Name: Kailee Tateman & Clayton Deible
Date: 2-23-17
Week#: 5R
Objective: In this lab we will be learning more about the procedural steps in gel electrophoresis. In addition, we will be applying this knowledge in order to make our own gel!
Procedures: (bullet point the procedures)

Make Gel Solution

- Combine in Erlenmeyer flask
  - 100mL TBE
  - 1.2 g Agarose
- Mix by flask
- Use beaker as lid
- Place in microwave
  - Power level 30 for 2 minutes
- Remove flask with heat resistant gloves
- Shake flask
- Recover with beaker (as lid)
- Place in microwave again (to remove air bubbles)
  - 1 minute
    - If too much boiling occurs stop
- Remove from microwave with heat resistant gloves
  - Ensure that concentration is uniform (no other densities present)

Transferring Gel to Electrophoresis Container

- Pour solution into gel container and cover about ⅔
- Place DNA separators*
- Let sit for about
- 30 minutes
- Immediately clean flask and beaker
  - Use distilled water

Set Up Electrode

- Fill gel container with TBE
  - Enough to submerge gel
- Connect Red (+) wire
- Connect Black (-) wire
- Turn on electrode
  - Look for bubbles

Putting gel in electrophoresis electrode:

- Use a little distilled water to gently remove dividers from tray
- Remove gel tray and place on a paper towel
  - If additional gel is left in container clean up immediately with paper towel
- Place gel in electrophoresis
  - Put DNA end towards black wire
- Dilute 5x loading dye
  - For example: 4 microliters of DNA means 1 microliters of dye
  - 8 microliters of DNA means 2 microliters of dye
  - Mix by using spinner
- Obtain 5 microliters of 100 bp ladder

Loading the DNA (from left to right)

Slot 1: C
Slot 2: B
Slot 3: ladder
Slot 4: B
Slot 5: A (ours)
Slot 6: A

Turn on power to 200V

Note:

- Gel made of
  - TBE
  - Agarose
    - Higher the concentration of agarose the smaller the spaces in the mesh
      - Even smaller particles of DNA will not be able to go through
      - Beneficial when determining difference between shorter stranded DNA (better resolution)
    - Optimized concentration desired
- Concentration
  - 1.0% w/v
  - 1 g/100 mL TBE
- Staining (in order to visually see DNA)
  - EtBr
    - Put under UV lamp to view
    - Problem: it can go into DNA by penetrating the lipid bilayer and further infiltrate the cell and allow for mutation
  - Gel Green
    - “10000 times safer” - Yoshi
    - Instead of UV use a different wavelength of light
      - 480 nm wavelength → blue
        - Use blue light to see green things
- Running gel
  - Rows
    - 100bp
    - 200bp
    - 300bp
    - 100bp ladder
    - DNA
  - Negatively charged at top
  - Positively charged at bottom
- DNA we have is between 300bp-500bp
  - Trying 1.2% gel
- To stain DNA, wait until after electrophoresis as EtBr will slow down DNA
  - Emits after contact with UV
  - Could also use Gel Green which is 10000x safer
    - Gel Green uses 480 nm instead of UV light which is blue which will cause Gel Green to emit 510 nm light which is green/yellow ish color

Name: Kailee Tateman & Clayton Deible
Date: 2-28-17
Week#: 6T
Objective: In this lab we will be reviewing the Central Dogma and cDNA synthesis. In addition, we will be learning and performing the procedure of PCR for our gene of interest.
Procedures:
Preparing Primer:

- Dilute to 100 µM
    - TC002498_F1
        - 0.20 mg primer (31.9 nmol) + 319 µL double distilled water
    - TC002498_F2
        - 0.17 mg primer (28.7 nmol) + 287 µL double distilled water
    - TC002498_R1
        - 0.24 mg primer (39.6 nmol) + 396 µL double distilled water
    - TC002498_R2
        - 0.19 mg primer (30.6 nmol) + 306 µL double distilled water
- Vortex
- Make 100 µL of 10 µM primer
    - TC002498_F1
        - 10 µL of primer solution + 90 µL double distilled water
    - TC002498_F2
        - 10 µL of primer solution + 90 µL double distilled water
    - TC002498_R1
        - 10 µL of primer solution + 90 µL double distilled water
    - TC002498_R2
        - 10 µL of primer solution + 90 µL double distilled water
- Vortex
- Combine materials as shown in Table 1 in notes section without the primers
    - Total 92 µL
- Split solution in half
    - 46 µL
- Add primers to each
- Vortex
- Transfer those solutions to PCR tubes

