Lecture 16

Temperate Bacteriophage Methods

Transduction as art.

A genetic switch Let us remember the split life cycle of the temperate bacteriophage lambda:

Cryptic infection by temperate bacteriophage

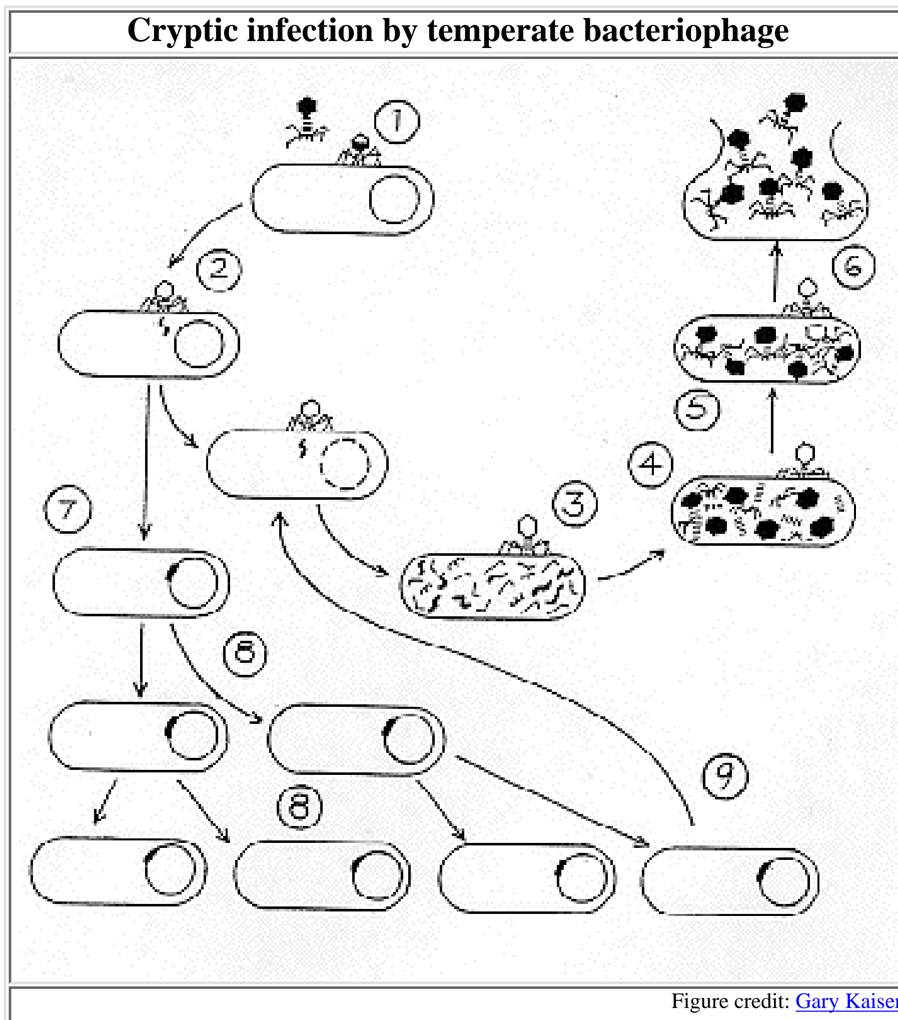


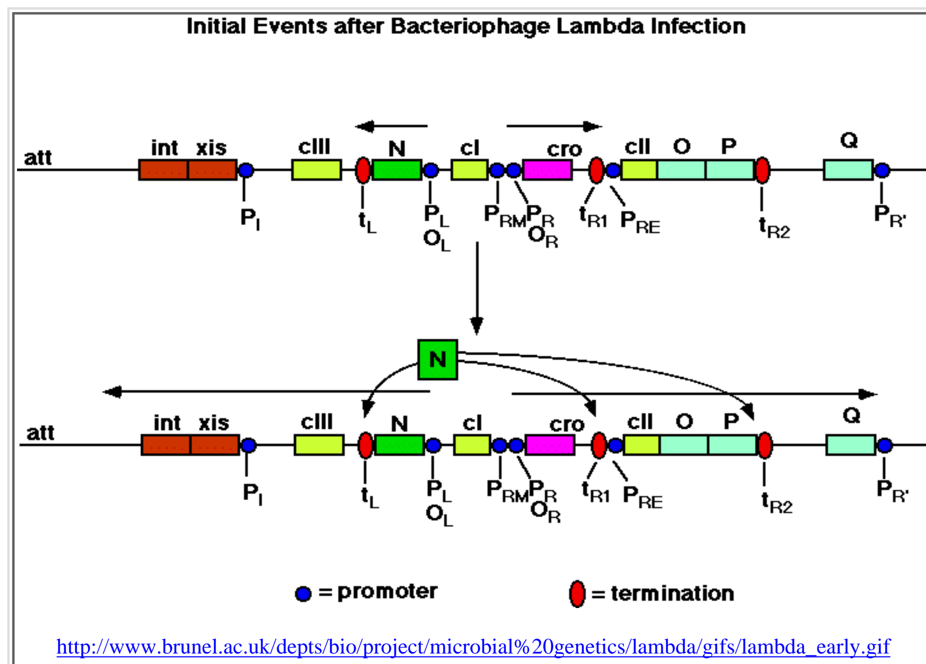
Figure credit: Gary Kaiser

As we learned in the previous lecture, the nature of immunity was discovered through the isolation of "clear mutants" of lambda that were unable to enter a lysogenic state. These mutated phage were completely virulent and only able to cause a lytic infection. Thus the name "clear" to describe the characteristics of their plaques on a plate. Three loci in the lambda genome could harbor mutations leading to a "clear" phenotype,

and these were named cI, cII, and cIII. Infection of a lysogenic bacterium with clear mutants of lambda did not lead to lysis, however! E. coli lysogenic for lambda was immune even to clear mutants.

