

CHAPTER 3

Recombinant DNA Technology and DNA Cloning

PART 1 of 4

3.1 Introduction to Recombinant DNA Technology and DNA Cloning

1970s: Gene cloning became a reality

Clone – a molecule, cell, or organism that was produced from another single entity

Made possible by the discovery of:

- 1. Restriction Enzymes** – DNA cutting enzymes (molecular scissors)
- 2. Plasmid DNA Vectors** – circular form of self-replicating DNA which can be manipulated to carry and clone other pieces of DNA
- 3. Other DNA manipulative enzymes**

The range of DNA manipulative enzymes

DNA manipulative enzymes can be grouped into four broad classes, depending on the type of reaction that they catalyze:

Nucleases are enzymes that cut, shorten, or degrade nucleic acid molecules.

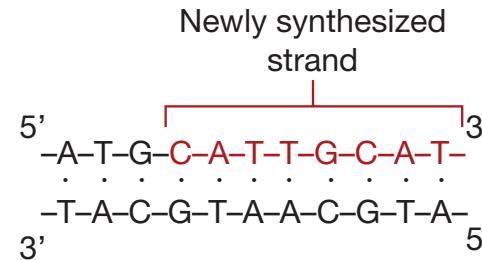
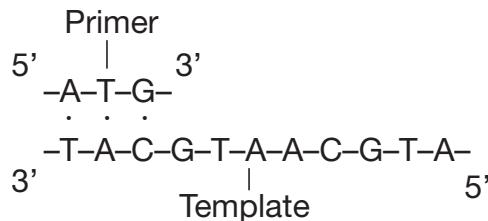
Ligases join nucleic acid molecules together.

Polymerases make copies of molecules.

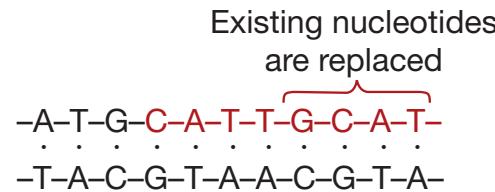
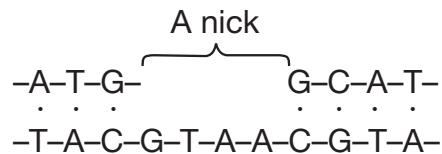
Modifying enzymes remove or add chemical groups.

Polymerases

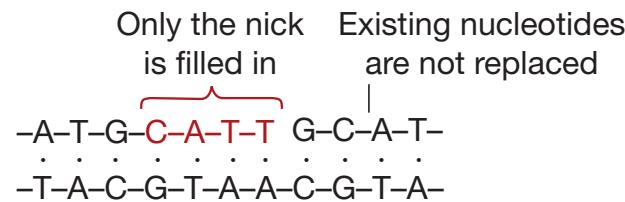
(a) The basic reaction



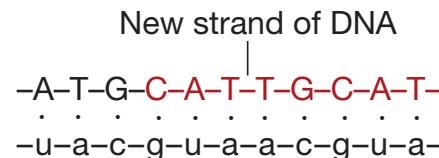
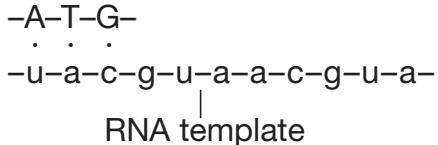
(b) DNA polymerase I



(c) The Klenow fragment

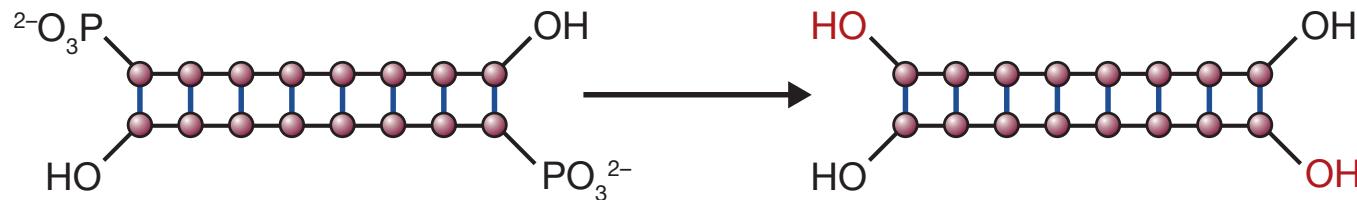


(d) Reverse transcriptase

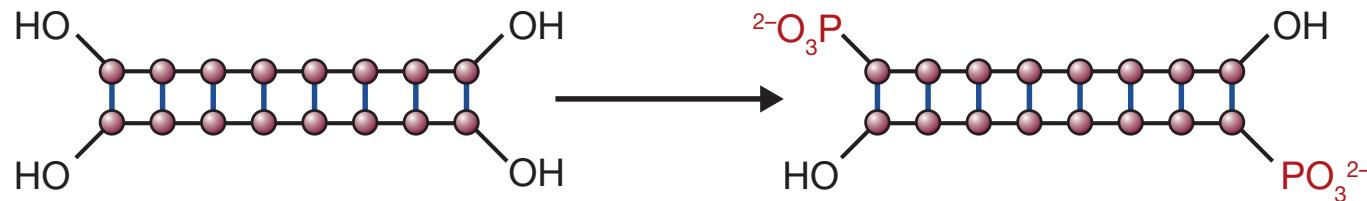


DNA modifying enzymes

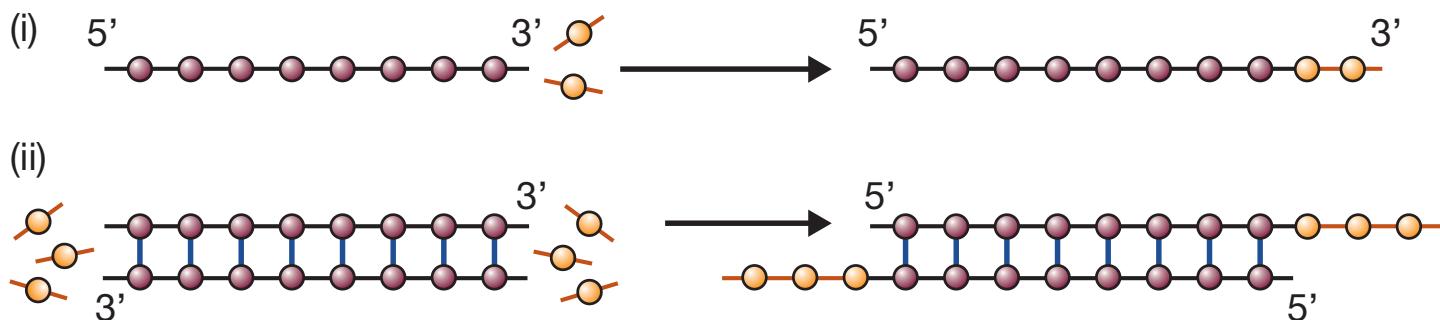
(a) Alkaline phosphatase



(b) Polynucleotide kinase



(c) Terminal deoxynucleotidyl transferase



Nucleases

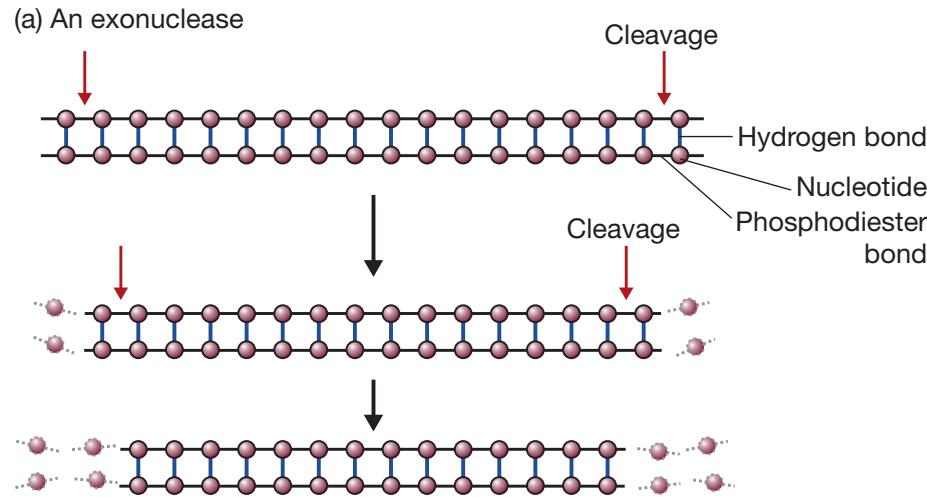
Nucleases degrade DNA molecules by breaking the phosphodiester bonds that link one nucleotide to the next in a DNA strand.

There are two different kinds of nuclease :

Exonucleases remove nucleotides one at a time from the end of a DNA molecule.

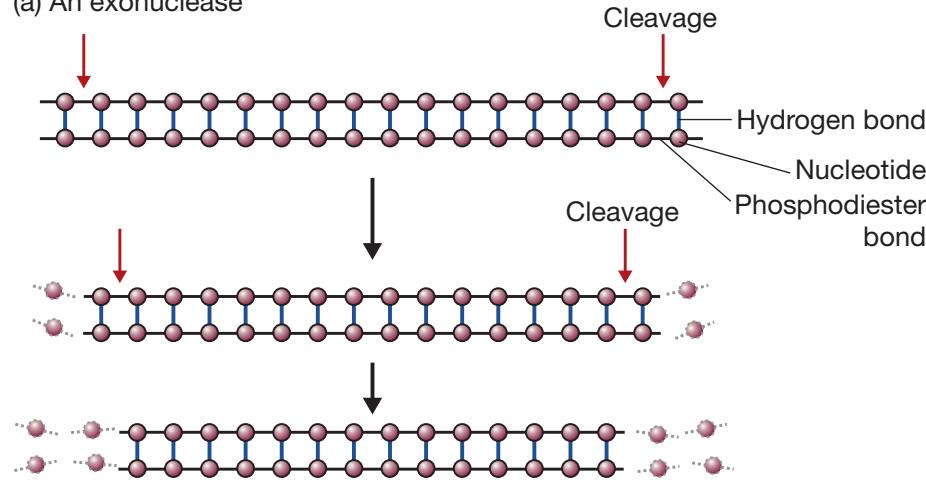
Endonucleases are able to break internal phosphodiester bonds within a DNA molecule.

Endonucleases VS Exonucleases

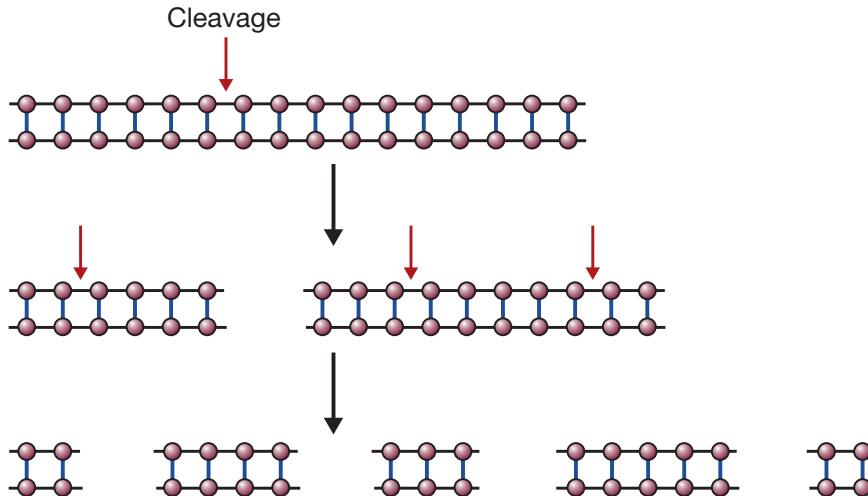


Endonucleases VS Exonucleases

(a) An exonuclease

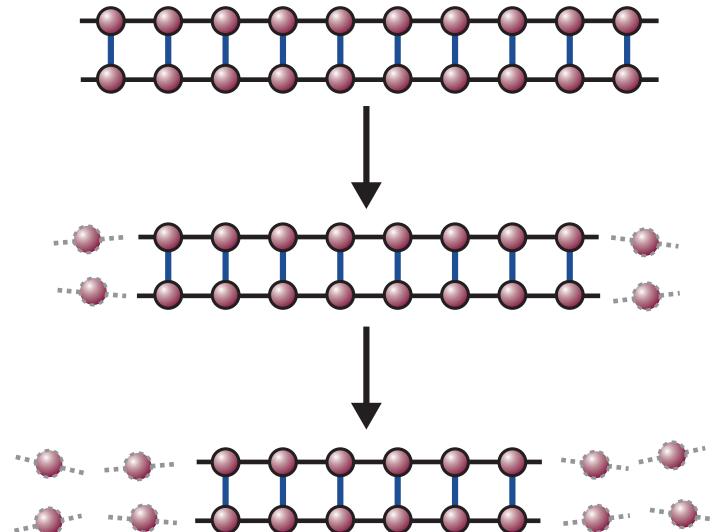


(b) An endonuclease

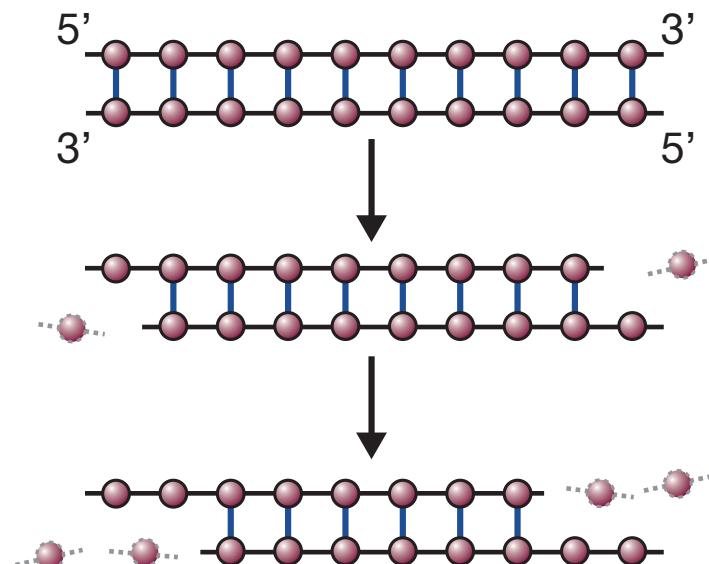


Types of Exonucleases

(a) Bal31

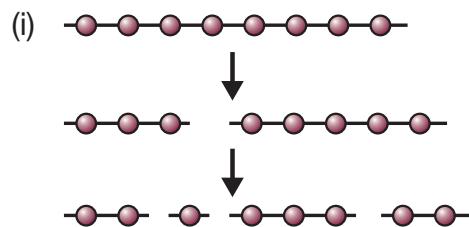


(b) Exonuclease III

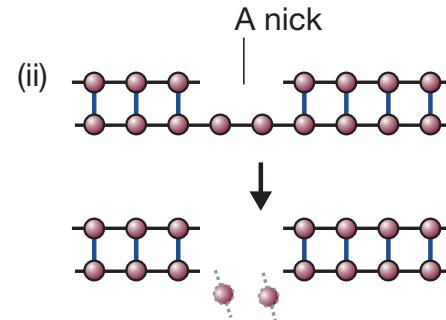


Types of Endonucleases

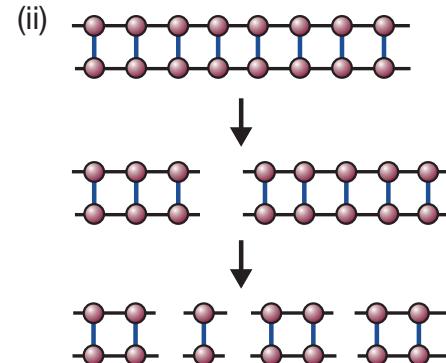
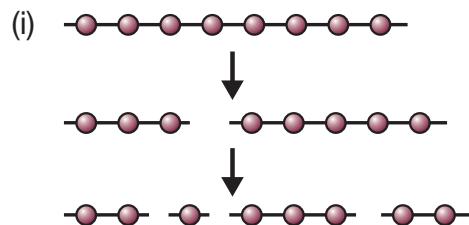
(a) S1 nuclease



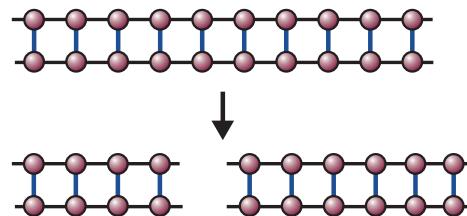
A nick



(b) DNase I



(c) A restriction endonuclease



Restriction Enzymes

Primarily found in bacteria (they use these for defense)

Cut DNA by cleaving the phosphodiester bond that joins adjacent nucleotides in a DNA strand

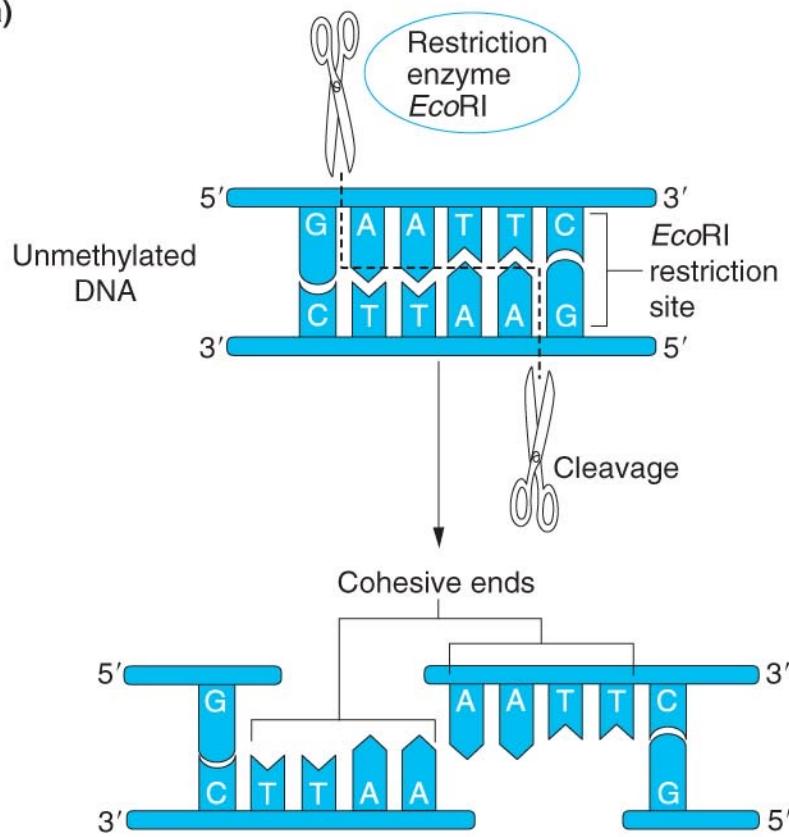
Bind to, recognize, and cut DNA within specific sequences of bases called a **restriction site**

Each restriction site is a **palindrome** – reads same forward and backward on opposite strands of DNA

There are 4 or 6 bp cutters because they recognize restriction sites with a sequence of 4 or 6 nucleotides

Restriction Enzymes

(a)

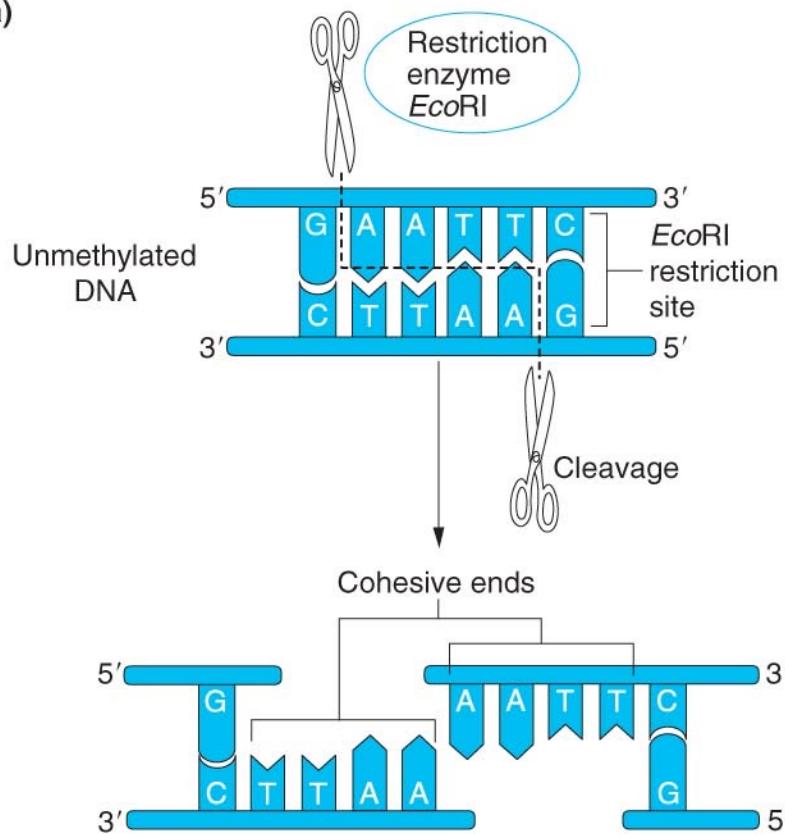


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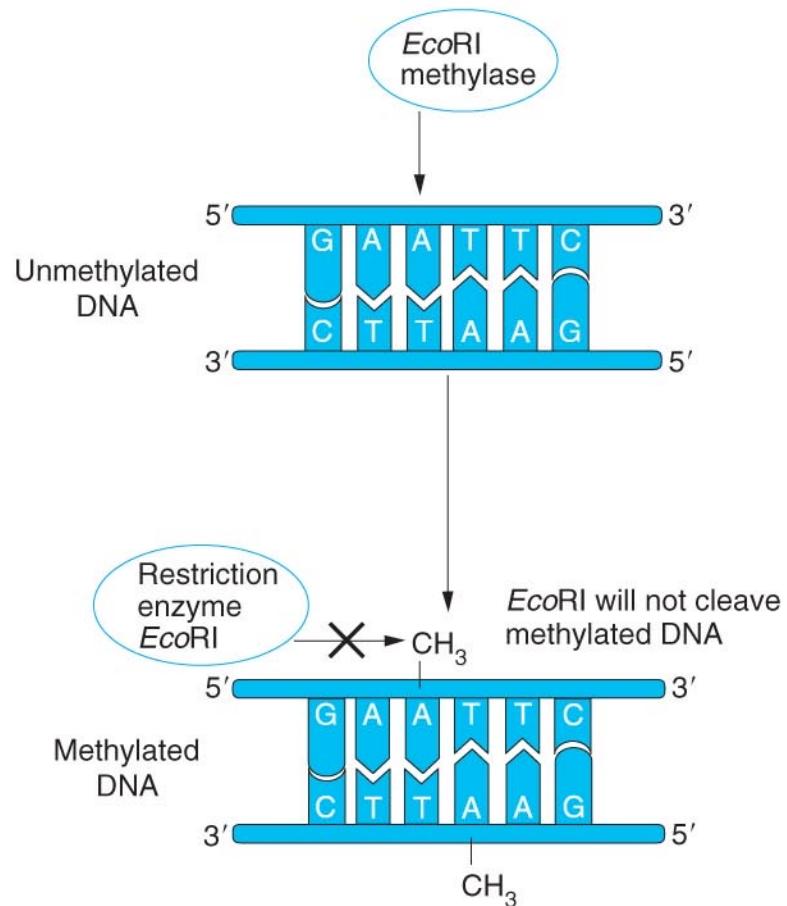
Why don't restriction enzymes digest bacteria DNA?

