Manipulation and Screening Strategy of Bacterial Artificial Chromosome DNA Recombinant

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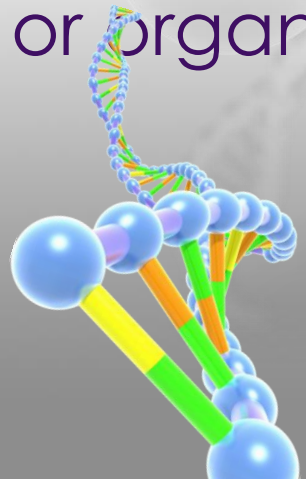
Introduction

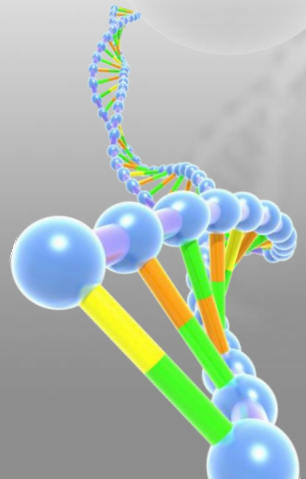
- **Clones**
  - Genetically identical molecules, cells, or organisms all derived from a single ancestor

- Cloning
  - The production of identical copies of molecules, cells, or organisms from a single ancestor



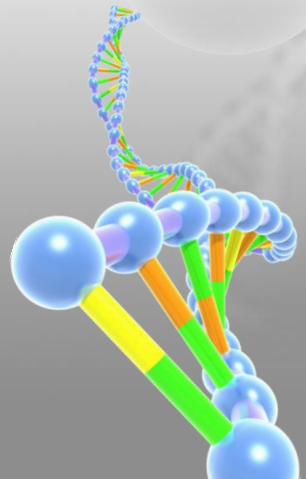
Cloning Genes Is a Multistep Process

- Technology was developed to clone segments of DNA molecules, based on enzymes (restriction endonucleases) that recognize and cut DNA at specific nucleotide sequences

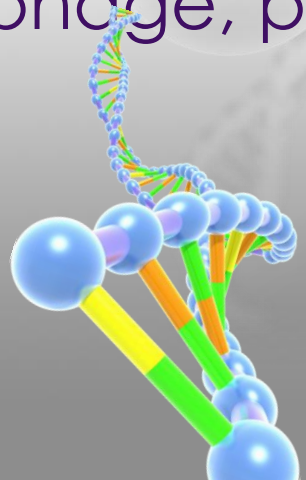


•Recombinant DNA Technology

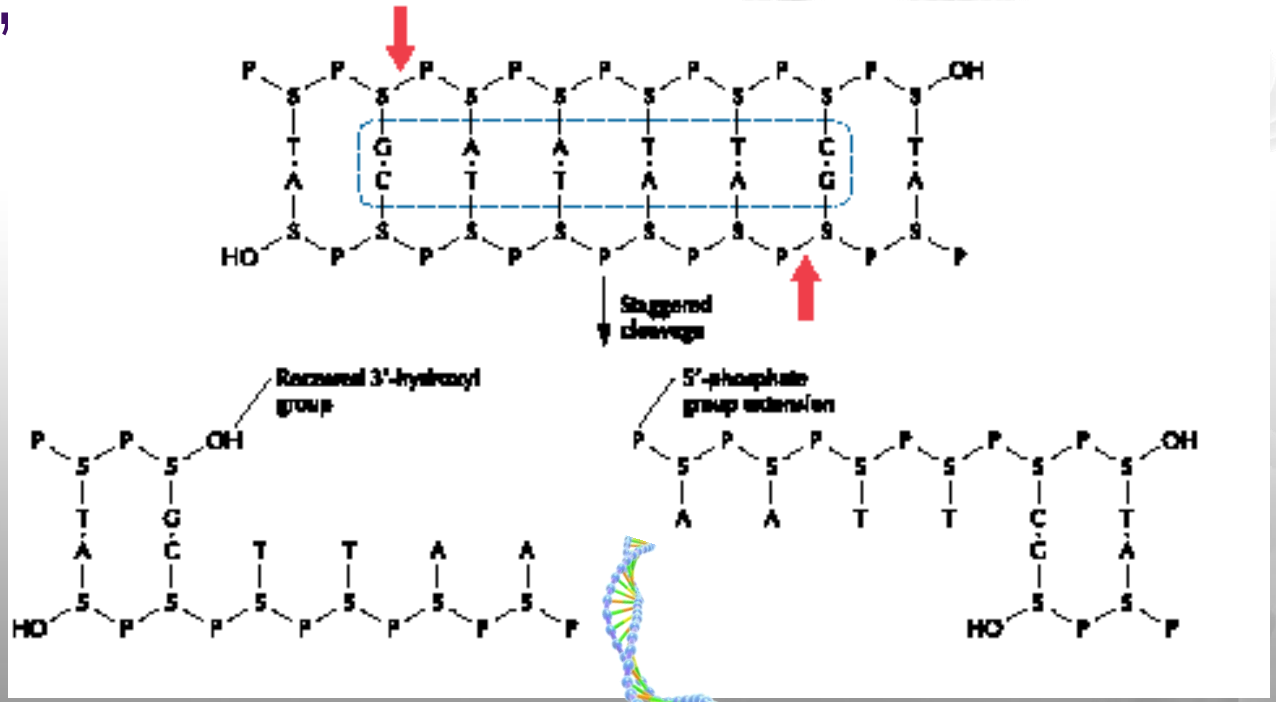
- **Recombinant DNA technology**
  - Techniques in which DNA fragments are linked to self-replicating vectors to create recombinant DNA molecules which are replicated in a host cell



- **Kinds of Libraries**
- Genomic Library: Stores a representation of the genome (at least one copy of a gene present)
- plasmid, bacteriophage, phagemid,
- cosmid vectors
- cDNA Library: Stores a representation of the mRNAs expressed at a certain time or stage by a microorganism or organism
- plasmid, bacteriophage, phagemid,
- cosmid vectors

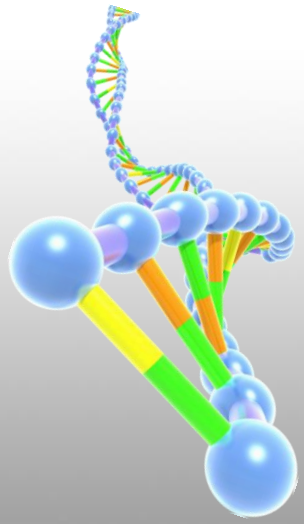


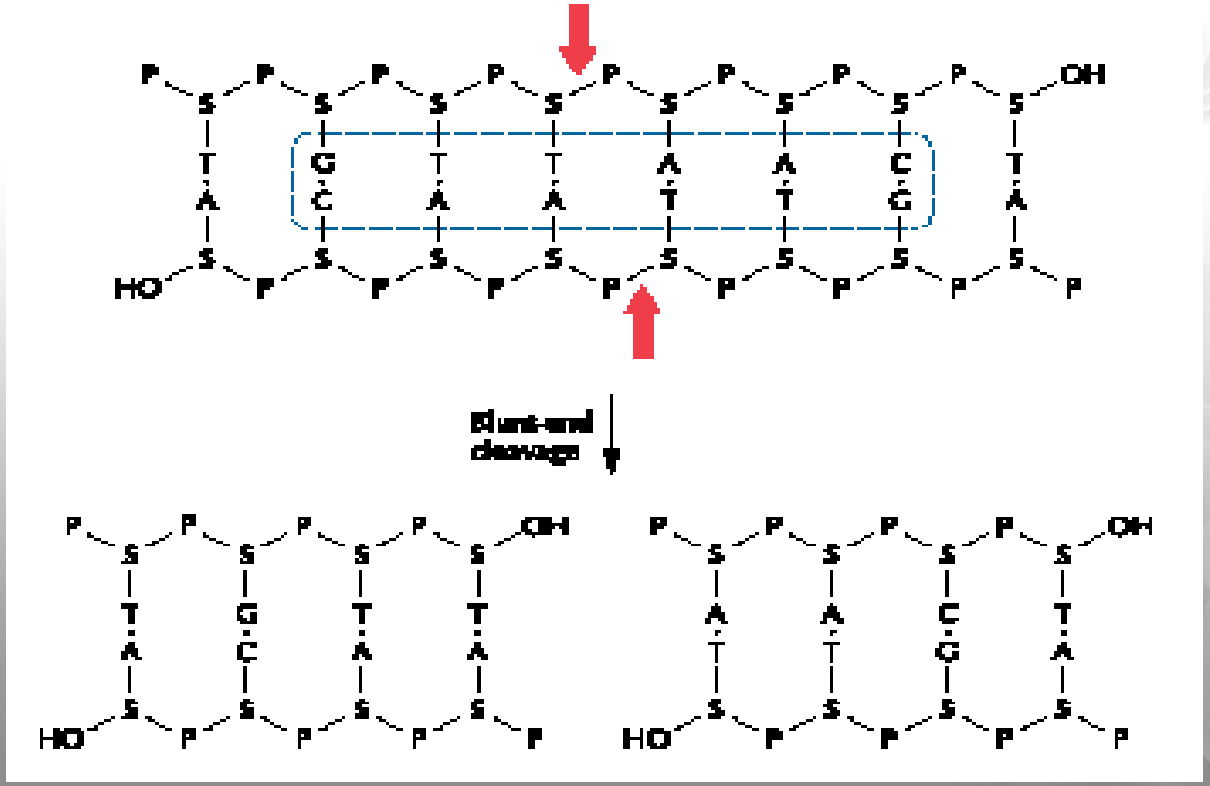
- **How is a Library Built:**
- Restriction Enzyme Mechanisms:
- Preparation of DNAs to be joined
- (a)Staggered cut: leaves "sticky ends"



•How is a Library Built:

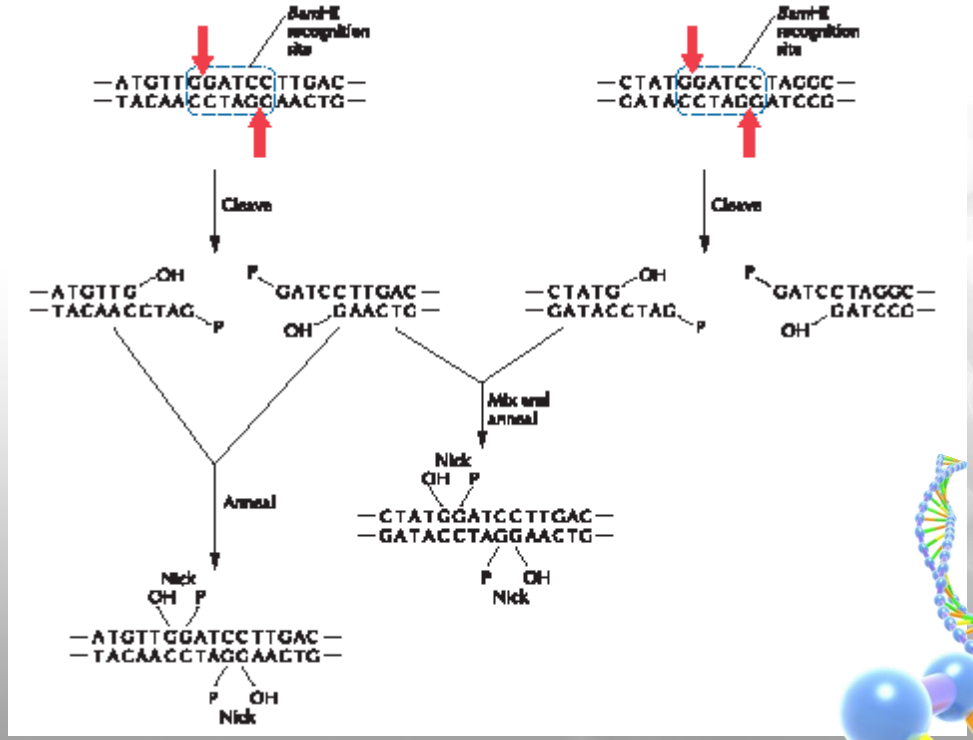
- Restriction Enzyme Mechanisms: Preparation of DNAs to be joined:
- (b) Blunt End





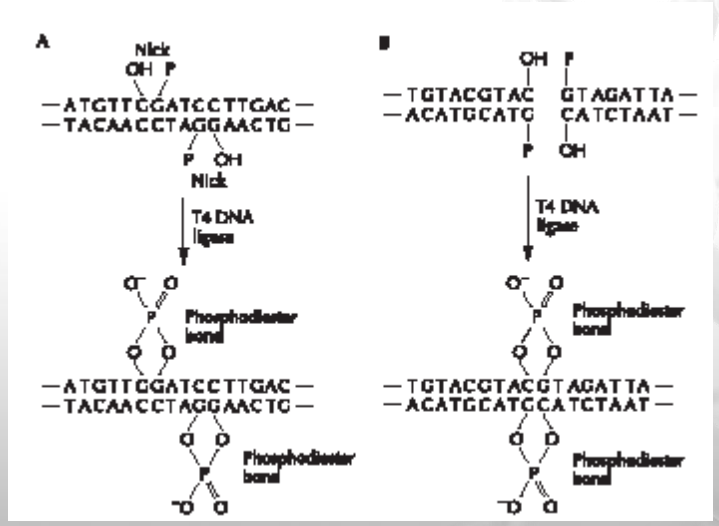
•Ligation of DNA cut with a Restriction Enzyme

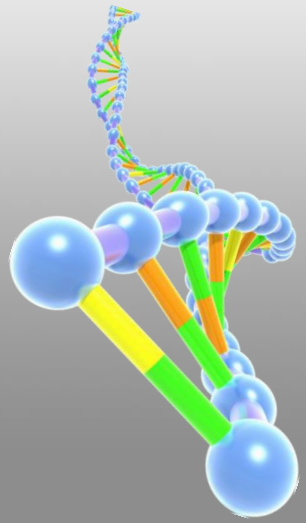
- Staggered "sticky ends"



•Ligation of DNA cut with a Restriction Enzyme

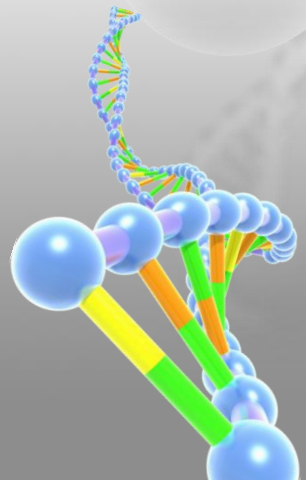
- Role of T4 DNA Ligase





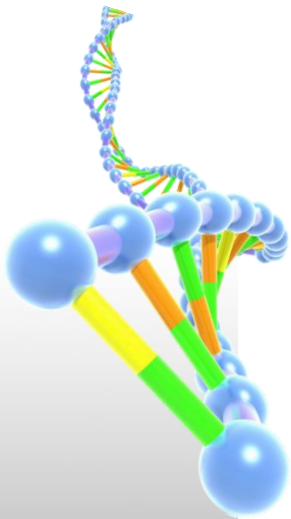
Choosing the Vector

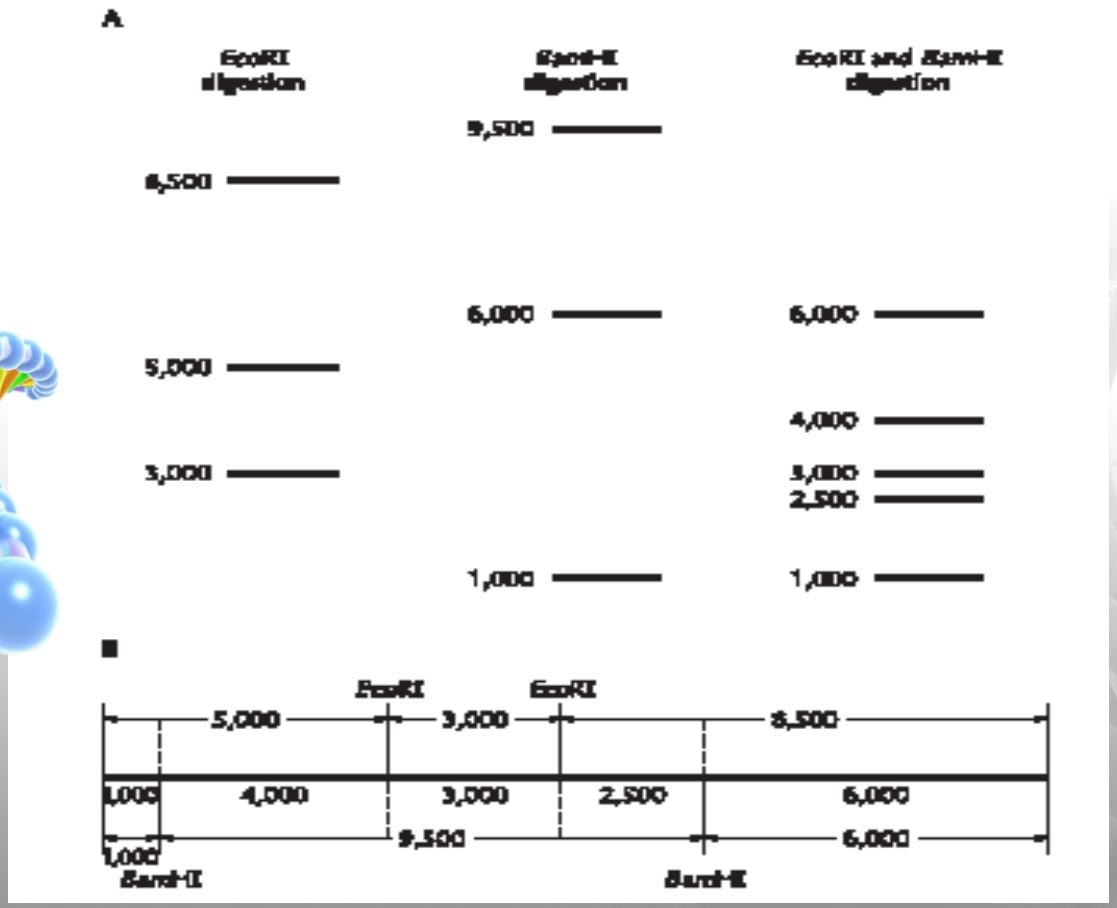
- Depends on the size of DNA to be cloned
- Is the protein encoded by the DNA going to be expressed in a prokariotic or eukaryotic cell?



•Restriction Enzyme Map

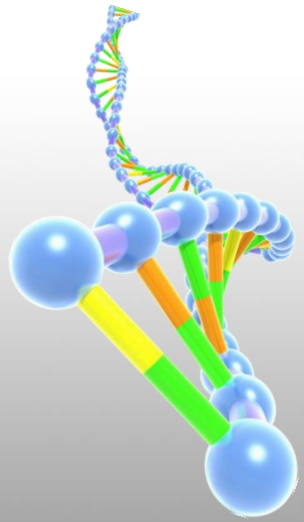
- Allows ordering of DNA fragments

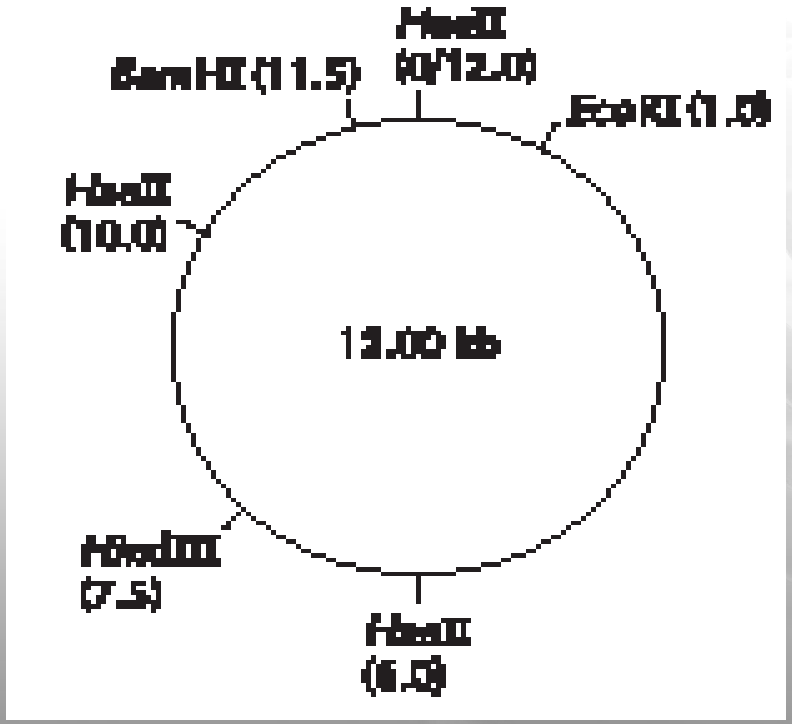




•Restriction Enzyme Map

- We can then build maps of linear and circular molecules



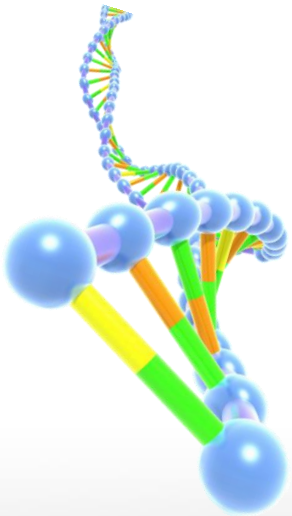


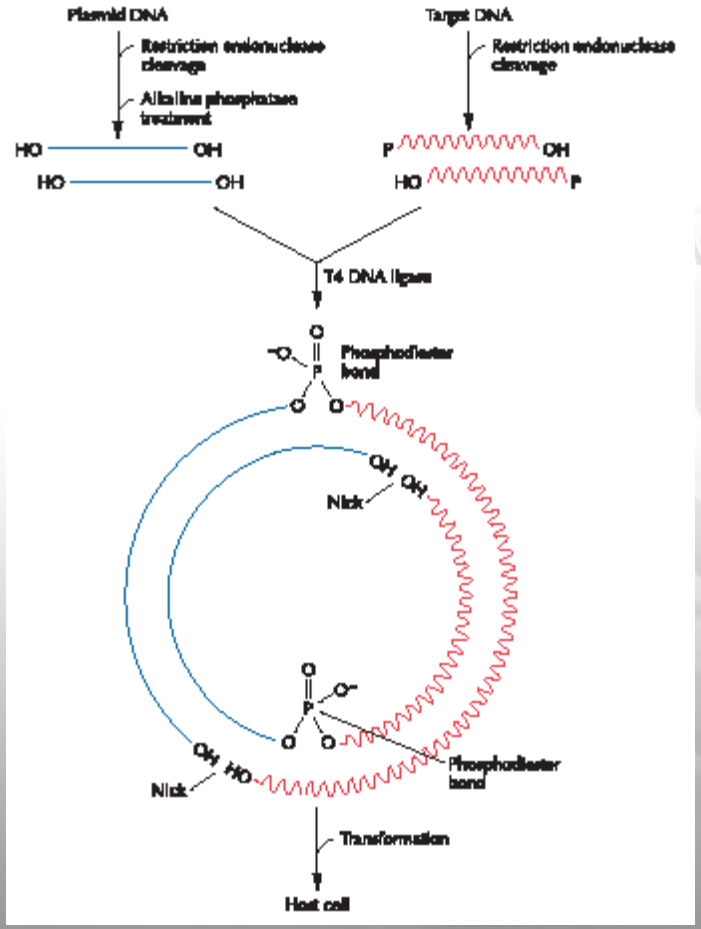
•Selectable Markers:

Table 4.4 Some antibiotics commonly used as selective agents

Antibiotic	Description
Ampicillin (Ap, Amp)	Inhibits cell wall formation; inactivated by β-lactamase
Hygromycin B (HygB)	Blocks translocation from amino acyl site to peptidyl site; inactivated by a phosphotransferase
Kanamycin (Km, Kan)	Binds to 30S subunit and prevents translocation from aminoacyl-tRNA site to peptidyl site; inactivated by a phosphotransferase
Neomycin (Nm, Neo)	Binds to 30S subunit and inhibits protein synthesis; inactivated by a phosphotransferase
Streptomycin (Sm, Str)	Blocks protein initiation complex formation and causes misreading during translation; inactivated by a phosphotransferase
Tetracycline (Tc, Tet)	Prevents binding of aminoacyl-tRNA to 30S ribosomal subunit; resistance gene encodes an inner cell membrane protein that passes the antibiotic out of the cell and blocks the passage of the antibiotic through the cell wall

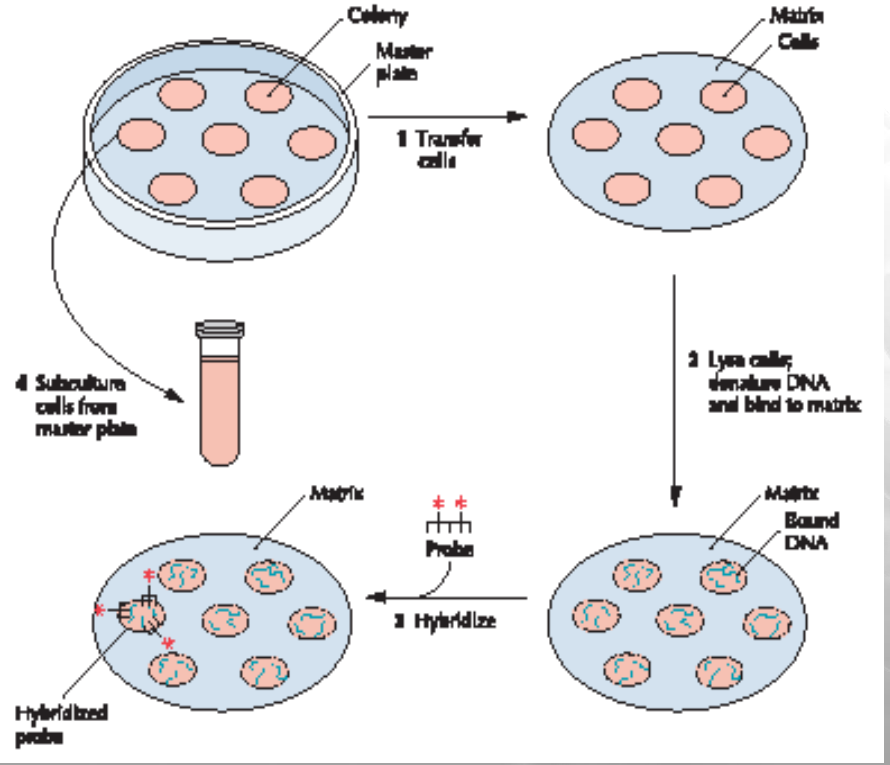
- Plasmid: a cloning vector or vehicle



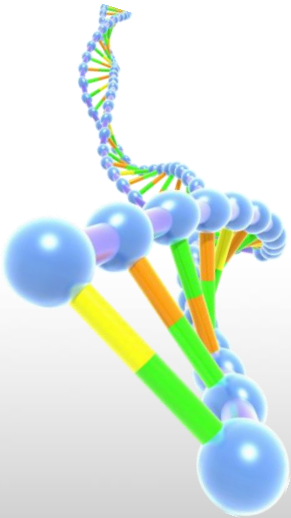


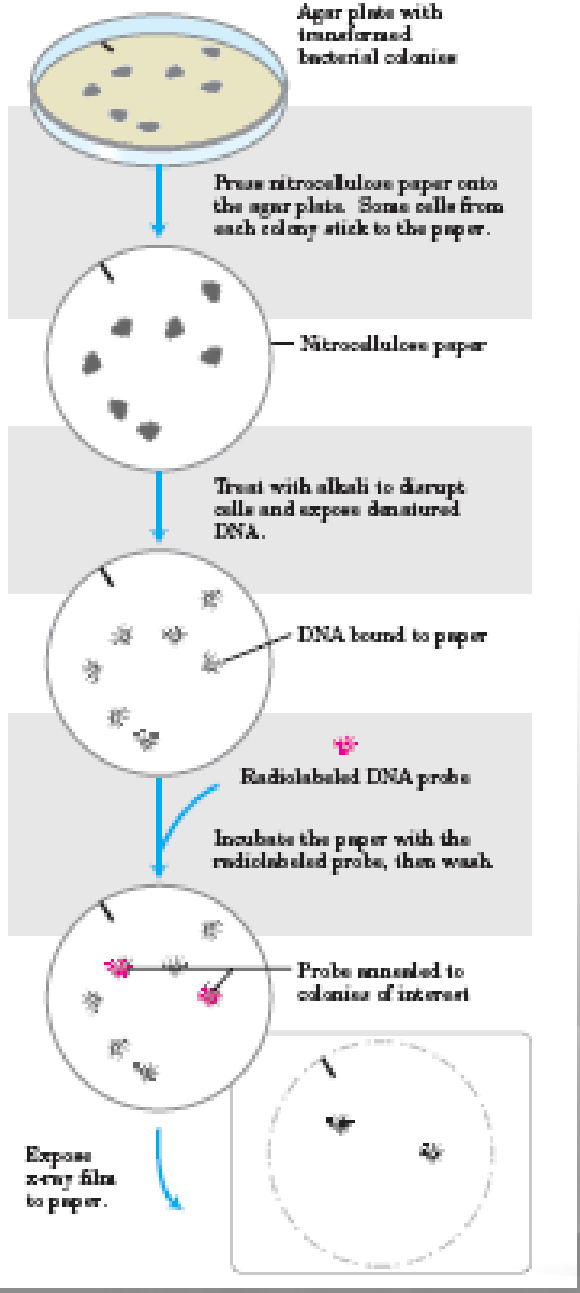
•Screening a Genomic Library

- Colony Hybridization

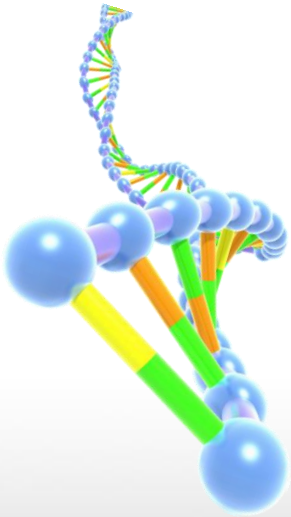


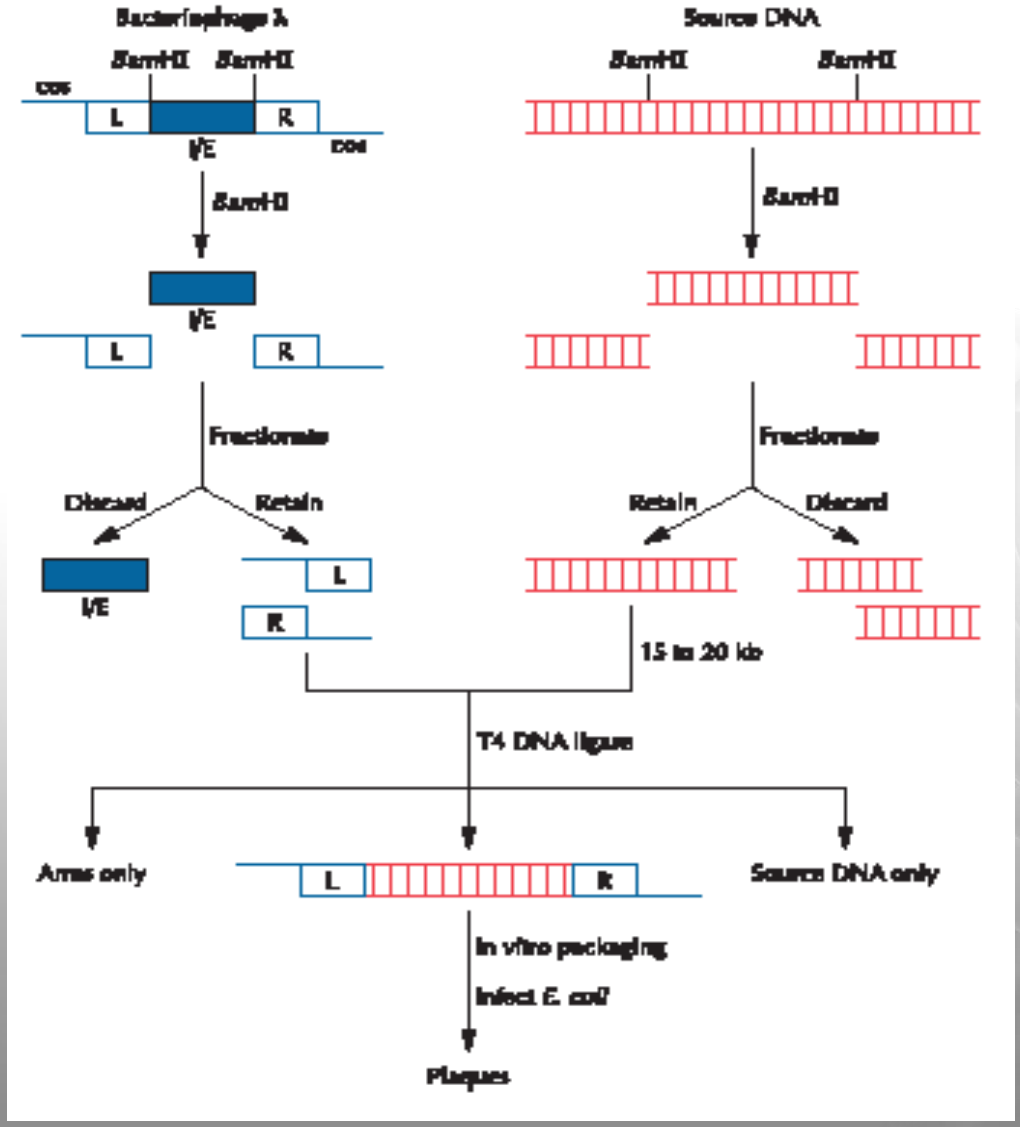
•Colony Hybridization:





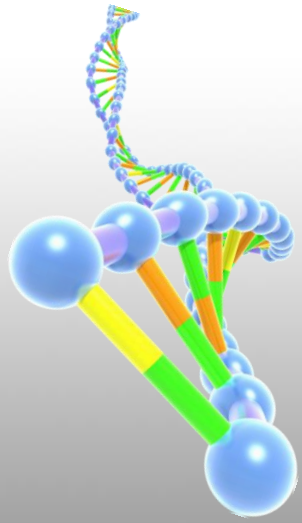
•Genomic Library: Bacteriophage λ

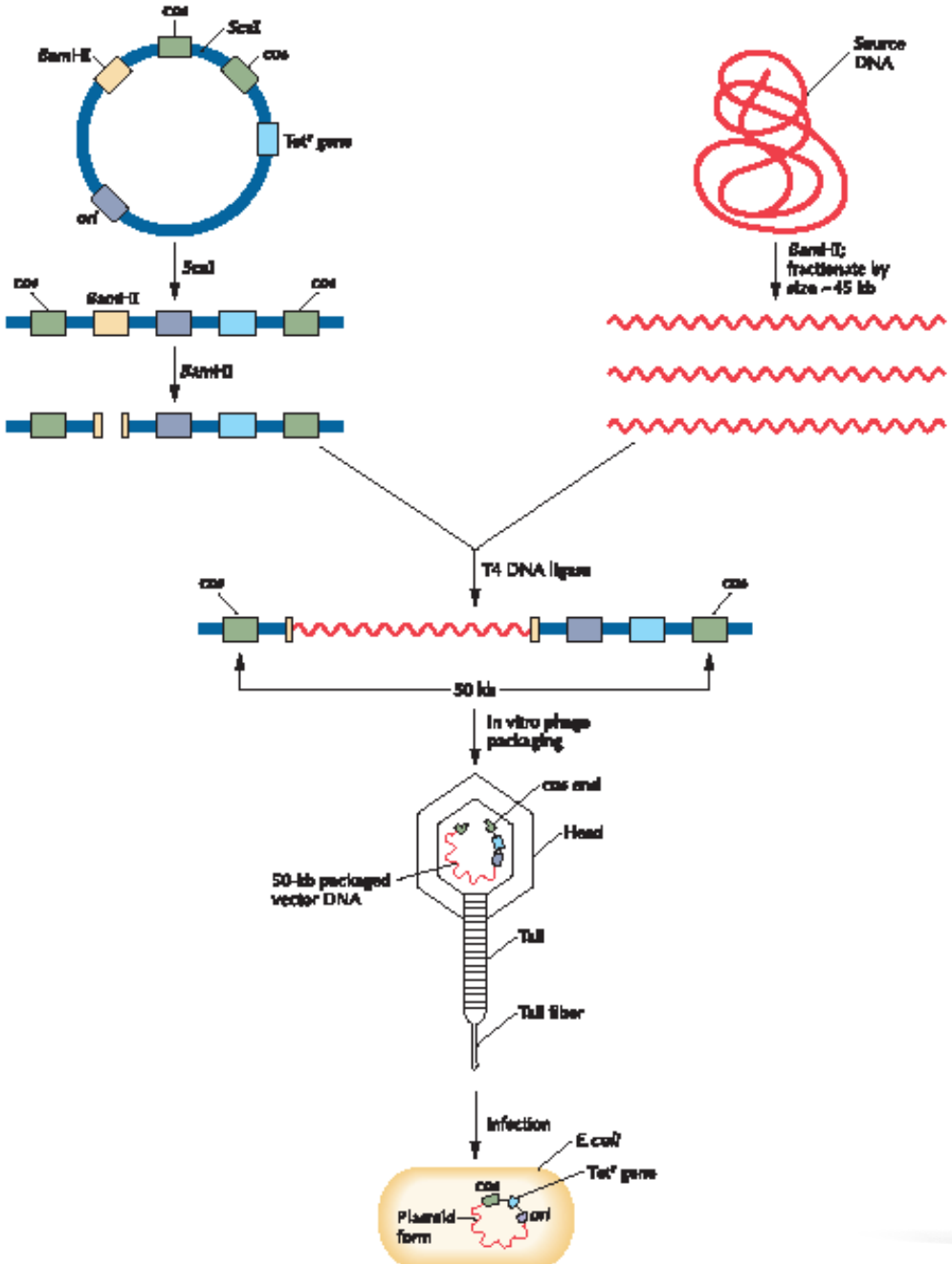




•Cosmid library

Allows cloning of 45 kb DNA fragments





•YACs:Yeast Artificial C s

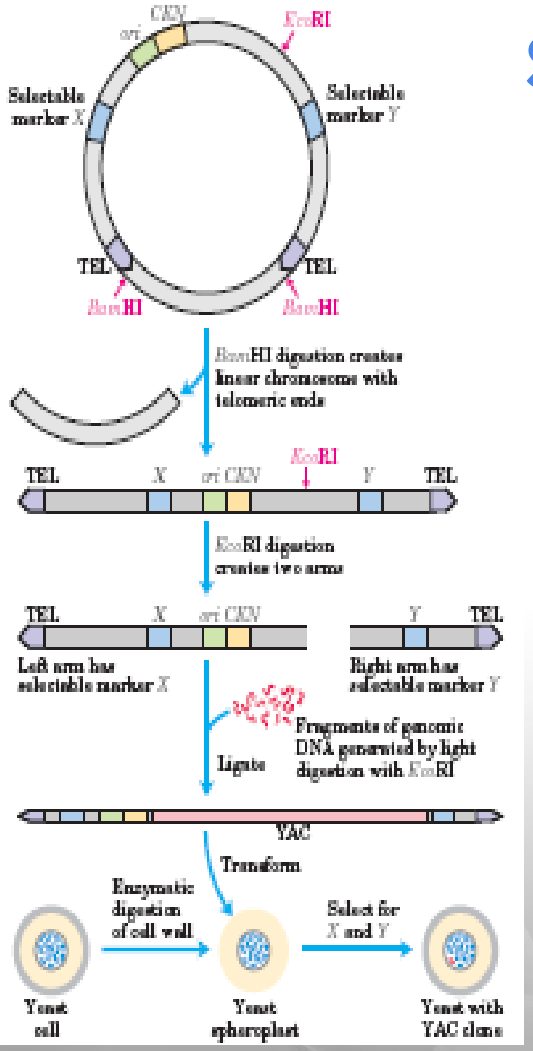
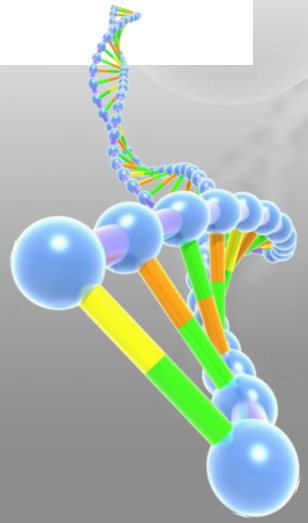
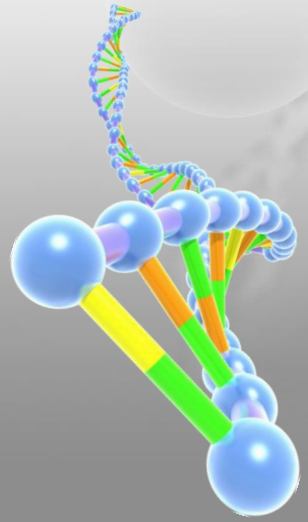


FIGURE 9–8 Construction of a yeast artificial chromosome (YAC). A YAC vector includes an origin of replication (ori), a centromere (CEN), two telomeres (TEL), and selectable markers (X and Y). Digestion with *Bam*H1 and *Eco*RI generates two separate DNA arms, each with a telomeric end and one selectable marker. A large segment of DNA (e.g., up to 2×10^6 bp from the human genome) is ligated to the two arms to create a yeast artificial chromosome. The YAC transforms yeast cells (prepared by removal of the cell wall to form spheroplasts), and the cells are selected for *X* and *Y*; the surviving cells propagate the DNA insert.

