Lab notebook template

5/10/18

How to do the final report

Date: 5/2/18
Week#: 13
Objective: (in one or two sentences)
Observe RNAi beetles.
Procedures: (bullet point structure)

- **Use microscope to observe beetles.**
- **Add ethanol to the beetles to keep them from drying up.**

Note:
There is one beetle that is alive. All of the others are dead. Total:11. 10 dead. We will observe our beetles again on Tuesday.

Alive 11
Dead 18
? 4
We have some beetles that died in the larval stage. We have some that have died in the pupal stage. They are currently still in the pupal stage and they are mostly in the process of dying. It appears that our beetles may not make it to their adult stage.
However it appears that the eyes are significantly smaller. We will check the size of the otidium today and compare it to the image we had on Tuesday since the eyes develop every 12 hours. Some are still alive.
Necrotizing tissue present.
Something morphologically wrong with the legs. - aberrant growth tarsi tibia, femurs look okay

Date: 4/26/18
Week#: 12
Objective: (in one or two sentences)
Observe RNAi beetles

Procedures: (bullet point structure)

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Note:
Alive 11
Dead 18
? 4
We have some beetles that died in the larval stage. We have some that have died in the pupal stage. They are currently still in the pupal stage and they are mostly in the process of dying. It appears that our beetles may not make it to their adult stage.
However it appears that the eyes are significantly smaller. We will check the size of the otidium today and compare it to the image we had on Tuesday since the eyes develop every 12 hours. Some are still alive.
Necrotizing tissue present.
Something morphologically wrong with the legs. - aberrant growth tarsi tibia, femurs look okay

Date: 4/24/18
Week#: 12
Objective: (in one or two sentences)
Observe RNAi beetles

Procedures: (bullet point structure)

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Note:

Date: 4/17/18
Week#: 11
Objective: (in one or two sentences)
Learn about Tribolium anatomy to determine any morphological changes

Procedures: (bullet point structure)

- **Draw the adult form of the tribolium from a dead model under the microscope**

Note:
Metamorphisis- animal changes body structure throughout life
    Hemimetabolous - incomplete metamorphis
        Egg, nymph, adult
            Nymphs lack wing and reproductive organs
    Holometabolous
    Egg, larvae, pupae, adult
        Drosophila and tribolium
            Juvenile and adults do not resemble eachother
Red flour beetle: tribolium castaneum
    Egg 1 week- larvae 3 weeks - pupa 1 week- adult 1 year

Why is the different shapes of the insects good for them?

Different living space and eat different things; not competing with offspring for food

Pupal stage if gene efects them becoming adult

If the bug will be disturbed in adult stage focus on that

Date: 4/12/18

Week#: 10

Objective: (in one or two sentences)

Remove everything but dsRNA from mix

Procedures: (bullet point structure)

- **Mix 20 uL from last time, 30 uL master mix**
- **Incubate 30 mins at 37 degrees**
- **Add 50 uL 10X binding buffer, 150uL nuclease-free water, 250uL 100% ethanol**
- **Move into Filter cartridge**
- **Centrifuge 2 mins at max speed**
- **Discard flow through**
- **Wash w/ 500 uL wash solution**
- **Centrifuge**
- **Discard**
- **Wash**
- **Centrifuge**
- **DIscard**
- **Centrifuge**
- **50 uL of elution, incubate at 70 degrees for 10 minutes**
- **Centrifuge, repeat elution**

Note:

RNA pol attaches to template strand to create a 5' -> 3' segment of RNA, which then anneals with another strand to create dsRNA

Want to remove salts, NTP, DNA, protein (polymerase), ssRNA.

Salts, NTP, and Protein can be removed by column

DNA removed by DNAse

RNA removed by RNAse

Exam now on 5/1

Date: 4/10/18

Week#: 10
Objective: (in one or two sentences)

Purify the PCR product. Use this as a template to make dsRNA in vitro transcription reaction. In vitro transcription

Redo sequence analysis

Procedures: (bullet point structure)

- **1combine 200 ul of the PCR reaction**
- **Add 1 ml of PB buffer**
- **Place QIAquick column on vacuum**
- **Pour 600ul of PCR/ buffer mix into the column**
- **Allow sample to pass through the column**
- **Pour 600 ul of the PCR buffer mix over the column**
- **Allow sample to pass through the column**
- **Add 750ul of PE buffer to the column and allow to flow through**
- **Place the QIA quick column back into tube**
- **Centrifuge 13,000rpm for 1 min**
- **Place in the a clean test tube**
- **Add 30 ul of EB to elute the DNA**
- **Centrifuge for 1 min at 13,000rpm**
- **Quantify DNA**
    - **Concentration: 343.8 ng/ul**

