Lab notebook

Name: Dani and Jordan
Date: 5/8/2018
Week #10

Objective: Final observation of beetles
Procedures: N/A
Notes: All beetles have matured. 17 survived to adulthood. A few adult mortalities but most look healthy. Appear to be of a normal size.

Name: Dani and Jordan
Date: 5/3/2018
Week #9

Objective: Observe injected pupae and larvae
Procedures: N/A
Notes: Most beetles have matured into adults and are healthy but do not appear to be smaller.

Name: Dani and Jordan
Date: 4/26/2018
Week #8

Objective: Observe injected pupae and larvae
Procedures: N/A
Notes: No new pupae deaths observed and 1 adult beatle. Unclear whether beetles will end up being smaller in size. 15 pupae, 2 larvae and 1 adult. 1 dead larvae.

Name: Dani and Jordan
Date: 4/24/2018
Week #8

Objective: Observe injected pupae and larvae
Procedures: N/A
Notes: 18 were injected, 15 living pupae, 2 living larvae, 1 dead larva
Pupae do not appear smaller than the wild type. GFP looked like wild type.

Name: Dani and Jordan
Date: 4/17/2018
Week #7

Objective:
1. Observe different stages of the red flour beetle

Procedures:
1. Place dish with the specific stage you want to observe under microscope
2. Focus to see clearly
3. Draw what you see

Notes: Got nice pictures of the adult beetles.

Conclusion:

Drawing can be hard, but at least we have photography. Should have used something to help provide a scale so we can compare the morphological changes later.

Up Next:

Name: Dani and Jordan
Date: 4/12/2018
Week #6

Objective:
1. Complete nuclease digestion to Remove DNA and ssRNA
2. Purify the dsRNA

Procedures:

1. Assemble RNase digestion reaction on ice

2ul	dsRNA
21 ul	Nuclease-free water
5ul	10x digestion buffer
2ul	DNase I
2ul	RNase

2. Gently mix the reaction by pipetting up and down
3. Assemble the dsRNA binding mix by adding 10x Binding buffer, water, and 100% ethanol to the dsRNA according to the table below

50ul	dsRNA
50ul	10x Binding Buffer
150ul	Nuclease-free water
250ul	100% ethanol

4. Gently mix the reaction by pipetting up and down
5. Place mix into a filter column and centrifuge for 2 minutes at 1300 power
6. Pipette 500 ul of wash solution onto the filter in the Filter Cartridge. Draw the wash solution through the filter as in the previous step.
7. Repeat with 500ul of wash solution.
8. After discarding the wash solution, centrifuge again for 1 minute to remove the last traces of liquid.
9. Apply room temperature elution solution , close the tube lid over the filter cartridge, and incubate in a heat block set to 70 degrees celcius or warmer for 10 minutes
10. Centrifuge for 2 minutes at maximum speed
11. Repeat steps 9-10

Notes:

DNase and RNase are used because they will get rid of DNA and ssRNA without getting rid of the dsRNA

Conclusion:
Up Next: Our beetles will be injected soon!

Name: Dani and Jordan
Date: 4/10/2018
Week #6

Objective:

1. Purify PCR

Procedures:

1. Add 1000ul Buffer PB to 200ul of the PCR reaction and mix. If the color of the mixture is orange or violet, add 10ul 3M sodium acetate, pH 5.0 and mix
2. Mix and vortex
3. Place a QIAquick column in a provided 2ml collection tube
4. To bind DNA, apply the sample to the QIAquick column of 600ul each and apply vacuum to the manifold until all the samples have passed through the column
5. To wash, add 0.75 buffer PE
6. Centrifuge for one minute at 13,000 rpm
7. Add 30ul of the dilution buffer, allow to sit for one minute, then centrifuge for one minute
8. Add the following together in a PCR tube

H2O	0ul
10x Buffer	2ul
Template	(1.5ug)
T7 RNA pol	2ul
NTPmix	8ul
Total	20ul

Notes:

To find the minimum concentration of the template needed to obtain 1.5ug ? ng/ml:

1500ng/8ul = 187.5 ng/ml

We obtained a concentration of 133.88. Due to this low concentration, we decided to not dilute the sample and add 8ul of the Template DNA.

