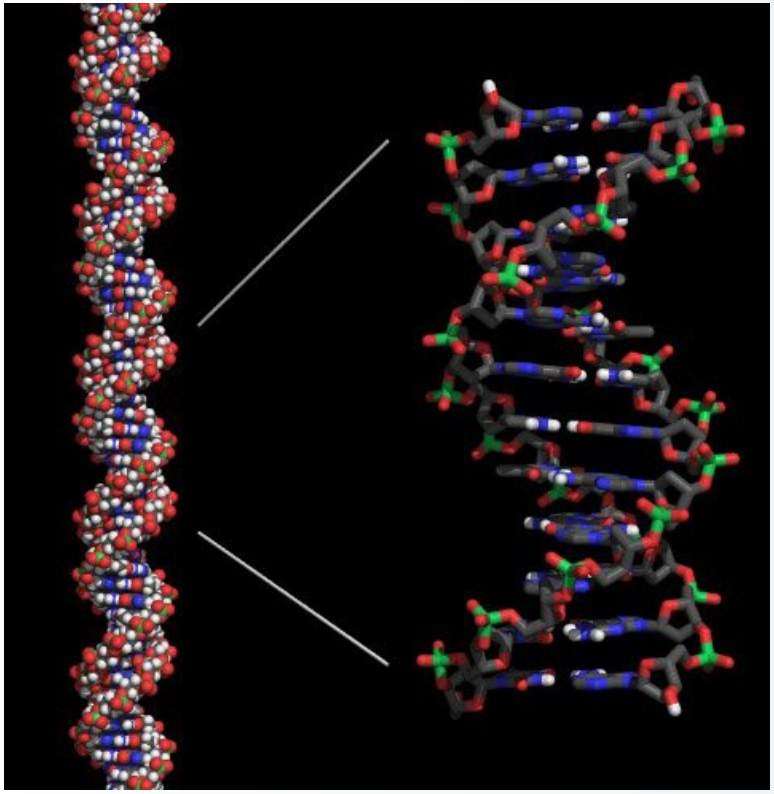
Cloning:Recombinant DNA



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Multistep Process

1. Produce fragments of DNA using enzymes that cut DNA at specific base sequences.
2. Link these fragments to self-replicating forms of DNA = vectors.

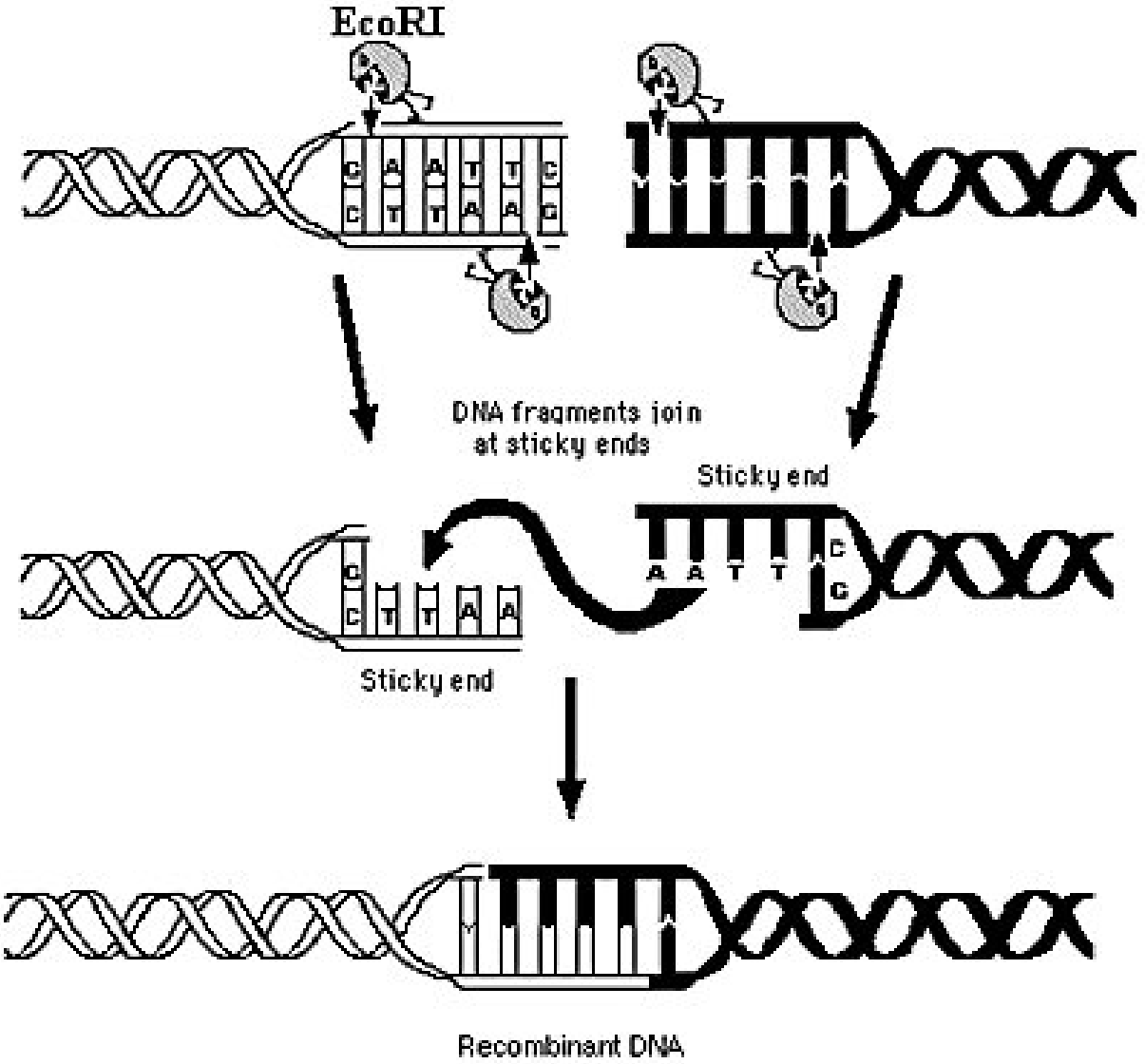
3. Replicate the recombinant DNA molecule in the host organism (1000's of copies).
4. Retrieve the cloned copies for use or modification.
5. Produce and purify gene product.

Restriction Enzymes

- Enzymes that recognize a specific base sequence in DNA and cleave at that site
- Isolated from bacteria that inactivated viruses via cutting their DNA
- “Molecular scissors”

Recognition sequence

- Palindrome - sequence is read the same on either strand, when read from 5’ to 3’
- Creates either sticky ends or blunt ends

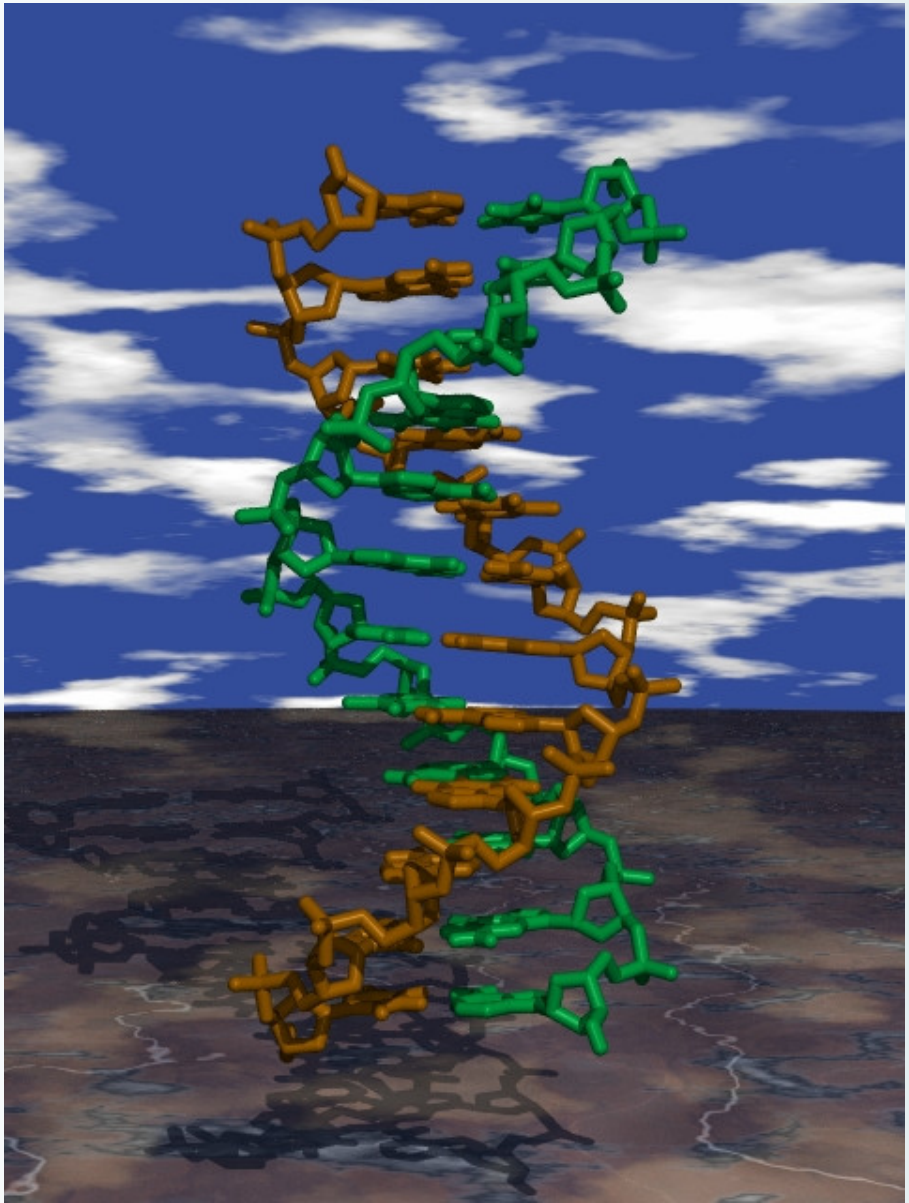


Restriction Enzyme Action of EcoRI

Vectors

- A self-replicating DNA molecule that is used to transfer foreign DNA fragments between cells.

Steps in Cloning



Steps in cloning - General

- Isolate vector DNA and gene of interest
- Cut both with the same restriction enzyme
- Mix DNA's and ligate = recombinant DNA

- Transfer recombinant molecule into host cell (transform)
- Grow/Select transformants
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- Grow/Select transformants

Types of Vectors and DNA delivery systems

Types of Vectors and DNA delivery systems

- Plasmid
- Phage (virus)
- Cosmid
- Yeast Artificial Chromosome (YAC)

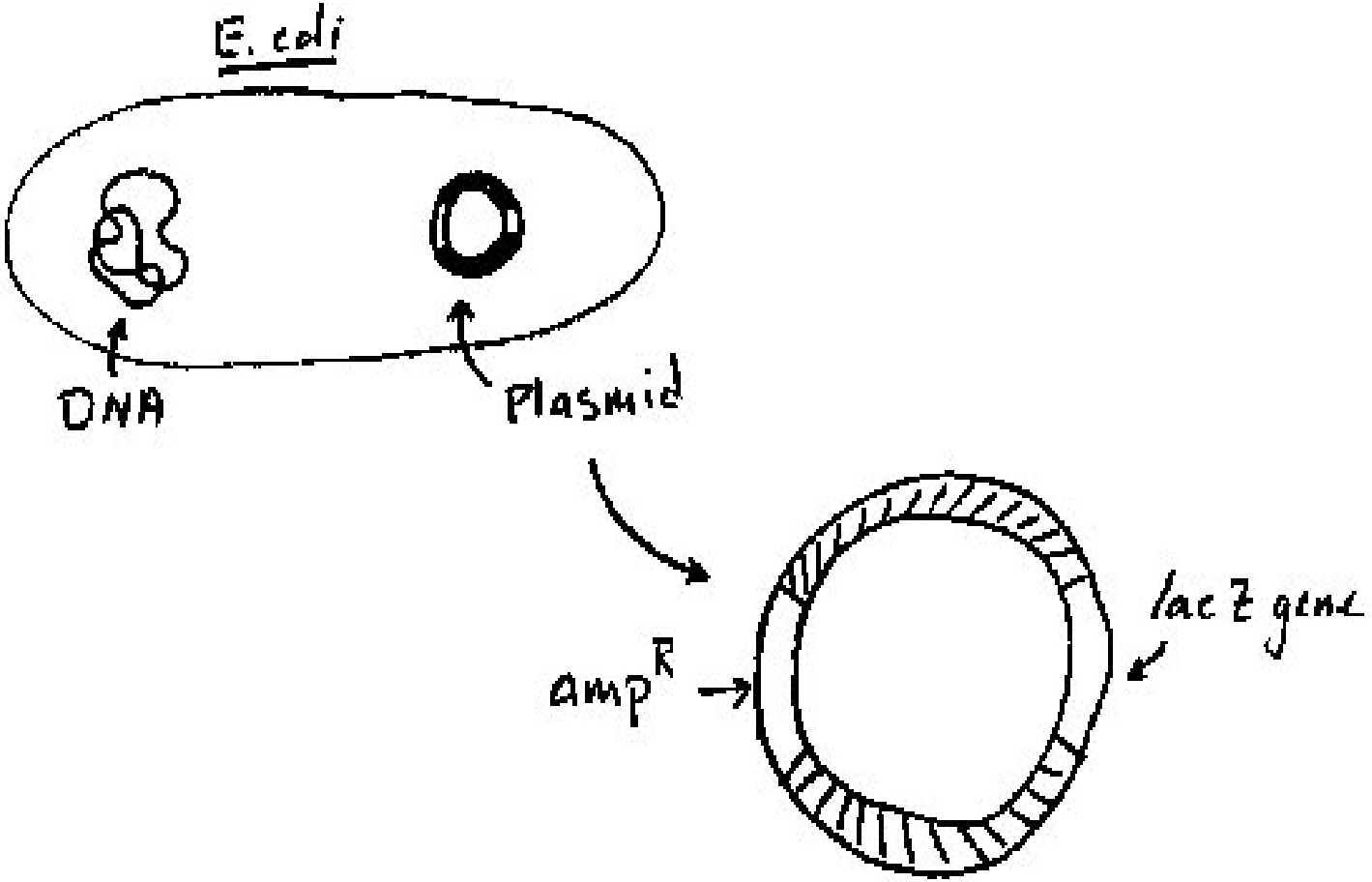
Plasmids

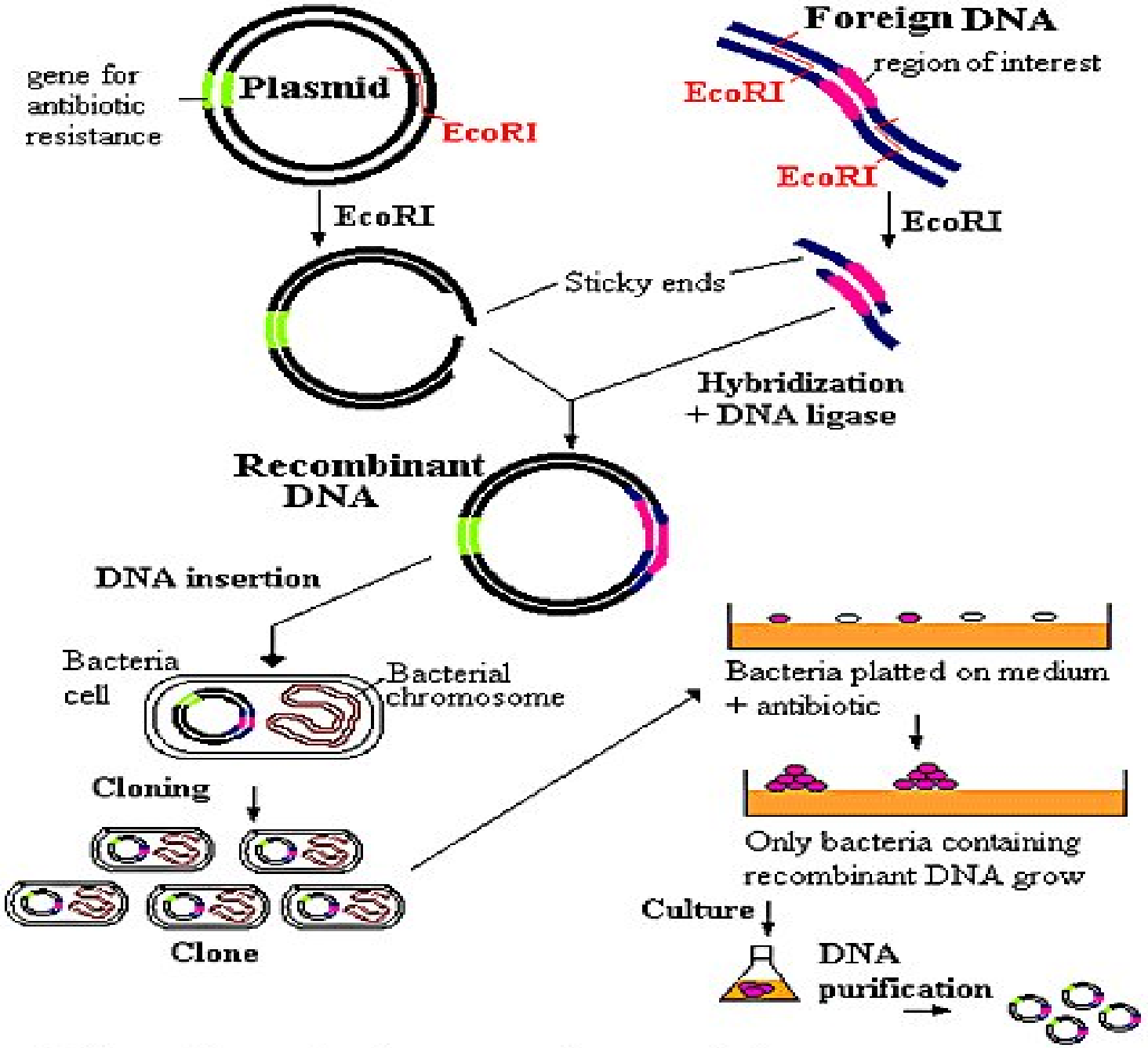
- Circular extrachromosomal DNA molecules naturally found in bacteria
- Self-replicating
- Can insert pieces up to 10kb

Plasmid vectors need…

- origin of replication
- selectable marker (antibiotic)
- unique restriction enzyme cleavage sites

Plasmid Placement in Cell



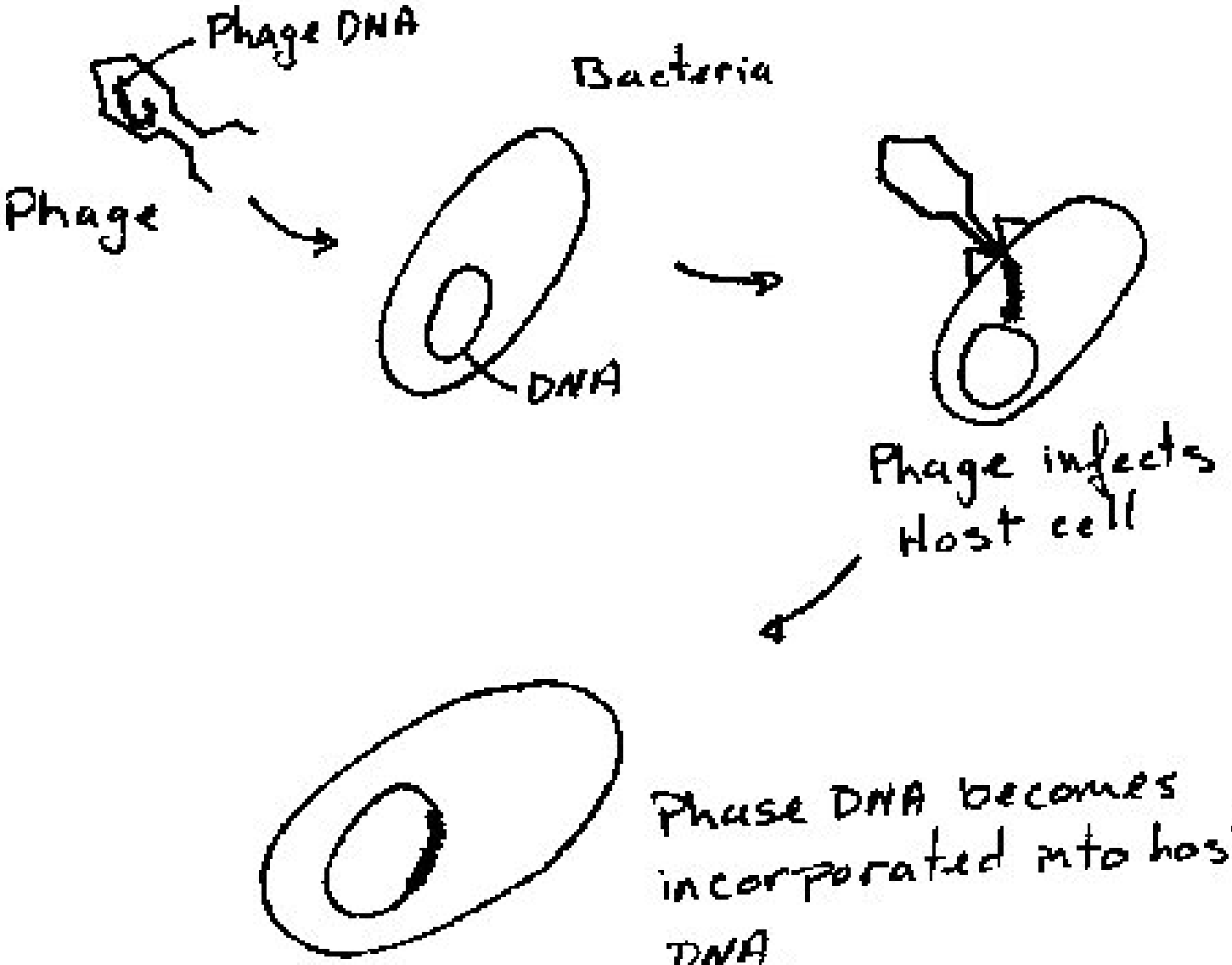


Cloning into a plasmid

Phage vectors

- Derivatives of phage ● (lambda)
- Linear DNA
- Can insert up to 15 kb fragments

Phage Insertion



Cosmids

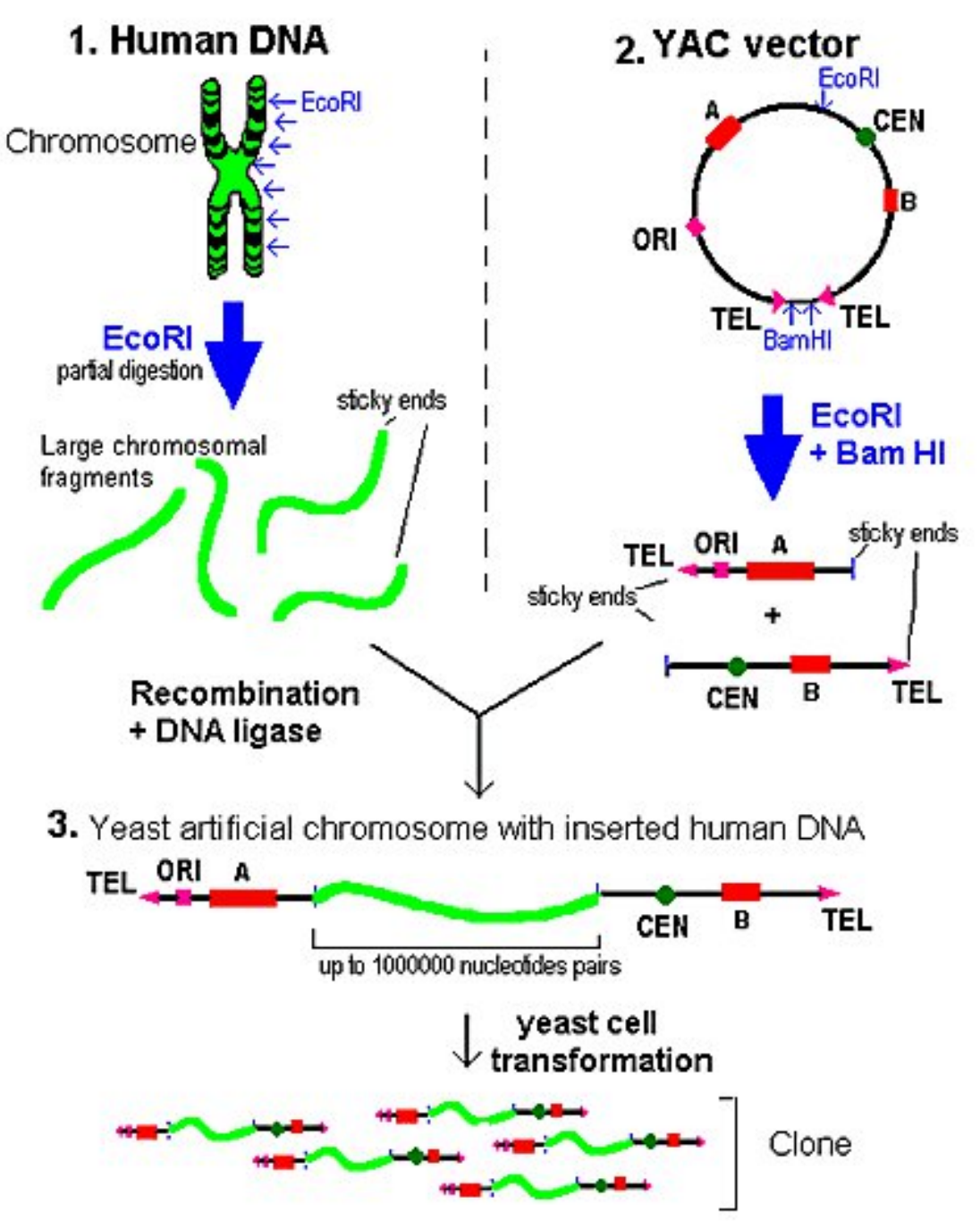
- Don’t occur naturally
- Constructed using features of both plasmids and phage
- Can carry inserts up to 45 kb

YACs

- Yeast artificial chromosome
- Self-replicating elements
- Can insert segments up to 1 million base pairs
- Can replicate any inserted DNA via transfer to yeast cells

Essential elements for YACs

- Tel - telomeres
- Cen - centromere
- Ori - Origin of replication
- Selectable markers
- Restriction enzyme recognition sites



Cloning into a Yeast Artificial Chromosome (YAC)

Particle Gun

- Usually using cell culture
- Shoot DNA coated objects into cells
- Tungsten pellets, Whiskers

We can insert the gene into cells – Now what?

Selecting for transformed cells and amplifying the product

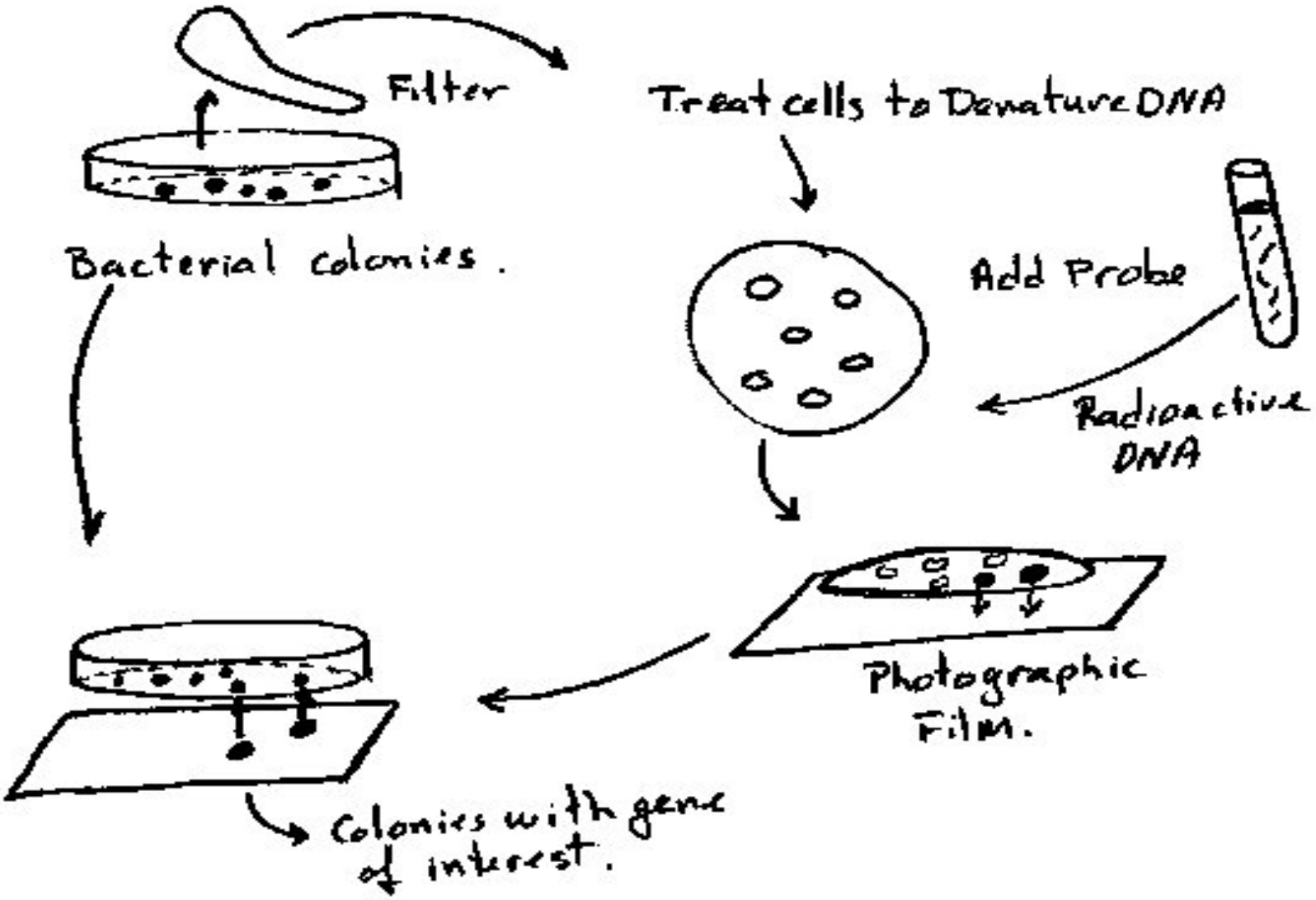
Basic Steps

- Identify the transformants
- Isolate transformed colonies
- Amplify the product

Identifying transformants

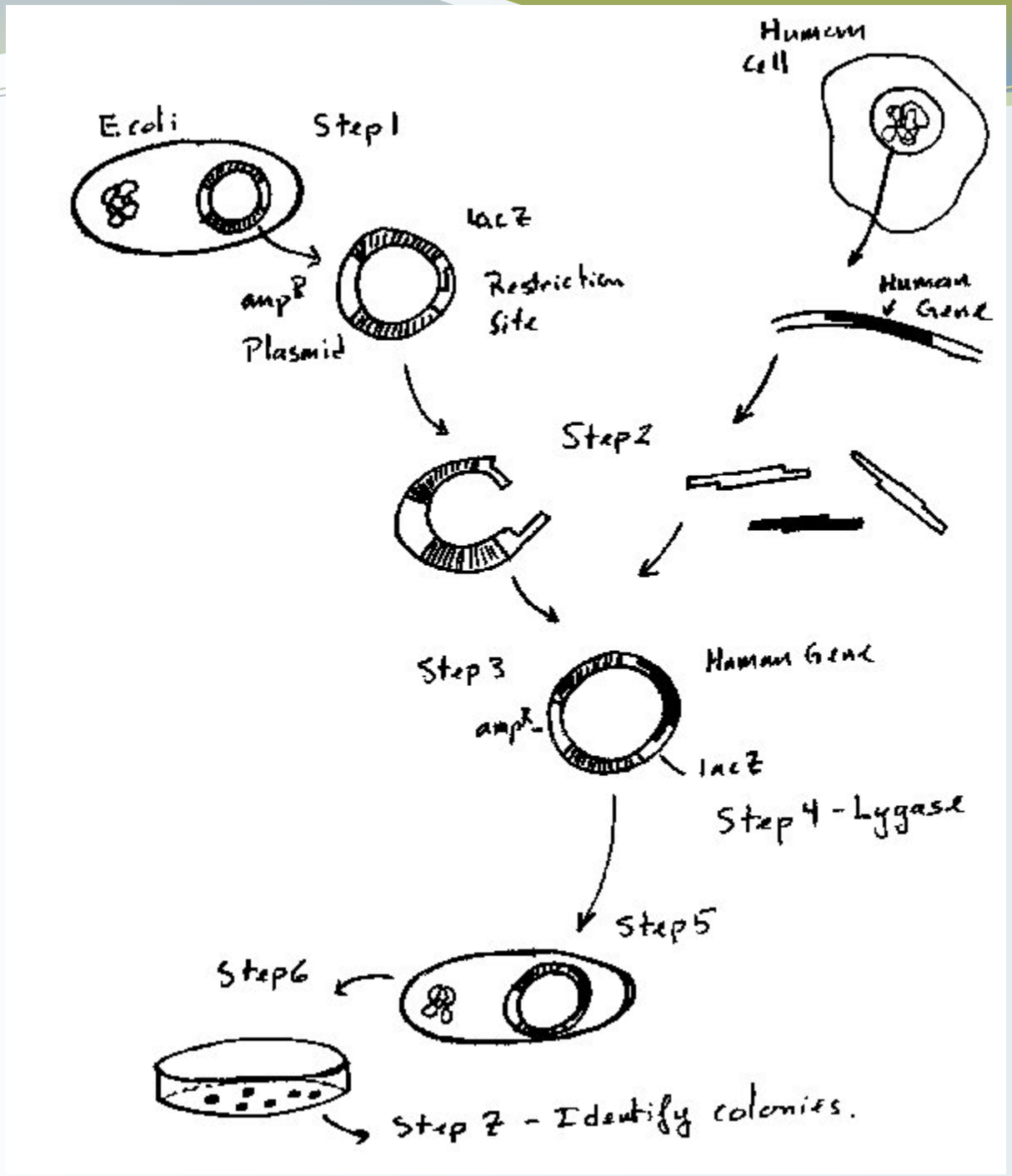
- Vectors containing antibiotic resistance genes can be used
- Those that took up the vector will now express antibiotic resistance
- Ability to metabolize substances included in media

Isolate Colonies of Interest

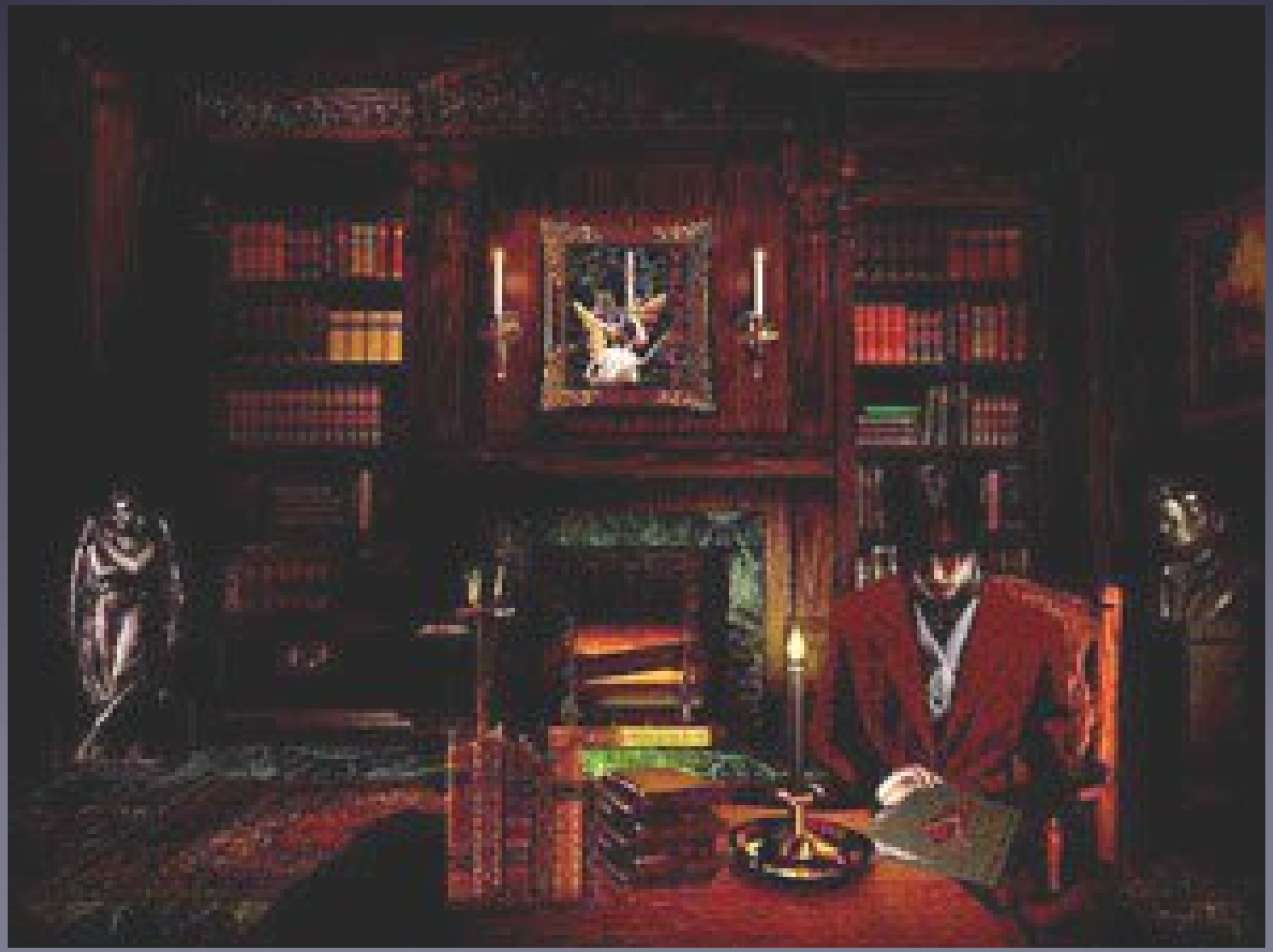


Amplify the Product

- Use bacteria (usually E. Coli) to amplify product
- Sometimes yeast cells, if the gene you are amplifying is a eukaryote specific gene


Human cell
E.coli
Step 1
lacZ
amp^R
Restriction Site
Plasmid
Human Gene
Step 2
Step 3
Human Gene
amp^R
lacZ
Step 4 - Lygase
Step 5
Step 6
Step 7 - Identify colonies.

Genetic Libraries



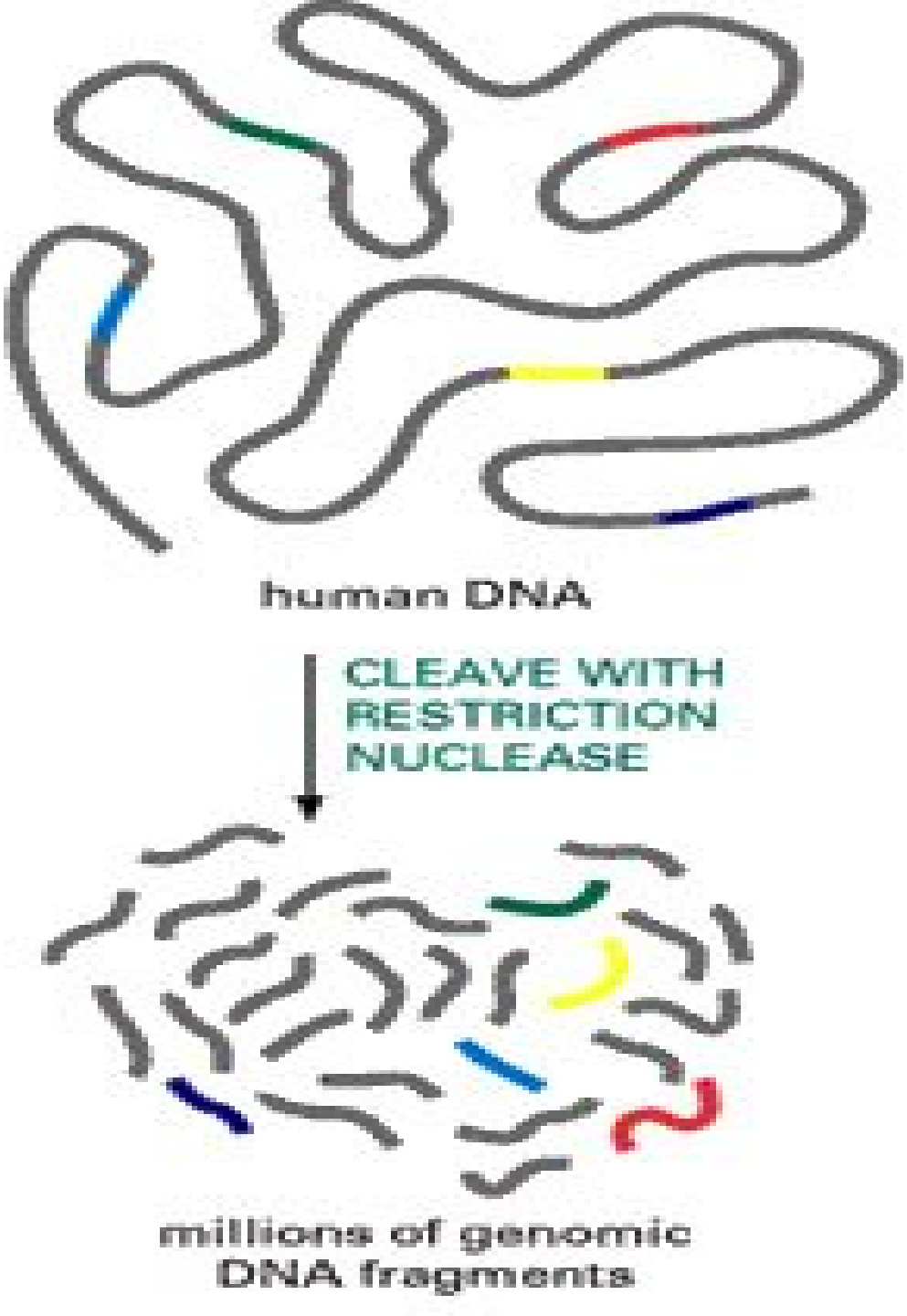
Genetic library

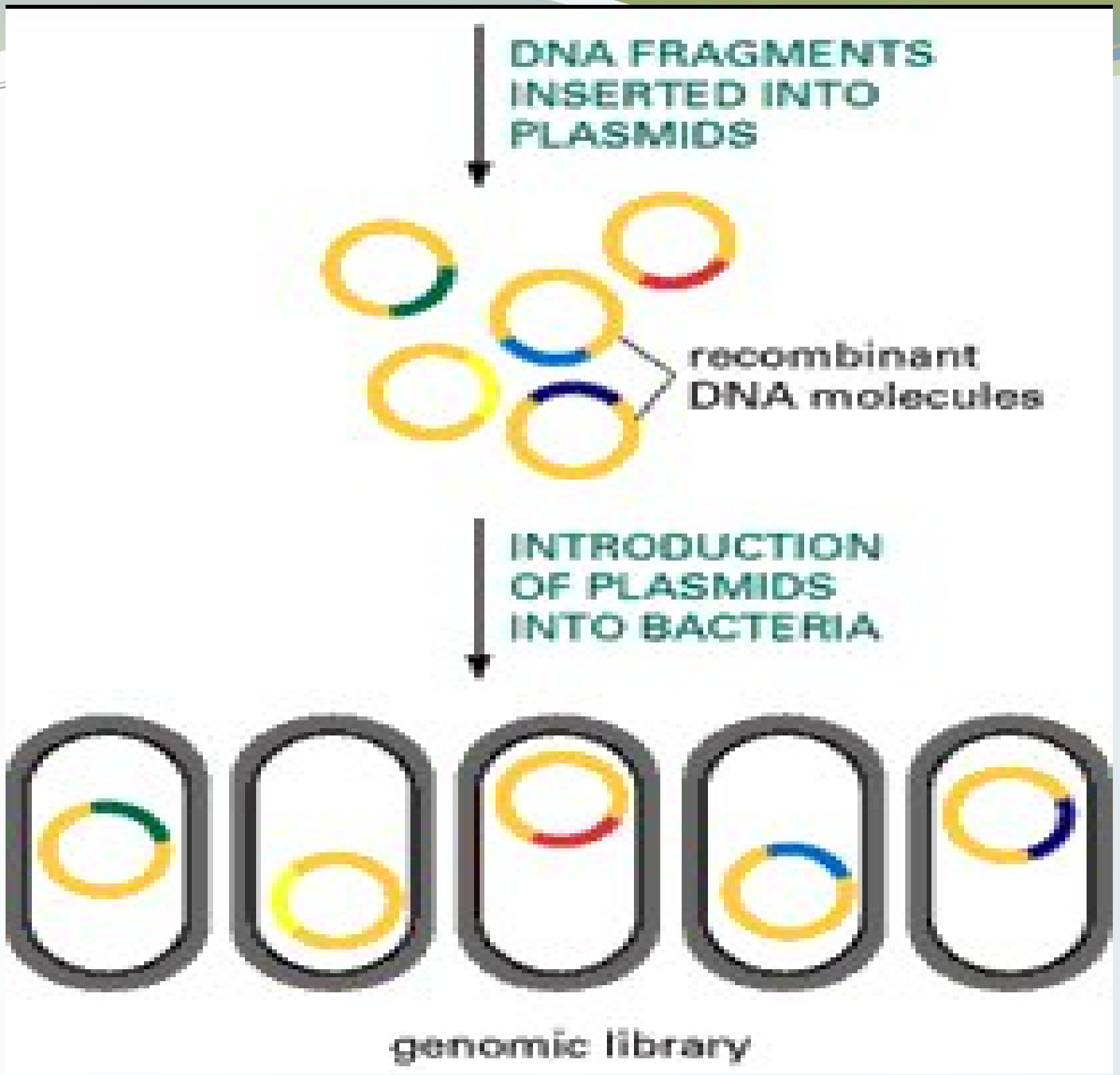
- Collection of clones that contains all the genetic information of an individual = genomic library - gene bank
- Chromosomes, set of genes of single cell type etc.
- cDNA - mRNA population made into cDNA. Produce clones

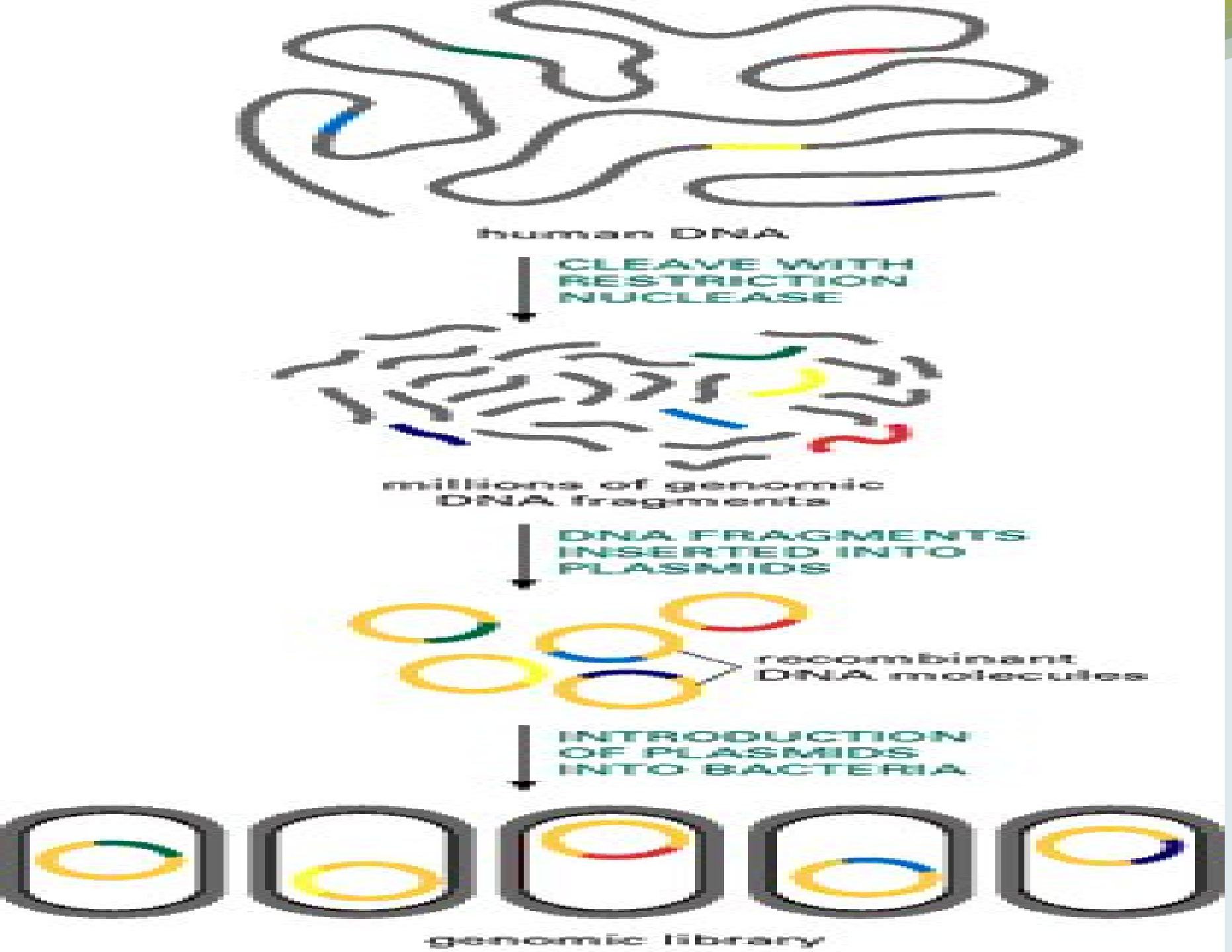
- Can recover genes of interest from libraries for
  - Clinical studies
  - Evolutionary comparison
  - Experimental studies
  - Commercial use

Construction of…

- DNA isolated from an organism
- Digest into smaller segments which can be inserted within vectors (size limitations)
- record of genome or portion of
- Can be screened, hybridization


human DNA
CLEAVE WITH
RESTRICTION
NUCLEASE
millions of genomic
DNA fragments


DNA FRAGMENTS
INSERTED INTO
PLASMIDS
recombinant
DNA molecules
INTRODUCTION
OF PLASMIDS
INTO BACTERIA
genomic library


human DNA
CLEAVE WITH RESTRICTION NUCLEASE
millions of genomic DNA fragments
DNA FRAGMENTS INSERTED INTO PLASMIDS
recombinant DNA molecules
INTRODUCTION OF PLASMIDS INTO BACTERIA
genomic library