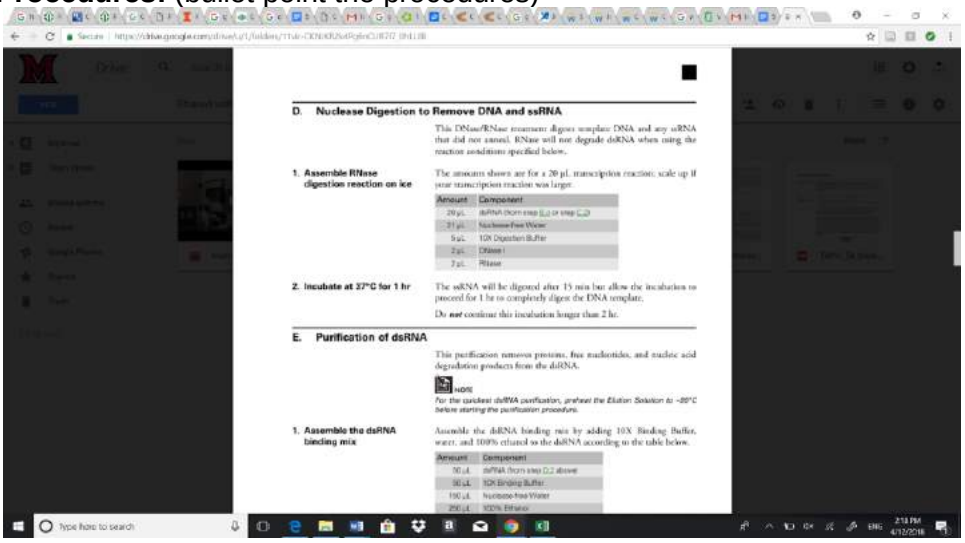
Lab notebook template

Name: Catherine and Lan
Date: 4/12/18
Week#: 10

Objective: (in one or two sentences)
Purify ssRNA before injection
Get rid of: salt, DNA, NTP, RNApolymerase, ssRNA
Salt, NTP, and polymerase is easy with column
DNA can be removed with DNase
ssRNA removed by RNase

Procedures: (bullet point the procedures)



D. Nuclease Digestion to Remove DNA and ssRNA

This DNase/RNase treatment digests template DNA and any ssRNA that did not anneal. RNase will not degrade dsRNA when using the reaction conditions specified below.

1. Assemble RNase digestion reaction on ice

The amounts shown are for a 20 µL transcription reaction; scale up if your transcription reaction was larger.

Amount	Component
20 µL	dsRNA (from step 6.D or step 7.D)
21 µL	Nuclease-Free Water
5 µL	10X Digestion Buffer
2 µL	DNase I
2 µL	RNase

2. Incubate at 37°C for 1 hr

The ssRNA will be digested after 15 min but allow the incubation to proceed for 1 hr to completely digest the DNA template.

Do **not** continue this incubation longer than 2 hr.

E. Purification of dsRNA

This purification removes proteins, free nucleotides, and nucleic acid degradation products from the dsRNA.

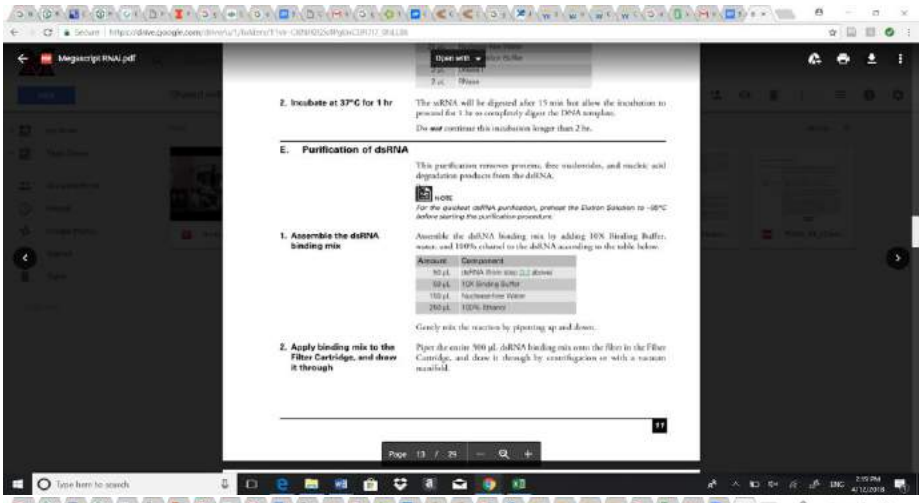
NOTE
For the quickest dsRNA purification, preheat the Elution Solution to ~55°C before starting the purification procedure.

1. Assemble the dsRNA binding mix

Assemble the dsRNA binding mix by adding 10X Binding Buffer, water, and 100% ethanol to the dsRNA according to the table below.

Amount	Component
50 µL	dsRNA (from step 6.E above)
30 µL	10X Binding Buffer
150 µL	Nuclease-free Water
250 µL	100% Ethanol

*only incubates for 30 minutes



2. Incubate at 37°C for 1 hr

The ssRNA will be digested after 15 min but allow the incubation to proceed for 1 hr to completely digest the DNA template.

Do **not** continue this incubation longer than 2 hr.

E. Purification of dsRNA

This purification removes proteins, free nucleotides, and nucleic acid degradation products from the dsRNA.

