

Induction of the Lambda Lysogen

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In this experiment, students will examine gene regulation in bacteria – the production of λ phage particles that occurs when the lysogenic relationship between a bacterial cell and its quiescent prophage is disrupted. The lysogenic state in the bacterial cell is maintained by a repressor protein specified by the λ virus; deactivation of the repressor results in de-repression of the regulated genes and production of λ phage. Normally, the lysogenic state is indefinitely stable once established and a lysogenic bacterial cell produces lysogenic progeny cells, but no free viruses. The particular strain of lambda we are using in this experiment has a temperature sensitive mutation (857) in the repressor gene (*cI*) that results in an altered repressor protein that can be inactivated by a shift to 42°C. Therefore, we can easily initiate the lytic or reproductive cycle of phage lambda in an entire population of lysogenic bacteria and follow the events of phage production. Students are given a lysogenic culture of CM58 bacterial cells that they will shift to 42°C. They will take aliquots of the culture after de-repression and measure the production of lambda phage particles by pour plating with *E. coli* C600 host cells on agar plates. The phage produced will kill the host bacteria and form a clear area called a plaque upon infection of the host cells. Students will observe the lysis of the lysogenic culture, and the production of lambda phage after de-repression.

Keywords: Lamda phage, induction, lysogenic, repressor protein, pour plating, *E. coli*

Introduction

In this experiment, students will examine another system of gene regulation in bacteria – the production of λ phage particles that occurs when the lysogenic relationship between a bacterial cell and its quiescent prophage is disrupted. The lysogenic state in the bacterial cell is maintained by a repressor protein (*cI*) specified by the λ virus; deactivation of the repressor results in de-repression of the regulated genes and production of λ phage-specified structural, regulatory, and catalytic proteins.

Normally, the lysogenic state is indefinitely stable once established and a lysogenic cell produces lysogenic progeny cells, but no free viruses. The repressor protein (*cI*) prevents transcription and is responsible for preventing infection from other lambda phages (superinfection immunity) (Ptashne, 1967). Experimentally one can inactivate the repressor by a number of techniques including chemical treatment and UV-irradiation. The particular strain of lambda we are using is a strain that contains a mutation (857) in the repressor gene (*cI*) that results in an altered repressor protein. As a result of this mutation, the repressor protein is temperature sensitive and can be inactivated by a shift to 42°C. Therefore, we can easily initiate the lytic or reproductive cycle of phage lambda in an entire population of lysogenic bacteria and follow the events of phage production. This experiment is similar to a classic experiment of Ellis and Delbruck (1939) which

demonstrated the unique nature of phage reproduction and provided a basis for many important experiments that used bacteriophage as a tool to understand molecular aspects of genetics. Our experiment is different from that landmark experiment because we induce phage production from within a lysogenic strain rather than infect cells from without. For additional reading see Burz et. al. (1994)

Students are given a 15 mL log phase lysogenic culture of CM58 bacteria cells that have been grown at 30°C to maintain the lysogenic state. Before experimentation, students first take samples of the culture to determine initial parameters of viable cell count, infectious centers, and free phage. The viable count will provide a measure of the number of bacterial cells initially in the culture. The viable count is performed by taking a one mL aliquot of cells, diluting and spreading onto LB agar plates and incubating at 30°C, to maintain the lysogenic state. Infectious centers are a measure of the number of lysogenic cells in the culture. The same dilutions that were used for viable count are pour plated with *E. coli* C600 host and soft top agar on top of an LB plate, and incubated at 42°C. During this incubation at 42°C, the induction takes place on the agar plate. The lysogenic cells enter the lytic cycle, and the phage particles are assembled and released, killing the *E. coli* host cells, and causing a clear area called a plaque on the agar plate to form. One plaque is a result

of one lysogenic cell. The number of lysogenic cells can be compared to the viable count of the culture to determine the number of lysogenic cells in the initial culture. Finally, it is necessary to determine if there were any fully assembled λ phage in the initial lysogenic culture, termed the free phage. This free λ phage value should be subtracted from the λ phage produced at each time point in the experiment, in order to obtain an accurate estimation of the number of phage produced. To determine this small amount of fully assembled λ phage, students are instructed to design an experiment that will determine the free phage value. It is critical that the students think about how to obtain this value on their own, in order for them to understand the lysogenic culture. They can seek guidance from the instructor or teaching assistant but each student must come up with the experiment on their own.

To solve this question, students can do the following: An aliquot of the initial culture can either be centrifuged to pellet the cells leaving the free phage in the supernatant, or the cells can be lysed open with SDS (sodium dodecyl sulfate, a strong ionic detergent). In either procedure, the free phage in solution are pour plated with *E. coli* C600 host and soft top agar on top of an LB plate, and incubated at 42°C. Each plaque produced is a result of one lambda phage particle. Some students have suggested filtering the culture with a sterile filter, but this often provides inaccurate results, as the phage can stick to a sterile filter, and you will not get an accurate phage count.

Once all the initial parameters have been determined, the induction can begin. The students will shift the log phase lysogenic culture that has been growing at 30°C to a pre-equilibrated 42°C water bath. During this time, students will take 0.5mL aliquots of the culture every 10 minutes, to monitor

the induction. At each 10 minute time interval, the culture is removed from the water bath, and its OD (optical density) is recorded, a 0.5mL aliquot is placed in a culture tube, and the culture is put back into the 42°C water bath. In order to measure when the first phage has been fully assembled, each 10 minute aliquot is immediately treated with SDS to rupture open the cells, and to release any fully formed λ phage particles. The lysed cells at each time point are then pour plated with *E. coli* C600 host and soft top agar on top of an LB plate, and incubated at 42°C. Each λ phage particle will result in the formation of a plaque on the agar plate. The de-repression will be completed in 60 - 70 minutes. Over this time period, students will observe a decrease in the OD of the initial lysogenic culture, with a concurrent increase in the amount of plaque formation observed on the agar plates. Students will continue to take time points for 10 more minutes after the turbid culture appears clear (indicating all cells have lysed), or until the OD has reached a consistent value of zero for two time points.

This lab can be done in one 4-5 hour lab, or in two separate 3-hour lab periods. The first part of the experiment is the de-repression, taking the samples and monitoring the decrease in growth until all the cells have lysed. There is an initial 20 minute period of growth immediately after de-repression, where the phage particles are using the bacterial host cells to reproduce more phage particles. The initial lysis phase (indicated by a drop in OD) begins after 40 minutes. During the first lab period, students can take lysogenic culture aliquots every 10 minutes, rupture the cells, and store the samples in the refrigerator. During the second lab period, students can make dilutions of the time point aliquots, and pour plate with the host *E. coli* C600 host bacteria.

Student Outline

Introduction

During today's experiment you will examine another system of gene regulation in bacteria - the production of phage particles that occurs when the lysogenic relationship between a bacterial cell and its quiescent prophage is disrupted. The lysogenic state is maintained by a repressor specified by the virus; deactivation of the repressor results in de-repression of the regulated genes and production of phage specified structural, regulatory and catalytic proteins.

Normally, the lysogenic state is indefinitely stable once established and a lysogenic cell produces lysogenic progeny cells, but no free viruses. Experimentally one can inactivate the repressor by a number of techniques including chemical treatment and UV-irradiation. The particular strain of lambda we are using is strain that has a mutation (857) in the repressor gene (cI) that results in an altered repressor protein. As a result of this change, the repressor protein is temperature sensitive and can be inactivated by a shift to 42°C. Therefore, we can easily initiate the lytic or reproductive cycle of phage lambda in an entire population of lysogenic bacteria and follow the events of phage production. This experiment is similar to a classic experiment of Ellis and Delbruck (1939) that demonstrated the unique nature of phage reproduction and provided a basis for many important experiments that used bacteriophage as a tool to understand molecular aspects of genetics. Our experiment is different from that landmark experiment because we induce phage production from within a lysogenic strain rather than infect cells from without.

An Overview

- Before initiating de-repression of the prophage, you will determine the number of viable cells by spread plating.
- You will also determine the number of cells capable of producing phage (called infectious centers) by pour plating at 42°C.
- You will reserve a sample for determining the number of free phage.
- De-repression will be initiated by placing the culture in the shaking water bath at 42°C.
- You will observe thermal de-repression of the *E. coli* λ cI857 lysogen by recording the turbidity of the culture.
- Samples of the cells in culture will be removed at regular intervals after de-repression, treated with SDS and saved.
- The production of phage particles will be determined by pour plating these samples of the culture with bacterial host cells.

Review the brief overview and be certain that you know how each of these steps is carried out, what materials or equipment is used, and what record must be kept. Plan accordingly.

Protocol - Initial determinations

Viable count

Remove a 1.0 mL sample of the culture and transfer to 9.0 mL of sterile dilution fluid (D.F.). Then carry out seven serial 10-fold dilutions that will yield about 10 to 50 colonies per 0.1 mL. Label the seven tubes 10^{-1} through 10^{-7} to indicate the dilution factor. Spread 0.1 mL of this dilution and each of the three previous ones in the series onto lambda plates. Incubate the plates upside down in the 30°C incubator.

Infectious centers

To determine the number of infectious centers or plaque forming units per unit culture, pour plate samples from the 10^{-4} through 10^{-7} dilution tubes outlined above. Note that the diluted samples from the culture are not treated with chloroform before plating with the host cells.

After 8-12 hours at 42°C each of the lysogenic cells from the culture will have been fully de-repressed and will have produced phage visible as a plaque on the C600 lawn.

Free phage

Every culture of cells that is lysogenic for a virus will also have some of the complete infectious virus particles present in suspension outside of the cells. These may not be very numerous, but it is reasonable to expect that they are present. Since they may contribute to our determinations of the number of virus produced by de-repression, we have to determine the numbers of these 'free phage.'

To do so, before you initiate de-repression, remove 1.0 mL of the culture to a sterile screw capped tube and keep the sample chilled. Devise and carry out a simple procedure for determining the number of free phage per unit of culture. Consult with the instructor or teaching assistant; while they won't tell you what to do, they may discourage you from following procedures that will not give you the information you desire.

Pour plating to determine infectious centers

- Pipet 0.1 mL volumes of each of the 10^{-4} through 10^{-7} viable count dilutions into sterile Wasserman tubes.
- Add 3-4 drops of a chilled log phase culture of the host (sometimes called a 'tester') bacteria, *E. coli* C600.
- Place the tubes in a rack in the 45°C water bath or heating block and add 3-4 mL of top agar to each tube. Incubate no longer than 5 minutes.
- Immediately mix each tube vigorously and pour the contents onto the surface of solidified sterile lambda medium. Touch the lip of the tube to the agar surface to draw out the last droplet.
- Allow the top agar to solidify (approximately 1-3 minutes), then invert the plates and put them in the incubator at 42°C

Kinetics of phage production

Once all the samples have been taken for the initial parameters of the culture, measure and record the turbidity of the *E. coli* λ c1857 culture and remove a sample (~0.5 mL) of the culture to a labeled tube containing a drop of SDS. Mix the contents of this tube vigorously and place it in an ice-water slurry. This is the $t = 0$ sample.

Place the flask in the shaking water bath at 42°C.

At ten-minute intervals, read and record the turbidity of the culture and continue to collect samples. Continue in this way until you have observed that induction has occurred and cellular lysis is complete. Collect an additional 1-3 samples before you stop. Dilute each SDS-treated sample you have reserved and pour plate the dilutions as outlined below.

Diluting and plating reserved samples

These samples containing bacteriophage are plated so that the numbers (and types) of phage per unit volume can be determined. As with bacterial cells, we plate or culture the virus under conditions that allow the invisible virus to proliferate so that each single biological entity forms a visible plaque which can be counted, and whose shape, size, turbidity, etc. help in identification. Since the virus is often very numerous – titers of 10^{11} phage/mL can easily be obtained – samples must be carefully diluted so that the numbers of plaques per plate is within a countable range.

The general methods for dilution and pour plating that are outlined below and include a sufficient range of dilutions so that you can get usable counts whether there are only 10^4 phage/mL or 10^{11} PFU (plaque forming unit)/mL. We dilute every sample extensively, and plate every dilution because we do not know in advance which dilution will give us the information we need.

Pour plating to determine progeny phage

- To dilute, transfer 0.1 mL of the CHCl_3 -treated sample into 9.9 mL of sterile dilution fluid. Carry out a total four such 100-fold serial dilutions of each of the CHCl_3 -treated samples.
- Remove 0.1 mL of each of the four dilutions and pipet each into a separate sterile Wasserman tube, add 3-5 drops of tester bacteria (*E. coli* C600), let stand on the bench for 5 minutes.
- Move tubes to water bath. Pipet 3.5-4 mL top agar (approximately 50°C) into each tube. **THEY SHOULD INCUBATE FOR NO LONGER THAN 10 MINUTES!!!!**
- Mix the contents of each tube vigorously and pour the viscous liquid onto the surface of a lambda plate. Touch the lip of the tube to the surface to draw out the last droplet.
- Tilt the plate to ensure the liquid flows over the entire surface.
- Allow the top agar to solidify (approximately 1-3 minutes), then invert the plates and incubate upside down at 42°C.

Follow-up activities*Reserving the remainder of the culture*

After your last reading and sample has been taken, you should have 10 mL or more of lysed cells. Add several drops of chloroform to the remaining culture and swirl to complete the lysis of the cells. Centrifuge 10 mL of the lysate in a 15 mL Corex tube in the Sorvall SA600 rotor at 8,000 rpm (7,700 x g) for five minutes at 0-5°C. Pour or pipet off the supernatant into a sterile screw capped tube. Store this lysate in the cold.

Students can carry out research projects with the stored lysate, as it is a solution of only lambda phage. Experiments can be performed to test for superinfection immunity (the lysogenic strain is immune from infection by lambda phage). Students can design experiments with the lambda phage and lysogenic cells to test this hypothesis. The lysogenic cells are pour plated with the lambda phage and the host cells and pour plated on the agar plates. No plaque formation should occur due to superinfection immunity.

Plate Counts

Plates are incubated for 10-18 hours then transferred to the cold room for storage. Count all plates with <500 units (colonies or plaques) per plate. Plates with only a few units can be held over a light box to get a simple count, marking each unit off on the back of the plate with a marker. Estimate counts for those with >500 cells or plaques by counting a portion, usually $\frac{1}{4}$, and record these counts as the actual count x 4. Note any peculiarities of color, size, number or morphology.

About Plates

- Label plates with indelible marker on the business part – the bottom. The lid is only a dust cover. Write around the edges so your view of the plaques or colonies is not obstructed.
- Include the identity of the sample, the variable (time, perhaps), your initials, and other essential information.
- Stack plates in the incubator and tape them completely around to keep them together. Plates may also be stacked in the plastic sleeves they come in. Do not tape the sleeves closed – too much moisture collects inside.
- Be sure all plates are upside down in the incubator. The medium gives off considerable moisture during incubation which will, if incubated right side up, collect on the lid and drip on the surface of the agar. This causes colonies or plaques to run together. If incubated upside down, moisture collected on the lid (now on the bottom) will do no harm.
- When moving the plates after incubation, be careful not to slop the moisture onto the agar surface.

Getting it Right

You should plan ahead and carefully allot your time. After the initial platings, you will have a relatively long period during which you need only sample and check the OD of your culture. Use this time to prepare tubes and label plates you'll need for titering the phage. You can also dilute and plate a group of the samples as soon as they are collected if you can do so and still maintain the sampling schedule.

Notice that the samples of the culture used to determine the infectious centers are not treated with SDS, while those samples used to determine the kinetics of phage production are treated with chloroform.

*Sample Lab Report Questions**Data Tables*

Transfer your plate counts to Tables 1 and 2 shown below. Complete footnotes c, Table 1 and footnotes a and c of Table 2, showing formulas for, and samples of, calculations. Carry out the calculations and enter the values thus determined into the tables. Provide additional footnotes if necessary. NOTE: If there are problems with your data, consult your instructor.

Table 1. _____

Culture Parameter	Plate Count ^a for Dilution					Phage Titer ^c (PFU/mL) ^b
	10 ⁻³	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	
Viable Count						
Infectious Centers						
Free Phage						

Data for calculations are circled

PFU = plaque forming units

Table 2. _____

Sample	Min at 42 ^o	Turbidity (Klett)	Plate Count ^a for Dilution				Phage Titer ^c (PFU/mL) ^b
			10 ⁻²	10 ⁻⁴	10 ⁻⁶	10 ⁻⁸	

Lambda plates are (description):

PFU = plaque forming units

Calculated from data circled as follows:

Observations

Briefly note relevant observations. (You should have considered, before you started, what observations might be necessary and relevant, so you would be ready!)

Figures

Plot the log of turbidity versus time and the log of phage titer versus time on the same graph (Figure 1 – see Appendix). Find a way to appropriately display both phenomena even though the scale and range of data encompassed by each are quite different. Provide key, labels, units, and title.

Results

Submit answers and discussion as requested, in numbered sections.

Describe your method and results for determining the number of free phage present in the culture before induction.

Define the fraction of cells in the culture that are lysogenic. Show your work. Should you correct this value to account for free phage?

Burst size is the average number of progeny phage per (infected or) lysogenic cell (it is analogous to “litter” size). Set up an equation defining burst size; the units in the general equation should be appropriate for your data. Calculate burst size. Discuss.

Use your data to determine the minimum (time when you have evidence that the first cell has been lysed) and average times (time when most cells have been lysed – the average time) required for cell lysis following induction. Be specific in citing the data that demonstrate this.

Use your data to define the minimum time (time when you have evidence the first phage has been assembled) required for synthesis and assembly of phage DNA and proteins into a complete virus particle. Cite specific data.

Briefly describe the kinetics of phage production as displayed in Figure 1(see Appendix). Your data should illustrate periods or phases termed latent, burst, eclipse, rise, and plateau. Discuss the kinetics in terms of the cellular and molecular events. Explain any irregularities in the data.

In some cases, the phage titer drops after lysis has occurred. This drop in titer may or may not be apparent in your data. Explain why such a drop occurs.

The aliquots you removed from the culture at intervals after induction were treated with SDS. Alternatively, one could simply dilute and plate those samples without chloroform treatment. The values for the phage titer would be different from, but related to, those you have found. On your Figure 1 draw in a curve that accurately represents the kinetics of phage production that would be observed in that experiment. Briefly discuss that shape of this curve and how and why it differs from your own experimental curve. Identify the portions of the curves that illustrate the phases termed latent, burst, eclipse, rise and plateau.

Notes for the Instructor

The *E. coli* CM58 lysogenic culture is always grown at 30°C to prevent any de-repression. To pre-test the culture in preparation for the experiment, you can take single colonies from your plates, and streak a portion of the same colony onto two separate plates, labeling one 30°C and the other, 42°C. Streak around 6 – 10 colonies to test. Place each plate at the indicated temperature overnight. After the overnight incubation, the colonies at 42°C should be lysed and appear as a clear streak, while the 30°C plate should show growth. Single colonies are streaked onto lambda plates, and individual colonies are inoculated into 5 mL LB broth tubes. The cultures are shaken overnight to saturation, and can be kept in the cold room until ready to use.

On the day of the experiment, calculate the amount of LB broth culture needed for your class. Inoculate ½ of this volume with 2 mL of the 5.0 mL starter culture, and shake at 30°C, monitoring the growth by measuring the OD at 340 nm in a Klett Summerson Colorimeter or other spectrophotometer. Once the culture is in log phase growth, it will double every 20-30 minutes. Keep the culture growing until class time, or until the OD reaches a value of 100 on the Klett Summerson, or .5 to .6 OD 600nm on a spectrophotometer. Dilute the culture appropriately with enough LB broth to give a final Klett reading of 40 OD, or .4 OD600nm. Each student is given one 15 mL culture to start.

The host cells: *E. coli* host C600 can be grown at 37°C in LB broth, however, maltose must be added to the LB broth at a final concentration of 0.2 % in order to produce the receptor protein on the host cells. Once the cell density has reach 80-100 Klett or .5 OD600, the culture is spun down at 6,000 rpm for 5 minutes, and the resulting *E. coli* pellet is resuspended in an equal volume of resuspension buffer.

The strain can be stored frozen at -80°C in LB broth with glycerol added to a final concentration of 30%. The lambda lysate is stored at 4°C, and is stable for a year.

The CM58 strain and answers to the questions can be obtained by contacting Carrie Doonan at the above address.

Materials

- 40 dry “lambda” plates (tryptone and NaCl)
- Dropper bottle of SDS
- 15 mL early log phase culture of strain CM58, *E. coli* λcI857 in a side-arm flask
- 15 mL of host bacteria - *E. coli* C600 - grown to mid-log phase, resuspended in Tris MgSO₄ buffer
- 1 large screw cap tube to store your λ lysate
- 32 sterile 12 x 100 mm (Wasserman) capped tubes
- 40 dilution tubes each with 9.9 mL of sterile dilution fluid (black caps)
- 10 dilution tubes each with 9.0 mL sterile dilution fluid (lavender caps)
- Sterile pipets, 0.1, 1.0, and 10.0 mL
- Melted sterile top agar
- Ice-water slurry

- Shaking water bath at 42°C
- Serological bath at 45°C
- Rubber-coated racks for bath
- Vortex mixer
- Klett-Summerson colorimeter and blank

Resuspension buffer, pH 7.4

1.82 g Tris-base, final concentration = 0.01M
 3.69 g MgSO₄•7H₂O, final concentration= 0.01M
 1500 mL H₂O
 Adjust pH to 7.4

Top Agar			
Ingredients	500mL	1 liter	2 liters
Sodium chloride	2.5 g	5.0 g	10.0 g
Nutrient broth	4.0 g	8.0 g	16.0 g
Agar	4.0 g	8.0 g	16.0 g
H ₂ O	500 mL	1 liter	2 liters

Heat to boiling to solubilize agar, stirring constantly on a stir plate. Dispense into small bottles. Autoclave for 25 minutes to sterilize. Remove from autoclave and store in oven or water bath at 55°-60°C until needed. We usually make this on the morning of the day it is to be used.

1X Dilution Fluid (DF)				
Ingredients	500mL	1 L	2 L	4 L
Potassium phosphate, monobasic	0.68 g	1.36 g	2.72 g	5.44 g
Potassium phosphate, dibasic	0.87 g	1.74 g	3.48 g	6.96 g
Magnesium sulfate heptahydrate, or 1.0M MgSO ₄	0.123 g (0.5 mL)	0.246 g (1.0 mL)	0.492 g (2.0 mL)	0.984 g (4.0 mL)
Calcium chloride dihydrate, or 0.1M CaCl ₂	0.0075 g (0.5 mL)	0.015 g (1.0 mL)	0.03 g (2.0 mL)	0.06 g (4.0 mL)
Sodium chloride	2.65 g	5.3 g	10.6 g	21.2 g
H ₂ O	to 500 mL	to 1 liter	to 2 liters	to 4 liters

LB Broth				
Ingredients	100 mL	250 mL	500 mL	1000 mL
Tryptone	1 g	2.5 g	5 g	10 g
Yeast extract	0.5 g	1.25 g	2.5 g	5 g
Sodium chloride	1 g	2.5 g	5 g	10 g
Thymine (2 mg/mL)	0.7 mL	1.75 mL	3.5 mL	7 mL
1N NaOH	0.2 mL	0.5 mL	1 mL	2 mL
H ₂ O	100 mL	250 mL	500 mL	1000 mL
For LB + amp, make stock solution of 20 mg/mL and add:	0.5 mL	1.25 mL	2.5 mL	5 mL

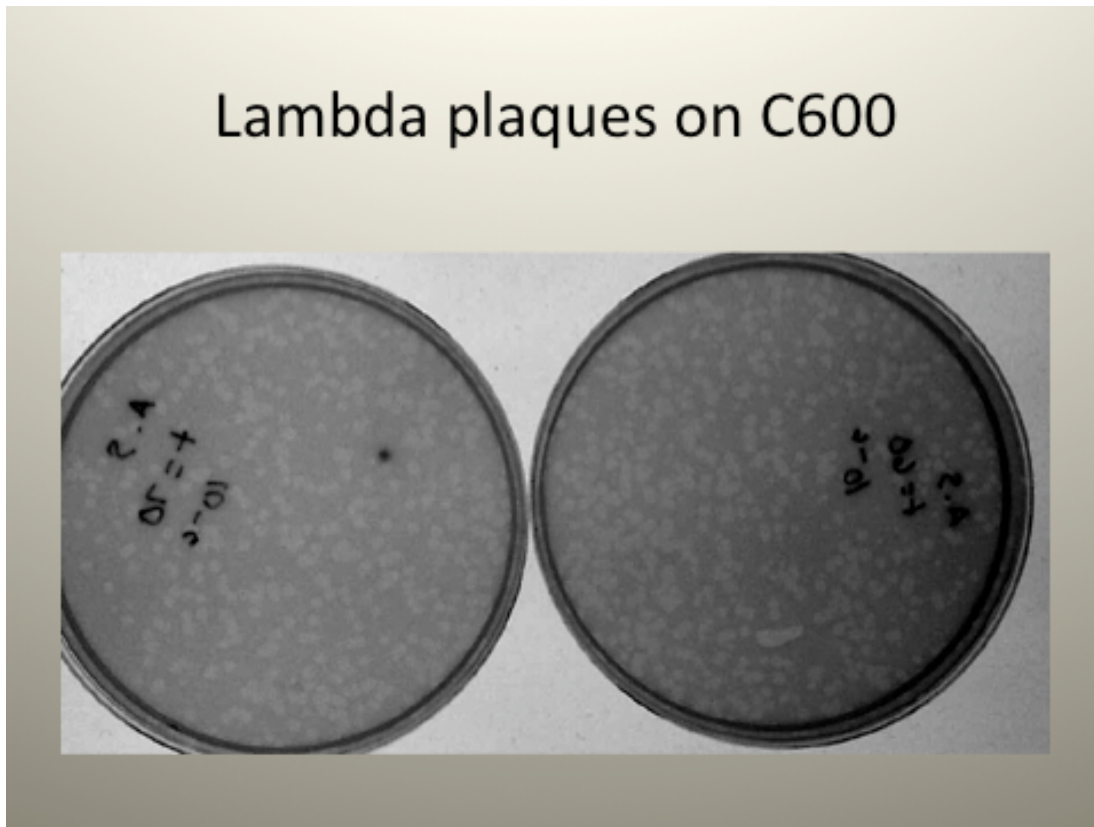
20% Maltose			
Ingredients	100 mL	250 mL	500 mL
Maltose	20 g	50 g	100 g
H ₂ O	to 100 mL	to 250mL	to 500 mL

Lambda Plates				
Ingredients	500 mL	1000 mL	2000 mL	3000 mL
Tryptone	5 g	10 g	20 g	30 g
Sodium chloride	2.5 g	5 g	10 g	15 g
1N NaOH	0.575 mL	1.15 mL	2.3 mL	3.45 mL
Agar	7.5 g	15 g	30 g	45 g
H ₂ O	500mL	1000mL	2000mL	3000mL
Combine reagents in flask and autoclave. Larger quantities (>1 liter) should be autoclaved 40-45 minutes. Smaller quantities should be autoclaved 20-25 minutes. Pour plates. Yields 35-40 plates/liter.				

Literature Cited

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- Burz, D. S.; Beckett, D.; Benson, N.; Ackers, G. K. (1994). Self-assembly of bacteriophage lambda cI repressor: Effects of single-site mutations on the monomer-dimer equilibrium. *Biochemistry* 33 (28): 8399-8405.

Appendix Sample Data



Data Tables

Table A1. Determination of Viable Count, Infectious Centers, and Free Phages for λ Phage Incubated with *E. coli* C600 at 42°C _____.

Culture Parameter	Plate Count ^a For Dilutions							Units/mL culture ^c
	Undiluted	10 ⁻²	10 ⁻⁴	10 ⁻⁵	10 ⁻⁶	10 ⁻⁷	10 ⁻⁸	
Viable count			~556	63	11	0		6.3 x 10 ⁷ CFU ^d
Infectious Centers			9	1		0		9.0 x 10 ⁵ PFU ^b
Free Phage	15	8	1		4		29 ^e	1.5 x 10 ² PFU

a. Data for calculations are circled, PFU = Plaque Forming Units

b. Calculated as in sample calculation: number of colonies/plaques counted from plate X 1/dilution factor = amount of colonies/phages in original culture / amount plated = CFU/PFU per mL. Using viable count as an example:
 $63 \times (1/10^{-5}) = 6.3 \times 10^6 / 0.1 \text{ mL} = 6.3 \times 10^7 \text{ CFU/mL}$.

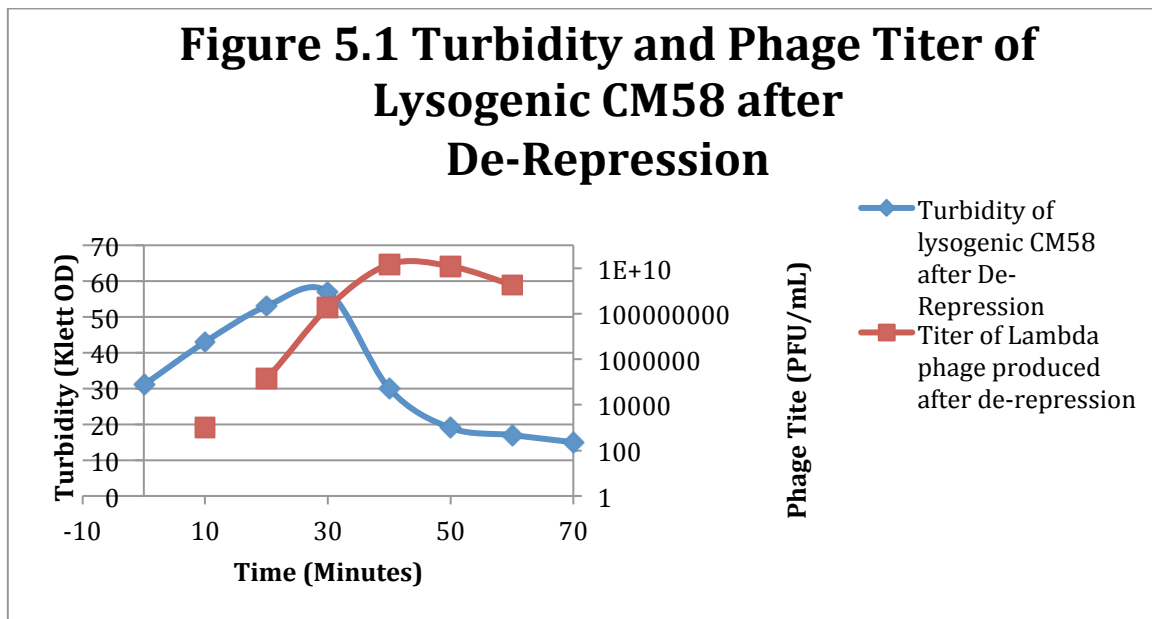
c. CFU = colony forming units

d. This value does not follow the trend for this set of data. We believe something may have happened during the experiment, such as twice the amount of free phage being added to the Wasserman before pour plating.

Table A2. Determination of Phage Titer of λ Phage^a Incubated with *E. coli* C600 at 42°C _____

Sample	Minutes at 42°C	Turbidity (Klett)	Plate 10 ⁻²	Count 10 ⁻⁴	At 10 ⁻⁶	Dilution 10 ⁻⁸	Phage Titer ^c PFU/mL ^b
0	0	31	Lawn ^d	Lawn	Lawn	Lawn	0
1	10	43	1	Lawn	Lawn	Lawn	1.0 x 10 ³
2	20	53	~480 ^f	Lawn	Lawn	Lawn	4.8 x 10 ⁵
3	30	57	CL ^e	CL	86	113	8.6 x 10 ⁸
4	40	30	CL	CL	~592	45	4.5 x 10 ¹⁰
5	50	19	CL	CL	~576	20	2.0 x 10 ¹⁰
6	60	17	CL	CL	~236	9	9.0 x 10 ⁹

- Lambda plaques are (description): small, circular plaques. The edges are very even and the circles are very symmetric circles; they are not lop-sided or deformed. They are very regular shaped circles.
- PFU = plaque forming units
- Calculated from data circled as follows: number of plaques counted from plate X 1/dilution factor = amount of phages in original culture / amount plated = PFU/mL. Using sample 3 as an example: 86 X (1/10⁻⁶) = 8.6 X 10⁷ / 0.1 mL = 8.6 X 10⁸ CFU/mL.
- A lawn indicates that no phage plaques were observed on the plate. What was observed on the plate was host bacteria and top agar.
- CL = confluent lysis. This means that there were so many phages that all or nearly all the bacterial cells were infected and lysed upon the plate. The top agar was nearly clear and the plaques ran together, such that they were unable to be counted.
- Even though the upper limits of the amount of plaques the eye can easily perceive is 200, I have no choice but to take this value, as it is the only value obtained for this time point out of the possible dilutions. This number was used to calculate the phage, with this reasoning in mind.

Sample F

About the Author

Carrie Doonan was educated at Chatham College (BS) and the University of Connecticut, (Ph.D) and began her teaching career at Carnegie Mellon University in 1993. Carrie is a Teaching Professor in the Department of Biological Sciences and Director of Undergraduate Laboratories. Her primary area of focus involves the teaching and administration of a range of experimental laboratories in the department. She is responsible for writing and developing experimental units, training of junior faculty and teaching assistants and is actively involved in all aspects of the undergraduate program. She has adapted many of her curricular innovations for use in K-12 outreach and has been invited to present this work at regional and national forums. She served as a Biotechnology Institute National Biotechnology Teacher-Leader in 2003 and

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