Lab Notebook
Name: Kristina Taynor and Mikey Solomon

Week#: 12
Date: 4/25/2017

Objective:
Observe pupae and beetle forms

Procedures:
- Visual inspection

Note:
All pupae are dead without fully transforming into beetles

Conclusion:
FOXO is essential for beetle development

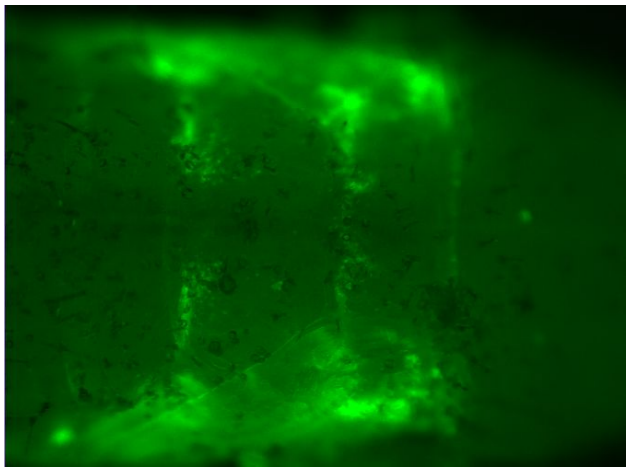
Next step:
Write-up results

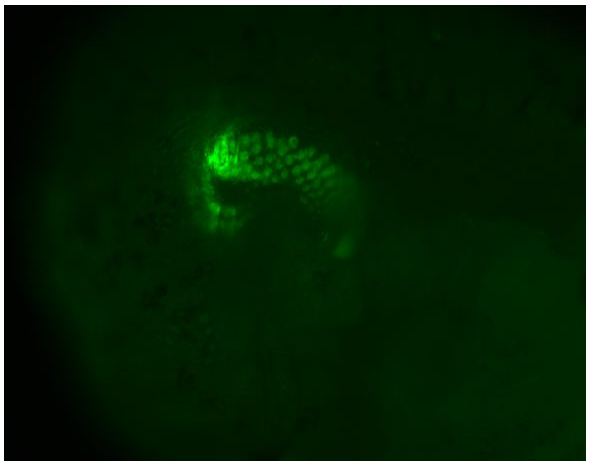
Week#: 11
Date: 4/20/2017

Objective:
Observe experimental pupae and larvae and compare morphology to WT.

Procedures:
- Visual observation and microscope pictures

Note:
Currently 10 pupae
Odd color - metabolic problems?
Odd GFP signal across back and around eyes





Next step:
Wait for beetles to transform

Week#:11
Date: 4/18/17

Objective:
Observe experimental larvae and compare morphology to WT larvae

Procedures:

- Visual observation of larvae and pupae

Note:
There are currently 3 larvae and 8 pupae

Next step:
Wait for beetles to develop

Week#: 10
Date: 4/6/17

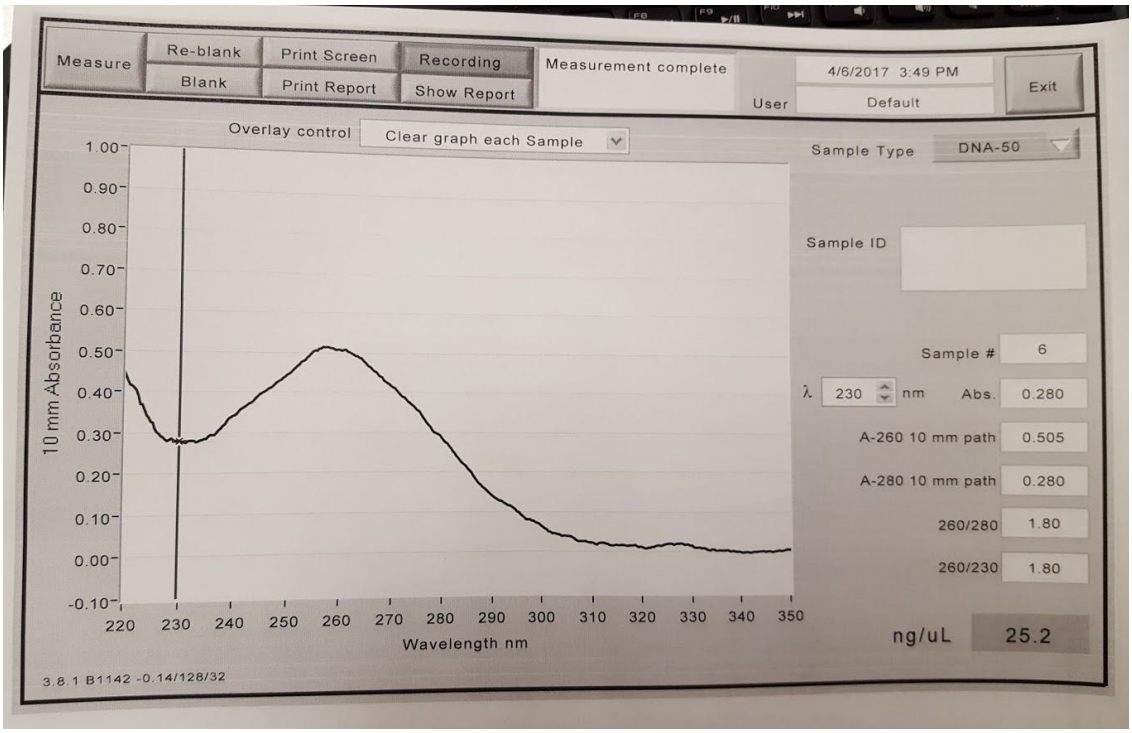
Objective:
We will clean up our dsRNA by removing DNA and ssRNA