Note:

Review of PCR:

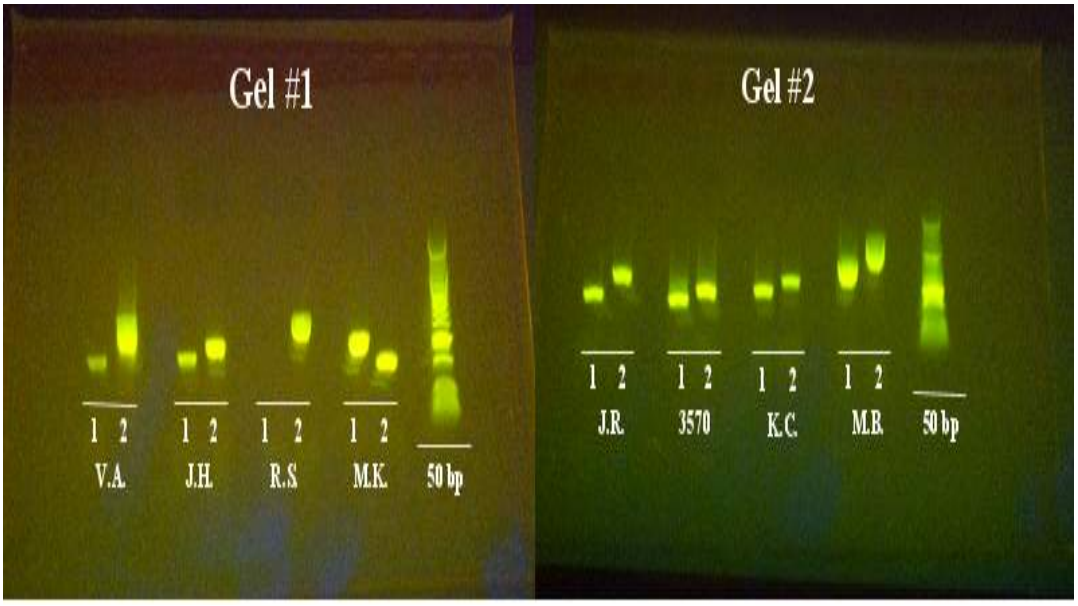
- Needs:
    - Taq polymerase
    - Primers (forward and reverse)
    - dNTP mix (nucleotides)
    - 10x Buffer
        - MgCl2 (to control specificity)
    - Template
        - Gene of interest
        - cDNA (more concentrated than genomic DNA
        - Genomic DNA
    - H2O (to adjust concentration)
    -

H_2O	59.5 µL
5 X PCR buffer	20 µL
dNTP mix (2.5 mM stock)	8 µL
Primer 1 (10 µM stock)	4 µL (add later)
Primer 2 (10 µM stock)	4 µL (add later)
Sample DNA	4 µL
Taq Polymerase (5U/µL)	0.5 µL
Total	100 µL

Table 1. Materials and their amounts.

When performing the PCR:

- At 95 degrees for 5 minutes
- At 94 degrees for 30 seconds
- At 57 degrees for 30 seconds
- At 72 degrees for 40 seconds
- 35 cycles
- At 72 degrees for 5 minutes
- At 4 degrees



Our bands are K.C. 1 and 2. Our K.C. 2 has a longer amplicon length so that is the primer set that we will use later on. (approx. 350 ish)

Conclusion:
By following the steps mapped out above we can successfully perform PCR on our own!

Next step:

Perform steps of PCR next class!