NOTE

For the quickest dsRNA purification, preheat the Elution Solution to ~95°C before starting the purification procedure.

1. Assemble the dsRNA binding mix

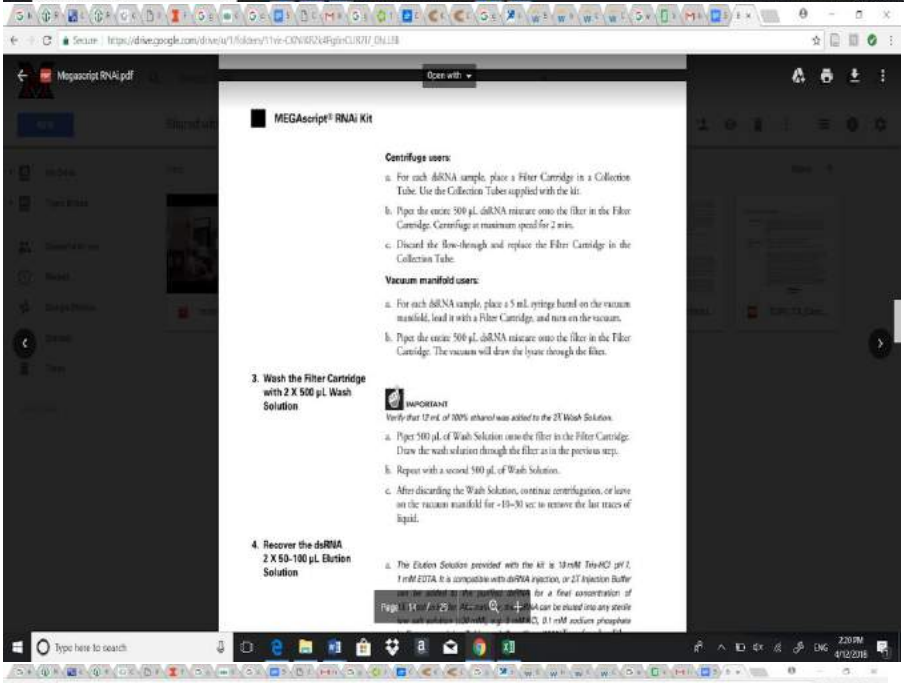
Assemble the dsRNA binding mix by adding 10X Binding Buffer, water, and 100% ethanol to the dsRNA according to the table below.

Amount	Component
50 µL	dsRNA from step 2.2 above
50 µL	10X Binding Buffer
150 µL	Nuclease-free Water
250 µL	100% Ethanol

Gently mix the reaction by pipetting up and down.

2. Apply binding mix to the Filter Cartridge, and draw it through

Pipet the entire 500 µL dsRNA binding mix onto the filter in the Filter Cartridge, and draw it through by centrifugation or with a vacuum manifold.



MEGAscript® RNAi Kit

Centrifuge users:

a. For each dsRNA sample, place a Filter Cartridge in a Collection Tube. Use the Collection Tubes supplied with the kit.

b. Pipet the entire 500 µL dsRNA mixture onto the filter in the Filter Cartridge. Centrifuge at maximum speed for 2 min.

c. Discard the flow-through and replace the Filter Cartridge in the Collection Tube.

Vacuum manifold users:

a. For each dsRNA sample, place a 5 mL syringe barrel on the vacuum manifold, load it with a Filter Cartridge, and turn on the vacuum.

b. Pipet the entire 500 µL dsRNA mixture onto the filter in the Filter Cartridge. The vacuum will draw the lysate through the filter.

3. Wash the Filter Cartridge with 2 X 500 µL Wash Solution

IMPORTANT

Verify that 12 mL of 100% ethanol was added to the 2X Wash Solution.

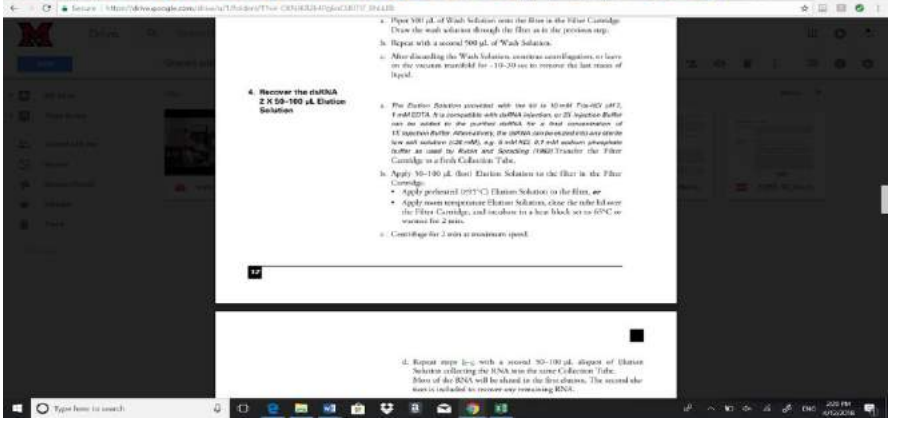
a. Pipet 500 µL of Wash Solution onto the filter in the Filter Cartridge. Draw the wash solution through the filter as in the previous step.

b. Repeat with a second 500 µL of Wash Solution.

c. After discarding the Wash Solution, continue centrifugation, or leave on the vacuum manifold for ~10–30 sec to remove the last traces of liquid.

4. Recover the dsRNA 2 X 50–100 µL Elution Solution

a. *The Elution Solution provided with the kit is 10 mM Tris-HCl pH 7, 1 mM EDTA. It is compatible with dsRNA injection, or 2X Injection Buffer*



a. Pipet 500 µL of Wash Solution onto the filter in the Filter Cartridge. Draw the wash solution through the filter as in the previous step.

b. Repeat with a second 500 µL of Wash Solution.

c. After discarding the Wash Solution, continue centrifugation, or leave on the vacuum manifold for ~10–30 sec to remove the last traces of liquid.

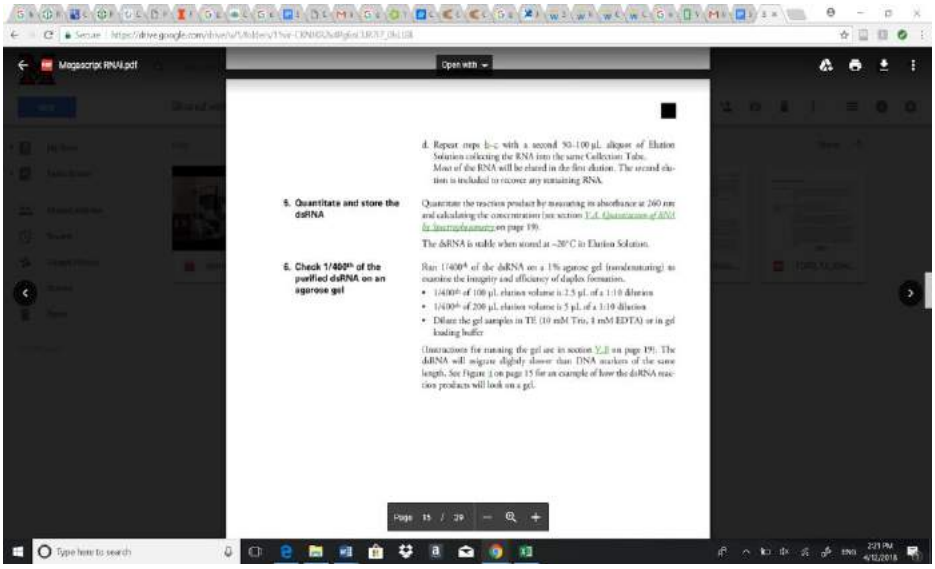
4. Recover the dsRNA 2 X 50–100 µL Elution Solution

a. *The Elution Solution provided with the kit is 10 mM Tris-HCl pH 7, 1 mM EDTA. It is compatible with dsRNA injection, or 2X Injection Buffer can be added to the purified dsRNA for a final concentration of 1X Injection Buffer. Alternatively, the dsRNA can be precipitated and resuspended in any sterile low salt solution (≤30 mM), e.g. 5 mM NaCl, 0.1 mM sodium phosphate buffer as used by Rubin and Spradling (1982) transfer the Filter Cartridge to a fresh Collection Tube.*

b. Apply 50–100 µL (but) Elution Solution to the filter in the Filter Cartridge.
- Apply preheated (95°C) Elution Solution to the filter, **or**
- Apply room temperature Elution Solution, close the tube lid over the Filter Cartridge, and incubate in a heat block set to 65°C or warmer for 2 min.

c. Centrifuge for 2 min at maximum speed.

d. Repeat steps b–c with a second 50–100 µL aliquot of Elution Solution collecting the RNA into the same Collection Tube. Most of the RNA will be eluted in the first elution. The second elution is included to recover any remaining RNA.



Note:
Put in water instead of the mastermix. The TA corrected for the error.
Step E4b. Did not warm it for 2 minutes but for ten minutes, as instructed by teacher.

Side note for studying: RNA polymerase attaches to the non-sense side and bind at 3' so creates copy 5'-3'

Conclusion:

Next step:

Lab notebook template

(Add new entry above of the previous entries)

Name: Catherine Smith
Date:
Week#: 10

Objective: (in one or two sentences)
To purify DNA and make dsRNA

Procedures: (bullet point the procedures)

1. Add 1,000 µl PB Buffer and 200 µl of the PCR'ed reaction to a 1.5 ml tube. If orange or violet, Add 10 µl 3 M sodium acetate and mix.
2. Place QIAquick column into vaccum, two separate times each 600 µl.
3. While still attached to vaccum, add 0.75 ml Buffer PE to column.

4. Centrifuge column for one minute to remove residual wash.
5. Place each QIAquick column into a clean 1.5 ml microcentrifuge tube,
6. Add 30 µl EB to the center of the column's membrane. Let column stand for 1 minute, then centrifuge

Later PCR Solution

ddWater	0 µl
5x Buffer	2 µl
dNTP	8 µl
Template	8 at least 187.5 ng/µl (so you have at least 1.5 ng)
RNApoly	2 µl
Total	20 µl

No primers for RNA

Note:
Ours appeared green so we did not alter the PH using sodium acetate
We used a vaccum verses a centrifuge during the washes
For vaccum we did not do it in even amounts for 600 µl into the vaccum. It did not overflow though so there should not be a difference.
Did not let it stand for 1 minute after the EB buffer.

Conclusion:

Next Step:

Lab notebook template

Name: Catherine and Lan
Date: 4/5/2018
Week#: 8

Objective: To get double stranded DNA from plasmid

Procedures: (bullet point the procedures)

PCR Batch

ddWater	102 µl
5x Buffer	40 µl
dNTP	16 µl
Primer	30 µl
Plasmid	10 µl
Go taq	2 µl
Total	200 µl

Note:
Making 40 µl of 10 µg/µl for plasmid (concentration of 2 µl plasmid)
Did it in 1.5 ml Tube
Split procedure into 8 tubes 25 µl each for PCR tubes

Conclusion:

Next step: synthesize dsRNA

Lab notebook template

Name: Catherine and Lan
Date:3/29/18
Week#:

Objective: (in one or two sentences)
Harvest plasmids. Remove proteins, RNA, lipids, sugar etc.

Procedures: (bullet point the procedures)
Centrifuge the tube to remove culture media (put 2 ml in 2 ml tube and remove liquid) x2
Resuspend into solution
Then increase pH to kill *E. coli* (alkaline treatment) it's how we keep plasmid intact but genomic DNA destroyed
Neutralize for 1 minutes (N3)
Then spin down again

Note:
We followed the protocol, except used a vacuum instead of centrifuging.

Conclusion:

Next step:
Sequence DNA

Lab notebook template

Name: Lan and Catherine
Date: 3/27/18
Week#:

Objective: (in one or two sentences)
Choose which colony you want to purify. This will prep which purified colony to be sent out for sequencing

Procedures: (bullet point the procedures)
Put PCR'ed solution into gel electrophoresis to verify which colony to choose

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

Ladder used: 50 bp
Small gel 20 ml and large gel has 30 ml (for making the gel)
Gel will be 1.5% gel concentration (we did not create gel)
5 µl of ladder
For each colony 2 µl each in each well

Conclusion:

Next step:

Lab notebook template

Name: Catheline and Lan
Date: 03/15/2018
Week#: 7

Objective:
Col. PCR

Procedures:

- Process colonies and incubate them
- Then Make master mix for PCR (4x quantity)
- Make 4 different tubes of this (18 µl each) and add colony
- PCR those tubes

Note:

1. Pick the colonies.

From the petri plate containing the *E.coli* colonies from last step, four colonies was took and put into 15 µl of LB + amp and mixing with the pipet. The chosen colonies need to single, smooth, round and medium size.

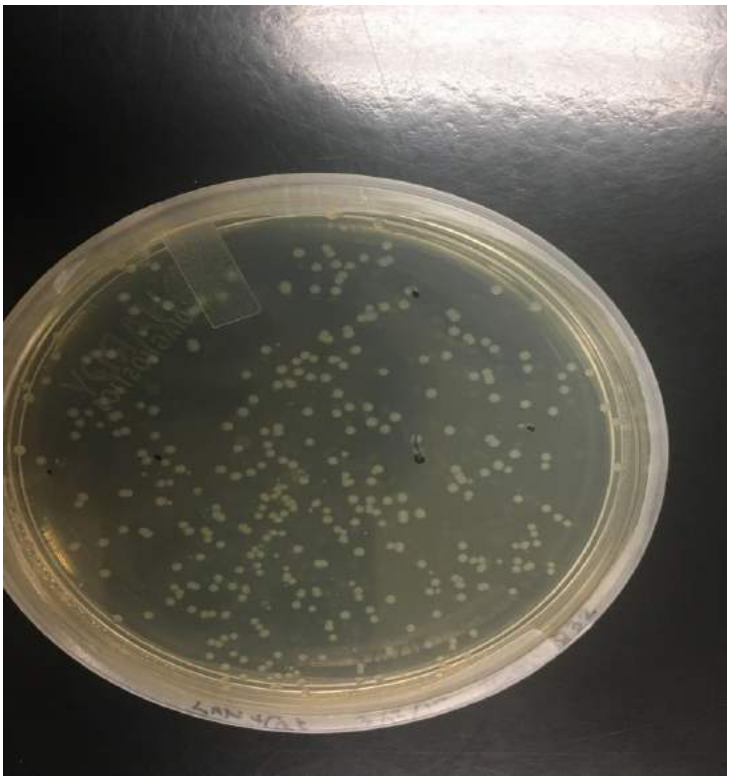


Figure 1: *E.coli colonies.*

The Tubes containing *E.coli* colonies solution were incubated at 37 degree celsius for 1 hour, and mixing them every 15 minutes. After incubation was done, these solution was used for col. PCR.

2. PCR Master Mix

H2O	36.6 µl
Green Buffer	16 µl
dNTP mix	6.4 µl
T3 Primer	8 µl
T7 Primer	8 µl
Go Taq	1 µl (DO NOT VORTEX!)
Colony	(not in master mix)

In each tube (n=4) 18 µl of master mix. Add 2 µl of different colonies to 4 separate tubes . Label the PCR tubes with #Tc 010832.

3. Col. PCR

Date: 03/15/2018

Aim: Col. PCR

Gene name: Big Brain

Reaction

H_20	___µl	8.15 µl
5 X PCR buffer	20µl	4 µl
dNTP mix (2.5mM stock)	8µl (0.2µM)	1.6 µl
T3 Primer (10µM stock)	___µl (__µM)	8 µl (__µM)
T7 Primer (10µM stock)	___µl (__µM)	8 µl (__µM)
E.coli Colony (_____)	___µl	2 µl
Taq polymerase (5U/ µl)	0.5µl (2.5U /100µl)	0.25 µl
Total	100µl	20 µl

Reaction Cycle

Program name: PCR4- TOPO

Denaturation: 95 ℃ X 5 min

↓

Denaturation: 94 ℃ X 30 sec

Annealing: 55 ℃ X 30 sec 30 cycles

Extension: 72 ℃ X 30 m / s

↓

Extension: 72 ℃ X 2 min

↓

Hold: 4 ℃

Result

Electrophoresis:__%agarose,__X TAE/ TBE

Loaded sample ___µl / lean

Conclusion:

Next step:

Run the gel electrophoresis to confirm the PCR.

Lab notebook template

Name: Catherine and Lan
Date: 3/13/2018
Week#: 7

Objective:

Cloning DNA (putting gene into the plasmid)
Putting linear DNA into plasmid (double stranded)

Procedures:

Overview

Clone DNA is the process of isolate a gene and put it in to the plasmid. In another words, cloning is putting DNA fragment into plasmid. Traditionally use to put into plasmid: Restriction enzymes (tool to cut bacterial DNA) and enzyme Ligase. Today we use another enzyme Topoisomerase (replicate DNA, have to unwind from one side, and then produce supercoiling. This one prevents the supercoiling). The advantage of this method that don't need any ATP. And This method called TA cloning because open plasmid has T overhang.

Select the *E.coli* for the plasmid: using antibiotic resistant which the only ones with these genes will survive if we put in antibiotics. Double check to make sure it has genes: check length, blue/white selection (now advanced it past that).

Note: The details about the cloning procedures.

1. Made solution of DNA:

Purified DNA 4 µL
Salt solution 1 µL
TOPO vector 1 µL

Total 6 µL

Put all solutions into a 1.5 tube, mixing with the pipet, and place at room temperature for 30 minutes.

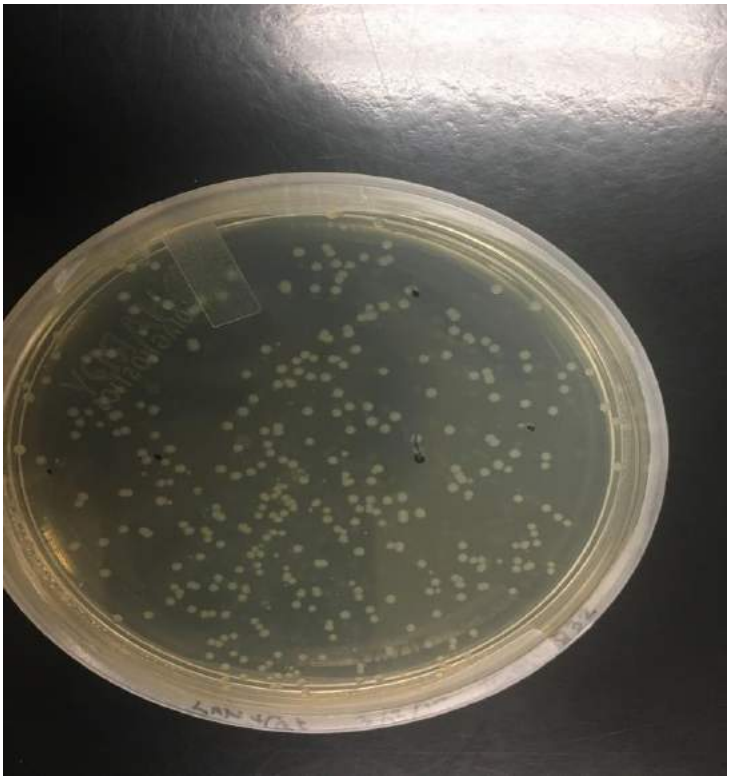
2. Putting the DNA to plasmid.

- Take 3 µL of the reaction and pipet it into the cell.
- Kept it on ice for 5 minutes.
- Heat shock for 30 seconds
- Put back on ice for 5 minutes
- Add 250 µL of SOC into the solution
- Incubate the solution at 37 celcius for 1 hour.

3. Culture the E.coli cell

Put 120 µL of the DNA/cell solution in to the petri plate containing cultured medium agar gel, and suspension spread the solution on the petri plate. Incubate the petri plate at 30 Celcius for 1-2 days.

After the petri plate incubated, the result of *E.coli* colonies shown as the below picture.



Conclusion:

Main reason to clone: linear DNA is not that stable. Best way to store them and keep them. Then whenever want to use it, put it back into *E.coli*, amplify, than harvest

Next step:

Colonies PCR

Lab notebook template

Name: Lan and Catherine
Date: 3/8/2018
Week#: 6

Objective:
Run gel for primers to verify successful PCR and get purified DNA.

