Recombinant DNA technology

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Pharmacology II

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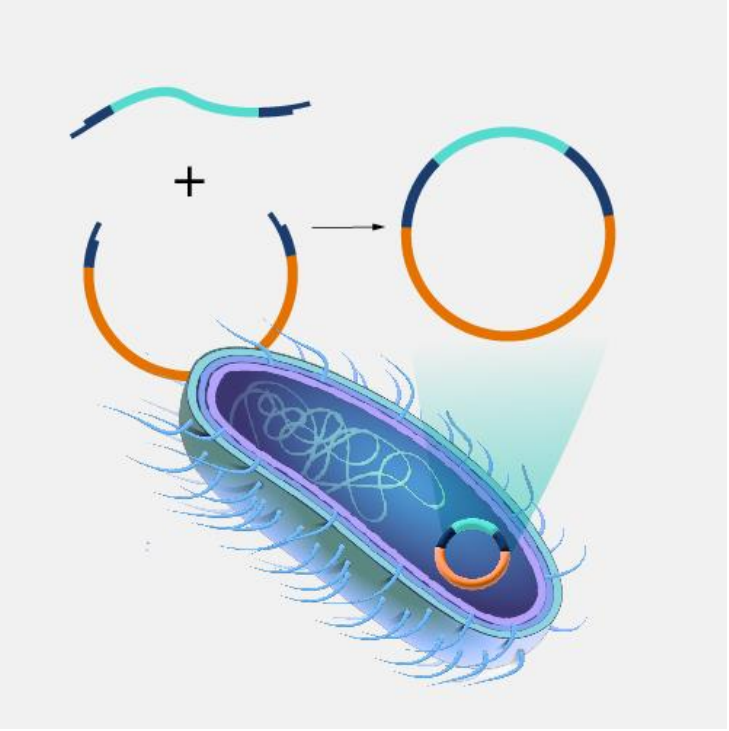
DAMCOP



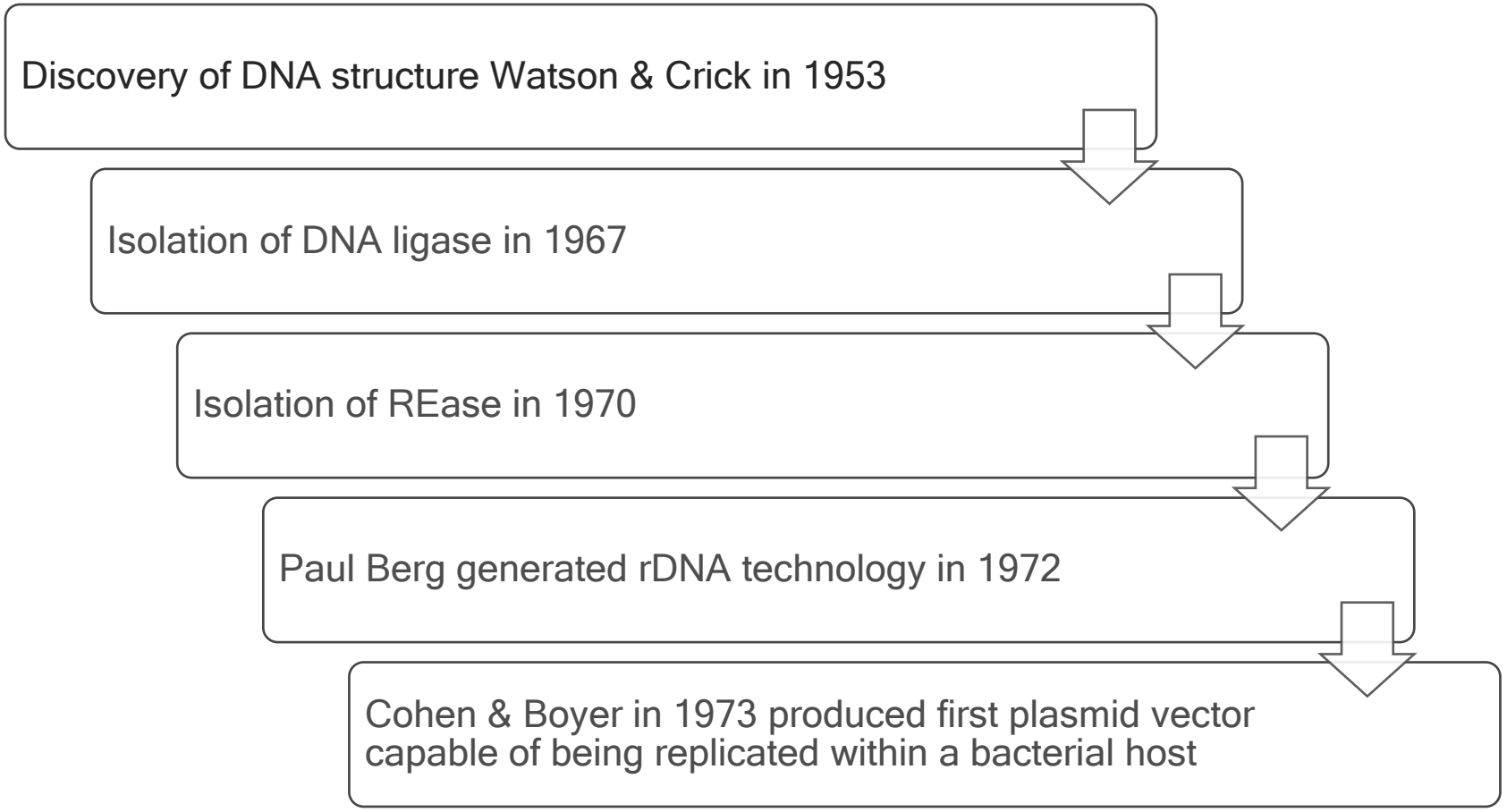
Contents

Recombinant DNA technology

DNA molecules that are extracted from differe nt sources and chemically joined together;

Eg:-, DNA comprising an animal gene may be recombined with DNA from a bacterium

Discovery of recombinant DNA technology



Goals of recombinant DNA technology

To isolate and characterize a gene

To make desired alterations in one or more isolated genes

To return altered genes to living cells

Artificially synthesize new gene

Alternating the genome of an organism

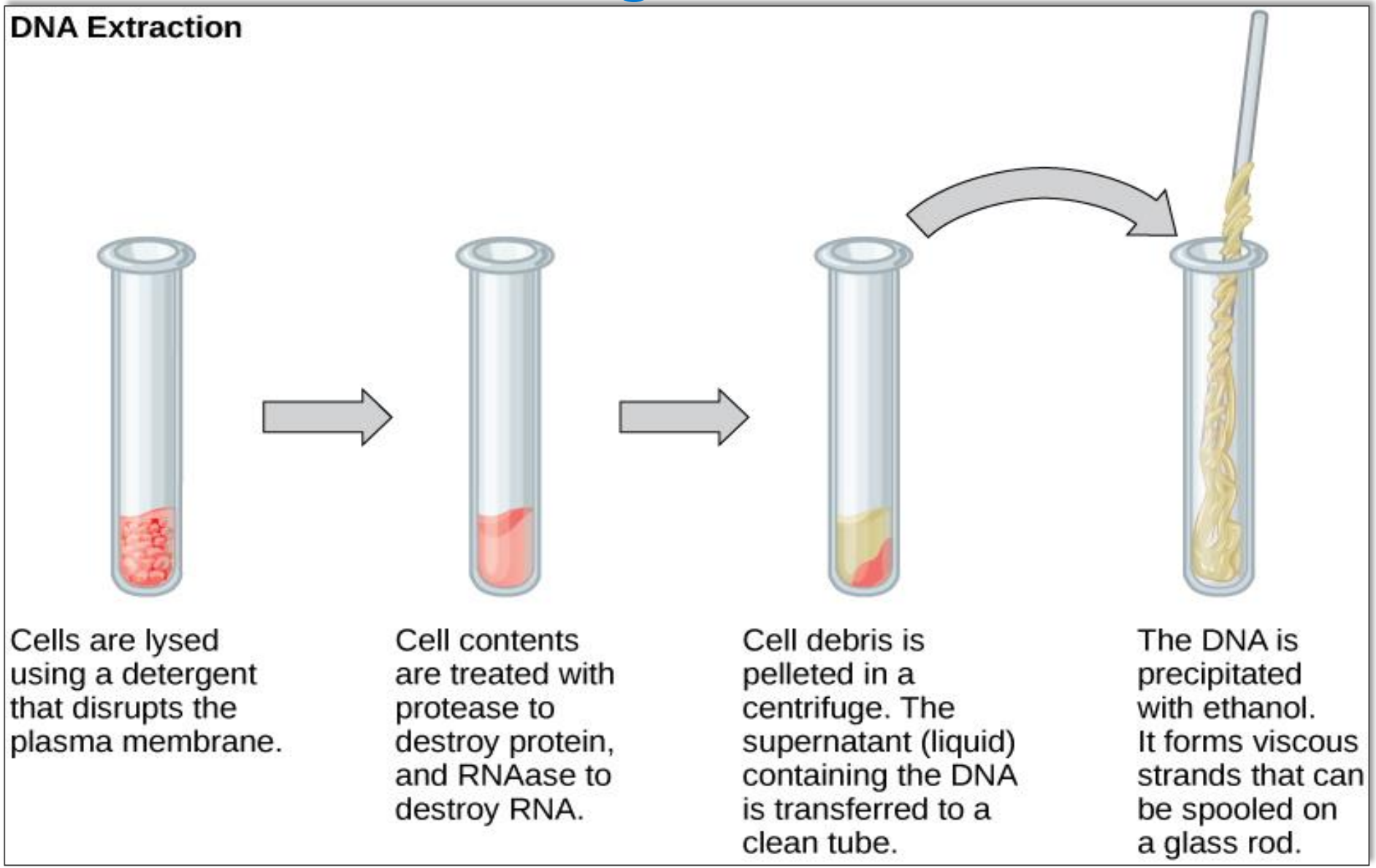
Understanding the hereditary diseases and their cure

Improving human genome

Procedure of making rDNA

- Isolating of DNA
- Cutting of DNA
- Joining of DNA
- Amplifying of DNA

Isolating of DNA



Cutting of DNA

DNA can be cut into large fragments by mechanical shearing.

Restriction enzymes are the scissors of molecular genetics.

Restriction enzyme

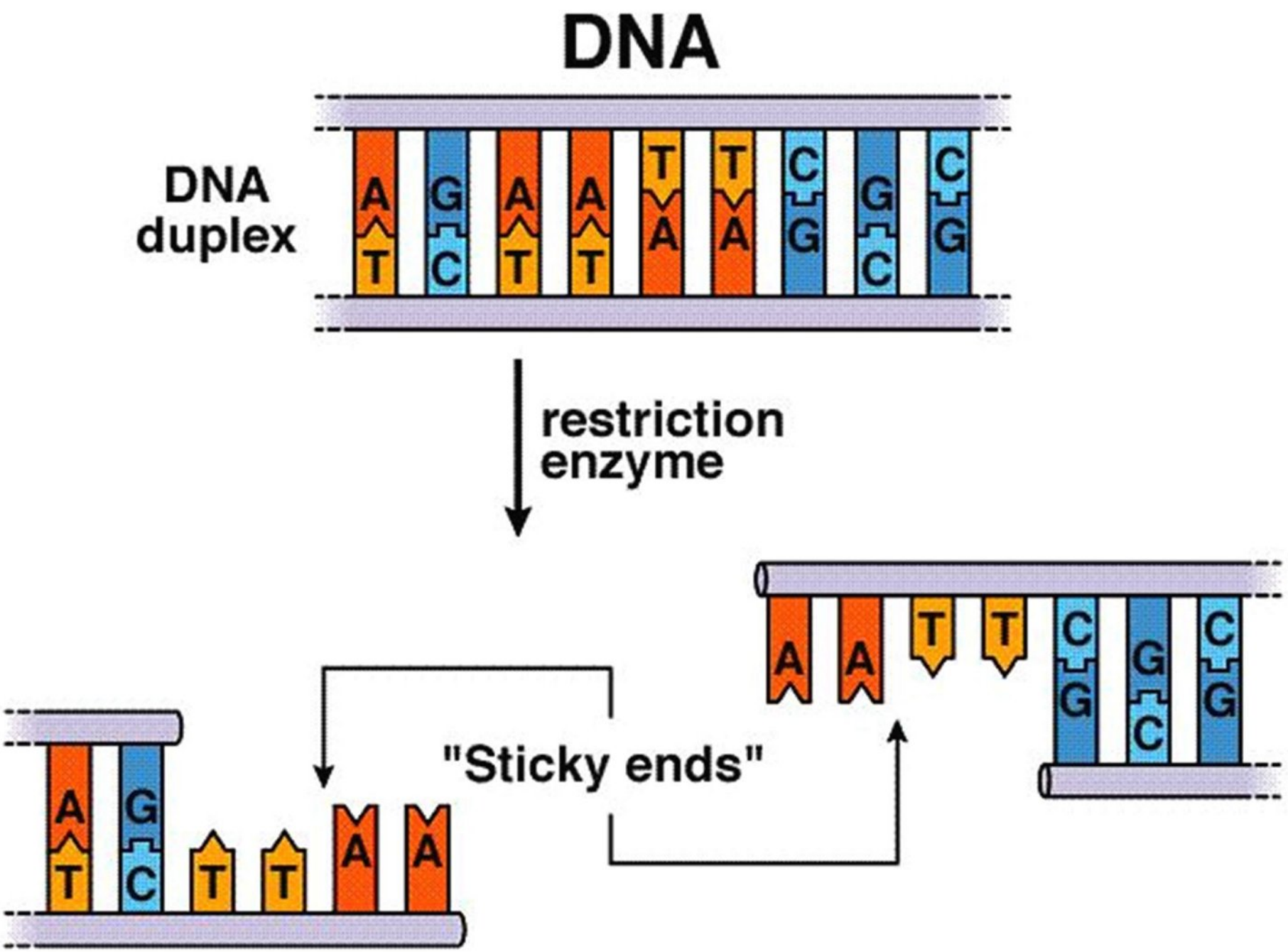
Found in bacteria

A special class of sequence-specific enzyme

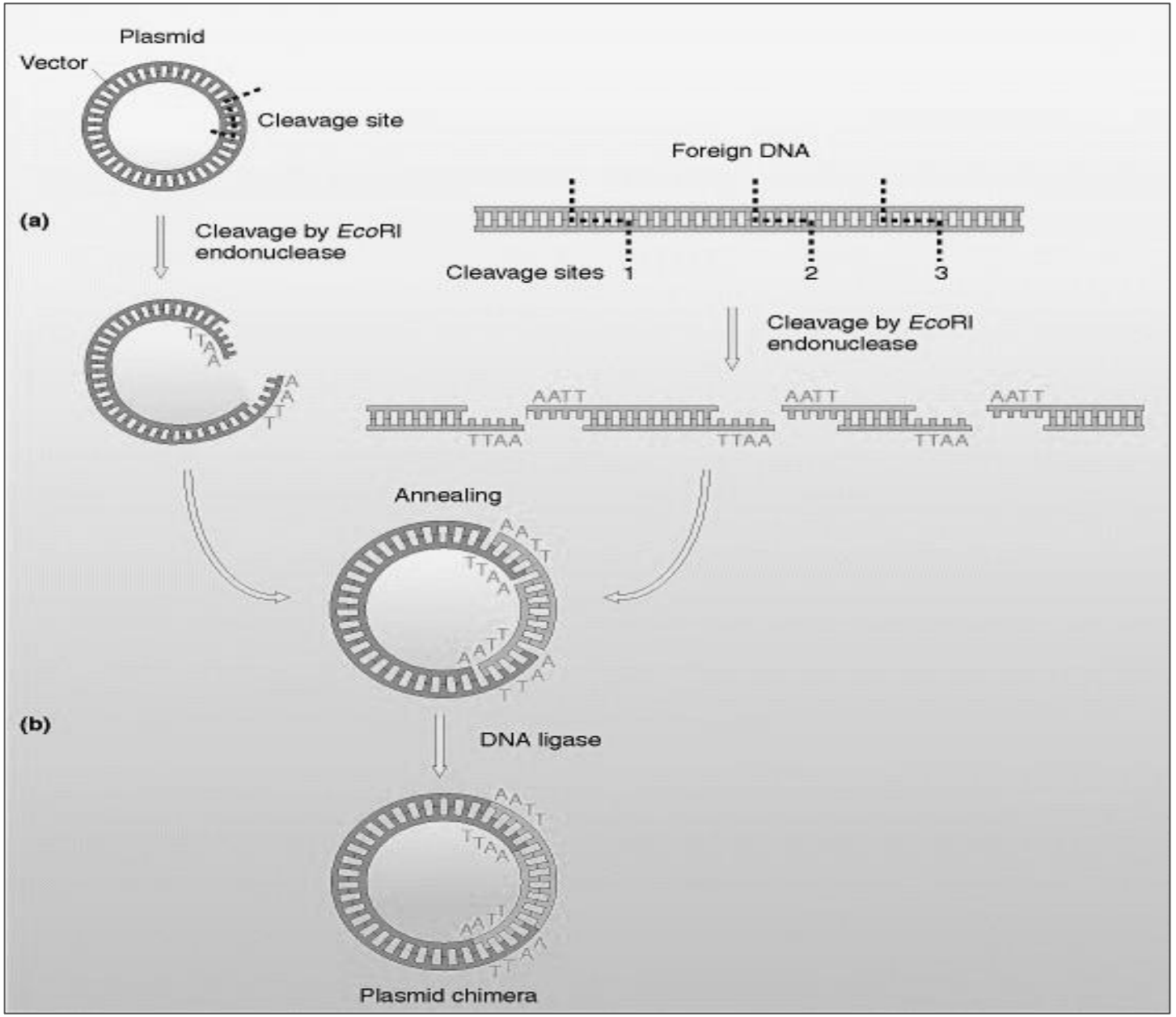
Site-specific-cleave DNA molecules only at specific nucleotide sequence

REases recognize DNA base sequence that are palindrome

REase make staggered cuts with complementary base sequences for easy circulization



Joining DNA


Plasmid
Vector
Cleavage site
(a)
Cleavage by EcoRI endonuclease
Foreign DNA
Cleavage sites 1 2 3
Cleavage by EcoRI endonuclease
AATT
TTAA
Annealing
(b)
DNA ligase
Plasmid chimera

Amplifying the recombinant DNA

Transforming the recombinant DNA into a bacterial host strain.

The cells are treated with $CaCl_2$

DNA is added

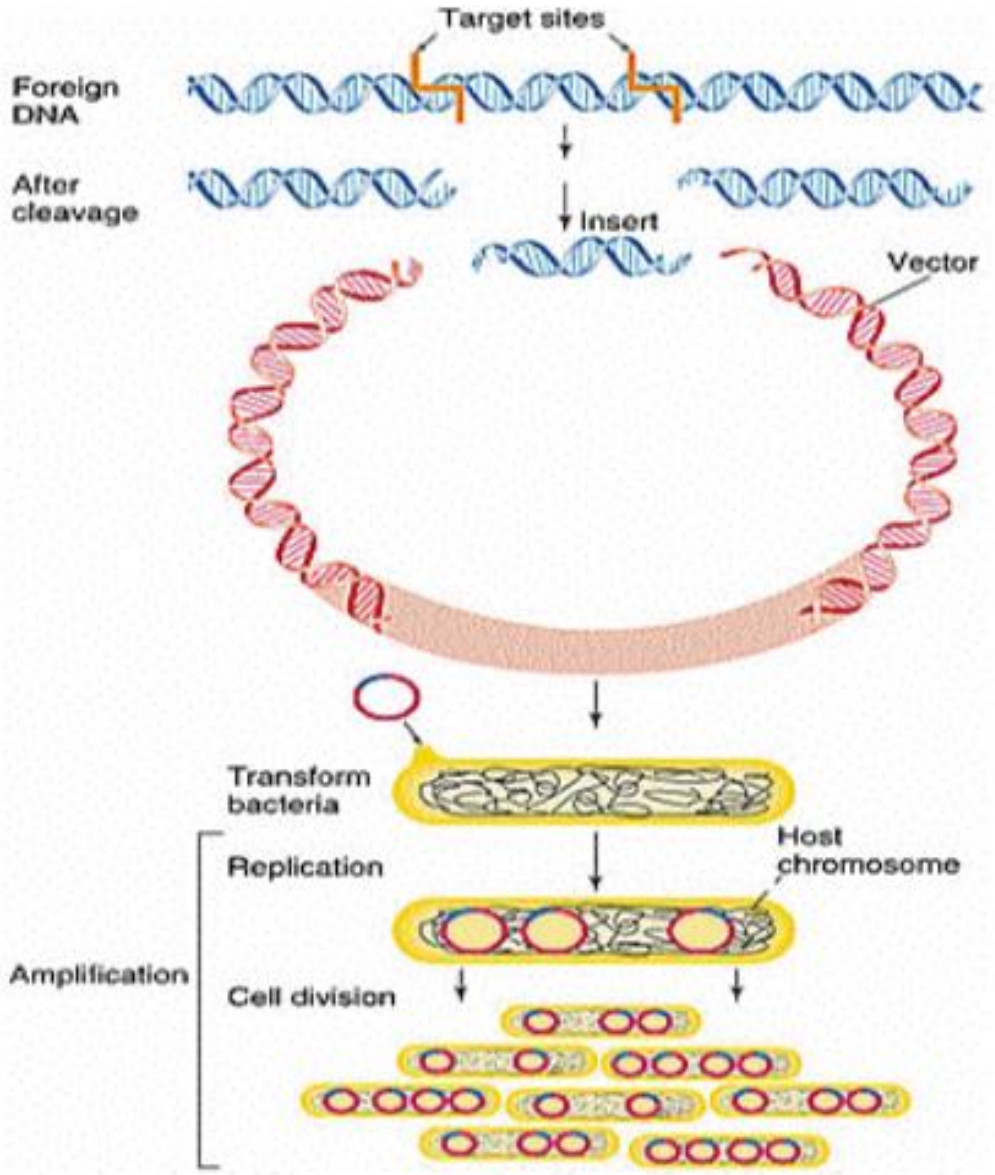
Cells are heat shocked at 42 C

DNA goes into cell by a somewhat unknown mechanism.

Once in a cell, the recombinant DNA will be replicated.

When the cell divides, the replicated recombinant molecules go to both daughter cells which themselves will divide later. Thus, the DNA is amplified

Amplifying the recombinant DNA



Enzymes used in recombinant DNA technology

DNA ligase	Bind to DNA molecules
Type II restriction endonuclease	Cleaves DNA at specific sites
Reverse transcriptase	Make a DNA copy of RNA molecule
DNA polymerase I	Fill single stranded gapes of DNA duplex
Polynucleotide Kinase	Adds a phosephate to the 5'-OH end of a polynucleotide
Terminal transferase	Adds homopolymer tails to the 3'-OH ends
Exonuclease III	Removes nucleotide residues from the 3' ends
Bacteriophage {lamda} exonuclease	removes nucleotides from the 5' ends
Alkaline phosphatase	Removes terminal phosphates

Vectors used in rDNA technology

A vector is an area of DNA that can join another DNA part without losing the limit for self-replication

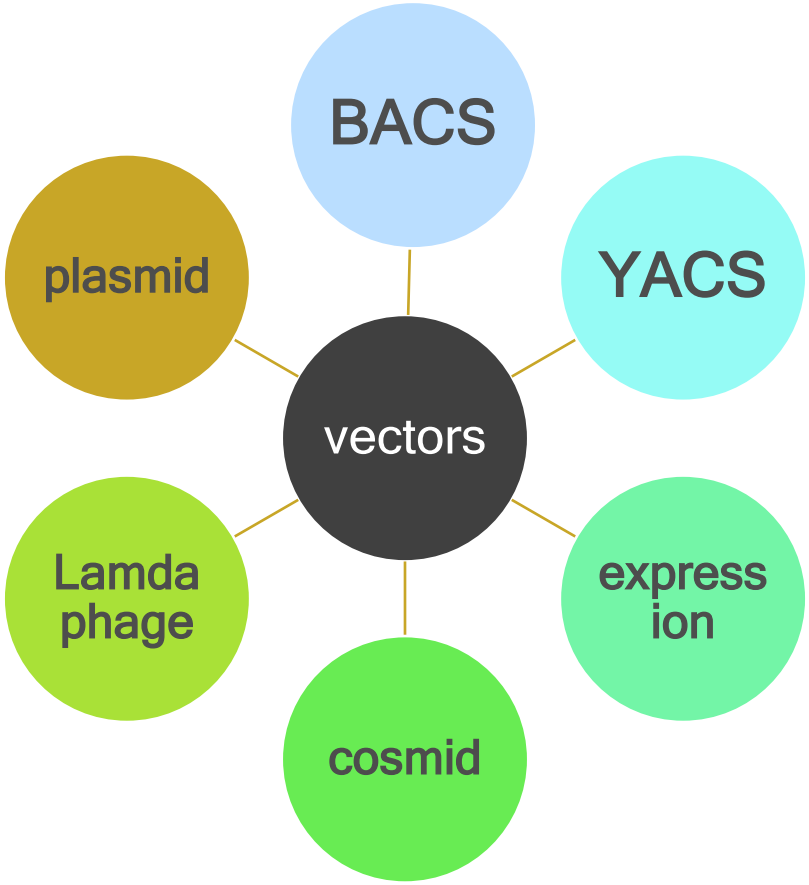
Should be capable of replicating in host cell

Should have convenient RE sites for inserting DNA of interest

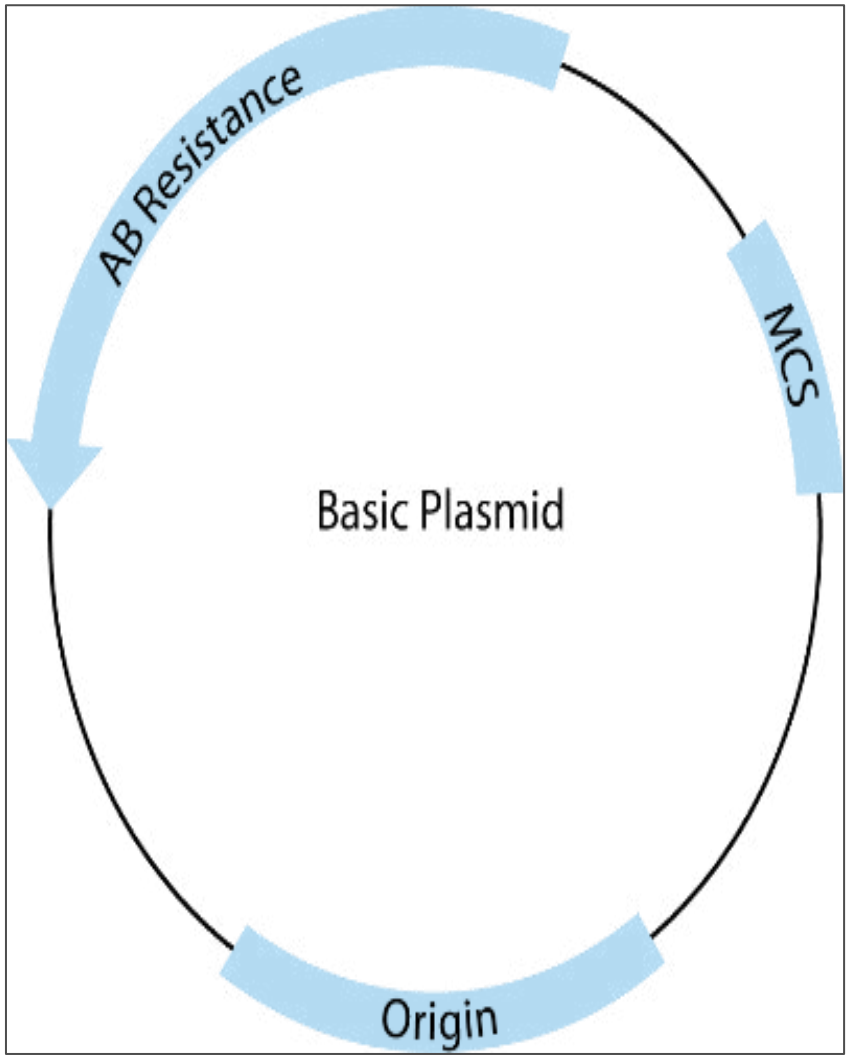
Should have a selectable marker to indicate which host cells received recombinant DNA molecule

Should be small and easy to isolate

Vectors used in rDNA technology



Plasmid vector



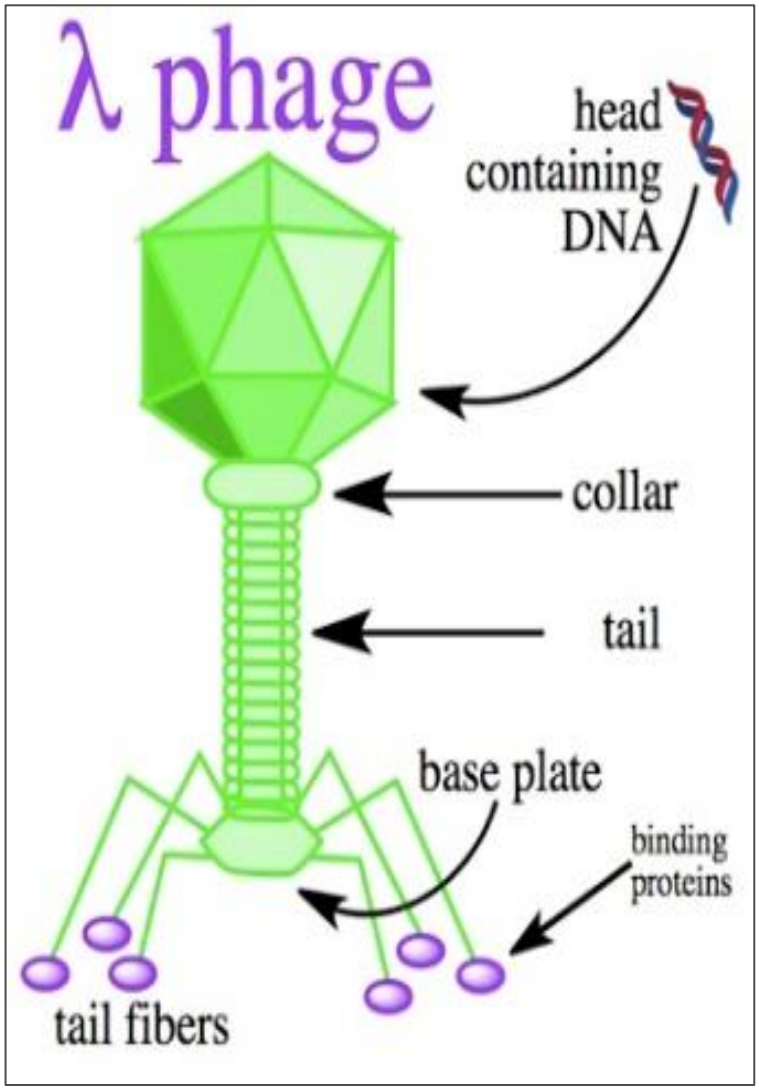
Plasmids are small, circular DNA molecules that are separate from the rest of the chromosome.

They replicate independently of the bacterial chromosome.

Useful for cloning DNA inserts less that 20 kb (kilobase pairs).

Inserts larger than 20 kb are lost easily in the bacterial cell.

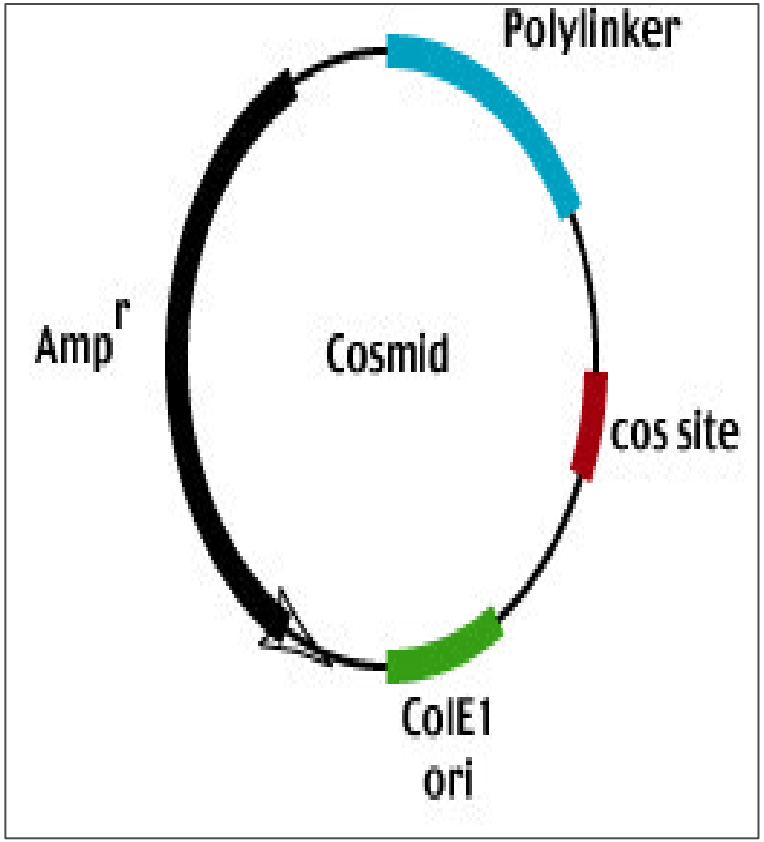
Lamda phage vector



Lamda phage vectors are recombinant infections, containing the phage chromosome in addition to embedded "outside" DNA.

All in all, phage vectors can convey bigger DNA groupings than plasmid vectors.

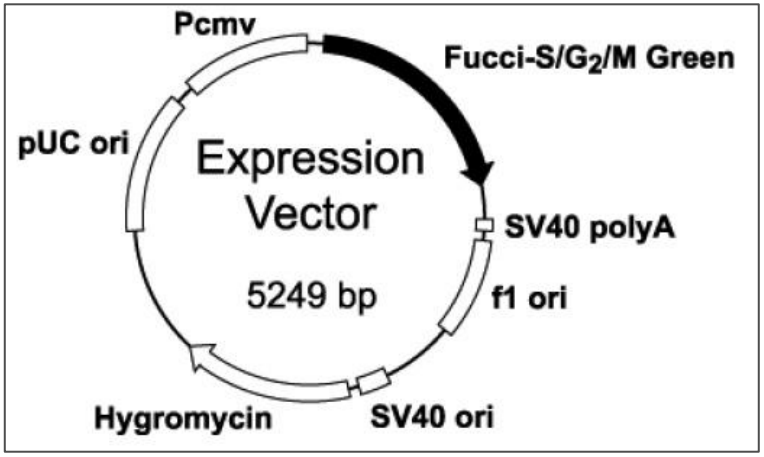
Cosmid vector



Cosmids are hybrids of phages and plasmids that can carry DNA fragments up to 45 kb.

They can replicate like plasmids but can be packaged like phage lambda

Expression vectors



Expression vectors are vectors that carry host signals that facilitate the transcription and translation of an inserted gene.

They are very useful for expressing eukaryotic genes in bacteria.

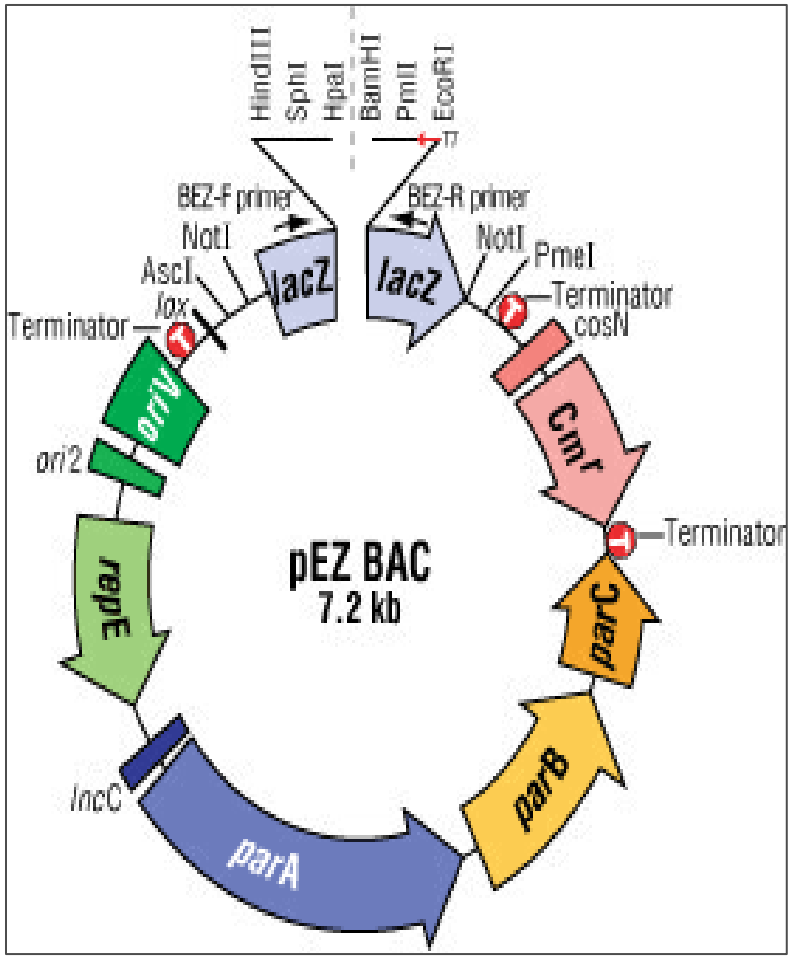
Yeast artificial chromosomes (YACS)

Yeast artificial chromosomes (YACS) are yeast vectors that have been engineered to contain a centromere, telomere, origin of replication, and a selectable marker.

They can carry up to 1,000 kb of DNA.

They are useful for cloning eukaryotic genes that contain introns.

Bacterial artificial chromosomes (BACS



Bacterial artificial chromosomes (BACS) are bacterial plasmids derived from the F plasmid.

They can carry up to 300 kb of DNA.

Techniques used in rDNA technology

1. Gel electrophoresis
2. Cloning libraries
3. Restriction enzyme mapping
4. PCR
5. Nucleic Acid Hybridization
6. DNA Microarrays

Gel electrophoresis

- **Gel electrophoresis** – DNA fragments of different sizes can be separated by an electrical field applied to a "gel".
- The negatively charged DNA migrates away from the negative electrode and to the positive electrode.
- The smaller the fragment the faster it migrates.

Cloning libraries

Libraries are collection of DNA clones in a certain vector.

The goal is to have each gene represented in the library at least once.

Genomic - made from RE DNA fragments of total genomic DNA

cDNA (complementary DNA) – made from DNA synthesized from mRNA

PCR

- Allows the isolation of a specific segment of DNA from a small DNA (or cell sample) using DNA primers at the ends of the segment of interest.

Restriction enzyme mapping

Frequently it is important to have a restriction enzyme site map of a cloned gene for further manipulations of the gene.

This is accomplished by digestion of the gene singly with several enzymes and then in combinations.

The fragments are subjected to gel electrophoresis to separate the fragments by size and the sites are deduced based on the sizes of the fragments.

Nucleic Acid Hybridization

A Southern allows the detection of a gene of interest by probing DNA fragments that have been separated by electrophoresis with a "labeled" probe.

Northern Blot (probe RNA on a gel with a DNA probe)

Western Blot (probe proteins on a gel with an antibody)

DNA Microarrays

Vast majority of the protein-encoding qualities onto a microarray chip, utilizing innovation in light of the DNA silicon chip industry.

The chip can be utilized to hybridize to cell RNA, and measure the statement rates of a substantial number of qualities in a cell.

Applications of rDNA technology

1. **Agriculture:** growing crops of your choice (GM food), pesticide resistant crops, fruits with attractive colors, all being grown in artificial conditions
2. **Pharmacology:** artificial insulin production, drug delivery to target sites
3. **Medicine:** gene therapy, antiviral therapy, vaccination, synthesizing clotting factors
4. **Other uses**:fluorescent fishes, glowing plants etc


Thank
you