Name: Kailee Tateman & Clayton Deible
Date: March 2nd
Week#: 6R
Objective: (in one or two sentences)
Today, the main goal is to purify our selected DNA, and then transform it into E. coli
Procedures: (bullet point the procedures)

- Make gel (150ml at 1.5%) (used 2.23g of agarose)
    - 1.5% because the lengths were shorter
- Run gel
    - Use 5 µL of 100bp Ladder and 20 µL of
    - Run for 30 minutes at 100V
    - 100bp ladder is in middle slot
    - KC 1 is in slot 4 and KC 2 is in slot 5
- Cut out DNA from gel & purifying it
    - We cut out the band with the longest DNA (Seen on Figure 1)
    - We then popped the DNA into the squeeze and freeze and put it in freezer for 5 minutes
    - Put the frozen product into a centrifuge for 3 minutes at 14,000 rpm
- Clone the DNA fragment into plasmid & transform E. coli with the plasmid
    - Use the following for Topo rxn mixture:
        - PCR product - 4 µL (after put through centrifuge)
        - Salt solution - 1 µL
        - Topo plasmid - 1 µL
    - Steps to do Topo rxn:
        - Let mixture rest at room temperature
        - Put 3 µL of mixture into E coli cell
        - Put E. coli mixture in 42 degree Celsius environment for 30 seconds
        - Then put E. coli mixture on ice for 5 minutes
        - Add 250µL SOC mixture into E. coli mixture
        - Put E.coli mixture on a shake plate for 40 minutes and incubate at 37 degrees
    - Plating the E. coli
        - Put 120µL of E. coli mixture on a plate and spread it around Luria Broth Agar and let incubate

Note:
Summary of what we did and why:

1. Make gels (150ml of 1.5%) (used 2.25g of agarose)
2. Run gels (20 µL)
    a. Use the primer pair with the longest Amplicon length
    b. We will be using Primer pair 2 (F2, R2)
3. Cut out DNA from gel
4. Purify DNA
5. Clone the DNA fragment into plasmid
    . Done to protect the DNA fragment
    a. Need an “open plasmid” and then perform TA cloning (have As at ends of DNA and Ts on open ends of plasmids) (there is more info on TA cloning in BIO 464 documents on google drive)

b. Need ligase to combine DNA and Plasmid but today we are using topoisomerase which is already conjugated on the plasmid that we are using

c. Just combine the DNA and plasmid

6. Transform E. coli with the plasmid

. To transform the E. coli, we use chemicals and heat shock to damage the membrane of E. coli and then put the plasmids into the E. coli

i. We incubate the newly transform E. coli on SOC so they can recover in great conditions

a. A problem with this is that sometimes you can get a plasmid without your gene of interest

b. The way to avoid this is to use a plasmid with a resistance gene (ours that we are using have ampicillin and Kanamycin resistance)

c. After transformation, you need to culture the E. coli on a plate with ampicillin and kanamycin

d. Another problem is that some plasmids will anneal themselves without your gene so we need to distinguish between E. coli with plasmids with our gene and without our gene

e. E. coli without the gene will turn blue with xgal because of Lacz gene. The E. coli with our gene will not be blue because the Lacz gene will be disrupted by our gene that we added.

. Another gene that the plasmids have is ccdB which will kill the E. coli if intact. So the E. coli that is alive on the plate all have our Gene of Interest because our gene disrupts the ccdB gene. (this is the type of plasmids that we are using)

