

5/8/18

Abnormal legs

Normal eye phenotype - inconclusive for now

Missing segmentation between the distal femur and proximal tibia

Pre tarsus is one instead of two

Basal insects (primitive) if they dont have wings they usually have only one claw

Phenotype goes back to the ancestral state

Study more the role of lim1 in leg developmetn, how does it effect the pre tarsal

Most distal of antennae is fused

### Lab notebook template

**Name:** Sarah Kosse and Bridget Dames

Date: 4/26/18

Week#:

**Objective:**

Observe injected beetles

**Procedures:**

**Note:**

Pupae- one eclosed (come out of shell)

Legs are weird - 11

One antennae is shorter and compact

RNAi is working because we can confirm because the antenna segments are fused similar to what we

**Conclusion:**

**Next step:**

### Lab notebook template

**Name:** Sarah Kosse and Bridget Dames

Date: 4/24/18

Week#:

**Objective:**

Observe injected beetles

**Procedures:**

**Note:**

Larva - 15 -cant tell how many are dead but there is alot of movement in the flour

Pupae- 15 injected- 11 alive

Legs longer than normal - 4

**Conclusion:****Next step:****Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 4/12

Week#:

**Objective:**

Invitro transcription

DNase treatment

To prepare double-stranded RNA, free pf protein and other contaminating molecules

**Procedures:**

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## 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  $\mu$ L transcription reaction; scale up if your transcription reaction was larger.

Amount	Component
20 $\mu$ L	dsRNA (from step <a href="#">B.4</a> or step <a href="#">C.2</a> )
21 $\mu$ L	Nuclease-free Water
5 $\mu$ L	10X Digestion Buffer
2 $\mu$ L	DNase I
2 $\mu$ L	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.

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## 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  $-95^{\circ}\text{C}$  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 $\mu$ L	dsRNA (from step <a href="#">D.2</a> above)
50 $\mu$ L	10X Binding Buffer
150 $\mu$ L	Nuclease-free Water
250 $\mu$ L	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  $\mu$ L dsRNA binding mix onto the filter in the Filter Cartridge, and draw it through by centrifugation or with a vacuum manifold.

**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  $\mu\text{L}$  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  $\mu\text{L}$  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 $\mu\text{L}$ Wash Solution



**IMPORTANT**

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

- a. Pipet 500  $\mu\text{L}$  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  $\mu\text{L}$  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 $\mu\text{L}$ 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 eluted into any sterile low salt solution ( $\leq 30$  mM), e.g. 5 mM KCl, 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  $\mu\text{L}$  (hot) Elution Solution to the filter in the Filter Cartridge.
  - Apply preheated ( $\geq 95^\circ\text{C}$ ) 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  $65^\circ\text{C}$  or warmer for 2 min.
- c. Centrifuge for 2 min at maximum speed.

d. Repeat steps **b–c** with a second 50–100  $\mu\text{L}$  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.

**5. Quantitate and store the dsRNA**

Quantitate the reaction product by measuring its absorbance at 260 nm and calculating the concentration (see section [V.A. Quantitation of RNA by Spectrophotometry](#) on page 19).

The dsRNA is stable when stored at  $-20^{\circ}\text{C}$  in Elution Solution.

**6. Check 1/400<sup>th</sup> of the purified dsRNA on an agarose gel**

Run 1/400<sup>th</sup> of the dsRNA on a 1% agarose gel (nondenaturing) to examine the integrity and efficiency of duplex formation.

- 1/400<sup>th</sup> of 100  $\mu\text{L}$  elution volume is 2.5  $\mu\text{L}$  of a 1:10 dilution
- 1/400<sup>th</sup> of 200  $\mu\text{L}$  elution volume is 5  $\mu\text{L}$  of a 1:10 dilution
- Dilute the gel samples in TE (10 mM Tris, 1 mM EDTA) or in gel loading buffer

(Instructions for running the gel are in section [V.B](#) on page 19). The dsRNA will migrate slightly slower than DNA markers of the same length. See Figure 4 on page 15 for an example of how the dsRNA reaction products will look on a gel.

**Note:**

Primer with T7 promoter on the 5' side

RNA polymerase binds to the promoter

After transcribed they will hybridize automatically

This will cause a dsRNA

The direction of RNA polymerase synthesizes 3 $\rightarrow$  5 of the antisense strand

RNA polymerase will use the template and make complementary RNA

Want to remove salts, NTP, DNA and protein (polymerase) and single stranded RNA

- We can use column to remove salts, NTP and protein
- We can use DNase to remove DNA
- We can use RNase to remove ssRNA

Some stay single stranded and we want double stranded

Need a column to remove everything except nucleotides

To remove only single stranded RNA= use RNAase

**Conclusion:**

**Next step:**

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 4/10/2018

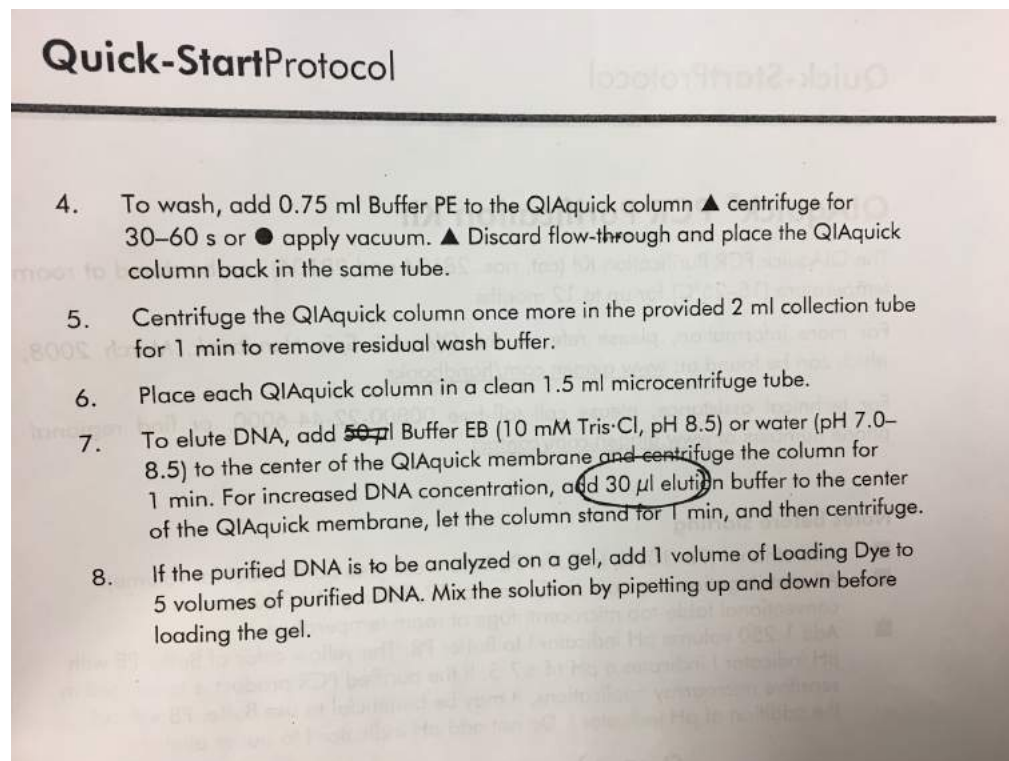
Week#: 9

**Objective:**

Purify DNA from PCR to use as template to make dsRNA  
Invitro transcription

**Procedures:**

1. Add 5 volumes Buffer PB to 1 volume of the PCR reaction and mix. If the color of the mixture is orange or violet, add 10  $\mu$ l 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn yellow.
2. Place a QIAquick column in ▲ a provided 2 ml collection tube or into ● a vacuum manifold. For details on how to set up a vacuum manifold, refer to the *QIAquick Spin Handbook*.
3. To bind DNA, apply the sample to the QIAquick column and ▲ centrifuge for 30–60 s or ● apply vacuum to the manifold until all the samples have passed through the column. ▲ Discard flow-through and place the QIAquick column back in the same tube.



**After purification → nanodrop to see if we eluted at least 189.5 ng/microliter of DNA**

**Note:**

T7 RNA polymerase to bind to the T7 promoter

10x buffer 2 microliters

Water to adjust entire volume

1.5 microgram of our template the minimum concentration we need is 187.5 ng/microliter

Found this by converting 1.5 micrograms to nanograms and divide by 8 microliters  
 8 microliters is the max volume that we can add for total to be 20

$$(314.8 \text{ ng/microliter})(V1) = (187.5 \text{ ng/microliter}) (8 \text{ microliters})$$

$$V1 = 4.76 \text{ microliters}$$

8 microliter dNTP  
 2 microliter enzyme

Total has to be 20 microliters

**Conclusion:**

**Next step:**

Injection

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 4/5/2018

Week#: 9

**Objective:**

Template synthesis via PCR  
 DNA fragment purification

**Procedures:**

**Reaction**

H <sub>2</sub> O	___ μl	102 μl
5 X PCR buffer	20 μl	40 μl
*MgCl <sub>2</sub> (25mM stock)	6 μl (1.5 μM)	___ μl (___ μM)
dNTP mix (2.5mM stock)	8 μl (0.2 μM)	16 μl
Primer1 ( 10 μM stock)	___ μl (___ μM)	30 μl (10 μM)
Plasmid DNA (_____)	___ μl	10 μl
Taq polymerase (5U/ μl)	0.5 μl (2.5U /100 μl)	2 μl
Total	100 μl	200 μl 8x 25 microliters tubes

$$46 / 2 = 23 + 1 \text{ microliter F1} + 1 \text{ R1} = 25$$

\*No MgCl<sub>2</sub> if using TAKARA Ex Taq or Promega GoTaq

40 microliter of 10 ng/microliter

**Reaction Cycle**

Program name: \_\_\_\_\_  
 Denaturation: 95 □ X 5 min

↓  
 Denaturation: 95 □ X 30 sec  
 Annealing: 61-62 □ X 30 sec  
 Extension: 72 □ X 30 sec

35 cycles

**Result**

Electrophoresis: \_\_\_% agarose, \_\_\_ X TAE/ TBE  
 Loaded sample \_\_\_ μl / lane

↓  
Extension: 72 □ X 5 min —  
↓  
Hold: 4 □

\*2: Primer Tm + 1-2 □

\*3: 500bp/30sec, 1,000bp/1min

**Note:**

Make DNA with T7

We want to add promoter sequence on both ends of our DNA

Make DNA from Plasmid DNA, use DNA polymerase = replication

-for this we need primer, dntp etc.

-this reaction is called PCR to make copies of this DNA

We have T7 on one side of our DNA, but we do have T3 on the other side

We need to use the primer and when we design primer we can add T7 promoter sequence to our primer, if we do this, then every single amplicon with our primer will have T7 promoter sequence

If we target plasmid with the t7 sequence = TOPO RNAi primer

**Conclusion:**

**Next step:**

**Lab notebook template**

**Name:** Sarah Kosse and Bridget Dames

Date: 4/3/18

Week#: 9

**Objective:** dry lab

How DNA sequences work

Sequence analysis

**Procedures:**

**Note:**

dNTP vs. ddNTP



-ddNTP lacs the oxygen so it leads to termination of synthesis because you cannot add a nucleotide onto the end of the ddNTP

**Conclusion:**

**Next step:**

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 3/29

Week#: 8

**Objective:** purify DNA to get rid of macromolecules

**Procedures:**

Add 2 ml and centrifuge

Remove liquid

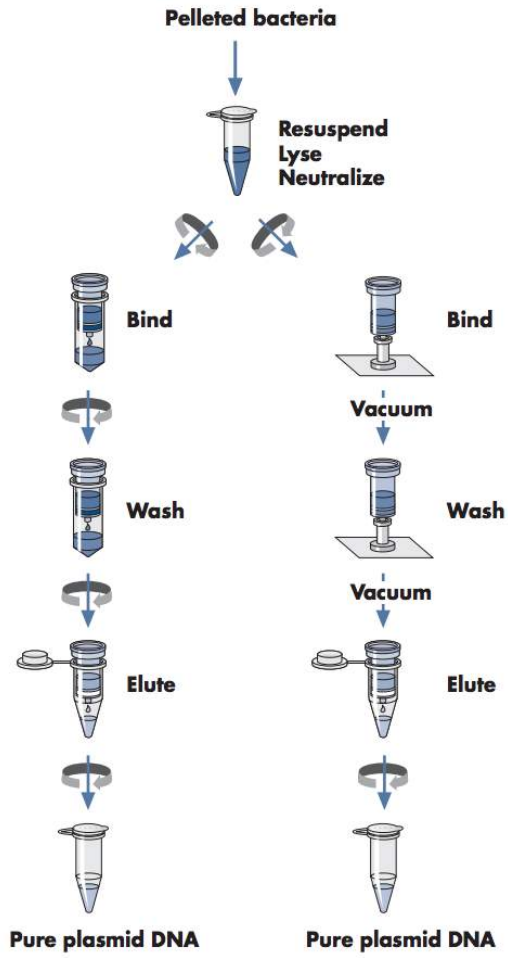
Repeat

Increase pH to kill cell by adding P2

1 min

Neutralize with N3

**QIAprep Spin Procedure  
in microcentrifuges on vacuum manifolds**



## Protocol: Plasmid DNA Purification using the QIAprep Spin Miniprep Kit and a Microcentrifuge

This protocol is designed for purification of up to 20 µg of high-copy plasmid DNA from 1–5 ml overnight cultures of *E. coli* in LB medium. For purification of low-copy plasmids and cosmids, large plasmids (>10 kb), and DNA prepared using other methods, refer to the recommendations on pages 35–36.

Please read “Important Notes” on pages 13–18 before starting.

Note: All protocol steps should be carried out at room temperature (15–25°C).

### Procedure

1. **Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.**

Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet.

If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.

2. **Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times.**

Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min.

If LyseBlue has been added to Buffer P1 the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.

3. **Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.**

To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.

If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

4. **Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.**

A compact white pellet will form.

5. Apply 800  $\mu$ l of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting.
6. Centrifuge for 30–60 s. Discard the flow-through.
7. **Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 ml Buffer PB and centrifuging for 30–60 s. Discard the flow-through.**

This step is necessary to remove trace nuclease activity when using *endA*<sup>+</sup> strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5 $\alpha$  do not require this additional wash step.

8. **Wash QIAprep 2.0 spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60 s.**
9. **Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual wash buffer.**

**Important:** Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

10. **Place the QIAprep 2.0 column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50  $\mu$ l Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 spin column, let stand for 1 min, and centrifuge for 1 min.**

**Note:**

Mix gently after adding P2 and Pipette gently

**Conclusion:**

**Next step:**

Analyze sequences

Lab notebook template

**Name:** Sarah Kosse and Bridget Dames

Date: 3/27/18

Week#: 8

**Objective:** Confirm size of gene by running gel

Chose 2 bands to culture

**Procedures:**

Make 1.5 % gel

Place 2 microliters of each PCR soln into 4 different wells

Run gel for 30 minutes

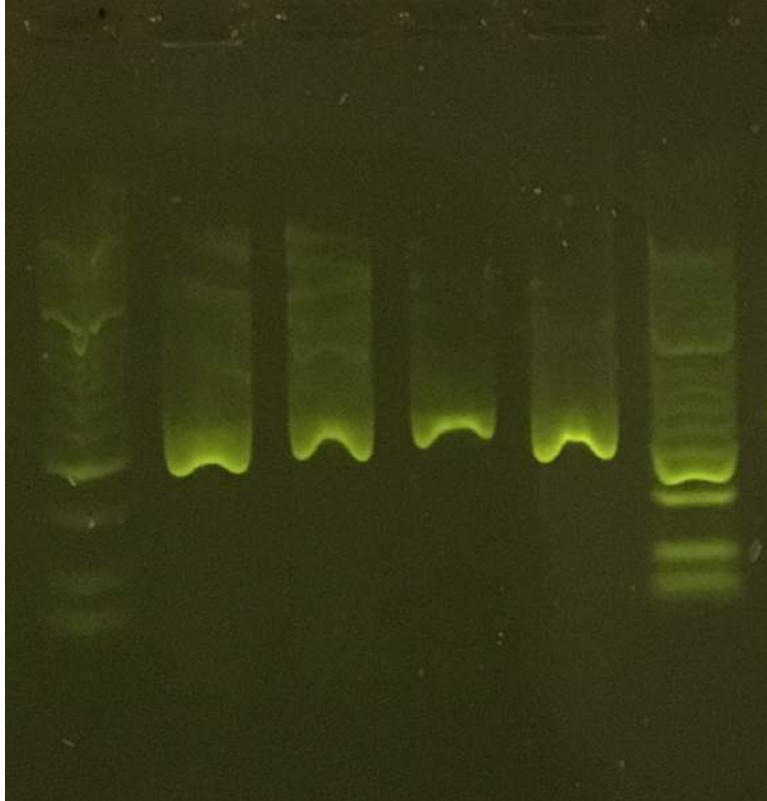
Choose 2 bands to culture

**Note:**

Try to culture two  
Use 50bp ladder  
1.5% because the sequences are longer  
Primer 1 amplicon length should be around 401 bp  
2 microliters in each well

**Conclusion:**

**Chose bands 1 and 2**

**Next step:**

Purify the plasmid

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 3/15/18 (Extra Credit word--- Pacific Rim 2)

Week#: 7

**Objective:**

To test if the kill gene system really works - take advantage of PCR to do so  
To pick up colonies and perform colony PCR

**Procedures:**

Pick colony from plate

Put into 15 microliter LB + amp

Culture 37 degree for 1 hour

Mixing every 15 minutes

Use for PCR

pCR 4-TOPO

Do the PCR then determine if the amplified product matches with our predicted length of our gene

--Water 8.15

--Green Buffer 4

-dNTP mix 1.6

-Primer1 2

-Primer2 2

Taq 0.25

Colony 2

Total 20

X4

PCR

95- 5 min

94- 30 sec

55- 30 sec

72- 30 sec

72- 2 min

4 C

**Note:**

Use kill gene to identify colony (ccdb)

All the ecoli that survive should have plasmid that survive

Using blue gene is usually pretty vague

Primer side on the plasmid, not your gene, so if we do PCR you will have the size of your gene

Easiest way is to use your pipette tip

Pick the colony that looks big

One tip gets MILLIONS of colonies

**Conclusion:**

Always have plan B

Microbiology is not always exact

Exact same PCR can sometimes work and sometimes not



**Next step:**

Run gel for colony PCR  
Start E.coli culture

**Lab notebook template**

**Name:** Sarah Kosse and Bridget Dames

Date: 3/13

Week#: 7

**Objective:** Cloning: put DNA fragment into plasmid

**Procedures:**

4 microliters purified DNA

1 microliter salt solution

1 microliter TOPO vector

In 1.5 microliter tube

Rt for 30 min

TOPO TA cloning short:

<https://drive.google.com/drive/u/0/search?q=lab%20tomoyasu%20topoisomerase>

Ecoli on ice  
3 microliter of our topo reaction mixture into cell  
5 min on ice  
30 sec in 42 degrees  
5 min on ice  
250 microliters of SOC into cell  
Wait 37 degrees in incubator for 50min-1 hr  
Then plate 120 microliter of bacteria  
Use "hockey stick" to spread the bacteria on the plate then close  
Leave 30 degree overnight

**Note:**

Do not vortex - it will denature the enzyme  
Can spin down

Put your DNA fragment into plasmid, then put your plasmid into E. coli  
Restriction Enzymes to produce sticky ends, then use ligase to attach  
Topoisomerase used to release tension  
Use antibiotics to choose if e coli has plasmid or not  
Use ccdB to see if the e coli has the kill gene

Keep the petri dish upside down

**Conclusion:**

Cloning a bacteria is very sensitive and can be contaminated very easily.

**Next step:**

Collect Bacteria cultures

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 03/08/18

Week#: 6

**Objective:**

Run the gel electrophoresis to purify the gene  
How to use a centrifuge

**Procedures:**

Make the gel (1.2%)

-0.6g agar

-50 mL gel green TBE (make 100 total)

- 3 min in microwave take out and stir 1 more minute in microwave on 3 power



Pour gel into cassette and let sit until hard with plastic grooved piece (6 wells) so that wells are created in the gel

TBE without green in big box - fill until covers the gel

100V for 25 min - stop running when the color of the loading dye reaches  $\frac{2}{3}$  of the gel

Wipe off gel

Load 10 microliters of the DNA + loading dye into the wells of the gel and run the gel

After you run the gel use blue light to see the size of our DNA fragments

Use Extractor and push down on the desired band to take out gel band.

put in the basket

Freeze for 5 minutes

Centrifuge for 1300 for 3 minutes

**Note:**

Extracted the larger band, the one that did not move as far through the gel

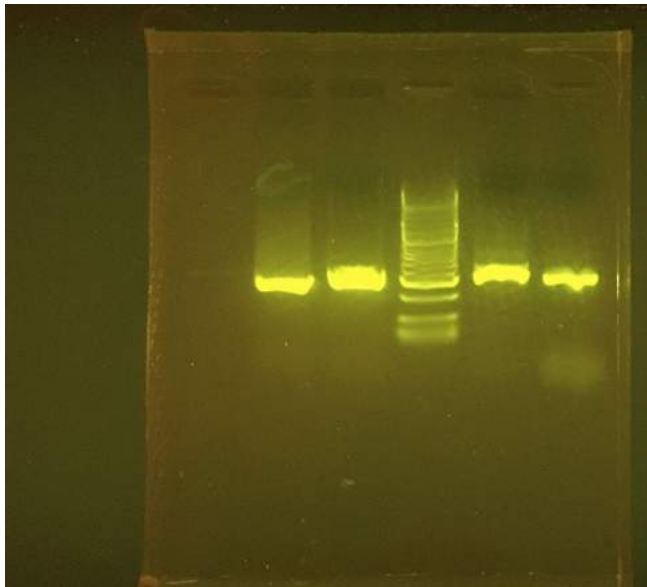
Extracted DNA with F\_1 and R\_1 primers

When the rotor is not balanced it can fly your sample out!

**Conclusion:**

Gel electrophoresis is used to purify the gene of interest. Used the fourth band from the left.

Primer set 1



**Next step:**

Put the DNA into a plasmid - DNA extraction

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 03/06/18

Week#: 6

**Objective:**

Learn how to dilute primers and prepare for a PCR

**Procedures:**

Dilute Primer- 50 microliter of 10 micromolar from 100 micromolar stock

TC014939\_F1 - 19.7 nmol; 0.12mg = 197 microliters

TC014939\_R1 - 28.6nmol; 0.17 mg = 286 microliter

TC014939\_F2 - 26.1 nmol; 0.16mg = 261 microliters

TC014939\_R2- 34.1 n mol; 0.21mg = 341 microliters

$$(28.6 \text{ nmol} \times 10^{-9}) / X \text{ L} = 100 \text{ microM} \times 10^{-6}$$

Dilute the stock:

$$(100 \text{ micromolar}) (x) = (50 \text{ microliters}) (10 \text{ micromolar})$$

Program to run the PCR:

**Note:**

Everything we get from the freezer, vortex and spin down except for the enzyme

Make the 46 microliter master solution in one tube then split in half before you add the forward and reverse primers

Date. \_\_\_\_\_

Aim: \_\_\_\_\_

Gene name: Lim1

Forward Primer: Tm=59.97 °C: sequence 5'--AGGCGGTACGGTACAAAGTG

Reverse Primer: Tm=60.03°C: sequence 5'-- CCGTGCAACGTGTGATGTTT

**Reaction**

H <sub>2</sub> O	___ μl	30.5 μl
5 X PCR buffer	20 μl	10 μl
*MgCl <sub>2</sub> (25mM stock)	6 μl (1.5 μM)	0 μl (___ μM)
dNTP mix (2.5mM stock)	8 μl (0.2 μM)	4 μl
Primer1 ( ___ 10 μM stock)	___ μl (___ μM)	2 μl (10 μM)
Primer2 ( ___ 10 μM stock)	___ μl (___ μM)	2 μl (10 μM)
Sample DNA (_____)	___ μl	1 μl
Taq polymerase (5U/ μl)	0.5 μl (2.5U /100 μl)	0.5 μl
Total	100 μl	50 μl

$$46 / 2 = 23 + 1 \text{ microliter F1} + 1 \text{ R1} = 25$$

\*No MgCl<sub>2</sub> if using TAKARA Ex Taq or Promega GoTaq

**Reaction Cycle**

Program name: \_\_\_\_\_

Denaturation: 95 °C X 5 min

**Result**

Electrophoresis: \_\_\_% agarose, \_\_\_X TAE/ TBE

Loaded sample \_\_\_ μl / lane

↓  
 Denaturation: 95 ° X 30 sec  
 Annealing:     61-62 ° X 30 sec  
 Extension:     72 ° X 30 sec     }     35 cycles  
 ↓  
 Extension:     72 ° X 5 min  
 ↓  
 Hold:     4 °

\*2: Primer Tm + 1-2 °

\*3: 500bp/30sec, 1,000bp/1min

**Conclusion:**

Listen better :)

PCR amplifies our gene of interest

**Next step:**

Run PCR then run the gel electrophoresis

**Lab notebook template**

**Name: Sarah Kosse and Bridget Dames**

Date: 03/01/18

Week#: 5

**Objective:** To learn how to run gel electrophoresis

To figure out the size of the DNA unknown fragment (Size A, B, C)

**Procedures:**

Make the gel (1.2%)

-0.6g agar

-50 mL gel green TBE

- 3 min in microwave take out and stir 1 more minute in microwave on 3 power

Pour gel into cassette and let sit until hard with plastic grooved piece so that wells are created in the gel

A- 82 ng/microliter

B- 118 ng/microliter

C- 115 ng/microliter

Diluter all 10 microliter of 10 ng/microliter

M1V1=M2V2

A- 1.22 microliter of DNA and 8.78 microliter of water

B- 0.85 microliter of DNA and 9.15 microliter of water

C- 0.87 microliter of DNA and 9.13 microliter of water

Mix DNA with 5x loading dye, then put the DNA into the gel

$$\frac{1}{5} = \frac{x}{10 + x} \rightarrow 2.5 \text{ microliters loading dye}$$

TBE without green in big box - fill until covers the gel

100V for 25 min - stop running when the color of the loading dye reaches  $\frac{2}{3}$  of the gel

Wipe off gel

Load 10 microliters of the DNA + loading dye into the wells of the gel and run the gel

After you run the gel use blue light to see the size of our DNA fragments

**Note:**

Gel- agar - make the gel with the TBE with gel green (aluminum around it)

Gel electrophoresis: separates DNA based on size

DNA is negatively charged so it will flow toward the positive probe; smaller the DNA fragment, the farther it moves through the gel

Ladder (usually 1kb)- mixture of DNA molecules that contains many different sized fragments of DNA to act as a control. Ladder tells you the size of your DNA fragment

Buffer- TAE and TBE (0.5x TBE)

Ethidium bromide goes into the DNA so that you can see the DNA with UV light

Gel green - use blue light to excite the dye

1% gel separates 1 kb - 5 kb (1g in 100mL buffer)

1.2-1.5% 100 kb - 1000 kb

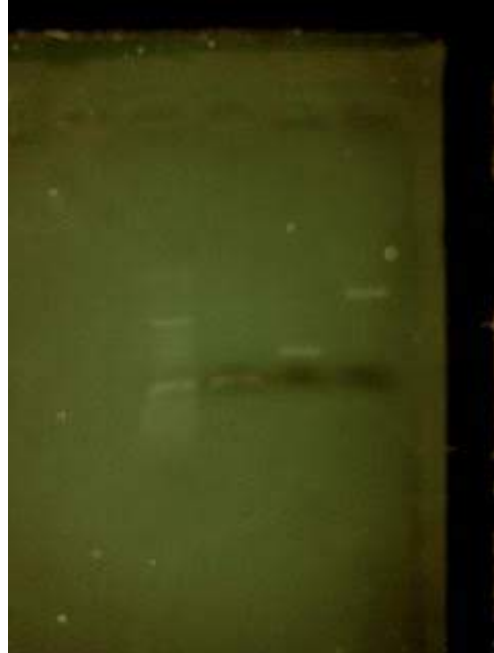
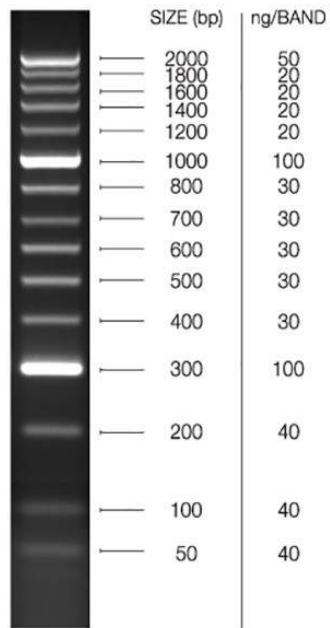
5x loading dye

Red is plus

10 microliter of ladder

**Conclusion:**

The size of A, B, and C fragment



**Next step:**

Dilute the primers for our gene.

Use the knowledge learned in this class to run a gel electrophoresis with our own gene.

**Lab notebook template**

**Name:** Sarah Kosse and Bridget Dames

Date: 02/27/18

Week#: 5

**Objective:** To learn basic lab techniques: how to pipette,

**Procedures:**

- use the pipette to transfer liquid from one tube to another
  - set amount on pipet, push down until first stop, place the pipet into the liquid, suck up the liquid
  - to push liquid out, push all the way down

**Note:**

- P1000 (200-1000 microliters)
- P200 (20-200 microliters)
- P20 (2-20 microliters)
- P10 (0.5-2microliters)
  - do not use p10 for 2-10 microliters (use p20)
- always keep pipet vertical
- keep tip box closed when not in use
- 

**Conclusion:**

Pipettes are fun!!!

**Next step:**

Pipette some real stuff

Lab notebook template

**Name: Sarah Kosse and Bridget Dames**

Date:

Week#:

**Objective:**

**Procedures:**

**Note:**

**Conclusion:**

**Next step:**