Lab Notebook

Name:Hunter Gazda and Connor Huelsman
Date:4.26.18
Week #:

Objective:
Examine the 18 surviving beatles we examined on 4.24.18

Notes:
Today we observe 3 larva which all seem to have normal morphological development
Today we observe 15 pupa alive where 13 have normal morphological development and 2 have morphological abnormalities

2 pupa with possible morphological developments
- 1 pupa has a developmental problem with its wings, left wing seems to be larger than normal, right wing possible morphological developmental problem
- 1 pupa has abnormal atena developmental problem

Conclusion:
Two pupa were confirmed to have morphological developmental problems as seen by the included image.

Next Step:
Continue to examine the development of the beatles

Lab Notebook

Name: Hunter Gazda and Connor Huelsman
Date: 4.24.18
Week#:

Objective:
Examine the tribolium under magnification in both the larvae and pupae stages at 1 week post injection.

Procedure:
A total of 20 tribolium larva were injected. Upon examination we had 5 beetles in the larva stage and 15 in the pupa stage. 4 larva were found to be alive with no noticeable morphological developmental problems. 14 pupa were found to be alive with no noticeable morphological developmental problems. 1 pupa and 1 larva was found dead with no noticeable morphological developmental problems.

Conclusion:
Of the surviving larva and pupa there was no noted morphological developments. However, one beetle in the larva stage was missing part of its wing development and a picture was take.

Next step:
The 18 remaining beetles will be re-examined in two days.

Lab Notebook

Name: Hunter Gazda & Connor Huelsman
Date: 4.12.18
Week#: 11

Recap:
We had our template with our gene which has T7 on the end of both the 5' and 3' strand. RNA polymerase will then polymerize. RNA polymerase will use the anti-sense strand and sense stand polymerizes in the 3 → 5 direction. → this reaction is not pure yet we still want to remove the salt (buffer), excesse NTP, DNA, RNA polymerase (protein),ssRNA (some RNA that didn't form dsRNA) → becasue we only want to inject dsRNA

Salts, NTP, and protein can be removed via a column. To separate DNA without losing the dsDNA we can utilize DNase. In order to remove only the single stranded RNA from the dsRNA we can use RNase because it only destroys sRNA.

Template is not the same as the sense strand, it is the antisense strand.

Objective:
Today we will begin purifying our dsRNA by removing salt (buffer), excess NTP, DNA, RNA polymerase (protein),and ssRNA.

Procedure:
- We will begin with a Nuclease Digestion to remove DNA and ssRNA
- We will incubate the reaction for 30 minutes
- Below are the reaction quantities
- Reaction mixture was mixed by pipetting up and down 10 times

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

Next we purified the dsRNA:

- Below are the reaction quantities for this step

Amount	Component
50 µL	dsRNA (from step D.2 above)
50 µL	10X Binding Buffer
150 µL	Nuclease-free Water
250 µL	100% Ethanol

- We will then pipet the entire 500µl dsRNA mix onto the filter in the filter cartridge and drew it through by centrifugation
    - Placed a filter cartridge in a collection tube
    - Pipetted the entire 500µl dsRNA mixture onto the filter cartridge
    - Centrifuged at max speed (13,000rpm?) for 2 min
    - Discarded the flow through and replaced the filter cartridge in the collection tube

- Washed the filter cartridge with 2 x 500µl Wash solution
    - Pipetted 500µl of the Wash solution onto the filter in the filter cartridge
    - Drew the wash solution threw via centrifugation
    - Repeated with a second 500µl of Wash solution
    - Centrifugation again

- Next we recovered the dsRNA*:*
- Transferred the filter cartridge to a fresh collection tube
- Applied 50 uL room temperature elution solution and incubated in a heating block for 10 min set at 70 degrees celsius
- Centrifuged at max (13,000rpm) speed for 2 min
- Repeated the previous two steps with a second 50 uL. The second elution is included when recovering any additional RNA

Conclusion:
We successfully purified and collected our dsRNA

Next Step:
Injection

Lab Notebook

Name:Hunter Gazda and Connor Huelsman
Date: 4.10.18
Week#: 10

Recap:
We now have the Tip60 gene with T7 on each end (both the 5' and 3') in dsDNA format

Objective:
Today we will purify the dsDNA from the previous PCR reaction so we can use this dsDNA to make dsRNA. We will then assemble RNA in vitro transcription.

Procedures:
dsDNA Purification

- Added 5 volumes Buffer PB to 1 volume of the PCR (200 uL) and mixed.
- Placed a QIAquick in a provided 2 ml collection tube or into a vacuum manifold.
- To bind DNA, applied the sample to the QIAquick column and applied vacuum to the manifold until all samples have passed through the column. Did this using 600uL twice.
- To wash, added 0.75 ml (750uL) Buffer PE to the QIAquick column back in the same tube.
- Centrifuged the QIAquick column once more in the provided 2 ml collection tube for 1 min to remove residual wash buffer.
- Placed each QIAquick column into a clean, dry 1.5 ml centrifuge tube.
- To elute the DNA, added 30 uL of elution buffer (EB) to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuged.
- Nanodrop analysis revealed a concentration of 197.1 ng/µl.