Procedures:

Make Gel (1.2% agarose, 50 ml/gel)

We did not make the gel, two other groups did today

Place two PCR's solutions as well as ladder (run 1-2 µL)

Remove DNA from gel from electrophoresis (Selected primer 2)

Squeeze and freeze kit (thing that took out DNA from gel: Extracta)

Note:

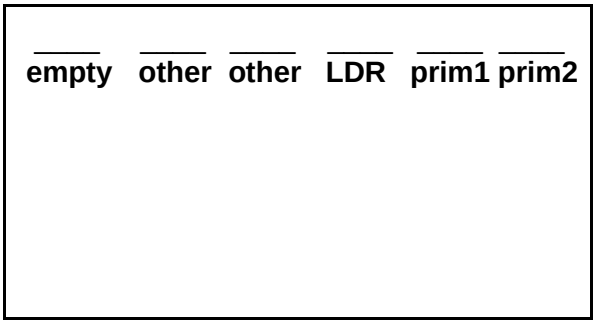
1. Gel making

2 group in class made the gel for all other group. The concentration of gels were 1.2 % and 5 ml/gel. Each group done with 100 ml for 2 gel.

In this time we need the bigger gel than last week and also need the bigger hole for 20 µL of solution.

2. Run gell

20 µL of 2 primers and 5 µL of 50 bp ladder were placed in the gel following the figure.



Where, other denotes another lab's sample, prim 1 denotes primer 1 with F1R1, and prim 2 was F2R2.

The gel was run at 100 V for 25 minutes. And the result of gel electrophoresis was show as the picture.

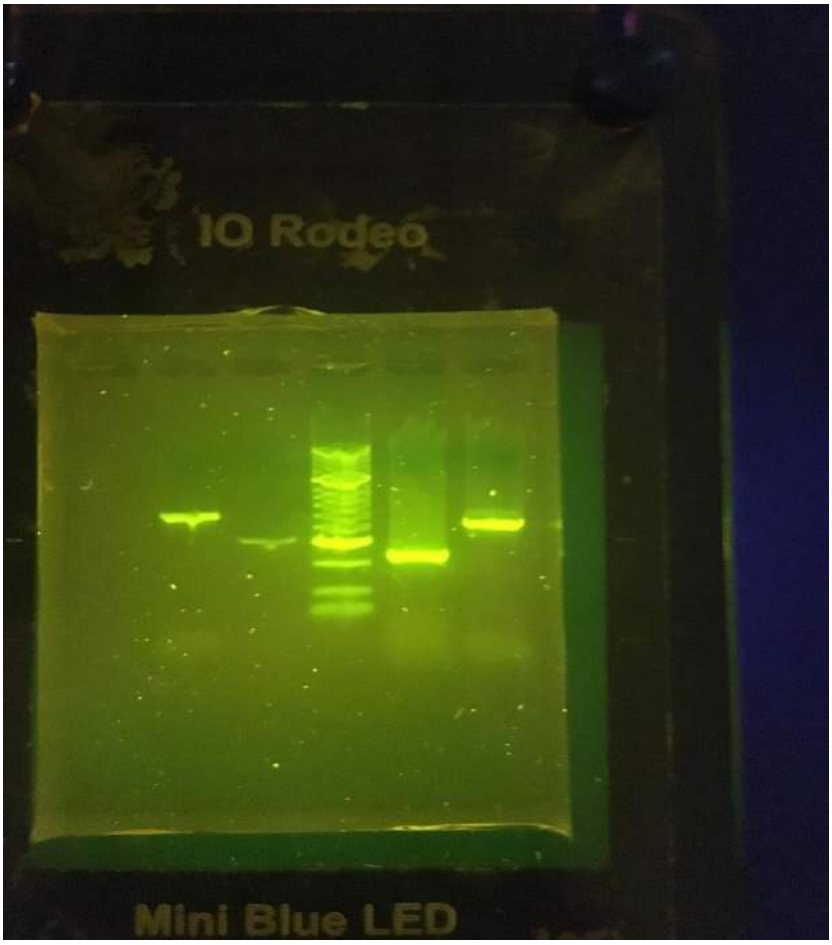


Figure 1: *Gel electrophoresis result*

3. Collecting the DNA

As the gel result shown on the figure 1, the F2R2 have the longer base and we selected this primer. To select the primer on the gel, We used the selected tube as the picture 2.

Figure 2: *The selected tube.*

The gel with DNA was centrifuged without maiming anyone. The centrifugation was set up with 3000 rpm of speed and run for 7 minutes. After done with centrifuging, the DNA was saved in the freeze with the tube have label of # Tc 0180342.

Conclusion:

Turns out we actually did a good job at preparing for the PCR (or good enough). PCR was successful. (as noted from professor. He ran PCR to check and see who should redo. W are running our own today for the rest of the experiment)

Next step:

Put this gene into plasmid

Lab notebook template

Name: Lan and Catherine

Date: 3/6/18
Week#: 6

Objective:

- Diluting Primer form 4 tubes of DNA fragment
- Prepare Primer for PCR

Procedures:

Diluting Primer

First dilute to 100 µM

Adding water to tube directly

Mix thoroughly using vortex then spin down

Then take a certain amount of that made solution to create 50 µL of 10 µM

Label all of these tubes you create (primer and new dilution)

TC # Primer name: TC010832_LetterNumber ; 10 µM concentration

Use green dots

Preparation PCR solution.

Note:

1. *Sanitizing*

Using a bottle of windex to spray on the table at worked area and using the napkins to clean the worked area before and after set up molecular biology reaction.

2. Diluting Primer

We need 2 primer set for our genes, so we need to take 4 tube of DNA fragment. These tube were prepared by Kevin and labeled R1 (M.W= 6155.0 g/ mol, 0.15 mg, 23.4 nmol), F1(M.W = 6117.0 g/ mol, 0.14 mg, 23.2 nmol), R2 (5923.9 g/ mol, 0.17 mg, 28 nmol), and F2 (M.W= 6084.0 g/ mol, 0.18 mg, 29 nmol)

All DNA fragment tubes were diluted following below figure:

First dilution calculations:

Dilute the DNA fragments to 100 µM of concentration (where, 100 µM = 100 µmol1 L = 100 nmol1 ml)

R1: the water volume needed = 23.4 nmol x 1000 µl100 nmol= 234 µl

R2: the water volume needed = 28 nmol x 1000 µl100 nmol= 280 µl

F1: the water volume needed = 23.2 nmol x 1000 µl100 nmol= 232 µl

F2:the water volume needed = 29 nmol x 1000 µl100 nmol= 290 µl

After dilution was finish, the tubes was labeled with Tc number (Tc # 010832), 100 µM, and R1, R2, F1 or F2 corresponding to the DNA fragment.

Second dilution:

5 µL of primer solution into 45 µL of dd Water.

All tubes labeled appropriately with the green labels with writing Tc #010832, 50 µM, and R1, R2, F1, or F2.

3. PCR preparation

Date. 3/6/18

Aim: start primer PCR

Gene name: Big Brain

Forward 1 Primer: Tm= 59.97 ℃: sequence 5'-- TCGCAGTCGGTTTACACGAA

Reverse 1 Primer: Tm= 59.97 ℃: sequence 5'-- GGCGCGCTATTTTTACTGGG

Forward 2 Primer: Tm= 59.90 ℃: sequence 5'-- CTCCGAGTGCATAGCGTCTT

Reverse 2 Primer: Tm=60.03 ℃: sequence 5'-- CATGCCCATCCACCTCCTTT

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)	4 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

Reaction Cycle

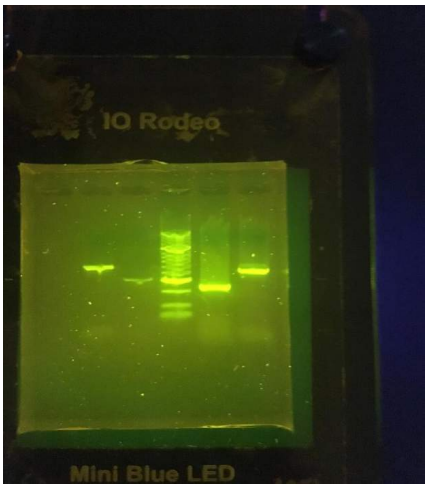
Program name: LC1

Denaturation: 95 ℃ X 5 min

Result

Electrophoresis: 1.2 %agarose, 5 X TAE/ TBE

Loaded sample 20 µl / lean



↓

Denaturation: 94 ℃ X 30 sec
Annealing: 59.90 ℃ X 30 sec 35 cycles
Extension: 72 ℃ X 30 sec
↓
Extension: 72 ℃ X 5 min
↓
Hold: 4 ℃

Because the water have the biggest volume, the amount of water was added first, then the small volume of other reagents was added later.

In a tube, 30.5 µL of water was added first followed by 10 µL of PCR buffer (blue color solution), 4 µL of dNTP and 1 µL of cDNA. The solution was rotated and spined down. Then, 0.5 µL of enzyme (Tag polymerase) was finally added. Now, The volume of solution was 46 µL, mixing solution well, then divided it to 2 tubes with equal volume. So, each tube contained 23 µL of buffer solution.

In tube 1: added 1 µL of F1 and 1 µL of R1, mixed solution well, and labeled Tc # 010832, F1R1.

In tube 2: added 1 µL of F2 and 1 µL of R2, mixed solution well, and labeled Tc # 010832, F2R2.

Place both tube 1 and 2 in to the freeze.

Conclusion:

Next step:

Purify the DNA.

Lab notebook template

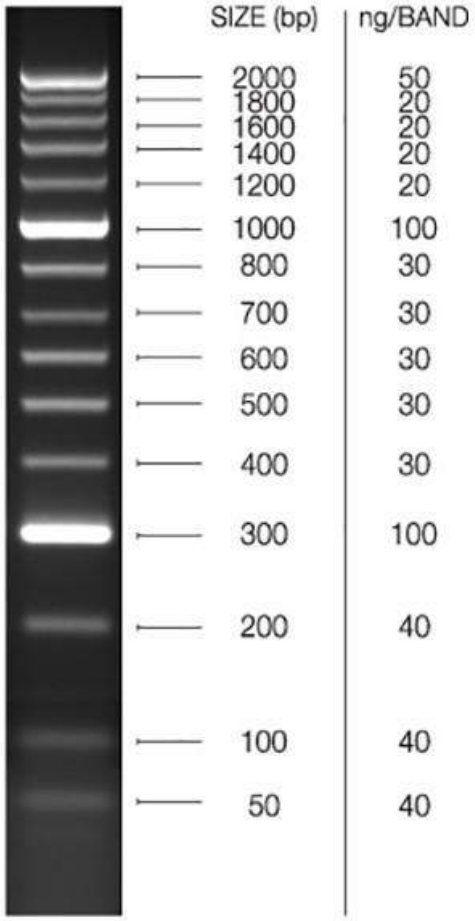
Name: Lan and Catherine
Date: March 1, 2018
Week#: 5