Procedures:

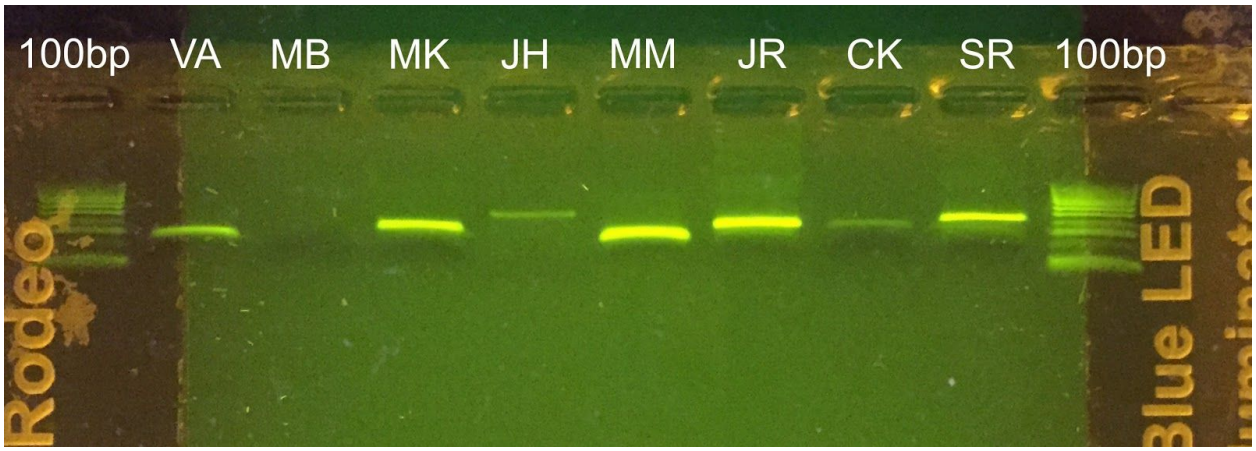
- Digest DNA and ssRNA
    - 20 uL dsRNA
    - 21 uL Nuclease-free water
    - 5 uL 10x digestion buffer
    - 2 uL DNase I
    - 2 uL RNase
    - Incubate at 37 C for 1 hour
- Purify dsRNA
    - Assemble dsRNA binding mix by adding 50 uL dsRNA, 50 uL 10X binding buffer, 150uL Nuclease-free water, and 250uL 100% ethanol then gently mix.
    - Pipet 500uL dsRNA mixture onto filter in filter cartridge then centrifuge at max speed for 2 min
    - Pipet 500uL wash solution onto filter and centrifuge 2 min @ 13,000rpm
    - Repeat 500uL wash and centrifuge for 2 min
    - After dumping flow through, centrifuge again for 1 min
    - Recover dsRNA by applying 60uL hot elution buffer
    - Centrifuge 2 min
-

Note:

Conclusion:
After nano drop analysis, our 20X dilution recorded a concentration of 25.2 ng/uL.
The concentration of our pure dsRNA sample is 504 ng/uL.
Because the RNA concentration was low, we did a second elution with 100 uL.



The product was also checked on a gel to confirm that there was RNA of the proper length (MK).



Next step:
We need to concentrate our eluted RNA

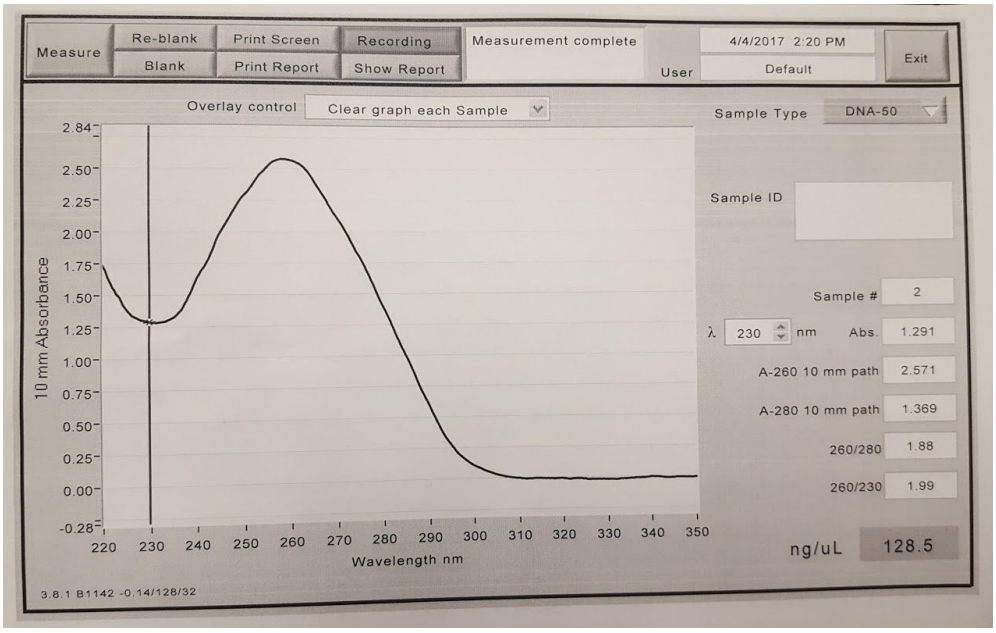
Week#: 10
Date: 4/4/17

Objective:
We will purify DNA and setup in vitro transcription to create RNA.

Procedures:

- Purify DNA
    - Mix 100 uL sample with 500 uL PB buffer
    - Vortex
    - Place in spin column and centrifuge 1 min
    - Discard flow through
    - Add 750 uL PE buffer and centrifuge 1 min
    - Discard flow through
    - Centrifuge to dry
    - Add 30 uL EB and centrifuge
- In Vitro Transcription
    - NTP mix 8 uL
    - 10x buffer 2 uL
    - Enzyme mix 2 uL
    - Template 8 uL

Note:



DNA template concentration

Conclusion:

The DNA step worked and provided a high enough concentration to continue with.

Next step:

Complete in vitro transcription.

Week#: 9

Date: 3/30/2017

Objective:
Run PCR

Procedures:
-take 25uL plasmid samples and combine in new 1.5mL tube
-Run 2uL on the 1.5% agarose gel (position #4)
-Afterwards, use 98uL plasmid for PCR purification
-5 volumes Buffer PB for every 1 volume of PCR Reaction
-Apply sample to the QIAquick column and vaccum.
-Wash with 750uL of Buffer PE and then centrifuge
-Place QIAquick column in a clean 1.5mL tube and elute DNA with 30uL of elution buffer

Note:
-Because our GOI is 325bp + 2(T7 primers (20bp)) = expected 365bp fragment
-First PCR run provided no results, so the PCR product was remade using procedures from last lab.

Conclusion:

Next step:

Week#: 9
Date: 3/28/2017

Objective: (in one or two sentences)
-Set up PCR for Thursday's Lab to add promoter site to GOI in plasmid so that RNA polymerase can bind and form dsRNA
-Analyze Sequence

Procedures: (bullet point the procedures)

- Analyze Sequence using 'form 7'
- 46uL H20, 20uL 5x buffer, 8uL dNTP, 15uL of PCR-4topo primer (10M stock), 10uL of 10ng/uL plasmid DNA, 1uL TAQ
- Our primer is 539.83 ng/uL and we needed a target concentration of 10 ng/uL; therefore I am making a 40uL solution using .74uL of plasmid diluted in 39.26uL of distilled water.

Note:

Conclusion:

Next step: complete PCR then *in vitro* transcription

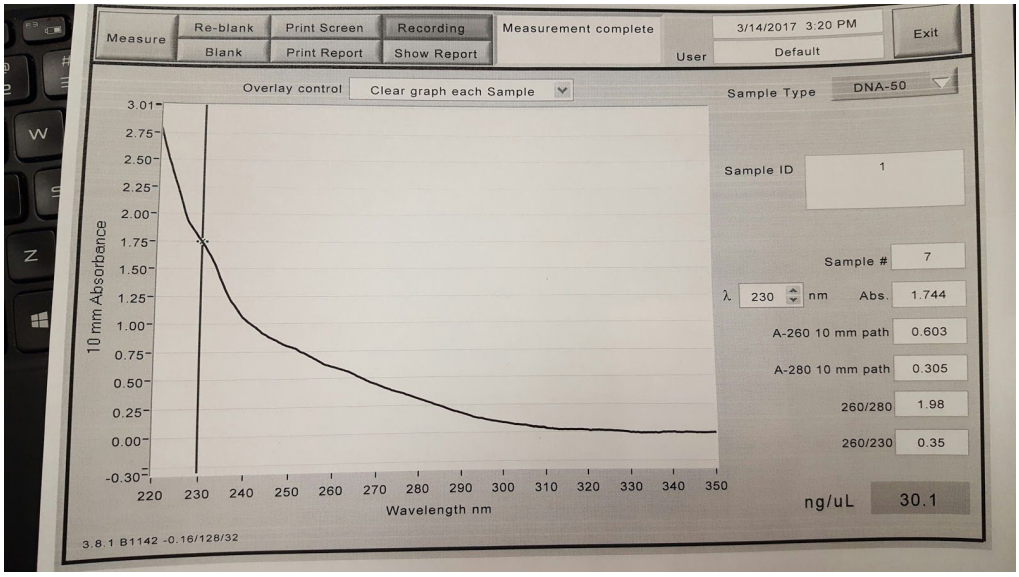
Week#: 8
Date: 3/14/17

Objective:
We will harvest the plasmids from our E. coli cells.

Procedures:

- Harvest E. coli using MiniPrep kit
    - Centrifuge culture to create E. coli pellet (12000 rpm, 2 minutes)
        - 2000 uL at a time, two rounds
    - Remove LB
    - Add 250 uL P1 (buffer) and vortex
    - Add 250 uL P2 (alkaline) and invert gently
    - Wait 3 minutes
    - Add 350 uL N3 to neutralize pH
    - Chill on ice for 5 minutes
    - Centrifuge, 12000 rpm, 10 minutes
    - Add 750 uL supernatant to column and vacuum
    - Add 500 uL PE to column and vacuum
    - Place column in clean 1.5 mL tube
    - Add 30 uL EB
    - Let sit 1 minute
    - Spin at 12000 rpm for 1 minute
    - Discard column

Note:
Wrong buffer was used initially, so pellet only had E. coli cells from 1000 uL, instead of 4000 uL.
On the second run, a different wrong buffer was used, so no DNA was acquired.



Very sad nanospec results.

Conclusion:
Instructions and names are important.

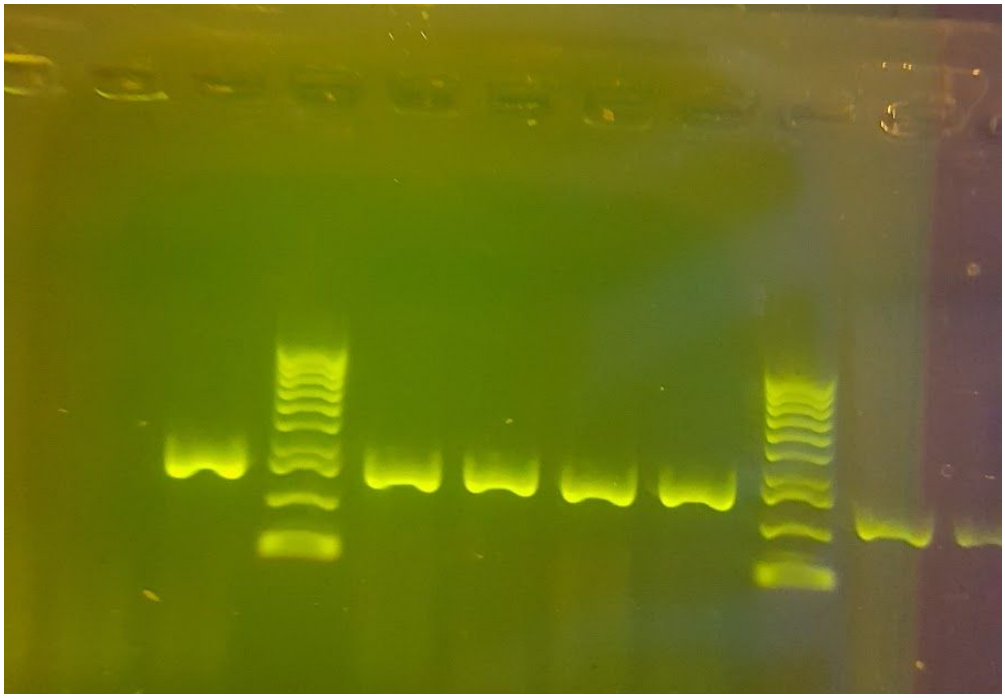
Next step:
Send the plasmid away to get sequenced.

Week#: 7
Date: 3/9/17

Objective:
We will run a gel on the products of our colony PCR to determine if we have a sequence of the correct length in our plasmids.

Procedures:
- Run gel on colony PCR
    - 1.5% agarose gel
        - 75 mL TBE
        - 1.125 g agar
    - 2 uL of each sample
        - Slots 6-9 on gel B

Note:
We used our F1 and R1 primers. We predict that the amplified fragment will be 325 bp long. From the gel, we estimate that the fragment is approximately 300 or just longer.



Conclusion:
We had bands with the expected lengths in all four PCR products. This indicates that all four of our selected colonies have the proper fragment in their plasmids.

Next step:
We can use any of our samples to extract plasmids from.

Week#: 7
Date: 3/7/17

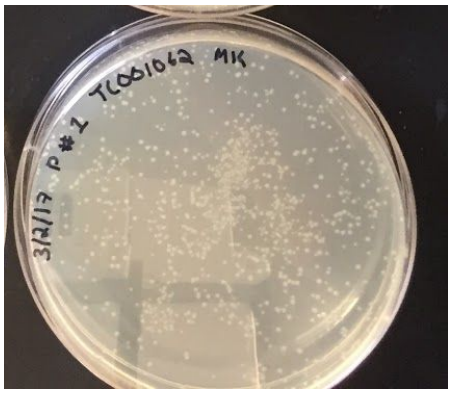
Objective:
Run colony PCR to determine the length of the fragment taken up by the plasmids.

Procedures:

- Pick 4 colonies to extract DNA from
    - Pipet 100 uL LB into a 1.5 mL microcentrifuge tube
    - In 4 PCR tubes add:
        - 15 uL LB+Ampr
        - Touch pipet tip to colony and add to tube
    - Incubate at 37 degrees for 1 hour (start at 2:40)
    - Samples are labelled MK1-4
- Colony PCR 1 reaction x5
    - H2O 8.2 uL 41 uL

○ 5x Green Buffer	4.0 uL	20 uL
○ dNTP mix	1.6 uL	8.0 uL
○ F1 Primer	2.0 uL	10 uL
○ R1 Primer	2.0 uL	10 uL
○ Taq polymerase	0.2 uL	1.0 uL
○ Culture	2.0 uL	
○ 20 uL reactions		

Note:



The plate with our transformed E. coli colonies.
There was a potential for small, untransformed satellite colonies to form around the transformed colonies.
In order to determine that the colony has the proper sequence in it, run PCR with our primer on the E. coli cells and run on gel to analyze and compare the size to our expected fragment.

Conclusion:
Our E. coli transformation seemed to work well, as we had many surviving colonies.

Next step:
After PCR, a gel will be run to determine the size of our fragment and make sure that it is the proper length.

Week#: 6
Date: 3/2/17

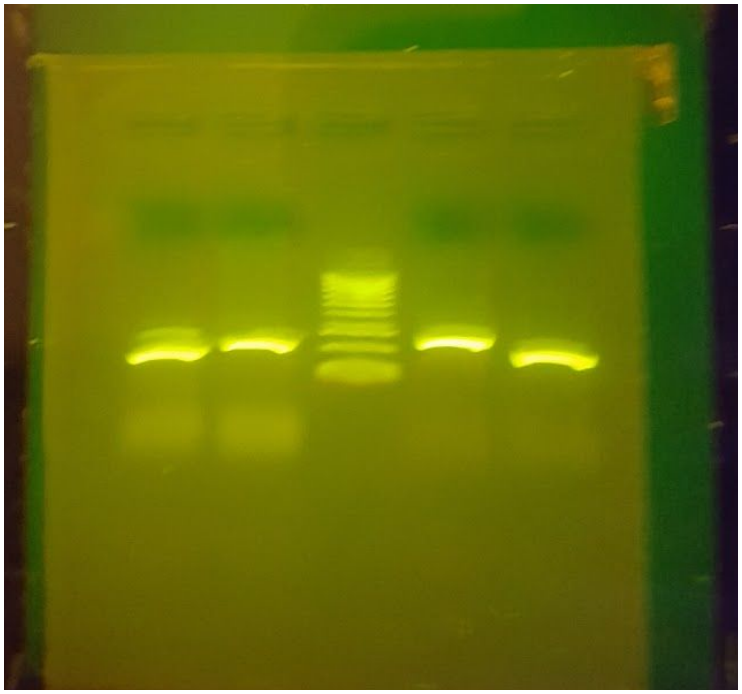
Objective:
Extract, purify, and clone DNA from primer 1 in order to transform E. coli.

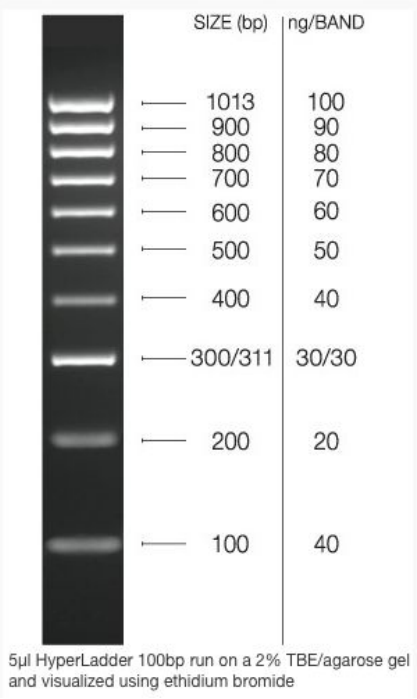
Procedures:

- Make gel (done before class)
    - 150 ml

    - 1.5% gel
- Run gel
    - Load 20 uL of each primer
    - Load 5 uL of 100 bp ladder
    - Gel 1
        - Slot 3 - ladder
        - Slot 4 - Primer 1
        - Slot 5 - Primer 2
- Cut out DNA
    - Using Freeze and Squeeze kit
- Purify DNA
    - Gel containing DNA placed in freezer for 5 minutes
    - Centrifuge for 3 minutes at 14000 rpms
- Clone DNA fragment into plasmid
    - TA cloning
        - As added to 3' end of PCR product
        - Open plasmid with T on ends
        - Other genes on plasmid:
            - Antibiotic resistance
            - ccdB - kills E. coli with empty plasmid
        - Use topoisomerase to combine PCR product and plasmid
    - 4uL PCR product
    - 1 uL salt
    - 1 uL Topo plasmid
    - Incubate at room temperature for 15 minutes
- Transform E. coli with plasmid
    - Chemical transformation
        - Change in salt concentration damages membrane
        - Allows plasmid to enter cell
    - Add 3 uL reaction into E. coli mixture (provided)
    - Heat shock: 42 C for 30 sec
    - Incubate at 4 C for 5 minutes
    - Add 250 uL SOC
    - Incubate at 37 C for 30 minutes with shaking
    - Plate E. coli with antibiotic
        - 120 uL E. coli mixture
        - Spread on plate
    - Only E. coli with proper plasmids will survive

Note:





Our samples were in wells 4 and 5. We used primer 1 in well 4 for the extraction and purification, as it is the longer of the two.

Conclusion:

Both of our primers work very well with no nonspecific amplification. Primer 1 is slightly longer (nearly 300 bps), so it is better to use for RNAi.

Next step:

Incubate E. coli and determine if any took up the proper plasmid.

Week#: 6

Date: 2/28/17

Objective:

Make primers and run PCR with both primer sets.

Procedures:

- Primers (TC001062)
    - Arrive dehydrated
    - Add H2O to make 100 uM
        - F1: 0.0276 umol + 276 uL H2O
        - R1: 0.0335 umol + 335 uL H2O
        - F2: 0.0297 umol + 297 uL H2O
        - R2: 0.0339 umol + 339 uL H2O

    - Spun on vortex mixer to ensure thorough mixing
    - Dilute in new tube, make 100 uL of 10 uM primer
        - 10 uL primer + 90 uL H2O
    - Spun on vortex mixer to ensure thorough mixing
- PCR
    - Taq DNA polymerase
    - dNTP mix
    - 10x Buffer
        - Includes MgCl2 and loading dye
    - H2O
    - Primer (F&R)
    - Template (genomic DNA or **cDNA**)

    - Heated (denature) at 95C for 5 minutes
    - Drop temp to 94C for 30 seconds to continue denaturation
    - Drop temp to 58C for 30 seconds to anneal
    - Raise temp to 72C for 40 seconds to extend
    - Repeat last three steps for 35 cycles
    - Maintain temp at 72C for 5 minutes to extend
    - Drop temp to 4C for storage

Date: 2/28/17

Aim: PCR with TC001602 primers 1 and 2

Gene name: dFOXO

Primer1F, Tm= 57.2℃: 5' AAGAAGAACTCCAGCCGACG 3'

Primer1R, Tm= 57.1℃: 5' TAGTCTCCATTGAAGCCGCC 3'

Primer2F, Tm= 57.1℃: 5' ATTAATGGACAGACGCCGCA 3'

Primer2R, Tm= 57.1℃: 5' GACGGCTCTGAATGCGGATA 3'

Reaction

H_20	**59.5 μl**	____μl
5 X PCR buffer	**20 μl**	____μl
~~*$MgCl_2$ (25mM stock)~~	~~6 μl (1.5 μM)~~	~~____μl (___μM)~~
dNTP mix (2.5mM stock)	**8 μl** (0.2 μM)	**46 μl (Master mix)**
Primer1 (10 μM stock)	0 μl (___μM)	**2 μl** (___μM)
Primer2 (10 μM stock)	0 μl (___μM)	**2 μl** (___μM)
Sample DNA (_____)	4 μl	____μl
Taq polymerase (5U/ μl)	0.5 μl (2.5U /100 μl)	____μl
Total	100 (**92**) μl	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: 57 ℃ X 30 sec] 35 cycles

Extension: 72 ℃ X 40 sec

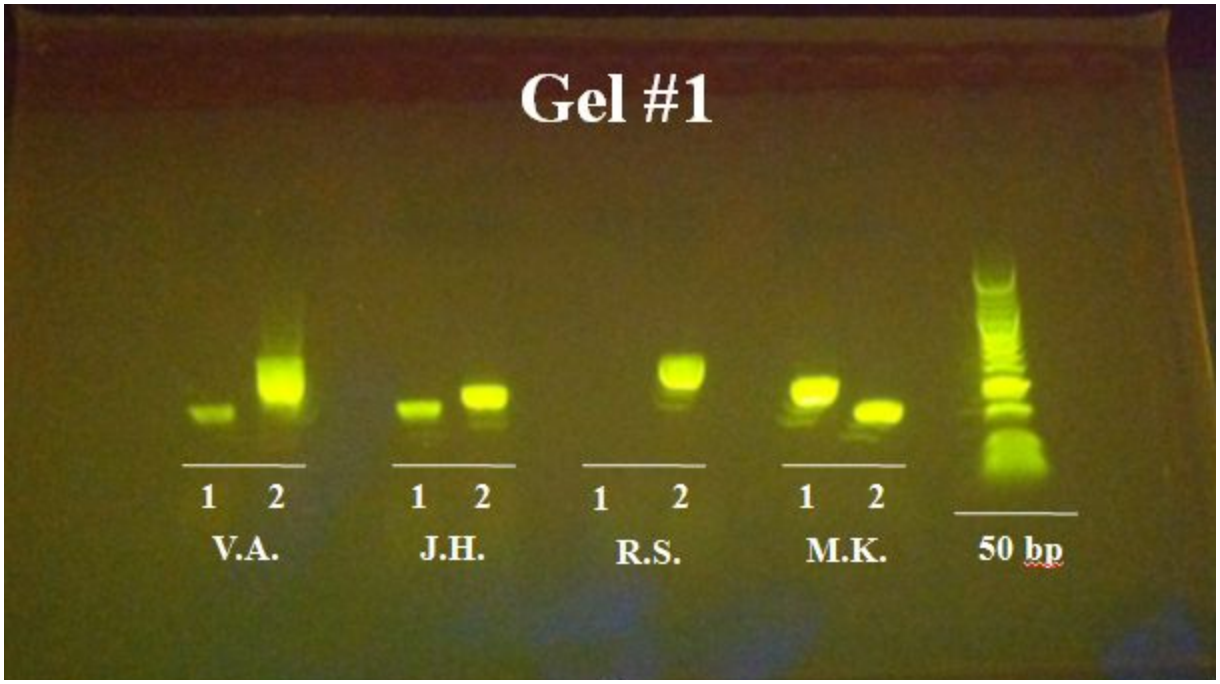
↓

Extension: 72 ℃ X 5 min

↓

Hold: 4 ℃

Note:
PCR mixture was created for both reactions, and split before adding primers.
PCR was started before leaving class and will be checked next class.



Our primers are labeled M.K. The gel shows that both primers worked and produce fragments with different lengths.

Conclusion:
Both of our primers worked and can be used in the next step. We will continue with the longer fragment, number one.

Next step:
Check PCR, run gel and extract to use in future steps.