Conclusion:

Up Next: Purify dsRNA

Name: Dani and Jordan
Date: 4/5/2018
Week #5

Objective:

1. Complete a miniprep of obtained colony samples to purify plasmid

Procedures:

Miniprep

1. Place 2ml of solution into a tube, centrifuge to obtain a pellet. Add 2 more ml and repeat to obtain a larger pellet.
2. Resuspend pelleted bacterial cells in 250ul Buffer P1 and transfer into a microcentrifuge tube.
3. Add 250ul buffer P2 and mix thoroughly by inverting the tube 4-6 times
4. Let P2 sit for 1 minutes at room temperature
5. Add 350ul buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times, then incubate on ice for 10 minutes
6. Centrifuge for 10 minutes at 13,000 rpm
7. Apply 750ul of the supernatant to the QIA 2.0 spin column by pipetting
8. Centrifuge for 30-60 seconds
9. Wash QIA spin column by adding 0.5ml buffer PB and centrifuging for 30-60 seconds, discard flow through
10. Wash QIAprep spin column by adding 0.75 ml Buffer PE and centrifuging for 30-60 seconds
11. Discard flow through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer
12. Place QIA column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 30ul Buffer EB or water to the center of each spin column, let stand for 1 min and centrifuge for 1 min

PCR

1. In a 1.5ml tube, make a solution of plasmid DNA with 40ul of 10 ng/ul
    a. Add the following to your primer to get a total volume of 200ul

Addition	Amount
H2O x	102ul
5x Buffer x	40ul
dNTP	16ul
primer	30ul
plasmid DNA	10ul
Go Taq	2ul (use p20)
Total	**200ul**

2. Split the 1.5ml tube into 8 samples of 25ul in a PCR tube

Notes:
Concentration 1- 317.73
Concentration 2- 308.92

Conclusion: We finally caught up!

Up Next: Purify plasmid

Name: Dani and Jordan
Date: 4/3/2018
Week #5

Objective: Sequence gene
Procedures:
Notes: The primers could not be found in the sequence provided so it's highly likely that the company did not send the correct sequence to us.
Conclusion:
Up Next: Miniprep and catching up

Name: Dani and Jordan
Date: 3/29/18
Week #4

Objective: To obtain a plate of colonies with our DNA plasmid

Procedures:

1. Vortex and spin down DNA solution
2. Add 4ul purified DNA, 1ul salt solution and 1ul TOPO vector to form 6ul of solution
3. Let set at room temperature for 30 minutes
4. Add 4ul of solution into e coli cells
5. Set on ice for 5 minutes
6. Heat shock for 30 seconds
7. Set on ice for 5 minutes
8. Add 250 ul SOC into cell
9. Let solution set for 45 minutes, label and tape it to the boogie woogie machine
10. Plate

Notes:

40(10ng) = 317.73v
1.26ul = 1.3ul

Conclusion:

Up Next:

Name: Dani and Jordan
Date: 3/27/18
Week # 4

Objective: Re-run gel electrophoresis with new PCR and extract sample to be plated

Procedures:
Making the gel

1. Mix 30ml of mixed died TAE and 0.45g powder
2. Microwave for 3 minutes at 30% power
3. Mix
4. Microwave for 1 minute at 30% power
5. Pour gel into mold and allow to sit for 30 minutes

Running the gel

1. Add 20 ml of each solution into each well
2. Add 5 ml of ladder to the well in between each group
3. Run the gel for
4. Extract the band from the gel
5. Freeze the extracted DNA for 5 minutes
6. Centrifuge the sample

Notes:

Make sure to hold the bottom of the flask to avoid breaking it!

Picture of the newly ran electrophoresis, our two samples are on the right

Conclusion: PCR reaction was successful and gel produced a much better result

Up Next:

Topo reaction

Name: Dani and Jordan

Date: 3/15/2018

Week #3

Objective:

1. Amplify dna using PCR and purified fragment as a template

Procedures:

1. Add solutions according to this chart

Addition	Amount
H2O x	61µL
5x Buffer x	20µL
dNTP Mix x	8µL
Primer F	4µL
Primer R	4µL
Template cDNA	2µL
Taq	1µL
Total	**100µL**

2. Vortex and spin down after each addition
3. Divide into four solutions, 25µL each.

Notes;
PCR procedures were redone to amplify the gene but used the DNA fragment instead of cDNA
Conclusion:

Up Next:
Hopefully PCR works so we can put the DNA in the plasmid!

Name: Jordan and Dani
Date: 3/13/2018
Week#: 3

Objective: (in one or two sentences)

1. Run gel electrophoresis to get pure DNA sample
2. Clone DNA

Procedures: (bullet point the procedures)

1. Put 20ul of each sample into the gel
2. Run electrophoresis for 25 min
3. Extract the gel with the chosen DNA fragment
4. Put extraction in the tube
5. Centrifuge for 3 minutes at 13000 rpm
6. Add 4ul purified DNA, 1ul salt solution and 1ul TOPOvector to form 6ul of solution
7. Let set at room temperature for 15 minutes
8. Add 4ul of solution into ecoli cells
9. Set on ice for 5 minutes
10. Heat shock for 30 seconds
11. Set on ice for 5 minutes
12. Add 250 ul SOC into cell
13. Let solution set for 45 minutes
14. Plate

Note:

Conclusion: Colonies did not form.

Next step: Try again.

Name: Jordan and Dani
Date: 3/8/2018
Week#: 2

Objective: Mix solution for PCR

Procedure:

4. Add solutions according to this chart

Addition	Amount
H2O x	30.5µL

5x Buffer x	10µL
dNTP Mix x	4µL
Primer F	2µL
Primer R	2µL
Template cDNA	1µL
Taq	0.5µL
Total	**50µL (w/o primer 46)**

5. Vortex and spin down after each addition
6. Divide into two solutions of 23 each and then add the 2µL of primer afterwards, keeping the solution in ice as much as possible

Notes:
PCR didn't go as expect so needed to be redone. But it worked the second time!

Date. 3/6/2018
Aim:

Gene name: TC002602

Forward Primer: Tm= 59.8 ℃: sequence 5'ACCTCCAGGAGCACAAACAG

Reverse Primer: Tm=59.97 ℃: sequence 5'CAAGATCCGAATACCGGCGA

Reaction

H_20	___µl	30.5µl
5 X PCR buffer	20µl	10µl
*$MgCl_2$ (25mM stock)	6µl (1.5µM)	0µl (__µM)
dNTP mix (2.5mM stock)	8µl (0.2µM)	4µl
Primer1 (10µM stock)	___µl (__µM)	2µl (__µM)
Primer2 (10µM stock)	___µl (__µM)	2µl (__µM)
Sample DNA ( )	___µl	1µl
Taq polymerase (5U/ µl)	0.5µl (2.5U /100µl)	0.5µl
Total	100µl	50µl

*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 __ cycles
Extension: 72 ℃ X _*3_ 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: PCR solution was prepared. The F1R1 primer set was not successful so the primer we will use is the F2R2 set.

Next Step: Run PCR and then use gel electrophoresis to separate DNA fragments

Name: Jordan and Dani
Date: 3/6/2018

Week#: 2

Objective:

1. Dilute primer to 100 µM then 50 µL of 10 µM
1. Mix solution for PCR

Procedure:

1. Dilute primer by adding specific amounts of water to form the stop solution that is 100µM/1L
2. Vortex each solution and then spin them down to ensure no solution is on the sides
3. Dilute once again by adding 5µ solution and 45ul of water to the working solution to have 50µl of 10µM
7. Vortex and spin down the solution once again

8. Add solutions according to this chart

Addition	**Amount**
H2O x	30.5µL
5x Buffer x	10µL
dNTP Mix x	4µL
Primer F	2µL
Primer R	2µL
Template cDNA	1µL
Taq	0.5µL
Total	**50µL (w/o primer 46)**

9. Vortex and spin down after each addition
10. Divide into two solutions of 23 each and then add the 2µL of primer afterwards, keeping the solution in ice as much as possible

Notes:

100µmol/1L
100 nmol/1ml

F1: 19nm/190µL
F2: 37.6nm/376µL
R1: b 21.5nm/215µL
R2: 27.3nm/273µL

Date. 3/6/2018
Aim:

Gene name:

Forward Primer: Tm= 59.8 ℃: sequence 5'ACCTCCAGGAGCACAAACAG

Reverse Primer: Tm=59.97 ℃: sequence 5'CAAGATCCGAATACCGGCGA

Reaction

H_20	___µl	30.5µl
5 X PCR buffer	20µl	10µl
*$MgCl_2$ (25mM stock)	6µl (1.5µM)	0µl (__µM)
dNTP mix (2.5mM stock)	8µl (0.2µM)	4µl
Primer1 (10µM stock)	___µl (__µM)	2µl (__µM)
Primer2 (10µM stock)	___µl (__µM)	2µl (__µM)
Sample DNA ( )	___µl	1µl
Taq polymerase (5U/ µl)	0.5µl (2.5U /100µl)	0.5µl
Total	100µl	50µl

*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 __ cycles
Extension: 72 ℃ X _*3_ 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: The primers were successfully diluted and the PCR solutions were prepared

Next Step: Run PCR and then use gel electrophoresis to separate DNA fragments

Name: Jordan and Dani
Date: 3/1/2018
Week#: 1

Objective: (in one or two sentences)
To determine the size of our sample using gel electrophoresis and get practice with it.

Procedures: (bullet point the procedures)

15. Dilute each sample to be 10 ul of 10ng/ul using C1V1=C2V2
16. Add the correct amounts of DNA and water to each tube
17. Add 2.5ul of 5x dye to each solution
18. Place 10ul of each solution into each well
19. Run the gel for around 25 minutes at 135 V
20. Take a picture of resulting bands and determine sizes.

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.

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

Get to concentration 10 ng/ul

C1V1= C2V2 (10x10)

A: 1.2ul DNA, 8.8ul Water
B:0.85ul DNA, 9.15ul Water
C: 0.87ul DNA, 9.13ul Water

Add 2ul of the dye because

$$\frac{x}{\text{Total volume} = 10 + x} = \frac{1}{5}$$

Malfunction with gel machine, results may be skewed because we were rushed.
Gels weren't very visible, probably due to degradation of dye. so used gel from another group.

Conclusion:

Next step:
Dilute Primers!