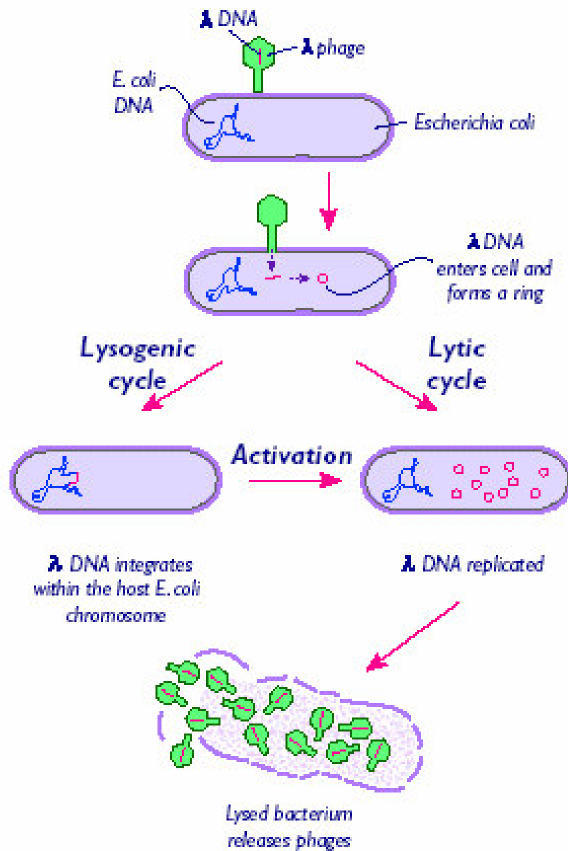
Bacteriophage Lambda

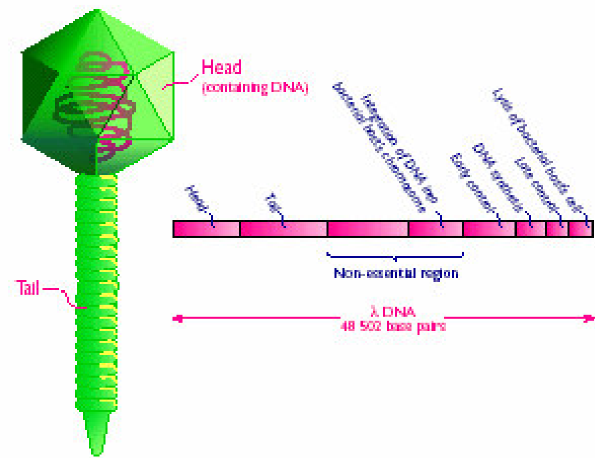
Bacteriophages (literally: 'bacteria-eaters') are viruses that invade bacteria. To reproduce, bacteriophages (or phages) must take over the molecular machinery of their bacterial hosts. The phage lambda (λ) preys upon *Escherichia coli*. Lambda enjoys a choice of life styles — it can either multiply within its host and destroy it (the lytic cycle), or the λ DNA can be inserted into the bacterial chromosome and remain dormant there for several generations (the lysogenic cycle). An environmental trigger *e.g., ultraviolet light* activates the lytic cycle.

Phage λ is a relatively simple organism. It consists of a double-stranded length of DNA wrapped around a core of protein and encapsulated in a proteinaceous coat.

The entire genetic make-up (genome) of λ has been sequenced and is 48 502 base pairs long. Within this genome are genes that code for the virus's protein coat, bursting (lysis) of the bacterial cell, integration of λ DNA into the host's chromosome and so on. The order in which these genes are activated is important. For example, it would be of little benefit to the virus if the host bacterial cell was broken open (lysed) before new virus particles had been assembled. Consequently, λ has evolved an elaborate system of gene regulation that has been studied in great detail.



▲ *Infection of the bacterium E. coli by bacteriophage lambda, showing the lytic and lysogenic (latent) cycles.*



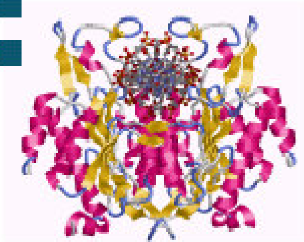
▲ *Bacteriophage λ and some of the main groups of the 60 or so genes in its genome.*

Relatively little of the λ genome is required to package DNA and deliver it into bacterial cells. About 20 000 base pairs can be deleted from its central region and replaced with DNA from another organism, without affecting the phage's viability. Several specially-constructed forms of λ can be used by molecular biologists to ferry new genes into bacteria.

Restriction enzymes

Enzymes that cut up DNA are called *restriction endonucleases*. They are made by bacteria to *restrict* the proliferation of invading viruses (such as the bacteriophage λ). Different restriction enzymes cut at specific sequences of bases in the DNA. The bacterium's own DNA is protected by the addition of methyl ($-CH_3$) groups to adenine (A) or cytosine (C) bases at the sites that normally are 'recognised' by the enzymes.

Source microorganism STRAIN Name	 Recognition site (5'→3')
Bacillus amyloliquefaciens H	
BamHI	G↓GATCC
Escherichia coli RY13	
EcoRI	G↓AATTC
Haemophilus influenzae Rd	
HindIII	A↓AGCTT



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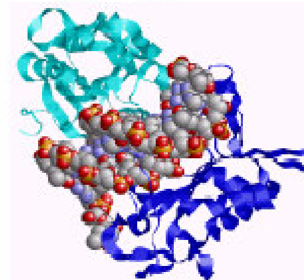
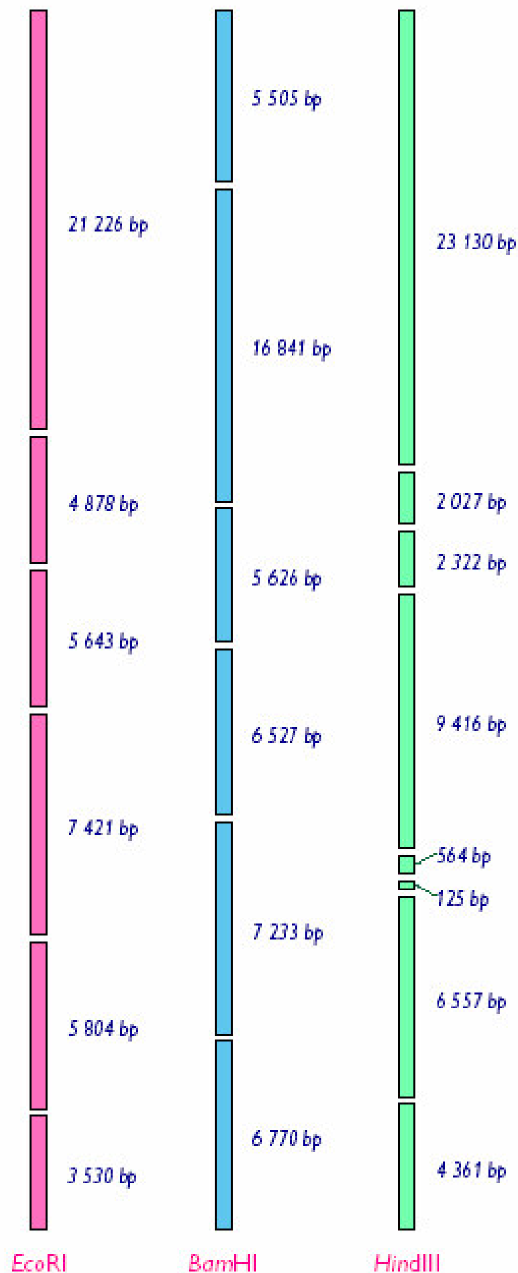
▶ *EcoRI attached to a length of DNA, seen down the axis of the DNA. β-sheets in the protein are shown in yellow; α-helices in magenta.*



PDB ID: 1BHM

▶ *BamHI attached to a short length of DNA. The protein is shown as a ribbon structure, with different shades of blue for the two chains that comprise the protein. The DNA is depicted as a space-filling model.*



▲ *These restriction maps show where EcoRI, BamHI and HindIII can cut λ DNA. The numbers are the sizes of the fragments, in base pairs (bp).*

Restriction enzymes take their names from the bacteria that produce them. *Eco*RI (the first such enzyme to be discovered) comes from *Escherichia coli*, strain RY13. The numeral I in the enzyme's name indicates that this was the first restriction enzyme found in *E. coli*. *Bam*HI is obtained from *Bacillus amyloliquefaciens* H, (with the numeral I again showing that this was the first restriction enzyme found in this bacterium). *Hin*dIII is made by *Haemophilus influenzae* R_d. Here the III indicates that this was the third restriction enzyme isolated from *H. influenzae*.

Many restriction enzymes are now obtained from high-yielding, genetically-modified production strains of bacteria.

DNA gel electrophoresis

Gel electrophoresis can be used to separate DNA fragments of different sizes. First, a gel is cast from agarose — a very pure form of agar, which is obtained from seaweed. At one end of the slab of gel are several small wells, made by the teeth of a comb that was placed in the gel before it set. A buffer solution is poured over the gel, so that it fills the wells and makes contact with the electrodes at each end of the gel. Ions in the buffer solution conduct electricity. The buffer also stops the gel from drying out.

The invisible DNA fragments are mixed with a small volume of loading dye. This dye is dissolved in a dense sugar solution, so that when it is added to the wells, it sinks to the bottom, taking the DNA with it.

An electrical current is applied to the electrodes, setting up an electrical field across the gel. Phosphate groups give the DNA fragments a negative electrical charge, so that the DNA migrates through the gel towards the positive electrode. Small fragments move quickly through the porous gel — larger fragments travel more slowly. In this way the pieces of DNA are separated by size. The loading dye also moves through the gel, so that the progress of the electrophoresis can be seen.

After electrophoresis, the gel is stained to reveal the DNA, either as a smear (many fragments of a wide range of sizes) or bands (each band is comprised of numerous DNA fragments of a similar size). Within a smear, specific bands can by highlighted using probes which bind to particular sequences of DNA (or RNA). Some probes are radioactive and for reasons of safety cannot be used in schools.

λ DNA, restricted with the enzyme *Hin*dIII, is often run on gels alongside other DNA. The λ fragments, which are of known sizes, can then be compared to other DNA fragments, thereby giving an indication of *their* sizes.