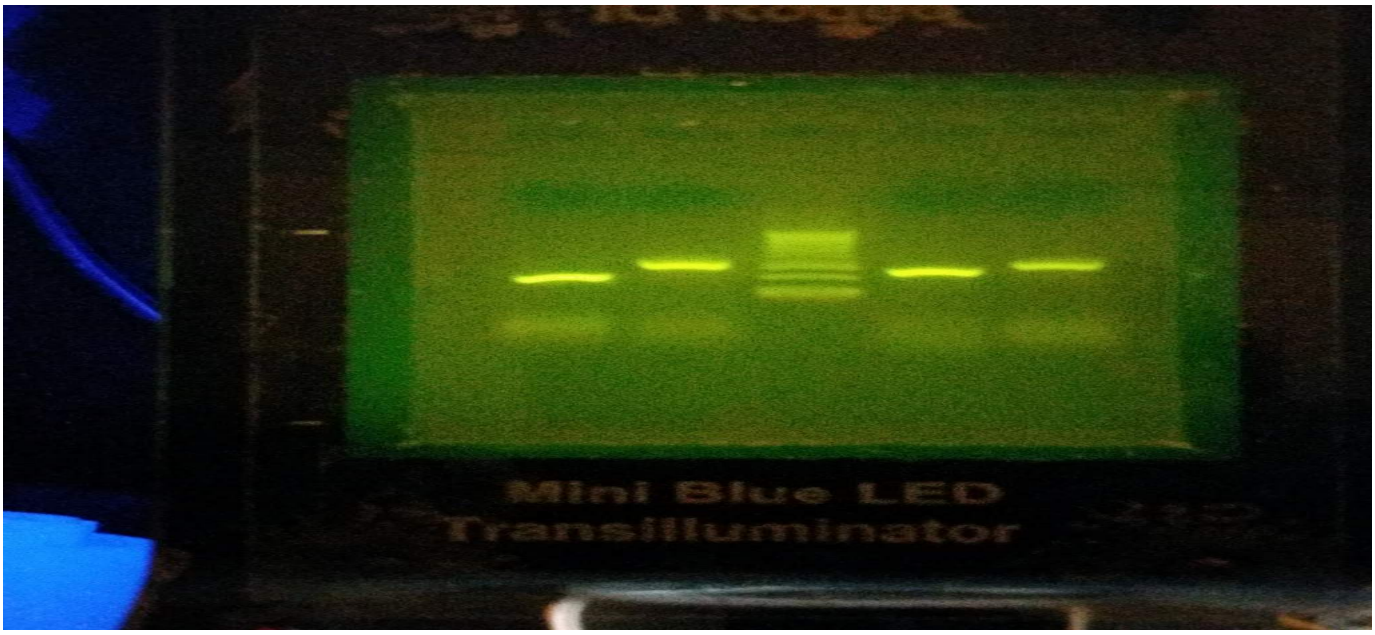


Figure 1: The 100bp ladder is in slot 3 (from left). The first 2 slots are from another group. K.C. 1 is in slot 4 and K.C. 2 is in slot 5. Since K.C. 2 is longer, we cut out the DNA from the band from slot 5.

Conclusion:
We performed these steps in order to set the DNA up to ready it to create double stranded RNA.

Next step:
The point of these processes that we did today is to set up the DNA to further transform it into double stranded RNA. In order to keep the linear DNA PCR products safe, we need to put the DNA into a plasmid and then transfer the plasmid into the E. Coli. We would then culture the E. coli, mini prep the plasmid, use the product of the mini prep as a template for PCR to make DNA fragments with T7 on both ends. This PCR product can then be made into double stranded RNA with Taq RNA polymerase.

