Lab notebook

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Date: 03/01/2018
Week#: 4

Objective: To create a gel and to practice gel electrophoresis

Procedures:

Making the gel

- 50 ml of gel green was added to a beaker (25 ml each)
- 6 g of agarose was measured and added to the beaker
- Mixture was heated in a microwave for 3 minutes at 30% power
- Flask was removed from microwave and was mixed by shaking flask
- Mixture heated in microwave again for 1 minute at 30% power to get rid of air bubbles
- Mixture was poured onto gel matrix and left to cool

Gel Electrophoresis and Prep

- 3 tubes were labeled “A,” “B,” and “C”
- 1.22 ul of 82 ng/ul DNA and 8.78 ul H2O was added to tube “A”
- 0.85 ul of 118 ng/ul DNA and 9.15 ul H2O was added to tube “B”
- 0.87 ul of 115 ng/ul DNA and 9.13 ul H2O was added to tube “C”
- 2.5 ul of EeBr dye was added to each tube
- Water was sprayed onto gel and well separator was removed by wiggling out
- 10 ul from each tube was added to a different well on the gel (Wells 1-3 right gel)
- 5 ul of DNA ladder added to center well (well 4 right gel)
- Add 0.5x TBE to gel box
- Gel was run at 100 V for 25 minutes
- After 25 minutes, remove gel and place over blue light to view results

Notes:

Math: M1V1=M2V2
Tube A
(82 ng/ul)(V2)=(10 ng/ul)(10 ul)
(V2)=(100 ng/ 82 ng/ml)
V2= 1.22 ul VH20=8.78 ul

Tube B

(118 ng/ul)(V2)=(10 ng/ul)(10 ul)
(V2)=(100 ng/ 118 ng/ul)
V2= 0.85 ul V H2O= 9.15 ul

Tube C
(115 ng/ul)(V2)=(10 ng/ul)(10 ul)
(V2)=(100 ng/ 115 ng/ul)
V2= 0.87 ul V H2O= 9.13 ul

Dye
X/(10+x)=
5x= 10+x
4x= 10
x= 2.5 ul dye

Our gel did not turn out well, it was essentially dim and a solid band across

Conclusion:
While just practice, this was important practice for when we perform gel electrophoresis on our beetle and fly genes.

Date: 3/6/2018
Week#: 5

Objective: To perform PCR on our gene

Gene name: COP9 signalosome complex subunit 1

Primer Set 1
Forward Primer: Tm=60.04: sequence 5'--CTGCGTCTGGAAGCCCTAAA

Reverse Primer: Tm=60.04: sequence 5'--TTCGAGTCTTTCGTCTGGGC

Primer Set 2
Forward Primer: Tm=59.90: sequence 5'--ACATGTACATCGCTCCGCAT

Reverse Primer: Tm=60.04□: sequence 5'--TTGCTTTTTAGAACCGCCGC

Reaction

H_20	___µl	___µl
5 X PCR buffer	20µl	___µl
*$MgCl_2$ (25mM stock)	6µl (1.5µM)	___µl (__µM)
dNTP mix (2.5mM stock)	8µl (0.2µM)	___µl
Primer1 (10µM stock)	___µl (__µM)	___µl (__µM)
Primer2 (10µM stock)	___µl (__µM)	___µl (__µM)
Sample DNA (_____)	___µl	___µl
Taq polymerase (5U/ µl)	0.5µl (2.5U /100µl)	___µl
Total	100µl	_____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 __ 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: 1.2%agarose,__X TAE/ TBE

Loaded sample ___µl / lean

Procedure:

- Each primer was diluted to 100 □M
  - 239 □l water added to primer F1
  - 290 □l water added to primer R1
  - 277 □l* water added to primer F2 (see notes)

    - 260 µl water added to primer R2
- Each primer was vortexed and centrifuged
- Clean tubes were labeled with TC# and R1, F1, etc.
- Each primer was diluted to 10 µM
    - 40 µl of water was added to tubes for F1, R1, and R2
    - 5 µl of 100 µM primers F1, R1, and R2 were pipetted into respective labeled tubes
    - 44.6 µl of water was added to the tube for F2
    - 5.4 µl of 100 µM primer F2 was pipetted into a labeled tube
- Primers were vortexed and centrifuged again
- Master mix was prepared in a clean tube on ice
    - 30.5 µl of water was added to the tube
    - 10 µl of concentrated PCR buffer was added to the tube
    - 4 µl of dNTP mix was added to the tube
    - 1 µl of template cDNA was added to the tube
    - 0.5 µl of Taq Polymerase enzyme was added to the tube

Notes:
*We accidently added 299 µl to the F2 primer. To compensate, we added 5.4 µl of the primer and 44µl of water to properly dilute to 10 µM.

Math:
F1:
100 µM = 23.8 nmol/(V)
V= 23.8 nmol / 100 µM
V = 239 µl of H2O

R1:
100 µM = 29.9 nmol/(V)
V= 29.9 nmol / 100 µM
V = 299 µl of H2O

F2:
100 µM = 27.7 nmol/(V)
V= 27.7 nmol / 100 µM
V = 277 µl of H2O
Added 299 µl on accident → diluted actually to 92.64 µM

R2:
100 µM = 26.0 nmol/(V)
V= 26.0 nmol / 100 µM
V = 260 µl of H2O

Dilution to 50 µl of 10 µM (For all primers except F2):

10 µM (50 µl) = 100 µM (V)
V = 5 µl of 100 µM solution
V of H2O = 45 µl

Dilution to 50 µl of 10 µM (For F2):
10 µM (50 µl) = 92.6 µM (V)
V = 5.4 µl of 92.6 µM solution
V of H2O = 44.6 µl

Conclusion:
We will be able to tell if PCR worked at the beginning of next lab, where we will run our amplified gene on gel.

Date: 03/08/2018
Week#: 5

Objective: To isolate DNA from our gel

Procedure:

- Gel was made using the procedure from Entry 1
- DNA was extracted from the gel using a freeze and squeeze extractor
- Sample was frozen in the tube by putting in the freezer
- Sample was centrifuged to remove gene from the sample

Notes:
We did not get enough DNA in our wells due to putting the tip of the pipette on the bottom. Thankfully, our first set of primers did have enough which we were able to extract.

Conclusion:
Next week we will be cloning our gene into plasmids. We should have enough DNA that it will not be an issue.

Date: 03/13/2018
Week#: 6

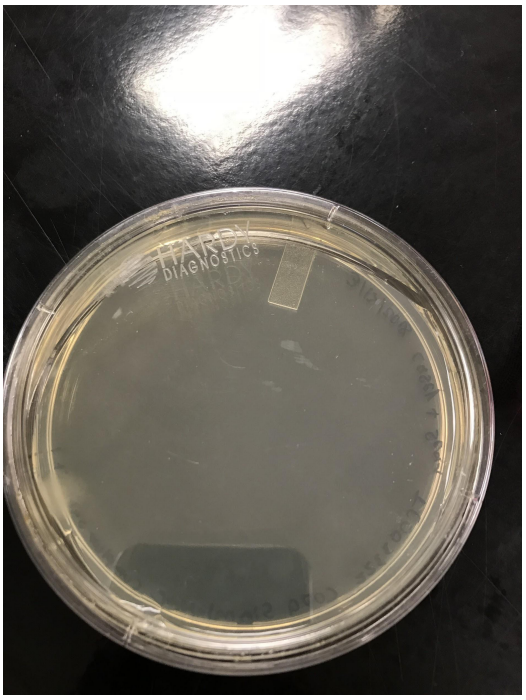
Objective: To clone our gene using TOPO cloning and then transform *E. coli*

Procedure:

- Frozen DNA was spun down
- 6 µl of solution was made in a 1.5 ml tube

    - 4 µl of purified DNA
    - 1 µl of salt solution
    - 1 µl of TOPO vector
- Reaction was allowed to sit for 30 minutes at room temperature
- *E. coli* was thawed on ice for 10 minutes
- 3 µl of reaction was added to the tube containing *E. coli*
- Tube was allowed to sit for 5 minutes on ice
- *E. coli* were heat shocked at 42°C for 30 seconds
- *E. coli* were left on ice for another 5 minutes
- 250 µl of SOC was pipetted into the tube containing *E. coli*
- *E. coli* were left at 37 degrees C for 45 minutes to one hour
- 120 µl of *E. coli* were plated on agar and left overnight

Results: We were not able to successfully grow any colonies (shown in picture of agar plate);



Conclusion
in the coming lab periods, we will redo our PCR, Gel electrophoresis and Isolation from Gel, and then finally cloning of our gene/transforming E. coli

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Date: 03/15/18
Week#: 6

Objective: To see if we successfully added our gene into the plasmid

Procedure:

- Colony PCR
- Pick colonies from plate into 15 l LB + amp

Results
No colonies grew

Conclusion
We will have to repeat the previous two entries

Date: 03/27/18
Week#: 8

Objective: To isolate our DNA again and run colony PCR on gel; determine which E. coli has correct fragment size (our gene)

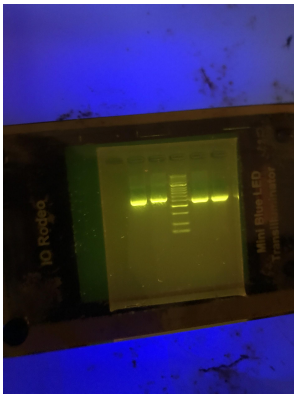
Procedure:

- Run all 4 colonies on gel with ladder (50 bp ladder)
- Choose two to culture in case one does not culture well

Notes:

- 0.7% gel = 3-10kbp; 1.0% gel = 1 - 5 kbp; 1.2 - 1.5% gel = 100 bp- 1 kbp;

Results:



Conclusion

We were able to successfully isolate our DNA and will run colony PCR again

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Date: 03/29/2018
Week#: 8

Objective: Purify plasmid from E. coli cells

Procedure:

- Harvesting plasmid
- Going to remove all macromolecules other than plasmid DNA and then purify plasmid DNA
- Add 2 ml of solution to 2 ml tube
- Centrifuge tube to collect all cells at bottom of tube → make pellet of cells
- Remove liquid
- Repeat to make pellet bigger (cells are still alive)
- Then suspend pellet in solution P1 (RNase)

- Increase pH by adding P2 to kill cells and degrade other macromolecules → control destruction by carefully timing how long pH is increased
- Increased pH for only 1 minute → keeps plasmid DNA intact
- After 1 minute, neutralize solution with N3
- Spin down again, plasmid DNA will be in solution and the other macromolecules will have precipitated to the bottom

Results:
Successfully purified and performed spec on our plasmid

Conclusion:
Next week we will be viewing our gene using SnapGene. This will show us if we did everything correctly.

Date: 04/03/2018
Week#: 9

Objective: Analyze sequence in SnapGene Viewer

Procedure:
- Sequences in FASTA format were uploaded to SnapGene Viewer
- Sequences were analyzed to determine which one was best to continue with

Results:
Our amplicon and primers were not present in our sequences.

Conclusion:
It is likely the company's fault rather than ours, since 3 other groups had the same issue.

Date: 04/05/2018
Week#:9

Objective: To purify plasmid from E. coli culture (miniprep) and then create a PCR master mix from the purified plasmid

Procedure: See above entry

Results
Successfully purified and performed spec on our plasmid

Conclusion
Our plasmid is isolated and we are ready to isolate our GOI

Date: 04/10/2018
Week#: 10

Objective: Purify our template DNA of our gene

Procedure:

- 200 microliters of PCR product was pipetted into a tube
- 1000 microliters of PB buffer was added to the tube
- Tube was vortexed to mix solution
- 600 microliters of solution was added to a QIAquick column and vacuumed
- 600 more microliters were vacuumed
- 750 microliters of PE buffer was used to wash
- QIAquick column was centrifuged for 1 minute
- DNA was eluted by adding 30 microliters EB buffer and centrifuged for another minute
- DNA was nanodropped and determined to be at 200 ug/ul
- Master mix was made by adding 8 microliters of NTPmix, 2 microliters of 10x buffer, 8 microliters of template DNA and 2 microliters of T7 RNA pol
- 8 microliters of purified plasmid and 12 microliters of master mix were added to a PCR tube and out on ice

Results:
Plasmid was purified to be about 200 ug/ul. The plasmid was added to the master mix and will be ready to use next lab.

Conclusion
The DNA containing our GOI was isolated and is now ready to undergo in vitro transcription

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Date: 04/12/2018
Week#: 10

Objective: To create double stranded RNA from our plasmid

Procedure:

- Master mix was created with the following:
    - 21 ul nuclease-free water
    - 5 ul 10x digestion buffer
    - 2 ul DNase I
    - 2 ul RNase

- 20 ul of dsRNA was added to the master mix
- Digestion reaction was incubated for 30 minutes
- dsRNA binding mix was made using:
    - 50 ul of dsRNA
    - 50 ul of 10x Binding Buffer
    - 150 ul of Nuclease-free water
    - 250 ul of 100% Ethanol
- Binding matrix was centrifuged at 1300 RPM for 2 minutes
- Flow through tube was discarded and replaced
- 500 ul of wash solution was added to the tube
- Tube was centrifuged for 2 minutes at 1300 RPM
- Last 3 steps were repeated
- Flow through was discarded and tube was centrifuged again at 1300 RPM for 2 minutes
- 50 ul of Elution buffer was added to the filter cartridge
- Cartridge was allowed to sit on a heat block (65 C) for 10 minutes
- Cartridge was centrifuged at 1300 RPM for 2 minutes
- Last 3 steps were repeated (DO NOT REPLACE TUBE)

Conclusion
Successfully created dsRNA and it is ready for injection into *Tribolium*

Date: 4/17/2018
Week#: 11

Objective: To observe the Tribolium life cycle for preparation to observe genetically modified beetles

Procedure:
- Beetles were observed under microscope at each stage of life cycle
- Sketches of adult beetles were made and leg/antenna segmentation was noted

Results: N/A

Conclusion
Gained a sound understanding of what wild type *Tribolium* look like at every larval stage. Important for attempting to observe LOF phenotypes

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Date: 4/24/18

Week#: 12

Objective: Review RNAi beetle phenotypes and compare to WT beetle phenotypes

Procedure:

- Beetles were observed under microscope at each stage of life cycle
- Phenotypes were compared

Notes:

- 18 beetle larvae were injected with double stranded RNA

Results:
There was no significant difference between the wild type pupae and our genetically modified pupae. This is expected, since our GOI affects leg and antennae development in adults.

Conclusion
Will observe beetles next week

Date: 4/26/18
Week#: 12

Objective: Observe RNAi beetle phenotype and compare to wild type beetle

Procedure:

- See previous entry

Notes:

- There are only 16 beetle pupae alive

Results:
2 beetle pupae had died since the previous lab. The remaining pupae have developed more, with some showing wing formation and more segmentation. There is still no obvious difference from the wild type pupae at this stage.

Conclusion
Will have to observe adult beetles next week

Date: 5/8/2018
Week#: 13

Procedure:

- Examine beetles under microscope

Notes:

- Beetle pigmentation is abnormal
- Elytra of beetles are translucent
- White mosaic pattern
    - Likely result of abnormality in another part of the beetle, not a direct effect on pigmentation
- Many beetles failed to eclose

Results:

Only 2 beetles managed to survive to adulthood. Those 2 beetles did not exhibit the RNAi phenotype. The ones that did exhibit the phenotype died before eclosing or right after. Elytra had a translucent/red striped pigmentation. It appears that the RNAi phenotype affects the development of the elytra attachment.

Conclusion

The white striped mosaic pattern appears in other beetles where the elytra were pinched and blood flow was stopped to the elytra. This could also explain the translucent pigmentation of the elytra, stopping pigment proteins from entering them.