Restriction Enzymes

(a)

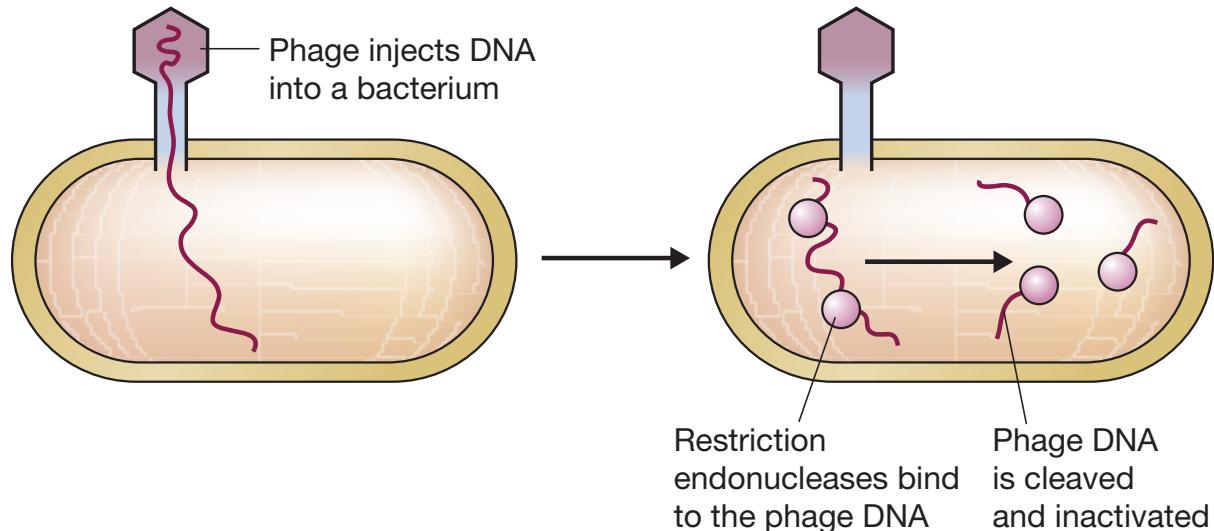


(b)

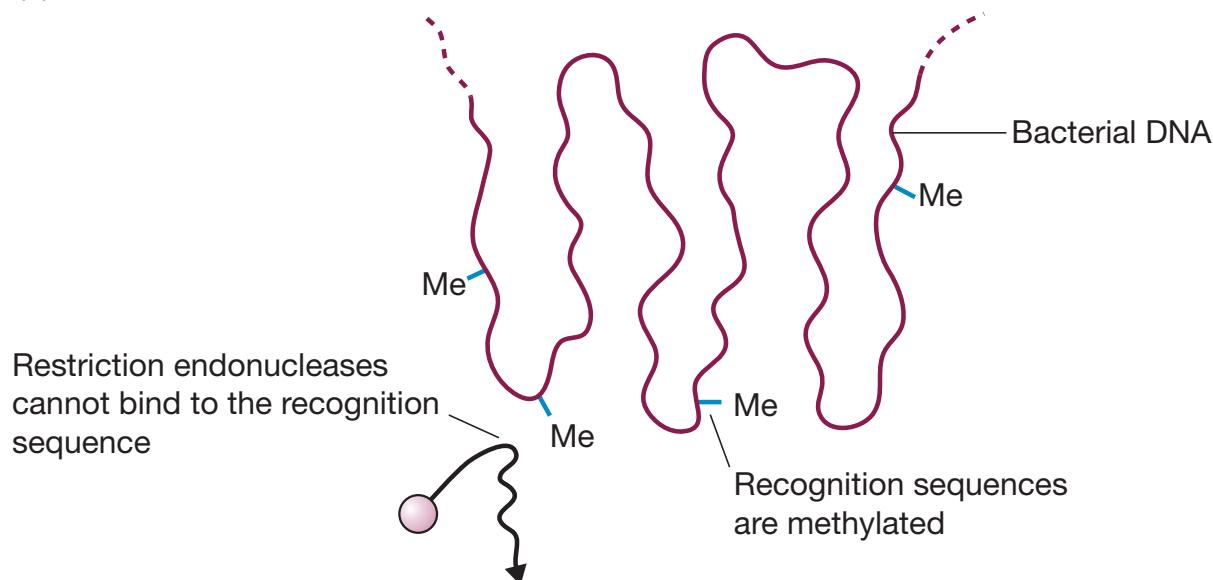


Why don't restriction enzymes digest bacteria DNA?

(a) Restriction of phage DNA



(b) Bacterial DNA is not cleaved



Restriction Enzymes

Some cut DNA to create DNA fragments with overhanging single stranded ends called "sticky" or "**cohesive**" ends

Some cut DNA to generate fragments with double-stranded ends called "**blunt**" ends

Restriction Enzymes

TABLE 3.1 COMMON RESTRICTION ENZYMES

Source Microorganism	Enzyme	Restriction Site
<i>Hemophilus influenzae</i>	HindIII	
<i>Escherichia coli</i>	EcoRI	
<i>Bacillus amyloliquefaciens</i>	BamHI	
<i>Thermus aquaticus</i>	TaqI	

Restriction Enzymes

TABLE 3.1 COMMON RESTRICTION ENZYMES

Source Microorganism	Enzyme	Restriction Site	
<i>Arthrobacter luteus</i>	<i>Alul</i>	5' A - G - C - T - T - C - G - A 3' 3' T - C - G - A - A - G 5'	5' A - G 3' 3' T - C 5'
<i>Haemophilus aegypticus</i>	<i>HaeIII</i>	5' G - G - C - C - C - C 3' 3' C - C - G - G - G - G 5'	5' C - C 3' 3' G - G 5'
<i>Serratia marcescens</i>	<i>SmaI</i>	5' C - C - C - G - G - G 3' 3' G - G - G - C - C - C 5'	5' C - C - C 3' 3' G - G - G 5'

Restriction Enzymes

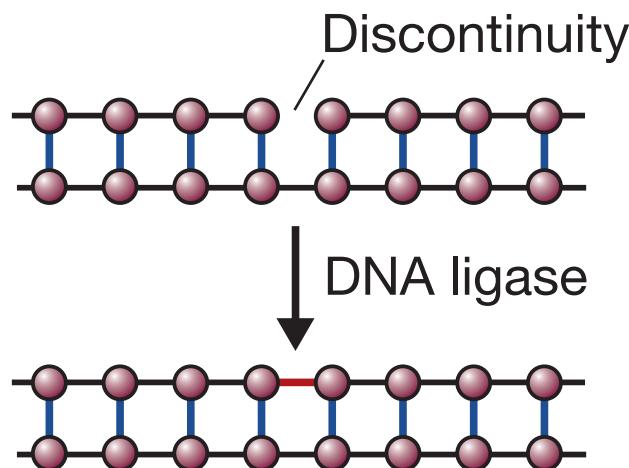
Enzymes that produce sticky ends are preferred for cloning because DNA fragments with sticky ends can be easily joined together because they base pair with each other by forming weak hydrogen bonds

Video (Restriction enzymes)

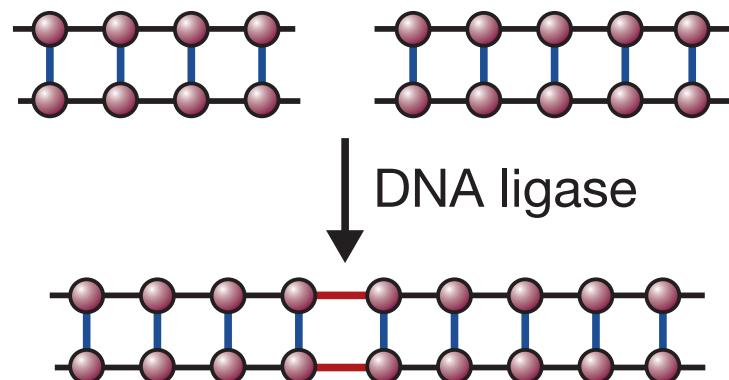
<https://mediaplayer.pearsoncmg.com/assets/secs-recombinant-dna-technology-restriction-enzymes>

DNA ligases

(a) Discontinuity repair



(b) Joining two molecules



Plasmid DNA vectors

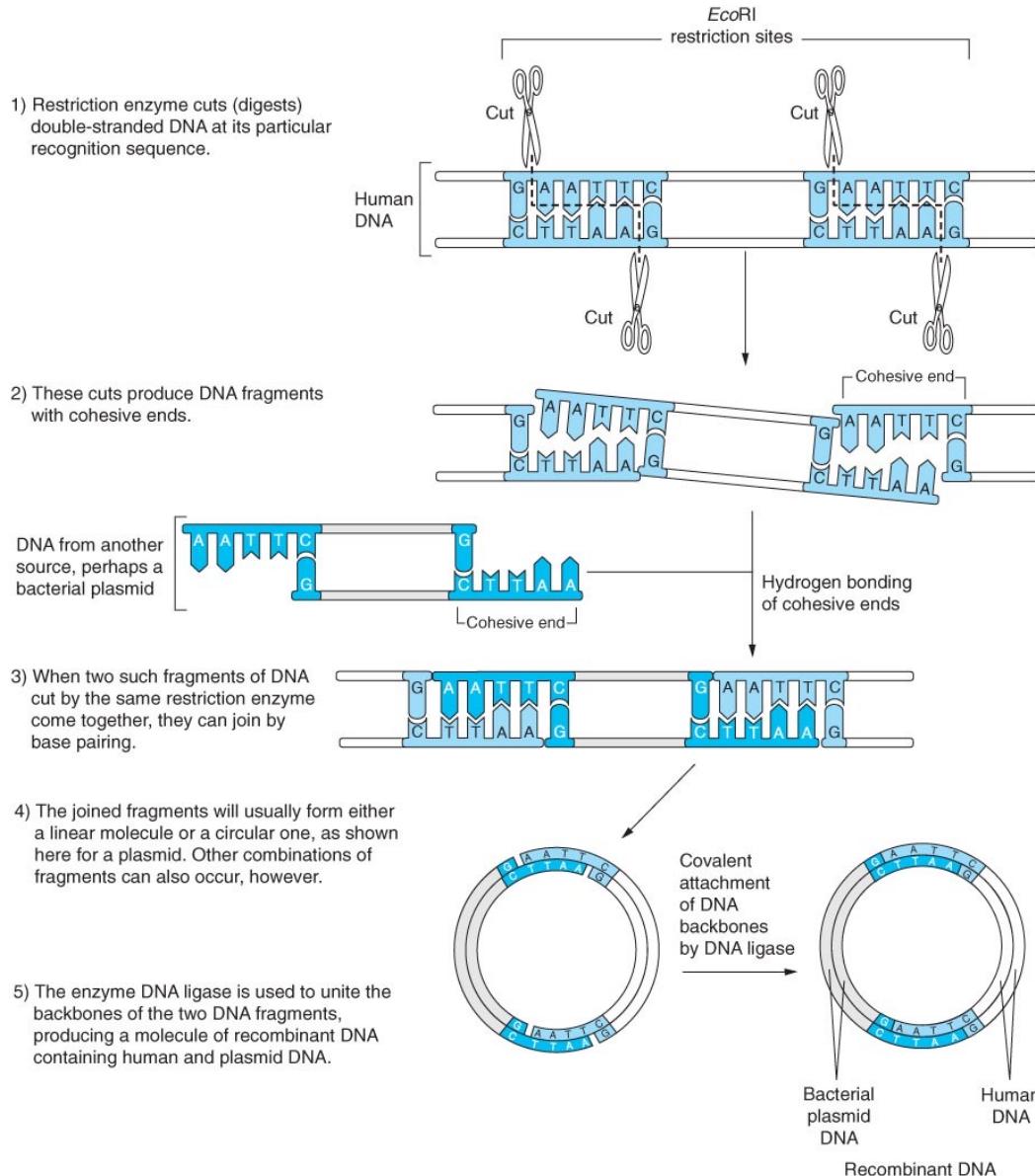
Plasmid DNA – small circular pieces of DNA found primarily in bacteria

Are considered extrachromosomal DNA because they are in the cytoplasm in addition to the bacteria chromosome

Can replicate independently of chromosome

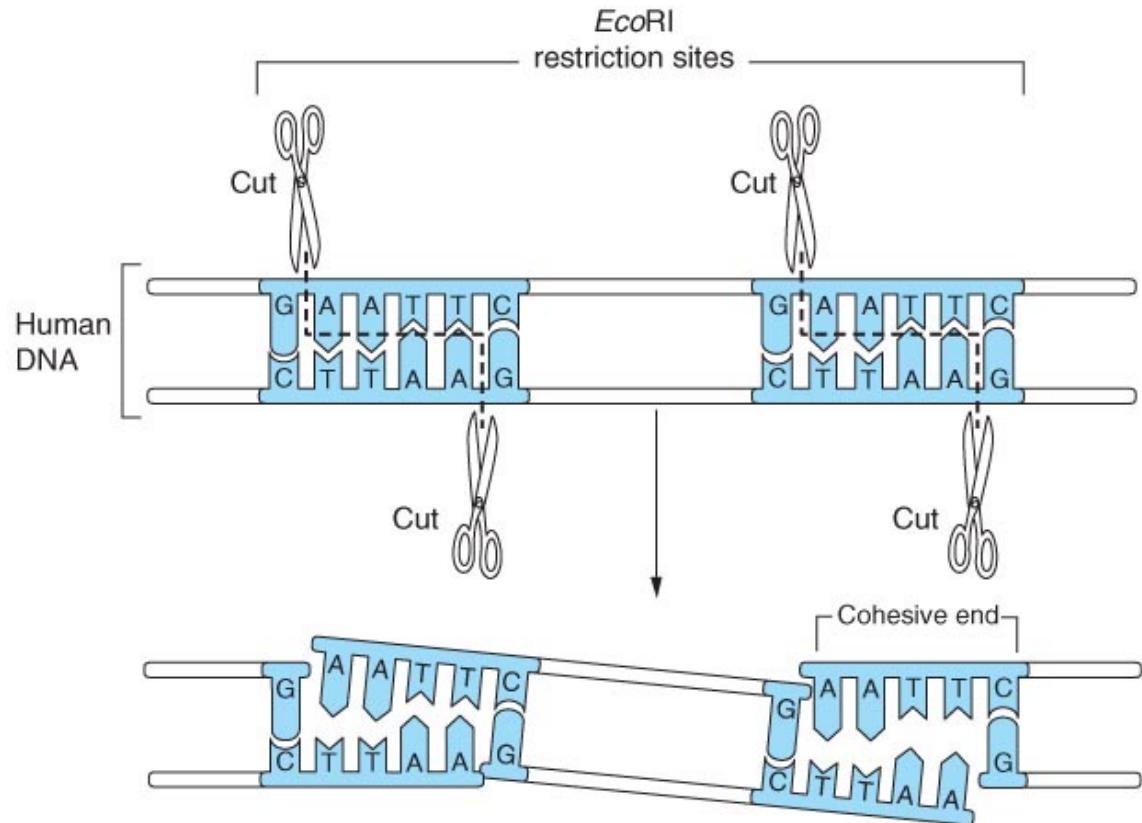
Can be used as **vectors** – pieces of DNA that can accept, carry, and replicate other pieces of DNA

Creating recombinant DNA



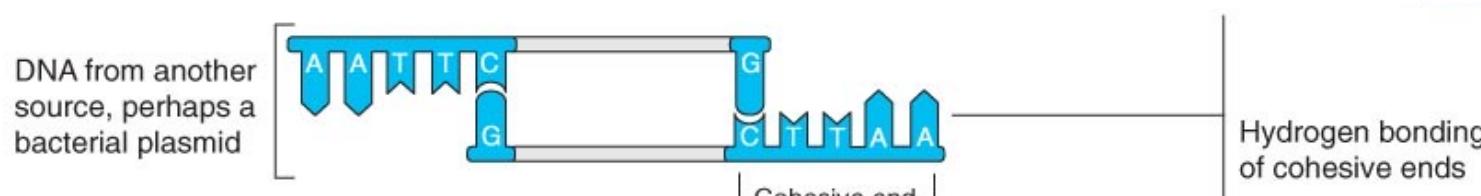
Creating recombinant DNA

- 1) Restriction enzyme cuts (digests) double-stranded DNA at its particular recognition sequence.



- 2) These cuts produce DNA fragments with cohesive ends.

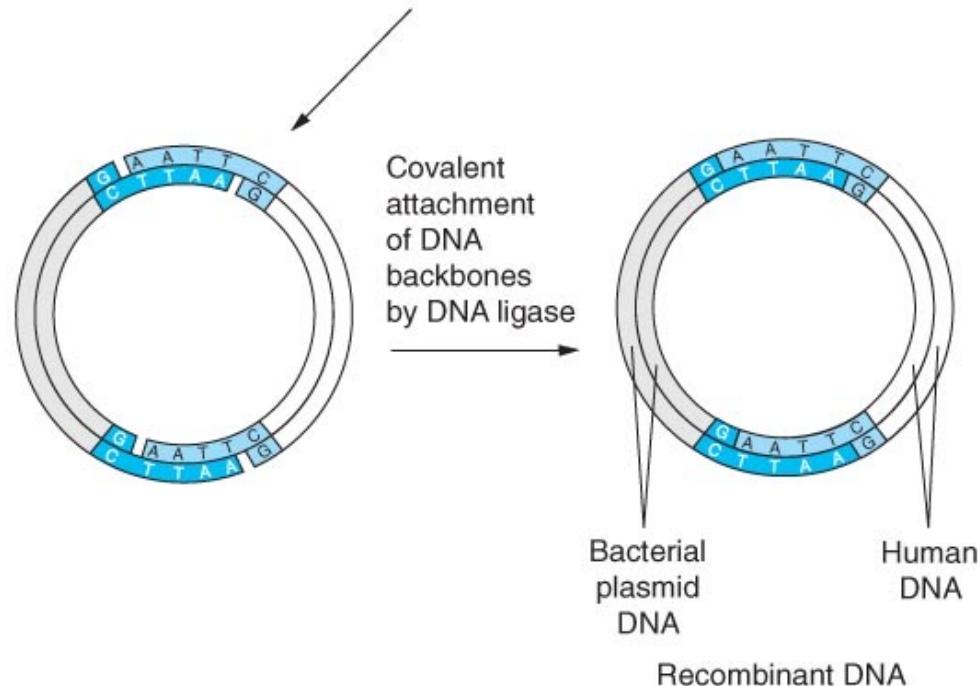
Creating recombinant DNA



- 3) When two such fragments of DNA cut by the same restriction enzyme come together, they can join by base pairing.



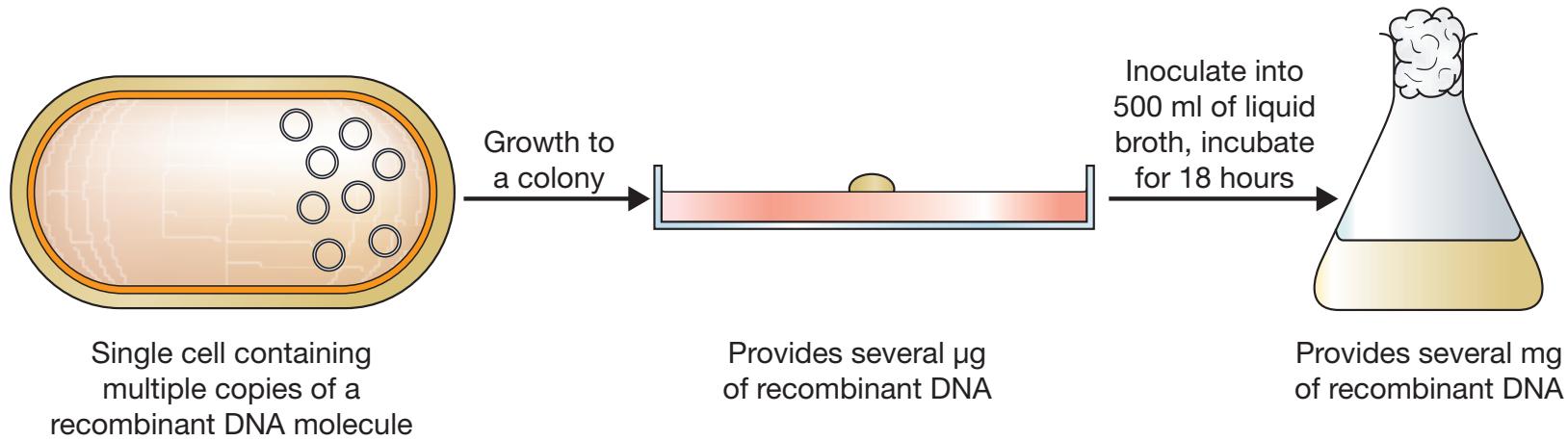
- 4) The joined fragments will usually form either a linear molecule or a circular one, as shown here for a plasmid. Other combinations of fragments can also occur, however.



Video (DNA cloning)

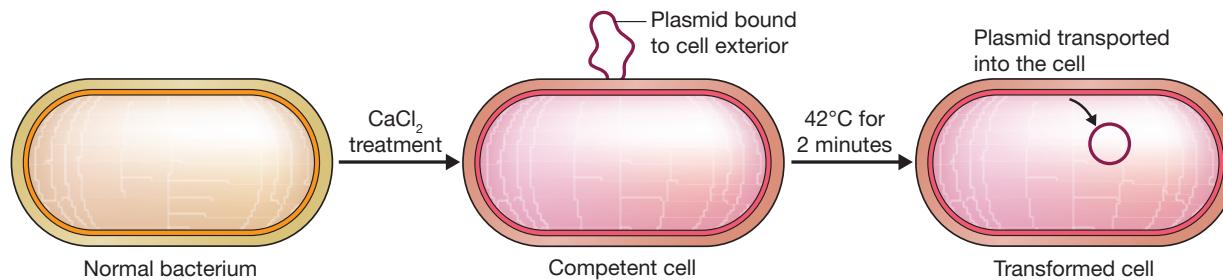
<https://mediaplayer.pearsoncmg.com/assets/secs-dna-cloning-in-a-plasmid-vector>

Cloning can supply large amounts of recombinant DNA



Transformation of bacterial cells and antibiotic selection of recombinant bacteria

Transformation of bacterial cells is a very inefficient process for inserting foreign DNA into bacteria



Procedure for chemical method:

- 1.Treat bacterial cells with calcium chloride
- 2.Add plasmid DNA to cells chilled on ice
- 3.Heat the cell and DNA mixture
- 4.Plasmid DNA enters bacterial cells and is replicated and express their genes

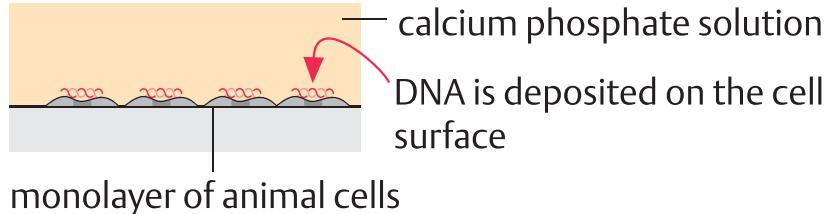
Transformation of bacterial cells and antibiotic selection of recombinant bacteria

Electroporation is also used to transform bacteria

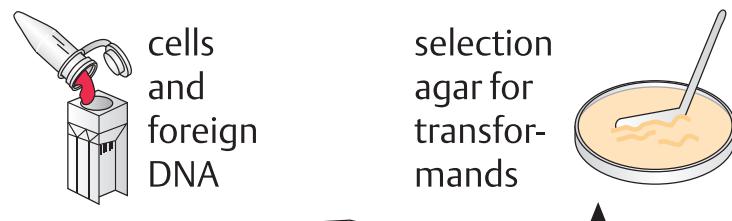
Electroporation involves applying brief pulse of high voltage electricity to create tiny holes in the bacteria cell wall that allow the DNA to enter

Transformations based on nonbiological methods

endocytosis

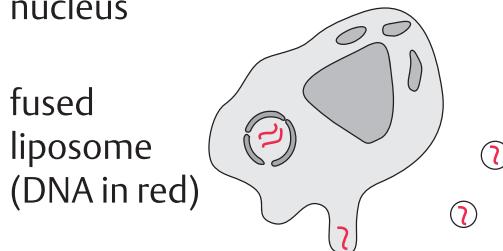


electroporation

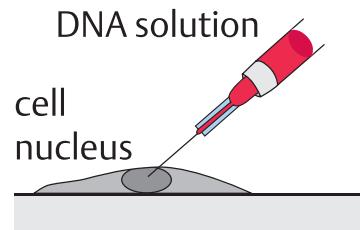


liposome fusion (lipofection)

transfer of DNA into the cell nucleus



microinjection



$\sim 10\ 000\ \text{V/m}^2$
for $\sim 10\ \text{ms}$

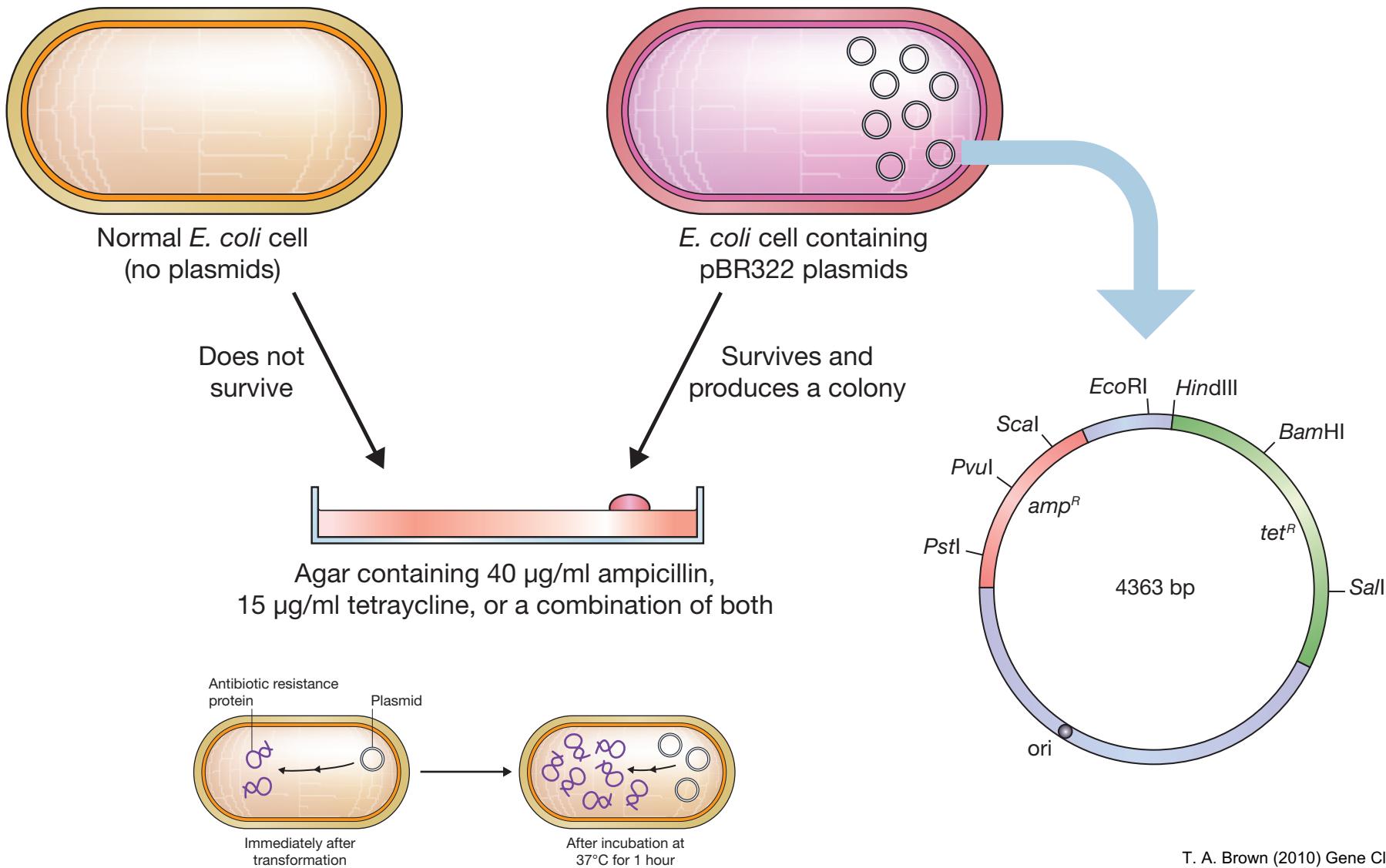
Transformation of bacterial Cells and Antibiotic selection of Recombinant bacteria

Selection of recombinant bacteria after transformation is a process designed to facilitate the identification of recombinant bacteria while preventing the growth of non-transformed bacteria and bacteria that contain plasmid without foreign DNA

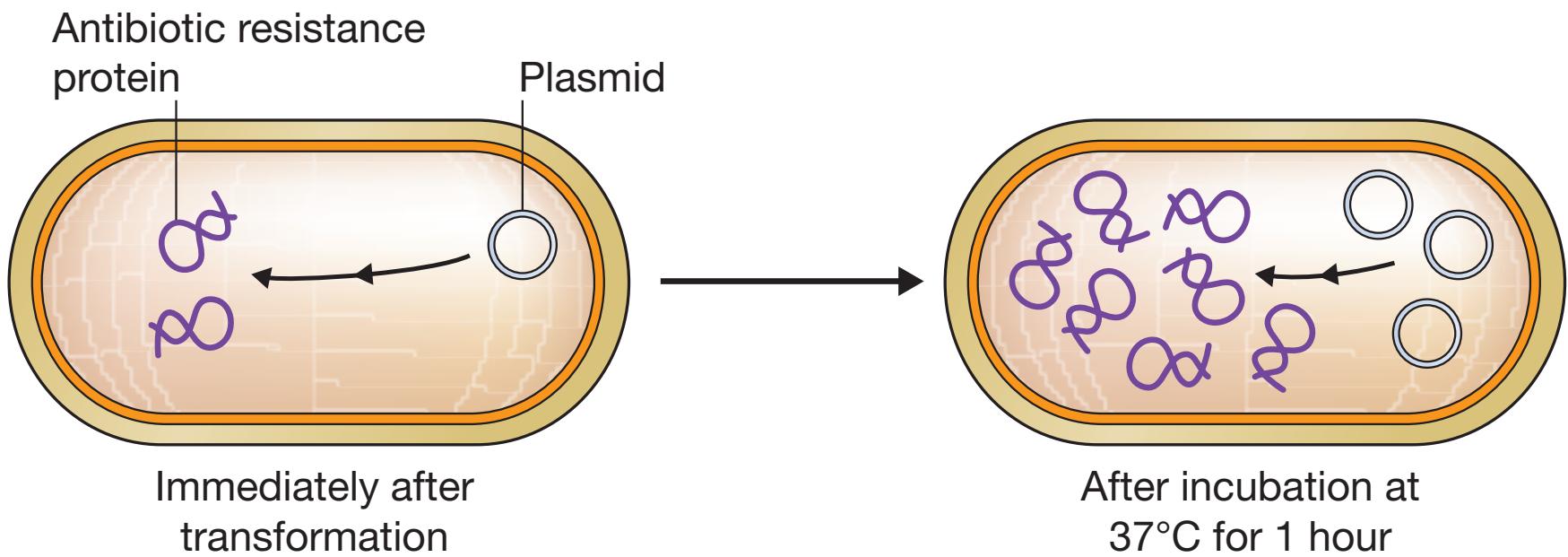
Antibiotic selection – plate transformed cells on plates containing different antibiotics to identify recombinant bacteria and non-transformed bacteria

Does not select for plasmid containing foreign DNA vs. recircularized plasmid

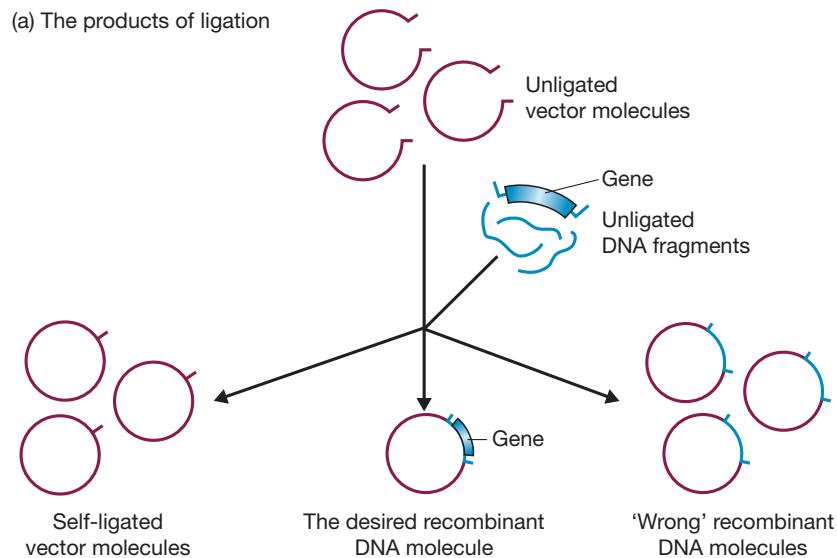
Antibiotic Selection



Antibiotic Selection

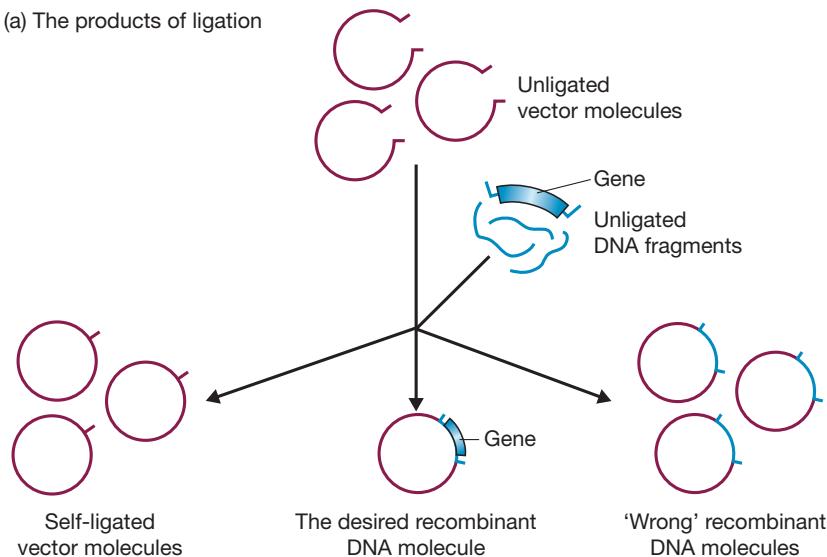


Antibiotic Selection

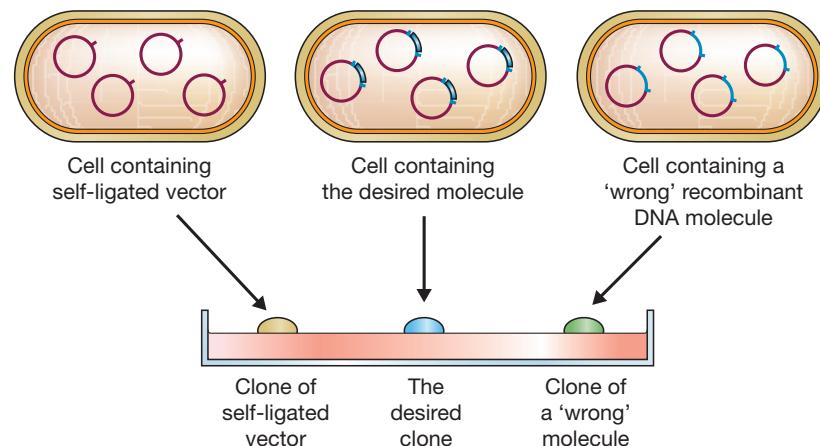


Antibiotic Selection

(a) The products of ligation



(b) All circular molecules will be cloned



Transformation of bacterial Cells and Antibiotic selection of Recombinant bacteria

Blue-white selection

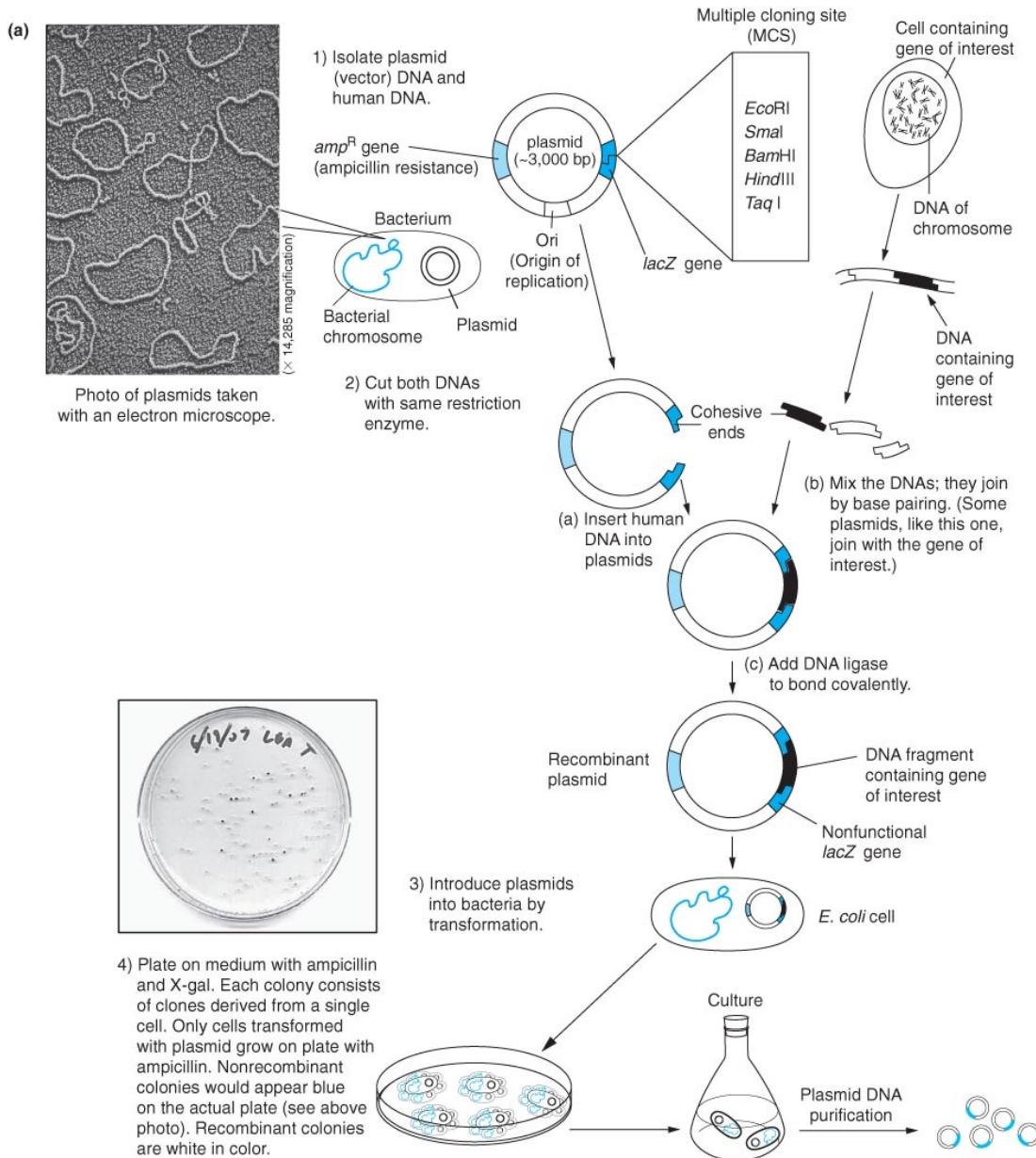
DNA is cloned into the restriction site in the *lacZ* gene

When it is interrupted by an inserted gene, the *lacZ* gene cannot produce functional β -galactosidase

When Xgal (artificial lactose) is added to the plate, if functional *lacZ* is present = blue colony

Non-functional *lacZ* = **white colony** = **clone** = genetically identical bacterial cells each containing copies of recombinant plasmid

Cloning a Gene in a Plasmid and Blue-White Selection



Cloning a Gene in a Plasmid and Blue-White Selection

(a)

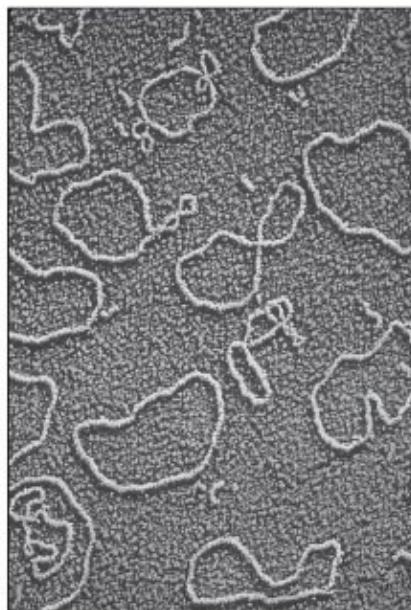
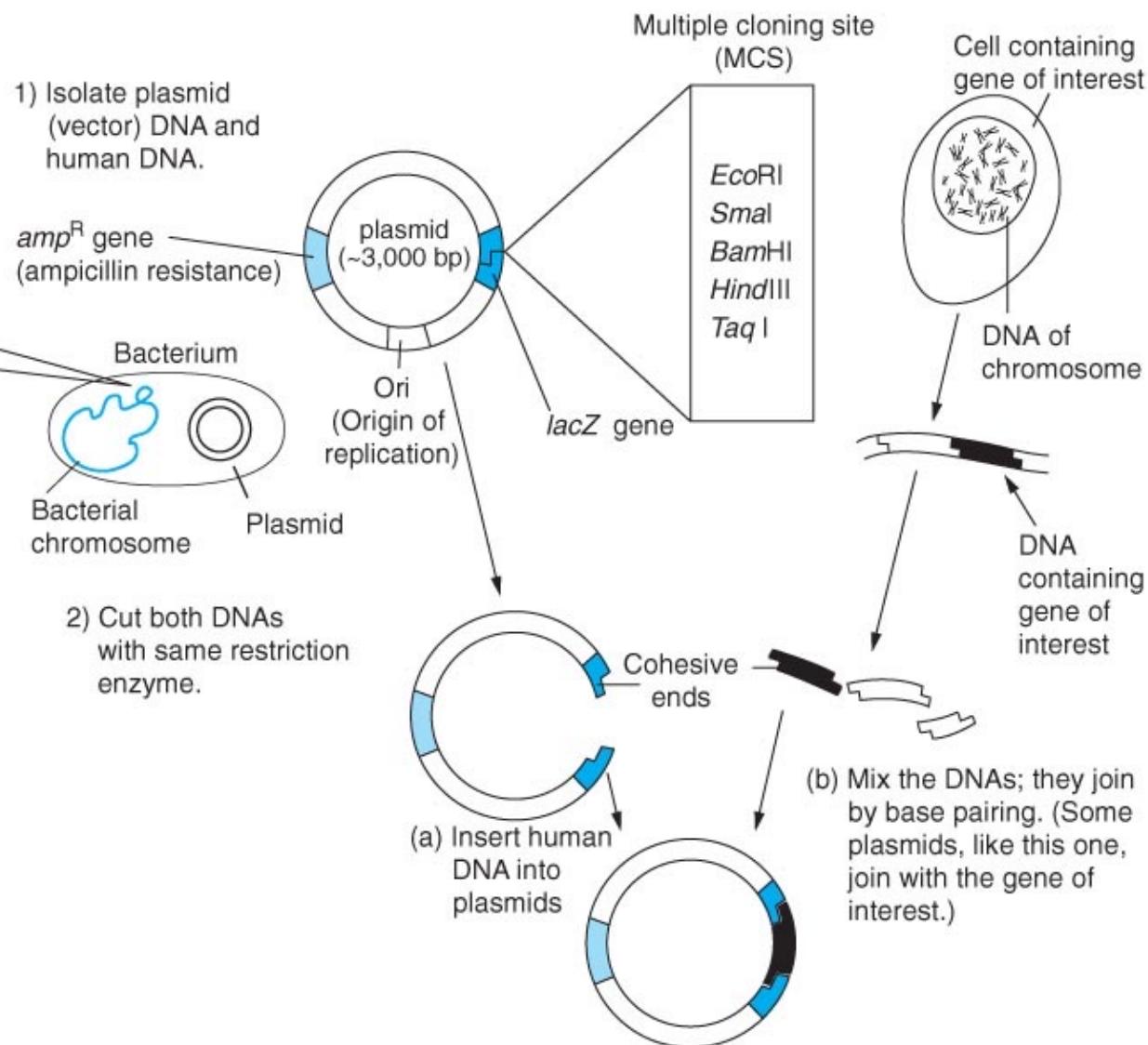


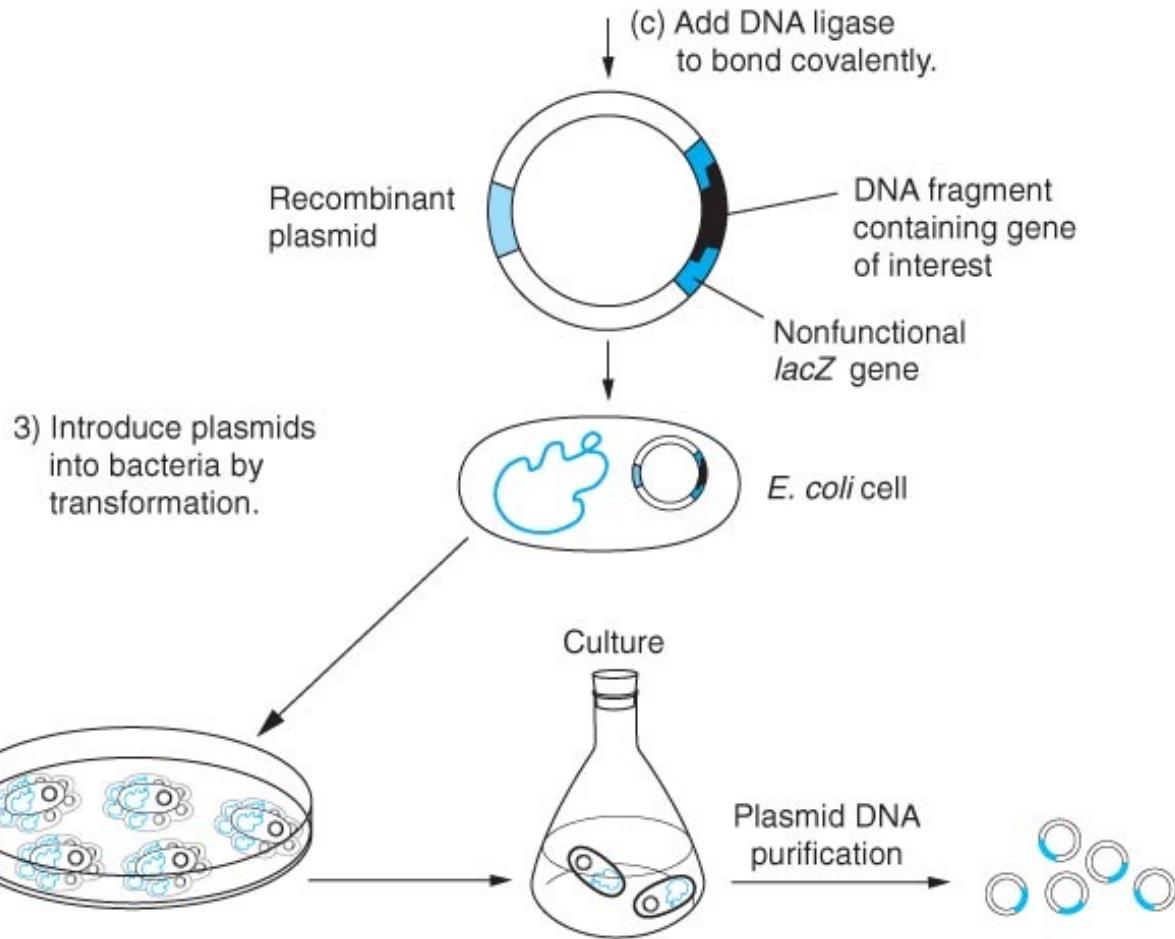
Photo of plasmids taken with an electron microscope.