Note:

- Before In vitro Transcription need to perform PCR purification of dsDNA and elute in 30µl (the determined concentration will determine how much template to add)

In vitro transcription:

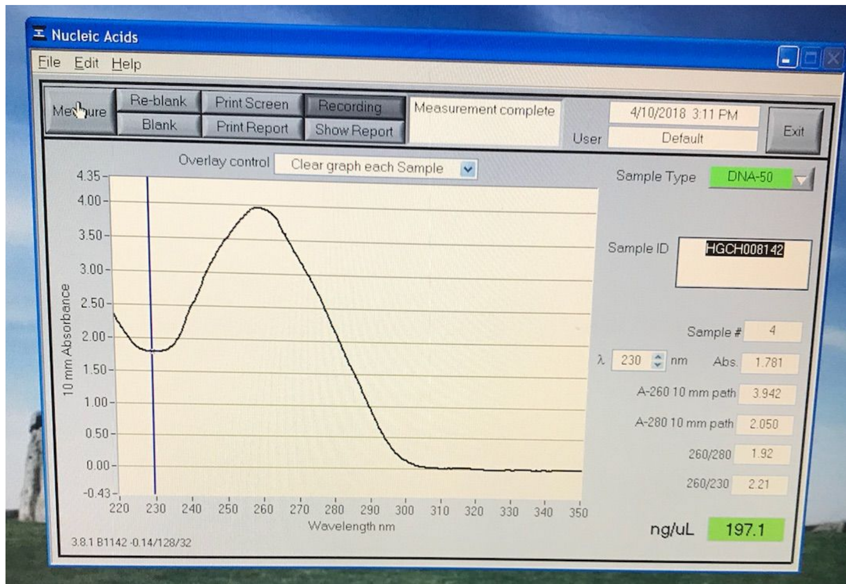
- Want to make dsDNA to dsRNA
- We need RNA polymerase (T7 RNA polymerase which will bind to T7 promoter), NTPmix (8µl), 10x Buffer (2µl), Template (want to use 1-1.5µg)(can only add a maximum of 8µl), T7 RNA pol(2µl)

Trying to figure out what is the minimum concentration we need to achieve 1.5 µg (need 1500ng/8µl) = 187.5 ng/µl - we need this concentration or above in order to go on with In vitro transcription

In vitro transcription:

Reagent	Volume of MasterMix (uL)
ddH_2O	0
NTP mix	8
10x Buffer	2
dsDNA template	8
T7RNApol	2
Total	20

- With a concentration of 197.1 ng/µl our calculation revealed we should add 7.61µl and 0.39µl H2O
  (1500ng)(1µl/197.1ng)=7.61µl
  8 - 7.61µl = 0.39µl ddH_2O
- Because we cannot accurately pipette 0.39µl we are going to add 8µl of our sample without diluting
- We incubated for 10 hours



Conclusion:
We purified our DNA and obtained our dsDNA concentration before preparing the In Vitro Transcription reaction.

Next Step:
The In vitro transcription reaction will be run outside of lab hours.

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 4.5.18
Week#: 9

Objective:
To make dsDNA of our gene using TOPO-RNAi primer containing T7 in the primer.

Procedures:
- Diluted our plasmid DNA to 40ul of 10ng/uL (calculations found in notes)
- Prepared Master PCR Mix with quantities in table in notes
- First pippeted H20 into 1.5ml tube
- Thawed buffer, vortexed and spun down before adding buffer quantity
- Added primer
- Added dNTP
- Added Plasmid DNA
- Vortexed and spun down mixture
- Added Go Taq and briefly vortexed
- Aliquoted 25ul of Master PCR Mix into 8 PCR tubes

Note:
PCR Master Mix Quantities:

Reagents	Quantity (uL)
H2O	102
5x Buffer	40
dNTP	16
Primer	30
Plasmid DNA	10
Go Taq	2
Total	200

Reaction Cycle

Program name:

Denaturation: 95 ℃ X 5 min

Denaturation: 94 ℃ X 30 sec
Annealing: _*2_ ℃ X 30 sec
Extension: 72 ℃ X _30_m / s

35 cycles

Extension: 72 ℃ X 5 min

Hold: 4 ℃

*2: Primer Tm + 1-2℃
*3: 500bp/30sec, 1,000bp/1min

Result

Electrophoresis:__%agarose,__X TAE/ TBE
Loaded sample ___µl / lean

- Our plasmid DNA concentration is 354.6ng/µl and we need to make 40ul of 10ng/uL in 1.5ml tube, will make PCR mixture in 1.5ml tube and then divide reaction into 8X25ul in PCR tubes
- Calculation: (354.6ng/uL) (V1) = (10ng/uL) (40uL)
  V_{DNA} = 1.13uL
  V_{ddH2O}= 38.87

Conclusion:

PCR reaction was prepared and handed off to be run later tonight.

Next step:

PCR reaction will be run overnight

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 4.3.18
Week#:9

Objective:
Analyzed our Sanger Sequencing results by completing a sequence analysis form.

