Lab notebook template

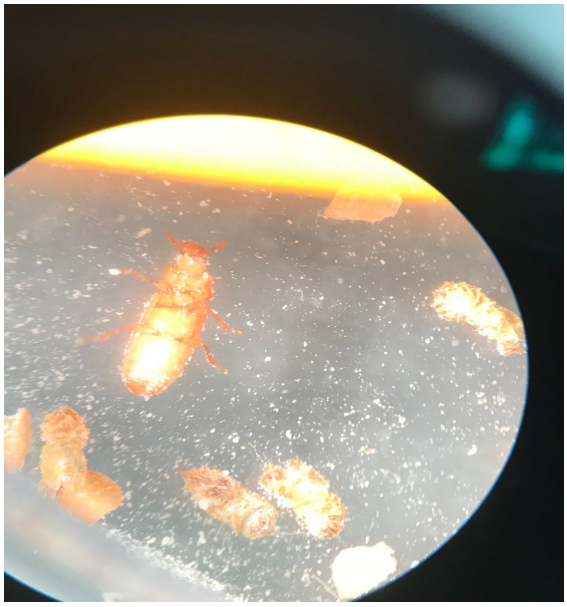
(Add new entry above of the previous entries)

NEW EXAM DATE: Tues 5/1/18 - i think

4/26/18

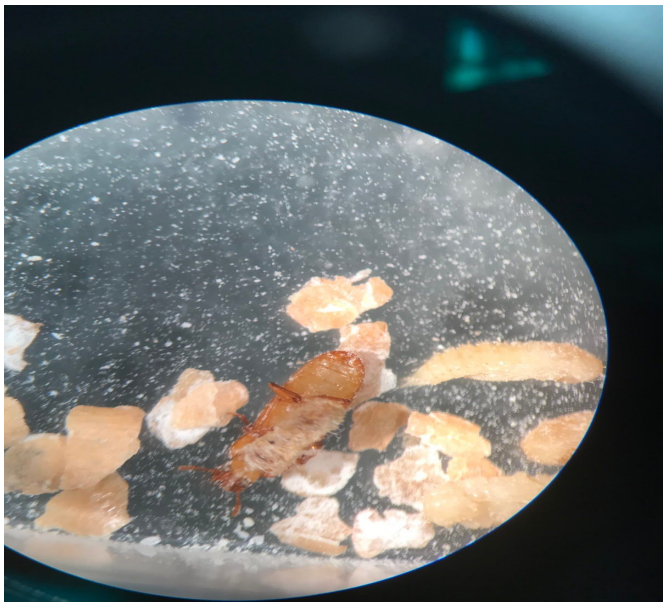
Concentrations:

1 ug/ml → 7 alive, 1 adult, 8 pupa (no noticable differences)



10 ng/ml → 9 alive, 0 adult, 9 pupa (no noticable differences)

100 ng/ml → 9 alive, 1 adult, 8 pupa (no noticable differences)



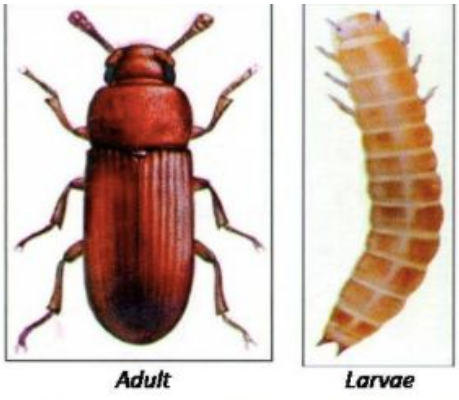
4/24/18

Three concentrations
Larval stage
1ug/ml
10ug/ml

All the larva are alive

Pupal stage
1ug/ul
7/10 alive no difference between the original form
10ng/u
9/10 alive no difference between the original form
100ng/ul
9/11 alive no difference between the original form

4/17/18
Lecture about insects

- Found everywhere on earth except ocean
- Why so many
    - Reproduce quick, small, size, protective colors
- Why beetles to study development
    - Same genes that pattern human limbs pattern insects
    - Good model organisms, easy to culture, develop quickly, available dna and genome sequences, molecular biology techniques available
- Metamorphosis = change body structure throughout life
    - Hemimetabolous (incomplete), 3 stages of metamorphosis: egg nymph and adult
    - Holometabolous (complete) metamorphosis, 4 stages: egg, larva, pupa, adult. (tribolium do this)
        - Inject RNAi in larva stage (2-3 weeks old)
        - The stage that is relevant/important for our gene is Adults
        - 

Name:
Date: 4/12
Week#: 12

Objective: (in one or two sentences)
RNA polymerase binds to the two 5' T7, will transcribe the gene template RNA into dsRNA. Rna polymerase moves 3' to 5'. But this isn't pure, must remove Mg salt from buffer, excess NTP, and DNA, protein (polymerase), and ssRNA leftover.

For salts, NTP, and protein, use the column to remove. Nucleotides (DNA ssRNA and dsRNA) are trickier to separate bc need to remove DNA and ssRNA without losing dsRNA. Remove DNA via DNase, and ssRNA via RNase (destroys only ssRNA, dsRNA is resistant) -- left with pure dsRNA.

Procedures: (bullet point the procedures)

*in D. step 2, proceed for 30 min not 1 hr. Add 20 uL of our dsRNA and 30 uL of Kevins master mix which consists of nuclease free water (21 uL), 10x buffer (5uL) , DNase and RNase (2 uL each).

*We can use 150 uL of our water, dont need water from up front

******For B do 50 uL, and do 10 min instead of 2 min incubation
Use fresh tube for the elution buffer, if you don't it's contaminated

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.

- (See above)

Conclusion:
Obtained pure dsRNA

Next step:

Name:
Date: 4/10/18
Week#: 11

Objective: (in one or two sentences)
Last week made template which has your gene with T7 on each 5' end -- this is dsDNA. Today we will make dsRNA. First we need to purify the dsDNA PCR product from last week to use as template to make dsRNA.

Procedures: (bullet point the procedures)

- Do the QIAquick PCR steps (See below)

- Start at step 1, do step 3 twice 600 uL two times, don't need step 8 - kevin already did

    - Combine all the small pcr tubes into one 1.5 mL tube

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.

- The **minimum** concentration of DNA template we need is 187.5 ng/uL on the nanodrop downstairs.
    - We want 1.5 ug of template, we have *at the mos*t 8 uL available. Convert 1.5 ug to ng = 1500ng, then divide this by 8uL to get 187.5 ng/uL. If the concentration comes out to be exactly this, we don't have to add any water since this would use 8 uL and we have 12 uL in the reaction mix already (20 uL total) → unlikely
        - If our concentration is greater than this, just do a proportion to determine the uL to use (which will be less than 8uL) and then fill in the rest with H20.
- Kevin already ran the gel of the dsDNA from last week's PCR product

Calculation of how much DNA template: (263.3 ng = 0.2633 ug)

$$uL = \frac{1\ uL}{0.2633\ ug} \times \frac{1.5\ ug}{1} = 5.7\ uL$$

How much water to add: 8 uL - 5.7 uL = 2.3 uL

Conclusion:

Purification and post-PCR in vitro (w/o cell) transcription to make RNA from DNA. Use T7-RNA polymerase (2uL) for this. Also need 8uL NTP (mix),10x buffer (2 uL), template (1.5 ug), and H20 if still need more volume to equal 20uL (uL).

PCR Master Mix:

NTP mix = 8 uL
10x buffer = 2 uL
T7RNA pol = 2 uL
Total = 12 uL

We add:

DNA Template = 5.7 uL
H20 = 2.3 uL

Total = 20 uL

Next step:

Obtain dsRNA

Name:

Date: 4/5/18
Week#: 10

Objective: (in one or two sentences)
To make dsRNA of our gene, need RNA polymerase (transcription DNA→ RNA). Besides the DNA, RNA polymerase also requires the promoter in the DNA sequence to bind to and show where to transcribe (where the gene is).
T7 promoter is used (T7 primer binds to the T7 promoter sequence).
Our gene fragment doesn't have promoter, need promoter on each side of the gene.

We will make dsDNA with T7 on both 5' end of our gene fragment.

From the plasmid DNA to normal DNA outside of plasmid need DNA polymerase (replication). Need primer for this, and dNTP. We will utilize PCR to make lots of DNA.

How to weave in the promoters into the gene: use the primer, design it such that you add T7 promoter to our primer. Then every single amplicon of the PCR will have the promoter. Would need to redesign primers so they have T7 → but instead will use only 1 paired primer for all groups since every group's plasmid is the same apart from the specific gene.