Objective:
To learn how to make dilutions for running a gel electrophoresis as well as how to make gel

Procedures:
- Make 25-50 ml per gel
- Calculate how much agarose is needed (depends on between .7%-1.2%
    - Today used 1.2% and made 50 ml of gel each Needed 0.6 g agarose
- Cook 3 min in microwave at 30% power
    - Take out, mix, 1 more minute at the same power
- Dilute DNA fragments and mix with loading dye
- Run gel electrophoresis for ~25 minutes

Note: We learned how to make dilutions today as well as running a gel electrophoresis
Struggled with seeing what the gel electrophoresis looked like.

a. *Gel electrophoresis*.
- Separating technique base on the molecule size.
- Control the concentration of agar and control mass run through
- DNA negative charge



Source: https://www.bioline.com/uk/hyperladder-50bp.html#RL

B. Making the gel

First, need to know how to decide the concentration of gell.

1% 1kb- 5kb
0.7% 3 kb- 10 kb
1.2- 1,5 % 100 kb- 1000 kb
2 % 100 bp

In this lab, we make 1.2 % gel with 25 ml/ gel. On one time of cooking agar, we make 2 gell. So, the total volume we need to do is 50 ml. The weight of agarose need for 50 ml of water was: (1.2g x 50ml)/ 100 ml = 0.6 g.

The progress to make 1.2% gel following these step:

1. Weighting 0.6 gram of agarose: put a bowl in to the balance, tare it to zero, add 0.6 gram of agarose to the bowl.
2. Puting the agarose in to a 125 ml erlenmeyer flask, then adding 50 ml of water. Using a small becker (25 ml becker) to cover the top of erlenmeyer flask
3. Place the erlenmeyer flask in to microwave, set up 3 minutes at 30% power. When the time finish, take it out and mix the solution gently. Then, continuous place the erlenmeyer flask into microwave with 1 minutes at 30% power, and mixing solution thoroughly to avoid air bubbles. If solution still have air bubbles, continuous cook it more 1 minutes at 30 % power.
4. Gently pour the agarose solution in to a tray and making a hole. In this lab, each group need to make 4 hole on the gel: 1 hole for the ladder and 3 hole for three sample A, B, and C.

C. Dilute DNA fragments and mix with loading dye

Dilute DNA fragments

Three sample of DNA fragments were provided: A (82 ng/ µl), B (118 ng/ µl), and C (115 ng/ µl). These samples were diluted to make 10 µl solution with concentration 10 ng/ µl. To do this dilution, the calculation using the equation, $C_1V_1 = C_2V_2$ was show as below:

- Needed volume for sample A = {(10 ng/ µl).(10µl)}/ (82 ng/ µl) = 1.22 µl
- Needed volume for sample B = {(10 ng/ µl).(10µl)}/ (118 ng/ µl) = 0.85 µl
- Needed volume for sample C = {(10 ng/ µl).(10µl)}/ (115 ng/ µl) = 0.87 µl

So, The dilution DNA fragments of three sample were done with volume:

- Sample A: 1.22 µl of sample and 8.78 µl of water.
- Sample B: 0.85 µl of sample and 9.15 µl of water.
- Sample C: 0.87 µl of sample and 9. 13 µl of water.

Mixing with loading dye

If the sample is put in the gel by itself, the solution of sample will go through every where in the gel because of its dilution. So the necessary to mix a sample with a loading dye, which is very heavy solution, to control the direction of sample loading. The loading dye is usually used with concentration 5x, 10x.

In this lab, we mixed samples with 5x dye. The 10µl of 5x dye was added to the samples A, B, and C. To dilute the 5x dye from 1x dye, we calculated follow:

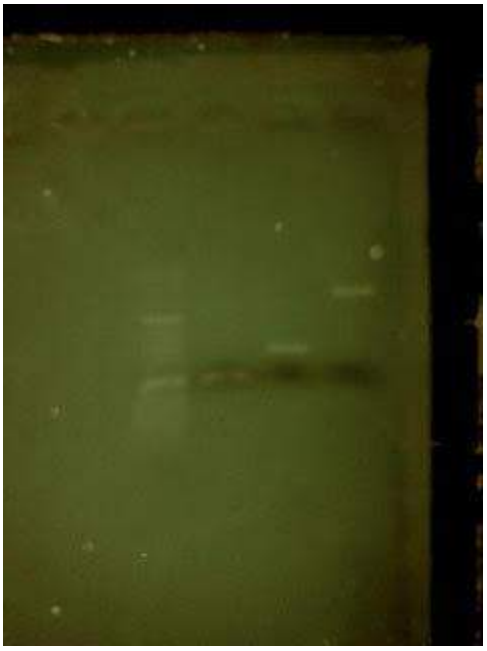
$$x10 + x = 15$$

The result gave x = 2.5.

So, 2.5 µl of dye was added into the sample.

D. Run gel electrophoresis

After put three samples A, B, and C plus 2.5 µl of dye in to the gel, and also put 5 µl in to the gel. The gel electrophoresis was run at 100 V for 25 minutes. The result of the gel electrophoresis was show as the picture.



Conclusion:
There was something wrong with the gel green.

Next step:

Next class we will be preparing our primers.