Procedures:
-See Sequence Analysis Form

Note:
- We have a plasmid (PCR4-TOPO) with ccdb and ampr – there is a cloning site within the ccdb gene that destroys the ccdb gene when our gene is inserted – the plasmid has T3 and T7 site. We sequenced plasmid from the T7 site (we used DNA polymerase to sequence this, also needed nucleotides, and primer)(if dNTP used you will simply form double stranded DNA without a chain reaction)(use ddNTP instead so the reaction will stop at certain points) and to do this you set up four reactions which all contain dNTP and then each of the four contains one of the (ddTTP, ddATP, ddCTP, ddGTP) (sanger sequencing/dideoxy-sequencing) and after performing this PCR you run the gel – gel will have four wells where samples from each of the four forms are loaded – the smallest strands/the ones that travel the farthest are that nucleotide (the ddNTP) next in the unknown sequence (don't need to do this with gel anymore – can now use colored ddNTPs and use a chromatograph method)

Conclusion:
The predicted size of our amplicon of 457 bp was observed in the Sanger Sequencing results. The nucleotide sequence we obtained from Sanger Sequencing was almost a complete match to our predicted Tip60 nucleotide sequence with only a few minor differences. The differences, however, were synonymous in that they coded for the same amino acids.

Next step:
To make dsDNA of our gene using TOPO-RNAi primer containing T7 in the primer.

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 3.29.18
Week#: 8

Objective:
Kill E.Coli cells and harvest plasmid DNA for sequencing. We will also determine the DNA concentration.

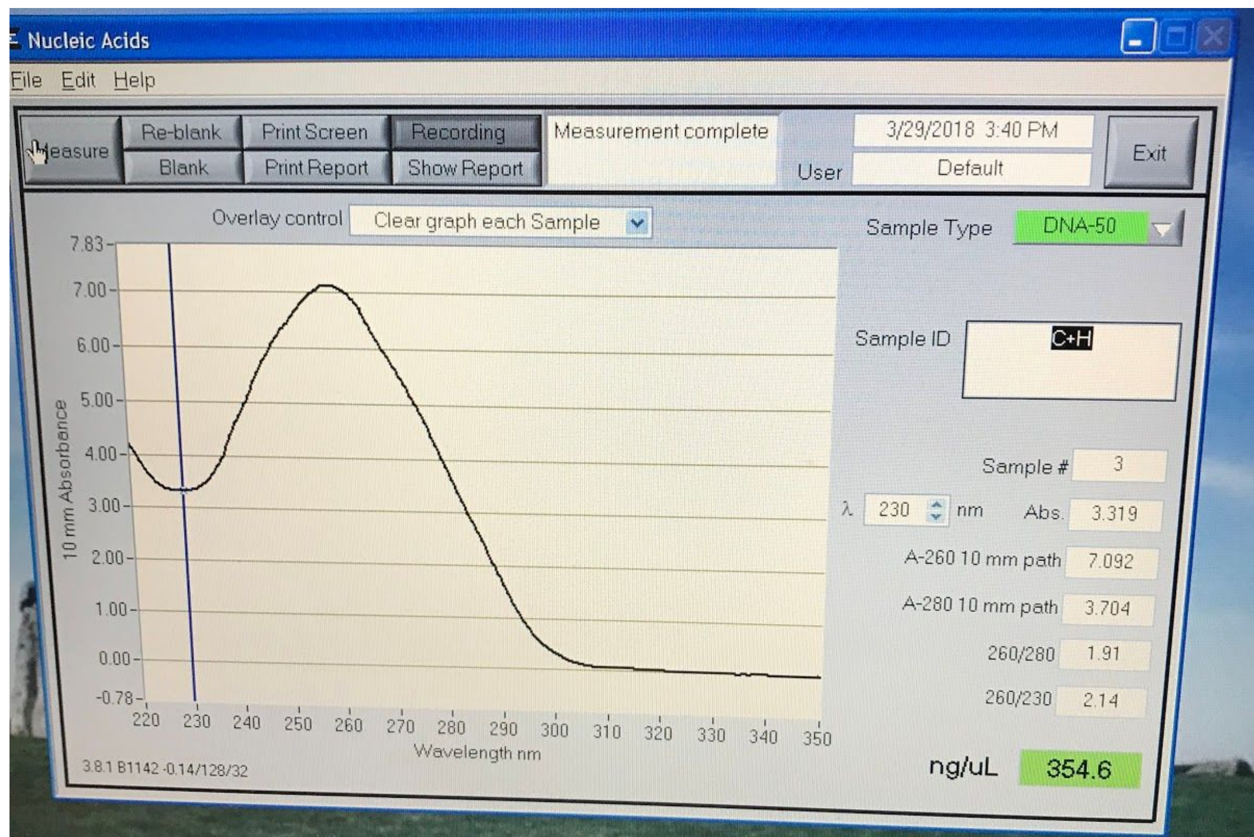
Procedures:
-we selected culture tube 2
-From larger culture tube 2000µl (2ml) of the E.Coli and culture media was transferred to 2ml and centrifuged for 3 min @ 12,000rpm
-after centrifuged we dumped excess liquid leaving only pellet in tube
-then added 2000µl from larger culture tube again on top of pellet and centrifuged for 3 min @12000rpm
-after centrifuge was completed the excess culture media was dumped off leaving only a pellet
-pellet was resuspended with 250µl of P1 and vortexed
-then 250µl of P2 was added and gently inverted to mix for 1 min
-after 1 min 350µl of N3 was added and tube was placed on ice for 10min
-after 10 min on ice the sample was centrifuged for 10min at 13,000 rpm
-added 750µl of supernatant to a QIAprep 2.0 spin column
-placed QIA prep 2.0 spin column on vacuum pump with vacuum manifolds
-centrifuged for 1 min @ 10,000 rpm
-placed QIA prep 2.0 column in a clean 1.5ml microcentrifuge tube and added 50µl buffer EB and let stand for 1 min before centrifuging @12,000 rpm for 1 minute
-threw out column and vortexed and centrifuged 1.5 ml microcentrifuge tube
- Performed NanoDrop of plasmid DNA to determine the concentration (2µl of sample was loaded onto the NanoDrop Apparatus)
- Our plasmid DNA concentration was 354.6ng/µl

