

Introduction

SC113603 Molecular Biology
Lect. Todsapol Techo

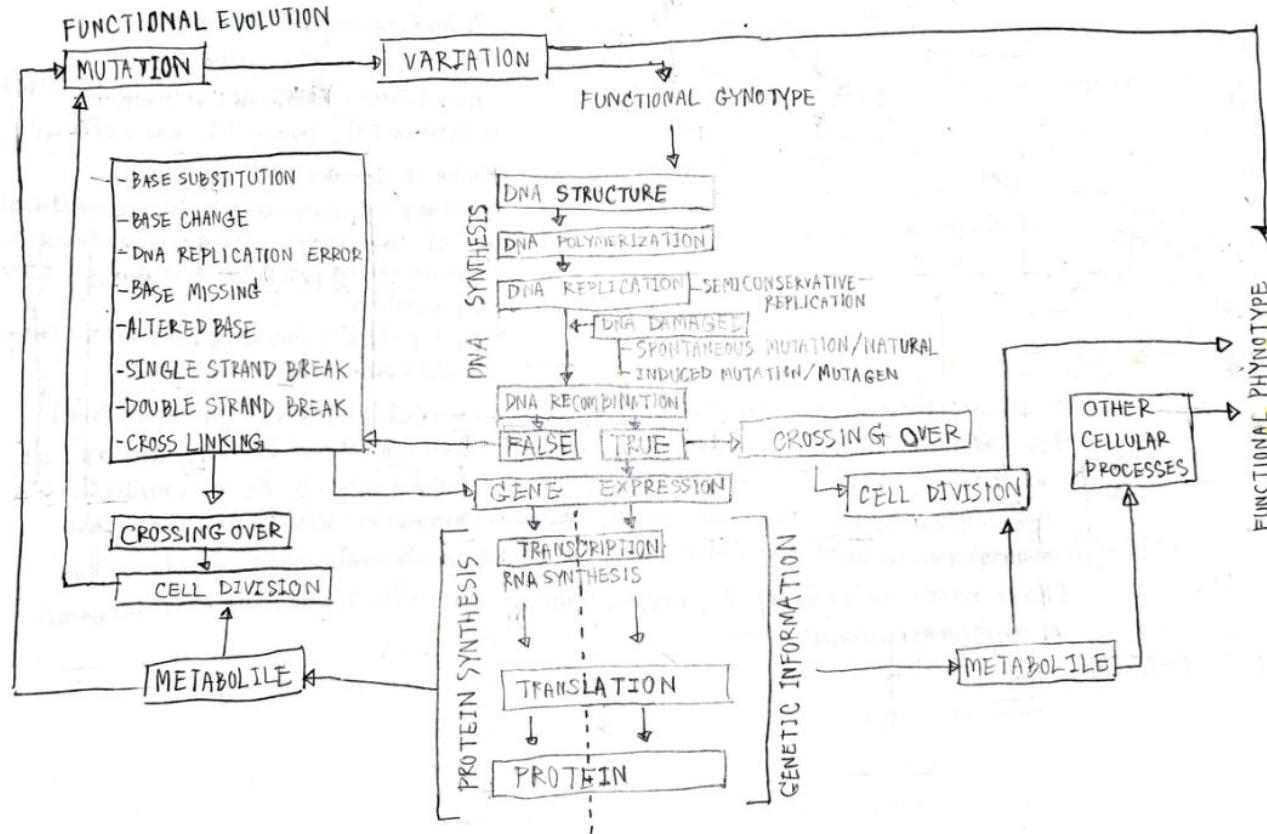
Scoring

- Full score for these parts = 29%
- Recombinant DNA Technology (13%)
 - Assignments 3%
 - Exam 10%
- Bioinformatics (16%)
 - Option 1
 - Assignments 6%
 - Exam 10%
 - Option 2
 - Assignments 8%
 - Exam 8%

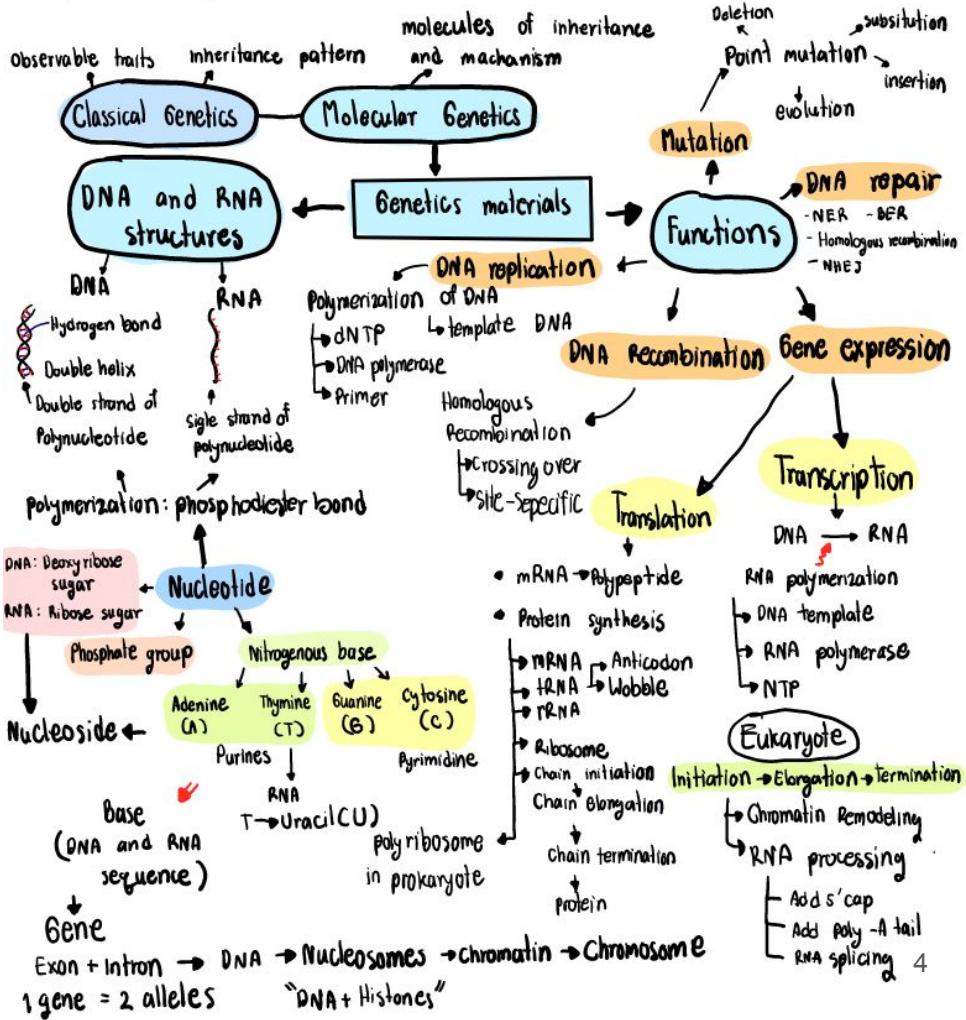
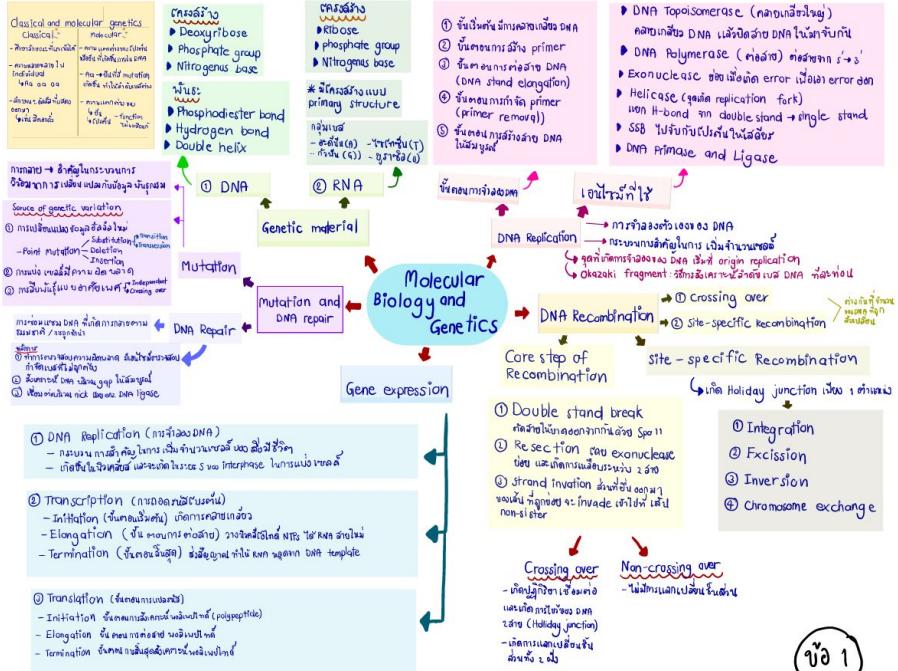
ผู้สอน	จำนวนชั่วโมงสอน	สัดส่วนคะแนน
ผศ.ดร.วัฒนชัย ลันทม	13.5	29%
อ.ดร.ทศพล เตชะ	13.5	29%
ผศ.ดร.วุฒิพงษ์ มหาคำ	18	42%
รวม	45	100%

3. เทคโนโลยีดีเอ็นเอสายพสมขั้นแนะนำ 3.1 หลักการเบื้องต้นของเทคโนโลยีดีเอ็นเอสายพสม, การโคลนยีน และการตรวจหาชิ้นดีเอ็นเอสายพสม 3.2 เทคนิคสำหรับศึกษาการแสดงออกของยีน 3.3 เทคนิค Polymerase Chain Reaction (PCR) พื้นฐาน	6	อ.ดร.ทศพล (คะแนน 13%)
4. ชีวสารสนเทศศาสตร์เบื้องต้น 4.1 การสืบค้นฐานข้อมูลชีวภาพ 4.2 กลุ่มโปรแกรม BLAST 4.3 ซอฟแวร์ในการวิเคราะห์ลำดับนิวคลีโอไทด์ และการออกแบบเพรเมอร์สำหรับ PCR 4.4 ชีวสารสนเทศศาสตร์ที่นำเสนอในปัจจุบันใน การศึกษาชีววิทยาเชิงระบบ	7.5	อ.ดร.ทศพล (คะแนน 16%)

Assignments (1)



Assignments (2)