        1ul / 343.8ng x 1500 ng = 1500ng/ 434.8ng = 4.36 ul

- **Elute dsDNA in 30ml for PCR purification**
    - **1.5 ug**
- **H2O 3.64ul**
- **NTP mix 8ul**
- **10x buffer 2ul**
- **Template 4.36 ul 1-1.5 ug (minimum of 187.5 ng/ul) (1500 ng/ 8ul)**
- **T7 RNA pol 2 ul**
    - **20 ul**

    H2O calculation: 20ul - 8ulNTP mix - 2ul of 10x buffer - 2ul T7 RNA pol - 4.36 ul template = 3.65ul H2O

They created the master mix for us.

- **12 ul of master mix**
- **3.64ul of H2O**
- **4.36 ul of template**

Note:

Make RNA from DNA so we take advantage of transcription in vitro by using T7 RNA polymerase which will bind to the T7 promoter.

Date: 4/5/18
Week#: 9
Objective: (in one or two sentences)

Procedures: (bullet point structure)

- **2ml of each ecoli in micro centrifuge tubes**
- **Centrifuge for 3 mins 13,00rpm**
- **Dump out liquid**
- **Add 2ml more of each e.coli into microcentrifuge tube with pellet**
- **Centrifuge for 3 mins 13,000rpm**
- **Dump out liquid and keep pellet**
- **Add 250ul P1 to resuspend pellet- contains RNAse**
- **Add 250ul of P2 mix thouroghly by inverting (highly alkaline denatures other macromolecules and it precipitates) - 1 min SHARP**
- **Add 350ul of N3 see white floats incubate for 10 minutes increases precipitation**
- **Centrifuge for 10 mins at 13,000rpm**
- **Add 750ul of supernatant to the QIA spin column**
- **Place on vacumn**
- **Wash with .5ml of Buffer PB**
- **Wash with .75ml oh Buffer PE**
- **Centrifuge 60 seconds**
- **Transfer to a 1.5 ml microcentrifuge tube tube**
- **Add 50 ul Buffer EB**
- **Let stand 1 min**
- **Centrifuge 1 min**

- **Quantify amount of plasmid DNA**
  - **Elute 1: 253.89 ng/ul**
  - **Elute 2: 81.28 ng/ul**

- **Make 40ul of 10ng/ul of the Plasmid DNA**
  - **253.89 ng/ul x V1 = 10ng/ul x 40ul**
  - **1.57ul = Volume**

- **H_2O 102 ul**
- **5x buffer 40 ul**
- **dNTP 16 ul**
- **Primer 30 ul**
- **Plasmid DNA 10 ul**
- **Go Taq 2 ul**

200ul

Place on ice

- **Divide into 8 25ul in 1.5ml PCR tube**

Use P20 to pipette enzyme

Note:
Need RNA polymerase for transcription of plasmid to dsRNA
RNA polymerase requires promoter in DNA sequence to bind (DNA needs primers)
T7 RNApol- promoter
Need promoter from both sides to make dsRNA
dsDNA- add promoter sequence to both and then mix with RNAT7 polymerase to make dsRNA next week

Plasmid DNA → dsDNA = needs DNA polymerase (replication) - need primers, dntp, PCR to make a lot of the DNA
Design primer with T7 and every amplicon will automatically have this sequence on both ends

Redesign the primer with T7 on it- instead we came up with a way to target plasmid T7 sequence this primer will be used for all groups (T7 same in every group, gene different)
TOPORNAIprimer anneal and

Only need one primer for this pcr because it will bind to both sides.
Date: 4/3/18
Week#: 9
Objective: (in one or two sentences)

Sequence didn't contain our gene, empty plasmid.

Procedures: (bullet point structure)

Note:
Plasmid - PCR4-TOPO
Unique sequence on plasmid - T7, T3

Use DNA polymerase to sequence
Primer against T7 anneals to complementary strand
dNTP makes another strand
ddNTP causes replication to stop, lacking Oxygen at 3'
Sanger method to sequence, ddNTPs stop replication at different points, decipher sequence by going from bottom to top of size separated sequences
Now, each dNTP is colored, chromatography analyzes color to determine sequence

Date: 3/29/18
Week#: 8

Objective: (in one or two sentences)

We did not refrigerate our bacterial cells over the two week break, so even though we could select the correct colony from the gels, our colonies were dead and we couldn't use them. We will have to repeat this step from the Thursday of week 7. We will select new colonies. We know that the kill gene system really worked. We will use colony pcr show the gene in our bacterial colonies. We denature and rupture the membrane and check the plasmid.

Procedures: (bullet point structure)

- Primer site on plasmid
- Pick colonies from plate into 15ul LB + amp
- Use pipette tip and pick a big colony
- Mark the chosen colonies on the plate
- 37 dgreees C- 1hr with mixing it every 15 minutes
- Use for pcr
  - H2O 8.15 32.6
  - Green buffer 4 16
  - dNTP mix 1.6 6.4
  - T3 primer 2 (10uM) 8
  - T7 primer 2 (10uM) 8
  - GoTaq 0.25 1
  - Colony (2ml)
    - Total 20ul x 4

Add 2ul of colony to each PCR tube set from each one

Don't vortex a lot after the enzyme is added

PCR

- 95C 5 min
- 94C 30 sec
- 55C 30 sec
- 72C 30 sec repeat these for 30 cycles
- 72C 2 min
- 4C

Make sure to refrigerate the 4 bacterial colonies.

Note:

The class is working on killing all of the bacterial cells that were amplified. All of the lipids of the bacterial cells are destroyed. Its proteins will be denatured. Increases the ph, we control the destruction by carefully timing the reaction. Have to have a balance so that the DNA doesn't also get denatured. 3 minutes sharp! Add P2 alkaline solution almost always associates with the membrane or lipid. 1 min neutralized with neuralized with N3. Spin down; you get chunks and solution. DNA is in the liquid. Lipid and protein is precipitated + genomic DNA and lipid membrane.

Unique polymer at the bottom that captures only dna, all liquid will go through except for plasmid. There are wash buffers to remove other thing. ELution buffer allows DNA to free from polymer. Go downstairs to check how much DNA you have.
4 or 6 hundred

Other Students:
12,000 rpm centrifuge for 3 mins

After adding P2, prepare pipette to add P3 after 1 minute

Make sure to label blue column

Date: 3/27/18
Week#: 8/9?

Objective: (in one or two sentences)
Run the PCR colonies on the gel. Select the correct size fragment to use for the downstream procedure aka the colonies with the plasmid in it.

Procedures: (bullet point structure)

Other group is making gel

1.5%
80ml/gel
Microwave 3 min 30% → shake well → 1 min 30%

50bp ladder

#1 #2 #3 #4 50bp ladder #1 #2 #3 #4 50bp ladder #1 #2 #3 #4
group A group B group C
Kayla and Bradley

- Rinse the gels off with water
- Remove the gels and wipe them down with a paper towel
- Place gels in the water
- Pipette 2uL of #1, #2, #3, #4 of the PCR e.coli colonies into each of the lanes on the gel (buffer ready included the loading dye/ it was mixed in the buffer without reacting with PCR reaction) - will produce a faint sharp band
- Add a 5uL 50 bp ladder to the right
- Run the 4 e. Coli colonies on the gel at 100volts for 30 minutes.
- Determine which colonies have the correct sized fragment: **614 bp**
- Mark the two clones to use on the tubes for the downstream procedures

Note:

PCRs were run on our 4 colonies.

We need to know our gene size plus 100bp to take into account the T7 and T3 primers that were added.

514bp + 100bp = 614bp

If bands appear much shorter or longer, then the e. coli doesn't have your gene. We picked 4 colonies to find the right gene.

Cannot culture today. Culture will start tomorrow night and on thursday we will purify the plasmid from e. Coli. Then we will sequence the gene and we can analyze the sequence.

How to determine which % gel to use?

Agarose is from seaweed, and the density being used to run the gel is important. For a fragment with several kb ex. 2kb, use a gel with larger holes that is less dense 1.2% is favored for large fragments in order to get good seperation. They will travel faster and spread better throughout the gel. In the case of a few hundred bp, 1.5% can be used. 1.8% is used for small fragments where you want them to travel through denser agarose, therefor slower. Use higher percentages to see the exact size of the band. Make it thicker to make the best possible.