Name of this primer is TOPO-RNAi primer. Do PCR with this one primer.

Procedures: (bullet point the procedures)

PCR mix: 200 uL total

- H_20 102 µl
- 5x Green PCR buffer 40 µl (200/5)
- dNTP mix (2.5mM stock) 16 µl
- 1 Primer binds to both sides 30 µl (µM)
- Our Plasmid DNA 10 µl
- Go Taq (5U/ µl) 2 µl
- Total 200 µl

1.) Make 40 uL total of 10 ng/uL of our plasmid DNA
2.) Make the reaction mixture in the 1.5 mL tube
3.) Split the mix from the 1.5 mL tube into 8 PCR tubes (25 uL each)

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.

- Our DNA concentration from mini-prep was ~ 100 ng/uL (96.6), we want 10 ng/uL → this is a 10x dilution. So for step 1 we will use 4 uL DNA (40/10) and 34 uL H20 (40-4) for our 10x dilution of 40 uL total.

Conclusion:

Next step:
Make dsRNA

Name:
Date: 4/3/18
Week#: 10

Objective: (in one or two sentences)
Determine your sequence
Procedures: (bullet point the procedures)

- Use snapgene viewer to complete sequence document 7

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.

- Cloning site destroys ccdB
- T3 and T7
- Sequence the gene from the T7 side
    - 50/50 chance whether it copies from t7 → t3 or t3 → t7
- THE NAME OF THE **SEQUENCING TOOL: DNA Polymerase**
    - use DNA POLYMERASE**, and a PRIMER to decide where to star**t
    - If design primer that is 3 bp then annealing site anneals to the complementary sequence on the 3' end
    - **dNTP** continues the double strand after the annealed nucleotides on the 3' end (**extends the prime**r)
    - **ddNTP lacks OH**, cant add new nucleotide, acts to **terminate the synthesis**
- Set up 4 reactions traditionally
    - All mix with DNA polymerase, primer, and dNTP
    - 1st reaction add: dd<u>T</u>TP to stop the reaction after hit <u>A</u>
    - 2nd reaction add: dd<u>A</u>TP to stop the reaction after hit <u>T</u>
    - 3rd reaction add: dd<u>C</u>TP to stop the reaction after hit <u>G</u>
    - 4th reaction add: dd<u>G</u>TP to stop the reaction after hit <u>C</u>
        - Then run the gel, and read the sequence bottom to top
        - Each gel slot will contain a different nucleotide
        - OURS will be color coded so we don't need to do the old fashion way
            - Height of peaks = strength of nucleotide that comes through
            - Each color corresponds to a particular nucleotide (either ATGC)
            - Open the T7.ab1 file

Conclusion:
See sequence document 7
Found our reverse and forward primers and our entire gene

Next step:

- Starting to make dsRNA

Name:
Date: 3/29
Week#: 9

Objective: (in one or two sentences)
Today we will harvest and purify the plasmid. We will remove all the other macromolecules in the e coli besides plasmid DNA, this includes proteins, lipids, sugars, and genomic DNA.

Procedures: (bullet point the procedures)

- Pick one of the cloudy tubes (have e coli), mark which you use
- Harvest and purify the plasmid
    - E coli has genomic dna as well as plasmids that contain our gene
    - E coli has proteins, lipids, sugars, and nucleotides (macromolecules)
        - Our mission is to remove all the macromolecules and purify only the plasmid DNA with our gene

    - 1st step: centrifuge and make pellet of cells, remove culture media
        - Use 2 mL tube. Add 2 mL (2000 uL) of cloudy e coli culture, centrifuge 3 min 12k rpm to make pellet at bottom. Remove liquid on top, pour into flask. Use kimwipe to clean tube. Then add another 2 mL cloudy tube 3, repeat centrifuge to make bigger pellet, then remove liquid again.
            - This step does not kill cells
    - 2nd step: re-suspend into solution using P1 buffer (250 uL), shake and vortex
    - 3rd step: increase pH using P2 (250 uL) to kill cells. Invert very gentle a few times. Rest @ room temp for 1 min.
        - Destroys lipids (cell membrane) and denatures proteins. Genomic DNA becomes fragmented Whole cell gets killed. Do not shake tube, be gentle
        - 1 min rest → keeps plasmid entact but destroys genomic DNA
    - 4th step: After 1 min, neutralize using N3 buffer solution (350 uL); then put on ice for 10 min.
    - 5th step: Centrifuge for 10 min @ 13k rpm to form another pellet
            - In this pellet will be lipids, proteins, and genomic DNA, RNA will be destroyed
            - In the liquid will be plasmid DNA → this is what we want
    - 6th step: put new column tube on vacuum manifold machine; Pipette 750 uL of the supernatant to the QIAprep 2.0 spin column tub.
    - 7th step: pipette 500 uL PB into tube on vacuum
    - 8th step: pipette 750 uL PE into tube on vacuum
    - 9th step: Centrifuge for 1 min @ 10k rpm
    - 10th step: take blue basket out of the tube that we used for the vacuum. and put into a new clean 1.5 mL tube. Add 50 uL of the EB elution buffer onto the membrane in the tube (not the plastic) → allows the DNA to peel off of the filter.

Let stand for 1 min. Centrifuge for 1 min @ 13k rpm. DNA will be in the liquid after. Vortex and spin down.

- 11th step: go downstairs and check the concentration using Nanodrop. use 2 uL @ 260 nm wavelength

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 initially used tube 3 of e coli
- When centrifuge, orientate the tube such that the folding tab is facing out not in. This will make it so that the pellet forms on that same side of the tube → makes pellet easy to find. In this case it isn't a problem bc the pellet is big and easy to see, but sometimes the pellet is small and hard to find → this eliminates this issue.
- ***our tube #3 didnt work so we needed to repeat the entire procedure with e coli from tube 4
-

Conclusion:

The concentration of our DNA from tube 4 was 96.6 ng/uL
Next step:
Sequence the DNA

Name:
Date: 3/27
Week#: 9

Objective: (in one or two sentences)
Make the gel. Run colony PCR result on gel electrophoresis to see if e coli has our gene (we have 4 e coli colonies to get this right). Determine which e coli has the right size of the fragment. (length of our gene + 100 BP from the T3 and T7 primers → 50 bp each primer)

Procedures: (bullet point the procedures)

- Make gel, 80 mL 1.5% (80ml*0.015 = 1.2g agarose). 50bp ladder. Make sure you *cradle the bottom* of the flask when take it out of microwave.
- Run gel, 2 uL e coli, 5 uL ladder
- Quantify length of plasmids
- Of the four colonies of e coli, mark 2 out of the 4 on the tube with red pen. This way if one doesnt work, will have backup
- Which amplicon did we use? **Amplicon** = **RNAi target sequence**
    - **Length of amplicon alone = 359**
    - **Length of plasmid including T7 and T3 primers = 459 bp**

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

- PCR4-TOPO = plasmid
- ccdB = kill gene
- T7 and T3 sequence → primers target this sequence
    - Use e coli as template and do PCR between T7 and T3
        - T7 and T3 are 50 BP each
        - Our gene is ~200 BP, so if the pcr worked you will see a length of 300 bp (200 from gene + 50 from T7 + 50 from T3)

Conclusion:
We are using E coli colony # 3 & 4. Both have a length of 459 bp.

Next step:
Purify plasmid, ship to company for sequencing or sequence downstairs

Name:
Date: 3/15
Week#: 7

Objective: (in one or two sentences)
Do colony PCR to verify if gene is in the e coli plasmids

Procedures: (bullet point the procedures)

- Use micropipette tip (which is already sterilized) to pick up a big colony
    - Big = has a plasmid most likely
    - Touch colony, dip pipette tip into tube and shake a little bit, do this 4 times for the 4 tubes we have - change tips between each colony, incubate at 37*C for 1 hour to prepare for colony pcr, every 15 min tap to shake and encourage growth

- Colony PCR
    - Pick colonies from plate into 15 uL LB + ampicillin
    - Culture 37*C for 1 hr with mixing/tapping tube every 15 min
    - Use this for PCR
    - PCR4-TOPO
    - T3 side and T7 side, gene should be in between

- **Colony for 1 PCR Reaction (make this 4x bc have 4 colonies)**

H_20	8.15 µl	32.6 uL
Green PCR buffer	4 µl	16 uL
dNTP mix (2.5mM stock)	1.6 µl	6.4 uL
Primer1 (T3 10 uM)	2 µl (__µM)	8 uL
Primer2 (T7 10 uM)	2 µl (__µM)	8 uL
Colony template	2 µl	*(dont add yet)
Go Taq (5U/ µl)	0.25 µl	1 uL
Total	20 µl	72 uL