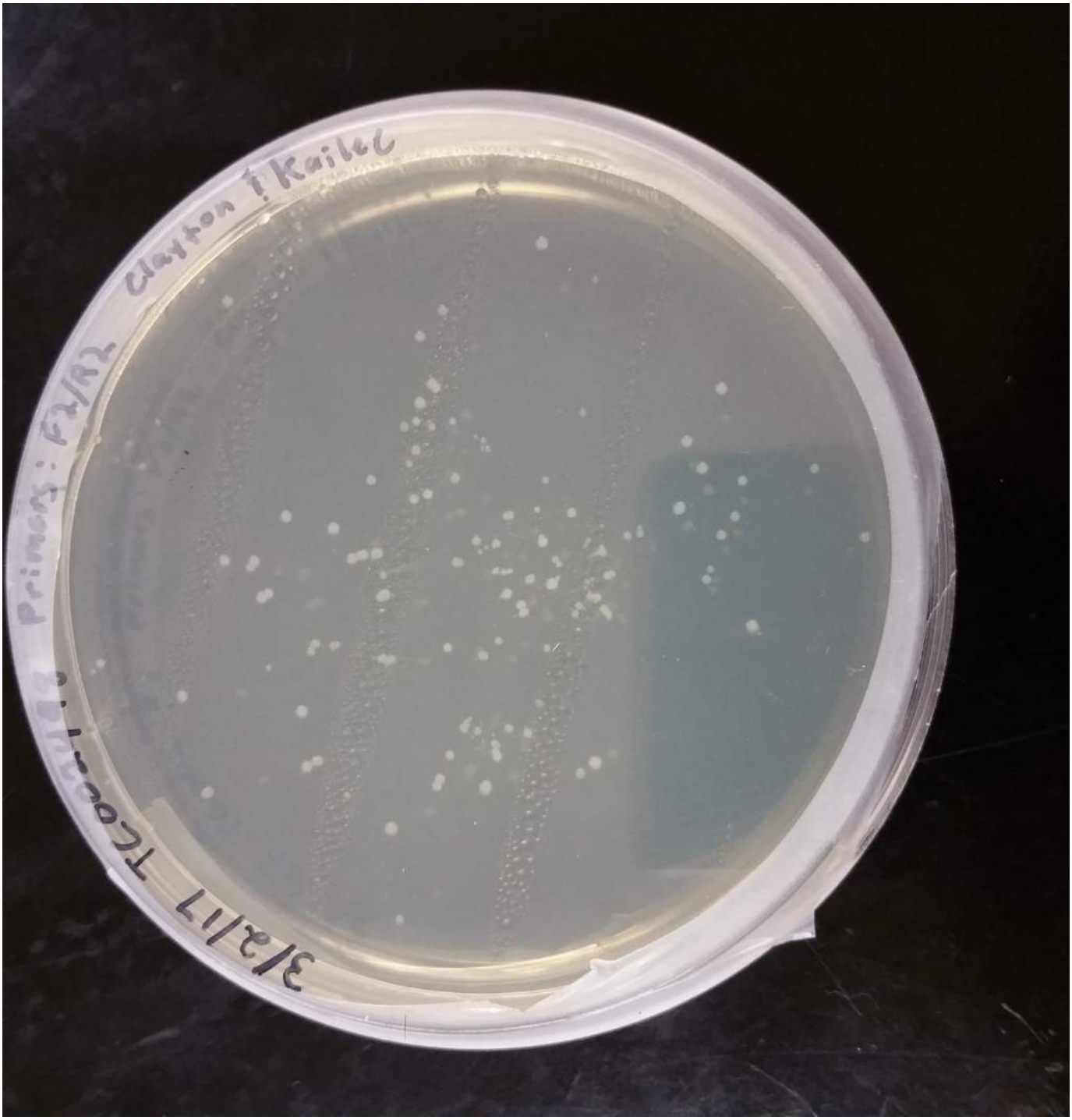
Name: Clayton Deibe & Kailee Tateman
Date: 3/7/17
Week#: 7T
Objective: In this lab we will be collecting our colonies from the last class, ensuring we have the colony with our gene, and performing colony PCR.
Procedures:
Selecting colony

- Add 15 microliters of LB + agar to 4 PCR tubes
- Using a medium sized pipette tip poke a large colony
- Insert the pipette tip into one of the 4 PCR tubes and stir around
- Repeat 3 more times with a different colony
- Incubate tubes at 37 degrees C for 1 hour

Preparing PCR

- Add the following:
    - 8.2 microliters of H20
    - 4 microliters of 5x green buffer
    - 1.6 microliters of dNTP mix
    - 2 microliters of primer 1 F
    - 2 microliters of primer 2 R
    - 0.2 microliters of taq
    - Total of 20 microliters
- Multiply these by 5 and then split up into 4
- Add in 2 microliters of culture into the PCR mix for the template

Note:



Colony PCR:

- Use this in order to determine that the E. Coli we chose does indeed have our gene in it
- Use E. Coli cells we choose as the DNA template

Conclusion:

We now have the colonies that contain our gene of interest. Since these colonies are alive, the ccdB gene was not active and did not kill the cell. So we are now ready to move further to create dsRNA

Next step:
Use the colonies on the plate and run a colony PCR. The products of this PCR will then be put through a gel to make sure the colonies we chose have our gene of interest

Name: Kailee Tateman & Clayton Deible
Date: 3/9/17
Week#: 7R
Objective: Run a gel to determine with colony has our gene in their plasmid

Procedures:

- Created gel
  - 1.5% with 75 ml of TBE
  - Used 1.125 g of agar
- Run gel with 2 microliters of each colony PCR product (slots 1-4)
  - Run with 100 bp ladder (slot 5)

Note:

Questions:

1. What are the primers you used?

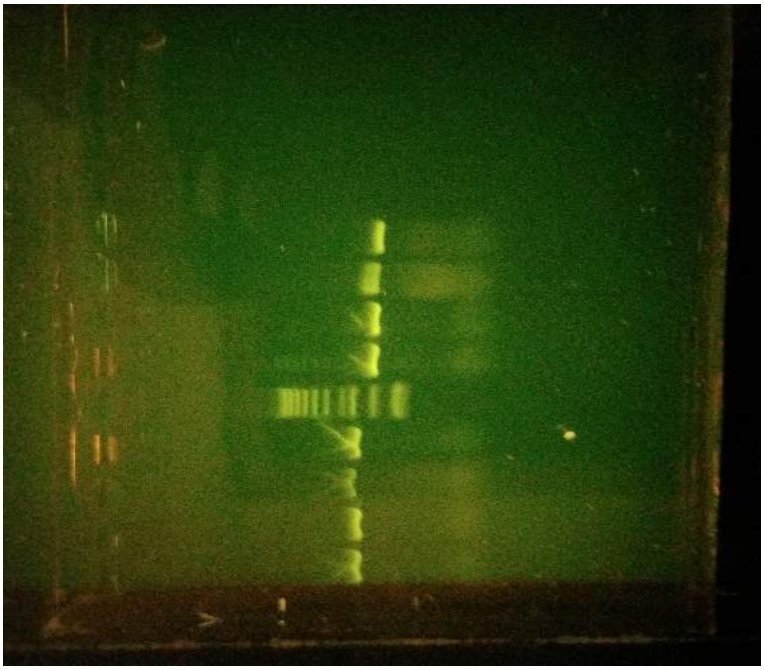
a. We used primers F2 and R2

2. What is the length of your PCR amplified fragment?

. Prediction: 326 bp

a. Actual: 300 bp

Conclusion:



Looking at this PCR, we can see that all 4 products are around the 300 mark on the 100bp ladder. Because of this, we can assume that all of these products represent our 326 bp primer in the *E. Coli*'s plasmid. However, we will be using the 1st colony (slot 1).
Next step: Next week we will be culturing our selected colony of E Coli. From here we will harvest the plasmid from the culture.

Name: Clayton Deible & Kailee Tateman
Date: 3/14/17
Week#: 8T
Objective: Harvest the plasmid with our Gene of Interest out of E. coli and purify it using a technique called “Mini Prep”.
Procedures:

- Pellet E. coli in liquid culture
  - Add 2 ml of culture in a 2ml tube
  - Spin down the 2ml in a centrifuge at 12k rpm for 2 mins
  - Repeat with another 2 ml of culture (same tube orientation)
- Add P1 (or PB)
  - 250 microliters
  - Vortex until pellet dissolves
- Add P2
  - 250 microliters
  - Wait three minutes
- Add N3
  - 350 microliters
  - Shake well to neutralize pH
- Put on Ice
  - 5 minutes (ish)
- Spin down again
  - 12k rpm for 10 minutes
- Obtaining the plasmids
  - Take 750 microliters of solution and put in column (DNA will bind)
- Vacuum out the liquid solution while DNA binds
- Add PE
  - 750 microliters
- Vacuum again
  - Then transfer to clean 1.5 ml tube
- Add EB
  - 30 microliters
  - Let sit 1 minute
- Spin down solution
  - Spin at 12k rpm for 1 minute
- Use Nanodrop to check concentration of product
  - Obtained 68 ng/microliter

Note:
Mini Prep procedure - Allows you to purify just the plasmid and not any of the E. coli genome, lipids, proteins, ect.