Virus	Host	Result
wild type lambda	lambda-free E. coli	turbid plaques
	lysogenic E. coli	no plaques
cI mutant of lambda	lambda-free E. coli	clear plaques
	lysogenic E. coli	no plaques

To understand the life cycle of bacteriophage lambda, we must first learn a bit about the regulation of its life cycle. Here is a diagram describing the initial leftward and rightward transcription patterns.



http://www.brunel.ac.uk/depts/bio/project/microbial%20genetics/lambda/gifs/lambda_early.gif

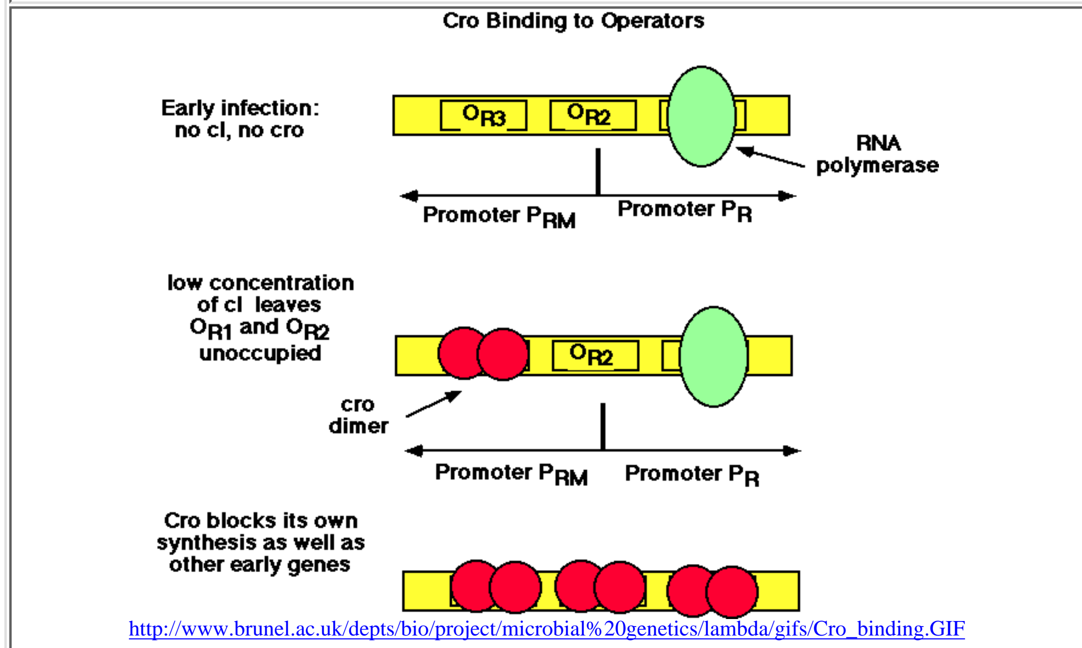
As shown in the diagram above, lambda DNA is initially transcribed from the promoters P_L and P_R, which direct synthesis of RNA in opposite directions (left and right respectively). Transcription is initially terminated at sites t_L and t_R, but expression of the N gene (in green) leads to "antitermination" and production of longer transcripts.

Here's where the idea of a "switch" comes into play ...