- *No $MgCl_2$ if using TAKARA Ex Taq or Promega GoTaq

- Spin down the 72 uL and pipette 18uL each into the 4 tubes which have the 2 uL colony in it

    - Parafilm plate and keep in freezer for 2 weeks upside down

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

- If no bacteria growth on the plate either forgot a component in the mixture, or low concentration of DNA
    - If low [DNA] use the faint band of DNA as a template to re-amplify
        - Ours worked well

- How to tell if gene is in the plasmid:
    - **Since used kill gene ccdB, if have plasmid the e coli will survive → if plasmid has your gene it will destroy the ccdB gene**
    - Use PCR to verify

        - Primer on plasmid (not your gene),
        - If do PCR from primer to primer on plasmid, if plasmid is empty (doesnt contain gene) it will be 100 bp (length of the two primers)
        - If have your gene in between primers, you will have the size of your gene + 100 bp from the primers on the plasmid
- Vortex and mix everything that comes from freezer, except for the taq enzyme
- **Pacific Rim is the movie he does not want to see in 2018 -- Midterm question**
- Colony PCR reaction cycle
    - 95 *C 5 min
    - 94 *C 30 sec Repeat 30x
    - 55 *C 30 sec Repeat 30x
    - 72 *C 30 sec Repeat 30x
    - 72 *C for 2 min
    - 4 *C keep until turn off PCR machine

Conclusion:
Our plate of bacteria colonies :

Next step:
Purify and sequence

Name:
Date: 3/13
Week#: 7

Objective: (in one or two sentences)
Cloning = isolate our gene and put our linear DNA fragment into plasmid (circular dna in E. coli) so that we can have the clone of our gene for the future.

Then put the entire plasmid back into E. coli, which will replicate/re-amplify it.

Procedures: (bullet point the procedures)

- Invitrogen TOPO PCR cloning technology → add components as described below into a new 1.5 mL tube, label
- How to put dna into plasmid
    - Gene 500 bp
    - Traditional way: need Restriction Enzyme to cut plasmid and produce sticky ends, then use ligase to attach 2 okazaki fragments together, and ATP
    - What we will do: use **topoisomerase1** (functions as both a restriction enzyme and a ligase) to unwind dsDNA and eliminate the super coil that would otherwise form, then topoisomerase will put it back together
        - Company sells plasmid that is already cut open and has TOPO attached on 3' end of plasmid, and has T on end. Topoisomerase will add A from our gene's 3' ends
            - T and A will match up to put gene into plasmid (T A cloning)
            - Let reaction happen over 10 min
- How to select the E. coli that has our plasmid:
    - **Add ampr gene** (antibiotic resistance gene), ours will stay alive when treated with antibiotic
- How to differentiate the E. coli that has our gene
    - **lacZ gene**, if add substrate makes colony blue, blue/white selection
    - **ccdB** = kills E coli when overexpressed
    - If have something in the plasmid that stops the ccdB gene, the e coli will survive
    - Selects only the e coli that has our gene
    - How? Heat shock E coli and put gene into plasmid, then put into ice, then plate onto antibiotics

- **<u>Steps</u>**:
    - put E coli from on ice 5 min, Pipette 3 uL of our topo rxn slowly onto e coli cell, put lid back on, then put on ice for 5 min, heat shock 42 *C for 30 sec, then put back on ice for 5 min, then pipette 250 uL SOC (food for E. coli) into tube, then 37 *C for 45-1 hr, then pipette 120 uL onto plate of antibiotic, use spreader to spread entire cell on the plate, flip upside down, 30 *C overnight to grow lots of colonies

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.

- From freezer add purified DNA (from gel) 4 uL (vortex and spin down), salt soln 1 uL, and TOPOvector 1 uL. Put in new 1.5 mL tube. Incubate for 30 min at room temp.

Conclusion:
Today we learned how to put our gene into the plasmid, and how to then put the modified plasmid back into the E. coli. Hopefully by next class, we will see a good amount of E. coli culture growth on the plate.

Next step:
Colony pcr

Name:
Date: 3/8/18
Week#: 6

Objective: (in one or two sentences)
Run gel to purify gene downstream

Procedures: (bullet point the procedures)

- Make 100 ml gel per group
    - 1.2% 50 ml/gel → make 2 gels for 100 ml total
    - 3 min 30% microwave
    - Shake well
    - 1 min 30% microwave
        - Dont need loading dye
- Use bigger comb indents (6 across / gel)
- Only use 5 of the indents: group 1's f1r1, f2r2, 50 bp ladder, group 2's f1r1, f2r2
    - Load 20 uL DNA into each slot, 5 uL ladder
- Use extractor to get your gene from gel
- Use freeze and squeeze gel purification kit (centrifuge to squeeze)
    - Gel stays in basket, dna goes into bottom of tube
    - 5 min freeze, then spin for 3 min >13000 rpm
-

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.

- Green buffer already contains loading dye, so just need to load and run the gel

We extracted the sample on the left which was f1r1
Why we chose it? It was longer (shorter fragments move farther) and has more DNA

[Here is a better picture that Kevin took. The left two samples are yours. --Yoshi]

-To centrifuge, it needs to be balanced!!
-Open it, screw off black lid, put sample in, balance it either with another equal sample or a tube with an equal amount of water, screw on black thing all the way, close

-If it is unbalanced or somethings wrong it will be louder so listen for a couple min

Next throw out basket, keep lid and put back in fridge

Conclusion:
We were able to obtain the largest DNA fragment from the gel and apply the freeze and squeeze to it. We also made our own gel for the first time and properly ran a gel electrophoresis.

Next step:
Clone fragment after freeze and squeeze procedure and put it into plasmid

Name:
Date: 3/6
Week#: 6

Objective: (in one or two sentences)
Primer dilution and PCR

Procedures: (bullet point the procedures)

- Clean lab benches before and after each class
- Dilute primers to 100 uM and to 50 uM (see notes below)
- Create PCR mixture (see notes below)

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.

- Dilute primers we designed (4 tubes for 2 sets of primers,1 set = 1 fwd primer & 1 reverse primer)
    - Dilute to 100 uM, add X uL H20 to make 100 uM, vortex, spin down using black centrifuge. DO NOT contaminate

    -
    - Next take Y uL and make 50 uL of 10 uM
    -
    - Green sticker label top of tube clear tubes with: 10 uM, TC#, & R1 / F1 / R2 / F2 (R1 = reverse primer 1, F1 = forward primer 1)
- PCR → denature, anneal, extension → repeated 35-40 times

Date 3/4/18
Aim: create the PCR mix

Gene name: dan

F1 Forward Primer: Tm= 59.96 ℃: R1 sequence 5'-- 59.89

F2 Reverse Primer: Tm= 60.04 ℃: R2 sequence 5'-- 59.97

Reaction

H_20	___µl	__30.5_µl
5 X PCR buffer	20µl	_10__µl
*$MgCl_2$ (25mM stock)	6µl (1.5µM)	_X_µl (__µM)

dNTP mix (2.5mM stock)	8µl (0.2µM)	___µl
Primer1 (10µM stock)	___µl (__µM)	__2__µl (__µM)
Primer2 (10µM stock)	___µl (__µM)	__2__µl (__µM)
Sample DNA ( )	___µl	__1__µl
Taq polymerase (5U/ µl)	0.5µl (2.5U /100µl)	__0.5__µl
Total	100µl	__50__µl

*No $MgCl_2$ if using TAKARA Ex Taq or Promega GoTaq

- Pipetted the volumes largest to smallest then pipette up and down a couple times to mix
- Only vortex for a couple sec; dont vortex the taq bc it will destroy
- Add primers after you add taq. When add taq you will have 46 uL, which you split into 2 tubes of 23 uL - one for each primer set. So the two sets of primers need to be 4 uL combined → 2 uL per set → 1 uL fwd, 1 uL rev (per primer set)
    - Master mix is 46 uL, split this into two tubes of 23 uL
- *Note: the buffer already has MgCl2 in it so for this PCR we don't need to add MgCl2
- Reaction Steps:
    - Prior to pcr denature at 95 degrees C for 5 min
    - Then do PCR, denature at 95 degrees for 30 sec, anneal at primer T.M. + 1-2 degrees, extension at 72 degrees 500 bp/30 sec depending on your length
    - Then do extension again 72 degrees for 5 min for taq polymerase to add
    - Finally hold at 4 degrees
- After label them, put into freezer
- Keep things on ice while waiting → Taq needs to stay cold

Conclusion:

Today we learned how to dilute primers and create the PCR mixture

Next step:
Run gel and separate DNA

Name:
Date: 3/1
Week#: 5

Objective: (in one or two sentences)
Gel electrophoresis

Procedures: (bullet point the procedures)

Make and run gel electrophoresis, tell size of A, B, C
A: 82 ng/ul
B: 118 ng/ul
C: 115 ng/ul
In new tube make 10 ul of 10 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.