Cloning a Gene in a Plasmid and Blue-White Selection



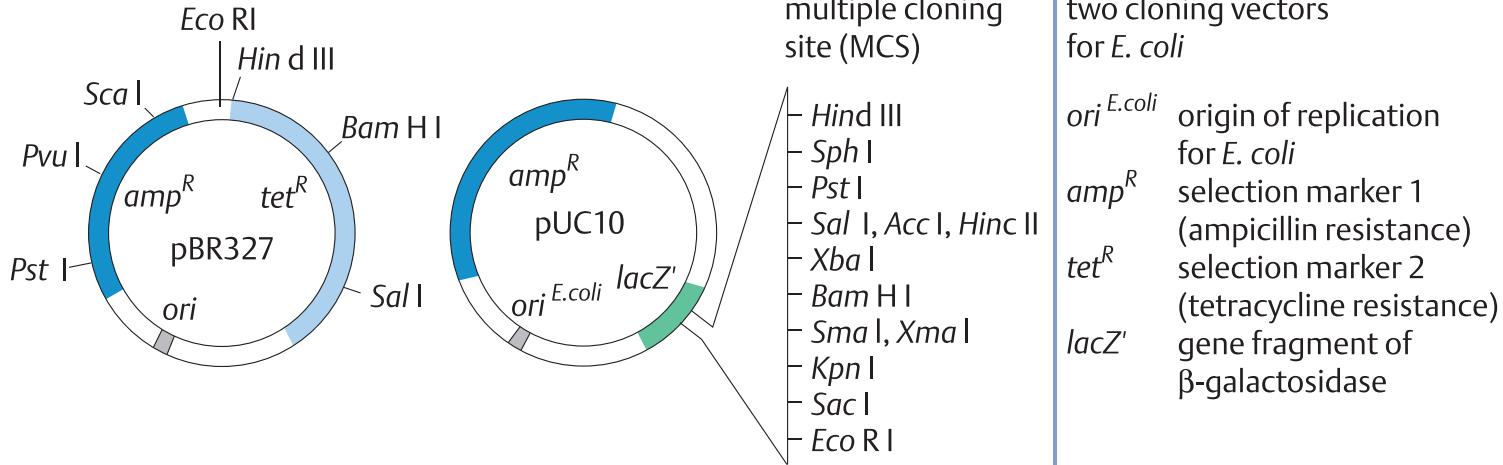
4) Plate on medium with ampicillin and X-gal. Each colony consists of clones derived from a single cell. Only cells transformed with plasmid grow on plate with ampicillin. Nonrecombinant colonies would appear blue on the actual plate (see above photo). Recombinant colonies are white in color.



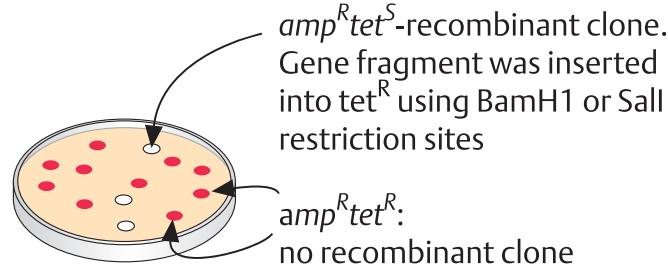
Video (DNA cloning using vector)

<https://mediaplayer.pearsoncmg.com/assets/secs-recombinant-dna-technology-vectors>

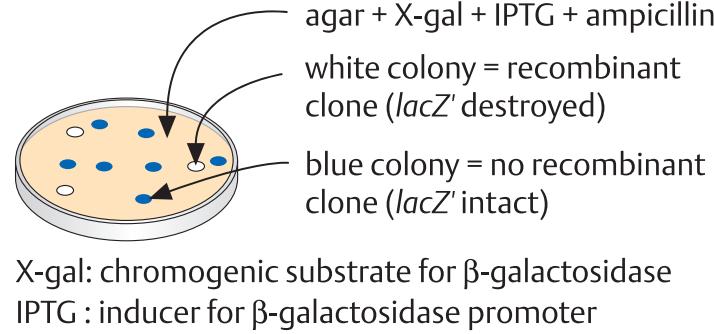
Cloning vectors



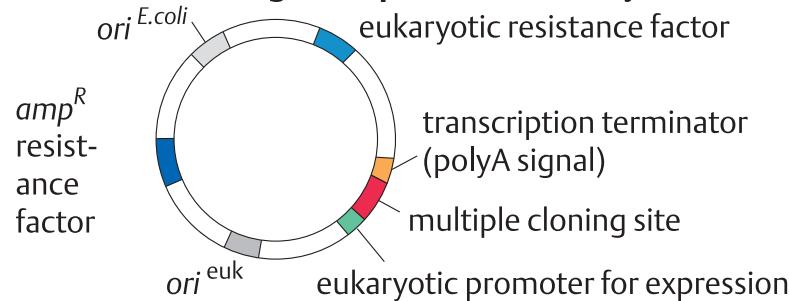
screening for antibiotic resistance



blue-white screening



shuttle vector for gene expression in eukaryotes



ori^{E.coli} origin of replication for *E. coli*
amp^R selection marker for *E. coli*
ori^{euk} origin of replication for eukaryotes (e.g., of 2μ plasmid for *S. cerevisiae*, of SV40 virus for animal cells)

Introduction to human gene cloning

First human protein expressed via recombinant techniques was insulin and next was growth hormone

Clone human insulin DNA sequence into a plasmid and the bacteria cells were then used to synthesize the protein product of the cloned gene

Can generate lots of pure protein via this technique

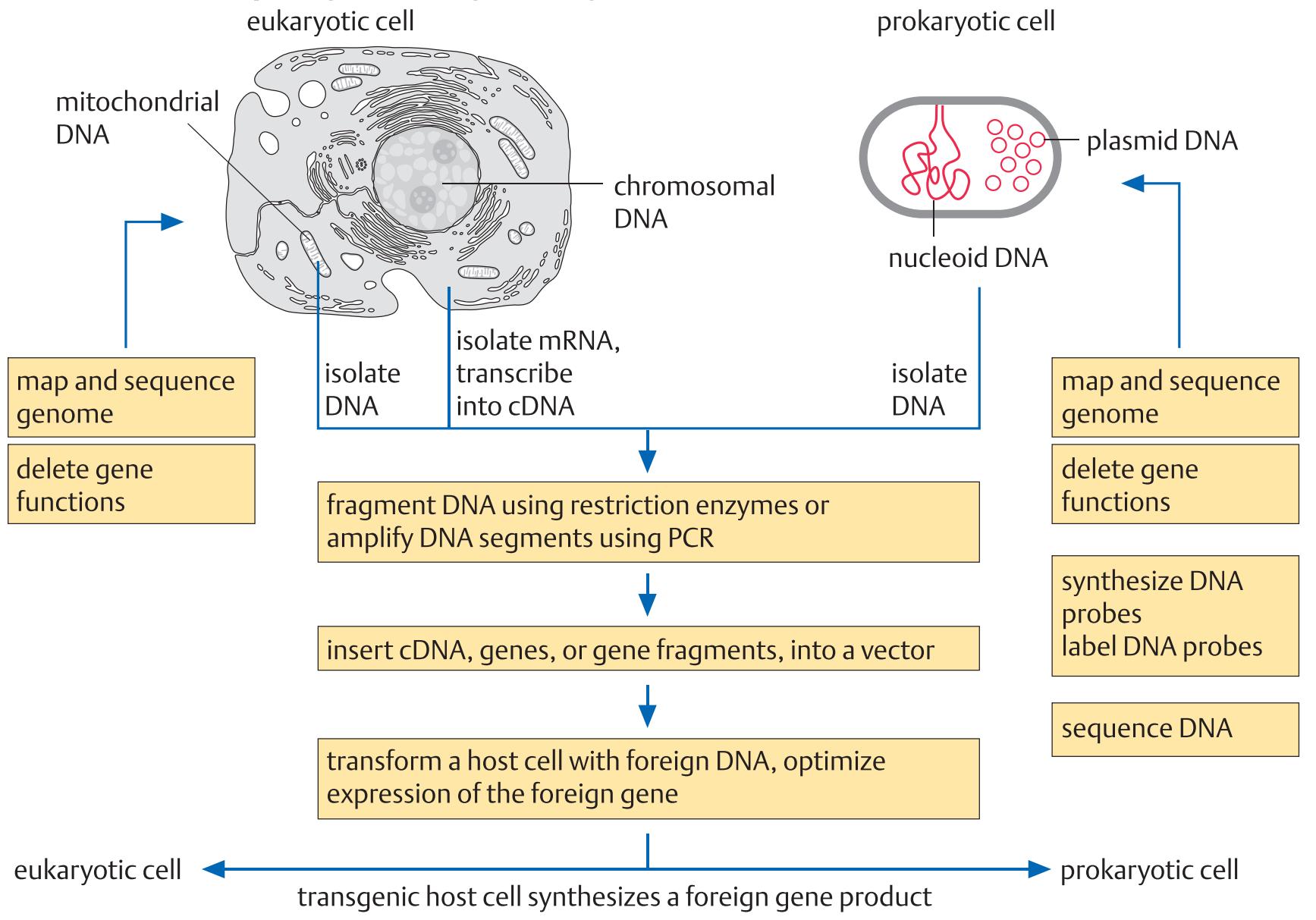
Prior to recombinant DNA technology, hormones like insulin and growth hormone had to be isolated from tissues and some time, from human cadavers.

Introduction to human gene cloning

In 1977, the insulin gene was cloned into plasmids, expressed in bacterial cells, and isolated by scientists at **Genentech** (named for **genetic engineering technology**), the first biotechnology company.

In 1982, the recombinant form of human insulin, called **Humulin**, became the first recombinant DNA product to be approved for human applications by the **U.S. Food and Drug Administration (FDA)**

Fundamental steps in genetic engineering



3.2 What Makes a Good Vector?

Practical Features of DNA Cloning Vectors:

1. **Size** – small enough to be separated from chromosomal DNA of host plasmid
2. **Origin of replication (*ori*)** – site for DNA replication that allow plasmids to replicate independently from host chromosome

Copy number: number of plasmids in the cell (normally small but plasmids have high copy numbers)

3.2 What Makes a Good Vector?

3. **Multiple cloning site (MCS)** – recognition sites for several restriction enzymes in which insert is cloned into
4. **Selectable marker genes** – allow to select for transformed colonies
5. **RNA polymerase promoter sequences** – used for transcription *in vitro* and *in vivo*
6. **DNA sequencing primers**

3.2 What Makes a Good Vector?

TABLE 3.2 A COMPARISON OF DNA VECTORS AND THEIR APPLICATIONS

Vector Type	Maximum Insert Size (kb)	Applications	Limitations
Bacterial plasmid vectors (circular)	~ 6–12	DNA cloning, protein expression, subcloning, direct sequencing of insert DNA	Restricted insert size; limited expression of proteins; copy number problems; replication restricted to bacteria
Bacteriophage vectors (linear)	~ 25	cDNA, genomic and expression libraries	Packaging limits DNA insert size; host replication problems
Cosmid (circular)	~ 35	cDNA and genomic libraries, cloning large DNA fragments	Phage packaging restrictions; not ideal for protein expression; cannot be replicated in mammalian cells
Bacterial artificial chromosome (BAC, circular)	~ 300	Genomic libraries, cloning large DNA fragments	Replication restricted to bacteria; cannot be used for protein expression
Yeast artificial chromosome (YAC, circular)	200–2,000	Genomic libraries, cloning large DNA fragments	Must be grown in yeast; cannot be used in bacteria
Ti vector (circular)	Varies depending on type of Ti vector used	Gene transfer in plants	Limited to use in plant cells only; number of restriction sites randomly distributed; large size of vector not easily manipulated

Types of Vectors

Bacterial plasmid vectors – can clone inserts that are smaller than 7 kb; some express eukaryotic proteins from genes poorly

Bacteriophage vectors

Cosmid vectors

Expression vectors

Bacterial Artificial Chromosomes (BAC)

Yeast Artificial Chromosomes (YAC)

Ti vectors

Bacteriophage vectors

Advantage: clone up to 25 kb λ genome is linear and 49 kb

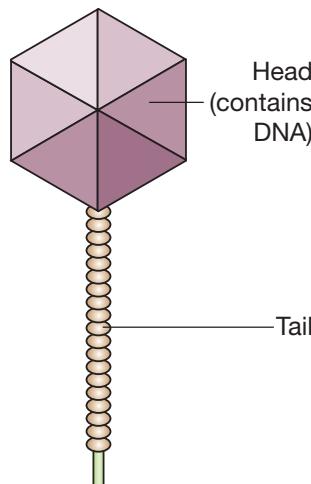
Recombinant chromosomes are packaged into viral particles *in vitro*

At each end of λ are 12 bp sites = COS which base pair together when they infect bacteria and circularize and replicate

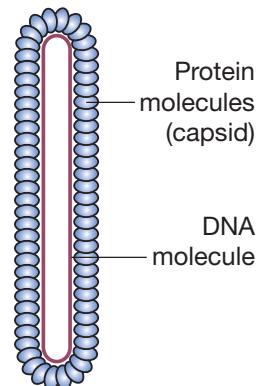
Obtain plaques – zones of dead bacteria which contain millions of recombinant phage particles

Bacteriophage vectors (λ and M13)

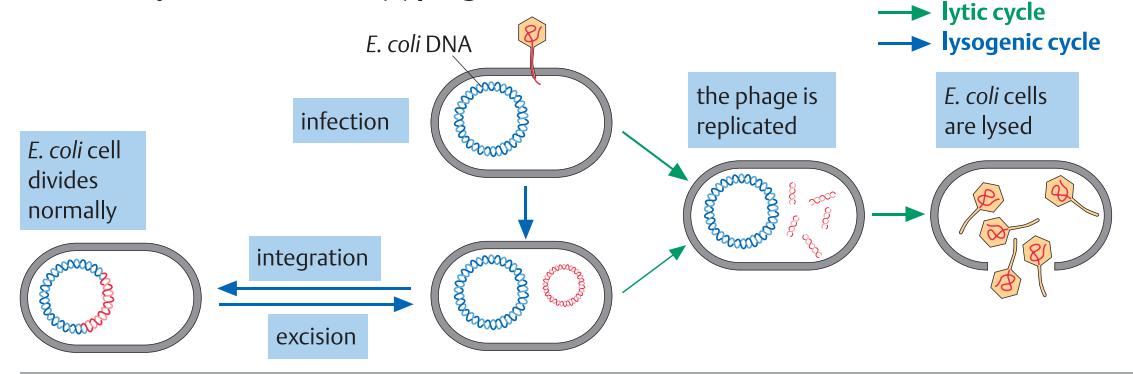
(a) Head-and-tail



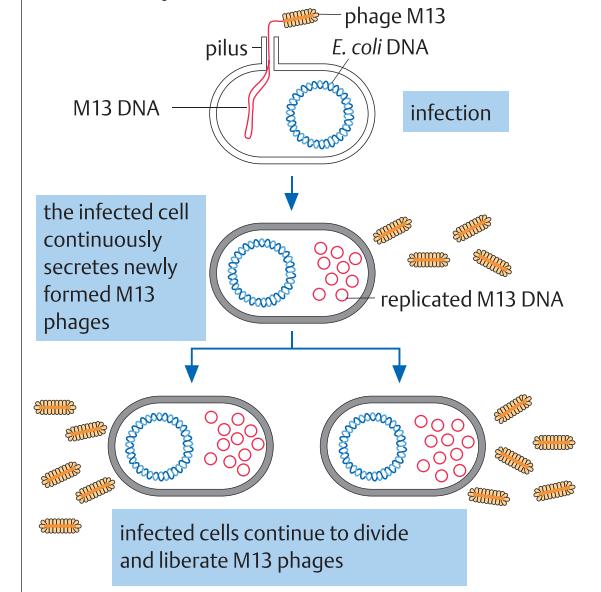
(b) Filamentous



Infection cycle of the lambda (λ) phage

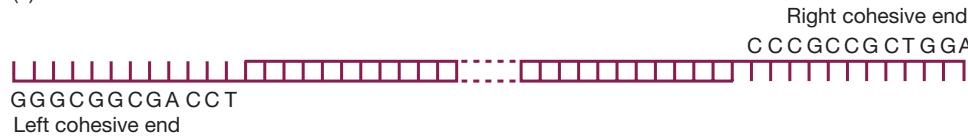


Infection cycle of M13

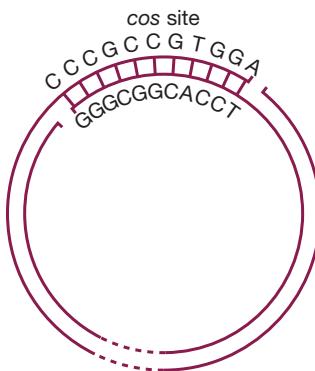


Bacteriophage λ genome replication

(a) The linear form of the λ DNA molecule



(b) The circular form of the λ DNA molecule

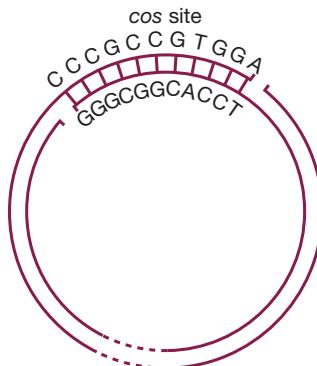


Bacteriophage λ genome replication

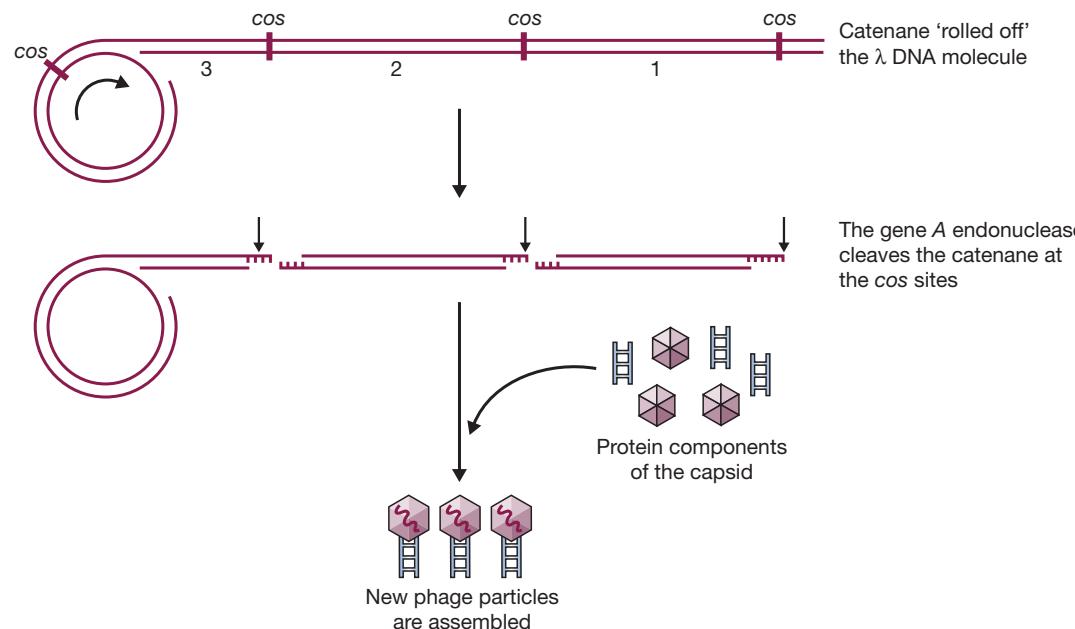
(a) The linear form of the λ DNA molecule



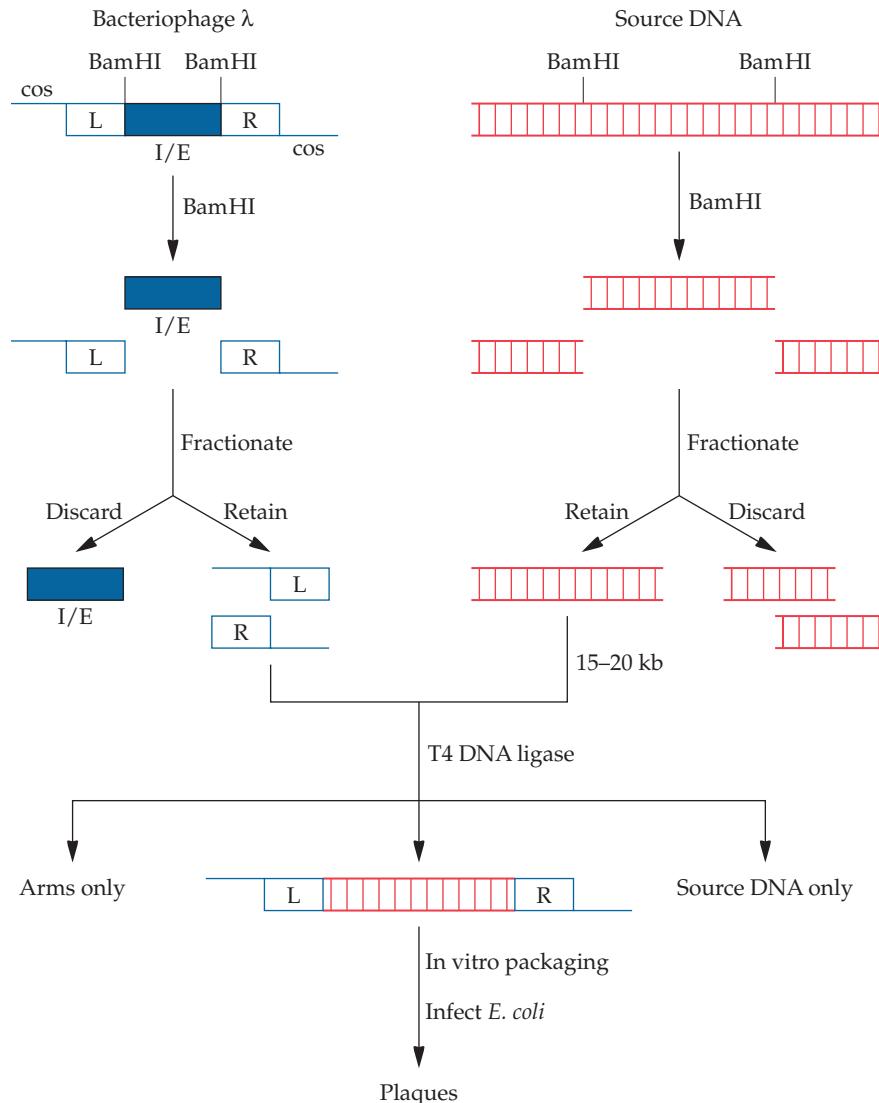
(b) The circular form of the λ DNA molecule



(c) Replication and packaging of λ DNA

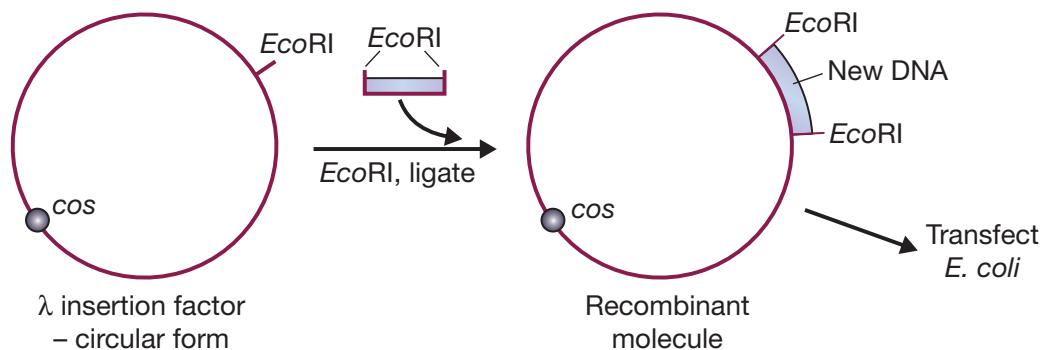


Cloning with a λ vector

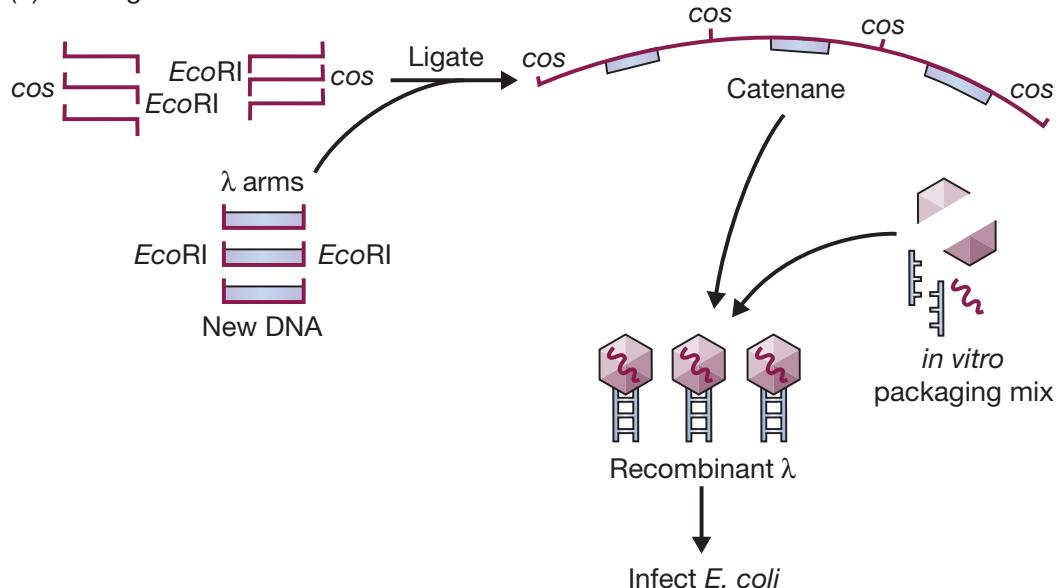


Cloning with a λ vector

(a) Cloning with circular λ DNA

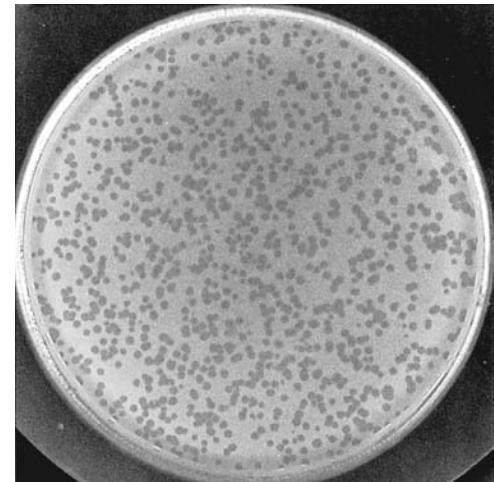
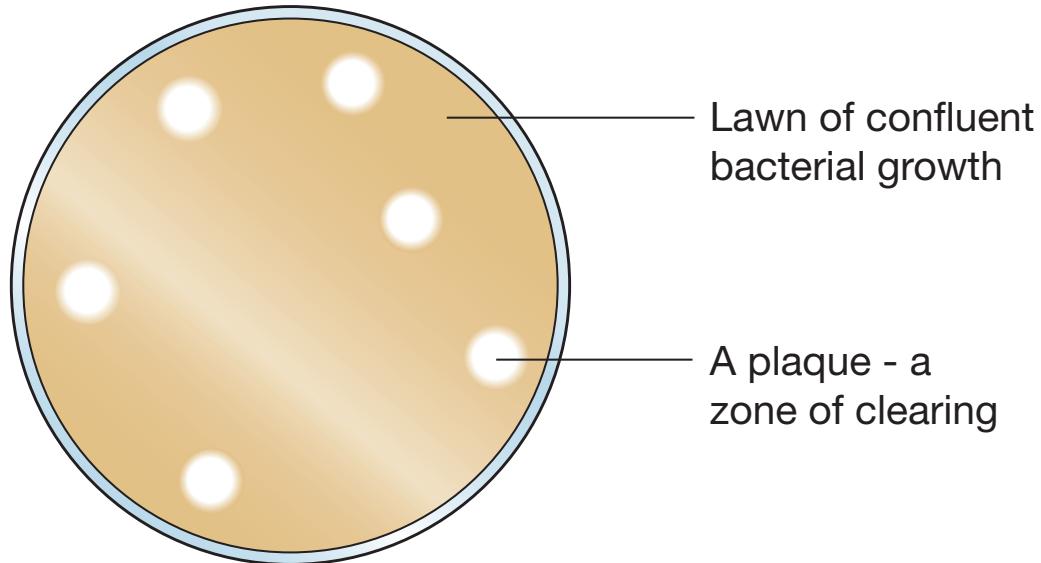


(b) Cloning with linear λ DNA

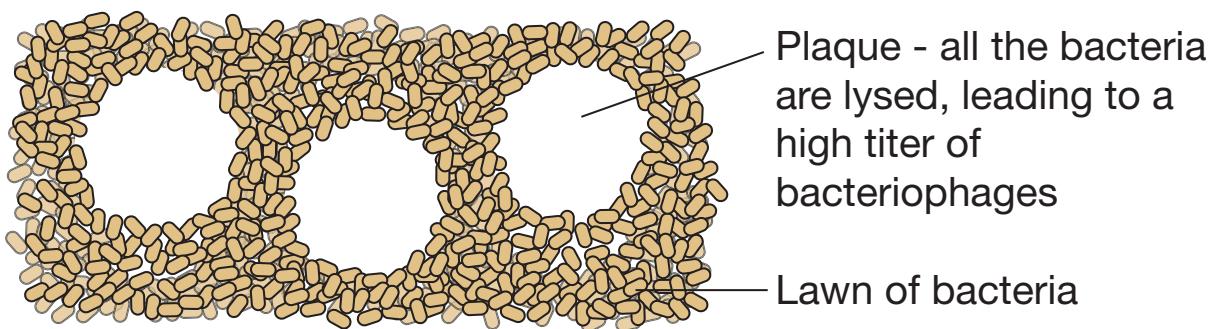


Bacteriophage vectors

(a) Plaques on a lawn of bacteria



(b) Lytic plaques



Cosmid vectors

Cosmid vectors contain:

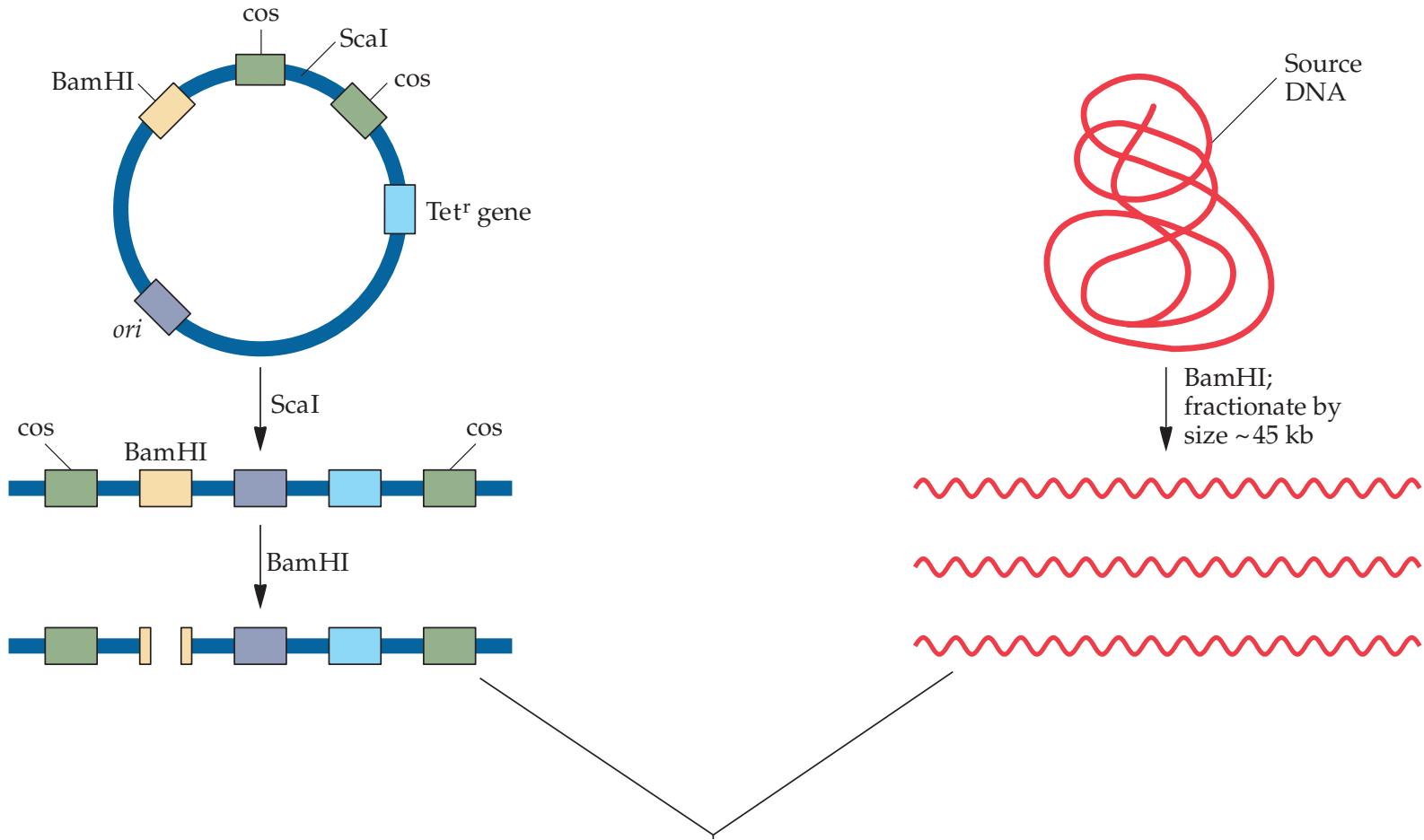
- 1.COS ends of λ DNA
- 2.Plasmid origin of replication
- 3.Gene for antibiotic resistance
- 4.DNA is cloned into restriction site

Then cosmid is packaged into viral particles and used to infect *E coli* cells at low copy number

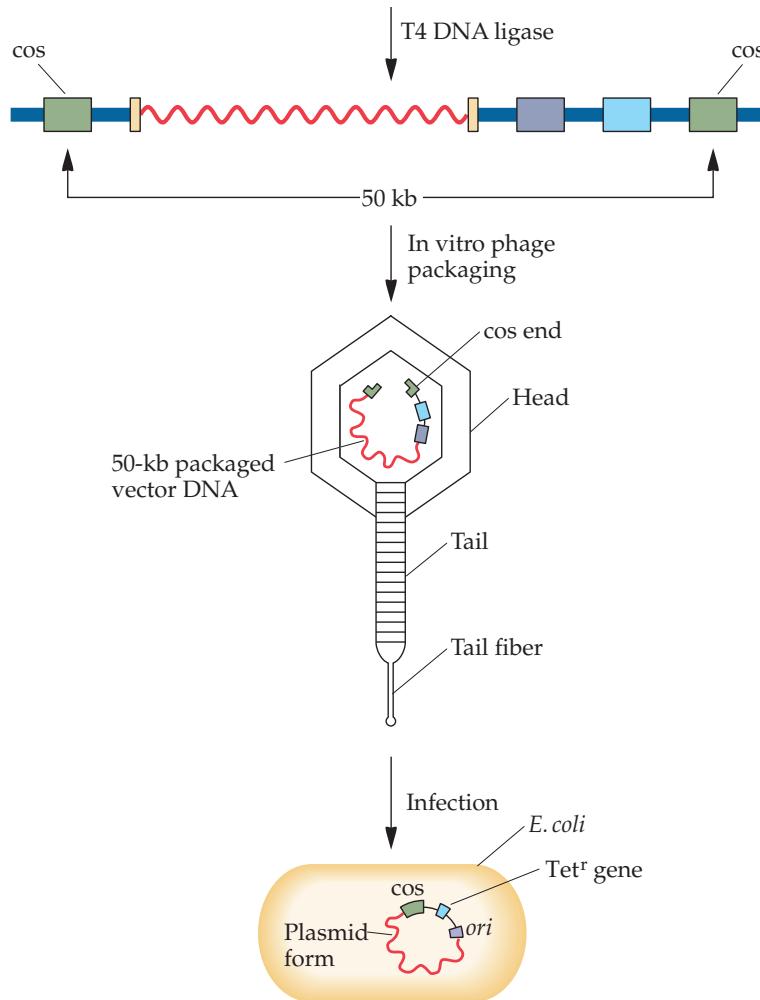
Bacterial colonies are grown on the plate and recombinants are screened by antibiotic selection

Advantages: clone fragments between 20–40 kb

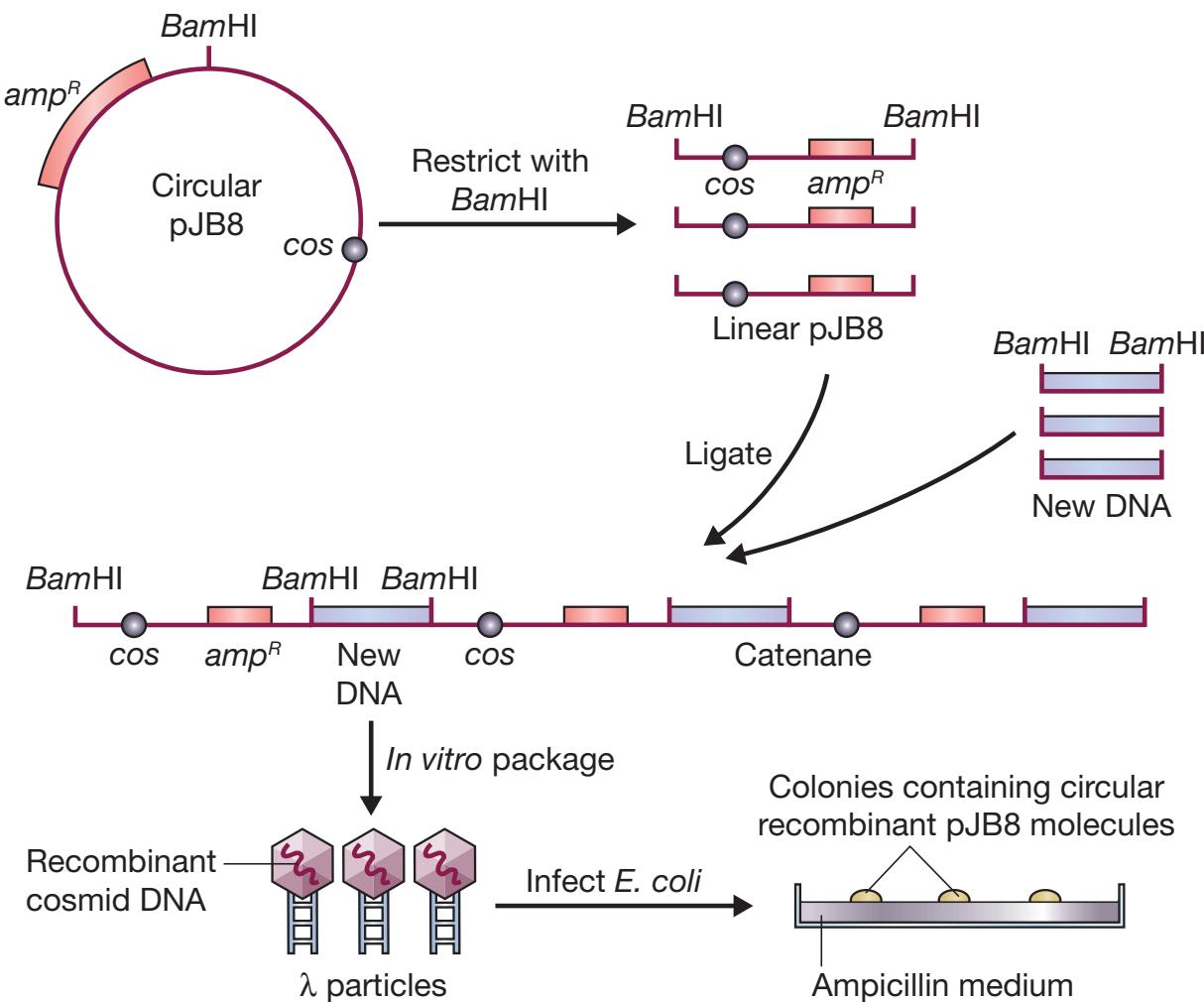
Cosmid vectors



Cosmid vectors



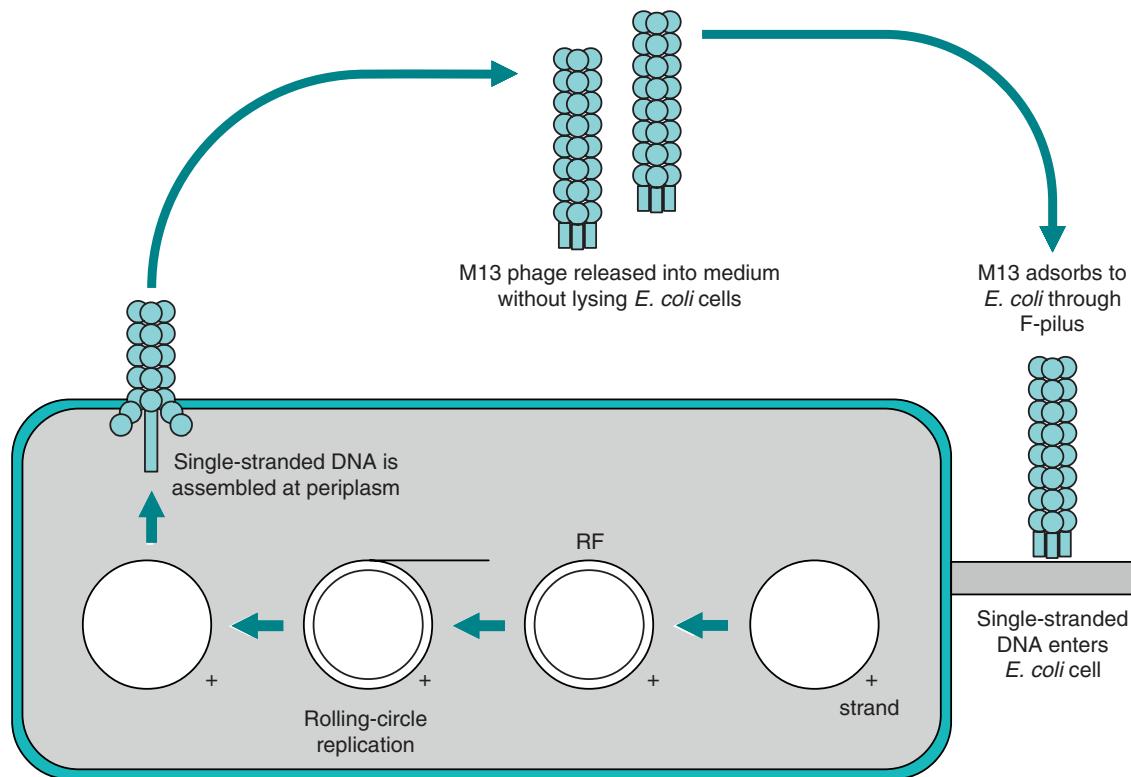
Cloning with a cosmid vector



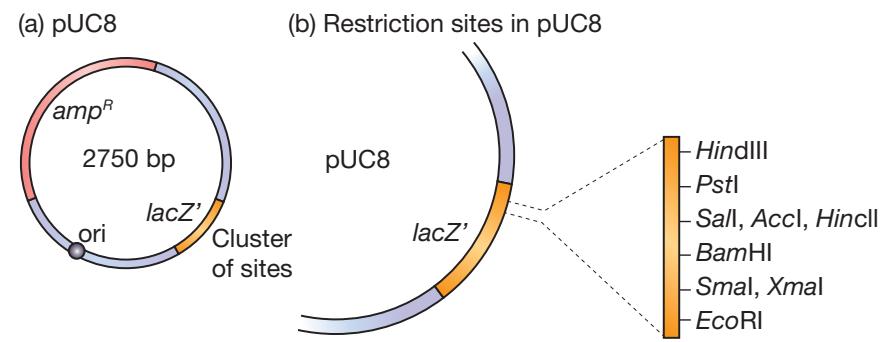
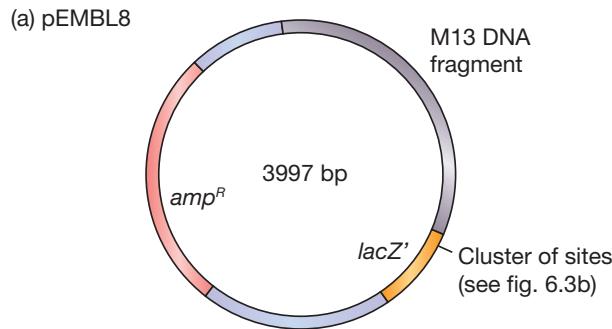
Phagemids

Vectors that have the combined properties of phage and plasmids

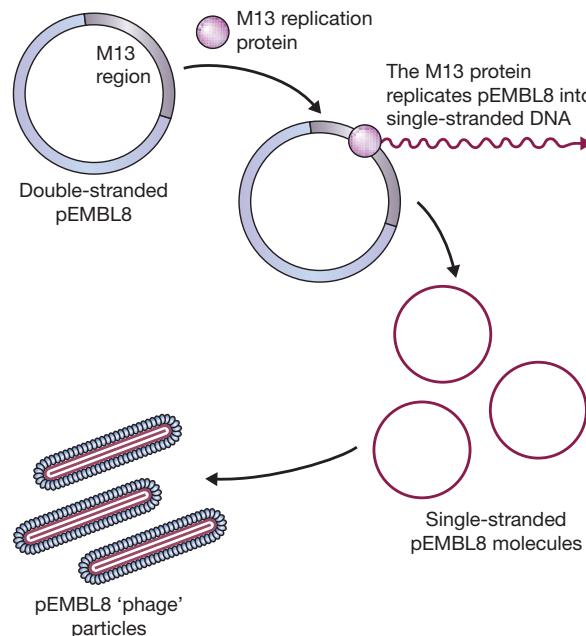
Phagemids are very similar to M13 and replicate in a similar fashion



Phagemids



(b) Conversion of pEMBL8 into single-stranded DNA



λ Zap system

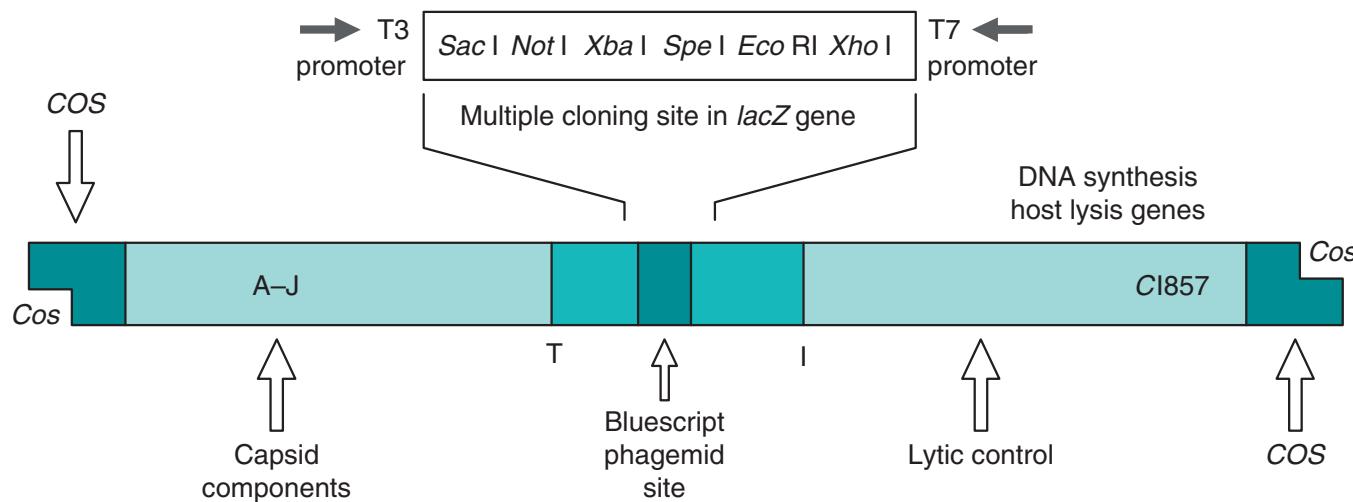


Figure 4.55 General map of λ Zap cloning vector, indicating important areas of the vector. The multiple cloning site is based on the *lacZ* gene, providing blue/white selection based on the β -galactosidase gene. In between the initiator (I) site and the terminator (T) site lie sequences encoding the phagemid Bluescript.