Note:
To Harvest Plasmid, Overview: (see protocol pdf for more detail)

- E.Coli cells have genomic DNA and a ton of plasmids that carry the Tip60 gene. Remember E. Coli cells have proteins, lipids, nucleic acids and carbohydrates(sugars). We want to remove all macromolecules and purify only plasmid DNA. Will first centrifuge E.Coli cells tube with culture media and form a pellet to remove culture media from cells. Will add 2ml of cells and media into 2ml tube and centrifuge will repeat this process since we have more than 2ml of cells and media. The cells were centrifuged at

12,000 rpm. Then will resuspend pellet and increase pH to destroy the lipid membrane of the cell (Alkaline treatment of E. coli, cell membrane destroyed, and genomic DNA becomes fragmented as well and degrades; only do pH increase treatment for 1 min in order to keep plasmid intact and destroy genomic DNA because pH destruction is not specific). Before increasing pH, P1 is added to resuspend the cells and kill RNA. pH is increased by adding P2 and after treatment neutralized with N3. The will spin down and get a pellet like substance and solution. The solution contains the plasmid DNA and the pellet contains lipids, protein, and genomic DNA.

Below is an image of the Nanodrop results:



Conclusion:

Today we successfully purified our plasmid and obtained a concentration of 354.6ng/µl. Nanodrop analysis also revealed via a single peak graph that our sample was pure.

Next step:

Sequence the purified plasmid.

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 3.27.18
Week#: 8

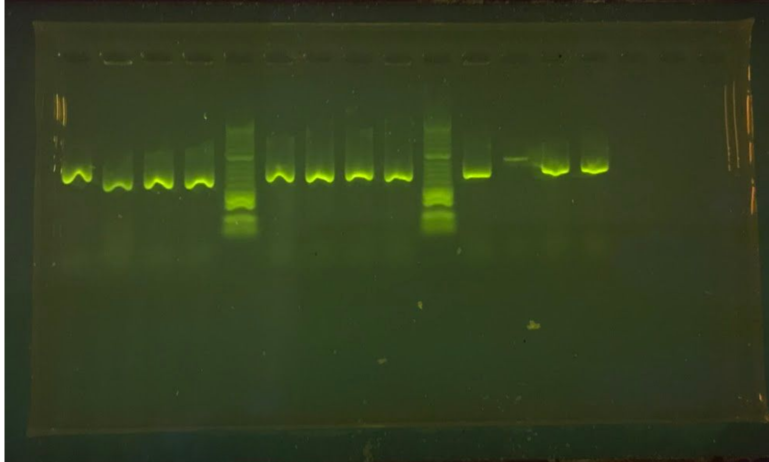
Objective:
Run gel electrophoresis to determine the results of TOPO cloning and colony PCR. If we receive a 100bp product after PCR our gene(Tip60) was not inserted into the plasmid. If we receive a product of 557bp our gene(Tip60) was inserted.

Procedures:
- Prepared 1.5% agarose gel (usually 1.2% agarose gel is prepared)
    - Mixed 1.2g of Agarose in 80ml of 0.5x TBE buffer with gel green
    - Microwave for 3 min at 30% power, take out and mix, then additional 1 min at 30% power (last min gets rid of air bubble) (do not grab by cap when mixing)
    - Pour into tray and waited __min to solidify
- 2µl of each of the four samples were loaded into PCR wells
- 5µl of a 50bp ladder was loaded into a well
- Gel was run at 100V for 30 min
- After analyzing samples we choose our reactions 2 and 3

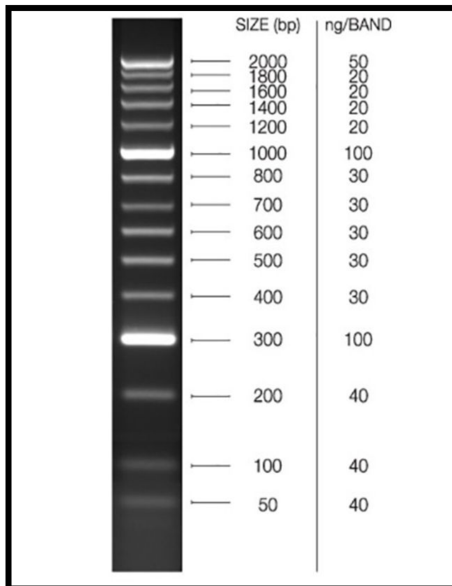
Note:
Gel Layout is found below:

1	2	3	4	ladder	1	2	3	4	Ladder	1	2	3	4
Angel and Saphira				50bp	Hunter and Connor				50bp	Kayla and Bradley			

Picture of gel results found below:



Picture of Ladder found below:



Conclusion:
We choose to continue with E.Coli colonies 2 and 3 after gel electrophoresis analysis. However any of 1-4 could have been used as they all came out to be around 600bp. With gel electrophoresis revealing 600bp large amplified regions the E.Coli likely contains the Tip60 gene.

Next step:
Purify plasmid from E.Coli and then Sequence. Overnight E.Coli cultures were spiked.

<u>**Lab notebook**</u>

Name: Hunter Gazda and Connor Huelsman
Date: 3.15.18
Week#: 7

Objective:
We will utilize the Colony PCR method to determine if our survived E.Coli cells contain a plasmid with our gene (TIP60).

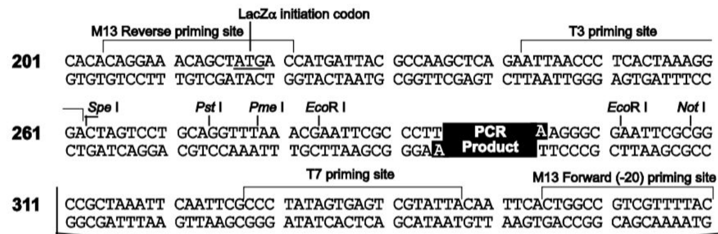
Procedures:
- picked large colony from plate and placed into 15μl LB+amp (1 colony into each tube) →4 tubes were prepared (labeled 1-4)→ cultured at 37 degrees Celsius for about 1 hr while mixing every 15min → will then use for PCR
- PCR reaction mixture was prepared with the below quantities.
- A master mix was created since we will be running four reactions (note: master mix contained everything except the colonies which will be added separately to each PCR tube)
- 18μl of the master mix was aliquoted into four separate PCR tubes

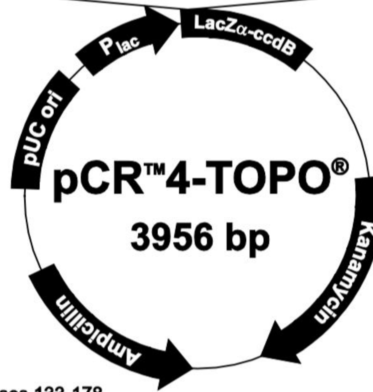
Item	Quantity (1rxn)	Quantity (4rxn)
H2O	8.15	32.6
Green Buffer	4	16
dNTP Mix	1.6	6.4
T3 primer(10μM)	2	8
T7 primer(10μM)	2	8
Go Taq	0.25	1
Colony	2	
Totals	20	

- After PCR aliquots were prepared 2μl of colony mixture were placed in the PCR tubes labeled (1-4)

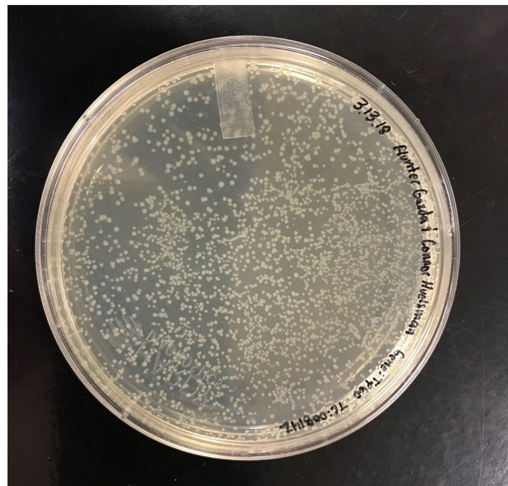
Note:
- The basis of Colony PCR is that the plasmids we used have two primer binding sites (T3 and T7) flanking the site where our gene would theoretically be inserted. Thus, we will use two primers to perform PCR amplification from the two primer binding sites. If we

receive a 100bp product after PCR our gene was not inserted into the plasmid. If we receive a product of 557bp our gene was inserted.





- Below is a picture of the agarose plate containing the surviving E.Coli colonies that were prepared and plated on 3.13.18



For PCR:

Reaction Cycle

Program name: Colony PCR
Denaturation: 95 ℃ X 5 min
↓
Denaturation: 94 ℃ X 30 sec

Annealing: _55_ °C X 30 sec 30 cycles
Extension: 72 °C X 30 sec
↓
Extension: 72 °C X 2 min —
↓
Hold: 4 °C

*2: Primer Tm + 1-2°C
*3: 500bp/30sec, 1,000bp/1min

Conclusion:
Colony PCR was set up and will be run after lab hours, which will determine if the selected E.Coli cells contain plasmids with the Tip60 gene. If we receive a 100bp product after PCR our gene was not inserted into the plasmid. If we receive a product of 557bp our gene was inserted.