We did not refrigerate our bacterial colonies over the two weeks, so they are most likely dead now. We will have to start over by re-selecting colonies from the plate. We must redo the work from 3/15/18 and make sure to label more accurately. We did not label the colonies chosen on our plate either. We also need to be more specific on labeling our tubes.

Date: 3/15/18

Week#: 7

Objective: (in one or two sentences)

Did the kill gene system really work? We use colony pcr to answer this question. We denature and rupture the membrane and check the plasmid.

Procedures: (bullet point structure)

- Primer site on plasmid
- Pick colonies from plate into 15ul LB + amp
- Use pipette tip and pick a big colony
- 37 dgreees C- 1hr with mixing it every 15 minutes
- Use for pcr
    - H2O 8.15 32.6
    - Green buffer 4 16
    - dNTP mix 1.6 6.4
    - T3 primer 2 (10uM) 8
    - T7 primer 2 (10uM) 8
    - GoTaq 0.25 1
    - Colony (2ml)
        - Total 20ul x 4

Add 2ul of colony to each PCR tube set from each one

Don't vortex a lot after the enzyme is added

PCR

- 95C 5 min
- 94C 30 sec
- 55C 30 sec
- 72C 30 sec repeat these for 30 cycles
- 72C 2 min
- 4C

Note: colony PCR kills bacterial cells

small

Date: 3/13/18
Week#: 7

Objective: (in one or two sentences)

The PCR was successful this time. We will catch up and then perform cloning.

Procedures: (bullet point the procedures)

Making gel:

- Need: Weigh boat, agarose. 25 mL/small gel (50 mL for both).
- Get 30 mL of gel green
- Weigh .36 g of agarose (for 30 mL of 1.2% gel)
- Mix in erlenmeyer flask, cover with small beaker

- Microwave 3 mins at 30%
- Mix
- 1 more min at 30%
    - Gets rid of air bubbles
- Pour into small compartment
- Use small pipette tip to move air bubbles to corners
- Insert hole/separator (smaller end for more samples)
- Wash flask
- Leave on bench for twenty minutes to harden
- Rinse with water
- Remove the separator
- Place the small container in the runner
- Pipette 20ul of each sample DNA to the rows
    - 1 on far left
    - 2 on second farthest left

Add 5 ul of ladder into the 3rd port

Run gel for 25 minutes.

- Remove gel
- Place on black light
- Selected band 2 to use
- Cut out DNA
- Squeeze DNA into the serpratory tube
- Freeze for 5 mins
- Centrifuge at 13,000rpm for 3 minutes

Cloning: (“Frozen” means you need to?)

- Add purified DNA 4 ul
- Salt solution 1ul
- Topovector 1ul

    Total of 6ul

        In 1.5ul tube

    Reaction Time 30min

(Solution has plasmid in it and DNA in it.)

Transformation:

    Heat shock to damage e. Coli to make it ready for plasmid

    Frozen at -80 degrees
    Thaw on ice (10 minutes) - we don’t want to shock

Pipette 3 ul of reaction into e.coli (Put plasmid into e.coli.)
5 min on ice
42 degrees C 30 sec
5 min on ice
250ul 50 degrees C into the cell
37 degrees for 45min- 1 hr (went in at 3:43)
Plate on antibiotic plate
Use hockey stick to spread out with turning to spread cells throughout plate
Leave upside down in 30 degrees overnight to get many colonies

- This is extremely fragile

Note:

The PCR was successful.
Cloning; put the DNA fragment into the plasmid. Insert into E. coli, which does replication for us.
Linear DNA is not stable so it goes into a circular plasmid.
Put fragment into plasmid. Then put plasmid into e.coli cell. Traditional way takes a few days, but if you pay more money you can shave off time and get it done in a day.
Traditional way to put gene(500bp) into plasmid by using ligase, restriction enzymes to cut and they produce sticky ends. Taking advantage of replication and bacterial immune response.
Digest plasmid, cut fragment, mix with ligase, ATP, overnight and add them together.
Today: We use topoisomerase which is usually used to unwind double stranded DNA. Super coiling builds up from other side of unwinding. Topoisomerase releases tension and puts it back. No ATP necessary and its easier to control. Company sells the plasmid already open with topoisomerase with a T over hang. You will add an additional A to your product.