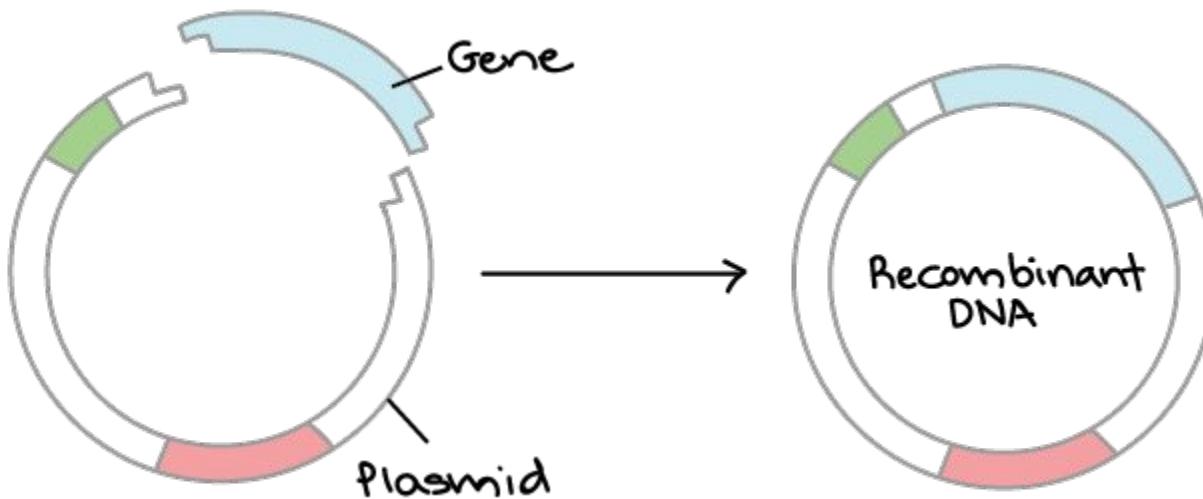
Recombinant DNA Technology

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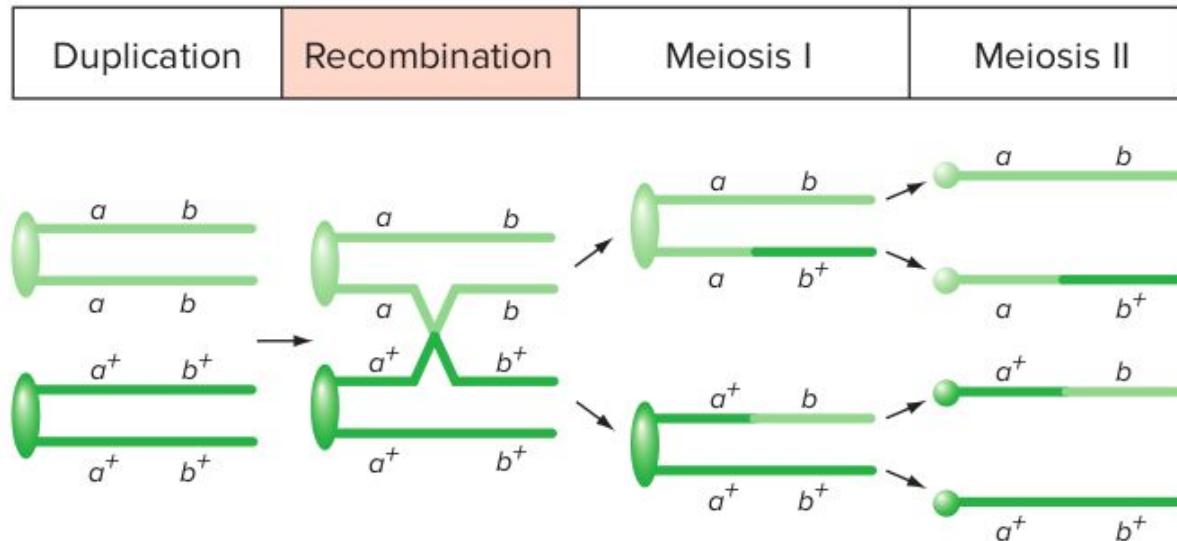
Objective

- Describe about the molecular techniques related to recombinant DNA construction
- Describe about the recombinant DNA construction and selection
- Describe about the application of recombinant DNA technology

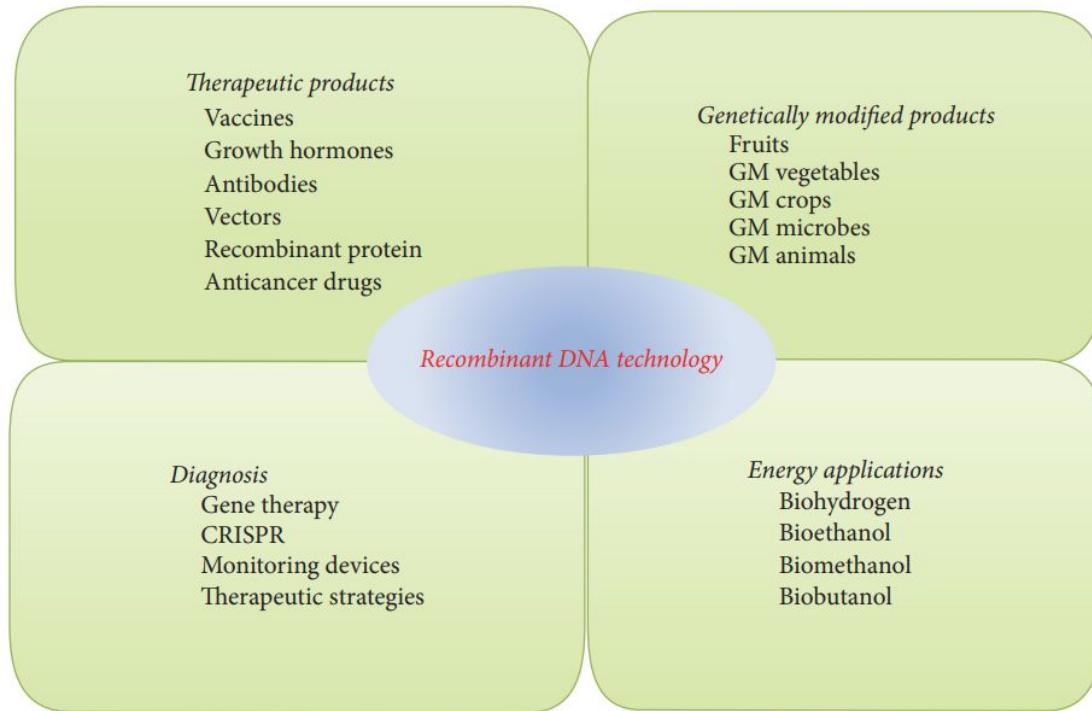
Recombinant DNA



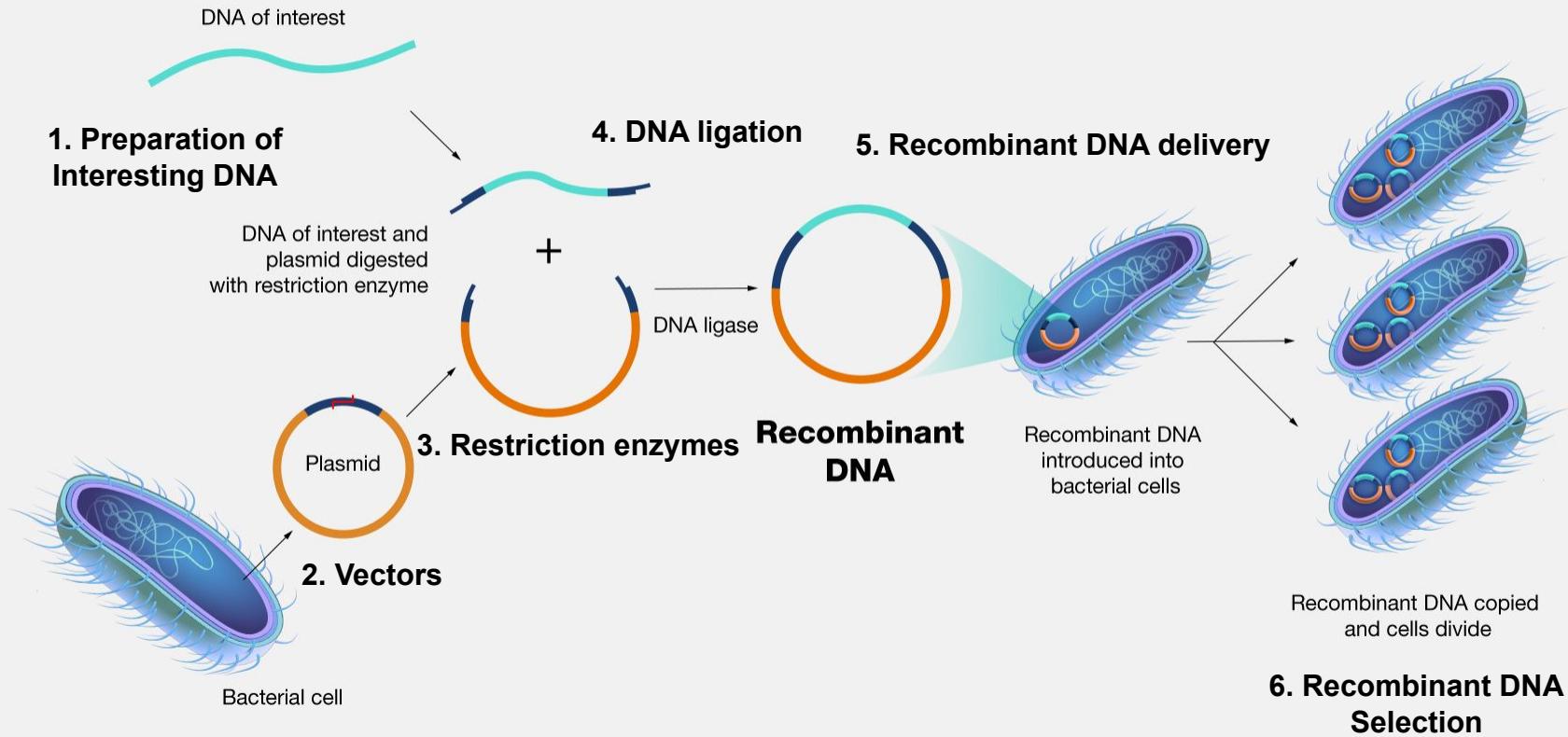
Genetic/DNA recombination



Why we have to know ?



Overview of Recombinant DNA Technology

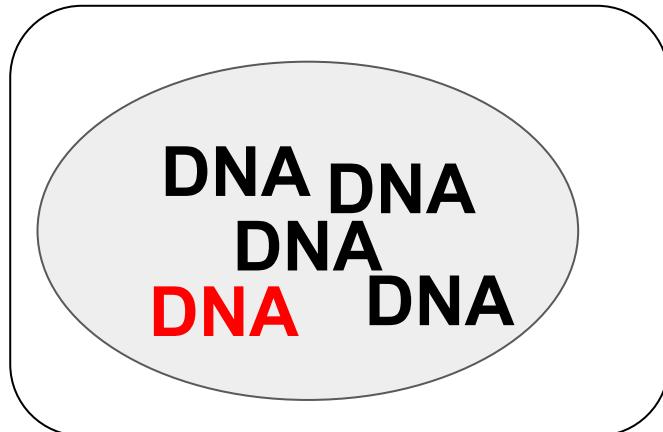


Molecular Techniques in recombinant DNA construction

- **DNA Isolation (1)**
 - Measurement of DNA quality, quantity, and integrity
 - Integrity by **Gel electrophoresis**
 - Quality and quantity by **spectrophotometer**
- **PCR (1)**
 - **Amplification of interesting DNA fragments**
 - Design Primers with recognition site of restriction enzyme
 - PCR
- **Restriction Enzyme Digestion (3)**
- **DNA ligation (4)**
- **Transformation of recombinant DNA to host (5)**
- **Selection of recombinant DNA by selective marker in bacteria and yeast (6)**
 - Antibiotic Marker
 - Essential amino acids marker

1. Preparation of Interesting DNA

- DNA Isolation
- Polymerase Chain Reaction (PCR)



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Molecular Technique: DNA Isolation

To obtain DNA, RNA, or Plasmid from living organisms

- **Cell or Tissue Lysis**
- **Remove other Biomolecules** such as Protein and Lipid
- **Separation DNA** by Centrifugation and Precipitation by alcohol
- **Cleaning the DNA**
- **Confirming the presence and quality of the DNA**

DNA Isolation: Lysis step

- **Prokaryote**

- Bacteria

- **Eukaryote**

- Cell wall
 - Fungi
 - Cell
 - Tissue
 - Plant
 - Cell
 - Tissue

- Non Cell wall

- Animal
 - Cell
 - Tissue

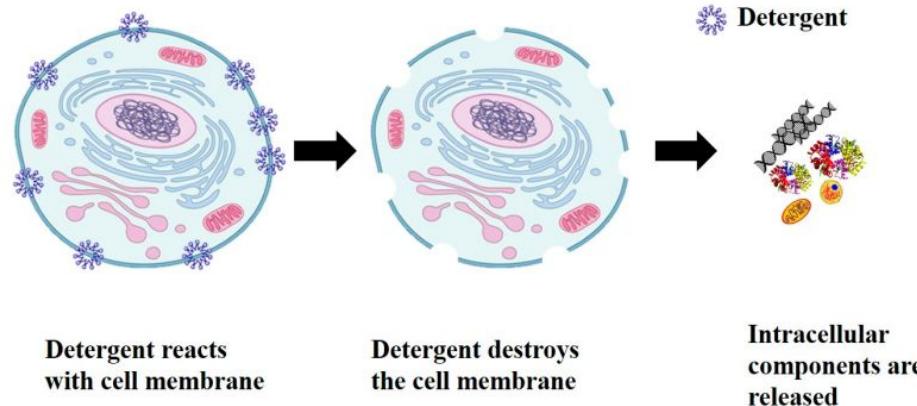


Figure 1. Cell lysis using detergent to open the cell membrane and release the intracellular components.
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DNA Isolation: Cell Lysis Methods

- **Mechanic Lysis**
 - High Pressure
 - Bead Mill
- **Non-Mechanic Lysis**
 - Physical
 - Heating
 - Osmotic Shock
 - Cavitation
 - Chemical
 - Alkali
 - Detergents
 - Biological
 - Enzyme

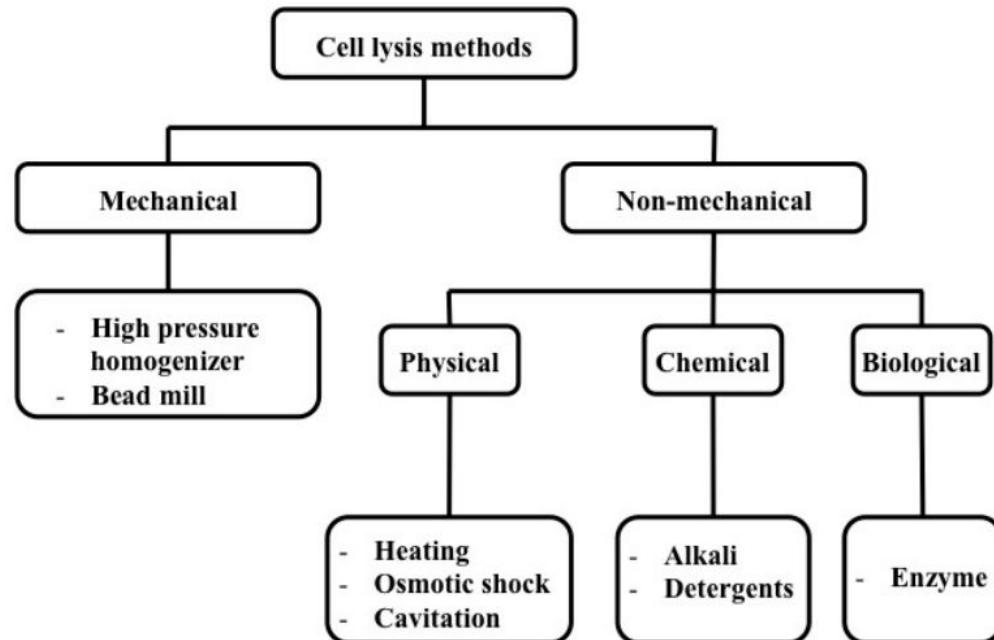


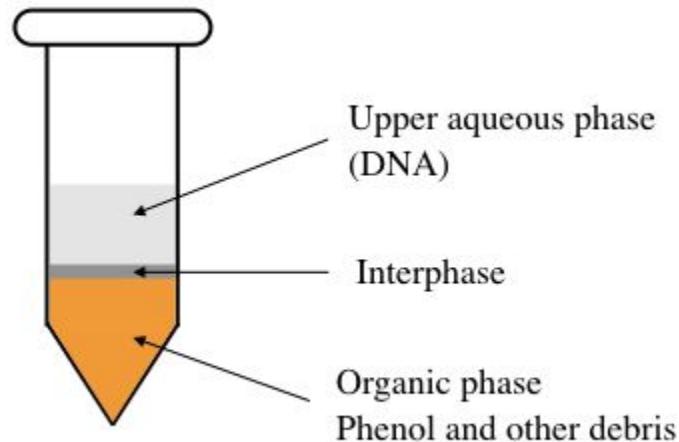
Figure 5. Classification of cell lysis methods.

DNA Isolation: Other steps

- **Remove other Biomolecules** such as Protein, Carbohydrate, and Lipid
 - Organic solvent (non polar) like Phenol/Chloroform/Isopropanol
- **Separation DNA by Centrifugation and Precipitation by alcohol**
 - Centrifuge to separate **Polar** (DNA), **Interface** (nonpolar protein), and **Nonpolar** (lipid, carbohydrate) layers
 - Precipitate DNA by **Isopropanol/ethanol** (separate DNA from polar protein)
 - Digestion **RNA** with **RNase**
- **Cleaning the DNA**
 - Wash DNA with **70% Ethanol**
 - Polar protein dissolve in aqueous (30% water) but DNA precipitated by Ethanol
- **Confirming the presence and quality of the DNA**
 - Run agarose to check integrity
 - Measure absorbance by spectrophotometer

Remove other Biomolecules

- Phase separation (Phenol/Chloroform/Isopropanol)



N
N A D A N A
D D N A N
N A A N D A

Bad
Quality

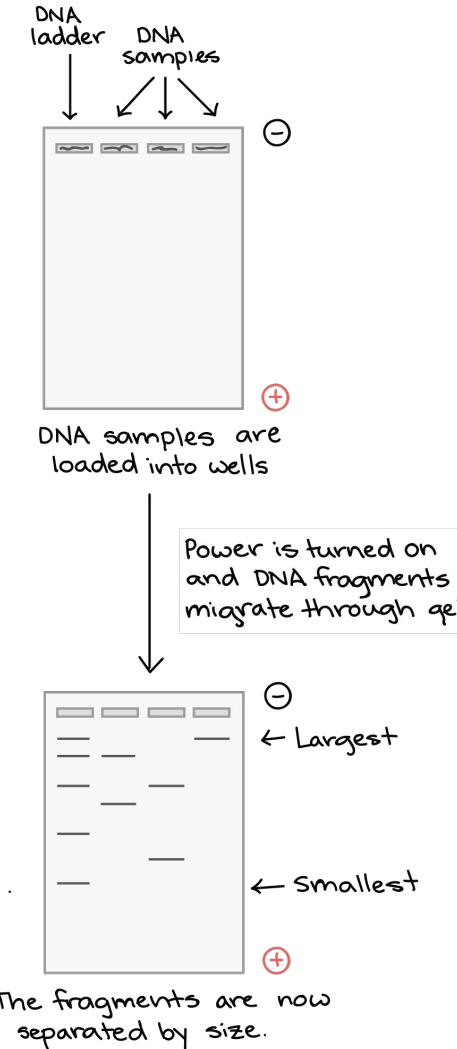
HOW?

DNA DNA DNA
DNA DNA DNA
DNA DNA DNA
DNA DNA DNA

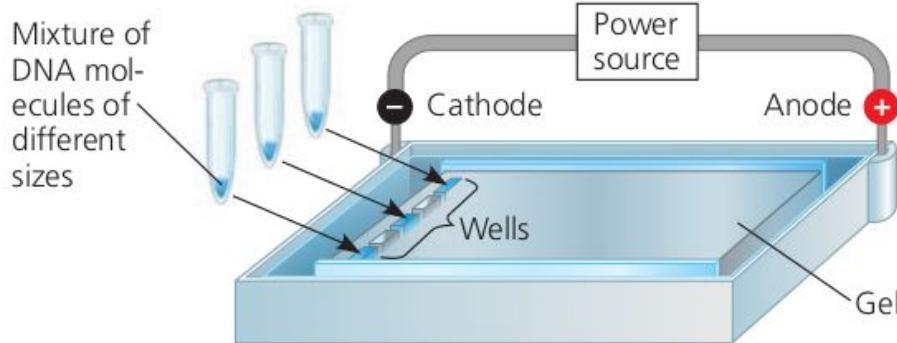
Good
Quality

Gel Electrophoresis

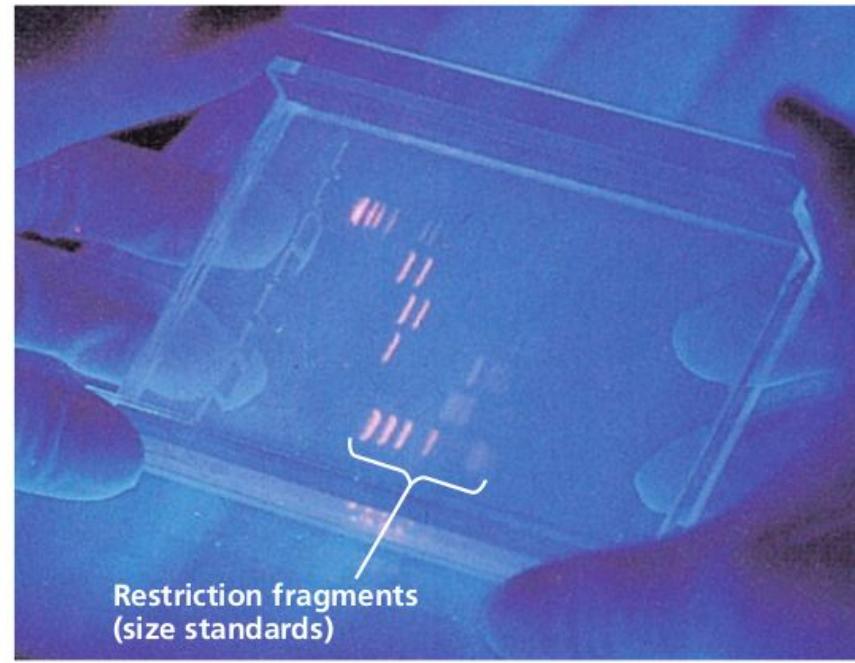
- **Gel Electrophoresis**
 - The movement of **charged molecules** in an **electric field**.
 - To **visualize** nucleic acid
- **Factors of DNA migration**
 - The strength of the electric field (Fix)
 - The composition of the gel (Fix)
 - Charge density (Fix)
 - **The Physical Size of the DNA molecules**
 - **DNA Forms:**
 - Linear < Circular < Supercoiled



▼ **Figure 20.6 Gel electrophoresis.** A gel made of a polymer acts as a molecular sieve to separate nucleic acids or proteins differing in size, electrical charge, or other physical properties as they move in an electric field. In the example shown here, DNA molecules are separated by length in a gel made of a polysaccharide called agarose.

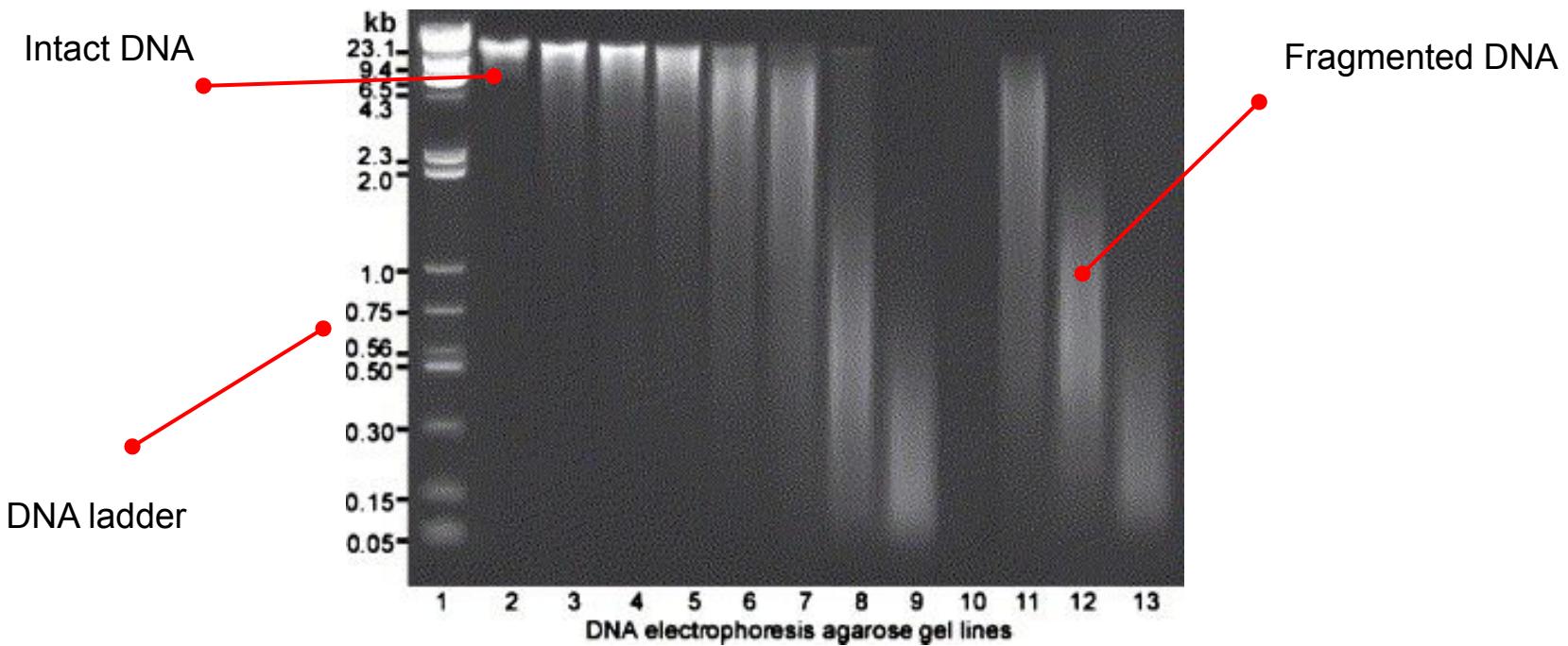


(a) Each sample, a mixture of different DNA molecules, is placed in a separate well near one end of a thin slab of agarose gel. The gel is set into a small plastic support and immersed in an aqueous, buffered solution in a tray with electrodes at each end. The current is then turned on, causing the negatively charged DNA molecules to move toward the positive electrode.



(b) Shorter molecules are slowed down less than longer ones, so they move faster through the gel. After the current is turned off, a DNA-binding dye is added that fluoresces pink in UV light. Each pink band corresponds to many thousands of DNA molecules of the same length. The horizontal ladder of bands at the bottom of the gel is a set of restriction fragments of known sizes for comparison with samples of unknown length.

Genomic DNA isolation



Access quantity of DNA by Spectrophotometer

- **Spectrophotometer**
 - UV Absorbance Technique
- **Quantity**
 - By Standard Curve
- **Quality**
 - Pure DNA = $A_{260}/A_{280} \sim 1.8$
 - Pure DNA = $A_{260}/A_{230} \sim 2.0-2.2$
 - Pure RNA = $A_{260}/A_{280} \sim 2.0$

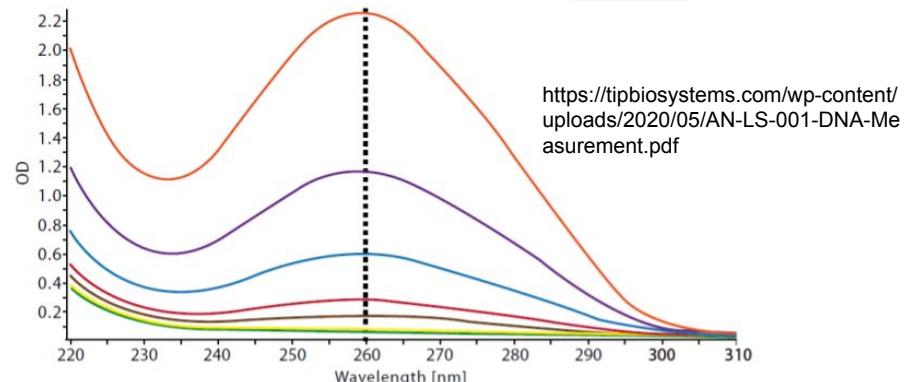
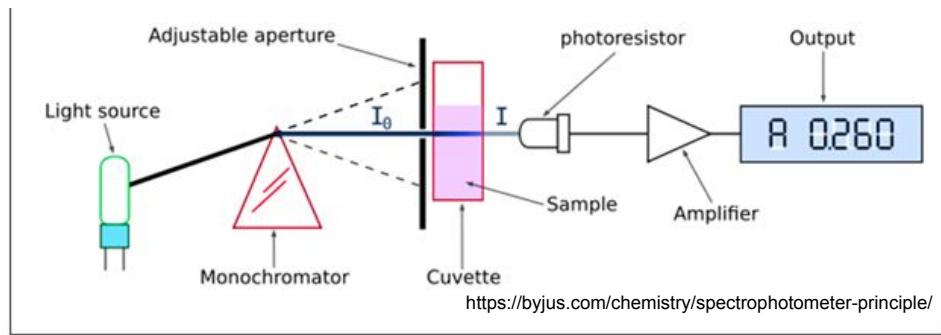


Fig 1: UV absorbance spectra for different DNA concentrations

DNA
DNA

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DNA
DNA

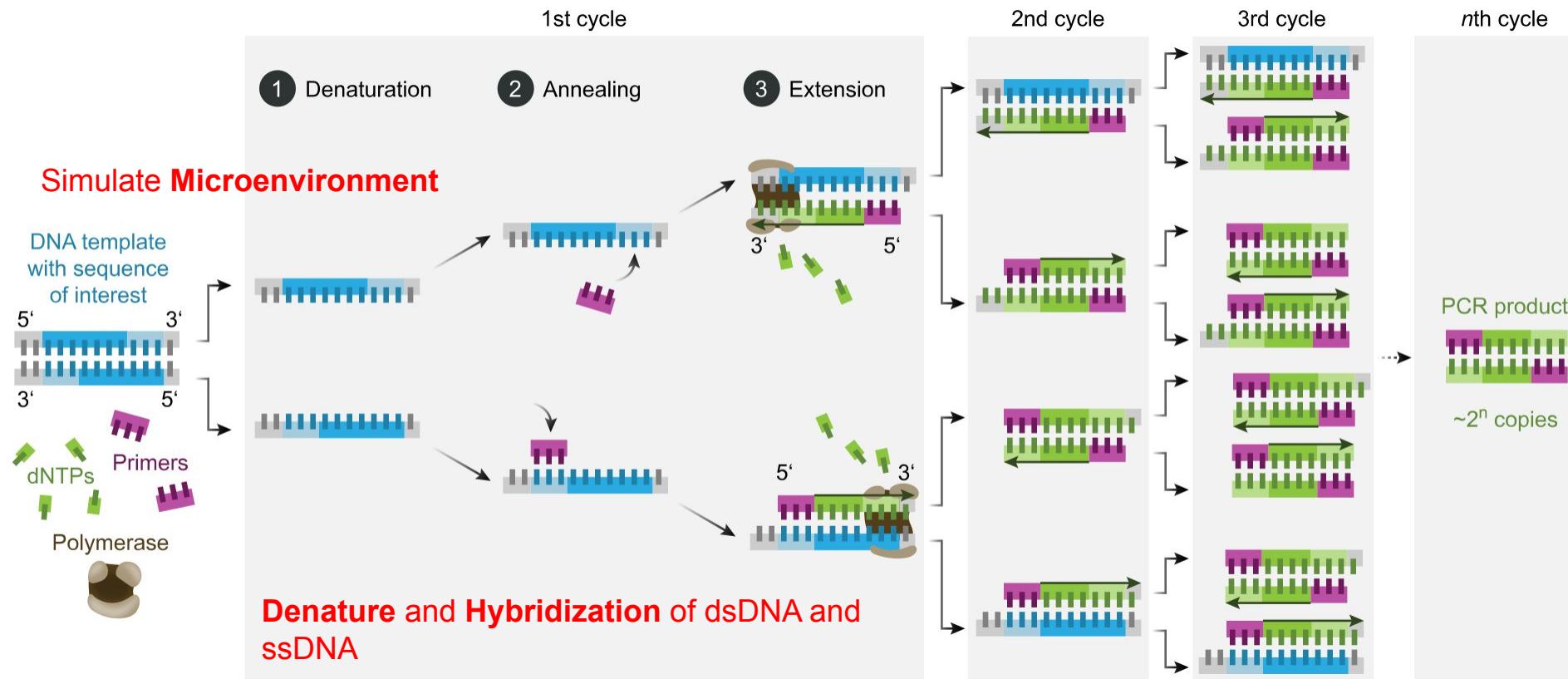
HOW?

Molecular Technique: PCR

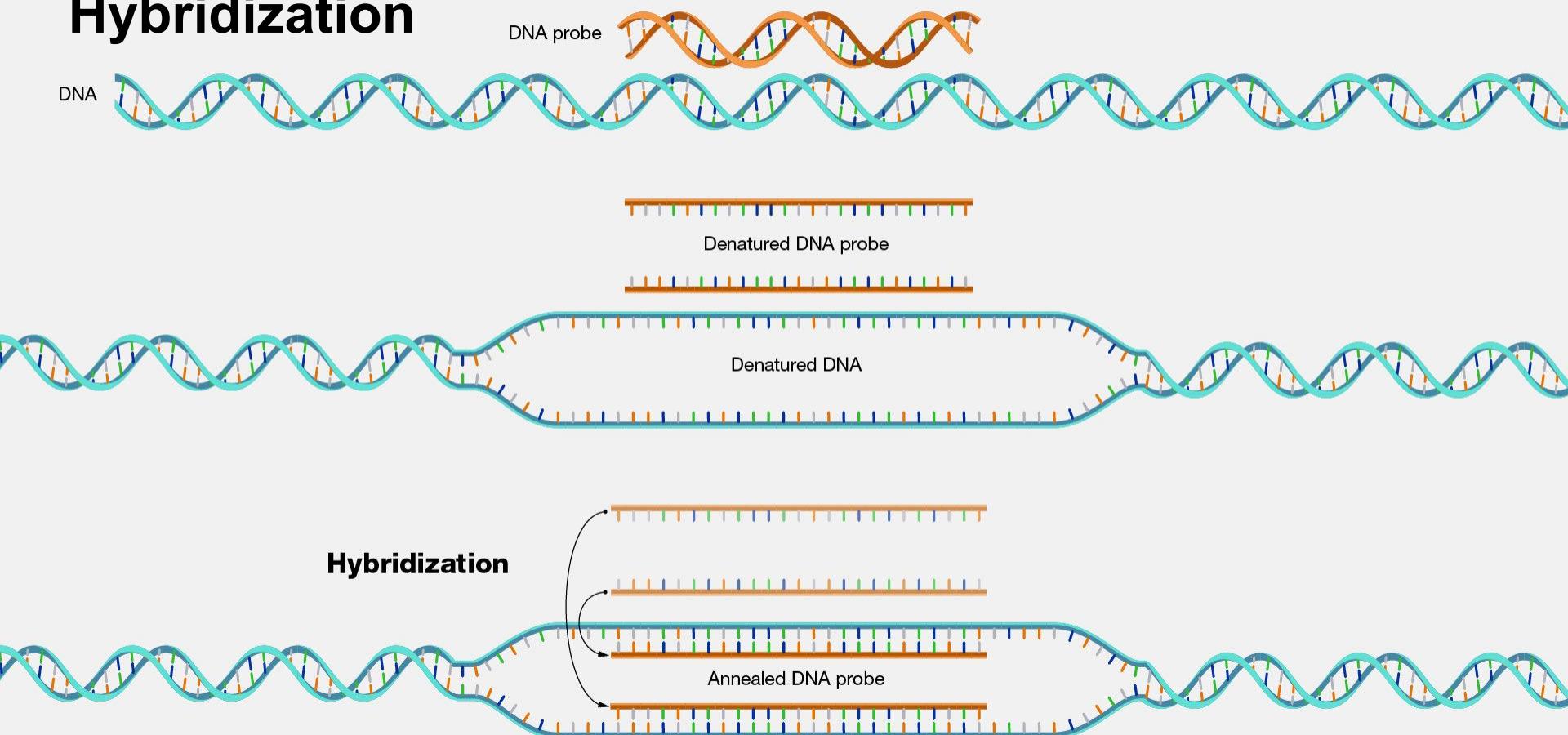
- Amplify **Specific DNA Fragment**
- *in vitro* method
- Required the knowledge of **DNA replication**
 - Simulate the **microenvironment** for the function of DNA polymerase
 - Use **Heat** to separate and deform dsDNA (double strand DNA)
 - Repeat the reaction
- **Hybridization** of Nucleotide

Polymerase Chain Reaction (PCR)

Repeat Reaction



Hybridization



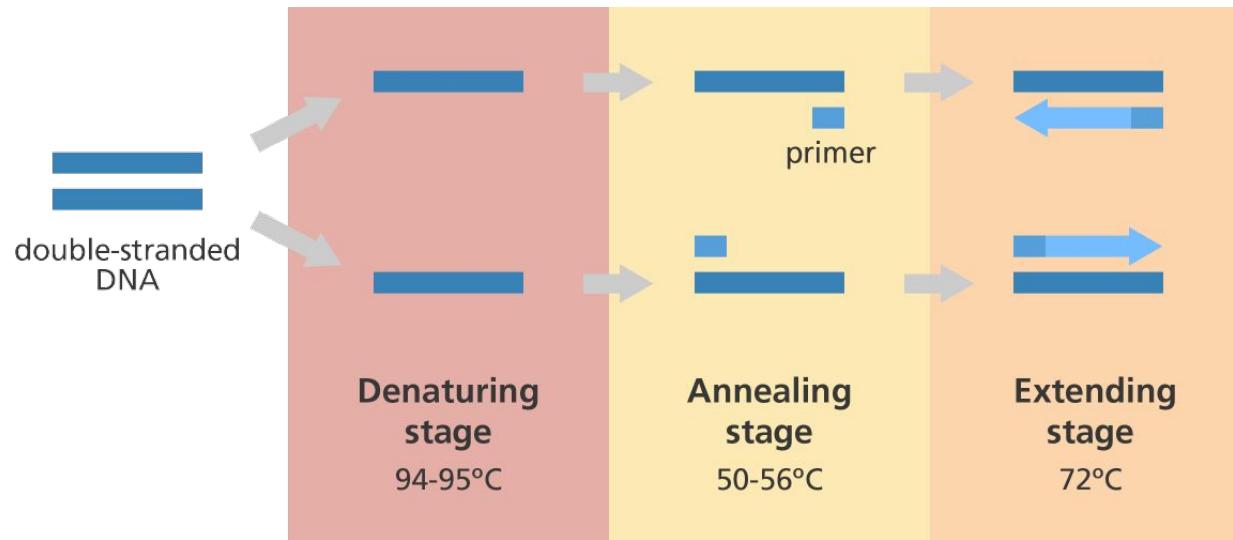
Setup: Microenvironment

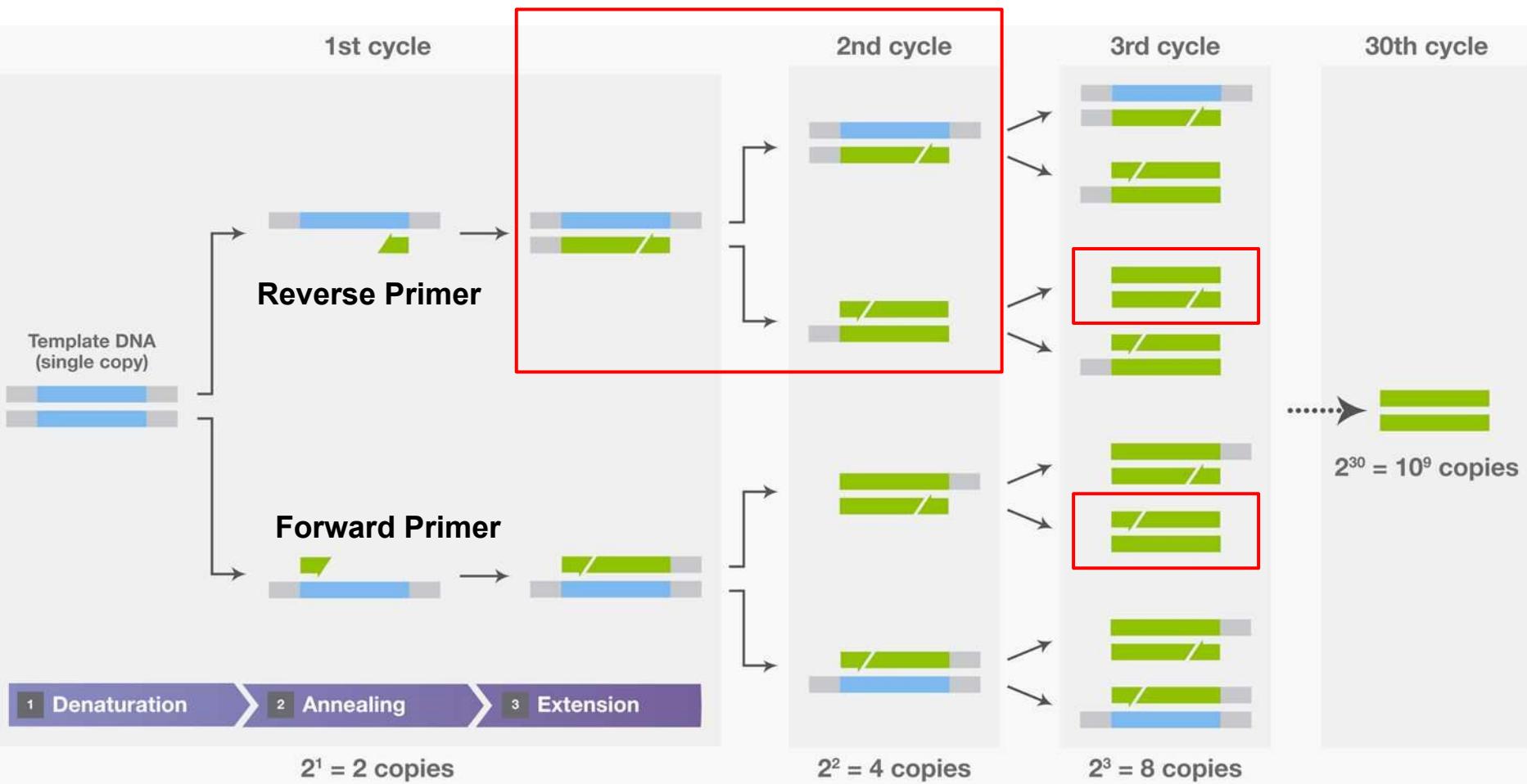
- **Enzymes and Chemical Reagents**
 - DNA polymerase (5'->3' polymerization): Thermotolerant enzymes
 - *Thermus aquaticus*: **Taq** polymerase: w/o Proofreading Function
 - *Pyrococcus furiosus*: **Pfu** polymerase :w/ Proofreading Function
 - etc.
 - DNA primers: Initiation for DNA polymerase
 - Forward Primer: Bind to antisense strand or template strand
 - Reverse Primer: Bind to sense strand or coding strand
 - Required **Hybridization** of complementary DNA
 - Deoxynucleotide Triphosphates (dNTP)
 - ATP, GTP, CTP, TTP
 - PCR buffer
 - Set microenvironment like in living cell: pH
 - **MgCl₂**
 - **Cofactor** of DNA Polymerase

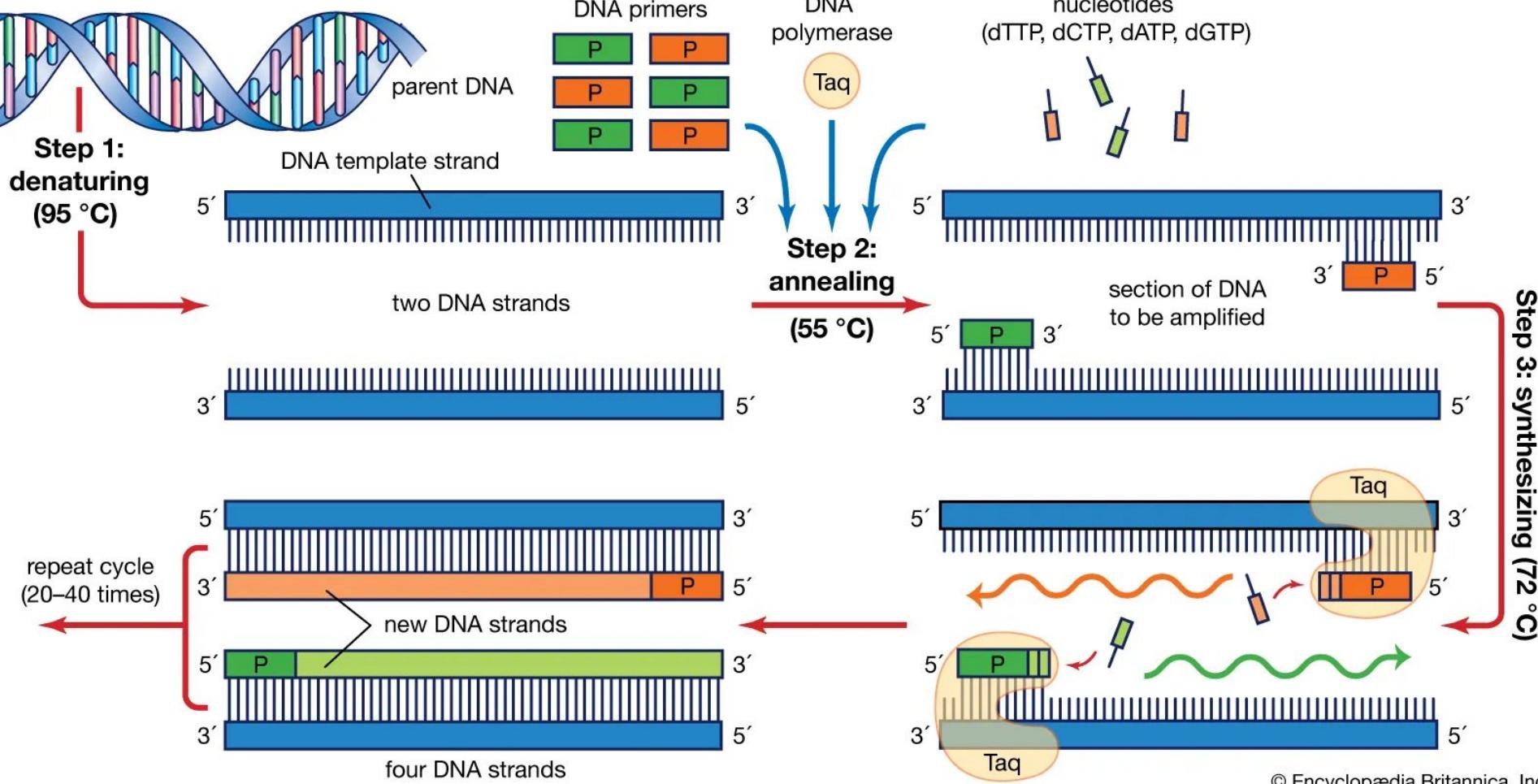
PCR Conditions: Denature and Hybridization

- **3 Steps of PCR**

- **Denature:** Break down double strand to single strand
- **Annealing:** Binding between primers and DNA template
- **Extension:** Polymerization

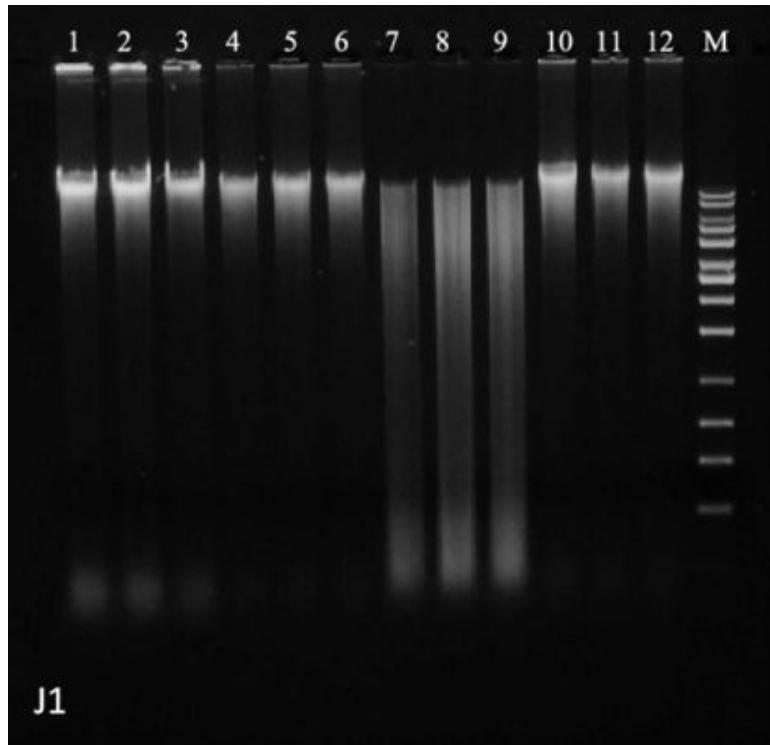




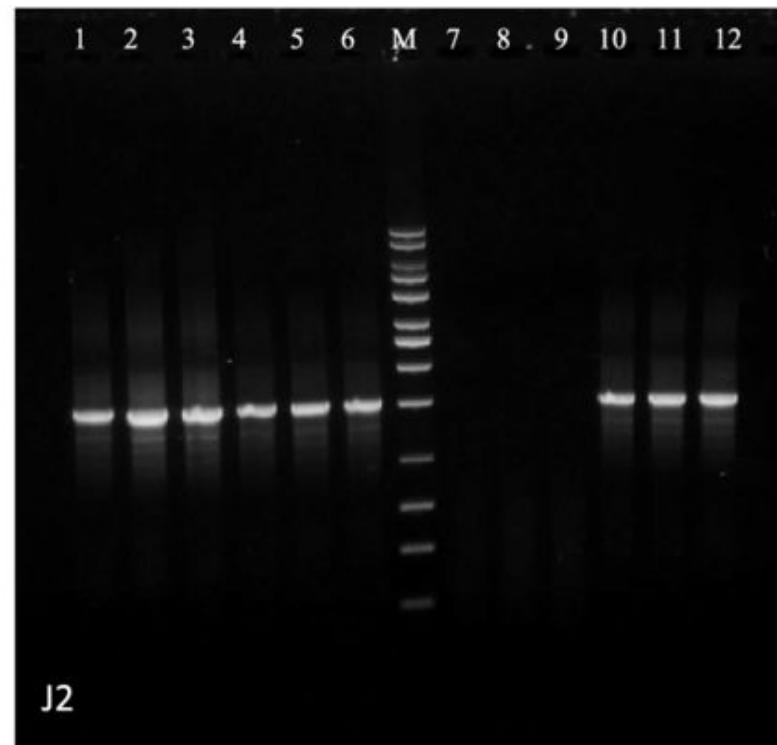


PCR product detection by Gel electrophoresis

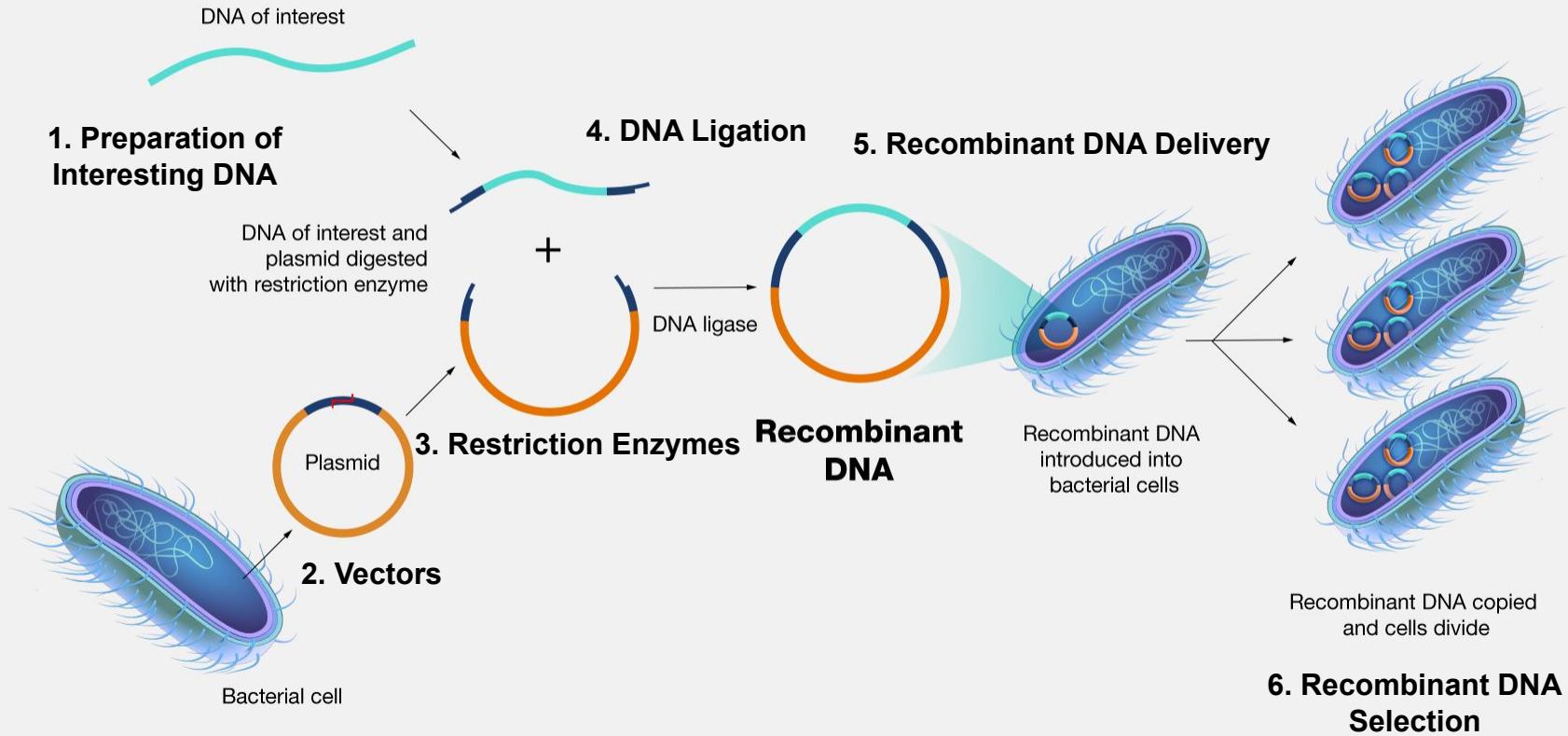
Crude DNA isolation



PCR product



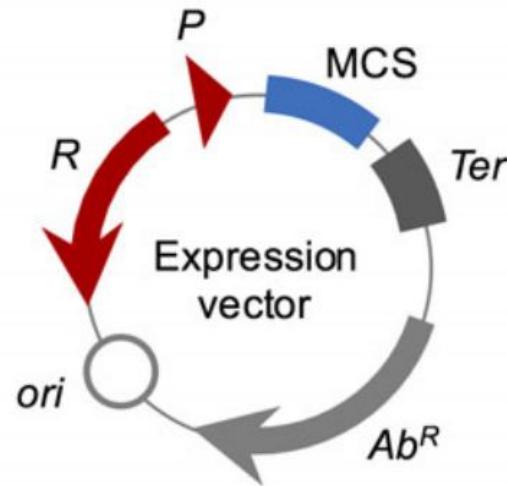
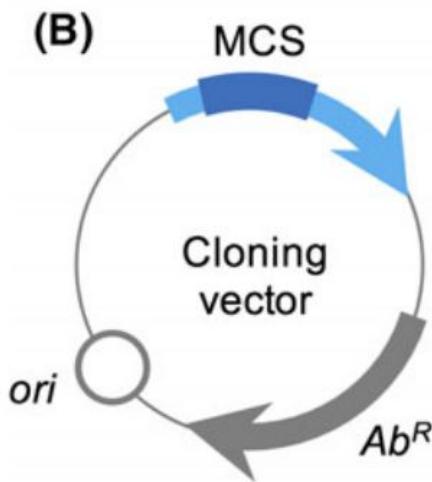
Overview of Recombinant DNA technology



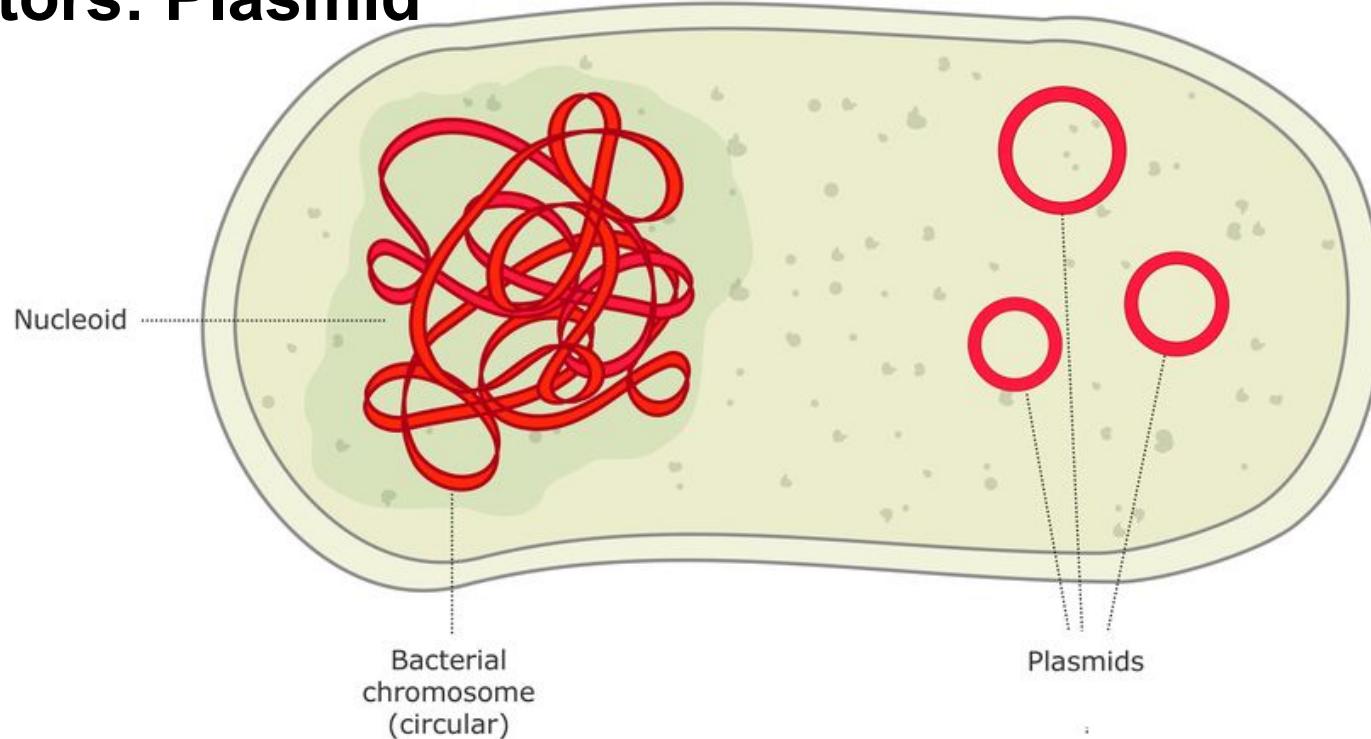
2. Vectors

A DNA molecule (often plasmid or virus) that is used as a vehicle to carry a particular DNA segment into a host cell as part of a cloning or recombinant DNA technique.

3 Functional Types



Vectors: Plasmid



2. Cloning Vectors

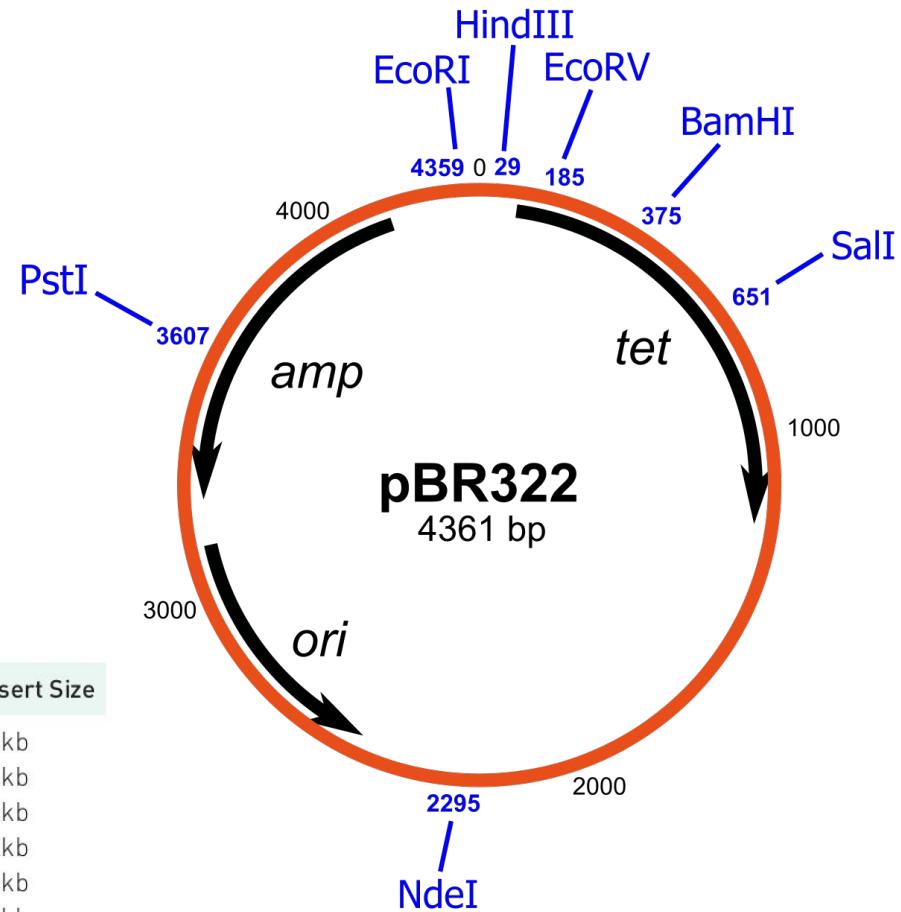
- Cloning Vectors

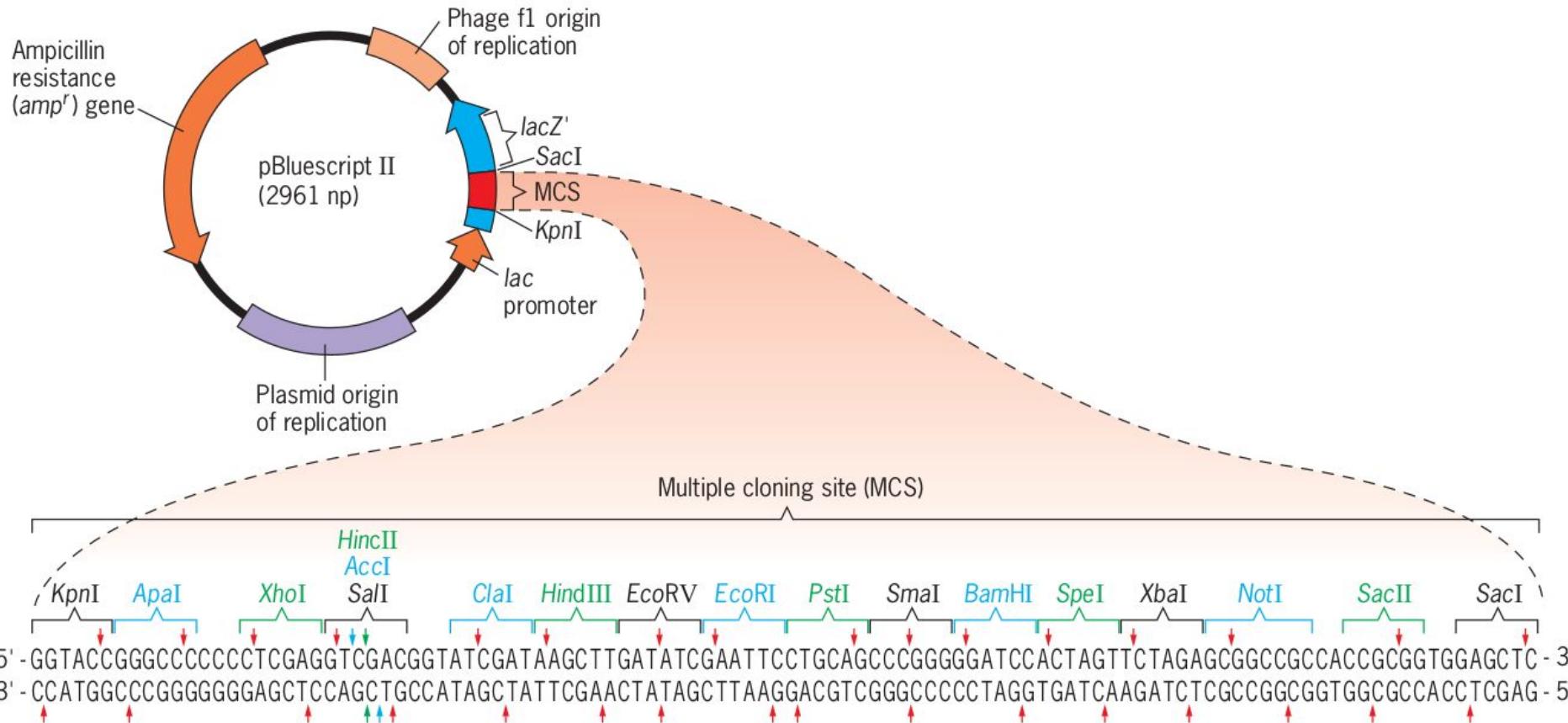
- Plasmids: small insert 20kb
 - Origin of replication (*ori*)
 - Selectable markers (*amp*)
 - Polylinker or Multiple Cloning Site (MCS)
- Artificial Chromosome: > 20kb
 - The largest capacity
 - Act like normal chromosomes
 - Bacterial Artificial Chromosome (BAC)

TABLE 14.2

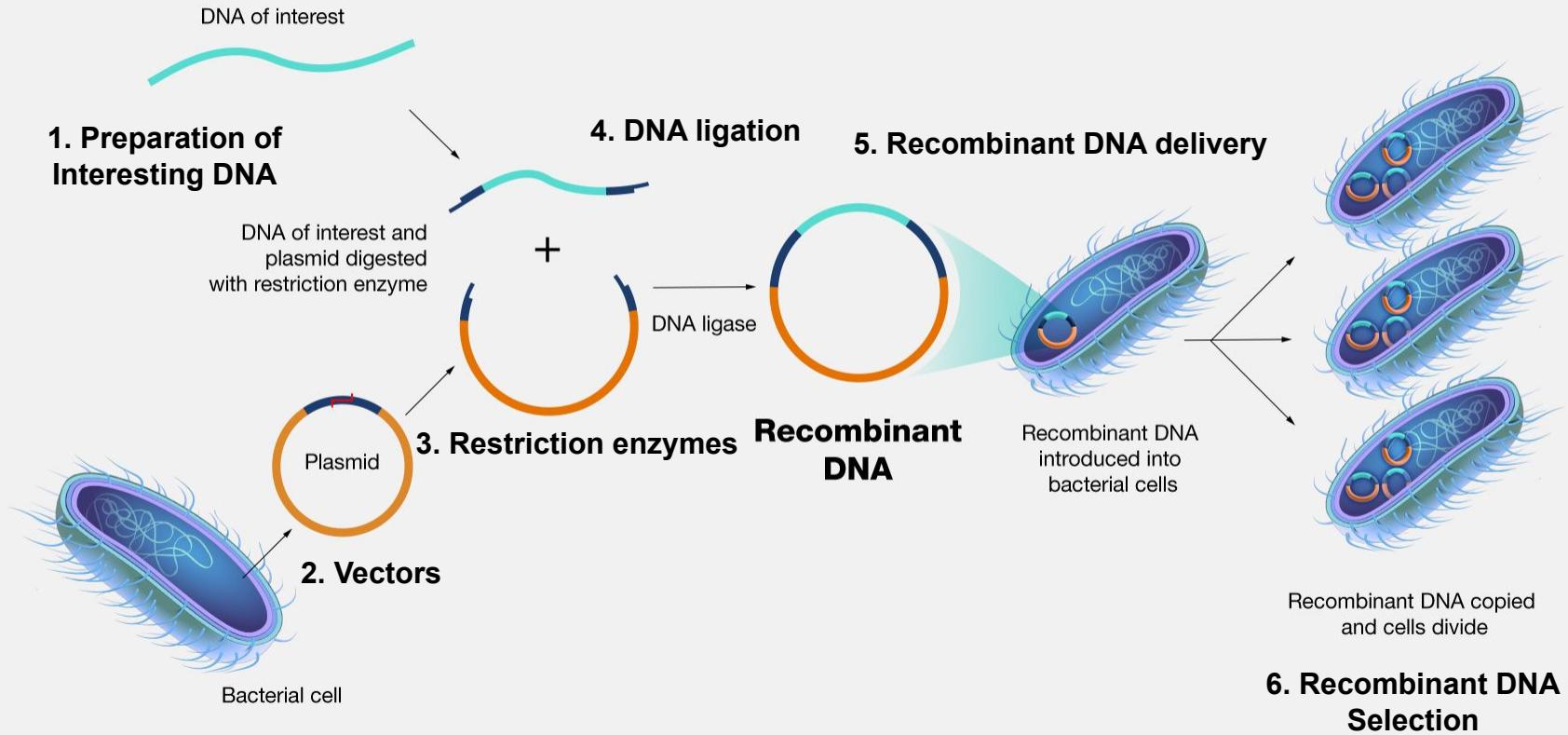
Selected Cloning Vectors and Maximum Insert Sizes