The control of the region between the cI gene and cro genes is crucial! There are three operator domains, as shown in the figure below, and these operators control promoters P_R and P_{RM} which initiate transcription in opposing directions. Only one of these two promoters is

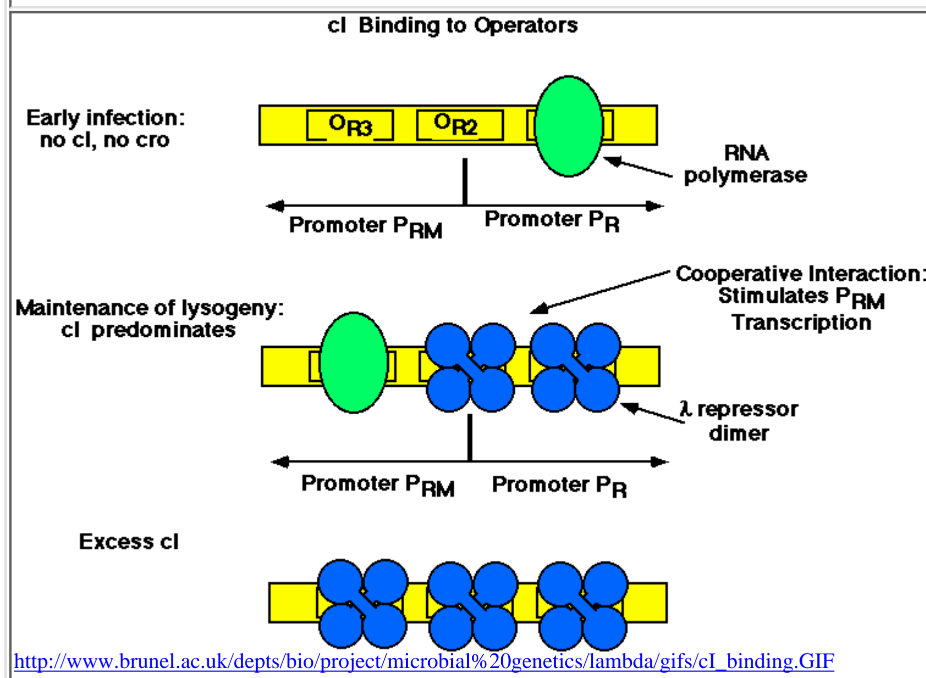
usually active, and it is a bit of a "contest" to see which one wins. If P_R wins and the protein **cro** is made, then production of **cI** will be repressed. If on the other hand promoter P_{RM} wins and the protein cI is made, then production of cro will be repressed.

If cro wins the "contest", P_R (and P_L) are active, and lytic growth ensues



http://www.brunel.ac.uk/depts/bio/project/microbial%20genetics/lambda/gifs/Cro_binding.GIF

If cI wins the "contest", P_{RM} is active, and lysogeny ensues



http://www.brunel.ac.uk/depts/bio/project/microbial%20genetics/lambda/gifs/cI_binding.GIF

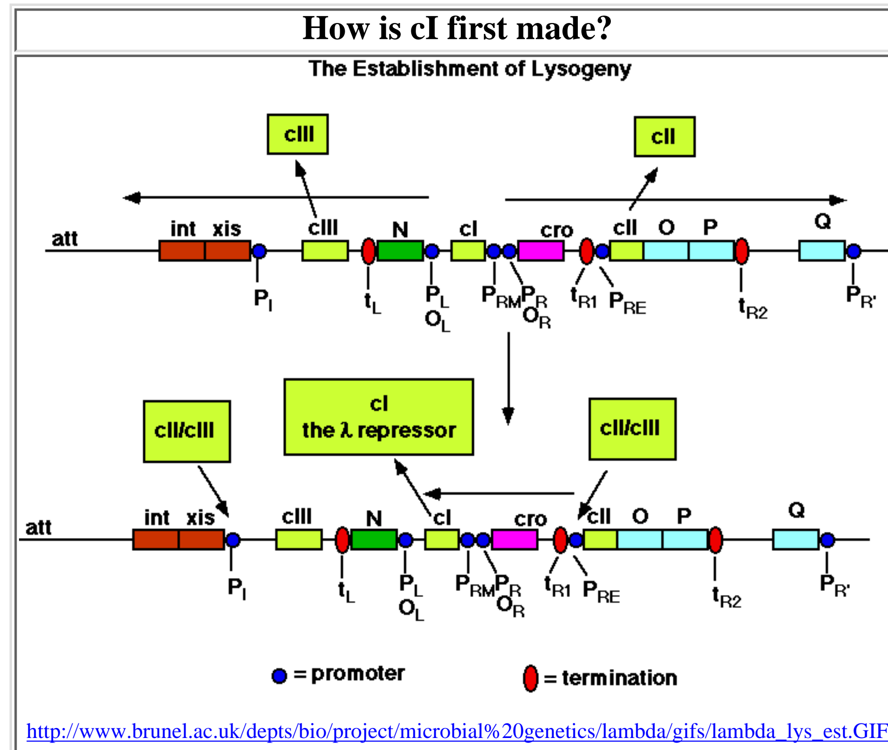
Wow! Is that simple or what!

If cro predominates, it hogs the operator region and prevents cI from being made. On the other hand if cI predominates, it hogs the operator

region, causing more of itself to be made (from the P_{RM} promoter).

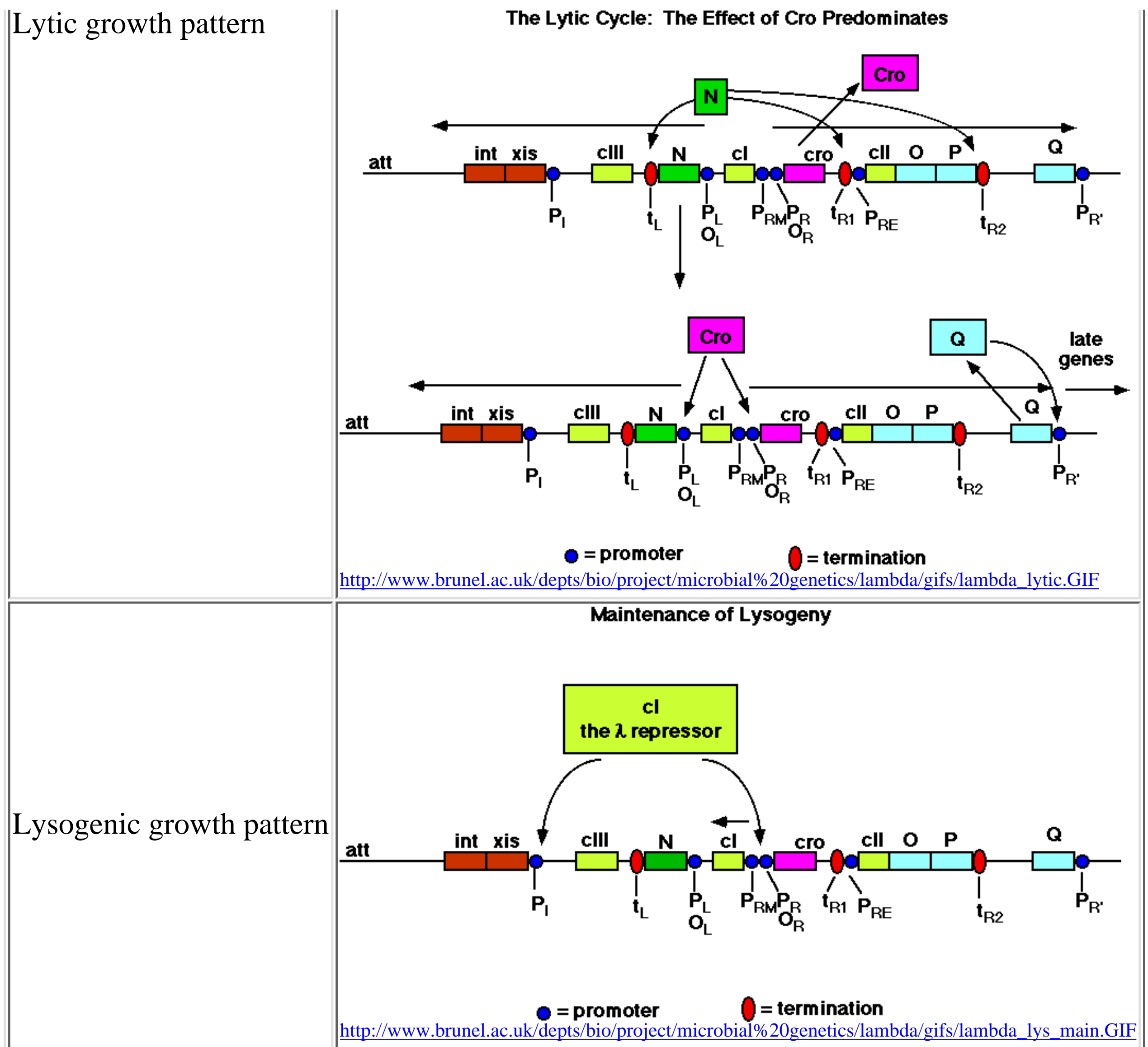
I can hear you asking... how is cI made, since the initial transcription from the genome seems only to be going away from cI? There is a promoter I haven't mentioned called P_{RE} that is activated by cII and cIII (which are produced after the anti-terminator N is made).

How is cI first made?



http://www.brunel.ac.uk/depts/bio/project/microbial%20genetics/lambda/gifs/lambda_lys_est.GIF

The "decision" on how much cI should be synthesized depends on the host of the cell and the number of infecting viral particles. Ultimately, either the cI or the cro will predominate, and one of the following two patterns of gene expression will result:



Obviously the first pattern leads to the growth of the virus (only a fraction of the genes involved in lysis are actually shown) and the death of the cell. The second pattern is the more interesting for our purposes. In the lower panel, cI is the only gene that is being expressed in the virus, and it is involved in a positive feedback loop to induce more of its own expression.

This explains why **the lysogenic state is stable**. The genome of the virus is essentially shut down during lysogeny, except for a single repressor protein. If another lambda happens to come along it's out of luck! The cI repressor from the first lambda simply prevents expression of the second lambda genome, and it fails to enter a lytic cycle. That explains **bacteriophage immunity**!

One allele of cI that is important in the laboratory is cI857, which is temperature sensitive (the protein is active at 32 degrees centigrade but inactivated at 39 degrees centigrade). We may therefore grow a lambda phage carrying cI857 as a lysogen at low temperature, then induce lytic growth by simply moving it to a warmer incubator. Very cool!

Now we understand why "clear" mutants of lambda produce clear plaques (i.e. lytic growth solely). Interruption of the function of the cI gene causes cro to win the "contest" hands-down! In a similar way, since cII and cIII work together to initiate expression of cI, mutations in those genes also give an edge to the cro gene.

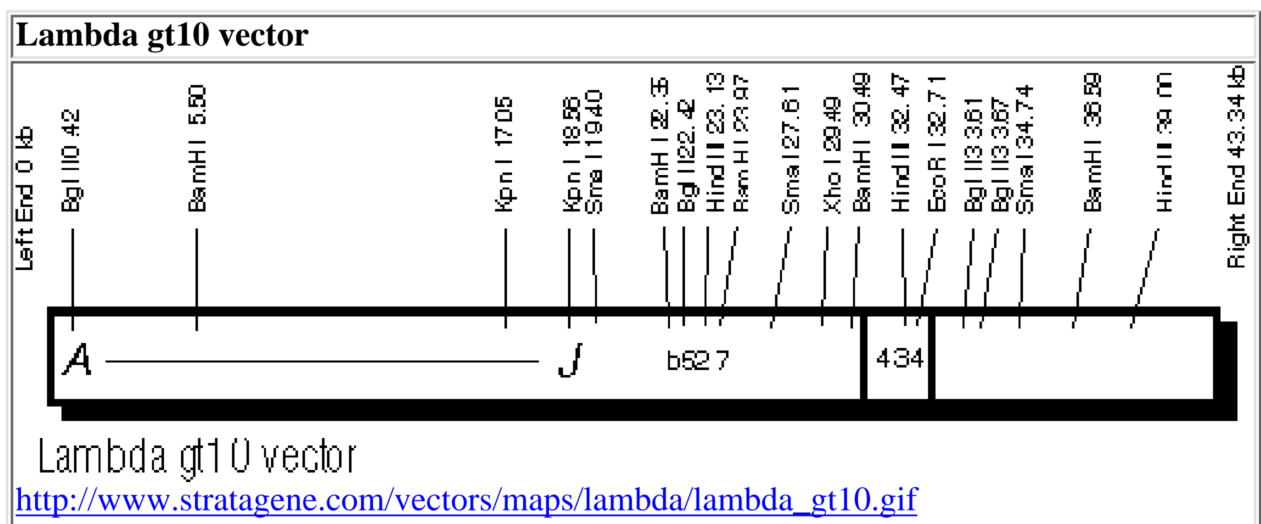
Lambda can be engineered to carry DNA into cells.

Do you recall the definition of bacterial **transformation**? That's when bacteria take up free DNA from their surroundings. If a virus injects its genome into a cell, that's clearly a different type of "uptake" of DNA (or RNA, as the case may be) - we would call that "**infection**" of course. What term do we use when the infecting virus is carrying DNA that is not normally its own? We call it "**transduction**" and with integrating viruses like lambda we distinguish between the transfer of genomic DNA adjacent to the normal integration site (specialized transduction) and tranfer of essentially random fragments from the E. coli genome (generalized transduction).

lambda gt10

Let us look at an example of a vector designed to carry foreign DNA - a sort of "engineered transduction". Note the gene marked "434" in the figure, and containing a unique EcoRI site.

Lambda gt10 vector



http://www.stratagene.com/vectors/maps/lambda/lambda_gt10.gif

The "434" refers to an immunity type of the cI repressor (there are different lambdoid phages in the world, with different classes of immunity, which you may be relieved to know isn't important for this

discussion). In any case, the unique EcoRI is a marvelous cloning site in this phage, and an insertion causes disruption of the cI (434) gene. How much foreign DNA can be inserted at the site? The packaging limit is 78% to 105% of the wild-type genome size of lambda, which is 48 kbp. Since lambda gt10 is 43.34 kbp in size, we can fit in an additional 0 to 9 kbp of foreign DNA. If the cI (434) gene is interrupted, then the phenotype will be "clear" plaques, right?

Very cool!

When we plate the recombinant phage, we can see whether each one carries a foreign DNA insert by simply looking to see whether each plaque is turbid or clear. A clear plaque indicates an interrupted cI gene, just as a white colony indicated an interrupted lacZ gene in our previous discussions. In fact, we can go one step further and plate the cells on an **hfl** mutant strain of E. coli to select against lytic growth of non-recombinant lambda gt10 phage. The "hfl" stands for "**high frequency of lysogeny**" and when temperate lambda is plated on an hfl strain, there is near certainty that it will grow lysogenically rather than lytically. If the cI gene is interrupted (in this case, by the DNA you have inserted at the EcoRI site) then it will grow lytically even on an hfl strain of E. coli. This is therefore a handy way to select against those phage that lack insertions - they will grow only lysogenically on hfl E. coli, and so will not generate any progeny phage.

Practical aspects

Lambda vectors can accept larger pieces of DNA than traditional plasmid vectors, and that is a tremendous advantage. On the other hand, one cannot efficiently introduce lambda DNA into a bacterium by transformation because the 50 kbp size makes it sensitive to shearing or breaking. (It should be noted that infecting a cell by transforming it with DNA, a process called **transfection** by bacteriologists, is not impossible -- it just isn't feasible on a grand scale)

How then, do we introduce the lambda DNA into cells? We can package the ligated DNA into viral capsids in vitro, then infect the cells with the newly created infectious particles. There are several commercial sources of "packaging extracts" that permit you to package ligated lambda concatamers into viral capsids.

Stratagene Corporation: Gigapack®
Promega Corporation: Packagene®

The process is detailed in the figure below:

Lambda cloning vector methodology
1. Ligate foreign DNA inserts and lambda arms into a concatemeric (multi-genome) segment
Detail of one lambda genome: Left arm, Insert, Right arm, cos, cos, ligate, ligate. Overall picture: cos, cos cos, cos cos, cos, ligate, ligate
2. Package into phage heads (capsids) in vitro.
cos, cos cos, cos cos, Phage assembly

The packaged lambda phage can then immediately be combined with E. coli, to introduce the recombinant phage vector efficiently into cells.

If that seems pretty complicated, look at this easy protocol that makes use of the Stratagene Gigapack kit (a two-lysate method):

How to package DNA into lambda phage capsids From: Mark Strom's protocol book

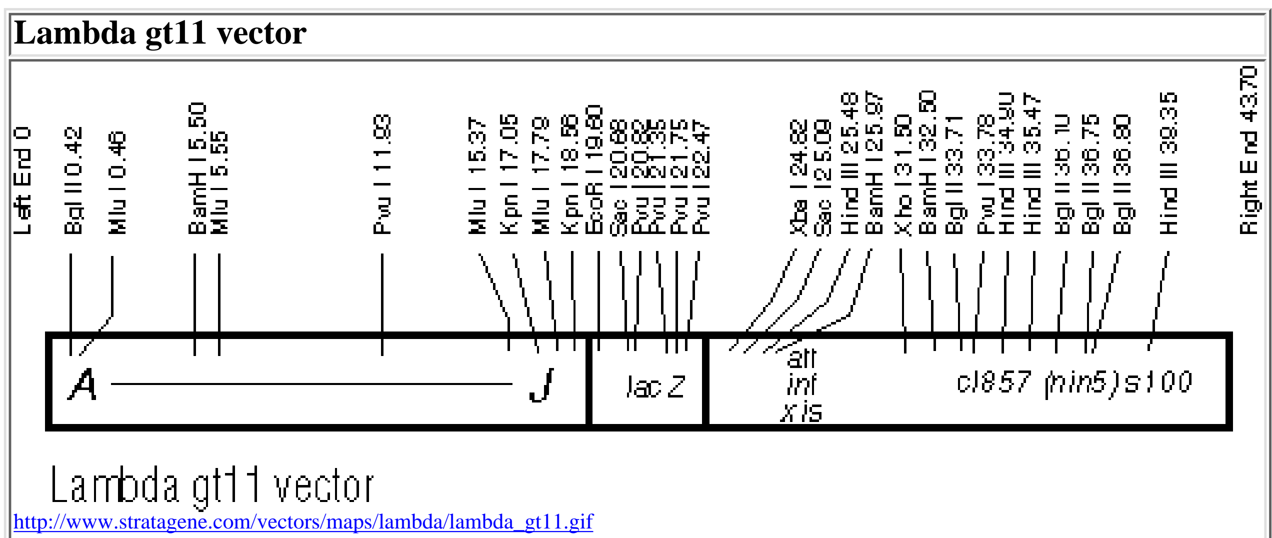
1. Remove extracts from freezer and place on dry ice. Start thawing sonic extract (yellow tube) first.
2. Quickly thaw freeze-thaw extract (red tube) until just beginning to thaw.
3. Add DNA immediately (1-4 ul containing 0.1-5 ug) to freeze-thaw extract. Place on ice.
4. Quickly add 15 ul sonic extract to DNA-freeze-thaw extract.
5. Stir or pipet to mix, avoiding introduction of air bubbles.
6. Incubate at room temperature for 2 h.
7. Add 500 ul phage dilution (SM) buffer. Store at 4 C. Supernatant is ready to be titered.

Now that you've seen an example of a lambda cloning vector (gt10), and have learned about the handling methods for packaging of DNA into viral capsids, let us go on to discuss another example of a lambda vector.

lambda gt11

Consider the vector lambda gt11, shown below, which has a cloning site at a unique EcoRI (at 19.60 kbp) in a lacZ gene:

Lambda gt11 vector



http://www.stratagene.com/vectors/maps/lambda/lambda_gt11.gif

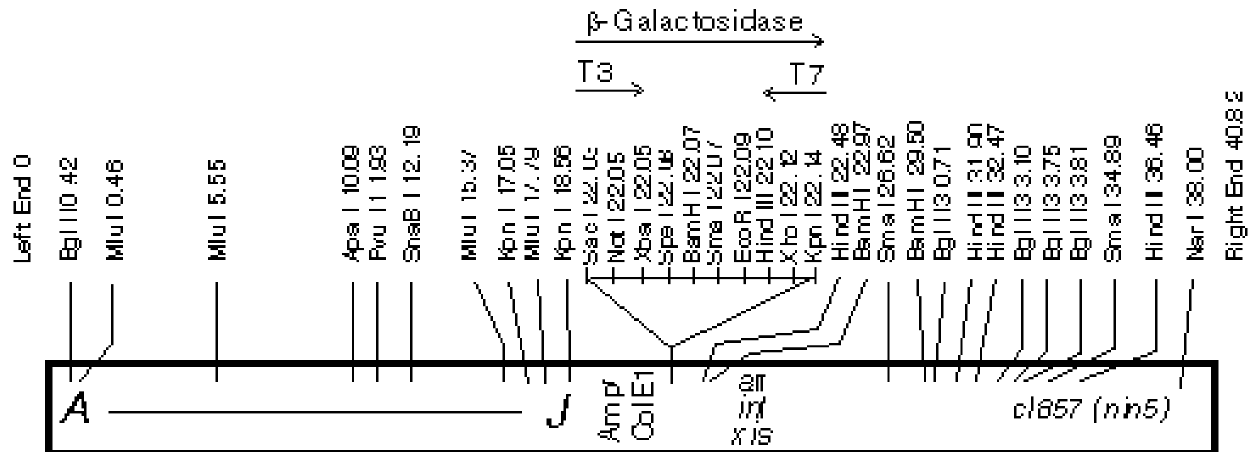
The size of gt11 is approximately 43 kbp, so the vector can accept insertions of 0 to 9 kbp.

There's our old friend lacZ again! In this phage vector, interruption of the lacZ gene is the hallmark of successful cloning, and we add IPTG and X-gal to the bacteriological plate to develop the blue-white screening method:

Phenotype	Caused by...	Meaning...
blue plaques	cleavage of X-gal substrate	an intact lacZ gene in vector
colorless plaques	no cleavage of X-gal substrate	an interrupted lacZ gene

The only difference between this assay and what we discussed in the previous lecture is that the color develops in a plaque (area of lysed bacteria) rather than in a colony.

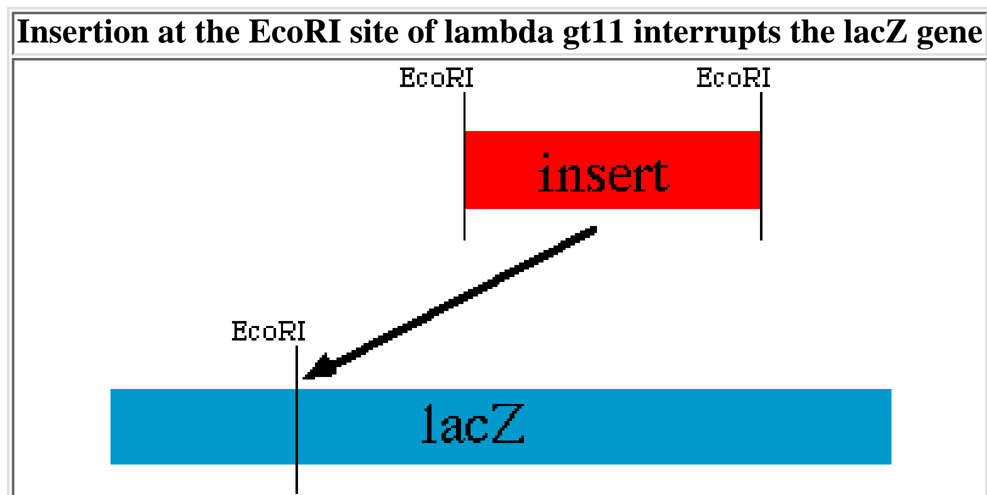
A slightly fancier version of gt11 is called lambda ZAP II, and it can be obtained from Stratagene.



http://www.stratagene.com/

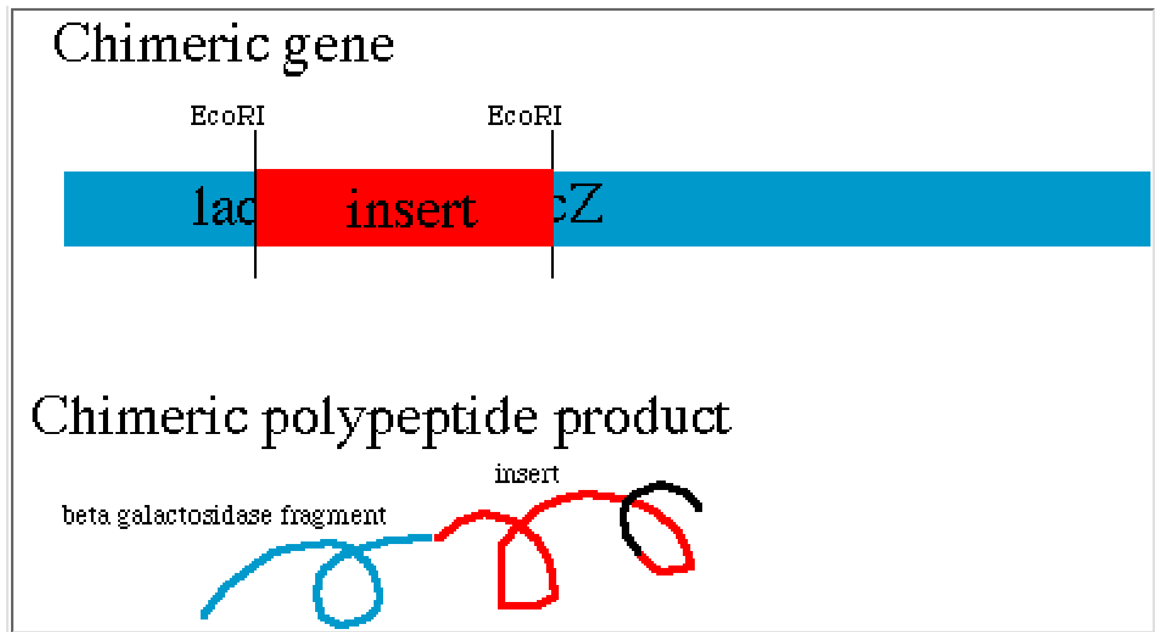
In either lambda gt11 or ZAP II, DNA sequences containing open reading frames are fused to the lacZ open reading frame.

Insertion at the EcoRI site of lambda gt11 interrupts the lacZ gene



The result is a chimeric (split) gene, which may encode a fusion protein (provided that the orientation and reading frames of the lacZ and insert are matched):

Possible creation of a fusion gene in lambda gt11 recombinants



If the inserted DNA is matched in orientation and reading frame with the lacZ gene, so that it becomes expressed as a **fusion protein** in E. coli, then we can screen the plaques using antibodies directed against the foreign protein (i.e. encoded by the inserted DNA) as a probe.

In which we find an answer

Here is a summary of how you can use this method practically to answer the BIG question.

Starting with a pure protein, how can you find the gene encoding it?

1. Isolate a protein of interest (perhaps an enzyme activity) in a fractionated cell lysate from your organism of interest.
2. Inoculate animals with the purified protein of interest, so that they will raise antibodies directed against it.
3. Plate a collection (library) of gt11 or ZAP II phage carrying genes from your organism of interest.
4. Screen the plaques for expression of your interesting protein (expressed as a lacZ

fusion) using the antibodies as a probe.
5. Pick the phage containing the gene that encodes your protein.

One important point to consider, when expressing foreign proteins in E. coli, is that many introduced sequences turn out to encode proteins that are toxic to the bacterium. That is, your clone may kill itself if the fusion protein is expressed! Yikes!!! There's a way to solve this little problem, and it has to do with keeping control of the operators.

1. When propagating the recombinant library, grow the infected cells at 32 degrees centigrade so that the cI857 repressor will be active. This will allow lysogeny, which will essentially shut down transcription of the lambda genes (other than the cI857 of course, as you recall from our previous discussion). Since the region containing the lacZ gene will be generally silenced and the DNA integrated into the E. coli genome as a single copy, no significant expression of the lacZ fusion will occur.

2. Keep the lac promoter repressed by not exposing the cells to IPTG. The lacZ gene is under the control of a lac operon promoter, as we have discussed previously for plasmid vectors.

The trick for screening is to plate the infected cells at 32 degrees centigrade and allow them to propagate as lysogens for a few hours. Then, move the plates to a 39 degree incubator and simultaneously apply a filter soaked in IPTG. The higher temperature destroys the cI857 protein (remember - it's temperature sensitive), leading to lytic growth of the virus, and the IPTG derepresses the lac operon so that lots of fusion protein is expressed and released into the developing plaque.

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