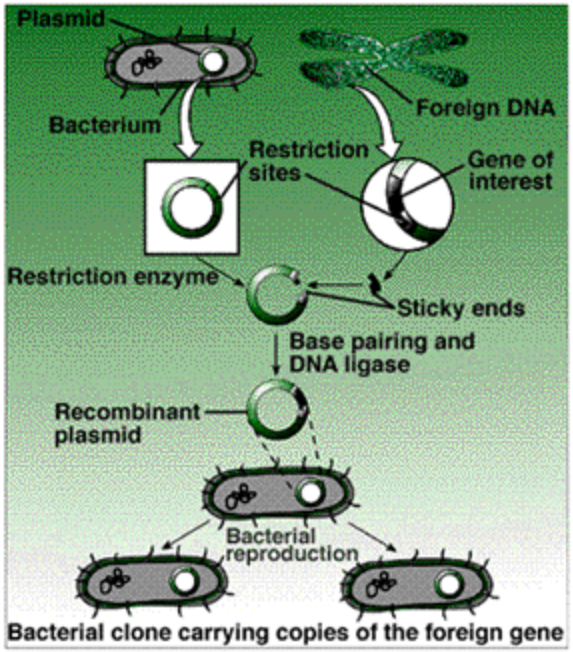
Recombinant DNA Technology

Key Lab of Molecular Medicine, Ministry of Education

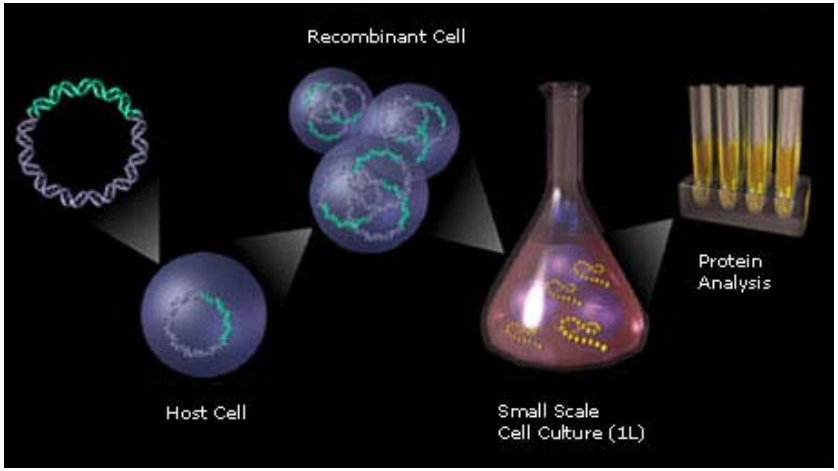
Duan Ma, Prof, MD, PhD



Recombinant DNA

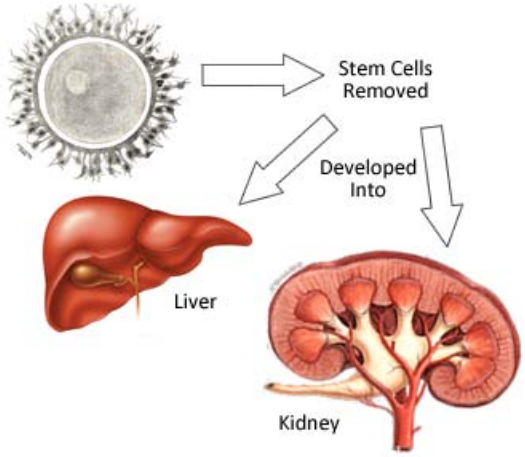
重组ＤＮＡ

- **Recombinant DNA** is a form of synthetic DNA that is engineered through the combination or insertion of one or more DNA strands.
- Through the use of recombinant DNA, genes that are identified as important can be amplified and isolated for use in other species or applications, where there may be some form of genetic illness.



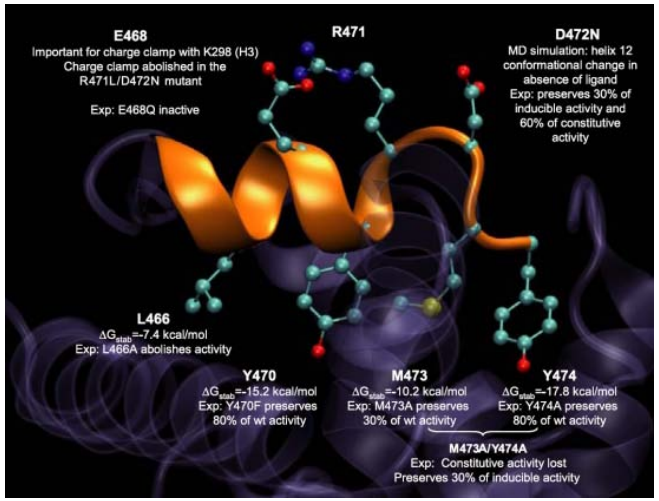
Some Definition

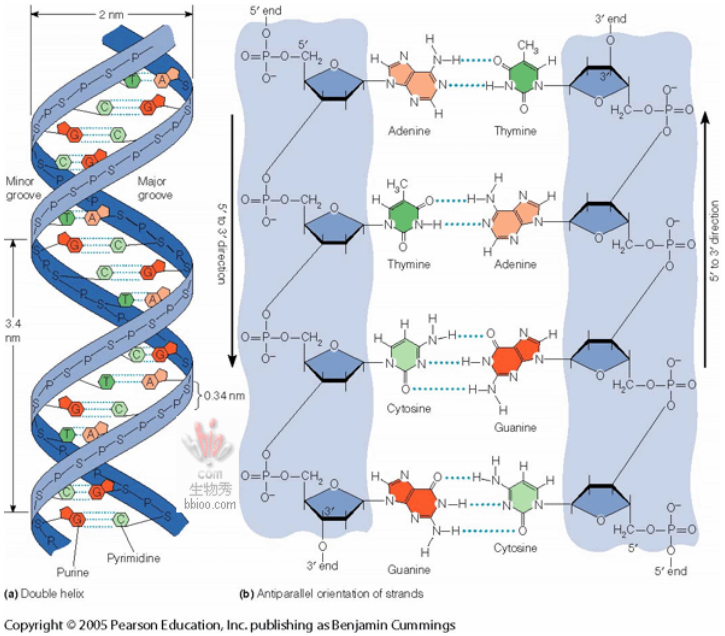
- **Clone**: A group of organisms or cells produced **<u>asexually</u>** from one ancestor or stock, to which they are genetically identical.
- **Cloning**: Propagation of a DNA sequence by incorporating it into a **<u>hybrid construct</u>** that can be replicated in a host cell.
- **Reproductive cloning:** Process of making a genetically identical copy of an organism.
- **Therapeutic cloning:** Process of making multiple copies of a cell to treat a disease.



What is Cloned DNA Used For?

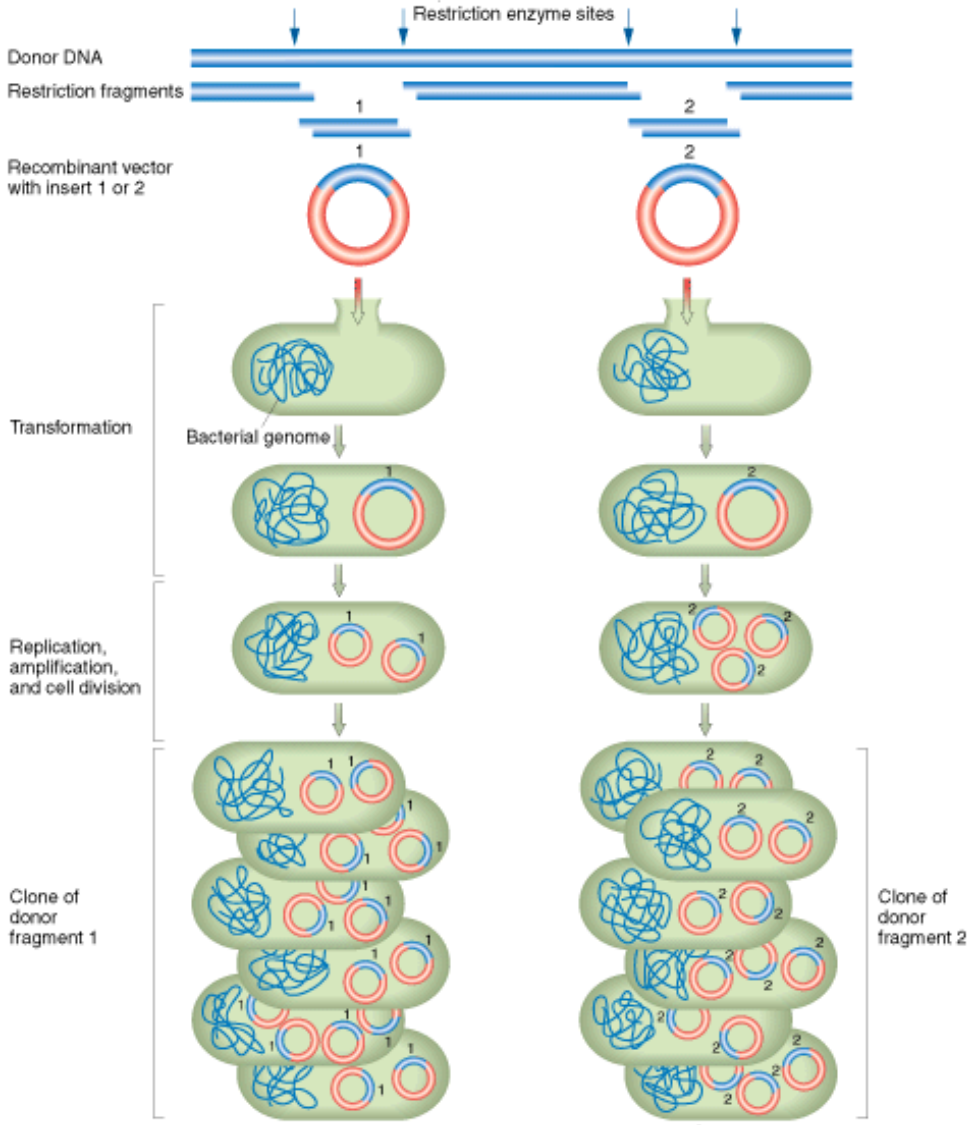
- Work out the **function of a gene**.
- Investigate a **gene's characteristics** (size, expression, tissue distribution).
- Look at **how mutations** may affect a gene's function.
- **Make large concentrations of the protein** coded for by the gene.



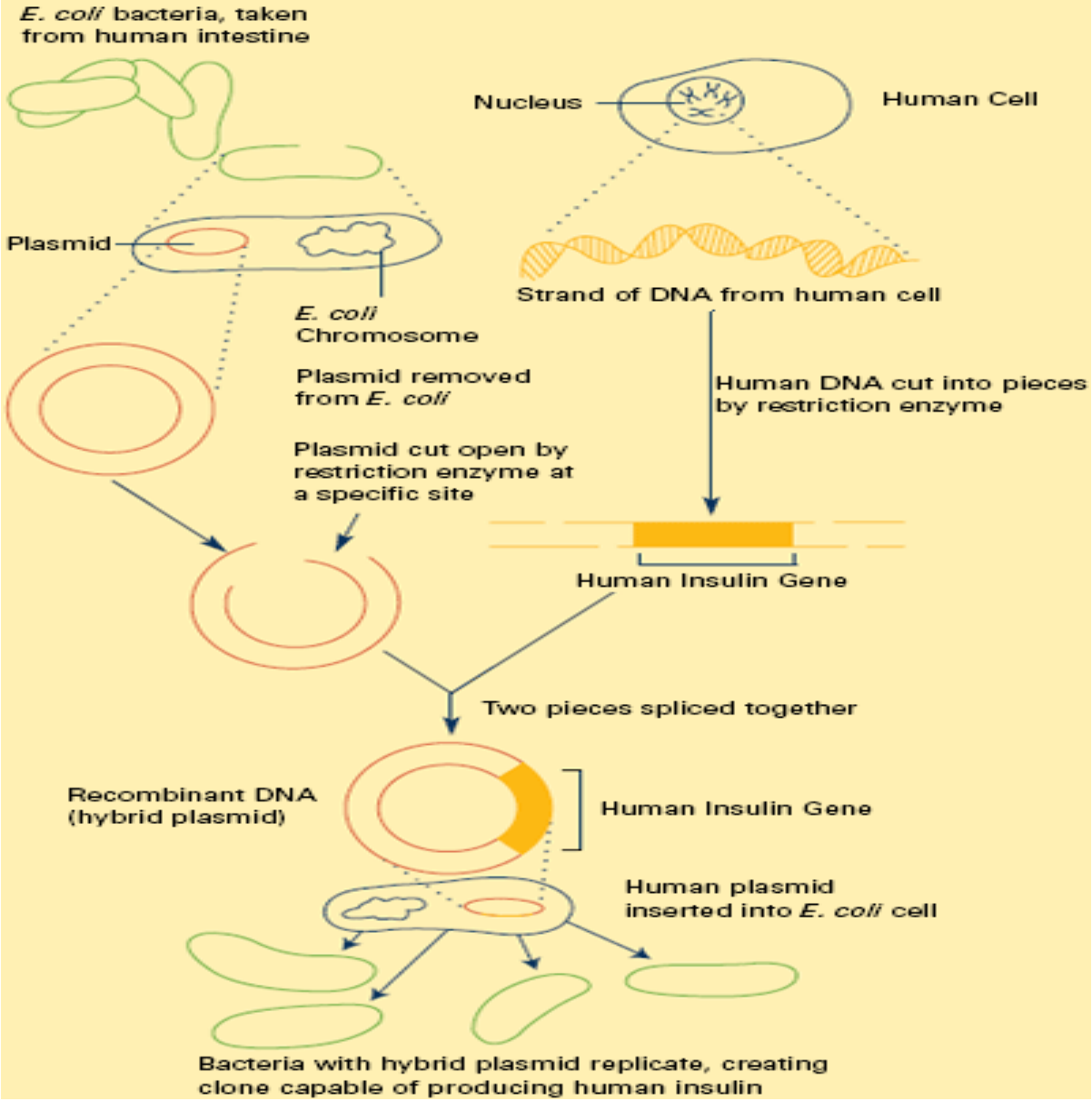


Main Steps in DNA Cloning

- The chosen piece of DNA is '**cut**' from the source organism using restriction enzymes.
- The piece of DNA is '**pasted**' into a vector and the ends of the DNA are joined with the vector DNA by ligation.
- The vector is introduced into a host cell, by a process called **transformation**. The host cells copy the vector DNA along with their own DNA, creating multiple copies of the inserted DNA.
- The vector DNA is **isolated** from the host cells' DNA and **purified**.



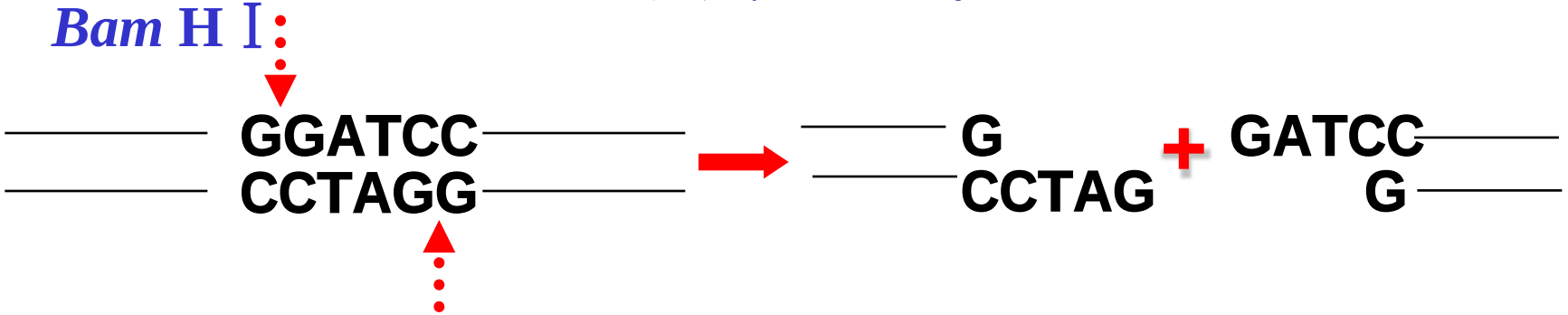
How rInsulin Produced?



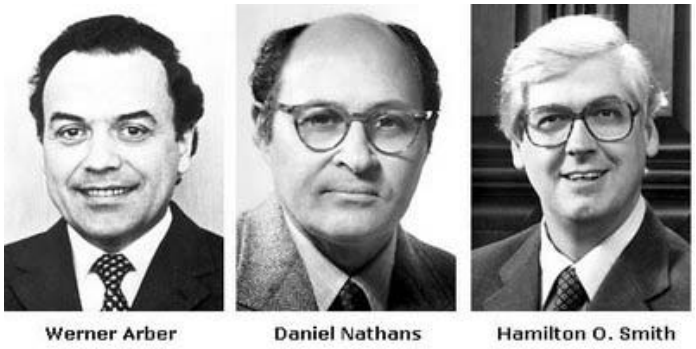
Molecular Tools in Recombinant DNA Technology

Restriction Endonuclease

限制性內切酶



A **restriction endonuclease** is an **enzyme** that cuts double-stranded or single stranded DNA at specific recognition nucleotide sequences known as **restriction sites**.



Along with **Werner Arber** and **Hamilton Smith**, **Daniel Nathans** received the Nobel Prize in Physiology or Medicine in 1978 for the discovery of restriction enzymes.

Name of Restriction Endonuclease

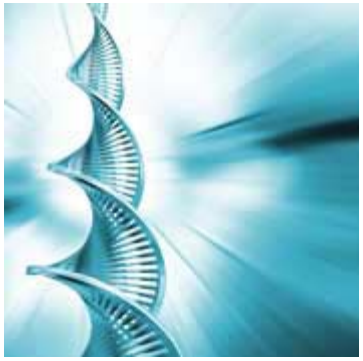
***Hin* dIII**

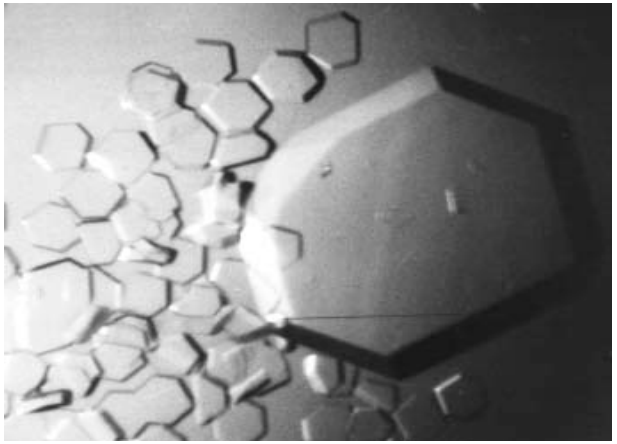
Haemophilus influenzae d strain III

Genus **Species** **Strain** **Order**

屬 **系** **株** **序**

AAGCTT





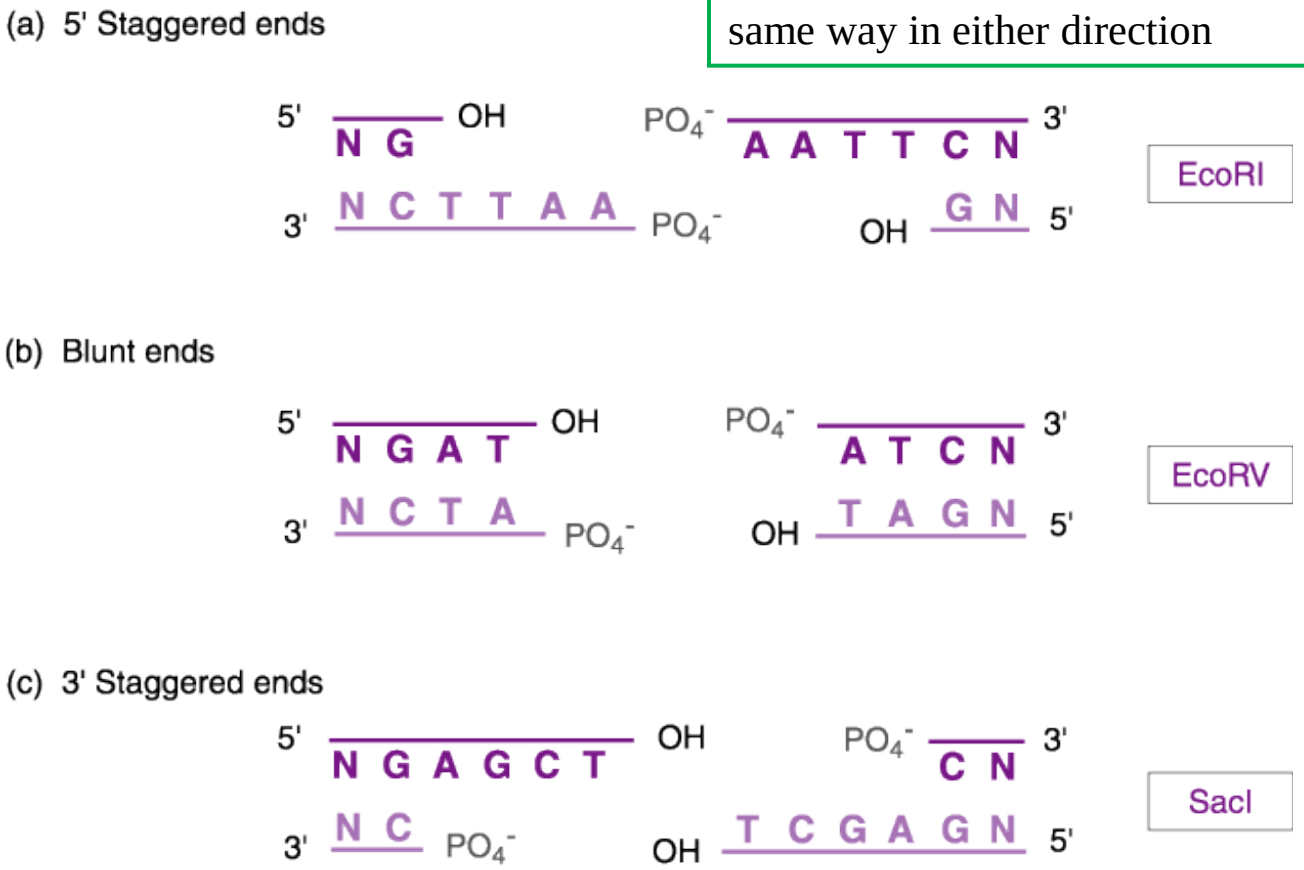
Characteristics of Restriction Endonuclease

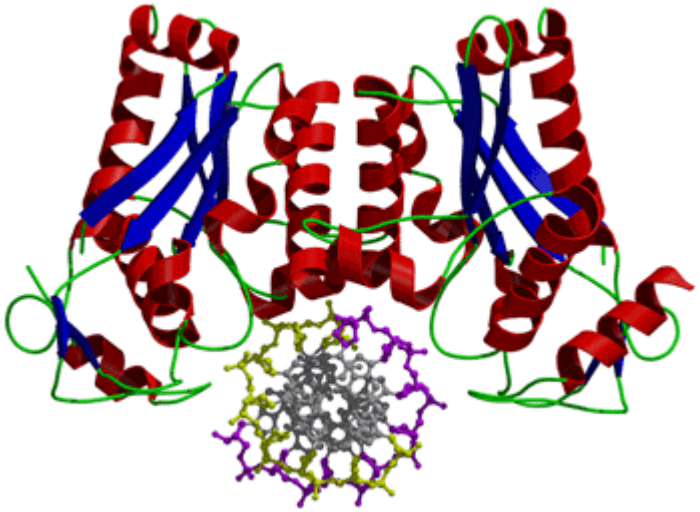
.....GGATCC.....

.....CCTAGG.....

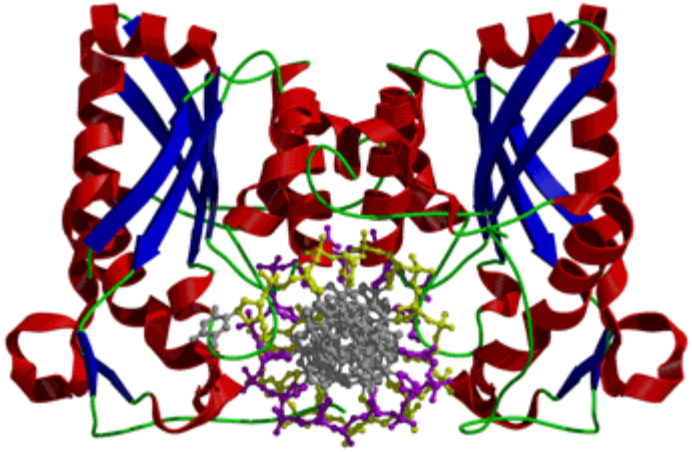
Palindrome 回文结构

A **palindrome** is a word, phrase, number or other sequence of units that can be read the same way in either direction





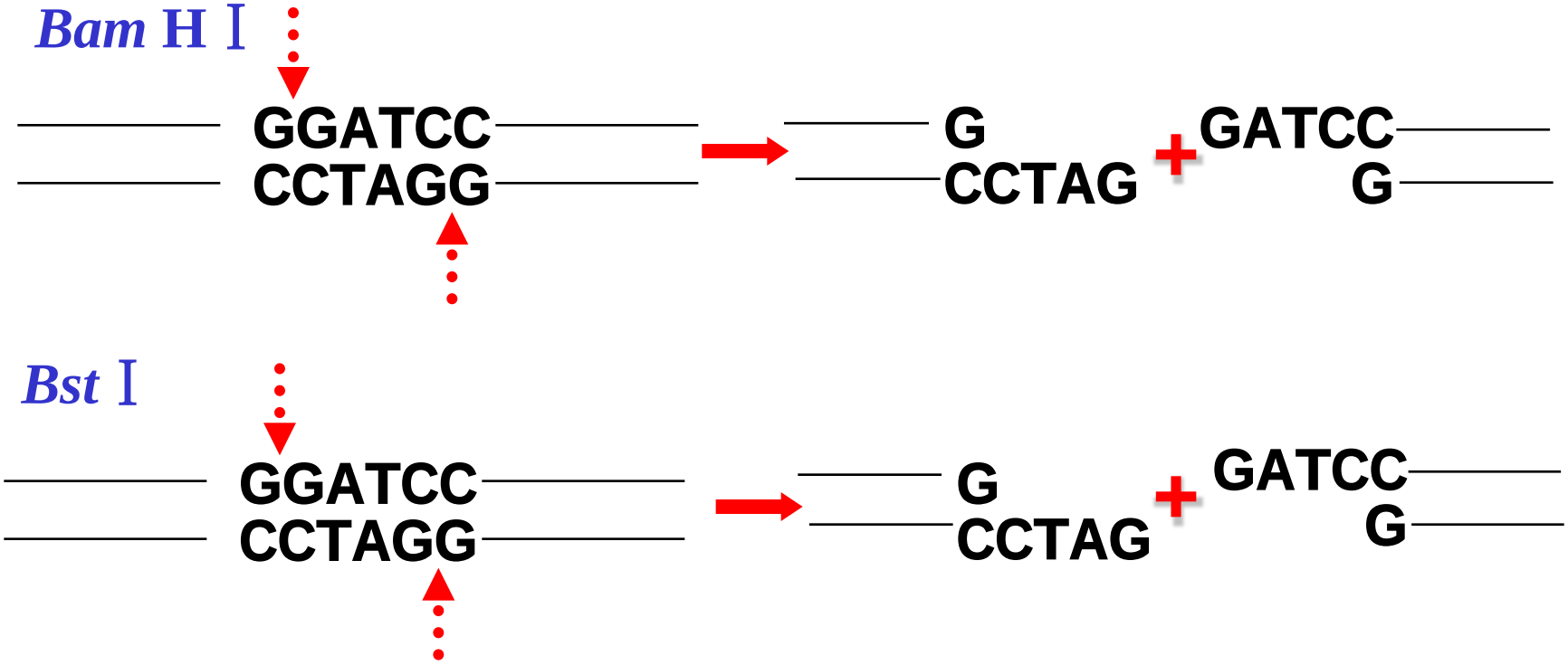
Structure of BamHI dimer at **non-specific** site



Structure of BamHI dimer at a **specific** site

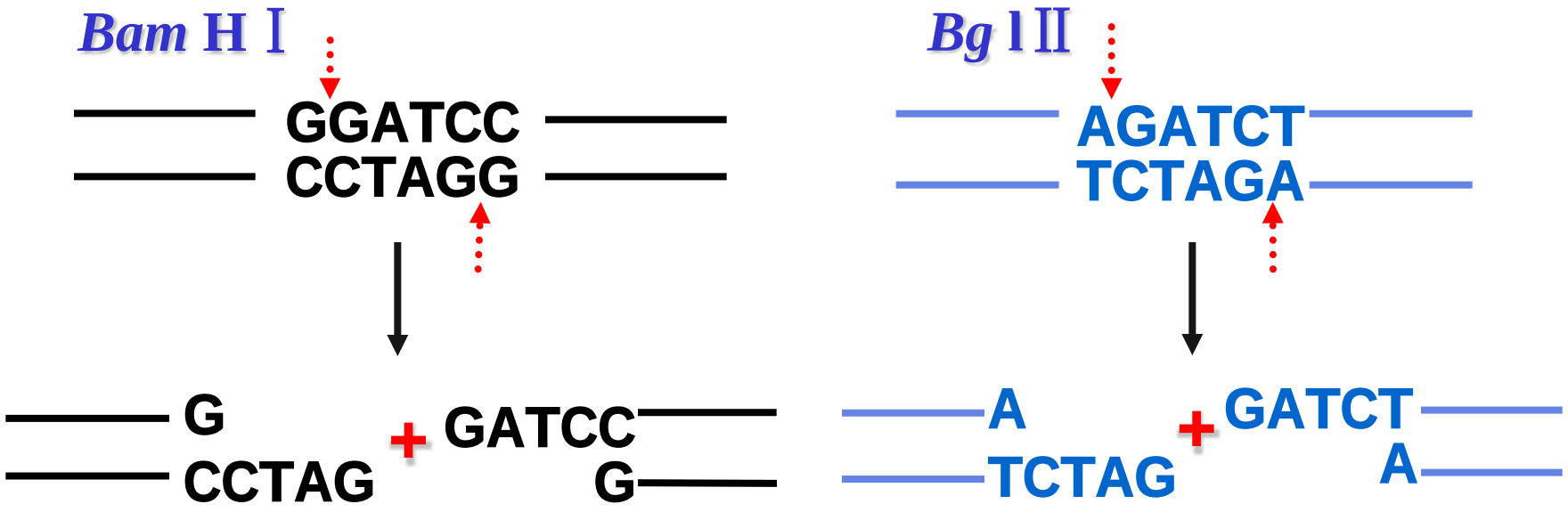
Isoschizomer 同识异切酶，同裂酶

Isoschizomers are pairs of restriction enzymes specific to the same recognition sequence and cut in the same location.



Isocaudarner 同尾酶

Isocaudarner (Isocaudomers) are pairs of restriction enzymes that have slightly different recognition sequences but upon cleavage generate identical termini.



Star Activity of Endonuclease

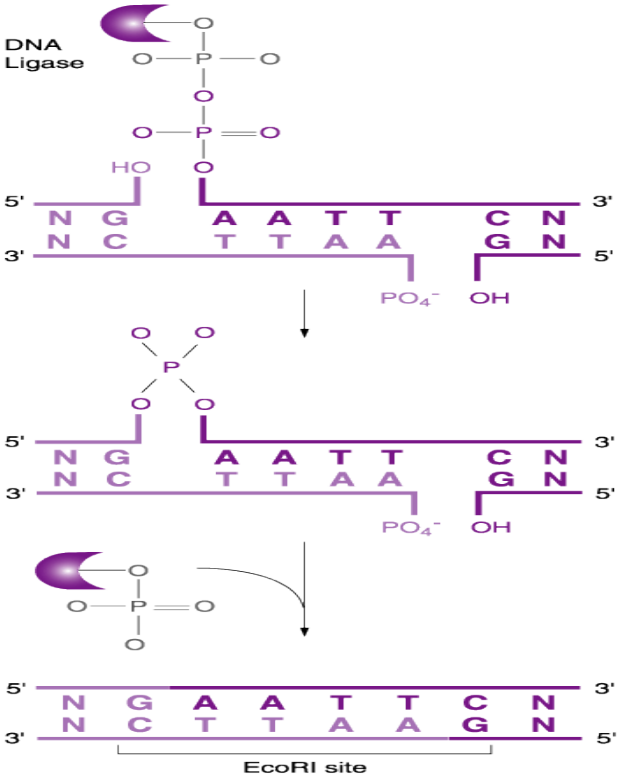
Star activity is a alteration of the specificity of restriction enzyme mediated cleavage of DNA that can occur under reaction conditions that differ significantly from those optimum for the enzyme. The result is typically cleavage at non-canonical recognition site, or sometimes complete loss of specificity.

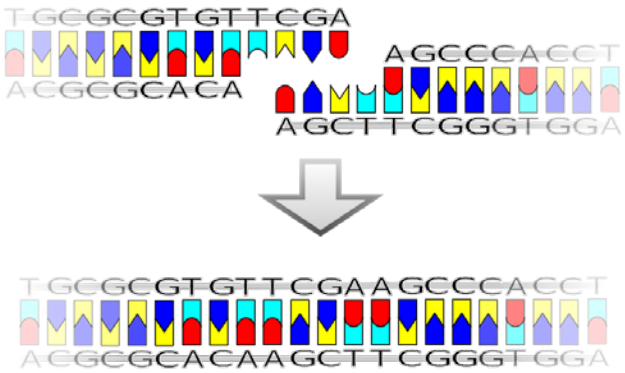
- **BamHI, BsiWI, BsoBI, DpnII, EcoRI, HgaI, PflMI, PvuII, SalI, ScaI, SspI**
- **Conditions that Contribute to Star Activity**
  - High glycerol concentration: >5% v/v
  - High units to µg of DNA ratio: Varies with each enzyme, usually >100 units/µg
  - Low ionic strength: <25 mM
  - High pH: >pH 8.0
  - Presence of organic solvents: DMSO (二甲亚砜), ethanol, ethylene glycol (乙二醇）
  - Substitution of Mg++ with other divalent cations [Mn++, Cu++, Co++, Zn++]

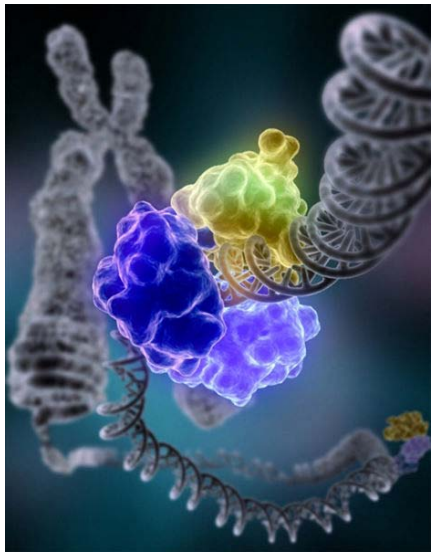
DNA Ligase

DNA ligase can link together two DNA strands that have single-strand breaks. The alternative, a double-strand break, is fixed by a different type of DNA ligase using the complementary strand as a template but still requires DNA ligase to create the final phosphodiester bond to fully repair the DNA.

DNA连接酶



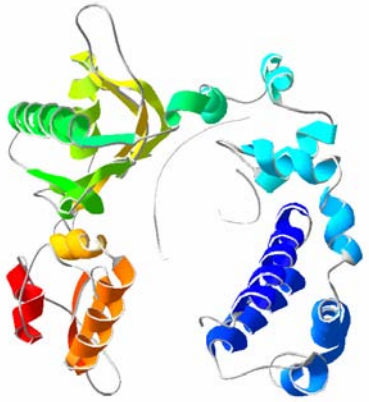




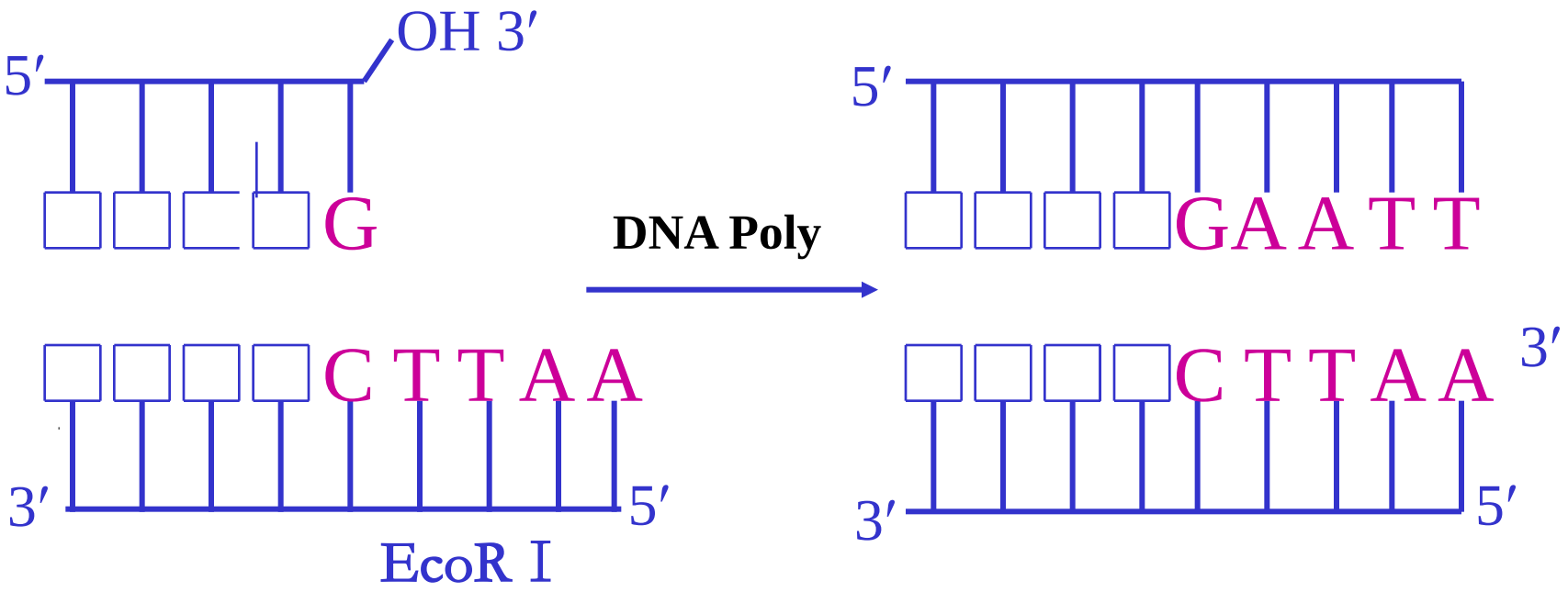
DNA ligase repairing chromosomal damage

DNA Polymerase

DNA聚合酶



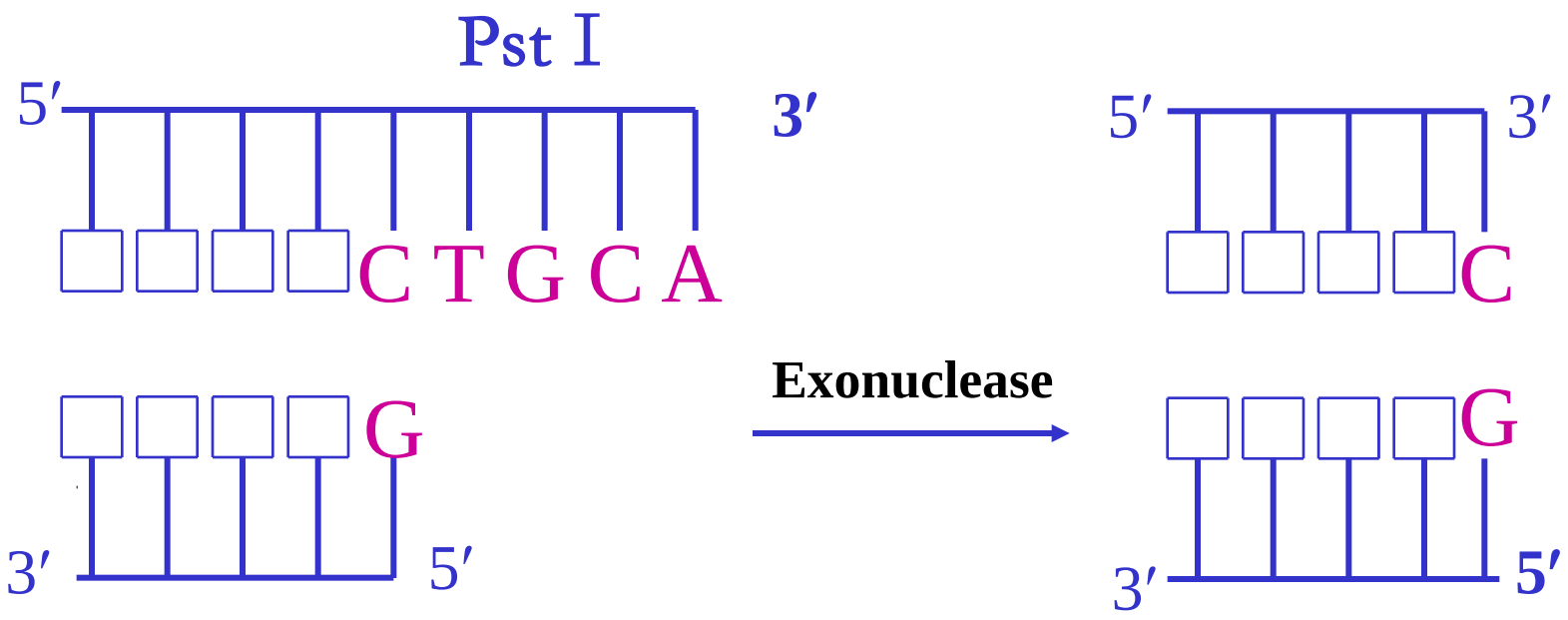
A DNA polymerase is an enzyme that catalyzes the polymerization of deoxyribonucleotides into a DNA strand. **DNA polymerase can add free nucleotides to only the 3' end of the newly-forming strand.**



Exonuclease

核酸外切酶

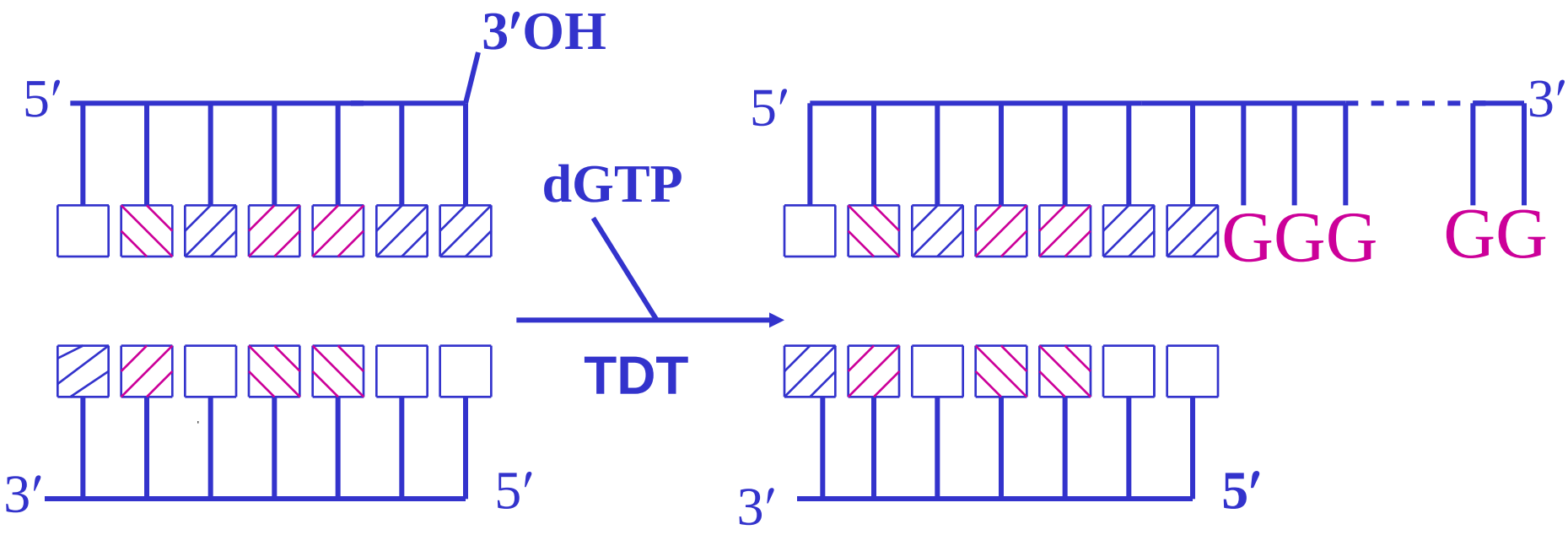
Exonucleases are enzymes that cleave nucleotides one at a time from an end of a polynucleotide chain. These enzymes hydrolyze phosphodiester bonds from either the 3' or 5' terminus of a polynucleotide molecule.



Terminal Deoxynucleotide Transferase （TDT）

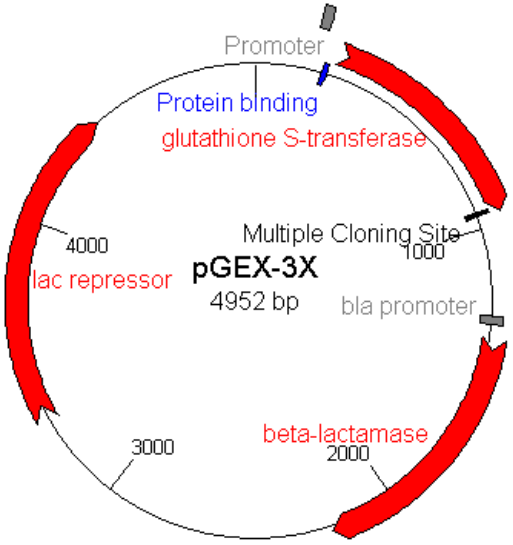
末端脱氧核苷酸转移酶

TDT is a polymerase which adds nucleotides at 3' -OH (hydroxy) end but does not require any complementary sequence and does not copy any DNA sequence



Vector （载体）

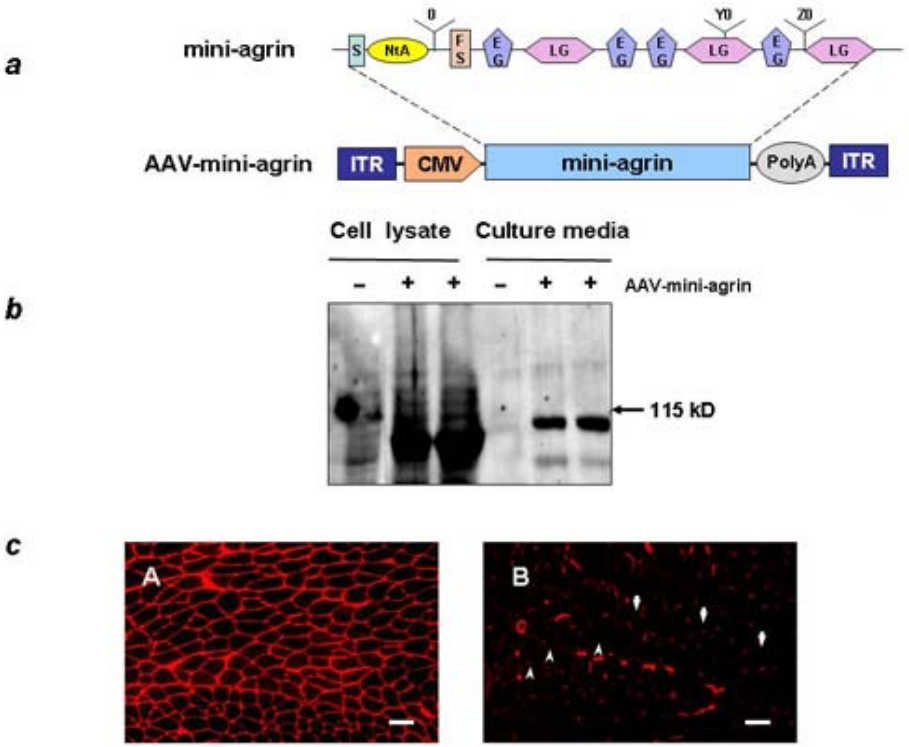
A **vector** is any vehicle used to transfer foreign genetic material into another cell. Common to all engineered vectors are **an origin of replication, a multicloning site, and a selectable marker.**



A cloning vector is a small piece of DNA into which a foreign DNA fragment can be inserted. The insertion of the fragment into the cloning vector is carried out by treating the vehicle and the foreign DNA with the same restriction enzyme, then ligating the fragments together.

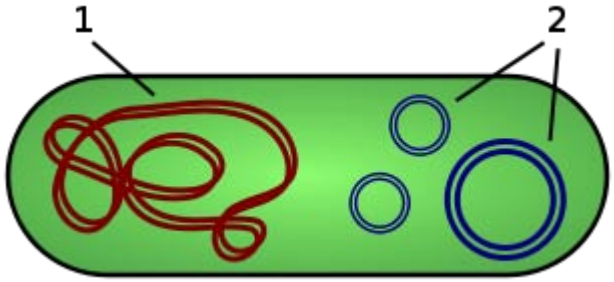
Expression Vector

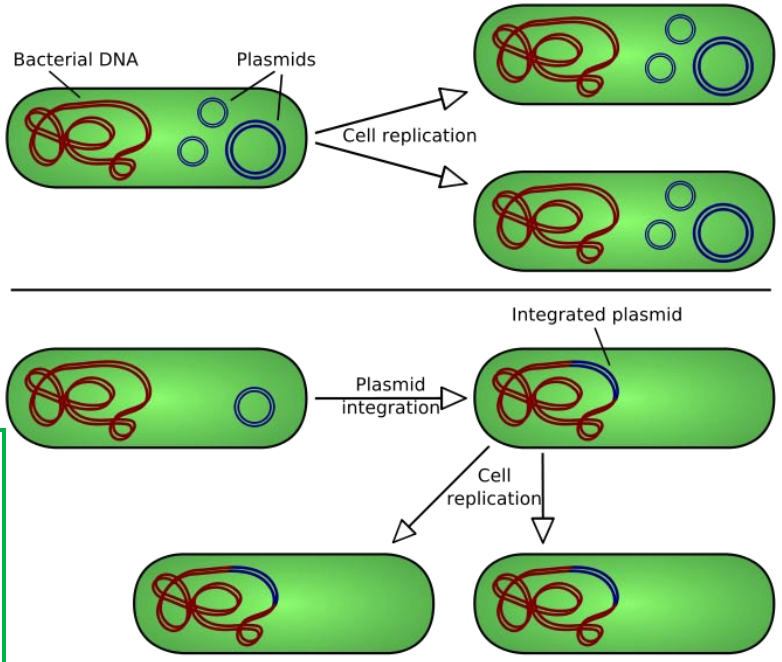
An expression vector is generally a plasmid that is used to introduce and express a specific gene into a target cell. Once the expression vector is inside the cell, the protein that is encoded by the gene is produced by the cellular transcription and translation machinery.



Plasmid （质粒）

A plasmid is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded.

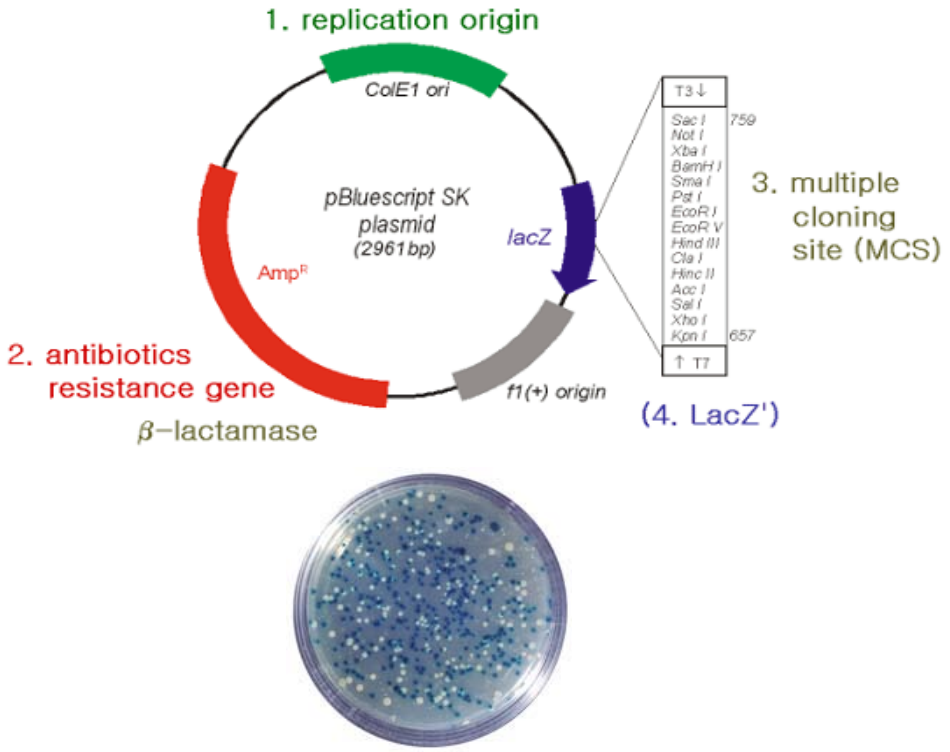


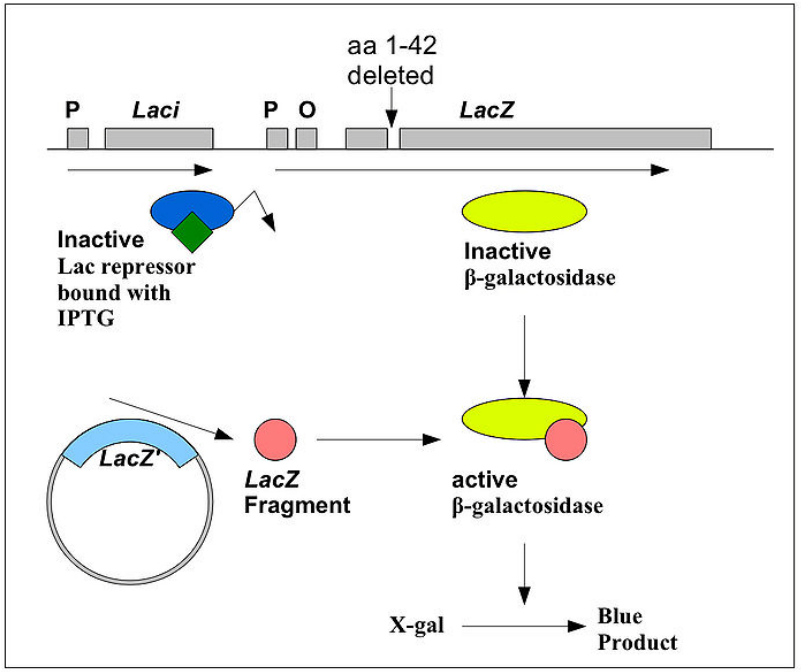


- **Cloning limit: 0.1-10 kilobases (kb)**

There are two types of plasmid integration into a host bacteria: **Non-integrating plasmids** replicate as with the top instance; whereas **episomes**, the lower example, integrate into the host chromosome.

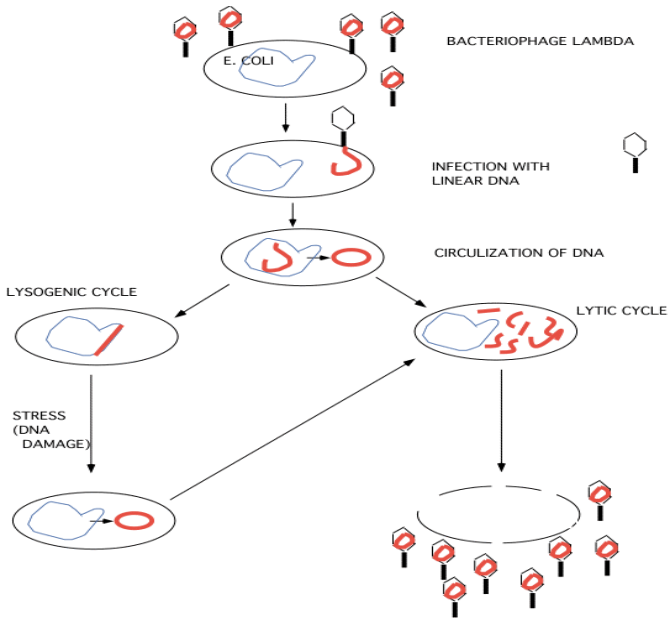
Component of Plasmid Vector

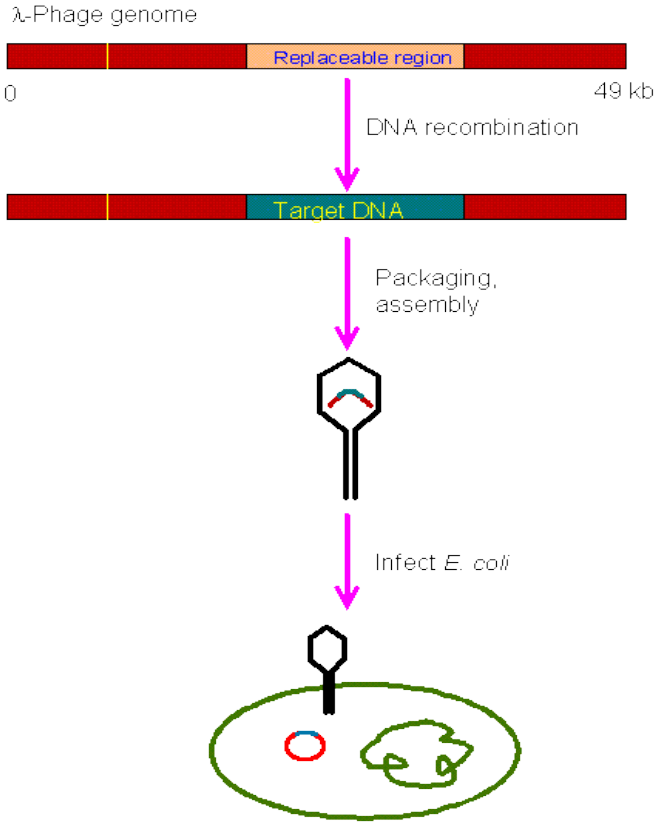




Insertion of foriegn DNA into the MCS located within the *lac Z* gene causes insertional inactivation of this gene at the N-terminal fragment of beta-galactosidase and abolishes intra-allelic complementation. Thus bacteria carrying recombinant plasmids in the MCS cannot hydrolyse X-gal, giving rise to white colonies, which can be distinguished on culture media from non-recombinant cells, which are blue.

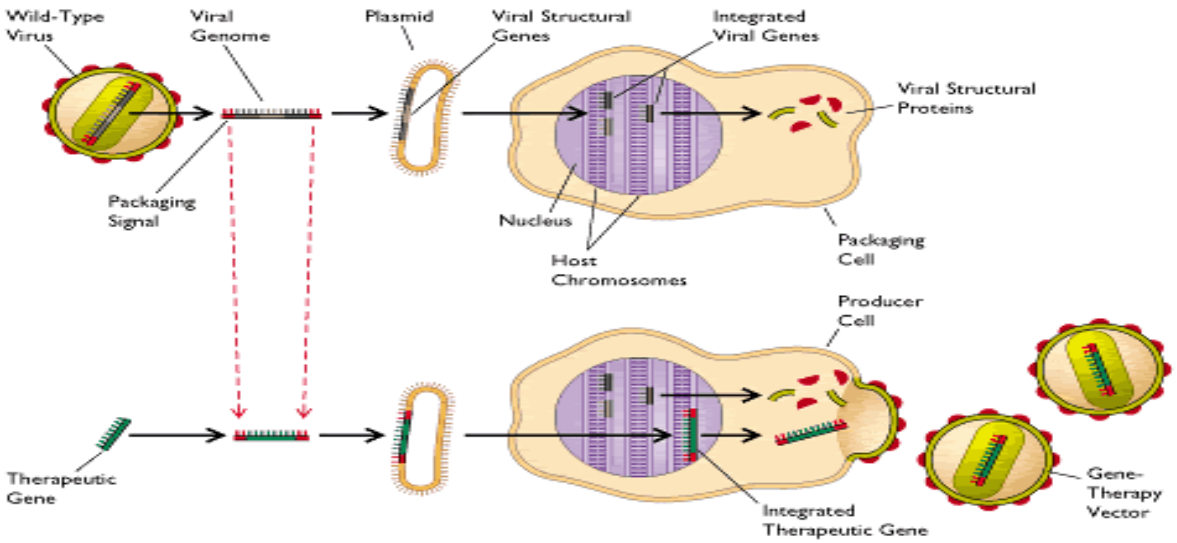
λPhage Vector

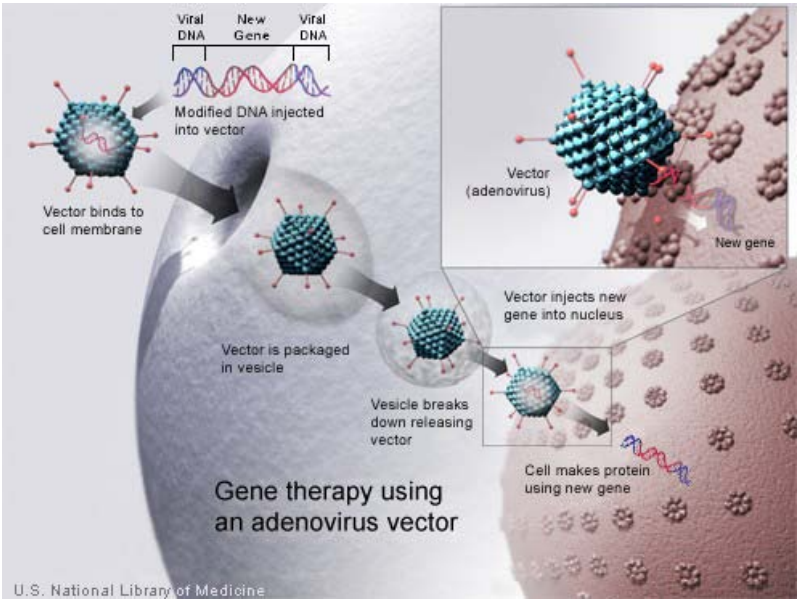




The rDNA to be cloned is first inserted into the λ DNA, replacing a nonessential region. Then, by an in vitro assembly system, the λ virion carrying the recombinant DNA can be formed. The **λ** genome is 49 kb in length which can carry up to **25 kb foreign DNA**. The major advantage of the λ phage vector is its high transformation efficiency, about **1000 times more efficient than the plasmid vector**.

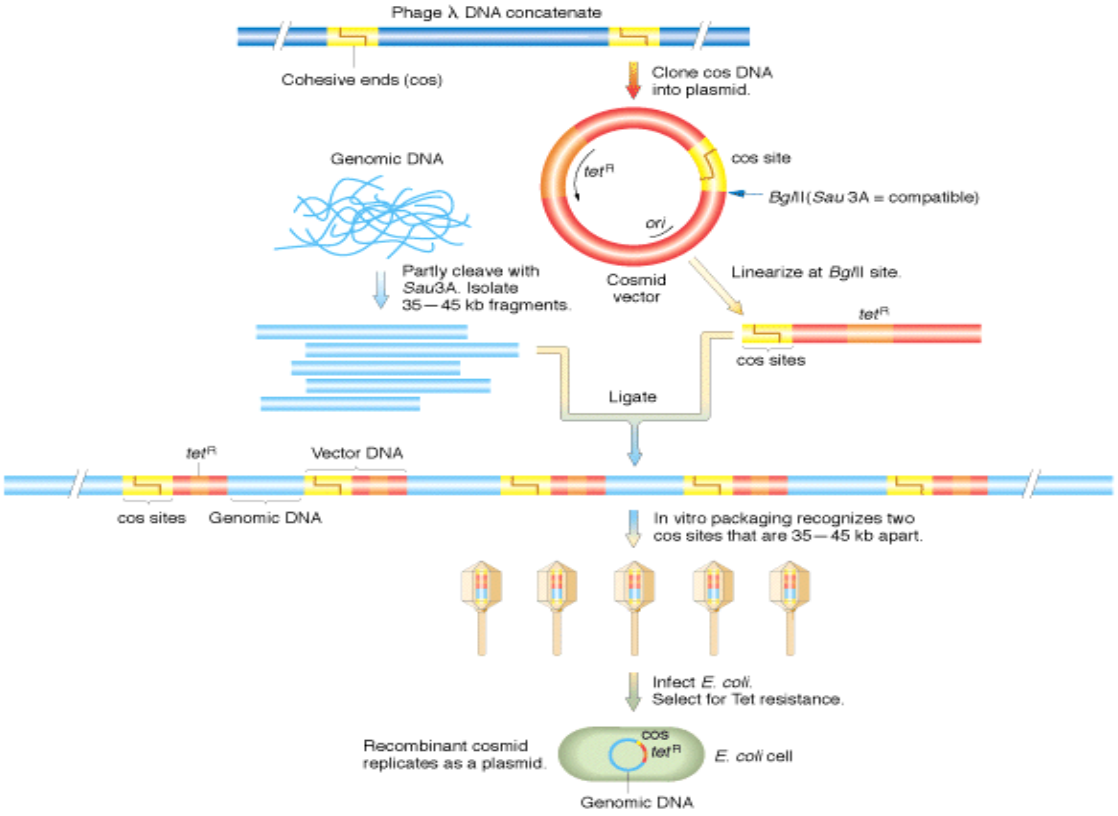
Development of Gene Therapy Vector





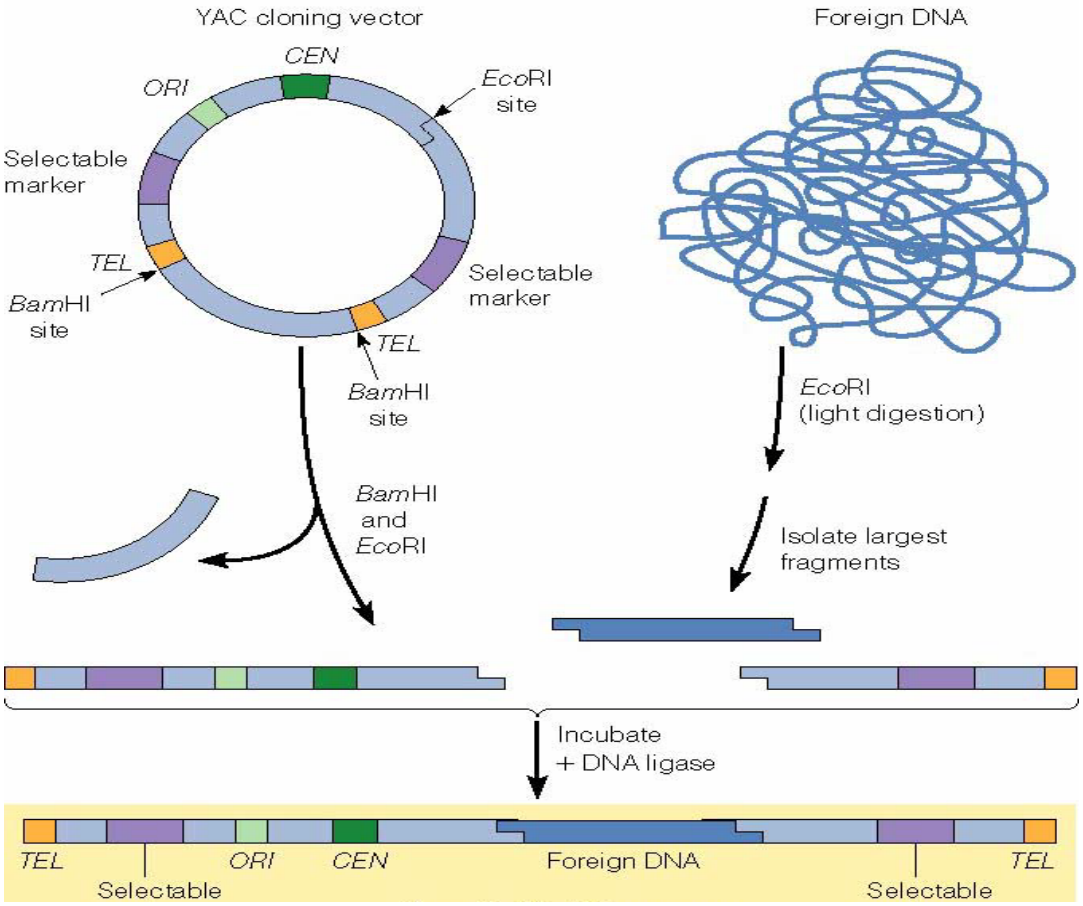
Cosmid 粘粒

A cosmid is a type of hybrid plasmid that contains cos sequences and DNA sequences originally from the Lambda phage. Cosmids can be used to build genomic libraries. **Cosmids are able to contain 37 to 52 kb.**



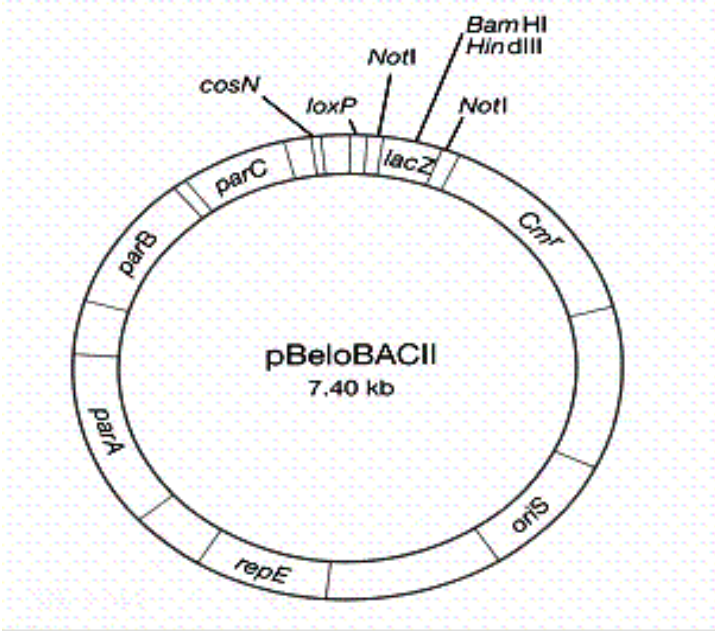
Yeast Artificial Chromosome, YAC

YAC is a vector used to clone large DNA fragments (larger than 100 kb and up to 3000 kb). It is an artificially constructed chromosome and contains the telomeric, centromeric, and replication origin sequences needed for replication and preservation in yeast cells.



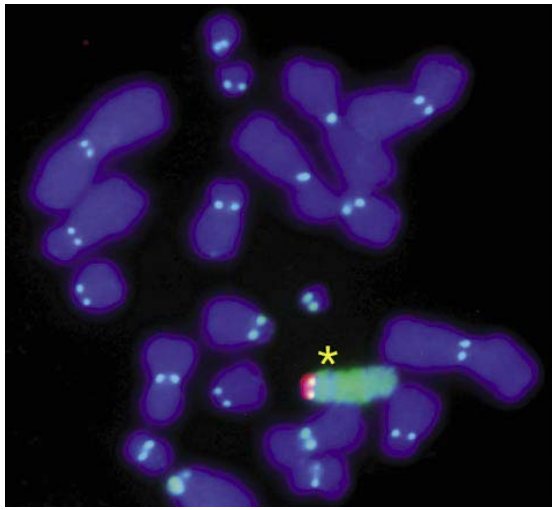
Bacterial Artificial Chromosome, BAC

BAC is a DNA construct, based on a fertility plasmid (or F-plasmid), used for transforming and cloning in bacteria, usually *E. coli*. F-plasmids play a crucial role because they contain partition genes that promote the even distribution of plasmids after bacterial cell division. **BAC's usual insert size is 150-350 kb, but can be greater than 700 kb.** BACs are often used to sequence the genome of organisms in genome projects.

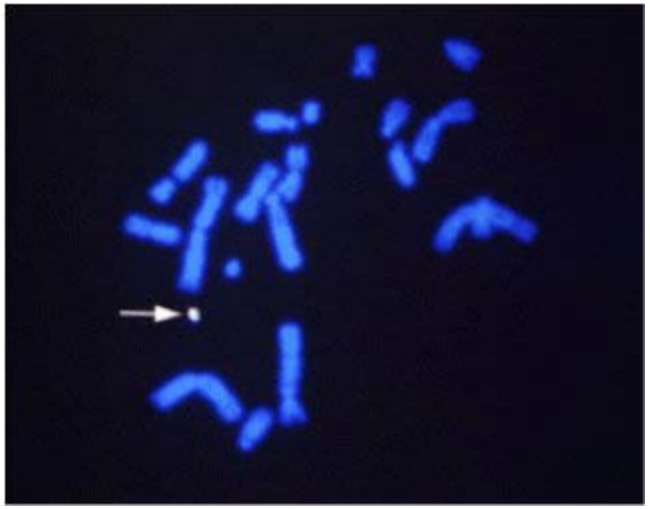


Human Artificial Chromosome

HACs are useful gene transfer vectors in expression studies and important tools for determining human chromosome function. HACs have been used to complement gene deficiencies in human cultured cells by transfer of large genomic loci also containing the regulatory elements for appropriate expression. And, they now offer the possibility to express large human transgenes in animals, especially in mouse models of human genetic diseases.

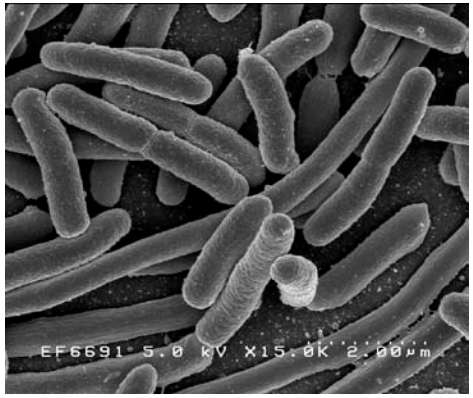


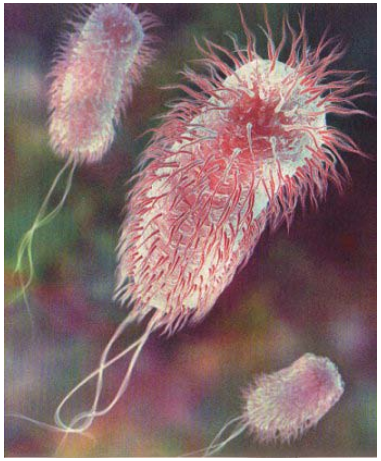
Human satellite DNA-based artificial chromosome



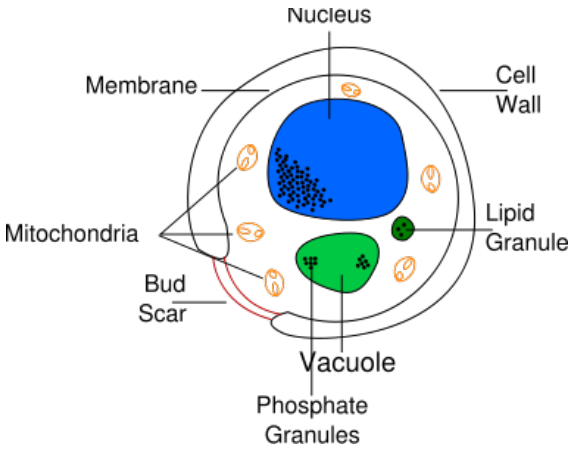
A fragmented human X chromosome in a CHO cell line

Host Cells

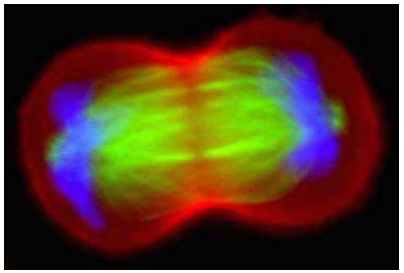


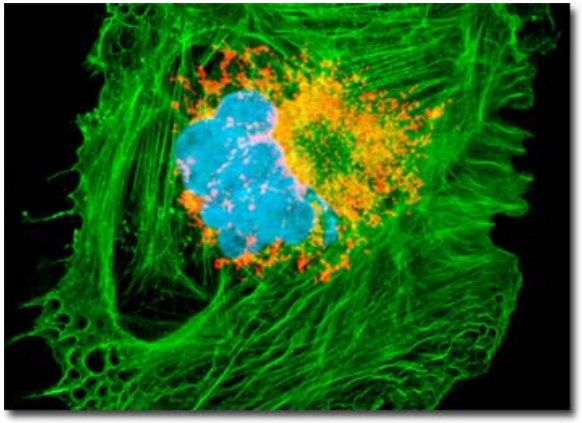


E.Coli

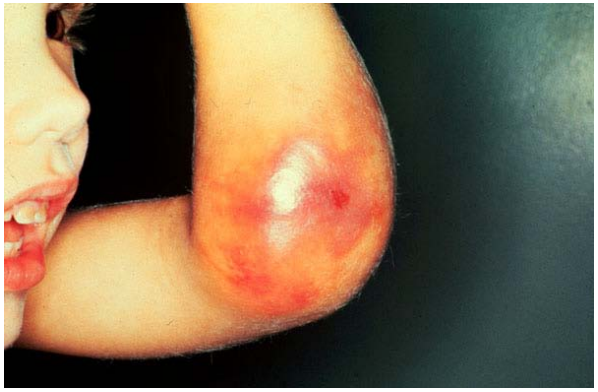


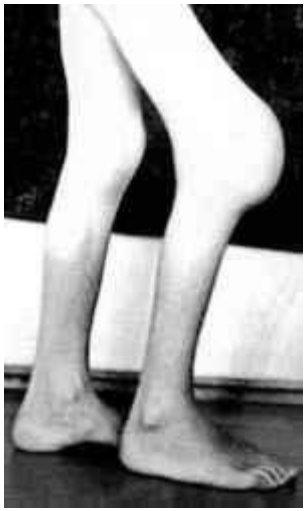
Yeast

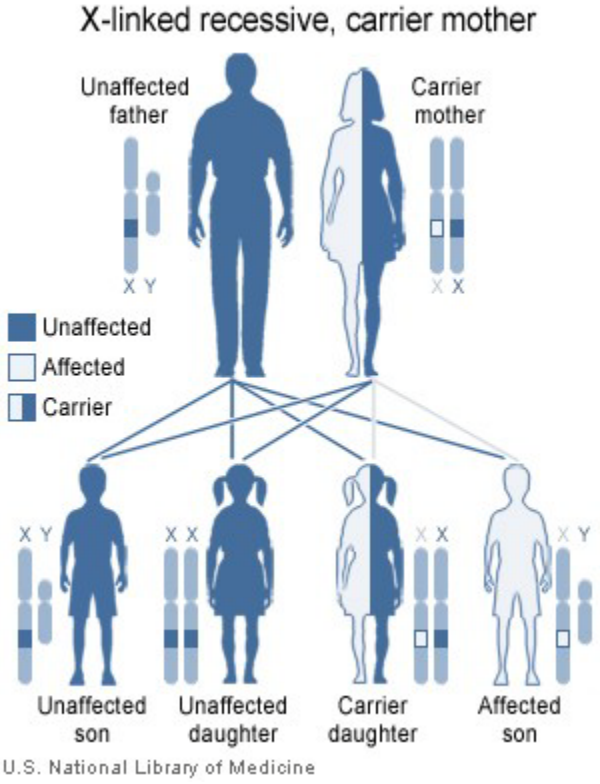




Chinese Hamster Ovary Cells, CHO







Hemophilia A is a blood clotting disorder caused by a mutation of the factor VIII gene, leading to a deficiency in Factor VIII. It is the most common hemophilia. Inheritance is X-linked recessive; hence, males are affected sexually while females are carriers or very rarely display a mild phenotype. 1 in 5,000 males are affected.

Summary

- **DNA Clone**
- **Enzymes: Restriction Endonuclease, ligase, DNA polymeryse, Exonuclease, Terminal Deoxynucleotide Transferase**
- **Vector: plasmid, Phage, virus, cosmid, YAC, BAC, HAC**
- **Host Cells: E.coli, Yeast, CHO**
- **Examples**

"Pleasure in the job puts perfection in the work."

-- Aristotle