1. Pellet E. coli (spin at 12 rpm for 2 mins)
2. Remove LB
3. Add P1 250 microliters
4. Add P2 250 microliters GENTLY for 3 minutes (if left any longer, will kill everything)
5. Add N3 350 microliters (neutralize pH)
6. On ice for 5 minutes
7. Spin down at 12k rpm for 10 minutes

8. Add 750 microliters supernatant to column
9. Vacuum
10. Add 500 microliters of PE
11. Vacuum

Conclusion:

Next step: Come back from Spring Break and learn about DNA sequencing and template synthesis!

Name: Clayton Deible & Kailee Tateman
Date: 3/28/17
Week#: 10T
Objective: Today we will be analyzing our sequence of DNA that was put into the plasmid and relating that to our predicted sequence from when we developed our primers.

Procedures:
Sequence Analysis:

- Obtained our sequence of the plasmid from 4peaks
- Compared the sequence from the plasmid with our predicted primers and sequence
- Ran a program to compare the predicted sequence with the sequence obtained from the plasmid (2 nucleotide differences)
- Then translated both the predicted sequence and the sequence from the plasmid
- Compared the translations (no differences)

Recipe for PCR reaction

- H2O → 46 microliters
- 5x buffer → 20 microliters
- dNTP → 8 microliters
- PCR_TOPO (primer) → 15 microliters
- Plasmid → 10 microliters (10ng/microliter)
- Taq → 1 microliter

Note:
Above procedures are self explanatory :)
Conclusion:
This was to make sure that our DNA was what we wanted and performed PCR to make multiple copies of it
Next step:

Name: Clayton Deible & Kailee Tateman
Date: 3/30/17
Week#: 10R
Objective: In today's lab we will perform PCR on our product from Tuesday. After this we will use mini prep to prepare the sample for the testing of concentration.
Procedures:

PCR

- Take all 4 PCR products and combine them into one tube (100 microliters total)
- Spin down the sample
- Run the sample on a 1.5% gel (40 ml of TBE and .6 g of agarose)

Mini Prep

- 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 microliters of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
- Place a QIAquick column in centrifuge with a 2 mL collection tube.
- To bind DNA, apply the sample to the QIAquick column and centrifuge for 30-60 seconds or apply vacuum to the manifold until all the samples have passed through the column. Discard the flow-through and place the QIAquick column back in the same tube.
- To wash, add 750 microliters Buffer PE to the QIAquick column. Centrifuge for 30-60 seconds or apply vacuum. Discard the flow-through and place the QIAquick column back in the same tube.
- Centrifuge the QIAquick column once more in the provided 2 mL collection tube for 1 minute to remove residual wash buffer.
- Place each QIAquick column in a clean 1.5 mL microcentrifuge tube.
- To elute DNA, add 30 microliter buffer EB or water to the center of the QIAquick membrane and centrifuge the column for 1 minute. For increased DNA concentration, add 30 microliters elution buffer to the center of the QIAquick membrane, let the column stand for 1 minute, and then centrifuge.
- 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.

Note:

- 1.5% gel → 40mL TBE + 0.6g agarose
- Mini Prep correction
    - Add 30 microliters of EB instead of 50 microliters

Conclusion: Got a concentration of 85.5 ng/microliter
Next step: Next week we will be doing in vitro transcription of the product.

Name: Clayton Deible & Kailee Tateman
Date: 4/4/17
Week#: 11T
Objective: Our objective in class today is to perform in vitro transcription.