Next step:
Run colony PCR and analyze length of amplified region to determine if the E. Coli cells contain plasmids with the Tip60 gene.

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 3.13.18
Week#: 7

Objective:
Today we will perform TOPO cloning by putting our DNA fragment into plasmid and then we will put the plasmid into E. coli.

Procedures:

- Mixed purified DNA (4µl), salt solution(1µl), and TOPO vector(1µl) by pipetting noted quantities into 1.5ml centrifuge tube to make our reaction mixture
- Incubated sample at room temperature for 30 min
- E.coli thawed on ice for approximately 10 min
- Pipetted 3µl of our reaction into thawed E.coli cells and slowly and gently mixed without pipetting up and down
- After 5 min of reaction mixture on ice we heat shocked reaction for 30 sec
- Then placed reaction mixture on ice for 5 min
- Pipetted 250µl SOC medium into reaction mixture containing E.Coli
- Incubated reaction mixture at 37C for 45min-1hr in the incubator
- Plated 120µl of our reaction mixture on antibacterial medium and spread around on it with hockey stick spreader
- Left plated colonies at 37C overnight

Notes:

- When taking a reaction ingredient from the freezer you always thaw, vortex, and spin down
- Cloning Isolates gene and puts it in a plasmid to have gene for the future
- Using TOPO cloning → We don't need restriction enzymes or ligase for this
- Plasmid contains ccdB gene which is disrupted when our DNA fragment is introduced into plasmid
- Our plasmid will have antibacterial resistance to ampr
- Thus, E.coli with our desired gene incorporated into a plasmid will be selected because only the E.Coli surviving ampr and not killed by the uninterrupted ccdB contain our desired plasmid

Conclusion:
TOPO cloning was performed to introduce our gene into a plasmid and then transformed into E.Coli

Next step:

The plasmids that took up the TIP60 gene and were introduced into the E.coli will survive overnight and then can be isolated.

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 3.8.18
Week#: 6

Objective:
Run gel electrophoresis to purify our gene Tip60
-We will choose one primer pair to extract
-Will run the gel, extract the DNA and gel, then freeze and squeeze

Procedures:

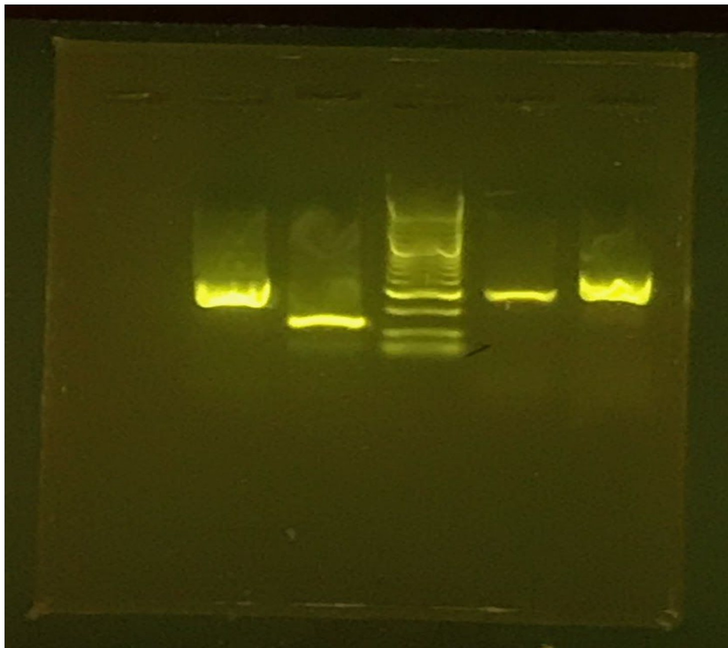
- Gel was made by another group (1.2% agarose)
- 20µl of primer pair 1 PCR reaction (F1R1) and 20µl of primer pair 2 PCR reaction (F2R2) were pipetted into two separate gel wells
- 5µl of of ladder was pipetted into wells
- Gel was run for 25 min @ 100V
- We chose to extract primer pair 2 (F2R2) fragment → using an extractor
- Then placed extracted sample in a basket centrifuge tube and placed in the freezer for 5 min
- Then centrifuged sample at 13000 rpm for 3 min ("squeeze step")
- After centrifuge the basket was removed and the DNA sample was placed in the freezer

Note:

- 1.2 % gel, 50ml/gel
- When preparing gel in microwave (3 min @ 30% → shake well → 1 min @ 30%)

Gel Layout:

		Blank	Other Group	Other Group	Ladder	F1R1 (Tc008142)	**F2R2 (Tc008142)**



Conclusion:

We chose to extract primer pair 2 (F2R2) because primer pair 2 has a longer amplicon length and it showed up very well on the gel indicating we had a large amount to work with

Next step:

Cloning

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 3/6/18
Week#: 6

Objective:
Today we will review how to calculate and then perform a primer dilution. We will then review PCR and set up a PCR reaction.