10/10
Very good note taking! So, what is the length of your DNA? --Yoshi

Week#: 5
Date: 2/23/2017

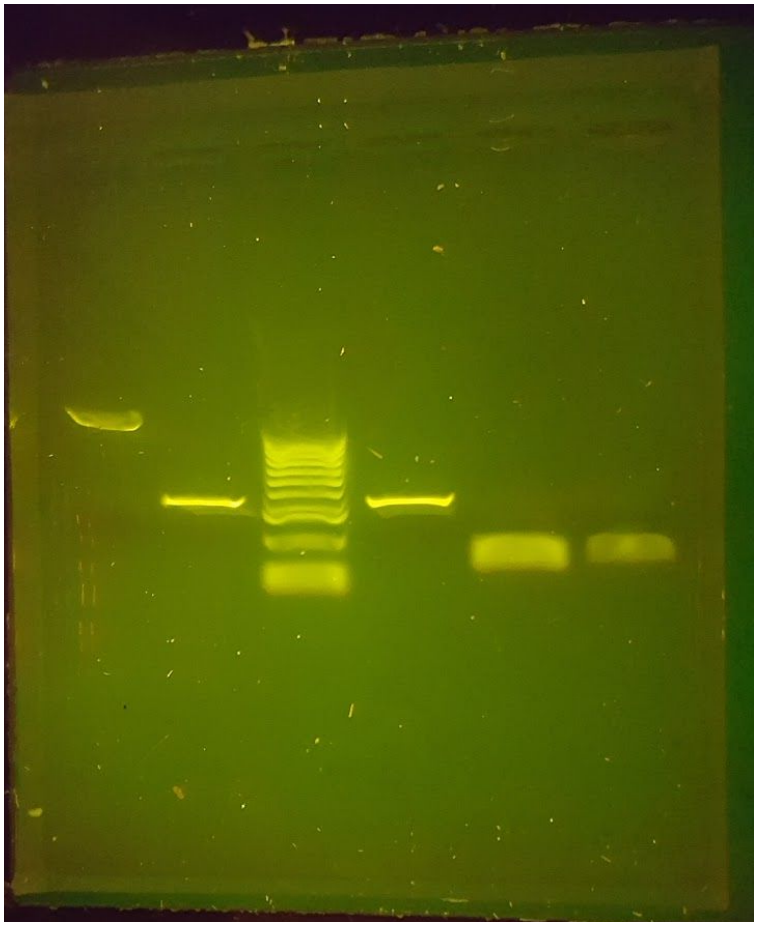
Objective:
We practiced making gels and analyzing the results.

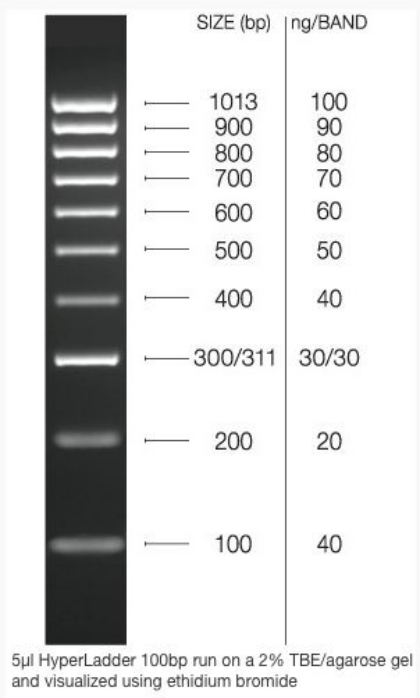
Procedures:

- Making gel:
  - 1.2% weight by volume
    - *Higher concentration, finer mesh, DNA runs slower
  - 1.2 g agarose powder/100 mL TBE
  - Add dye to make visible DNA
    - Ethidium bromide - have to use UV light to view
    - Gel Green (safer) - use blue light to see green wavelengths
  - Microwave for 2 minutes on power level 30
  - Swirl mixture
  - Microwave for 1 minute on power level 30 (removes air bubbles)
  - Pour into tray and allow to solidify (~30 min)
- Running gel:
  - Add enough TBE buffer to submerge gel
  - Submerge gel tray in rig
  - Load DNA samples
    - Add 5x loading dye to DNA samples
    - 8 uL DNA to 2 uL dye
  - Load 100 bp ladder to analyze DNA
    - 5 uL ladder

Note:
The gel was made by another group, following the procedure above. The Gel Green dye was already mixed into the TBE buffer used to make the gel. The gel was allowed to set up while we watched a demonstration of larval injections.
Gel rigs were checked to see which were working, and then loaded as follows:
1: Sample C
2: Sample B

3: Ladder
4: Sample B
5: Sample A
6: Sample A
The gel was run for approximately 20 minutes, then analyzed.





Conclusion:
Sample A is approximately 150-200 base pairs long, sample B is approximately 450 base pairs long, and sample C is approximately 1000 base pairs long.

Next step:
Starting work with our primers

Week#:
Date:

Objective: (in one or two sentences)

Procedures: (bullet point the procedures)

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.

Conclusion:

Next step: