RAMA UNIVERSITY

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FACULTY OF AGRICULTURE SCIENCES AND ALLIED INDUSTRIES

(Principles of Cytogenetics)

For

M.Sc. Ag (GPB)

Course Instructor

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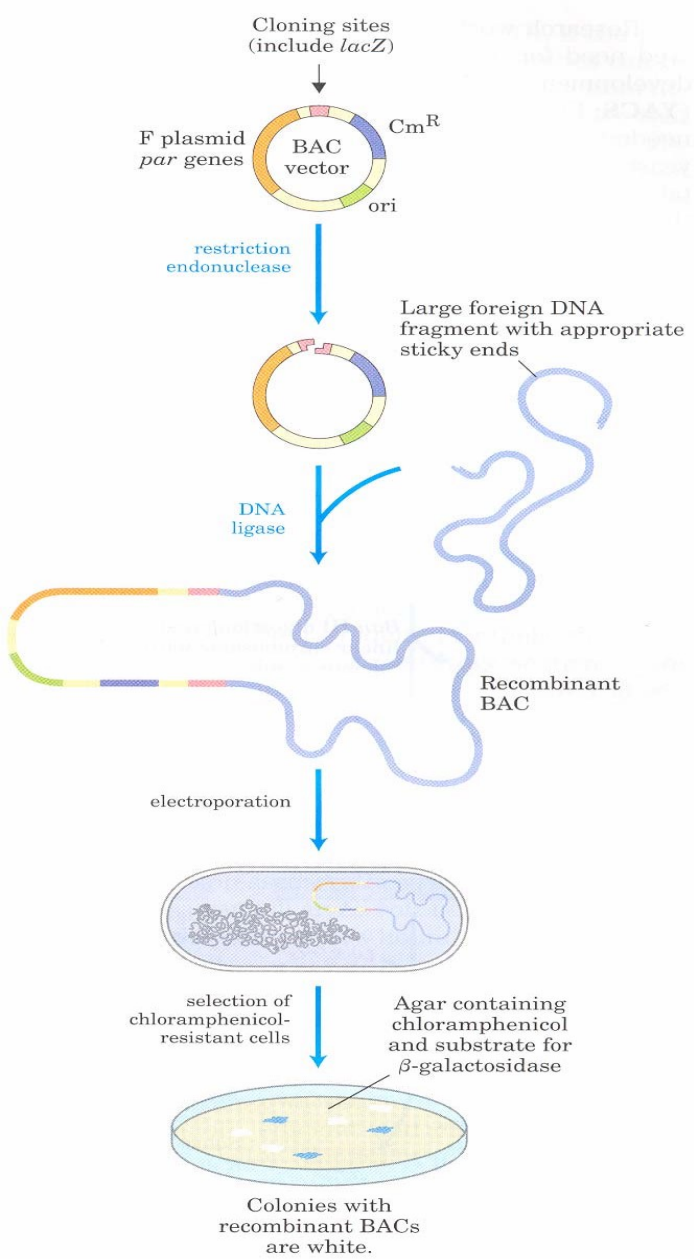
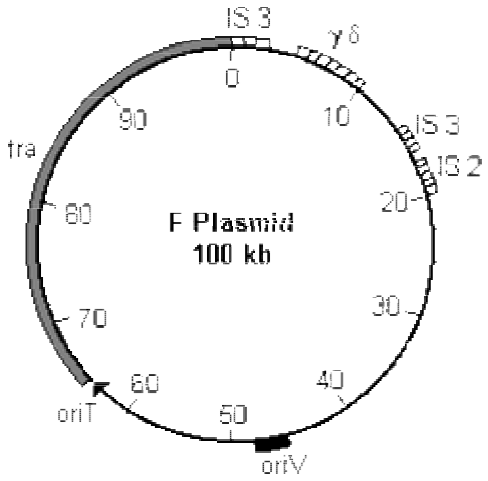
BAC vectors (Bacterial Artificial Chromosome)

BAC vectors are plasmids constructed with the replication origin of *E. coli* F factor, and so can be maintained in a single copy per cell. These vectors can hold DNA fragments of up to 300 kb. Since they are present in low copies, recombination between the high- copy plasmids.



BAC vectors (Bacterial Artificial Chromosome)

The F (fertility) factor is a plasmid that can be mobilized from F+ male bacteria and F- female bacteria. The gene transfer from one to another bacterial cell is called conjugation. The **F** factor controls its own replication. It has two origins of replication: ***oriV*** is the origin for bidirectional replication; ***oriS*** is the origin for unidirectional replication. The **F** factor also has genes that regulate DNA synthesis so that its copy number is kept at a low level; and, genes that regulate the partition into the daughter cells after ***E. coli***divides.



IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions

Tra region codes for proteins and enzymes essential for DNA mobilization

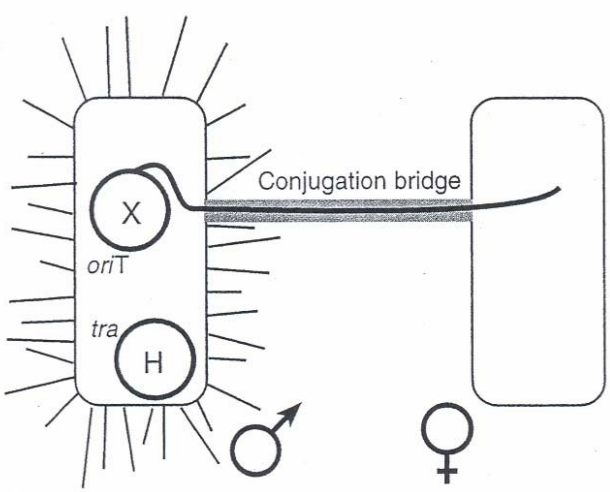
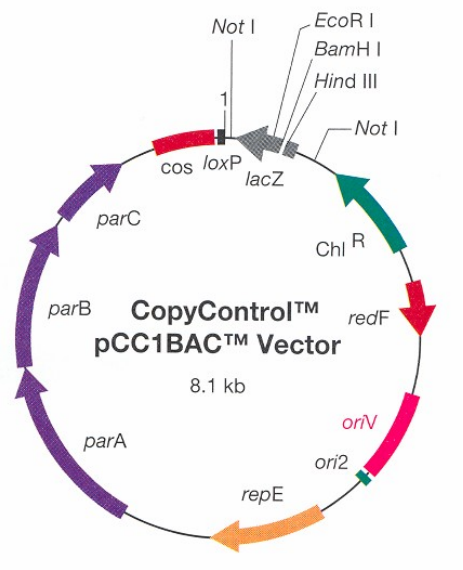


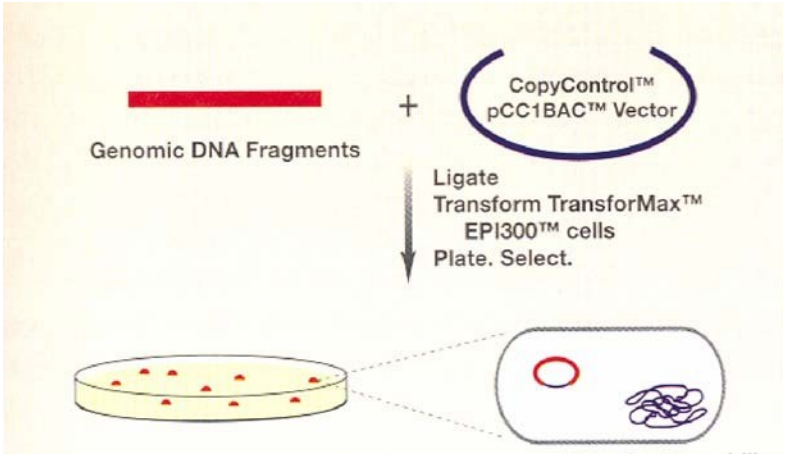
Figure 4.9

Transfer of a plasmid between bacteria by conjugation. Schematic diagram of a conjugation bridge between two *E. coli* bacteria, with transfer of a single stranded 'X' DNA from the pili-coated male (left) to female (right). The *trans*-acting factors that mobilize the plasmid 'X' are shown as genes (*tra*) on a separate helper plasmid 'H'. The plasmid 'X' has a *cis*-acting factor (*oriT*) from the F episome that is required for transfer. The male bacterium retains a double-stranded copy of the plasmid 'X'. DNA entering the female may recombine with the chromosome (not shown) or be retained as a replicon if transfer is complete.

BAC vectors (Bacterial Artificial Chromosome)

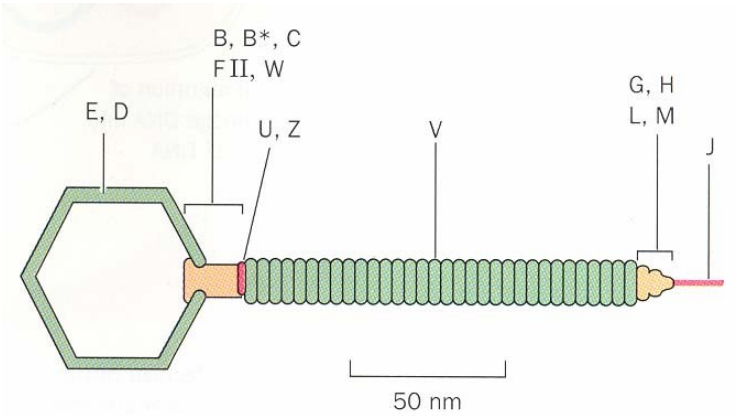
A modification on BAC vectors is to add a second replication origin to the vectors. The second origin, the oriV, will generate multiple copies of BACs, if a trans-acting factor, the TRF, is present. The expression of the trf gene, which has been integrated into the chromosome of the bacteria is controlled by a tightly-regulated inducible promoter.

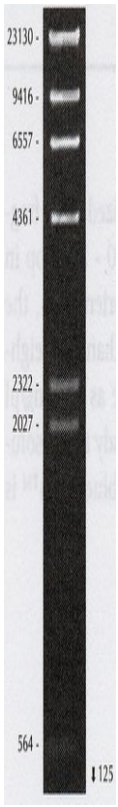




BAC vectors developed by EPICENTRE

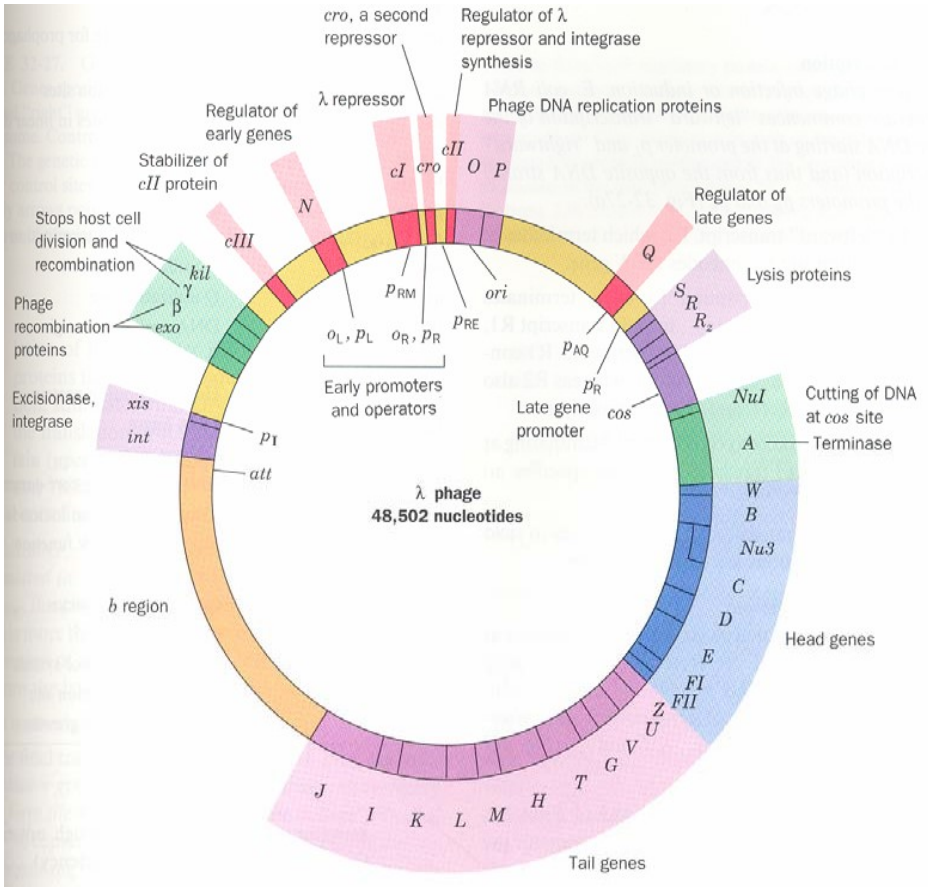
Lamda phage vectors





Bacteriophage λ, a midsized (58 million D) coliphage, has a 55 nm diameter icosahedral head and a flexible 15- to 135- nm long tail that bears a single thin fiber at its end. The virion contains a 48,502-bp linear double-stranded B-DNA molecule of

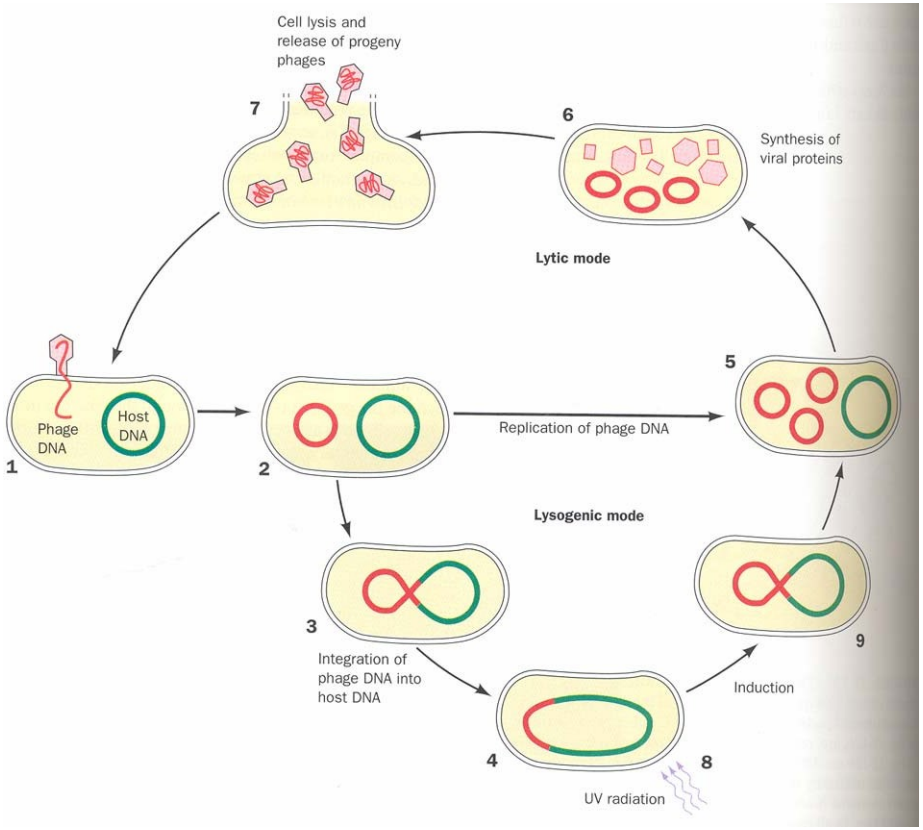
known sequence.



The bacteriophage λ encodes ~50 gene products, which include structural genes and control sites. Upon packaging into the virion, the circular chromosome is cut at the cos site yielding a linear DNA.

Lamda phage vectors

Bacteriophage λ adsorbs to E. coli through a specific interaction between the viral tail fiber and a maltose transport protein (the product of the E. coli lamB gene) that is a component of the bacterium's outer membrane. This interaction initiates a complex and poorly understood process in which the phage DNA is injected through the viral tail into the host cell. Soon after entering the host, the A. DNA, which has complementary single-stranded ends of 12 nucleotides (cohesive ends), circularizes and is covalently closed and supertwisted by the host DNA ligase and DNA gyrase.

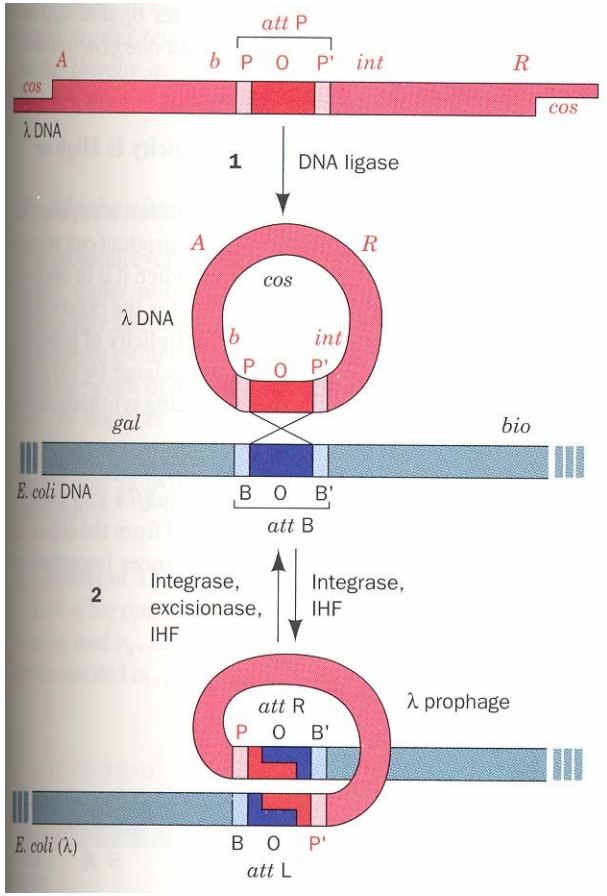


Once the λ chromosome is circularized, it can either be integrated into the bacterial chromosome or replicate itself independently to form new virions. These two processes are referred to as lysogenic and lytic pathways, respectively.

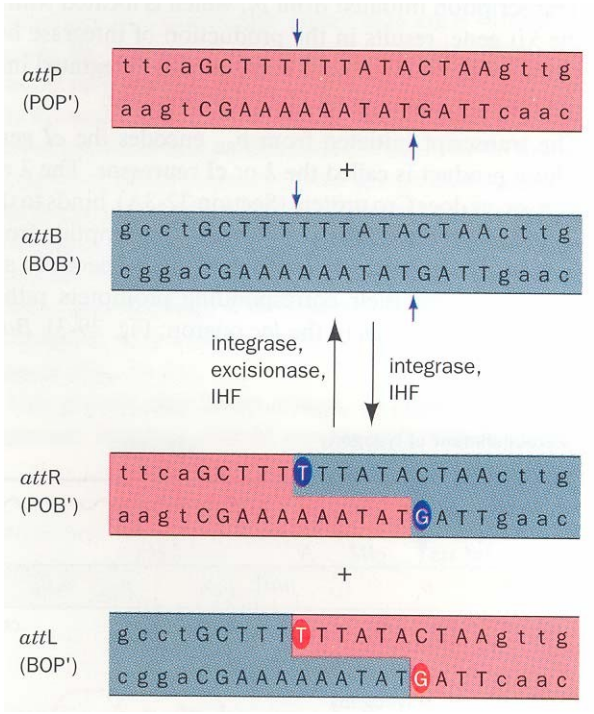
DNA CLONING

The Lysogenic Mode

Lamda phage vectors



Lysogeny is established by the integration of viral DNA into the host chromosome accompanied by the shutdown of all lytic gene expression. With phage λ, integration takesplace through a site-specific recombination process that differs from general recombination in that it occurs only between the chromosomal sites designated attP on the phage and attB on the bacterial host.

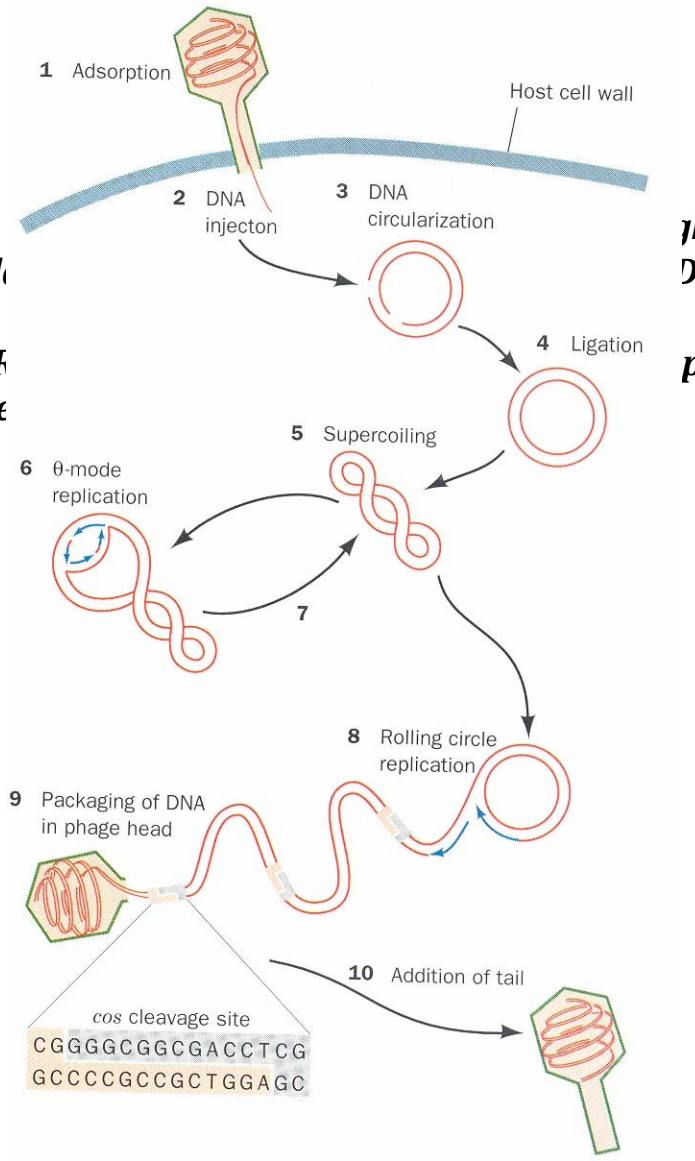


The site-specific recombination process that inserts/excises phage A. DNA into/from the chromosome of its E. coli host. Exchange occurs between the phage attP site (red) and the bacterial attB site (blue), and the prophage attL and attR sites. The strand breaks occur at the approximate positions indicated by the short blue arrows. The sources of the more darkly shaded bases in attR and attL are uncertain. The upper case letters represent bases in the 0 region common to the phage and bacterial DNA's, whereas lower case letters symbolize bases in the flanking B, B', P, and P' sites.

DNA CLONING

Lamda phage vectors

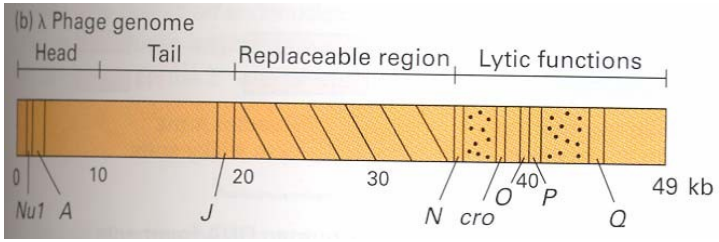
The Lytic Mode



In the early stages of lytic infection, λ DNA replication occurs both ***gle replication origin (ori), and by the rolling circle (σ) mode. By the l*** ***DNA replication has completely switched, via an unknown mechanism,*** ***accompanying synthesis of the complementary strand). The host*** ***pidly fragment the resulting concatemeric (consisting of tandemly linke*** ***inactivated by the phage r protein.***

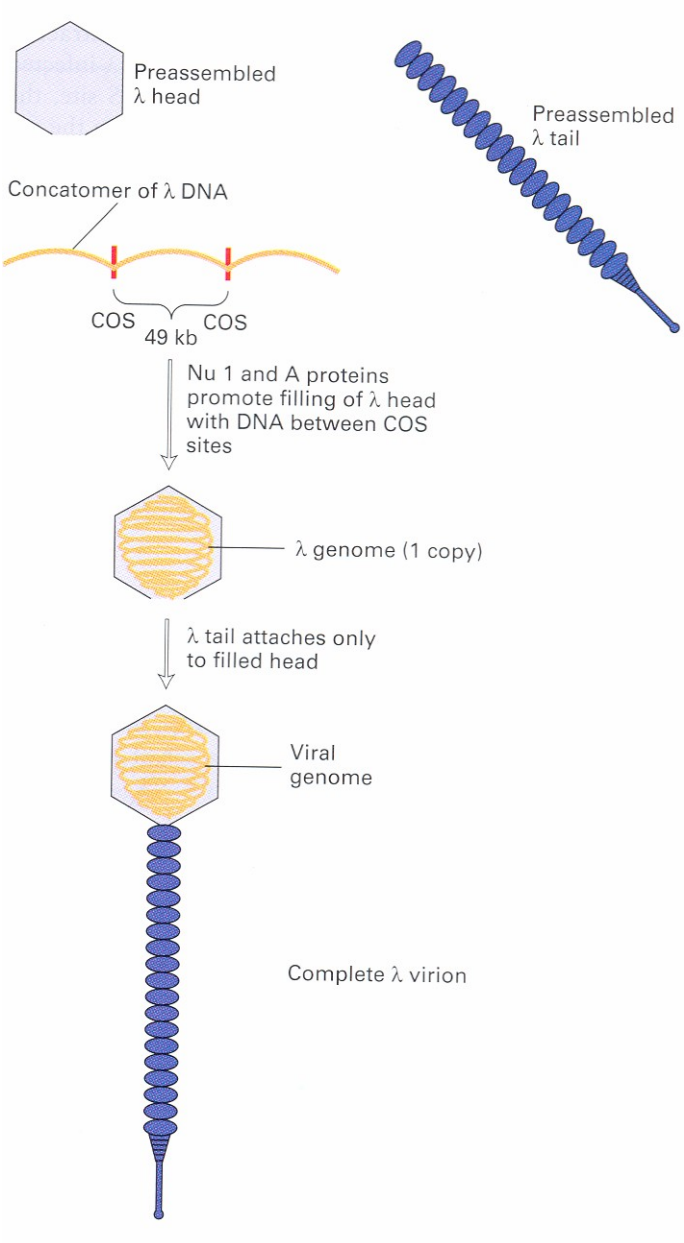
In the process of phage assembly, the concatemeric DNA is specifically cleaved in its cos (for cohesive-end site) site to yield the linearduplex DNA with complementary 12-nt single-stranded ends that are contained by mature phage particles. The staggered double~tranded scission is made by the so-called terminase, which is a complex of the phage proteins gpA and gpNuJ.

Lamda phage vectors



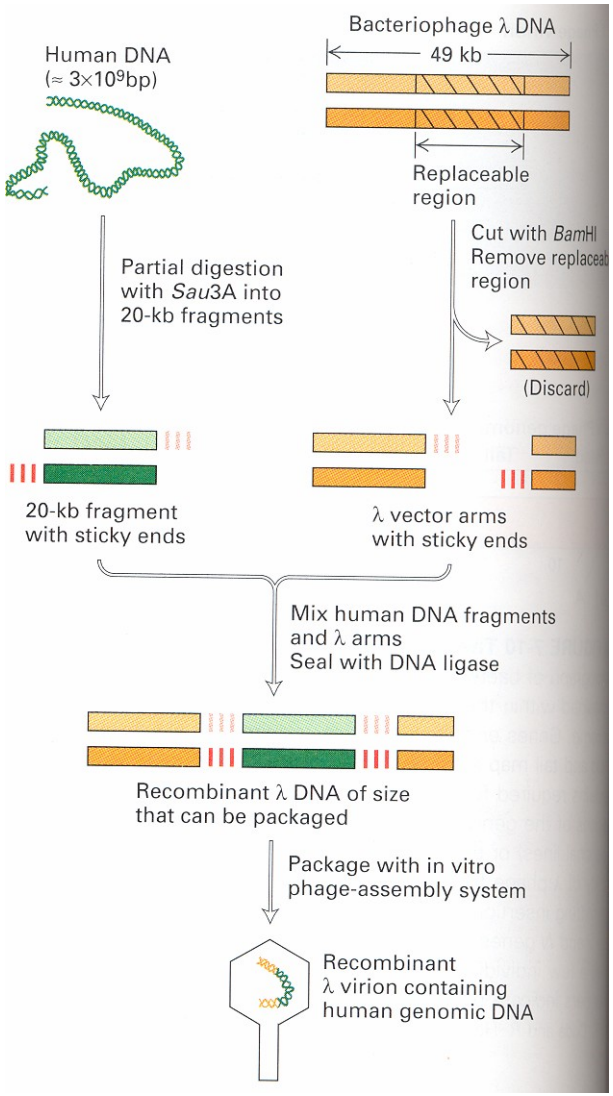
The genes involved in lysogenic pathway and other viral genes not essential for viral growth are removed from the viral DNA and replaced with the DNA to be cloned. Up to ~25 kb of foreign DNA can be inserted into the λ genome, resulting in a recombinant DNA that can be packaged in vitro to form virions capable of replicating and forming plagues on *E. coli* hostcells.

To prepare infectious λ virions carrying recombinant DNA, the phage assembly process is carried out in vitro. In one method, E. coli cells are infected with a mutant λ defective in A proteins, one of the two proteins required for packaging λ DNA into preassembled phage heads. These cells accumulate preassembled“empty” heads; since tails attach only to heads filled with DNA. Preassembled tails also accumulate in these cells. An extract containing high concentrations of empty heads and tails is prepared by lysing the cells infected with the λ A mutant. When this extract is mixed with isolated A protein (obtained from λ-infected cells) and recombinant DNA containing a COS sit, the DNA is packaged into the empty heads. The tail in the extract then combine with the filled heads, yielding complete virions carrying the recombinant λ DNA.



DNA CLONING

Lamda phage vectors and genomic libraries



Advantages with λ phage cloning:
1). Large capacity for inserts to cloned 2). High titers (10^7 plagues/µg λDNA vs 10^6 transformants/µg plasmid

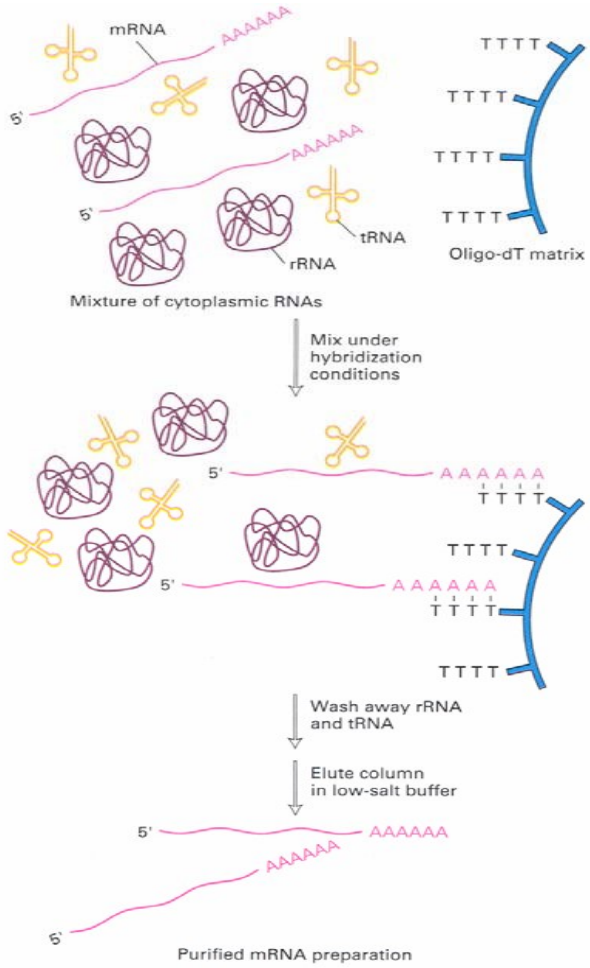
What is a genomic library?
It is a collection of clones that covers the complete genome of any given organsim.

How many λ phages are required to hold the complete human genome?
$3X10^9/2X10^4 = 1.5X10^5$ clones

So, about 10^6 recombinant phages are necessary to assure that each region of human DNA has a 90-95 percent chance of being included.

Lamda phage vectors and cDNA libraries

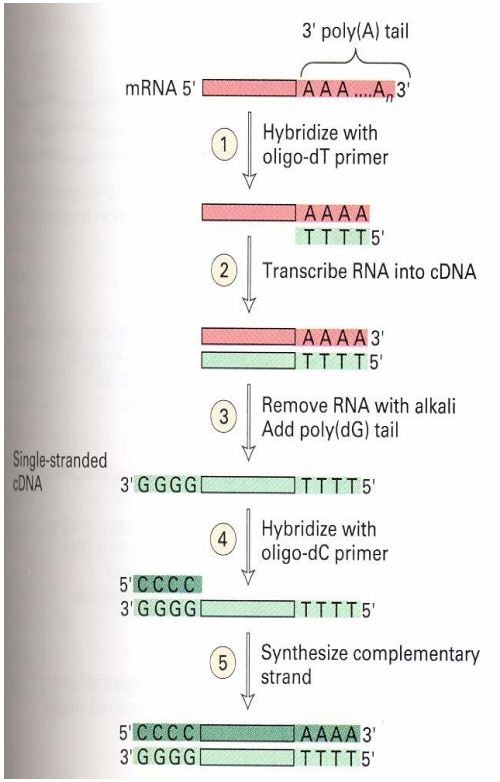
Mssenger RNA (mRNA) carries has multiple adenylate nucneotides (i.e. polyA) at 3'-ends. The poly(A) tail allow the mRNA to be attached to oligo dT matrix by forming hydrogen-bonds at high ionic concentrations. After other RNAs are washed away at moderate ionic concentrations, mRNA can be eluted in low-salt buffer.

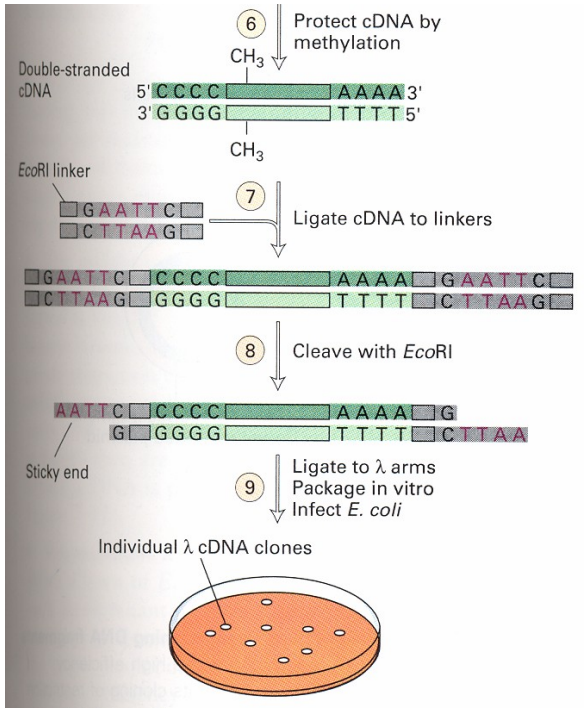


DNA CLONING

Lamda phage vectors and cDNA libraries

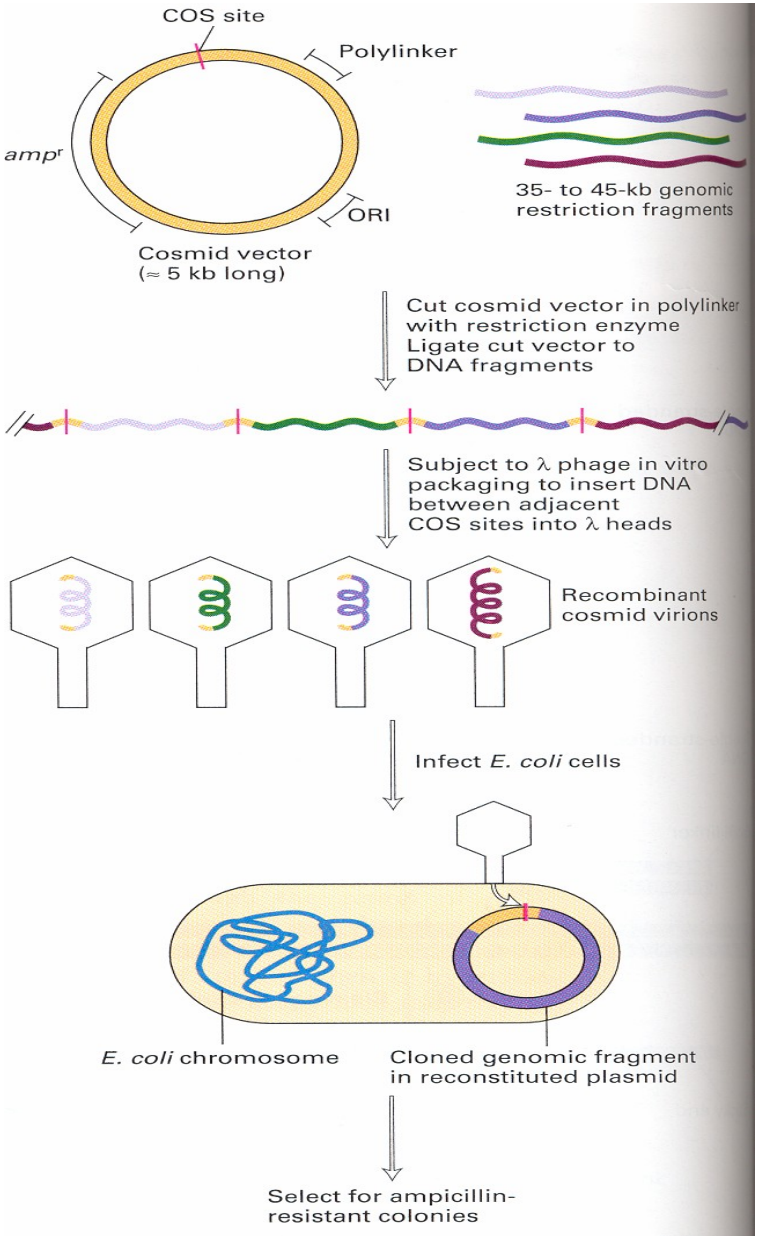
A cDNA library is a complete set of clones representing mRNA expressed in the cells or tissues.





At step 1 and 2, an oligo dT is added to anneal with mRNA poly(A) tail and acts as a primer for a reverse transcription reaction catalyzed by reverse transcriptases to sythesize the first strand cDNA. These enzymes are RNA-dependent DNA polymerases capable of using RNA as a template to synthesize the DNA. There are two major types of reverse transcriptases are currently in use: AMV(Avian Myeloblastosis Virus) and MuLV (Moloney Murine Leukemia Virus) reverse trancriptases. RNA is degraded by NaOH and the first strand DNA are then used for the synthesis of the second strand DNA to produce a double-stranded DNA, a process catalyzed by DNA polymerase (step 3 and 4). The ds-DNA is methylated, attached with linkers, and cloned into the vectors.

cosmid and PAC vectors



A cosmid vector is a hybrid containing both plasmid and phage vectors, in which the COS site from λ DNA phage DNA is inserted into a plasmid vector about 5 kb long. Up to 45 kb DNA fragments can be cloned into cosmid vectors.

P1 phage replicon can also be placed into a plasmid to create so called PAC vectors (P1 artificial chromosmes). The P1 phage head can accommodate larger DNA molecules (~100 kb) than the λ head.

TABLE 7-2 Approximate Maximum Length of DNA That Can Be Cloned in Vectors

Vector Type	Cloned DNA (kb)
Plasmid	20
λ phage	25
Cosmid	45
P1 phage	100
BAC (bacterial artificial chromosome)	300
YAC (yeast artificial chromosome)	1000

DNA CLONING

YAC (yeast artificial chromosome) vectors