Procedures:
- Performed Primer Dilution for primer pair 1 (F1 and R1) and primer pair 2 (F2 and R2) (table for dilution values included in notes section) to make four stock diluted solutions each with a concentration of 100µM
    - 242µl of H_2O was pipetted into 24.2 nmol of primer F1
    - 190µl of H_2O was pipetted into 19 nmol of primer F2
    - 266µl of H_2O was pipetted into 26.6 nmol of primer R1
    - 317µl of H_2O was pipetted into 31.7nmol of primer R2
- After performing initial dilutions, 50µl of 10µM aliquots of each 100µM diluted solution was prepared (details on dilution math included in notes section)
    - To create aliquots 5 µl of each of the four 100µM stock solutions was pipetted into separate microcentrifuge tubes each containing 45 µl of H_2O
- To begin PCR need: (H_2O, Buffer, dNTP mix, Primer F1/F2, Primer R1/R2, template cDNA, Taq)
- Prepared two PCR reactions which consisted of primer pair 1 (F1 and R1) and primer pair 2 (F2 and R2) → Specifics on quantities of substrates added are included in notes
- When preparing PCR reactions tubes, a master mix was first created by pipetting 30.5µl of H_2O to a microcentrifuge tube. Then, 10µl of 5X PCR buffer was pipetted followed by 4µl of dNTP mix and 1µl of cDNA. The microcentrifuge tubes were then vortexed and spun down before pipetting 0.5µl of Taq polymerase into the microcentrifuge tube. 23µl of the master mix was then pipetted into two PCR tubes before 2µl of a primer pair was pipetted into one of the two PCR tubes. (note: the master mix solution was kept on ice during preparation)

Note:
- When talking about primer pairs F(1 or 2) and R (1 or 2) refers to the forward and reverse primer respectively.
- Dilution values for the primer dilutions for primer pair 1 and primer pair 2 to make 100µM solutions below:

	TC008142_F1	TC008142_F2	TC008142_R1	TC008142_R2
Initial amount	24.2 nmol	19 nmol	26.6 nmol	31.7 nmol
ddH20 added	242 µL	190 µL	266 µL	317 µL

These values were found based on the nmol that was given from the manufacturer, and so this was the ratio we came up with to get the samples to 100µM.

- To create 50µl of 10µM concentrated aliquots 5µl of each 100µM concentrated F1, F2, R1, and R2 solution was mixed with 45µl of H2O → To calculate how much of each 100µM concentrated F1, F2, R1, and R2 solution to add along with how much water the equation: C1V1=C2V2 was used:

C1V1=C2V2
(100µM)(V1)=(10µM)(50µl)
V1=5µl=amount of 100µM concentrated solution
50µl - 5µl = 45µl = amount of H2O added

- **Specifics on quantities of substrates added for PCR reactions found below:**

PCR Reaction 1

Date: 3/6/18
Aim: Amplify the primers F1 and R1

Gene name: Tip60

Forward Primer (F1): Tm= 59.90 ℃: sequence 5'--TGTGTTTGTTGGCGAAGCTG

Reverse Primer (R1): Tm= 59.96 ℃: sequence 5'--GCTTGTGCCCAGTAACTCCT

Reaction

H_20	___µl	30.5 µl
5 X PCR buffer	20µl	10 µl
dNTP mix (2.5mM stock)	8µl (0.2µM)	4 µl
Primer1 (10µM stock)	___µl (__µM)	2 µl (10 µM)
Primer2 (10µM stock)	___µl (__µM)	2 µl (10 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

Reaction Cycle

Program name:
Denaturation: 95 ℃ X 5 min
↓
Denaturation: 94 ℃ X 30 sec
Annealing: _*2_℃ X 30 sec
Extension: 72 ℃ X _30_m / s
↓
35 cycles

Result

Electrophoresis:__%agarose,_X TAE/ TBE
Loaded sample ___µl / lean

Extension: 72 ℃ X 5 min —
↓
Hold: 4 ℃

*2: Primer Tm + 1-2℃
*3: 500bp/30sec, 1,000bp/1min

PCR Reaction 2

Date: 3/6/18
Aim: Amplify the primers F2 and R2

Gene name: Tip60

Forward Primer (F2): Tm= 60.11 ℃: sequence 5'--GGAGGAAATCGACGACAGCA

Reverse Primer (R2): Tm= 60.03 ℃: sequence 5'--CATCTGTGTGGGCCCATCTT

Reaction

H_20	___µl	30.5 µl
5 X PCR buffer	20µl	10 µl
dNTP mix (2.5mM stock)	8µl (0.2µM)	4 µl
Primer1 (10µM stock)	___µl (__µM)	2 µl (10 µM)
Primer2 (10µM stock)	___µl (__µM)	2 µl (10 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

Reaction Cycle

Program name:
Denaturation: 95 ℃ X 5 min
↓
Denaturation: 94 ℃ X 30 sec
Annealing: _*2_℃ X 30 sec — 35 cycles
Extension: 72 ℃ X 30 m / s
↓
Extension: 72 ℃ X 5 min
↓
Hold: 4 ℃

*2: Primer Tm + 1-2℃
*3: 500bp/30sec, 1,000bp/1min

Result

Electrophoresis:__%agarose,_X TAE/ TBE
Loaded sample ___µl / lean

Conclusion:

Initially, the four primers (F1, R1, F2, R2) were successfully diluted to 100µM and then each 100µl primer was used to create a 50µl of 10µM aliquot. Two PCR reactions, one for each primer pair, was successfully set up and stored until PCR will be run later at night.