How do we select the e.coli with your plasmid?
Add gene into plasmid that has antibiotic resistance. Amp gene included
We plate on antibiotic agar.
How do we differentiate the e. coli with the gene cloned with the empty gene?
Length
Blue white selection
Lac z produces blue colony
Gene cloned breaks gene and then the colony is white instead
Modified idea… what if gene kills e. Coli
With gene inserted it breaks the kill gene and the e.coli survives
ccdB = kill gene

Name: Kayla & Bradley
Date: 3/8/18
Week#: 6

Objective: (in one or two sentences)
Redo our PCR because when it ran, it was blank. It appears that we missed either the taq or the dNTPS, but we both clearly recall adding those into our master mixed solution.
Procedures: (bullet point the procedures)

Other teams
Making gel
1.2%
50ml/gel
100ml/group
3 min 30% → shake well → 1 min 30%
Use bigger size to use 20uL

50bp
F1R1 F2R2 ladder F1R1 F2R2

Team up for running the gels.

We will come an hour before lab on Tuesday in order to make our gel and run it and hopefully catch up with the other group.
The PCR will run again and hopefully we get a result.

After running the gels you take the gels out and put it on the glow plate. You look at the gels under blue light and pick one of the two primer sets. Whichever one you choose you cut it out of the gel. Then you squeeze it into the tube and label it and put it in the freezer.
Put in freezer for 5 minutes. Take out and centrifuge.
Freeze and Squeeze

Squeeze with centrifuge
(make sure that it is balanced)
13,000 rpm
Write down the primer pair you chose.

Note:

Name: Kayla & Bradley
Date: 3/6/18
Week#: 6

Objective: (in one or two sentences)
Dilute the primers.

Procedures: (bullet point the procedures)
Dilute primer to 100uM
From dried pellet in tube
Add certain amount of H_2O
2 forward primer and 2 reverse primers (4 tubes)

Take nmol of primer tube
Convert to umol by dividing by 1000
Set equation - umol/L=uM
Ex. .0265umol/xL=100uM ; xL=.0265/100 ; xL=.000265L
Convert to uL by multiplying by 10^6

F1 - add 265uL H_2O
R1 - add 266uL H_2O
F2 - add 271uL H_2O
R2 - add 319uL H_2O

After you add the sterile water
Mix using a vortex
Do not touch the liquid
Spin down so you don't have any liquid on the side using a centrifuge
Keep it balanced

Label the primers
On top of tube with sharpie. Write down TC#009967 on top of the F1, R1, F2, R2
Write down the concentration
Use green sticker TC#009967 F1R1 or F2R2 10uM on other tubes

In other tubes make 50ul of 10uM = working solution

Add 1 part solution, 9 parts H_2O to make 10 uM
To make 50uL of 10uM, add 5uL solution and 45 uL H_2O

Add 30.5uL of H2O
Add 10uL of buffer
Add 4uL dNTP mix
Add 1 uL template cDNA
    Pipette in and out to mix
  Vortex and spin down
Add 0.5 uL of the enzyme taq polymerase
    Do not spin
Keep on ice
Split the solution into two 23 uL
    Add primer set to test tube
        1uL of each primer F1, R1 and F2, R2 to make a total of 25uL in each tube

PCR program

    Denature 95 degrees C for 5 minutes
    Denature 95 degrees C for 30 seconds
    Anneal
    Elongation 72 degrees C for 30 seconds   1kb=1min 700 bp was 30secs

    Elongation 72 degrees C for 5 minutes
35 cycles

Note:

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

We made 100uM dilutions for each of the four primers and labeled it for the bank and made 10uM dilutions for each of the four primers in 50uM to serve as the working solution.

We then created our master mix for our PCR, split them into two for each of the primer sets, and added the primer pairs.
They are ready for PCR.

Conclusion:

We learned to calculate dilutions and applied them to our four primers. We made a working solution to use throughout the lab class.

We created the PCR solutions with primers.

Next step:

(Add new entry above of the previous entries)

Name: Kayla & Bradley
Date: 3/1/18
Week#: 5

Objective: (in one or two sentences)
Identify size of 3 Unknown DNA segments using gel electrophoresis, dilute RNA primer

Procedures: (bullet point the procedures)
Making gel:

- Need: Weigh boat, agarose. 25 mL/small gel (50 mL for both).
- Get 50 mL of gel green
- Weigh .6 g of agarose (for 50 mL of 1.2% gel)
- Mix in erlenmeyer flask, cover with small beaker
- Microwave 3 mins at 30%
- Mix
- 1 more min at 30%
    - Gets rid of air bubbles
- Pour in two smaller compartments
- Use small pipette tip to move air bubbles to corners
- Insert hole/separator (smaller end for more samples)
- Wash flask
- Leave on bench for twenty minutes to harden
- Rinse with water
- Remove the seperator
- Remove gel from the container
- Place in the runner

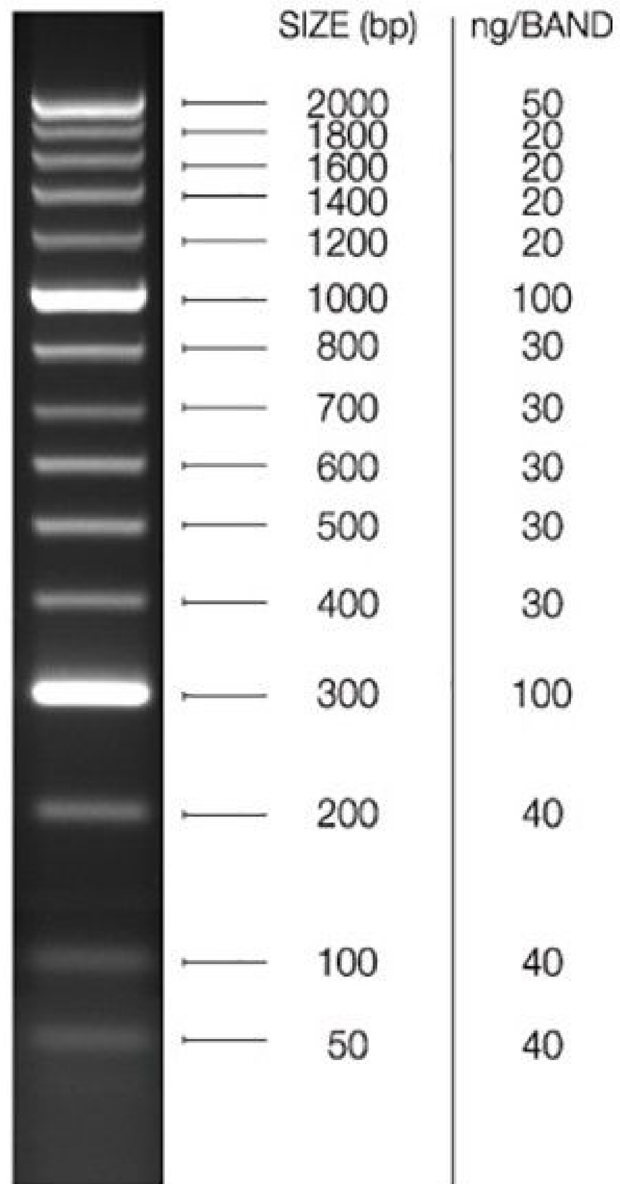
- A: 1.22 uL DNA, 8.78 uL ddH_2O
- B: .85 uL DNA, 9.15 uL ddH_2O
- C: .87 uL DNA, 9.13 uL ddH_2O

- 5x dye, added to 10 uL, need 2.5 uL of dye

- 100V at 25 min
- Stop running when color of loading dye reaches ⅔ of gel
-

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% gel for 1kb-5kb
.7% gel for 3kb - 10kb
1.2-1.5% for 100bp-1000bp



50 bp ladder

A: 82 ng/uL
B: 118 ng/uL
C: 115 ng/uL

Need 10 uL of 10 ng/uL

Conclusion:

Next step:

Name: Kayla & Bradley
Date: 2/27/18
Week#: 5

Objective: (in one or two sentences)
Learn how to use micropipettes.
4 pipette sizes

P1000
  1ml= biggest blue
  200- 1,000 microliter
P 200 yellow
  20-200 microliter
P 20 yellow
  2-20 microliter
P10 white
  1microliter- 10 microliter smallest white
    We'll treat thik a P2 (0.5-2 microliter)

P2
  .1-2 microliter smallest very fragile

Procedures: (bullet point the procedures)

- Select correct size pipette
- Set the pipette by spinning top or black piece to desired amount
- Push down to first stop and place into liquid
- Slowly release up to suck in the liquid
- Do not turn pipette sideways
- Push down to second stop to release the pipetted liquid

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 tested out each size pipette and learned the proper pitpetteing techniques to use in the lab.

Conclusion:

We now understand the differences between the four different pipettes. We know which ranges correlate with each pipette. We understand the proper pipetting techniques, and what not to do with the pipettes.