- Gel electrophoresis = separate molecules based on size/length
    - Gel is a polymer mesh, higher concentration smaller the mesh
    - Higher voltage = faster DNA run, **use 100V for 25 min, when reach ⅔ down the gel stop it even if it hasn't been 25 min**
    - Put dna into well
    - DNA is neg charged, put pos charge on other end, DNA is attracted to it
    - Smaller base pairs move farther
    - Allows us to tell the size of our DNA
    - DNA ladder allows for more precise comparison, **50 bp ladder** means that each rung in the ladder is 50 bp apart starting from the positive end
    - TAE and TBE buffers
        - 1x, 5x, 10x = concentration level, ex.) 10x means need to dilute 10 times before you have a usable → 100 ml + 900 mL H2O
        - For TBE buffer can use 0.5x
    - Cant put DNA directly into well with buffer bc DNA would distribute all over, so mix with dense solution called the Loading Dye which is usually 5x, mix this with your DNA for 5-10 min beforehand and the DNA will be good
    - After run the gel how to see DNA? DNA is not visible, so use EeBr dye which binds b/w double helix, then add energy (UV) to EeBr/DNA complex, then run the gel
    - In our lab, we use Gel Green dye instead of EeBr bc it is safer, bc its green dont need UV either, just use blue light to excite it and itll show the green colour.
        - You can use your phone to take picture
    - 1% weight/volume (g/100mL) gel, 1 - 5 KB DNA → 1%
    - longer fragments need to increase concentration, **100-1000 KB → 1.2-1.5%**
    - For our DNA use 1 g/100 mL (1%)
    - Make gel with the aluminum foil TBE/green, and gel box with the 0.5x TBE
    - Steps to make gel:
        - Use 25 mL/gel of gel green (in aluminum foil bottle)
        - 0.6 g agarose for **1.2% if using 50 mL** (50*0.012 = 0.6)
            - Multiply percentage as a decimal x mL needed to get mass agar
        - Put agarose in flask, then the gel, swirl, then do 3 min at 30% power in microwave, mix really well to make gel uniform then do another 1 min at 30% which will remove the air bubble, use mitts to remove flask from microwave
        - If making 2 gels do ABC then 50 bp ladder in middle then the next ABC
        - Pour flask contents into white gel template, get rid of bubbles
        - Insert well comb into liquid in template

- Wash flask when done
- When gel is solidified, squirt some water on the gel then remove the well comb

- Ladder: 5 uL, our sample is 10 uL

Calculation for A, B, and C dilution

Calculation for How much loading dye to add to mixture

Conclusion:
Today we learned how to make the gel and how to run gel electrophoresis.

Ladder A B C

Size of the fragments
A: 300 bp
B: 500 bp
C: 1400 bp

Next step:
Diluting Primers

Name:
Date: 2/27
Week#: 5

Objective: (in one or two sentences)
Familiarize ourselves with micropipettes.

Procedures: (bullet point the procedures)

- Use the 4 different sizes of pipettes, practice

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.

- Micropipettes:
    - 1 Blue = biggest 1000 ul (1ml) → P1000 (200-1000 ul)
    - 2 yellows = 20 and 200 ul → P200 (20-200 ul) & P20 (2-20 ul)
    - 1 white = 1-10 ul → P10 (0.5-2 ul) - use P20 for 2-10 not this one
- Adjust volume turning black rotating knob or top circle knob
- Stop at first notch, slowly suck up liquid you want to measure
- When dispense, push all the way down (dont just stop at first notch)
- DON'T TURN THE PIPETTES on side, KEEP VERTICAL
- Close tip box whenever not using
- Use gloves
- ALWAYS PICK UP THE TUBES
- Red number is the first digit, ex) on the blue one, 1000 ul, the red number would show "1", for 500 ul the red number would show 050

Conclusion:
Be careful, follow above techniques
Never turn pipette sideways or you will lose points

Next step:
Gel electrophoresis