λ Zap system

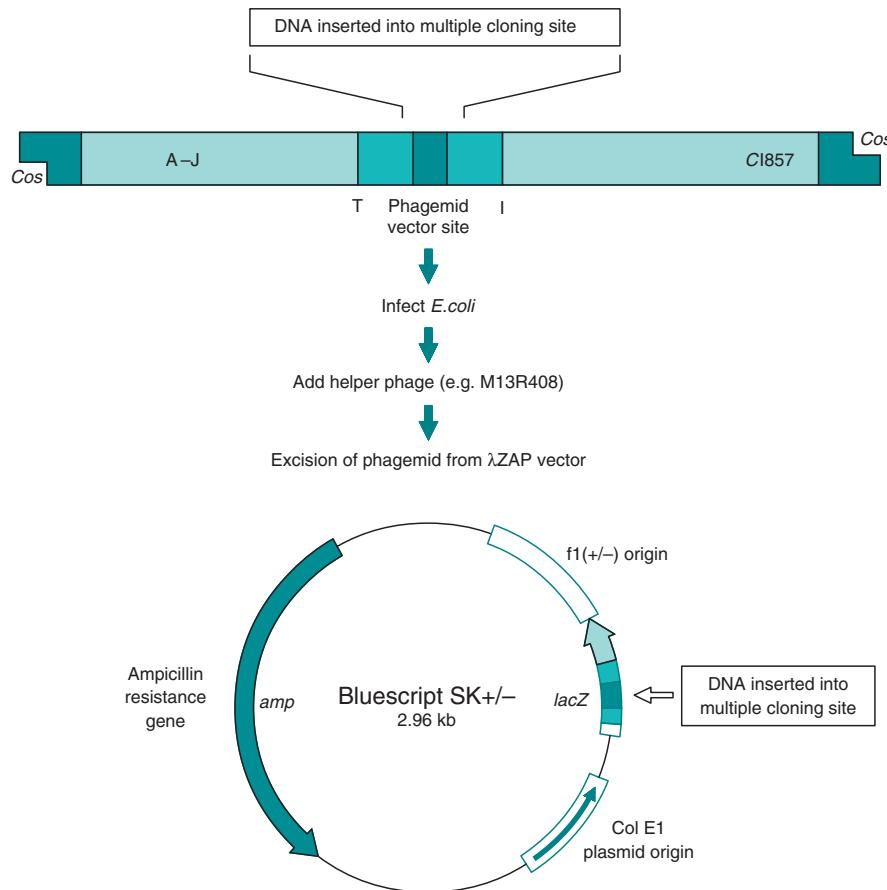


Figure 4.56 Single-stranded DNA rescue of phagemid from λ Zap. The single-stranded phagemid pBluescript SK may be excised from λ Zap by addition of helper phage. This provides the necessary proteins and factors for transcription between the I and T sites in the parent phage to produce the phagemid with the DNA cloned into the parent vector.

Expression vectors

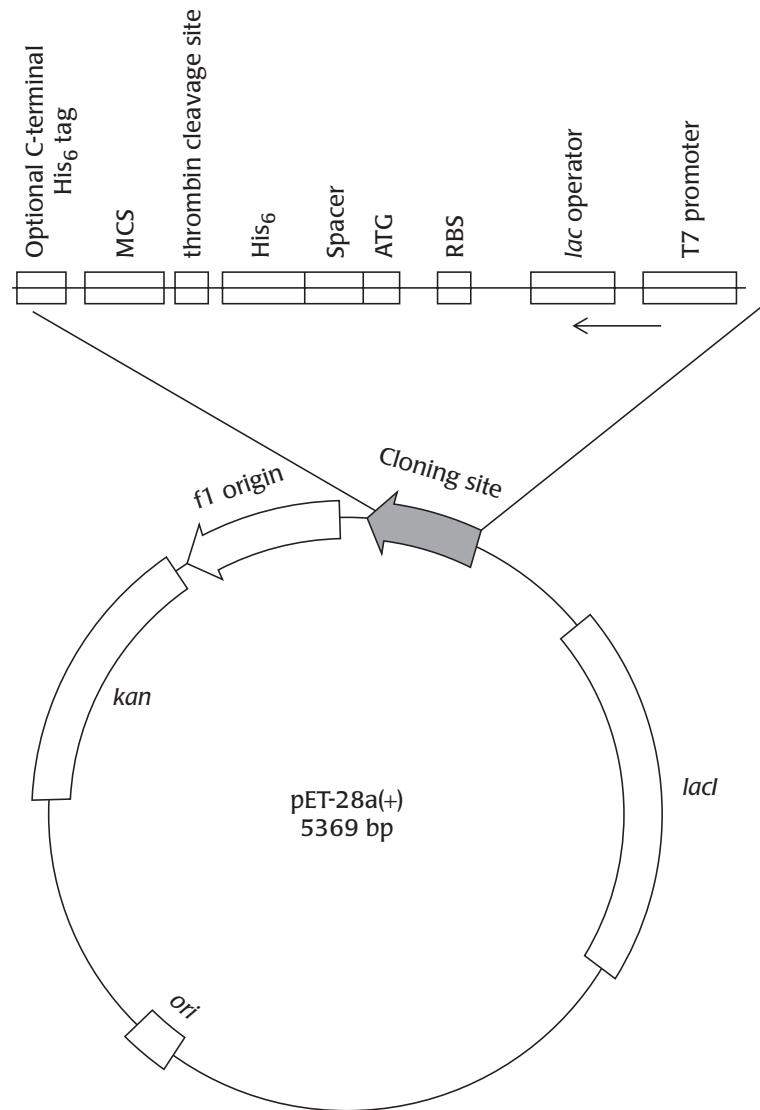
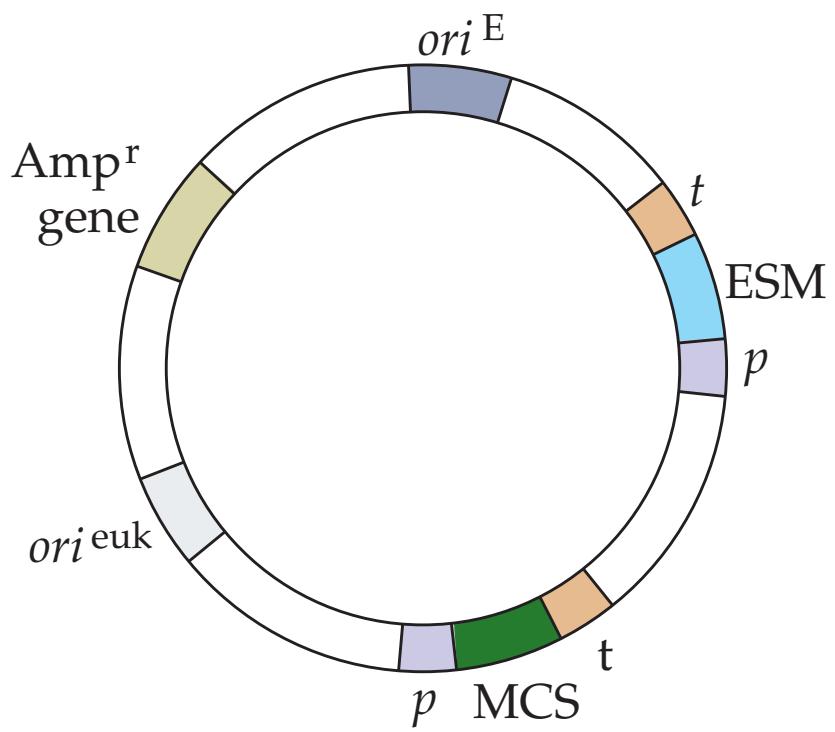
Allow high level protein expression in bacterial cells because they have a prokaryotic promoter site next to the MCS

Bacterial RNA polymerase can then bind to promoter and transcribe the insert's sequence which is then translated into protein

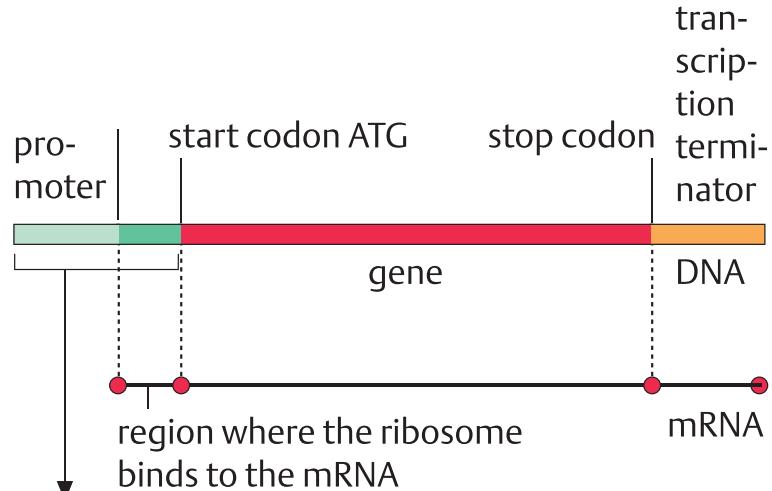
Protein is then purified using biochemical techniques

Sometimes bacteria ribosomes cannot translate eukaryote sequence or protein is not folded correctly since bacteria does not have organelles for processing.

Expression vectors



Expression vectors



a *E. coli*

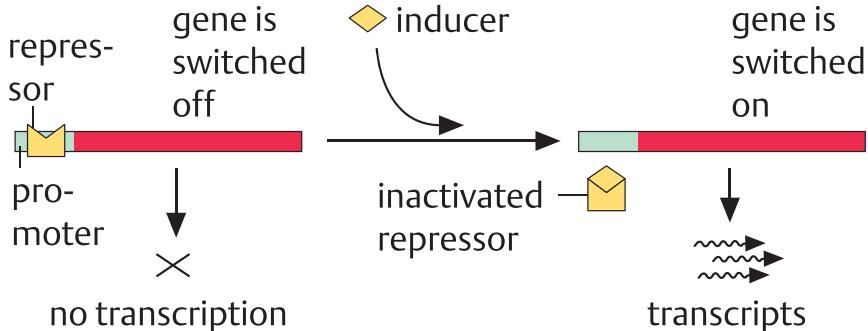
..... TTGACA TATAAT → gen
– 35-Box – 10-Box

b animals

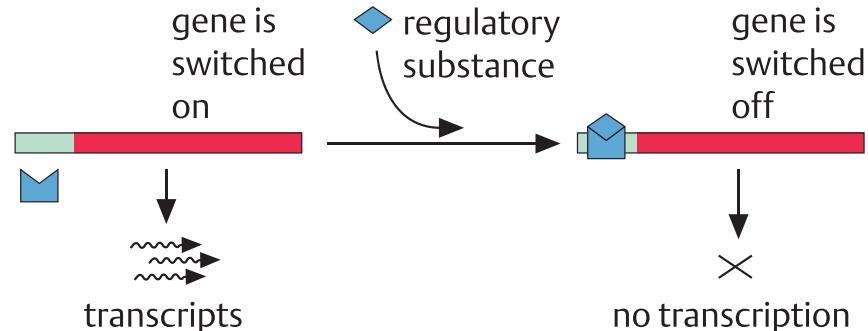
..... various TATATAT → gen
signal sequences – 25 box

Induction and repression

a inducible gene



b repressible gene

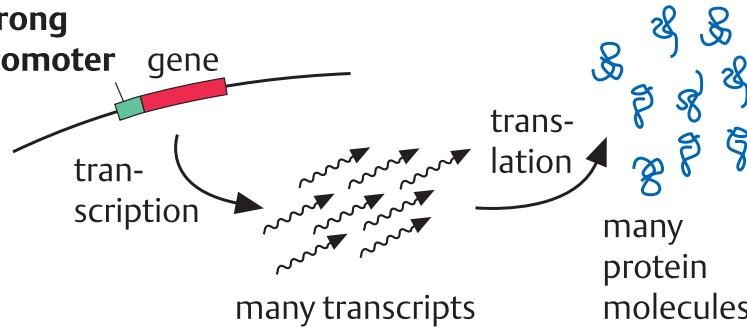


Inducible promoters (examples)

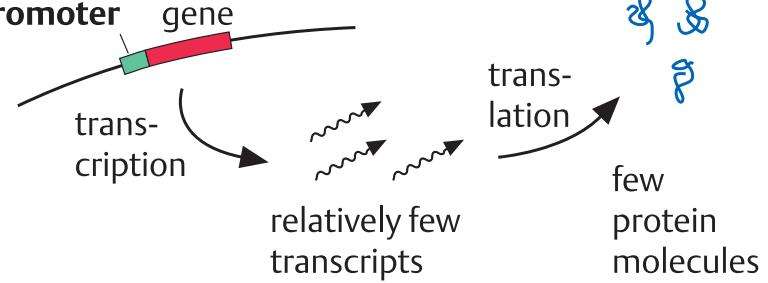
promoter	protein	inducer	host organism
<i>lacZ</i>	β-galactosidase	IPTG	<i>E. coli</i>
λP_L	–	temperature rise 30 – 42 °C	<i>E. coli</i>
<i>GAL10</i>	β-galactosidase	galactose	<i>S. cerevisiae</i>
<i>AOX</i>	alcohol oxidase	methanol	<i>Pichia pastoris</i>
<i>metallothionein</i>	metallothionein	Zn ²⁺	animal cells

Promoters

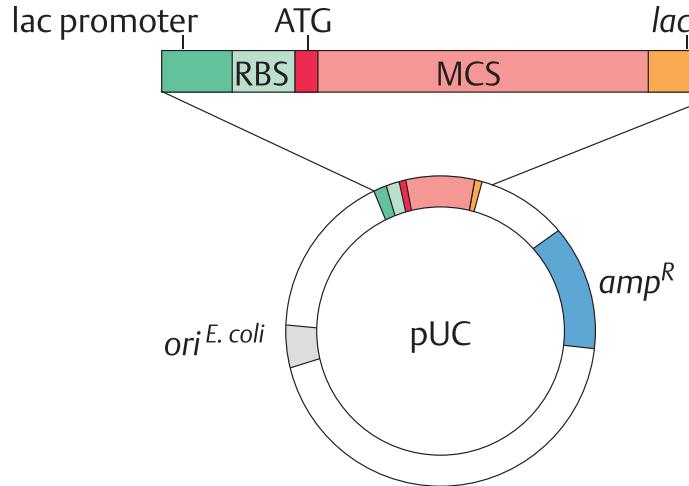
strong promoter



weak promoter



Expression cassette for *E. coli*



RBS

ribosome binding site

ATG

start codon

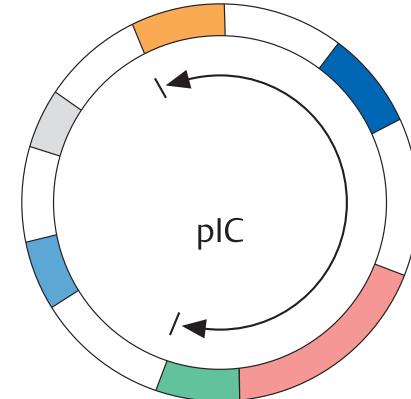
MCS
ori^{E. coli}

3' end of the alcohol oxidase gene
multiple cloning site with recognition
sequences for restriction enzymes

selection marker

origin of replication for *E. coli*

Expression cassette for *Pichia pastoris*



3' end of the alcohol oxidase gene

origin of replication for *E. coli*

selection marker amp^R for *E. coli*

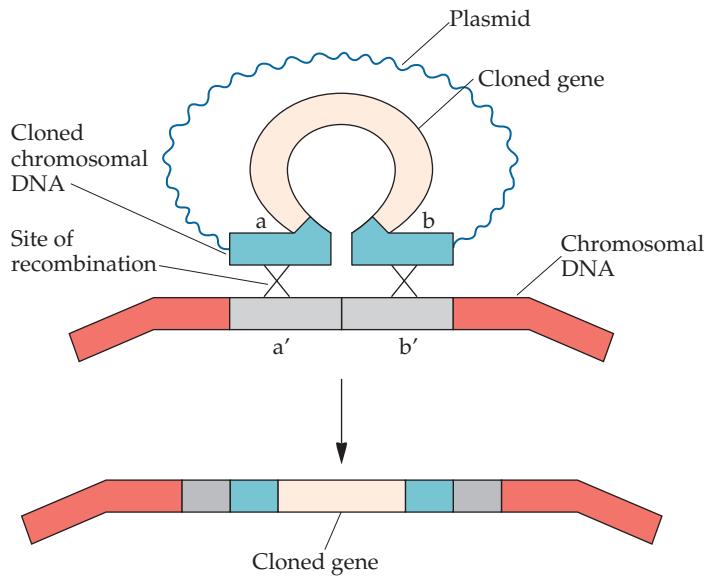
alcohol oxidase promoter

multiple cloning site with recognition sites
for restriction enzymes

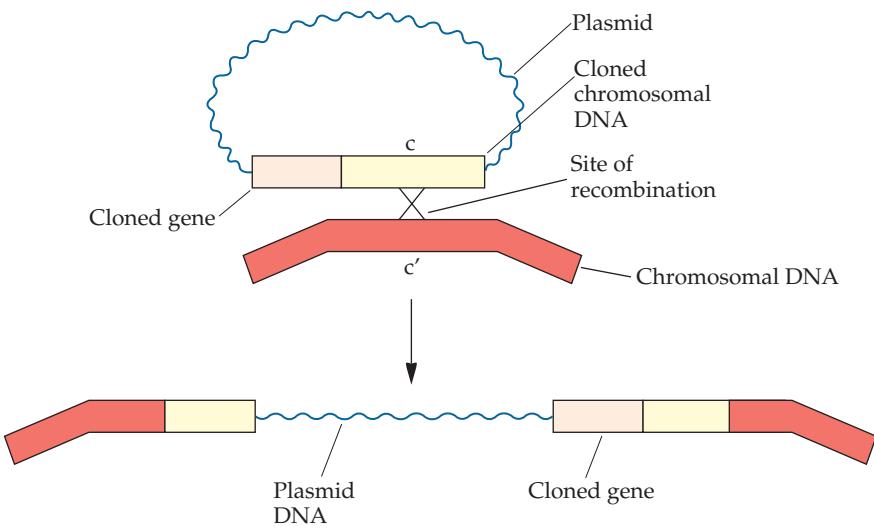
His4 auxotrophic marker for *Pichia pastoris*

|←→| vector integrates into *Pichia pastoris*
chromosome

Integration of a cloned gene into a chromosomal site



Integration of the
cloned gene only



Integration of the
whole plasmid

Bacterial artificial chromosomes (BAC)

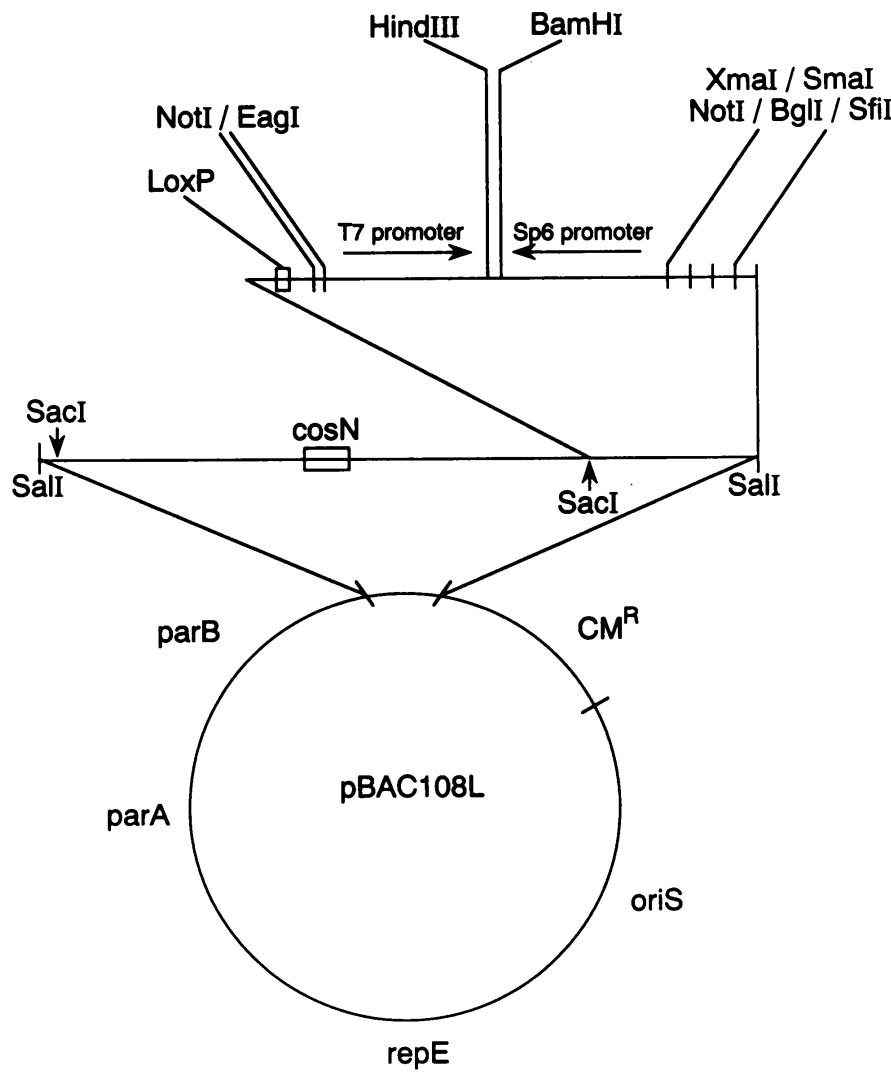
Large low copy plasmids

Contain genes that encode the F factor (unit of genes controlling bacterial replication)

Can accept large sizes of DNA inserts ranging from 100–300 kb

Were used during the human genome project to clone and sequence large pieces of chromosomes

Bacterial artificial chromosomes (BAC)



Yeast artificial chromosomes (YAC)

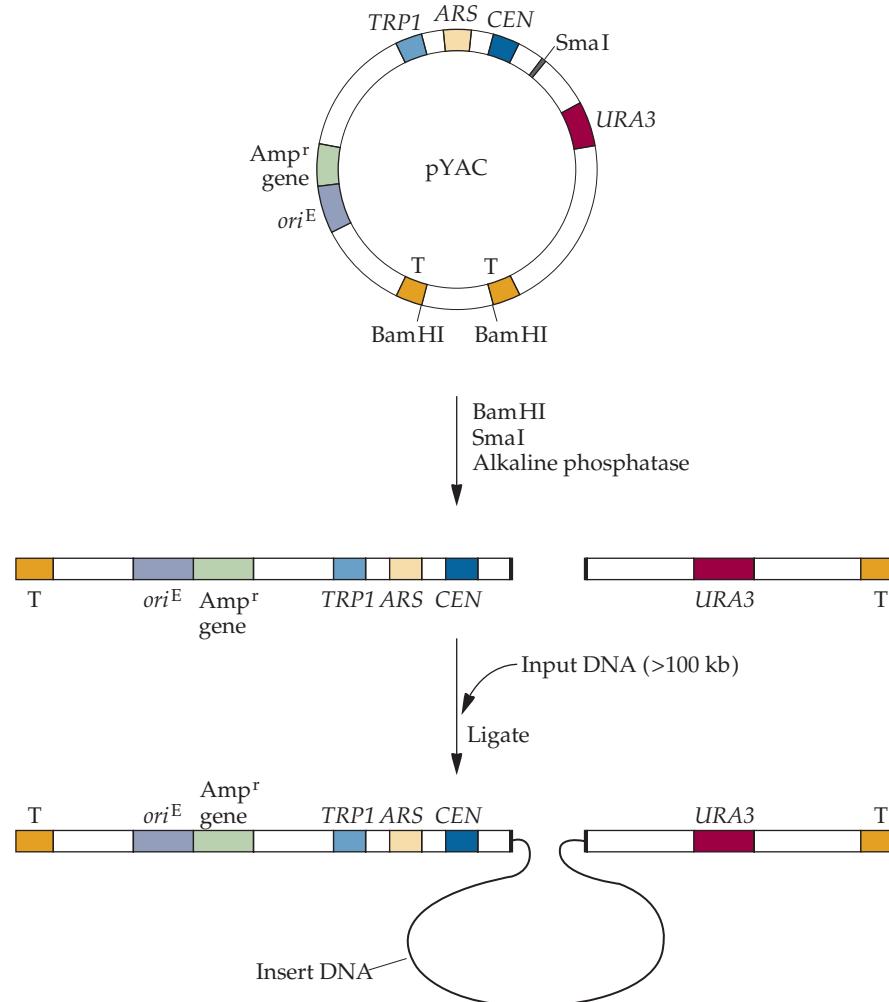
Small version of eukaryotic chromosome containing:

1. Origin of rep. (autonomously replicating sequence)
2. Two telomeres
3. Selectable markers
4. Centromere that allows replication of YAC and segregation of daughter cells

Best for cloning very large DNA inserts from 200 kb to 2 megabases

Were used for human genome project

Yeast artificial chromosomes (YAC)



Ti vector

Naturally occurring plasmids isolated from the bacterium that is a soil plant pathogen *Rhizobium radiobacter* causing disease in plants

When the bacteria infects plant cells, the Ti DNA from the Ti plasmid inserts into the host chromosome

T DNA codes for auxin hormone that weakens plant cell wall and infected plants divided and enlarge to form a tumor (gall)

Scientists use Ti vectors to deliver genes to plants by removing toxic gene for auxin

3.3 How Do You Identify and Clone a Gene of Interest?

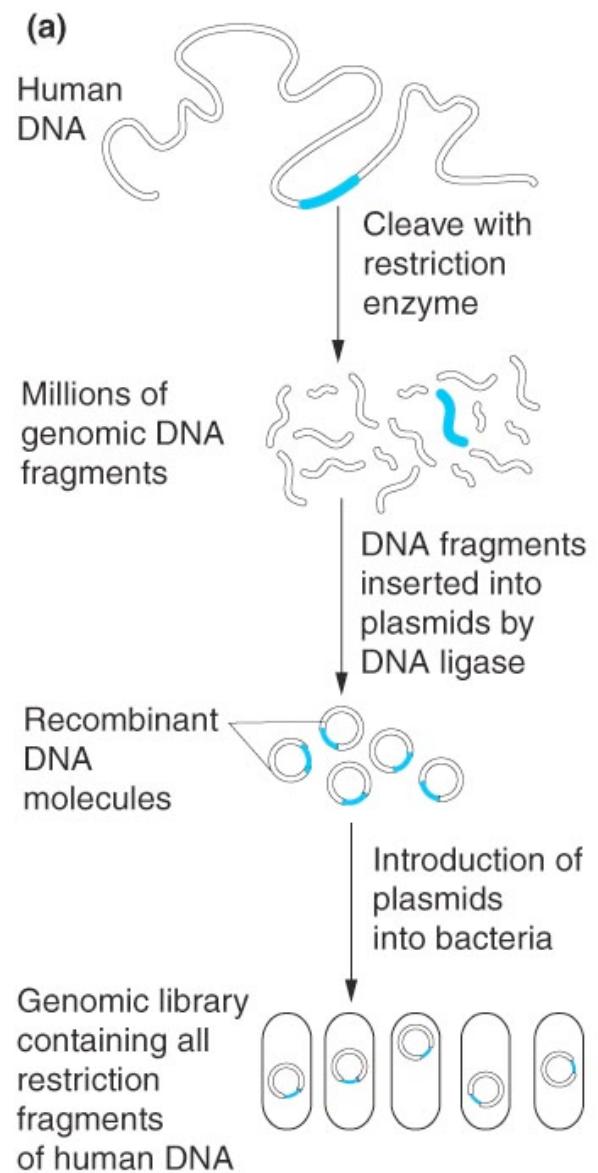
DNA Libraries are collections of cloned DNA fragments from a particular organism contained within bacteria or viruses as the host

Screened to pick out different genes of interest

Two Types of Libraries:

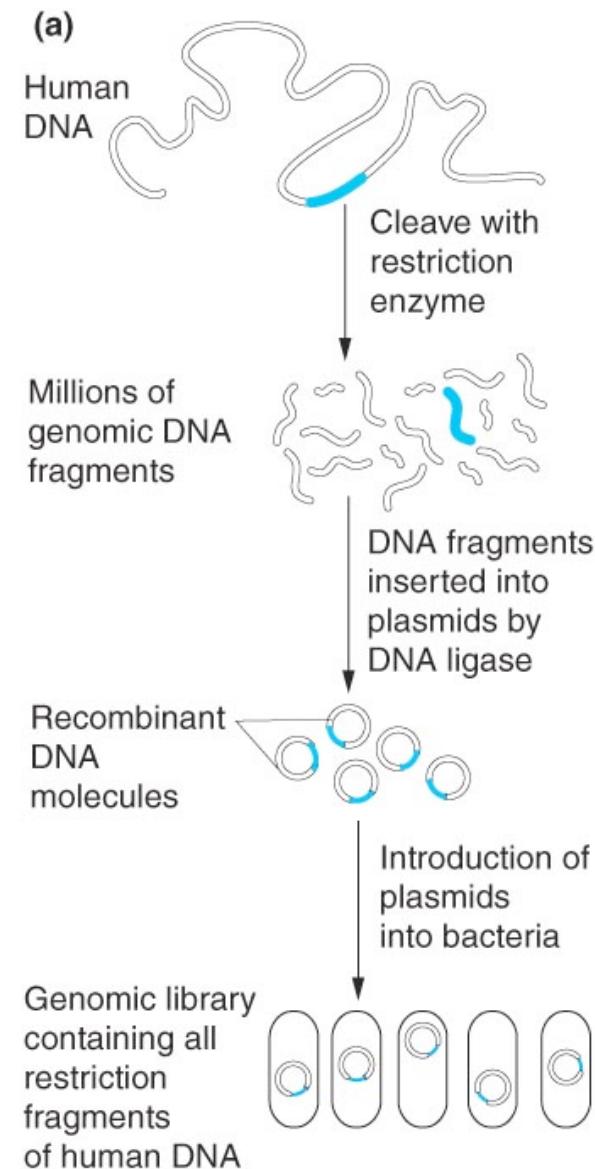
- 1. Genomic DNA libraries**
- 2. Complementary DNA libraries (cDNA libraries)**

Genomic DNA Library



Genomic DNA Library

$$N = \frac{\ln(1 - p)}{\ln\left(1 - \frac{a}{b}\right)}$$



Genomic DNA Library

Chromosomal DNA from the tissue of interest is isolated and digested with a restriction enzyme which produces many fragments that include the entire genome

Vector is digested with same enzyme

DNA ligase is used to ligate genomic DNA fragments and vector DNA

Recombinant vectors are used to transform bacteria and theoretically each bacteria will contain a recombinant plasmid

Disadvantages Genomic DNA Library

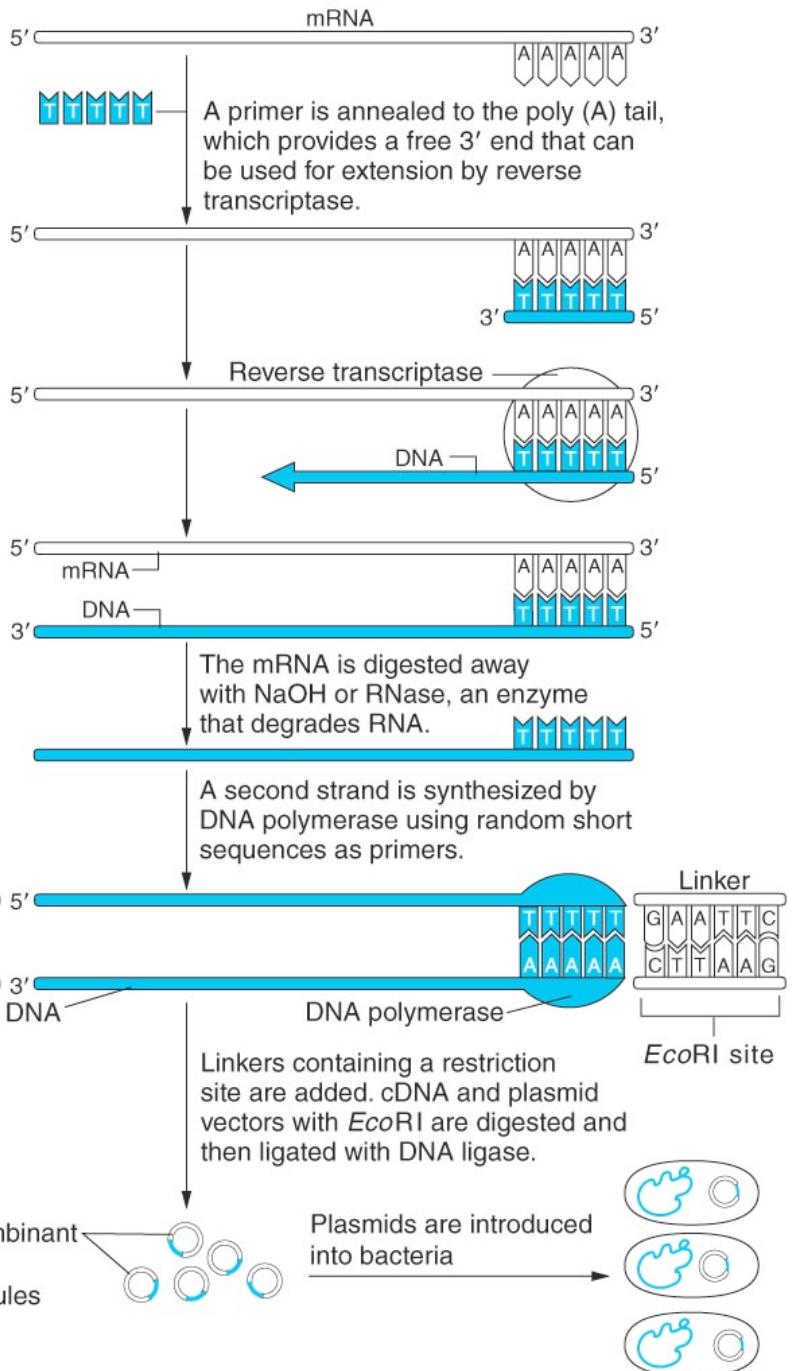
Introns are cloned in addition to exons (Majority of genomic DNA is introns in eukaryotes so majority of the library will contain non-coding pieces of DNA)

Many organisms have very large genome, so searching for gene of interest is difficult

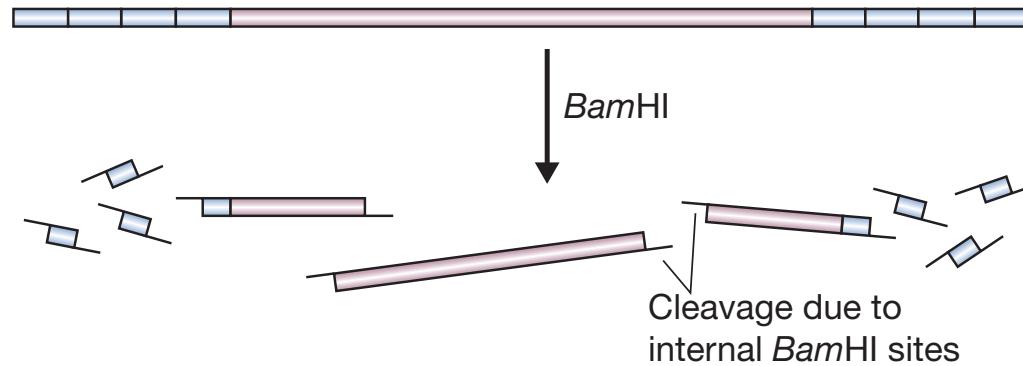
Time consuming!

cDNA Library

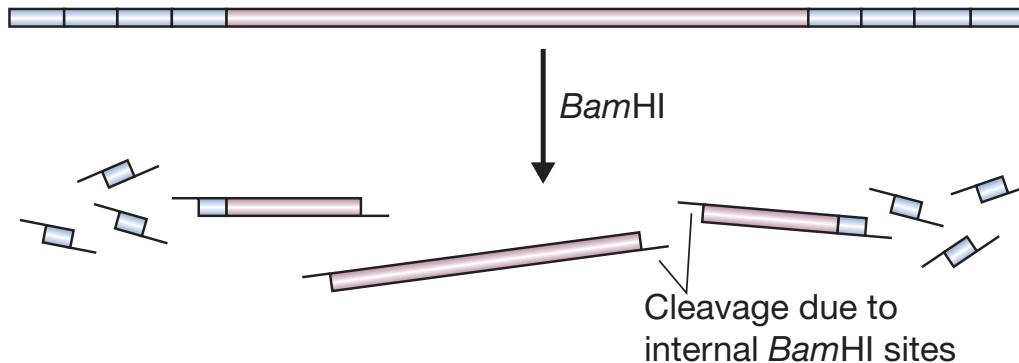
(b)



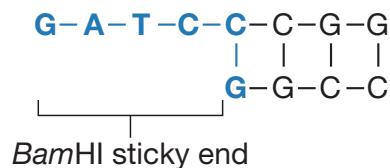
Problems with linkers



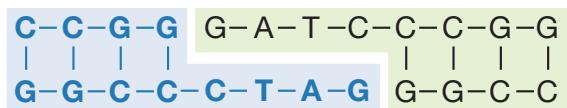
Problems with linkers



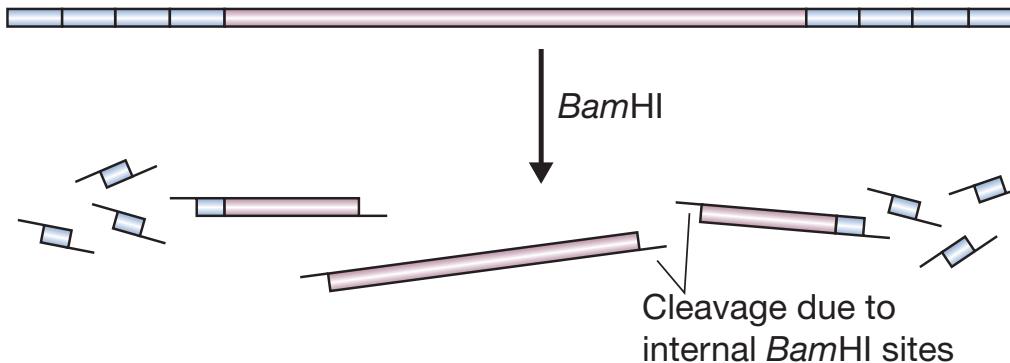
(a) A typical adaptor



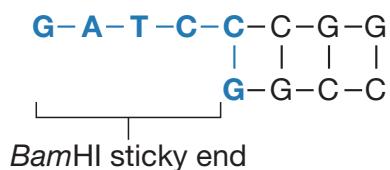
(b) Adaptors could ligate to one another



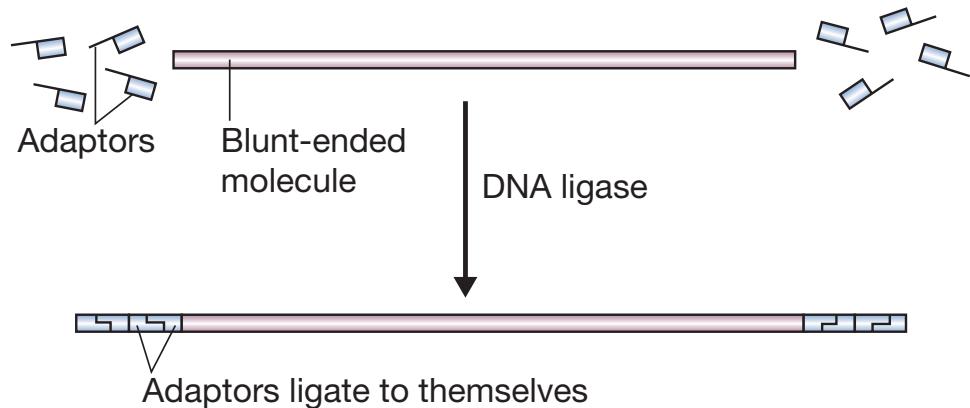
Problems with linkers



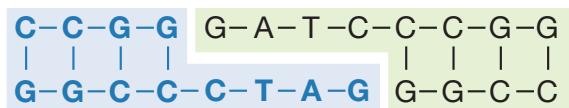
(a) A typical adaptor



(c) The new DNA molecule is still blunt-ended

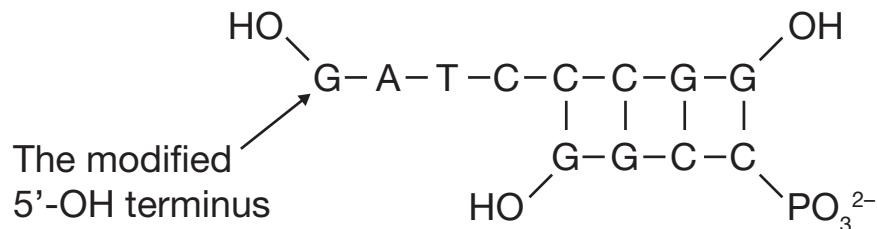


(b) Adaptors could ligate to one another

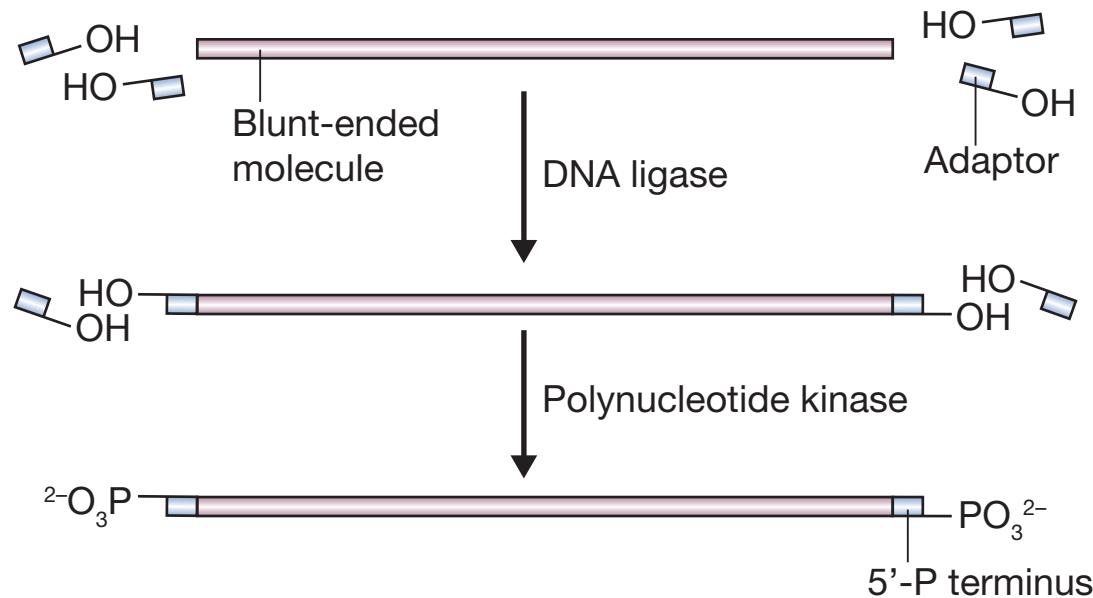


Problems with linkers

(a) The precise structure of an adaptor



(b) Ligation using adaptors



cDNA Library

mRNA from tissue of interest is isolated

The enzyme **reverse transcriptase** catalyzes synthesis of complementary single stranded DNA from mRNA called **complementary DNA** (cDNA) because it is an exact copy of the mRNA

mRNA is degraded either with an enzyme or alkline solution

cDNA Library

DNA Polymerase is used to synthesize second strand of DNA to create double stranded cDNA

Short linker double stranded DNA sequences which contain restriction enzyme recognition sites are added to the ends of the cDNA

cDNA and vector are with the same **restriction enzyme**, and then ligated to create recombinant vectors

Advantage of cDNA Library over genomic libraries

Collection of actively expressed genes in the cells or tissues from which the mRNA was isolated

Introns are NOT cloned

Can be created and screened to isolate genes that are primarily expressed only under certain conditions in a tissue

Disadvantage

Can be difficult to make the cDNA library if a source tissue with an abundant amount of mRNA for the gene is not available

Library screening to identify the gene of interest

Colony hybridization procedure:

1. Bacterial colonies containing recombinant DNA are grown on an agar plate
2. Nylon or nitrocellulose filter is placed over the plate and some of the bacterial colonies stick to the filter at the exact location they were on the plate
3. Filter treated with alkaline solution to lyse the cells and denature the DNA

Library screening to identify the gene of interest

Colony hybridization procedure:

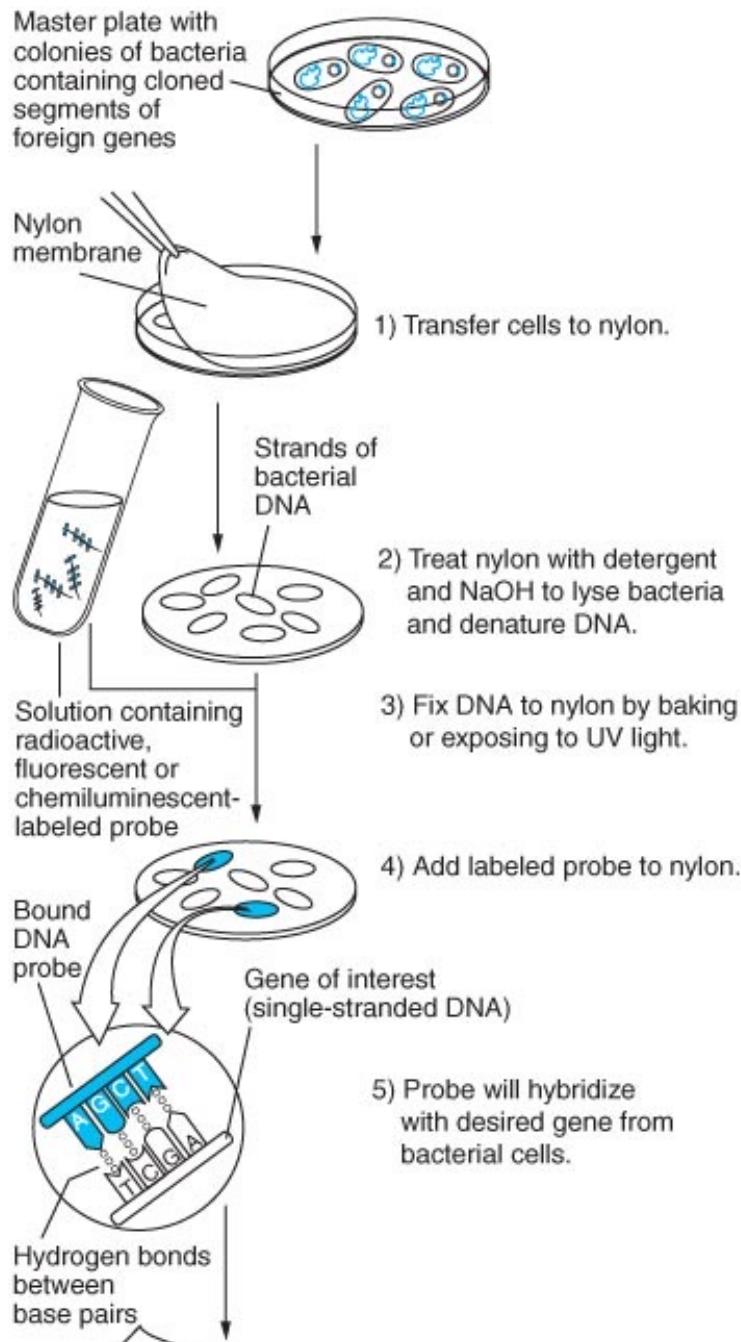
4. Denatured DNA binds to filter as single-stranded DNA
5. Filter is incubated with a **probe** (DNA fragment that is complementary to the gene of interest) that is tagged with a radioactive nucleotide or fluorescent dye
6. Probe binds by hydrogen bonding to complementary sequences on the filter = hybridization

Library screening to identify the gene of interest

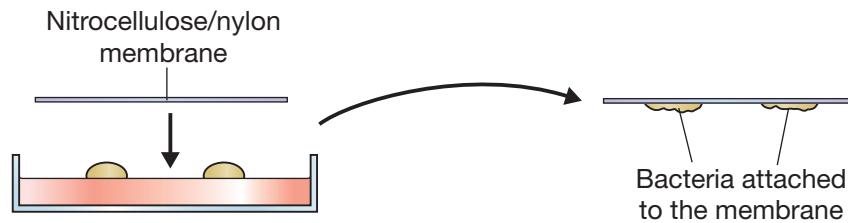
Colony hybridization procedure:

7. Filter is washed to remove excess unbound probe
8. Filter is exposed to film – autoradiography, and developed to create a permanent record of the colony hybridization
9. Film is then compared to the original agar plate to identify which colonies contained recombinant plasmid with the gene of interest

Colony hybridization



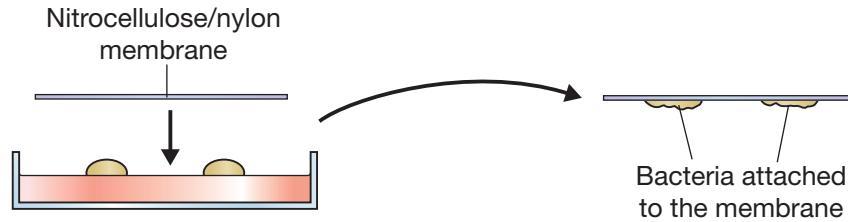
(a) Transfer colonies to nitrocellulose or nylon



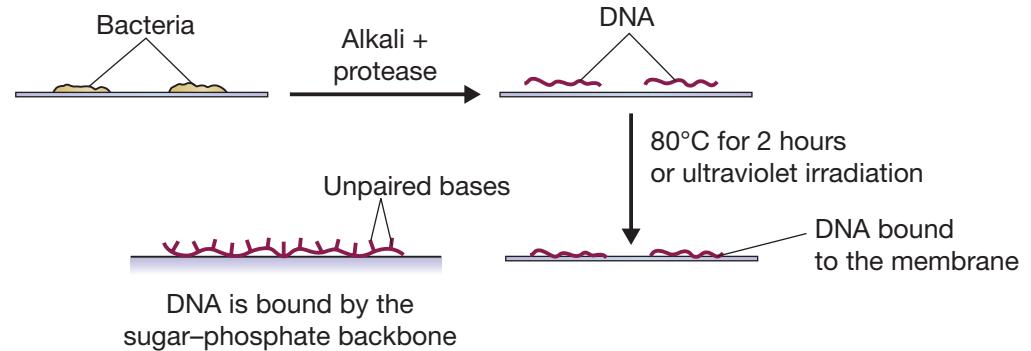
Colony Hybridization

Colony Hybridization

(a) Transfer colonies to nitrocellulose or nylon

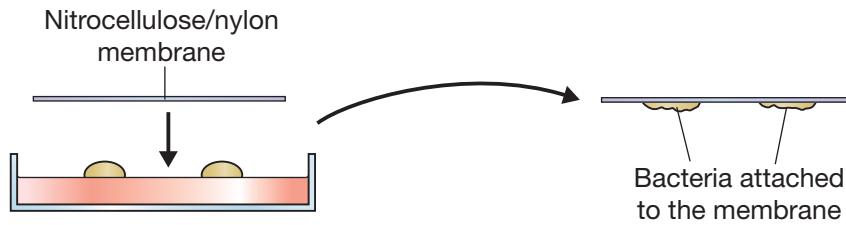


(b) Degrade cells, purify DNA

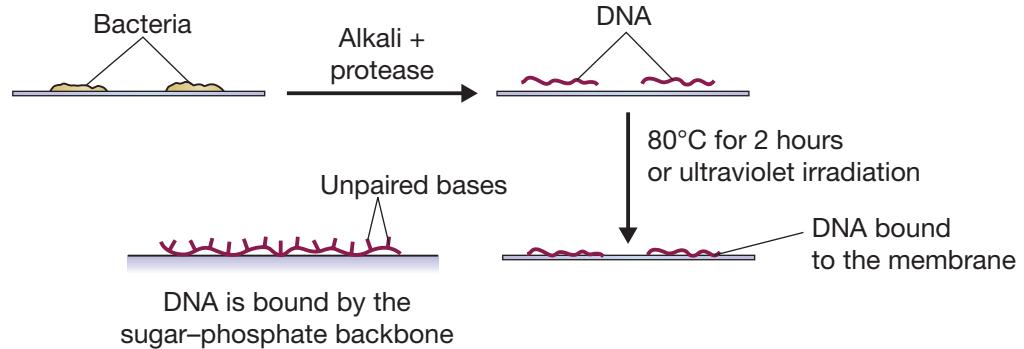


Colony Hybridization

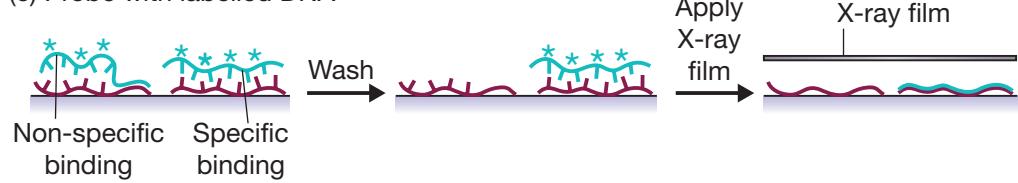
(a) Transfer colonies to nitrocellulose or nylon



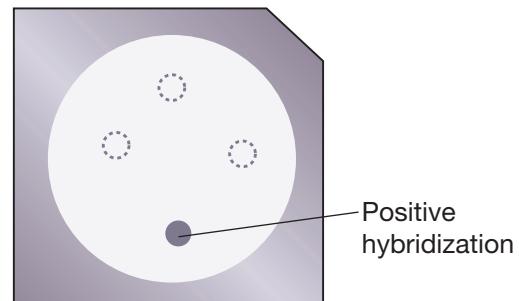
(b) Degrade cells, purify DNA



(c) Probe with labelled DNA



(d) The resulting autoradiograph



Library screening to identify the gene of interest

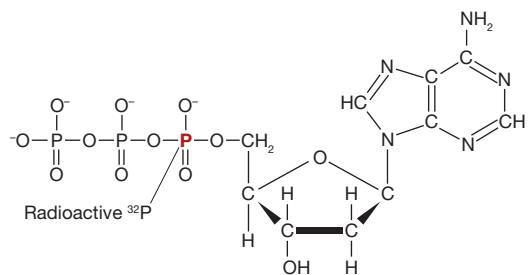
Type of probe used depends on what is already known about the gene of interest. For example, mouse or rat probe is used to screen a human library

Library screening rarely results in the cloning of the full-length gene

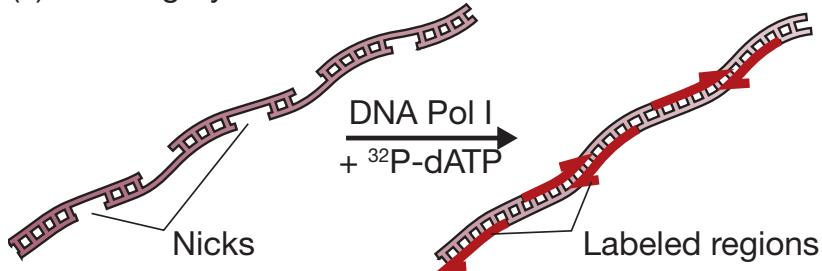
Usually small pieces of the gene are obtained; the pieces are sequenced and scientists look for overlapping sequences

Start and stop codons to are looked for to know when the full length of the gene is obtained

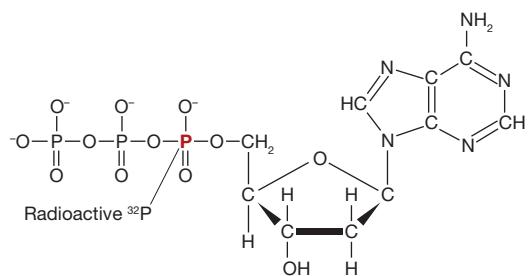
Methods for making radiolabeled probes



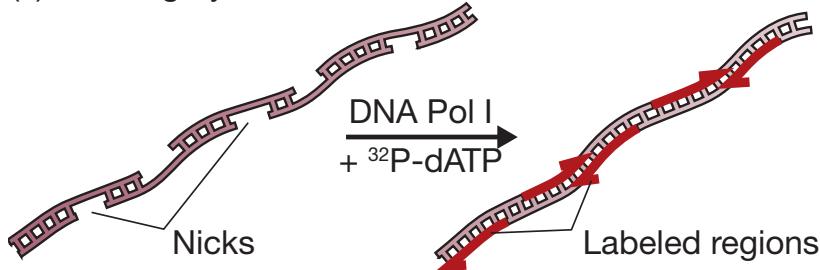
(a) Labeling by nick translation



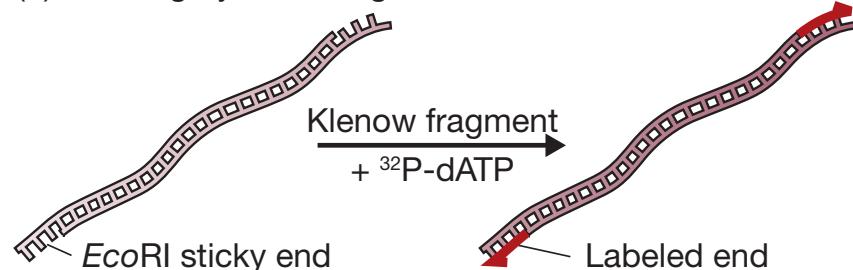
Methods for making radiolabeled probes



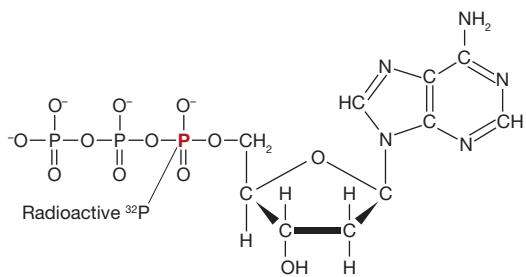
(a) Labeling by nick translation



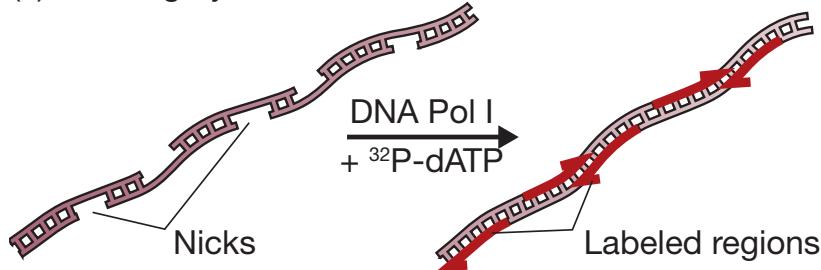
(b) Labeling by end-filling



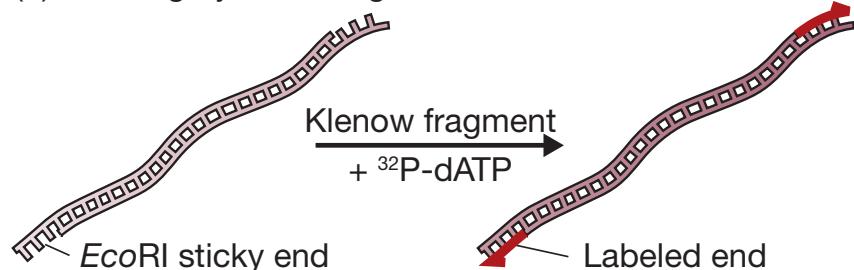
Methods for making radiolabeled probes



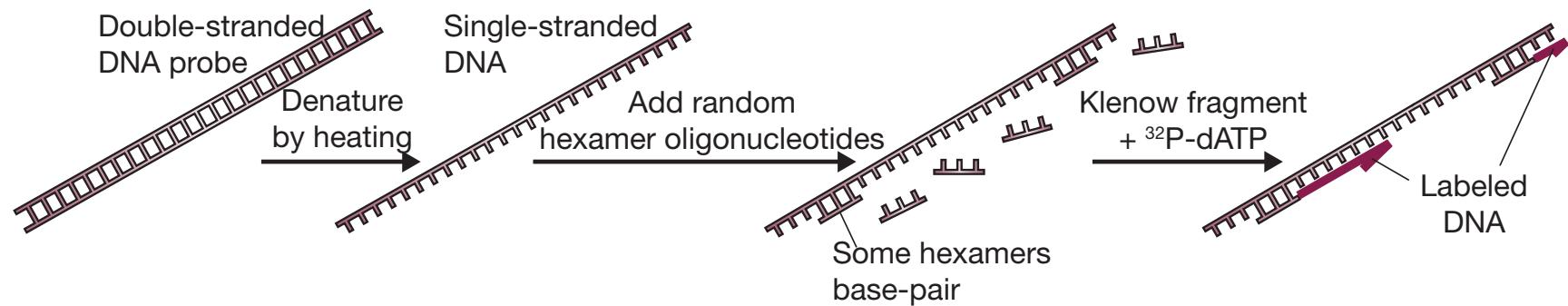
(a) Labeling by nick translation



(b) Labeling by end-filling

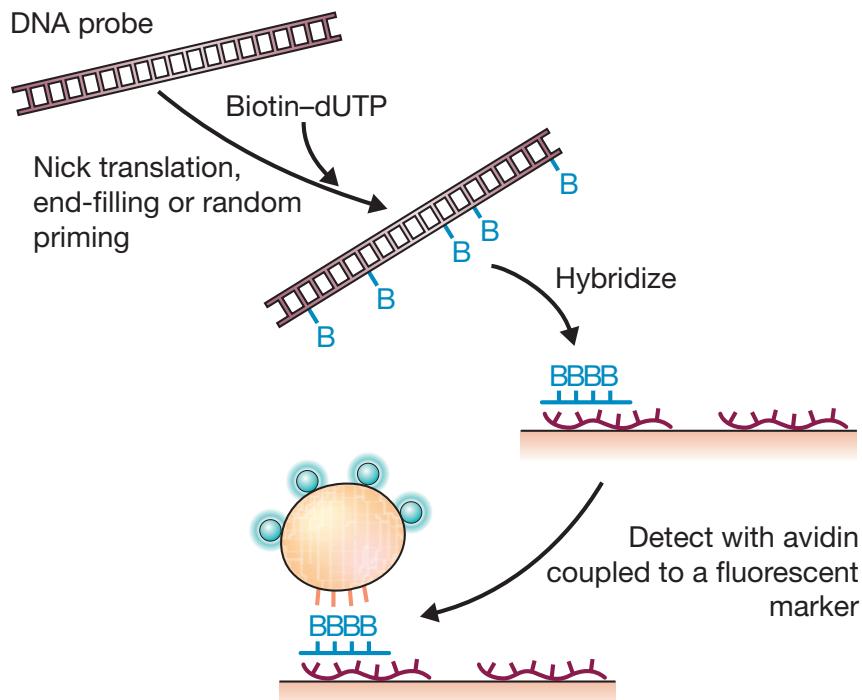


(c) Labeling by random priming



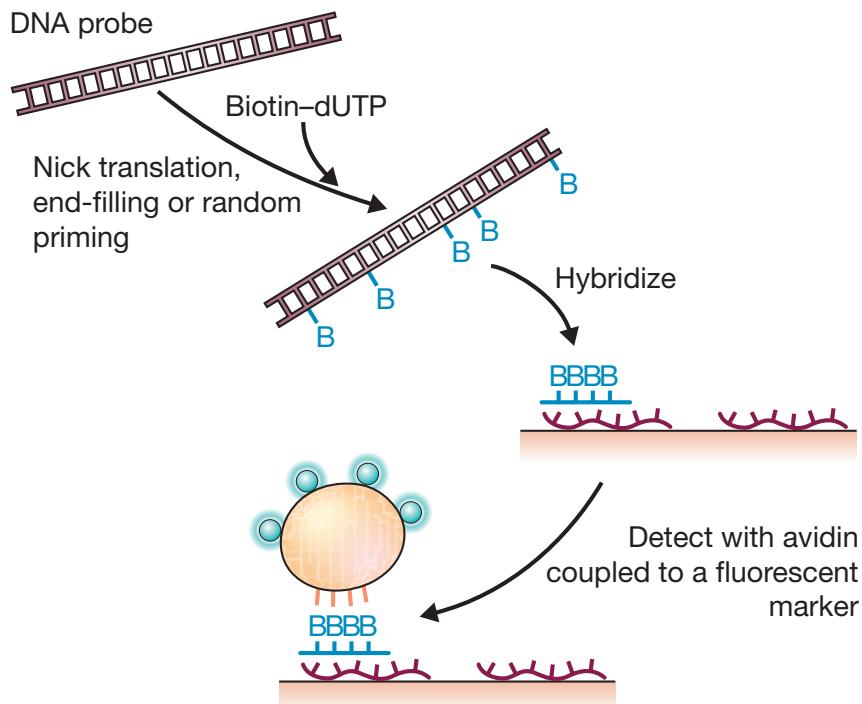
Methods for making Non-radiolabeled probes

(a) Labelling with a biotinylated nucleotide

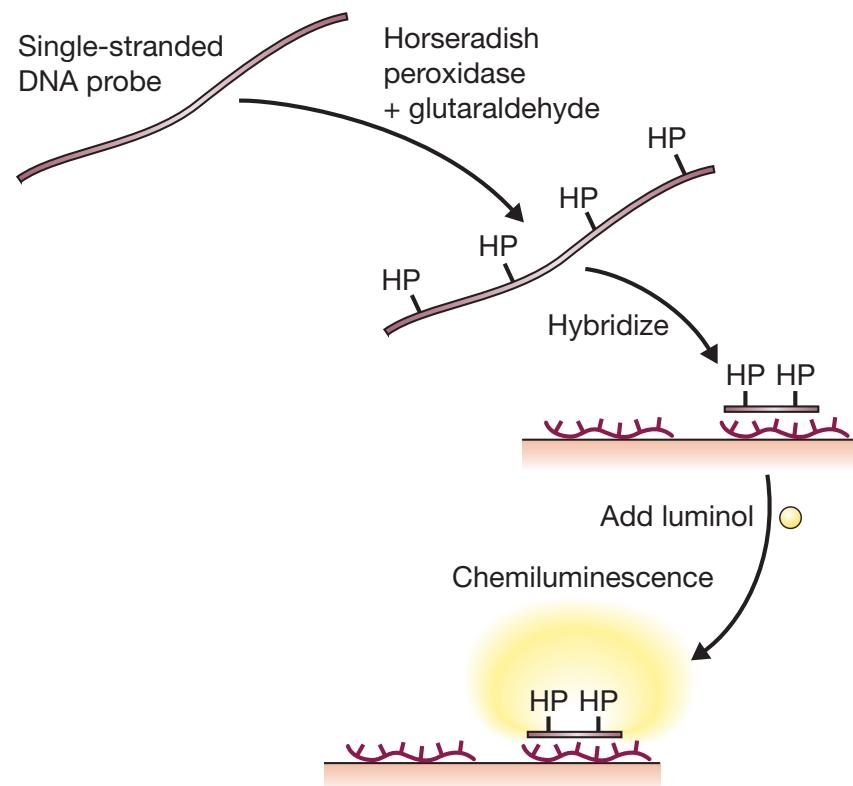


Methods for making Non-radiolabeled probes

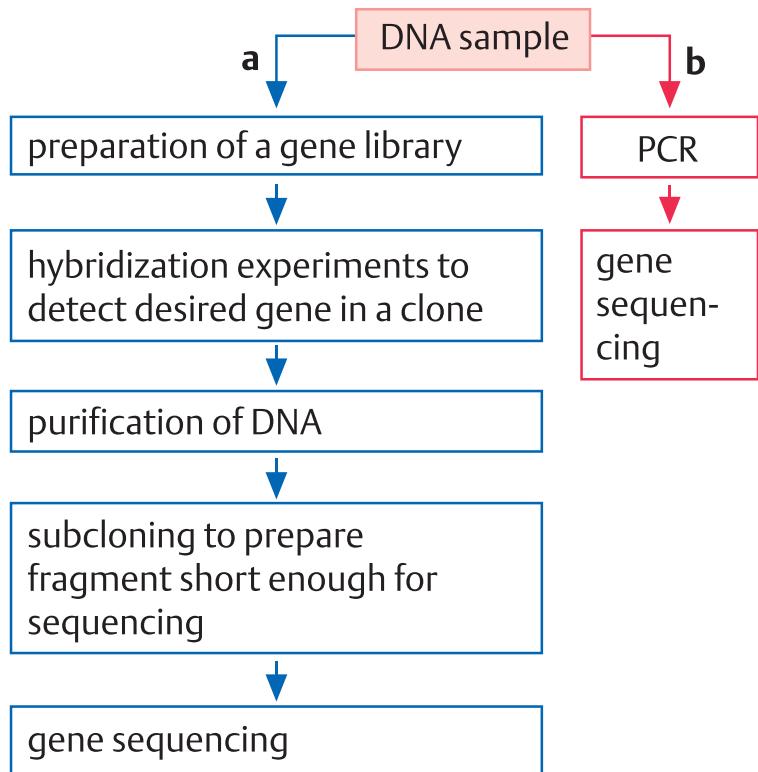
(a) Labelling with a biotinylated nucleotide



(b) Labelling with horseradish peroxidase



Cloning of genes



Detection methods

Southern blot	detection of DNA	hybridization with labeled DNA or RNA probe
Northern blot	detection of mRNA	hybridization with labeled DNA or RNA probe
Western blot	detection of proteins	immunological detection by labeled antibodies
reporter groups	detection of regulatory elements	expression of genes coding for reporter proteins