Next step:
Run the two PCR reactions.

Lab notebook

Name: Hunter Gazda and Connor Huelsman
Date: 3/1/18
Week#: 5

Objective:
To review and better acquaint ourselves with the details of gel electrophoresis, as well as practice the techniques. We will determine the size of three unknown DNA sample sizes.

Procedures:
Making the gel: (will make a 1.2% gel)

- 1.2% gel made using 0.5x TBE buffer.
- 0.3g (0.6g for two) agarose in 25 mL (50ml for two gels) of 0.5x TBE buffer and gel green.
- Microwave for 3 min at 30% power, take out and mix, then additional 1 min at 30% power (last min gets rid of air bubble)
- Pour into tray
- DNA samples loaded into 8 different wells: A, B, C, 50 bp ladder, A, B, C, and one left blank

Diluting DNA samples: (want 10uL @ 10ng/uL)
A: 82ng/uL → (100ng) (1uL/82ng)= 1.22
B: 118 ng/uL → (100ng) (1uL/118ng)= 0.85
C: 115ng/uL → (100 ng) (1uL/115ng)= 0.87

Diluting DNA samples:

-

Sample	vol/sample (uL)	vol/ sample ddH20 (uL)
A	1.22	8.8
B	0.85	9.15
C	0.87	9.13

- Calculating the amount of loading dye added (5x):
  (x/10+x) = ⅕
  x= 2.5 uL

Note:

Gel Electrophoresis review:

- Separates molecules by size and charge
- Essentially a mesh network - can control size of mesh by changing concentration of the gel (1% gel = 1kb-5kb; if bigger fragment then 0.7% = 3kb-10)(the percent is the weight per volume: 1g/100ml)
- DNA is negatively charged (PO_4^{3-} group)
- Ladder is used a reference to determine size of your loaded molecules
- We want to use 1x TAE or 1xTBE(for TBE could further dilute to 0.5x)
- Need to mix sample with loading die(which is usually 5x)
- Use ethidium bromide (EtBr) to die DNA after it has run (EtBr is supposed to get in between the double helix of the DNA)
- 5 uL of the ladder was used
- Order from top right of gel to the left: A, B, C, ladder

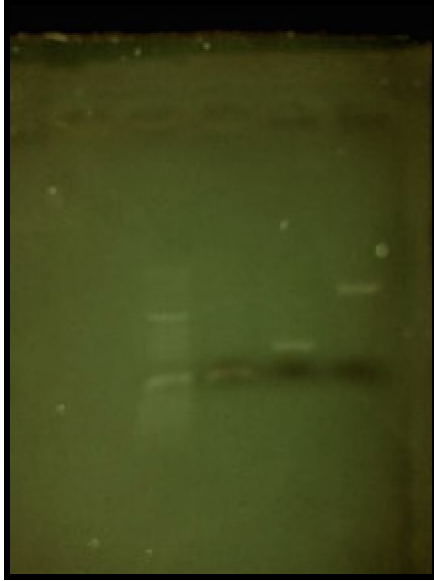
Conclusion:

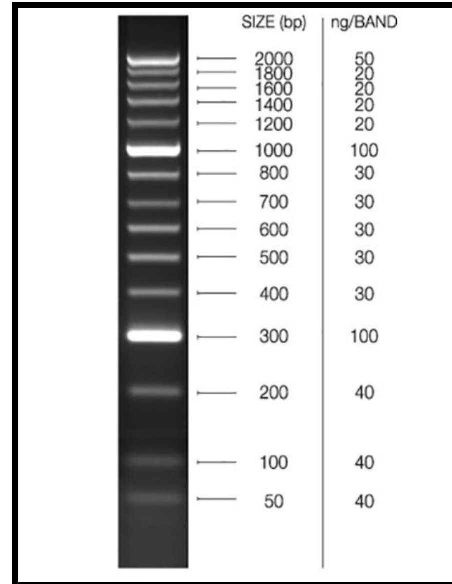
Our results did not come out very clearly due to the gel green not working very well.

Next step:

Will dilute our primers to 100 uM and possible re-run a gel to verify correct conditions

Images:





Lab notebook

Name: Hunter Gazda & Connor Huelsman
Date: 2/27/18
Week#: 4

Objective:
To be familiar with and practice with a pipette, and understanding the technique of proper pipetting and the correct pipettes in situations.

Procedures:

- Began practicing with p1000 by transferring 400ul into a different tube
- Then practiced with the p200 by transferring 100ul into a different tube
- Then practiced with p20 by transferring 10ul into a different tube
- The practiced with p10 by transferring 1ul into a different tube

Note:

P1000: (200-1000)
P200: (20-200)
P20: (2-20)
P10: (.1-2)

- Practiced pipetting different amounts of water and food coloring into test tubes.

Conclusion:
Basic pipetting procedures were reviewed and practiced

Next step:
Start working with real experimental materials