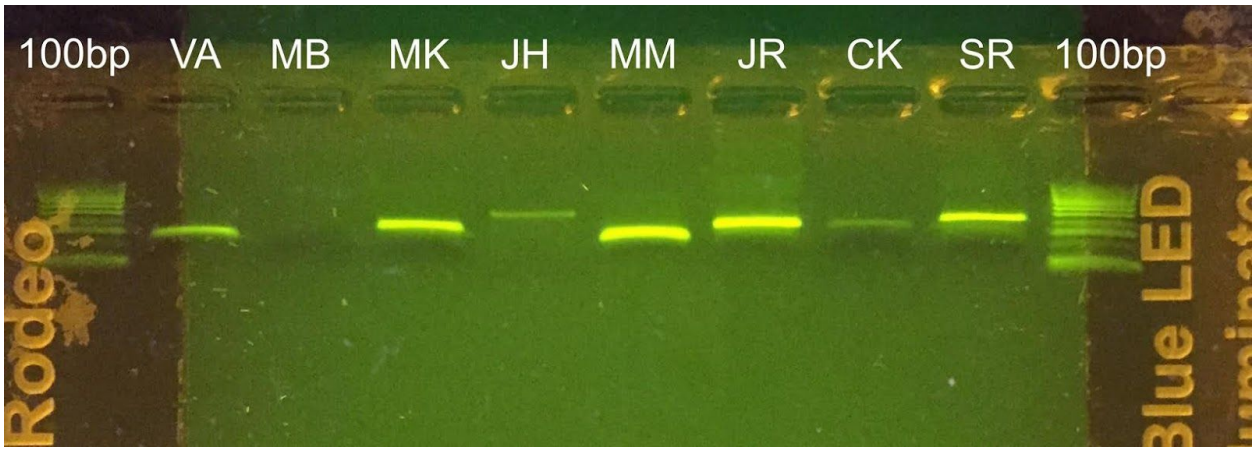


Figure 1. Purified dsRNA product after gel electrophoresis (aka it worked and we are awesome).

Next step: INJECT dsRNA INTO PUPAE :) :) :)

Name:Rebekah and Jessica
Date: 4/4/2017
Week#:10

Objective: Perform in vitro transcription and make double stranded RNAi

Procedures:

1. Mix components of transcription
    a. 8 microliters NTP mix
    b. 2 microliters 10x Buffer
    c. 2 microliters Enzyme mix
    d. 8 microliters template (1.33 micrograms) (1 to 1.5 micrograms) (Concentration was 166.27 nanograms/microliter)
    e. 0 microliters RNase Free H20
    f. Total 20 microliters

Notes:
Currently we have pure double stranded PCR (DNA) product with T7 promoter. We will use polymerase to produce double stranded RNA.

Conclusion:

Next step:
dsRNA purification.

Name: Rebekah and Jessica
Date: 3/30/17
Week#:9

Objective: Determine that our PCR was successful and purify PCR products.

Procedures:
Combine products from 4 PCR tubes of 25 microliters tubes to produce 1 tube with 100 microliters.

Make 1.5 % gel with 40 milliliters of water and 0.6 g of agarose
We anticipate our fragment should be 387 bp long

Run 2 microliters of PCR product on gel (in column 7).

Purify the 98 microliters of PCR products

1. Add 5 volumes (490 microliters) Buffer PB to 1 volume of PCR reaction and mix
2. If the mixture is orange or violet, add 10 microliters 3M Sodium acetate, pH 5.0 and mix (color will turn yellow)
3. Place mixture in a QIAquick column and onto a vacuum manifold and discard flow
4. Wash by adding 750 microliter Buffer PE to the column while in vacuum
5. Centrifuge 1 minute to remove residual buffer.
6. Place QIAquick column in a clean 1.5 ml microcentrifuge tube.
7. To elute DNA, add 30 microliters Buffer EB to the center of the QIAquick membrane and centrifuge the column for 1 minute.

Notes: (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:

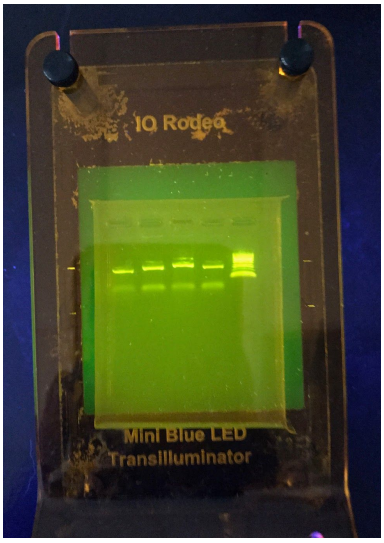


Figure 1. Gel electrophoresis of the PCR product from Tuesday.
Our sample is the 2nd from the left.

Our purified DNA gene segment with promoter was 166.27 nanograms/microliter.

Next step: Do transcription to make double stranded RNA.

Name: Jessica and Rebekah
Date: 3/28/17
Week#:9

Objective: We will check to make sure our sequence is correct. Will set up PCR to incorporate a promoter into our gene section of interest (no need for primers) so that we can do transcription and produce double stranded RNA. This PCR will read in the promoter.

Procedures: Procedure for PCR
46 microliter H2O
20 microliter 5x Buffer
8 microliter dNTP
15 microliter Primer (PCR4-TOPO)
10 microliter (of 10 nanogram per microliter) Plasmid
1 microliter Taq
100 microliter reaction

20 microliters* 10 nanograms/microliter /353.1 ng/microliter = 0.566 microliters

Notes:
Sanger sequencing: DNA obtained in single stranded form with primer DNTPs and ddntps and DNA polymerase. (sed T7 primer.) ddntps terminate strand so gel separates by length can determine the dntp at each strand.
Next Gen: uses different method from sanger. Can count which is coming next.
(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: Purification of PCR products.

Name: Jessica and Rebekah
Date: March 14, 2017
Week#: 8

Objective: (In one or two sentences.) Complete miniprep. The culture was spiked, then killed. Objective is to extract the plasmids (not the lipids, proteins, or genome) from the E.coli.

Procedures: (Bullet point the procedures.)

1. **Harvest E. coli**
    a. Centrifuge to pellet E. coli at 12 rpm for 2 minutes
    b. Remove LB
    c. Add P1 250 ml (suspended in buffer by vortex)
    d. Add P2 alkaline solution destroy membrane (but too long will destroy everything)
        i. Be very gentle, slowly tip back and forth a few times
        ii. 3min
    e. Add N3 350 uL which will neutralize it.
    f. Place on ice for 5 min
    g. Centrifuge (spin down) 12k RPM for 10 min

2. **Miniprep**
    a. Pipette 750 microliters of supernatant into column
    b. Vacuum out solution
        i. DNA will be retained in column
    c. Add 500 microliters PE
        i. Washes DNA to remove excess proteins, etc
    d. Vacuum
    e. Column to clean 1.5 microliter tube
    f. Add 30 microliters of EB
    g. Let sit 1 minute
    h. Spin 12 krpm 1 min

3. **Measure amount of DNA in solution**
    a. Take 2 microliters of solution
    b. Using Nanodrop that was calibrated on H2O, measure DNA quantity
    c. Results below. Usual results look like bell curve and have a peak of approx. 500 copies.
    d. Results showed peak of 61.6 ng/uL

Notes:
(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.

The main goal of today's miniprep procedure was to isolate our plasmid from the E. coli cells. Because we added PB in step 1, c. instead of P1, our DNA concentration was not nearly high enough. Most likely, Yoshi and David will repeat the miniprep procedure for us and we will continue on after spring break.

Conclusion:

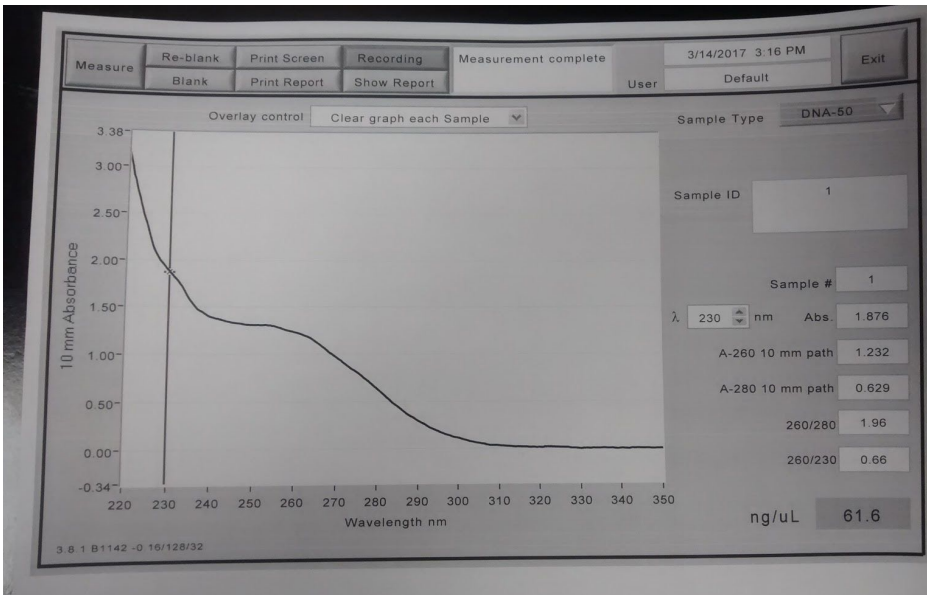


Figure 1. Results of NanoDrop machine. (Miniprep did not work since we used PB instead of P1.)

Next step:

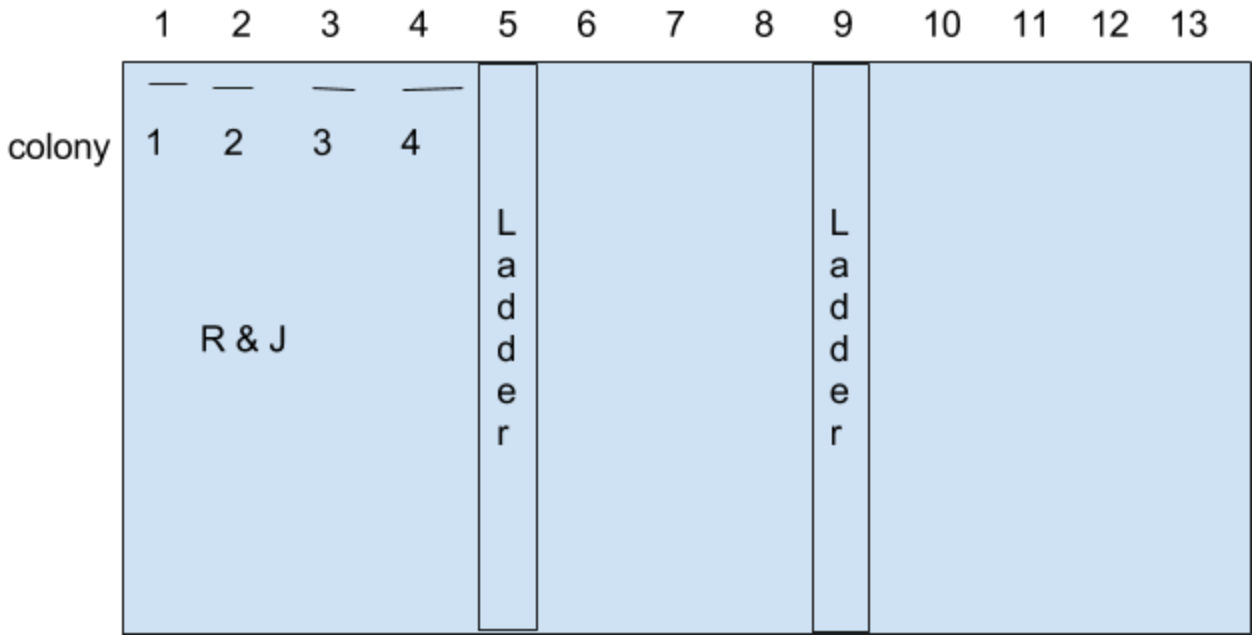
During the next class period we'll receive our sequencing results, and then set up PCR to incorporate a promoter into our gene of interest. (This will be necessary for in vitro transcription.)

Name: Jessica and Rebekah
Date: March 9th, 2017
Week#: 7

Objective: (In one or two sentences.)
Last class, we cultured four colonies and ran colony PCR. Today we will run gel electrophoresis to determine that at least one of the colonies was successfully transformed (plasmids contain our gene of interest).

Procedures: (Bullet point the procedures.)

- Gel electrophoresis was run
    - Gel made with 1.5% gel (1.125 g agar, 75 mL TBE)
    - 2 microliters of each of the 4 colonies were placed in one slot (each colony in a separate slot).
    - From colony 1-4 from left to right.



Notes:(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.

In order to determine if the E. coli cells were successfully transformed, we ran electrophoresis using a 1.5% gel. We used the second set of primers (F2 and R2) to amplify our gene fragment and transform the E. coli cells. Because of this information, we predict that the length of our PCR product should be about 347 bp.

Colony 4 was the colony that worked.

Conclusion:
Figure 1 shows the four colony PCR products that we ran through gel electrophoresis. Only one of our colonies worked well, but based on the ladder, we can be fairly certain that the fragment is around our predicted length of 347 bp.

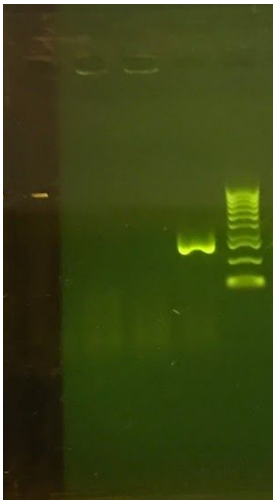
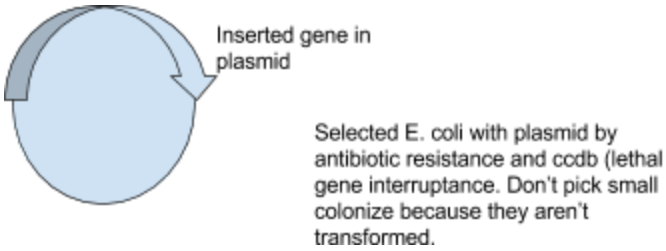


Figure 1. Gel electrophoresis result of colony PCR products.

Next step:
Our next step is to isolate our plasmids from the E. coli cells.

Name: Jessica and Rebekah
Date: March 7th, 2017
Week#: 7

Objective: We are currently in the process of making double stranded RNA. This week, we want to ensure that we have E. coli colonies with the correct gene sequence. We will perform colony PCR and gel electrophoresis today to do so.



Procedures: (Bullet point the procedures.)
Perform PCR with the colony and run gel electrophoresis.
Use our second primer set.

1. **Culture**
    a. Set up PCR, culture 1 hour (2:41 pm -), complete PCR
        i. Transfer 100 microL of LB into 1.5 ml tube in order to minimize contamination of source LB
        ii. 15 microliters to each of the 4 tubes--LB+Ampr
        iii. Open colony plate upside down
        iv. Use pipette tip to touch colony on plate, no need to scoop
        v. Gently tap the colonies off the tip into the tube of LB
        vi. Incubate at 37 degrees C for an hour
2. **Colony PCR**
    a. 5x Master Reaction Mixture
        i. H20…….41 microliters
        ii. 5x PCR Buffer……...20 microliters
        iii. dNTP mix (2.5 mM stock).....8 microliters
        iv. Primer 1 F.....10 microliters
        v. Primer 2 F.....10 microliters
        vi. Taq polymerase (5U/ microliter).....1 microliters (2.5U/100 microliters)

    b. Reaction specific
        i. H20…….8.2 microliters
        ii. 5x PCR Buffer……..4 microliters
        iii. dNTP mix (2.5 mM stock).....1.6 microliters
        iv. Primer 2 F.....2 microliters

v. Primer 2 F.....2 microliters
vi. Taq polymerase (5U/ microliter).....0.2 microliters (2.5U/100 microliters)
vii. Total 18 micrometers of Mastermix
viii. Culture……..2 microliters

Notes: (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.

After culturing our E. coli colonies using the above procedure, we created a master reaction mix in preparation for PCR.

Conclusion:
To be determined on Thursday.

Next step:
Our next step is to perform gel electrophoresis to confirm that the right gene was cloned into the plasmids.

Name: Jessica and Rebekah

Date: March 2nd, 2017
Week#: 6

Objective: (In one or two sentences.)
Transform E. coli with plasmid containing our GOI.

Procedures: (Bullet point the procedures.)
Overall Plan

1. **Make Gels (150 ml)**
    a. 1.5% gel for 200-300 bp primers
2. **Run Gels (20 microliters)**
3. **Cut Out DNA from Gel**
4. **Purify DNA**
5. **"Clone" the DNA Fragment into Plasmid**
    a. Because our DNA is currently linear, we will lose it over time. In order to preserve the fragment, we must insert it into a plasmid (E. coli) in order to amplify for future use. We will use E. coli plasmid to make double stranded RNA.
    b. Plasmid → put in E. coli → culture → mini prep plasmid → use this as template → PCR to make DNA fragment with T7 on both ends
    c. Methods:
        i. Plasmid and DNA fragment must be digested in order to insert DNA fragment
            1. Classic Way (restriction enzyme and ligase)
            2. New Way (TA cloning, plasmid with topoisomerase already conjugated
        ii. TA Cloning
            1. T placed at each 5' cut of plasmid, A placed at each 3' end of DNA
6. **Transform E. coli with the Plasmid**
    a. Plasmid has gene for antibiotic resistance
    b. How do we differentiate normal E. coli plasmid, E. coli plasmid with antibiotic resistance but no GOI, and E. coli plasmid with antibiotic resistance and GOI?
        i. ccdB gene (which kills E. coli) is placed on either side of where GOI insertion is supposed to take place (lacZ is also included)
        ii. Any plasmid that did not undergo GOI insertion will survive the antibiotic resistance, but not the intact ccdB gene

Notes:
(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.

1. **Make Gels**
    a. To begin, Clayton and I made a 1.5% gel for electrophoresis.
2. **Run Gels**

a. Our DNA was already mixed with a loading dye, so we were able to put our PCR product directly into the agarose gel. 20 microliters of each reaction mixture (1 and 2) were placed in wells, as was 5 microliters of a 100 bp ladder. The gel was run for 30 minutes at 100 V.

3. **Cut Out DNA From Gel**
    a. Our longer DNA segment (2) was removed from the gel and placed in a tube with filter.
4. **Purify DNA**
    a. After 5 minutes in the freezer ("Freeze") the tube containing gel/DNA was centrifuged ("Squeeze")
5. **Clone the DNA Fragment into Plasmid (Topo Reaction) and...**
6. **Transform E. coli with Plasmid**
    a. Components
        i. PCR product (4 microliters)
        ii. Salt (1 microliter)
        iii. Topo plasmid (1 microliter)
    b. Intubate for 15 min at room temp
    c. Transfer 3 microliters of reaction mixture into E. coli cells with pipette
    d. Heat shock at 42 degrees C for 30 sec
    e. Rest at on ice for 5 min
    f. Transfer Add 250 microliters SOC (super optimal culture)
    g. Shake for 40 min at 37 degrees C
    h. Pipiette 120 microliters of E. coli cells onto LB Agar plate and spread
    i. Incubate overnight

Conclusion:

Figure 1 shows the end product of gel electrophoresis run with the PCR products of both reaction mixtures from Tuesday's class. The reaction mixture with our first set of primers is in the first well from the left, and the reaction mixture with our second set of primers is in the second well from the left. The third well contains the 100 bp ladder.

Figure 1. Gel electrophoresis results using products from Tuesday's PCR.

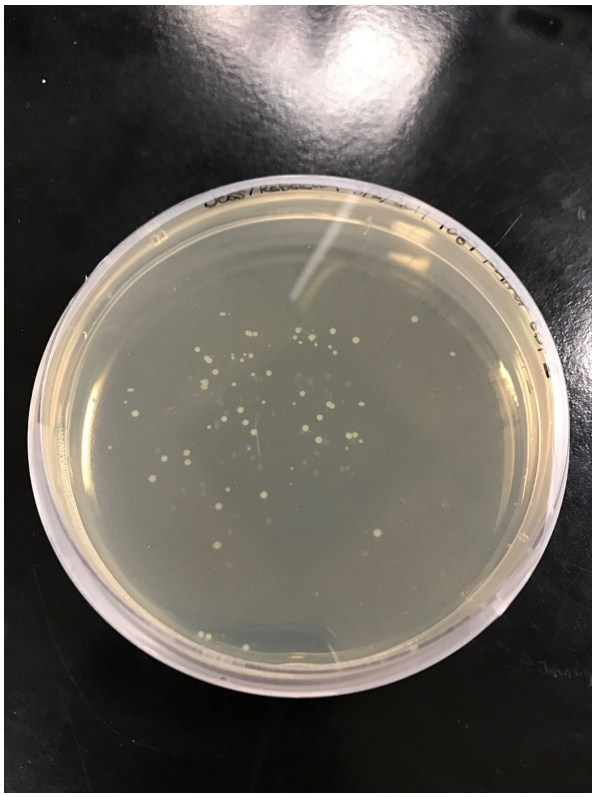


Figure 2. E. coli colonies.

Next step:
Pick up E. coli colonies and perform colony PCR.

Name: Jessica and Rebekah
Date: February 28th, 2017
Week#: 6

Objective: (In one or two sentences)
Amplification of gene through PCR.

Procedures: (Bullet point the procedures)

1. **Primer Dilutions**
   Primer was obtained dry. Must add water to adjust concentration: Primers diluted to 100 micromolar, then 10 micromolar.
   1 molar = 1mole/1liter (of fluid or of water)
   Samples were vortexed after each dilution.

2. **Polymerase Chain Reaction**
   a. Materials:
      Taq DNA Polymerase (DO NOT VORTEX)
      Forward and Reverse Primers
      dNTP mix
      10x Buffer, MgCl2
      Template (Options: GOI, cDNA, Genomic DNA)
         Genomic DNA does not work if primer crosses an intron
         cDNA can work across introns and includes isoforms due to mRNA obtained from larva, pupae, and adults.
      Water (to adjust concentration)
   b. Methods:
      Mix every component conserved between two reactions.
      Split mixture, and add primers accordingly.

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.

1. **Primer Dilutions**
   Our primers were obtained dry, and needed to be diluted in order to perform PCR. We first prepared primer samples with a concentration of 100 micromolar. To do so, we calculated how much water each sample needed, and that volume was added to each primer container using a micropipetter. An example of the first dilution calculations and how much water was added to each primer container can be found below (Table 1). Next, we created a working solution with a concentration of 10 micromolar. To do so, 10 microliters of our primer samples were combined with 90 microliters of water. Samples were vortexed after each dilution to ensure homogeneity of our solutions.

First Dilution Calculation, Example: 156199024 R1

(24.7 nmol)(1 micromol / 1.0x10^3 nmol) = 0.0247 micromol

(0.0247 microliters) / (X L) = (100 micoliters) / (1 L)

X = 247 micoliters H20

Table 1: Primary Calculations

Sample #	nmol	microliters (of H20)
156199024 R1	24.7	247
156199025 F2	30.8	308
156199026 R2	28.1	281
156199023 F1	23.2	232

2. Polymerase Chain Reaction

To set up the reaction, we combined all components that were used in both reactions (i.e. the primers), and all volumes can be found under “Reaction”. The solution was vortexed briefly (prior to the addition of the enzyme). This gave us a total volume of 92 microliters, which we divided in half (46 microliters) and placed in separate 1.5 mL tubes. 2 microliters of F1 and 2 microliters of R1 were added to the first reaction tube, and the same was done for F2 and R2 in the second reaction tube. After mixing, both reaction mixtures were transferred to small, PCR tubes (named with initials and “1” or “2”).

a. Reaction

H20…….59.5 microliters

5x PCR Buffer……...20 microliters

dNTP mix (2.5 mM stock).....8 microliters

Primer 1 (10 microM stock).....4 microliters

Primer 2 (10 microM stock).....4 microliters

Sample DNA ( ).....4 microliters

Taq polymerase (5U/ microliter).....0.5 microliters (2.5U/100 microliters)

Total……...100 microliters (50 microliters in each PCR tube)

b. Reaction Cycle

i. Denature at 95 degrees C for 5 min

ii. Denature at 94 degrees C for 30 sec
iii. Anneal at 57 degrees C (for now) for 30 sec
iv. Extend at 72 degrees C for 40 sec* (Note: Determined using rate of 1kb/min)
Repeat for 35 times
v. Extend at 72 degrees C for 5 min

vi. Hold at 4 degrees C

Conclusion:
During today's class we created stock solutions and performed dilutions of our primers. Additionally, we set up the polymerase chain reaction. Figure 1 depicts the gel electrophoresis results of our PCR.

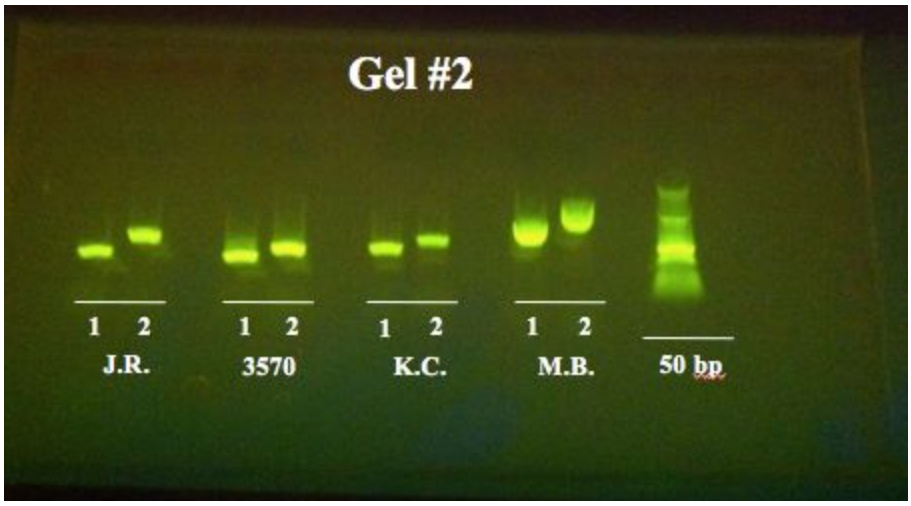


Figure 1. Gel electrophoresis results of PCR products, obtained 3/1/2017.

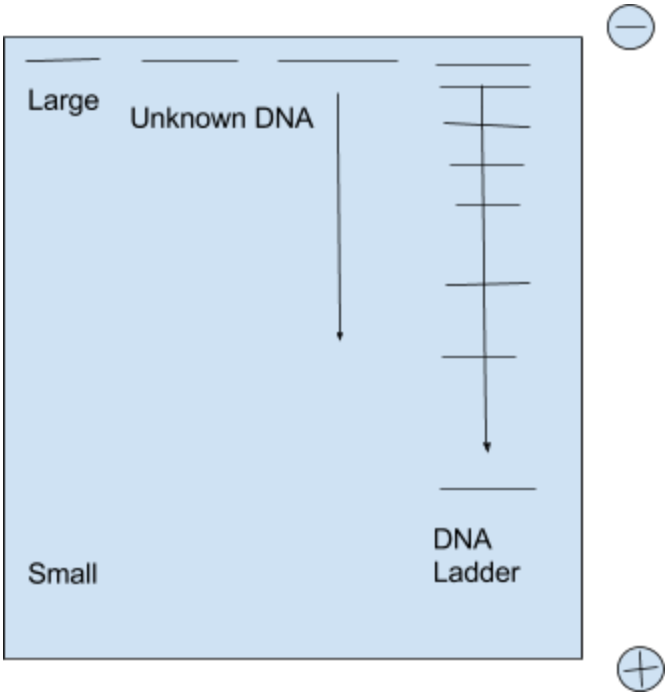
Next step:
Evaluate success of PCR via gel electrophoresis.

Name: Rebekah and Jessica
Date: 23.2.2017
Week#: 5

Objective: (in one or two sentences)
Prepare agarose gel and run two unknown DNA samples to determine length (bp). Observe injection of larvae for RNAi.

Procedures: (bullet point the procedures)

1. Overall Gel Procedure:
   Mix TBE (buffer)
   Agarose (Concentration: 1% weight/volume)
   Note: Concentration can deviate by length of DNA. The longer the DNA, the harder it is for it to travel through the gel. If gel is "too tight", i.e., high concentration of agarose, even small base pair lengths won't travel through.



   (Example gel above.)
   Mix TBE and Agarose (1g Agarose/100ml TBE)
   100 bp DNA Ladder (We can compare the ladder and different lengths to estimate the length of DNA.)
   Unknown DNA
   Example Concentrations for Various DNA Sizes:

1 kb-10 kb 0.7%
300 bp-1kb 1%
50 bp-300 bp 1.5%

We will attempt 1.2% gel.
Red is plus; black is minus.

2. Prepare Gel

As a class, make 4 gels. Each gel has 6 slots. Two groups will share a gel and put a ladder in the middle slots.
1.2 g + 100 mL of TBE for 1.2% gel.Mix and then microwave
TBE + EtBr (DNA staining chemical, UV light detection). DO NOT TOUCH, INDUCES MUTATIONS/APOPTOSIS.
Now use Gel Green, 1000x safer. Still a DNA intercultural but may not be able to go through lipid bilayer. Don't need to use UV light. STILL USE GLOVES.
480 nm, blue light, excites Gel Green. Excites to green or yellow.

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.

To Prepare 1.2% Gel:

1.27 g agarose were mixed with 100 mL Gel Green/TBE in an Erlenmeyer flask.
Using a beaker as a lid, mixture was microwaved at 30% power for two minutes.
Remove from microwave with gloves, swirl vigorously to mix.
Place back in microwave and cook for one more minute at 30% power.
Any air bubbles were removed.
Once the solution had an equal consistency, it was poured into the mold (about ⅔ of way to the top).
(Any remaining air bubbles were popped or moved to the side with a pipette tip.)
Comb was added to produce slots.
Remaining solution in Erlenmeyer flask was diluted with water to prevent solidification, poured out, and the flask was rinsed with double distilled water. .

To Run Gel:

Filled electrophoresis box with TBE (enough to submerge the gel).
Connected red and black power cords.
Tested power source, 100 V; bubbles should appear on one side.
Comb was removed from the gel using a little DI water so not too damage slots.
(Slots should be directed towards black side of gel, and then placed in the TBE. TBE should cover gel completely, but beware of too much excess buffer.)

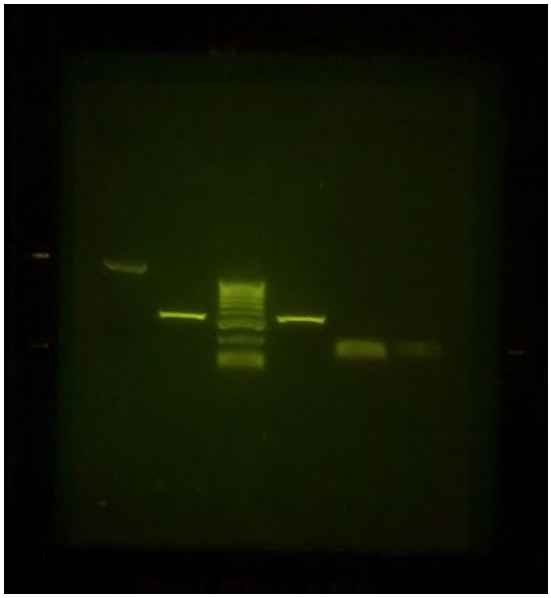
Need to mix DNA with 5x loading dye (heavy sugar) and ladder.
For example, 4 microliter DNA + 1 microliter dye (5x) = 5 microliter solution.

Sample 1:
8 microliter of DNA (Sample C) + 2 microliter of 5x dye
Sample 2:
5 microliter of 100 bp ladder

Sample Order From Left (10 microliter for each sample, 5 microliter for ladder):
Slot 1: Our sample, C
Slot 2: B
Slot 3: Ladder
Slot 4: B
Slot 5: A
Slot 6: A

Due to the gel box not working correctly, we transferred the gel to a new box shortly after starting.
Gel was removed from solution and placed on a paper towel. Gel ONLY was placed on Mini Blue LED box to visualize.

Conclusion:



Sample C is in the first slot. It is greater than 1 kb, because it didn't travel as far as the 1 kb ladder.

Next step:
Perform polymerase chain reaction during Tuesday's class period..