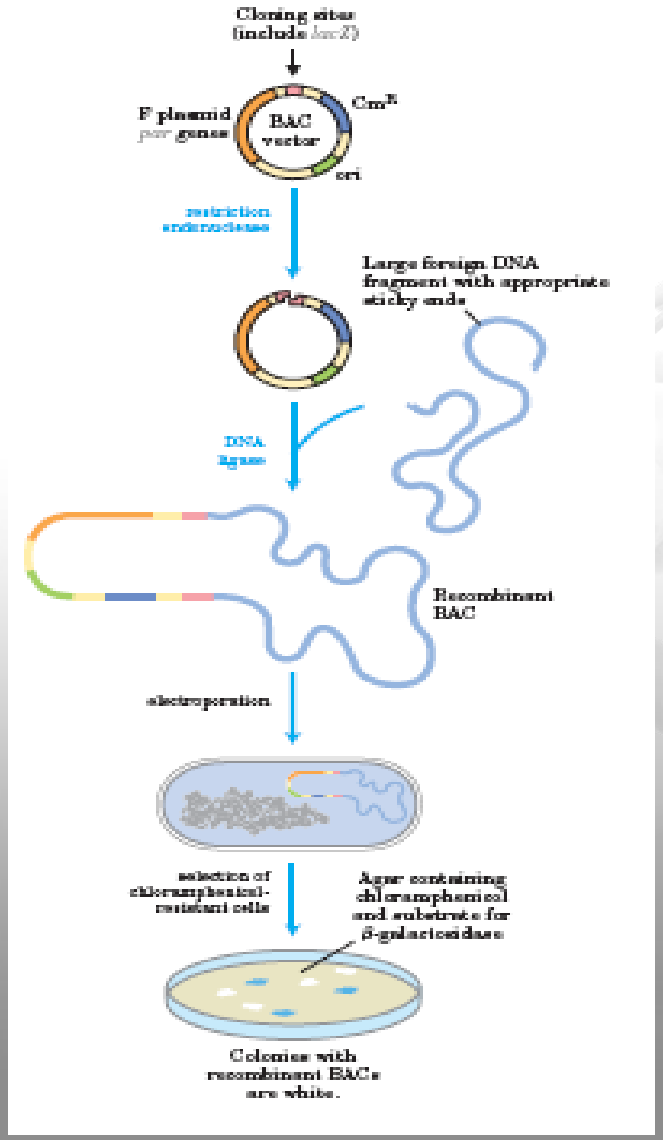


•BACs: Bacterial Artificial Chromosomes

- Based on P1 bacteriophage, the F plasmid and the lacZ region of pUC plasmids
- It's a low copy number plasmid
- Carries 50-300kb fragments



•BACs: Bacterial Artificial Chromosomes



Bacterial Artificial Chromosome DNA Recombinant

- Manipulation of BAC has been widely useful method to produce genomic DNA fragments for studying gene expression and function *in vitro* and *in vivo*
- In order the to accurately recapitulate the endogenous pattern of gene expression, it is necessary that all regulatory elements for each gene is observe
- The proper expression of large DNA transgene in transgenic mammal was shown by their ability to rescue mutant phenotype after reintroduction into the genome

Design BAC Recombinant Manipulation

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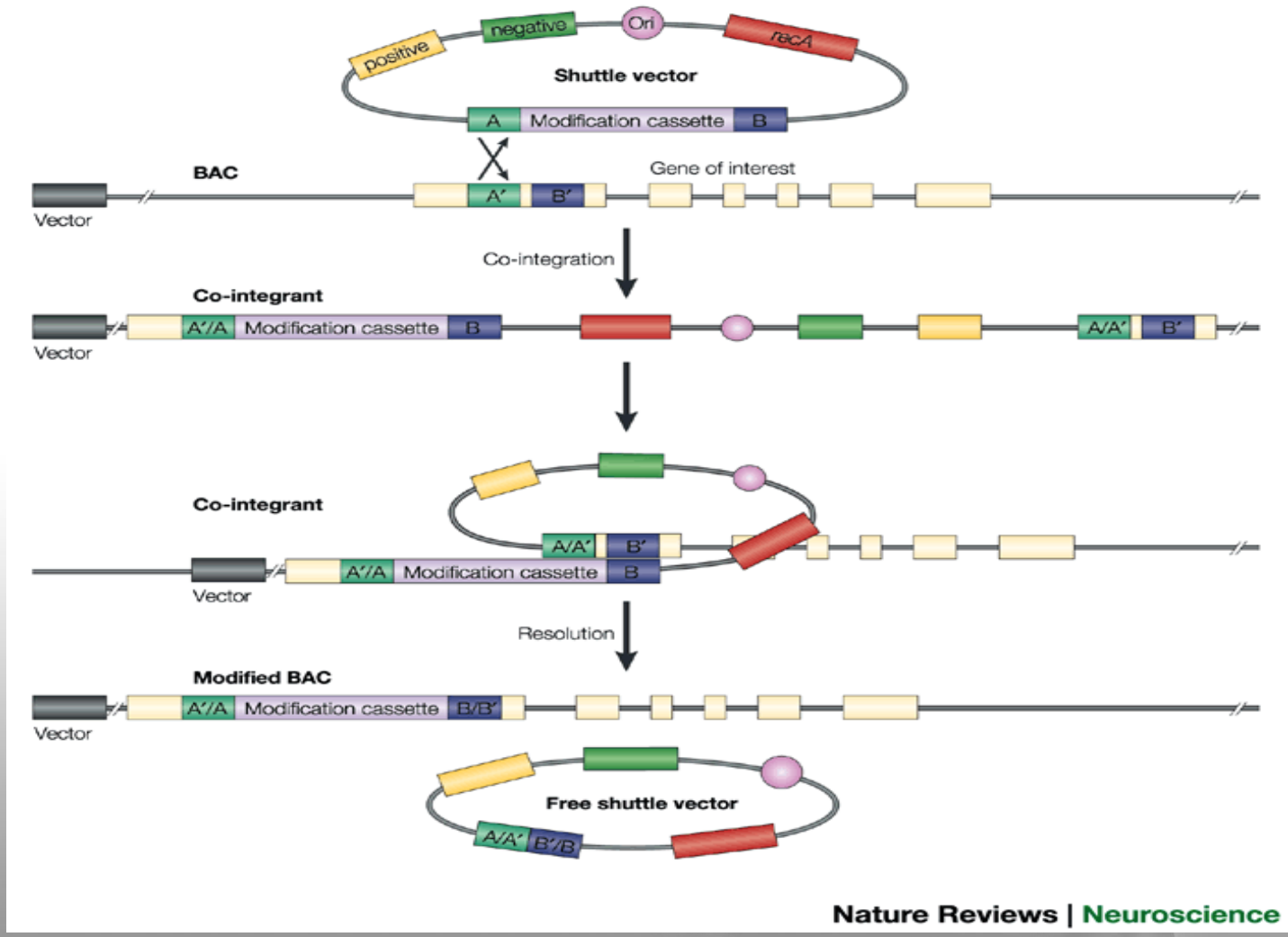
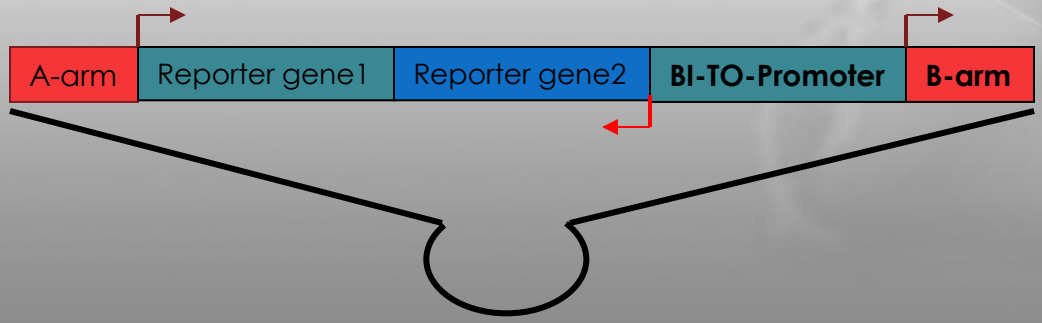
An accent, click to edit the text inside.

BAC Recombinant Manipulation

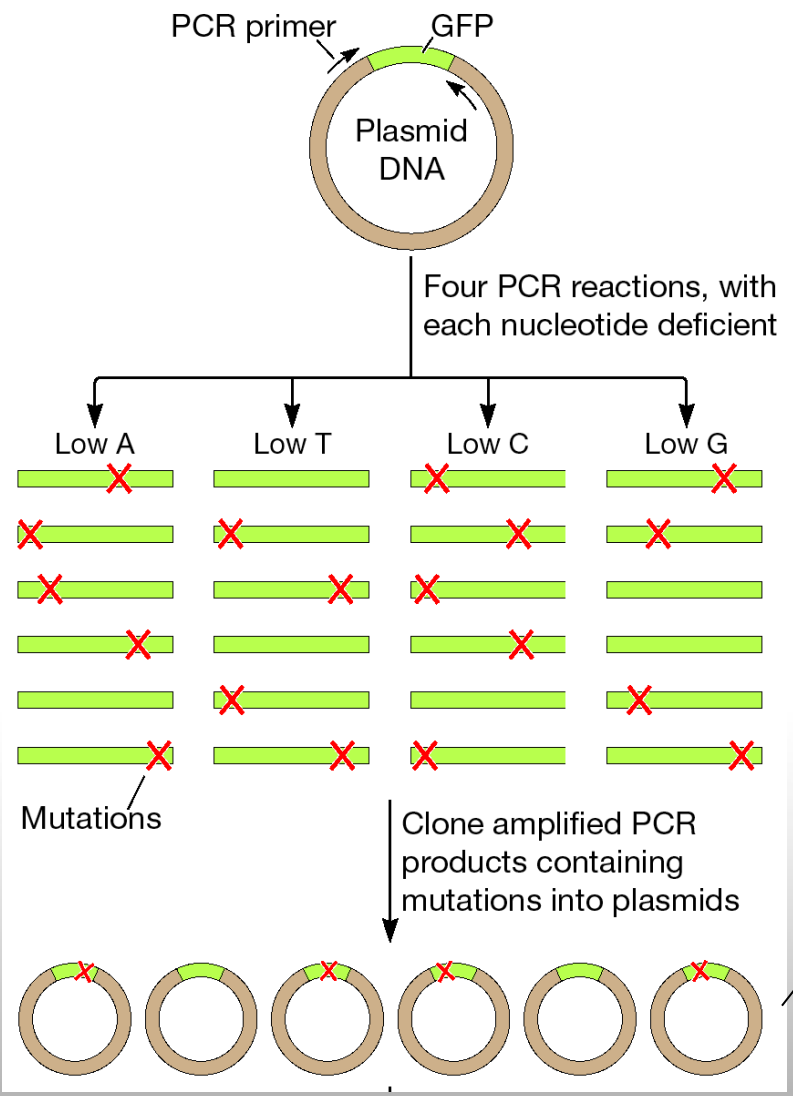


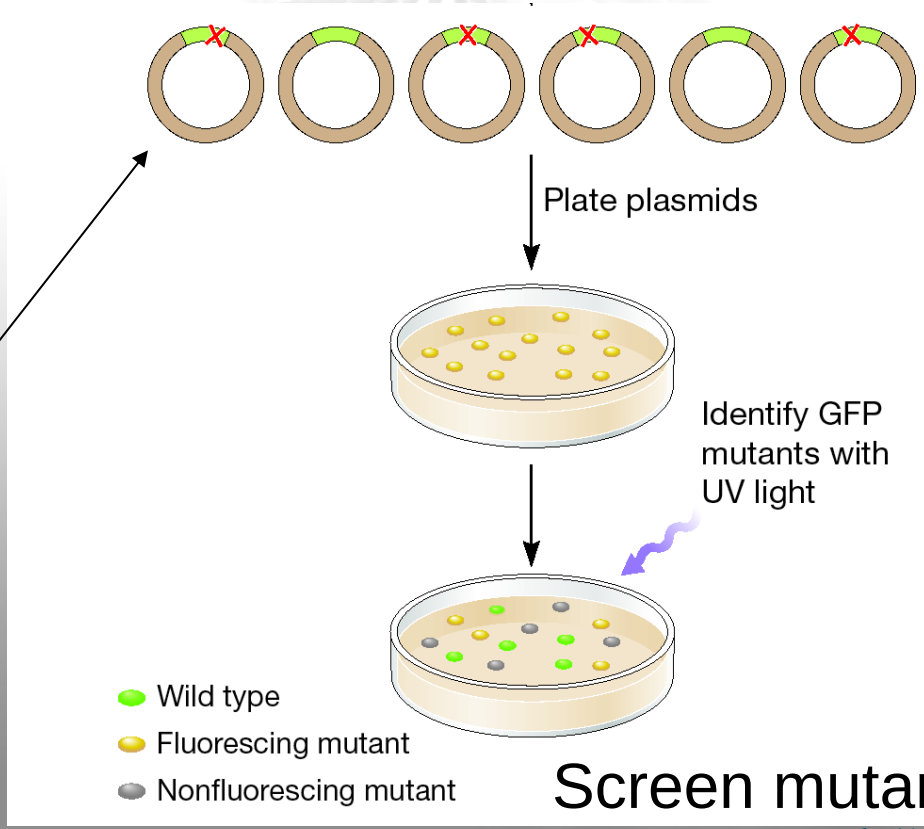
Modification Cassette

1. There are many functional element include: reporter gene, recombination enzyme, regulatory component, etc.
2. The use reporter gene can allow the polysistronic mRNA of target gene which synthesize under the control of the endogenous regulatory sequences.
3. Multiple cloning and combined with PCR to recognize the junction of start codon from endogenous regulatory and reporter genes.



Random mutagenesis by PCR: the Green Fluorescent Protein

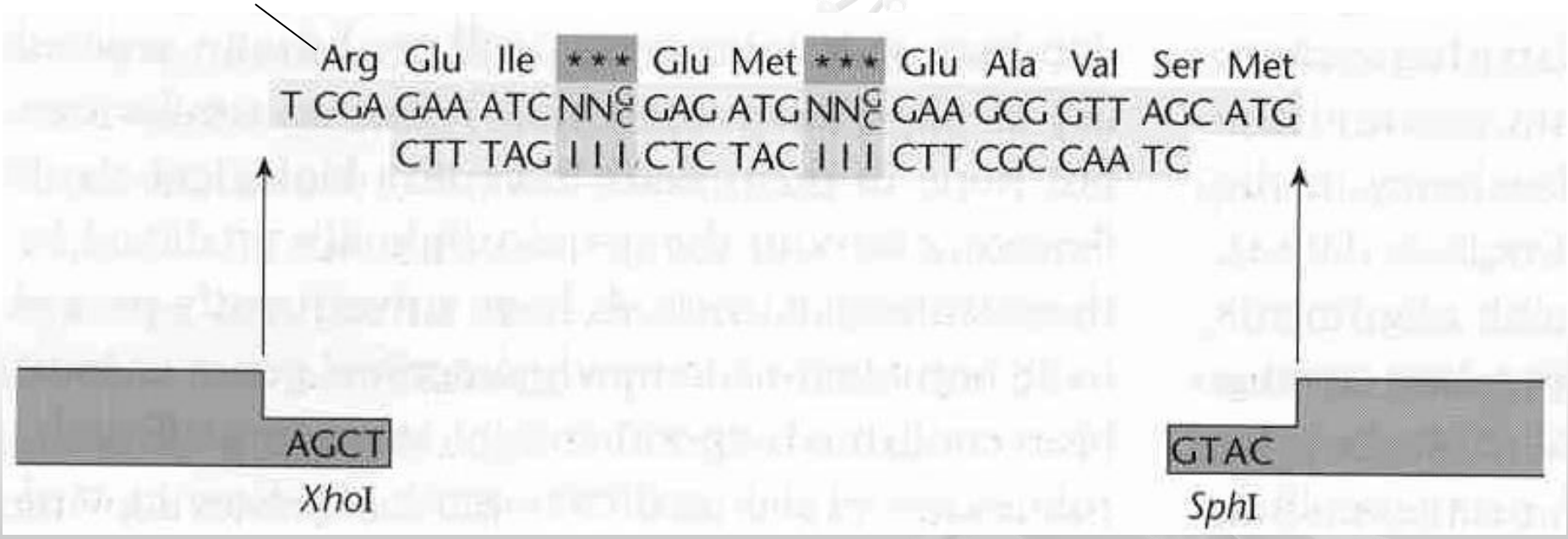




Screen mutants

•Cassette mutagenesis (semi-random)

Translation of sequence

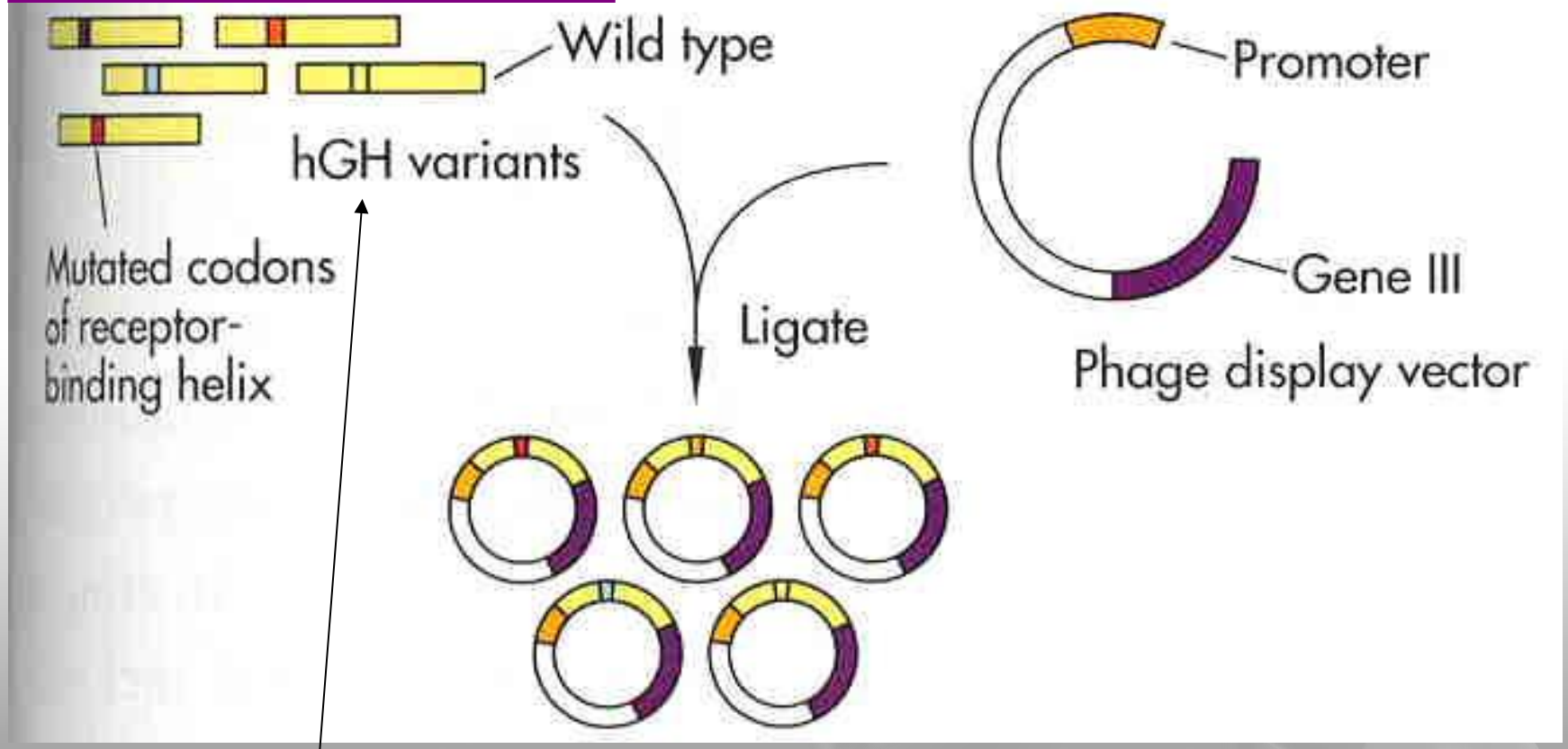


Strands synthesized individually, then annealed

Allows random insertion of any amino acid at defined positions

•Screening by phage display: create library of mutant proteins fused to M13 gene III

Random mutagenesis



Human growth hormone: want to generate variants that bind to hGH receptor more tightly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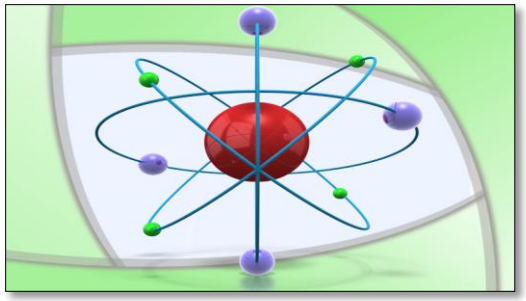


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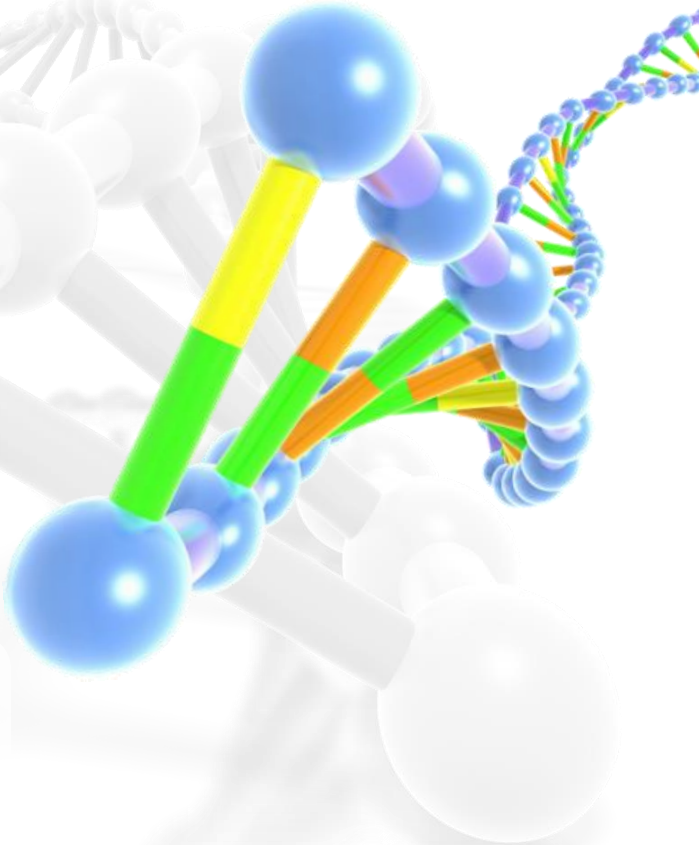
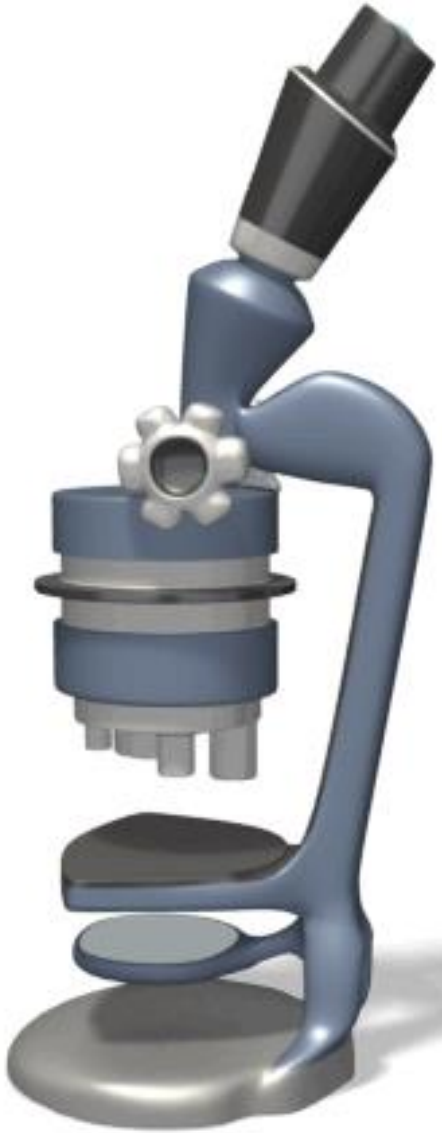


Thank you



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