Procedures:

In Vitro Transcription:

- NTP mix → 8 microliters
- 10x buffer → 2 microliters
- Enzyme mix → 2 microliters
- Template → 8 microliters (1 - 1.5 microgram)
- RNase free H2O → 0 microliters
- Total → 20 microliters

Note:

Again, self explanatory

Conclusion: Created dsRNA
Next step:

Name: Clayton Deible & Kailee Tateman
Date: 4/6/17
Week#: 11R
Objective: Our objective for this class is purify dsRNA from the solution from last class which included template DNA and ssRNA fragments

Procedures:

- Digestion of DNA template and single stranded RNA
  - Mix together:
    - dsRNA → 20 microliters
    - Nuclease-free water → 21 microliters
    - 10x digestion buffer → 5 microliters
    - DNase I → 2 microliters
    - RNase → 2 microliters
  - Incubate at 37 degrees celsius for 45 minutes
- Purification of dsRNA
  - Mix together:
    - dsRNA → 50 microliters
    - 10x binding buffer → 50 microliters
    - Nuclease-Free water → 150 microliters
    - 100% Ethanol → 250 microliters
  - Apply mix to filter Cartridge
  - Centrifuge for 2 minutes at 13,000 rpm
    - Empty contents
  - Add 500 microliters of Washing Buffer
  - Centrifuge for 2 minutes at 13,000 rpm
    - Empty contents
  - Add another 500 microliters of Washing Buffer
  - Centrifuge again for 2 minutes at 13,000 rpm
    - Empty contents
  - Centrifuge for 1 minute at 13,000 rpm to get rid of excess flow through
  - Apply 60 microliters of hot elution solution

Note:

1. The first step that we needed to do is to digest the DNA template using DNase and the single stranded RNA byproducts with RNase.

Conclusion:

Got a concentration of 2 ng/microliter…….yippee

Next step:

Inject dsRNA into tribolium

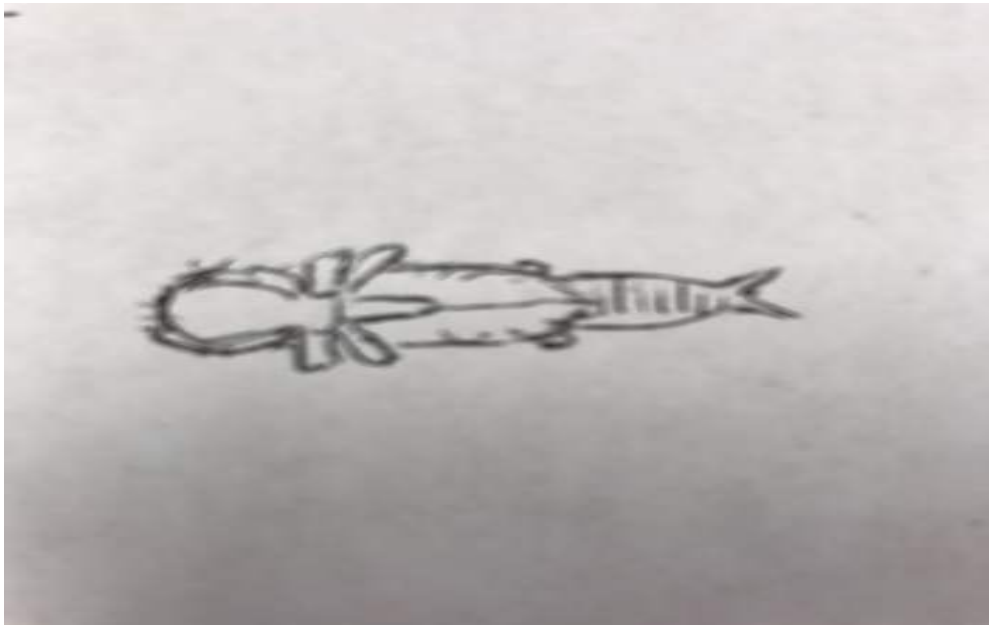
Name: Clayton Deible & Kailee Tateman
Date: 4/6/17
Week#: 11R
Objective: Our objective for this class is to observe Tribolium and sketch them from an eye's view and under the microscope!

Procedures:

Note:

Larvi Stage:

Pupi Stage:



Adult Stage: