# Restriction endonuclease

**Molecular scissor.**

**Recognise and cut specific nucleotide sequence (4-8 bp long) in DNA.**

| Microbial origin | Enzyme | Recognition site |
|---|---|---|
| 1. *Escherichia coli* | EcoRI | 5' G↓AA TTC 3'<br>3' CTT AA↑G 5' |
| 2. *Hemophilus influenza* | HindIII | 5' A↓AG CTT 3'<br>3' TTC GA↑A 5' |
| 3. *Serratia marcescens* | SmaI | 5' CCC↓GGG 3'<br>3' GGG↑CCC 5' |
| 4. *Hemophilus parainfluenzae* | HpaII | 5' C↓C GG 3'<br>3' GG C↑C 5' |
| 5. *Hemophilus aegyptius* | HaeIII | 5' GG↓CC 3'<br>3' CC↑GG 5' |

# Examples of Restriction Enzymes

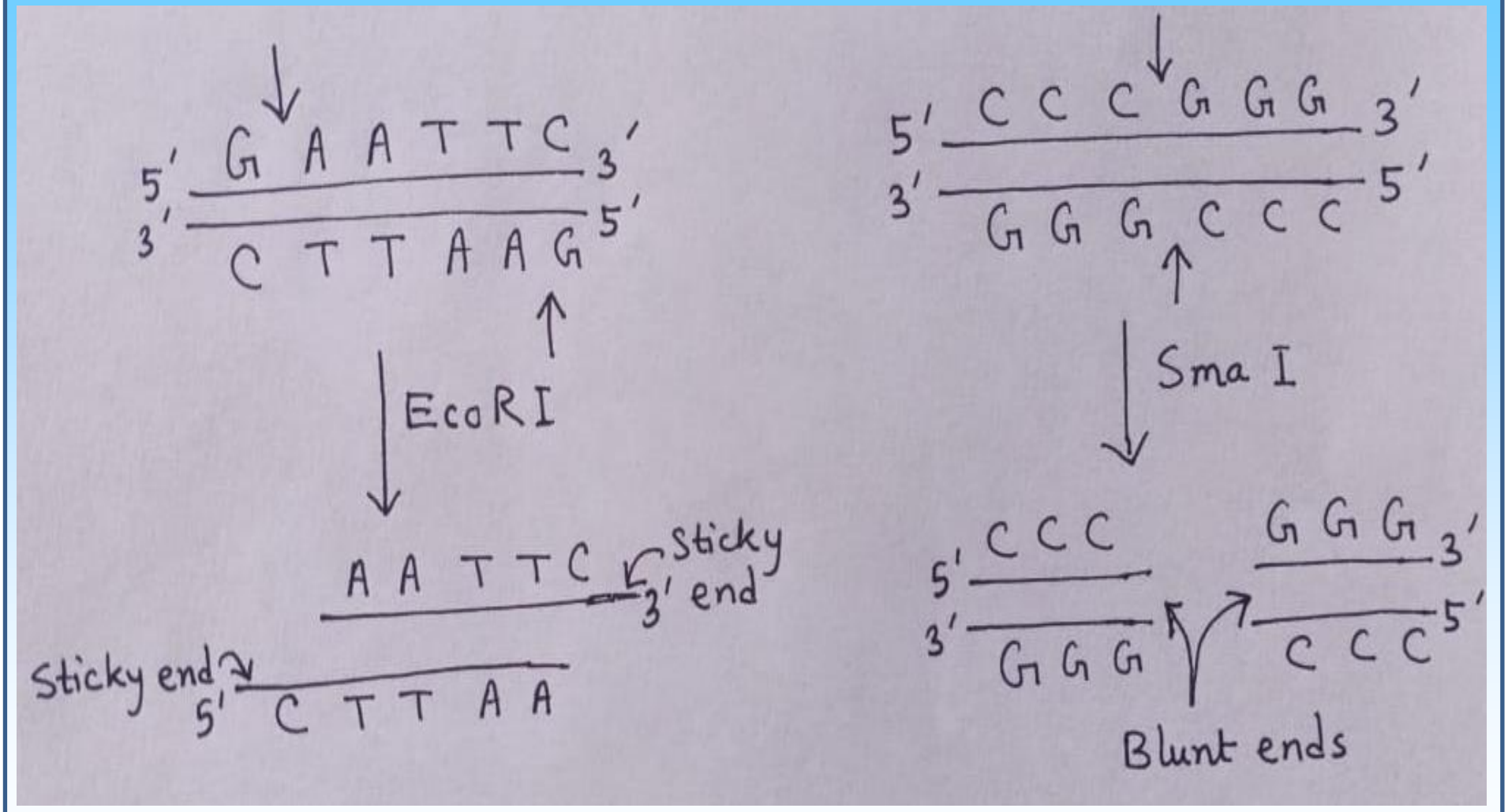
| Enzyme | Organism source | Recognized Sequence |
|---|---|---|
| TaqI | *Thermus aquaticus* | 5' **TC**GA 3'<br>3' AG**CT** 5' |
| BamHI | *Bacillus amyloliquefaciens* | 5' **GG**ATCC 3'<br>3' CCTA**GG** 5' |
| AluI | *Arthrobacter luteus* | 5' A**GC**T 3'<br>3' T**CG**A 5' |

| | | |
|---|---|---|
| *Mbo*I | *Moraxella bovis* | /GATC<br>GATC/ |
| *Nde*II | *Neisseria denitrificans* | /GATC<br>GATC/ |
| *Sau*I | *Staphylococcus aureus* | CC/TNAGG<br>GGANT/CC |
| *Bgl*I | *Bacillus globigii* | GCCNNNN/NGGC<br>CGGN/NNNNCCG |
| *Not*I | *Nocardia otitidis-caviarum* | GC/GGCCGC<br>CGCCGG/CG |
| *Dra*II | *Deinococcus radiophilus* | RG/GNCCY<br>YCCNG/GR |

## Depending upon mode of action 3 different types -

| Type I | Type II | Type III |
|---|---|---|
| DNA modification (methylation) capacity and DNA cleavage activity | Separate enzyme molecule for modification and restriction cleavage | DNA modification (methylation) capacity and DNA cleavage activity |
| Recognises specific base sequence but cut in DNA in a non-specific manner away from that sequence | Recognises specific palindromic sequence and make cut within that sequence | Recognises specific palindromic sequence and make cut within that sequence |
| No need of ATP | No need of ATP | ATP needed |
| EcoB, EcoK etc. | EcoRI, EcoRII, HindIII etc | HpaI, MboII, FokI etc. |

# Some enzymes introduce staggered cuts, others generate blunt ends



# Vector

Cloning of one DNA molecule with the help of another (vector) DNA molecule, which is capable of replicating in the host. Example- plasmid, a bacteriophage, a desired cosmid, a phagemid (phage+plasmid) or even a virus.

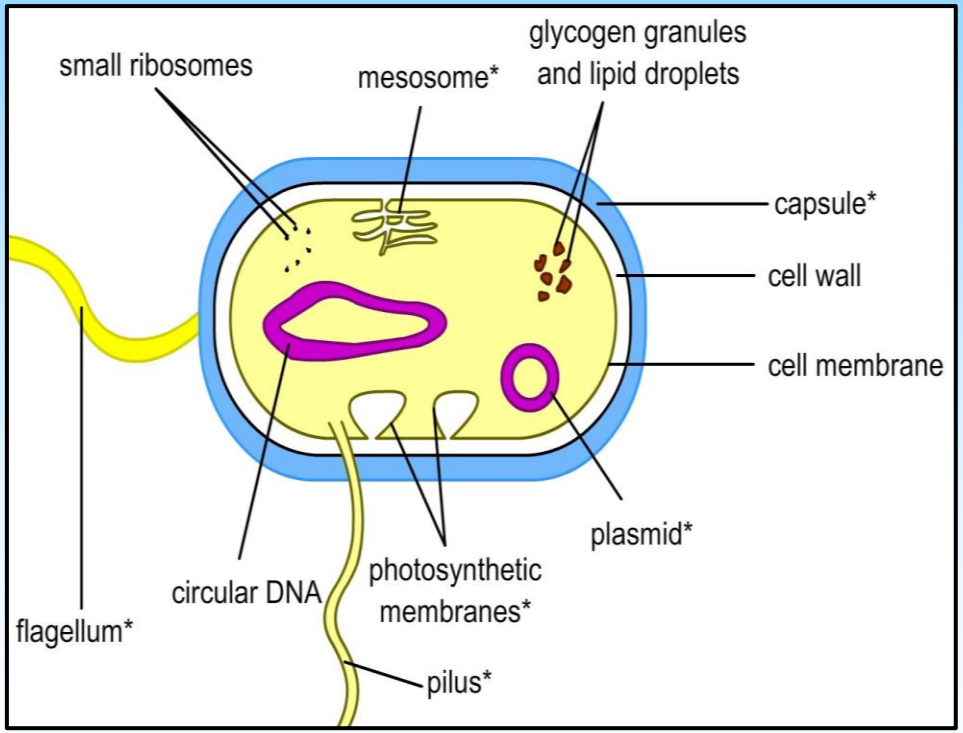
## Properties of a DNA molecule to serve as a cloning vector:

- Self replicating capacity of both inserted DNA and own DNA
- Should have number of restriction sites
- Should possess number of selectable marker sites for example different antibiotic resistant genes
- Should be easily purifyable

# Plasmid vector

Extrachromosomal, circular dsDNA (1-200) kb, replicate autonomously, accumulated upto 1000 copies.

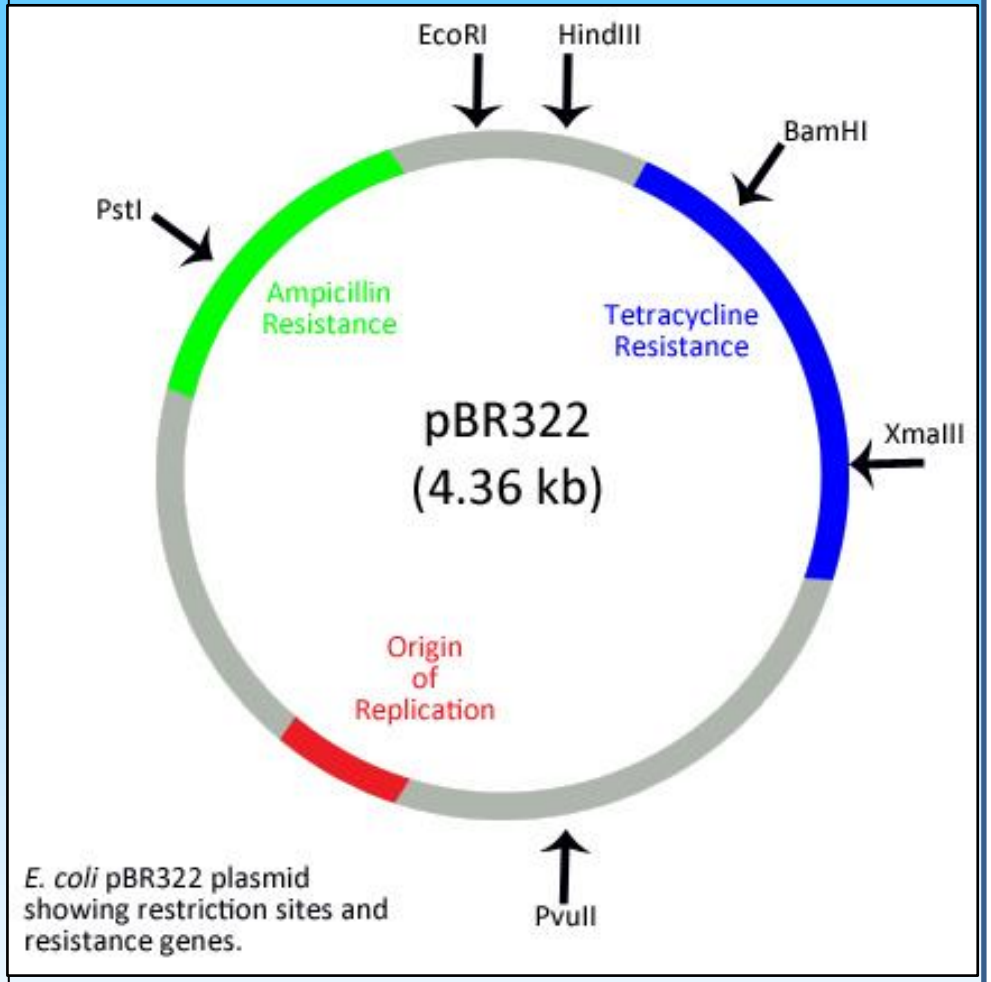
Restructured to make suitable cloning vector - Inserting genes for relaxed replication and/or by genes for antibiotic resistance.



## pBR 322

First artificial cloning vector developed from *E. coli* plasmid ColE1.

Most widely used. 4362bp long, have one site for origin of replication, two selectable marker gene and many unique restriction cleavage sites. Can accumulate upto 15-20 copies; may be amplified more.




*E. coli* pBR322 plasmid showing restriction sites and resistance genes.

Ti plasmid (from *Agrobacterium tumefaciens*) and Ri plasmid (from *A. rhizogenes*) are used to gene transfer in higher plants.

# Marker Genes

The genes which mark the transformed cells or tissues from the non-transformed one

Antibiotic resistance, herbicide resistance marker genes - *cat* gene, *npt* II gene, *bnl* gene

Plant metabolism related marker gene - phosphomannose isomerase, xylose isomerase

Two categories: selectable marker genes and nonselectable (also referred to as scorable marker or reporter) genes.

# Reporter Genes

Reporter genes usually report which cells are transgenic.

Such genes are cloned into the vector in close proximity to the gene of interest, to facilitate the identification of transformed cells as well as to determine the correct expression of the inserted gene. Example – beta-glucuronidase gene (gusA gene), green fluorescent protein (gfp), luciferase gene

## Steps of cloning technique

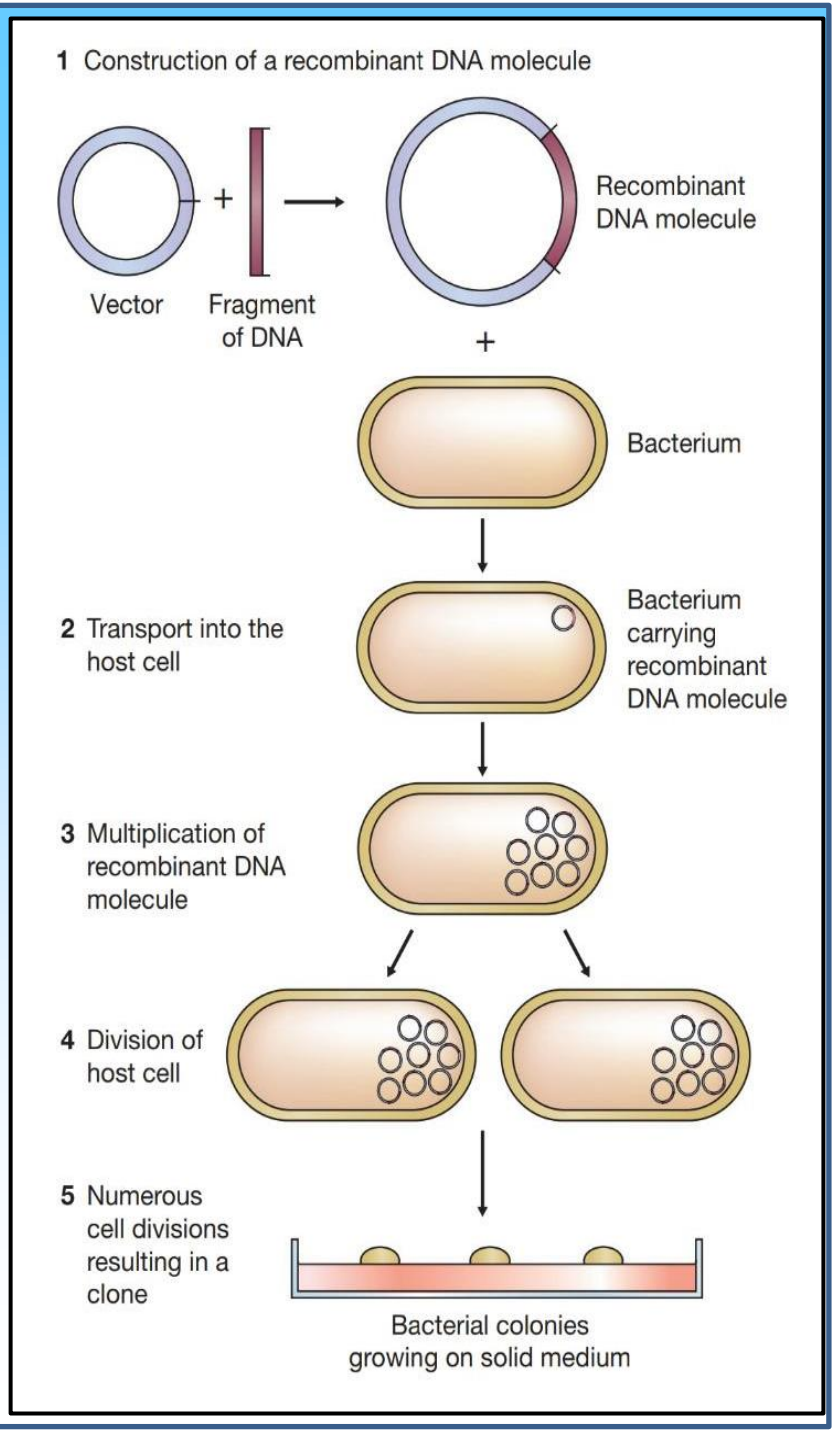
1. Fragment of DNA, containing the gene to be cloned, is inserted into a circular DNA molecule called a vector, to produce a recombinant DNA molecule.

2 Vector transports the gene into a host cell, which is usually a bacterium, although other types of living cell can be used.

3 Within the host cell the vector multiplies, producing numerous identical copies, not only of itself but also of the gene that it carries.

4 When the host cell divides, copies of the recombinant DNA molecule are passed to the progeny and further vector replication takes place.

5 After a large number of cell divisions, a colony, or clone, of identical host cells is produced. Each cell in the clone contains one or more copies of the recombinant DNA molecule. The gene carried by the recombinant molecule is now said to be cloned.

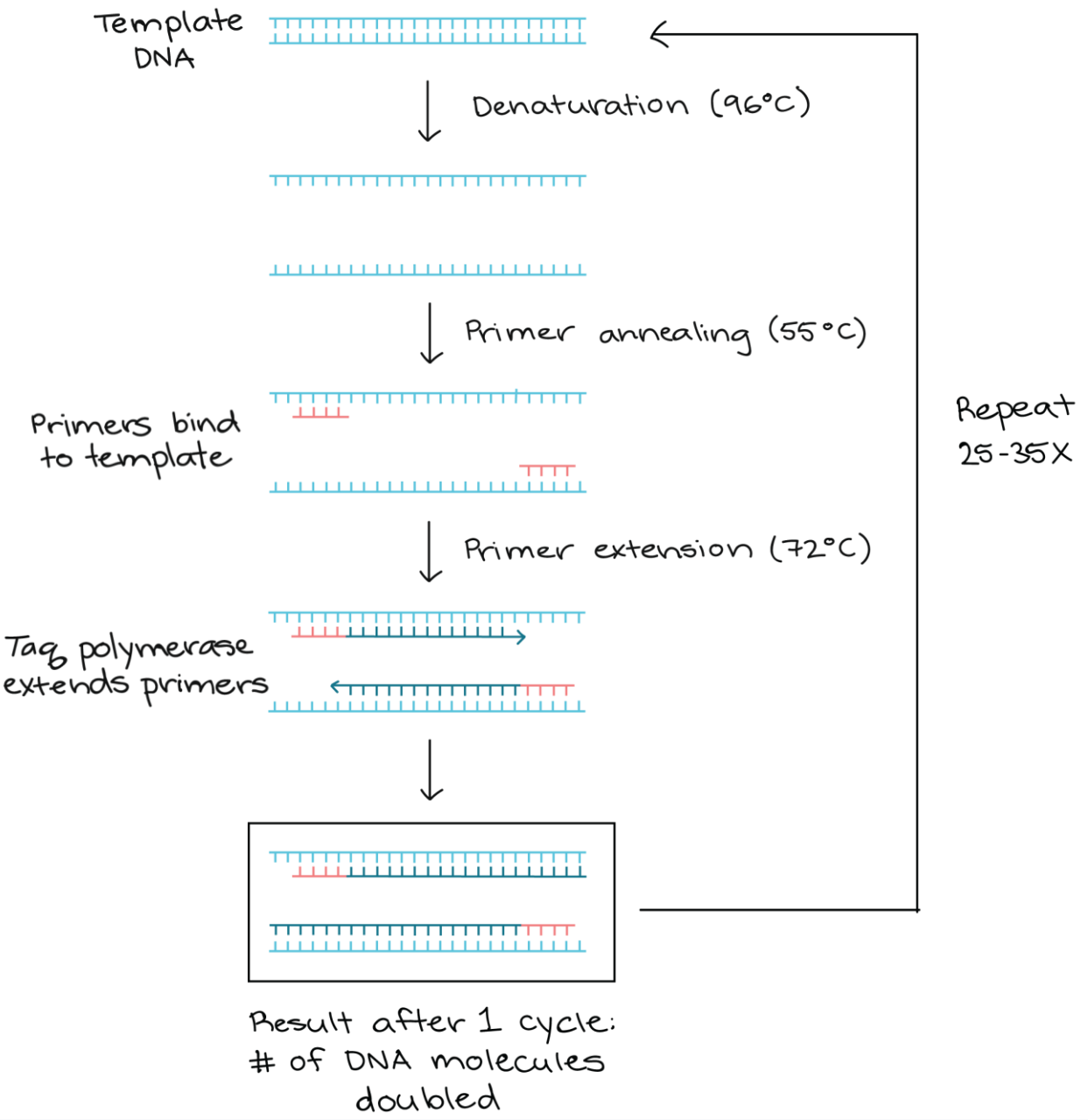


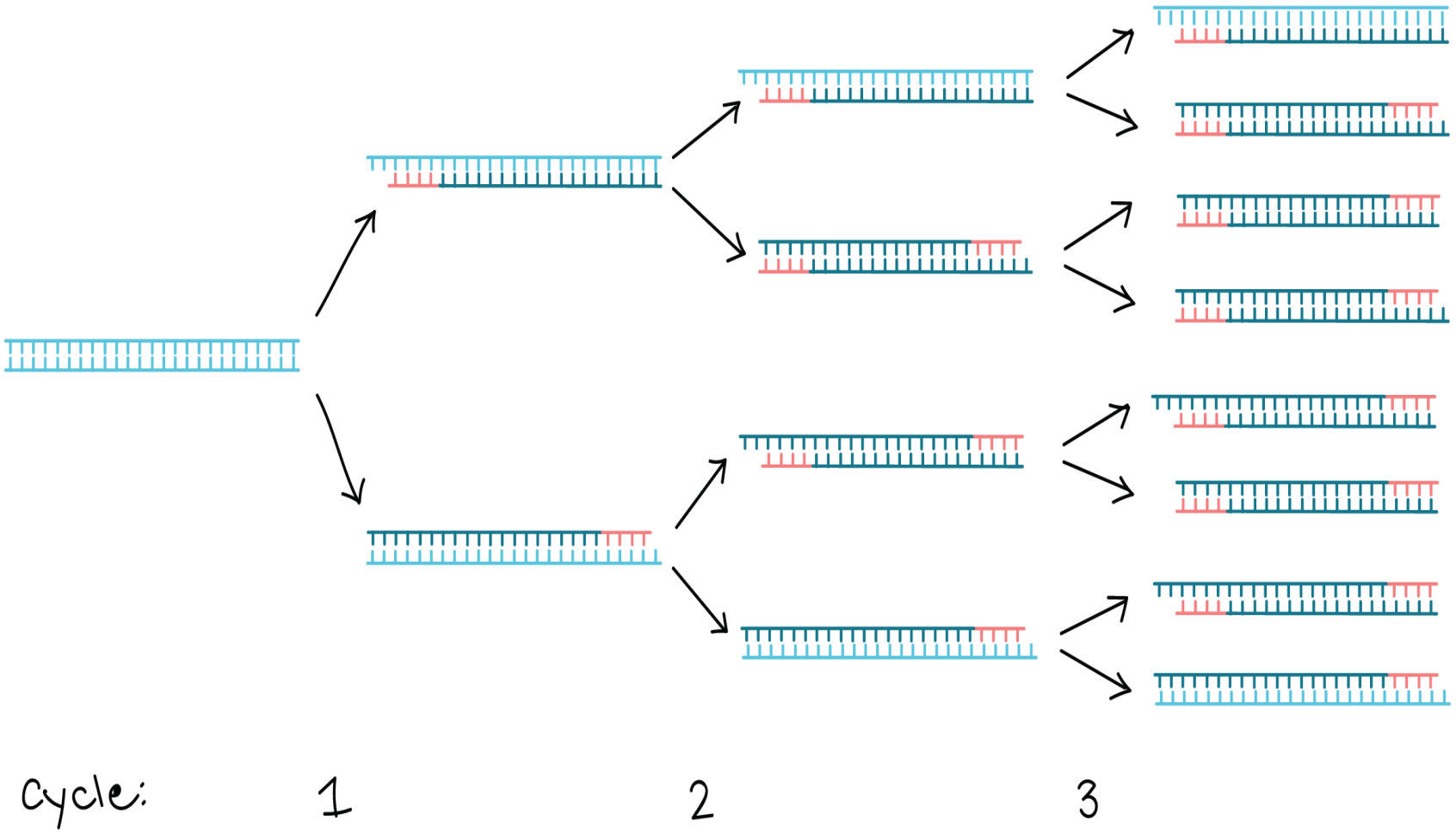
# PCR (Polymerase Chain Reaction)

Revolutionary method developed by Kary Mullis to amplify DNA

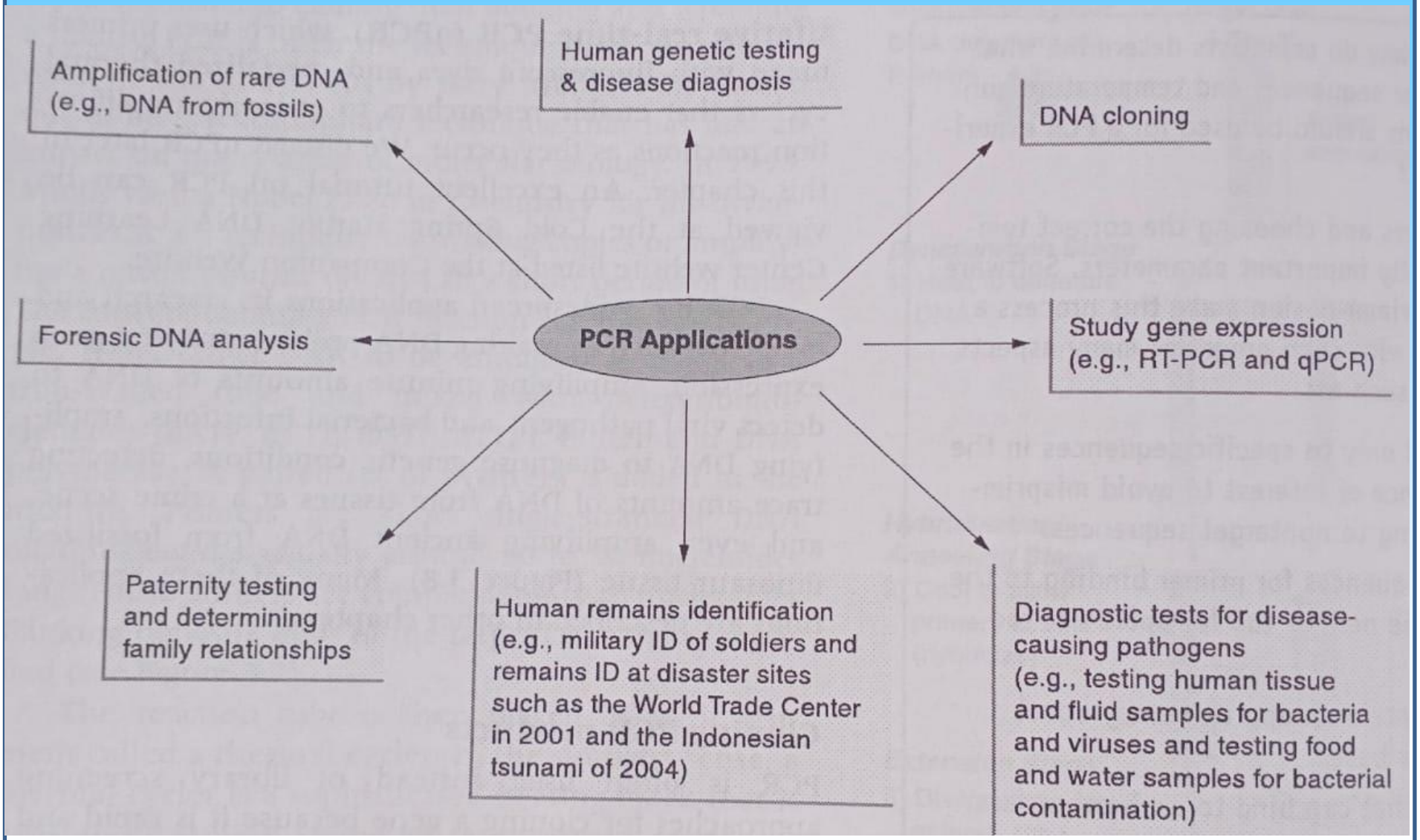
## Components of PCR

1. DNA template
2. DNA polymerase- Taq polymerase
3. Primers- short ss DNA complementary to target sequence.
4. Nucleotides (dNTPs)




Cycle:
1
2
3

# Application of PCR



# Genomic DNA and cDNA library

A DNA library is a collection of clones of DNA designed so that there is a high probability of finding any particular piece of the source DNA in the collection

- There are two types of DNA library

***GENOMIC LIBRARY:***

- The genomic library contains DNA fragments representing entire genome of an organism

***cDNA LIBRARY:***

- The cDNA library contains only complementary DNA molecules synthesized from mRNA molecules in a cell

# Genomic Library

- ***"A genomic library is a collection of bacteria which have been genetically engineered to hold the entire DNA of an organism"***
- A genomic library is a collection of genes or DNA sequences created using molecular cloning
- These libraries are constructed using clones of bacteria or yeast that contain vectors into which fragments of partially digested DNA have been inserted

# Genomic Library

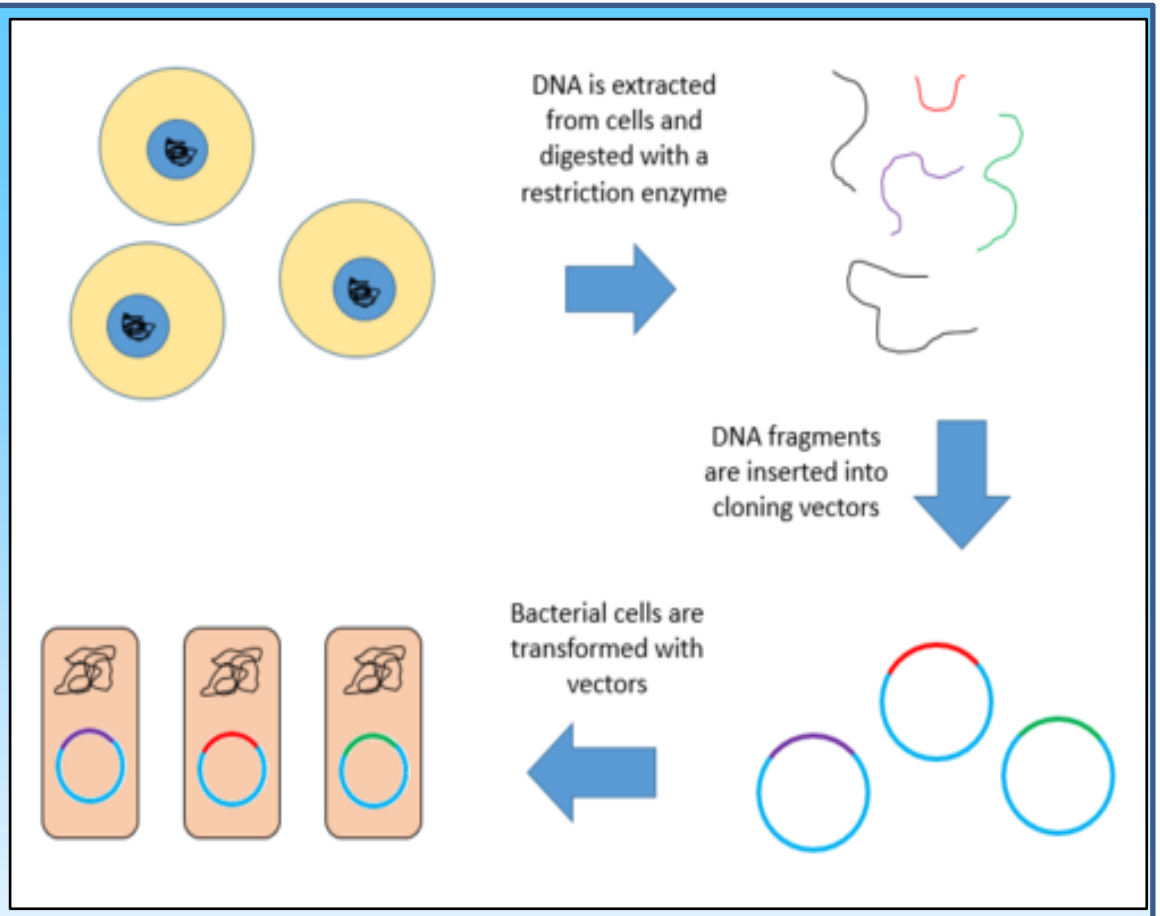
- These bacteria and yeast are subsequently grown in culture and when these microorganisms replicate their genome, they also replicate the vector genome contained within them, that is, they replicate DNA fragments that had been inserted in vectors producing clones of the original genome
- This collection of clones, in theory, contains all sequences found in the original source, including the sequence of interest
- Genomic libraries can be constructed using various hosts like plasmids, bacteriophage lambdas and many more

# Uses of Genomic library

- Researchers can explore the genome of an organism to learn more about *genomic structure* and *function*
- They can also map the genome, identifying the locations of specific genes
- A genomic library can also be used for the purpose of *cloning segments* of DNA

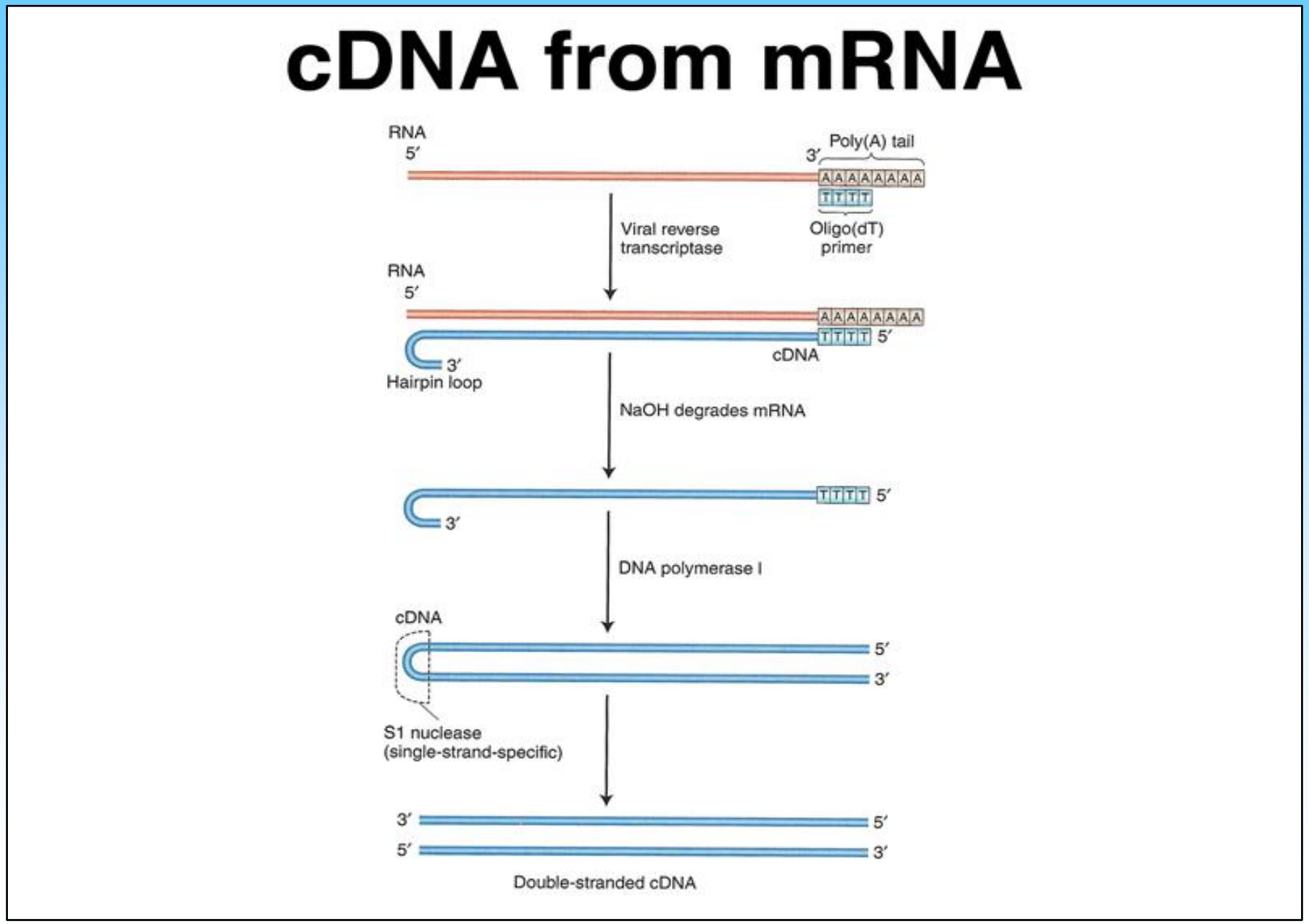
# Steps of construction

- Extract and purify DNA
- As purified DNA are extremely long, so must cut into manageable size
- DNA digested with restriction enzymes, which produce smaller fragments – each containing one or more gene

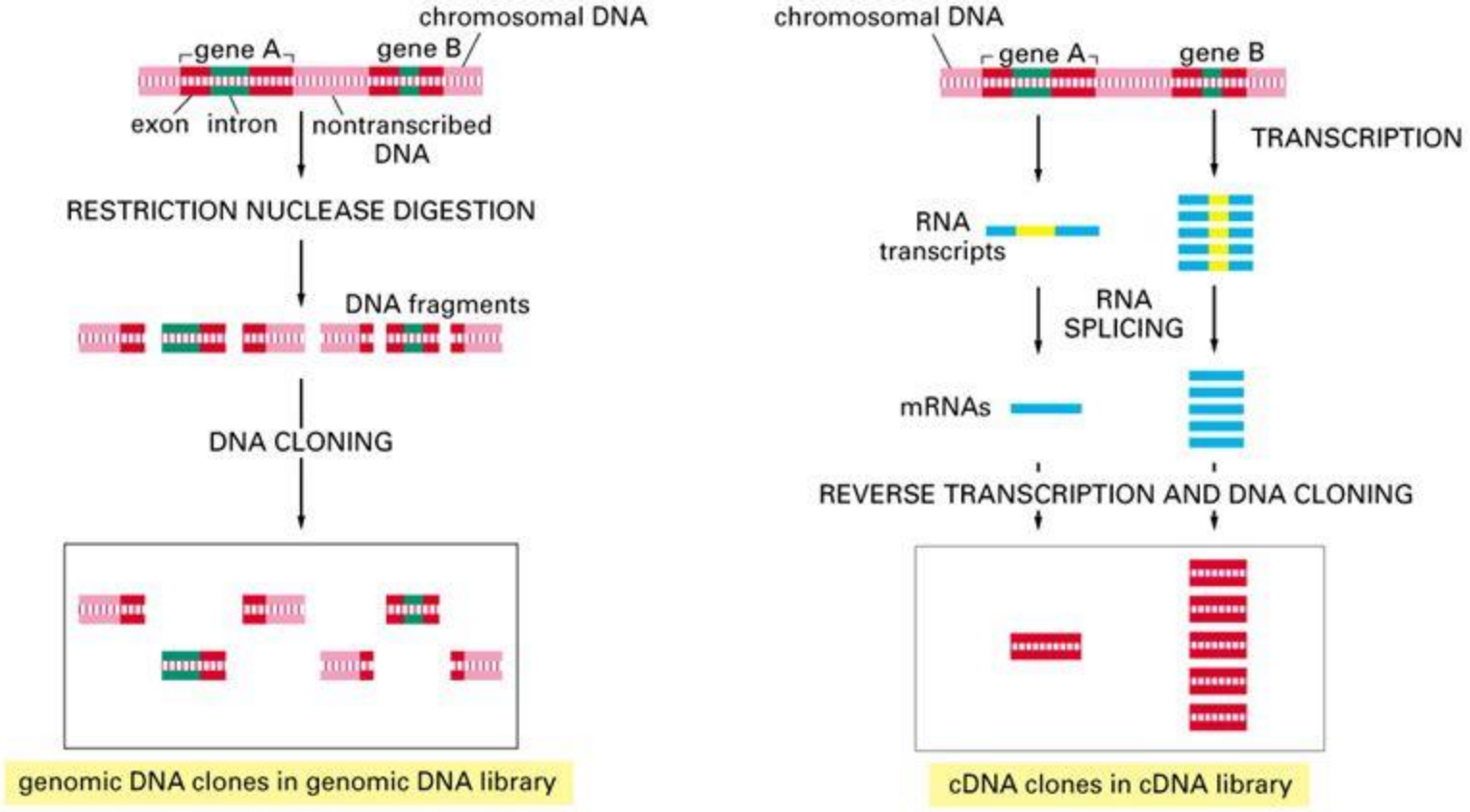



- Each fragment is different and contain unique DNA sequence
- Each fragment is inserted to plasmids
- Plasmids are also digested with restriction enzymes and then sealed to source DNA fragments using DNA ligase producing recombinant DNA
- When bacteria taken up the DNA, the entire collection of cells and DNA represents a genetic library

# cDNA library



# cDNA Vs. Genomic DNA Clones




- cDNA clones useful for deducing protein sequences
- Genomic clones useful for obtaining noncoding and total genome sequences
- Library screening to select clones of interest

An additional sequence-specific endonuclease, XmaIII, has been partially purified from Xanthomonas malvacearum. XmaIII recognizes ten cleavage sites in adenovirus 2 DNA, two sites in bacteriophage lambda and no site in either simian virus 40 DNA or ϕX174 DNA. It recognizes the sequence  and cleaves at the sites