Vector	Maximum Insert Size
Plasmids	15 kb
Phagemids	15 kb
Phage lambda	23 kb
Cosmids	44 kb
Bacterial artificial chromosomes (BACs)	300 kb
Phage P1 artificial chromosomes (PACs)	300 kb
Yeast artificial chromosomes (YACs)	600 kb





Overview of Recombinant DNA technology



DNADNAD**N**ADNA



HOW?

DNADNAD**N**

ADNADNA

TABLE 10.1 Ten Commonly Used Restriction Enzymes

Fragmenting DNA

- To cut the DNA (Digestion) at **specific region on DNA sequence**
- Restriction Enzymes (RE):** Mostly from Microbe
 - Restriction Endonucleases (Inside Cut)**
 - Recognition Sites**
 - Palindromes**

A
 EVE
 RADAR
 REVIVER
 ROTATOR

 LEPERS REPEL
 MADAM I'M ADAM
 STEP NOT ON PETS
 DO GEESE SEE GOD
 PULL UP IF I PULL UP

 NO LEMONS, NO MELON
 DENNIS AND EDNA SINNED
 ABLE WAS I ERE I SAW ELBA

 A MAN, A PLAN, A CANAL, PANAMA
 A SANTA LIVED AS A DEVIL AT NASA

 SUMS ARE NOT SET AS A TEST ON ERASMUS
 ON A CLOVER, IF ALIVE, ERUPTS A VAST, PURE EVIL; A FIRE VOLCANO

Enzyme	Sequence of Recognition Site	Microbial Origin
<i>TaqI</i>		<i>Thermus aquaticus</i> YT1
<i>RsaI</i>		<i>Rhodopseudomonas sphaeroides</i>
<i>Sau3AI</i>		<i>Staphylococcus aureus</i> 3A
<i>EcoRI</i>		<i>Escherichia coli</i>
<i>BamHI</i>		<i>Bacillus amyloliquefaciens</i> H
<i>HindIII</i>		<i>Haemophilus influenzae</i>
<i>KpnI</i>		<i>Klebsiella pneumoniae</i> OK8
<i>ClaI</i>		<i>Caryophanon latum</i>
<i>BssHII</i>		<i>Bacillus stearothermophilus</i>
<i>NotI</i>		<i>Nocardia otitidisca��iarum</i>

Restriction Enzymes

- Restriction Endonuclease
 - Blunt ends
 - Sticky ends
 - 5' overhangs
 - 3' overhangs

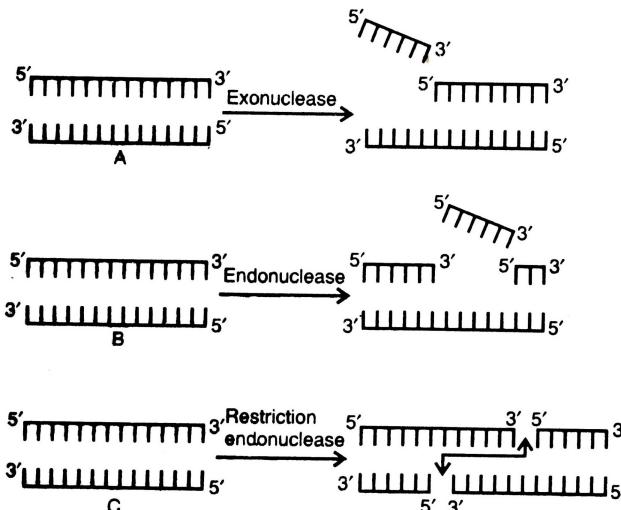
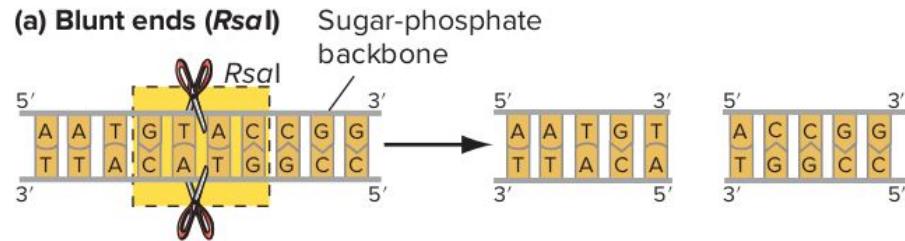
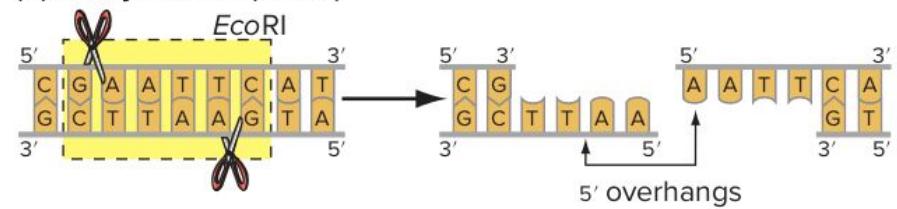


Figure 10.1 Restriction enzymes produce DNA fragments with either blunt or sticky ends. (a) The restriction enzyme *RsaI* produces blunt-ended fragments. (b) *EcoRI* produces sticky ends with a 5' overhang. (c) *KpnI* produces sticky ends with a 3' overhang.

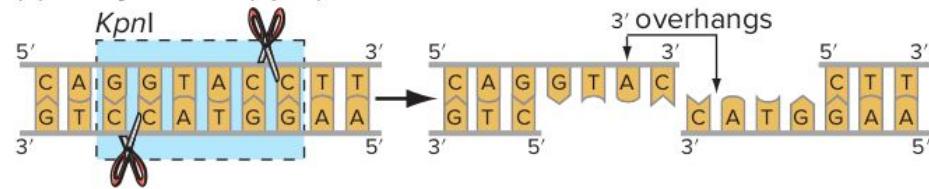
(a) Blunt ends (*RsaI*)



(b) Sticky 5' ends (*EcoRI*)



(c) Sticky 3' ends (*KpnI*)



Recognition Site of Restriction Enzymes

- Short recognition site (4 bp) produce Small fragment size with Large number of fragments
- Large recognition site (8 bp) produce Large fragment size with Small number of fragments

(a) Calculating average restriction fragment size

1. Probability that a four-base recognition site will be found at a given position in a genome =

$$1/4 \times 1/4 \times 1/4 \times 1/4 = 1/256$$

2. Probability that a six-base recognition site will be found =

$$1/4 \times 1/4 \times 1/4 \times 1/4 \times 1/4 \times 1/4 = 1/4,096$$

3. Probability that an eight-base recognition site will be found =

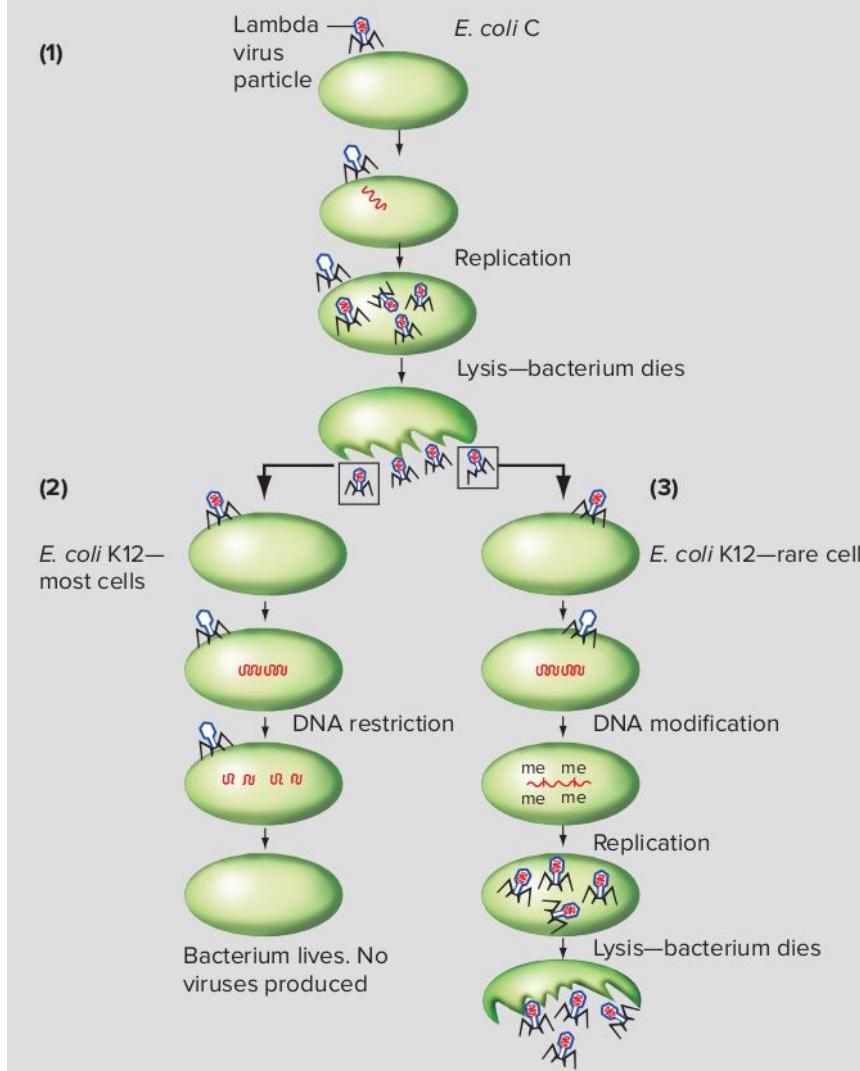
$$(1/4)^8 = 1/65,536$$

(b) Restriction map of a 200 kb region of chromosome 11



The Discovery of Restriction enzymes

- **Restriction**
 - Digest foreign DNA molecule
- **Modification**
 - DNA methylation
 - Protect DNA from digestion



Sequence-specific cleavage of DNA by EcoRI and protection from cleavage by methylation.

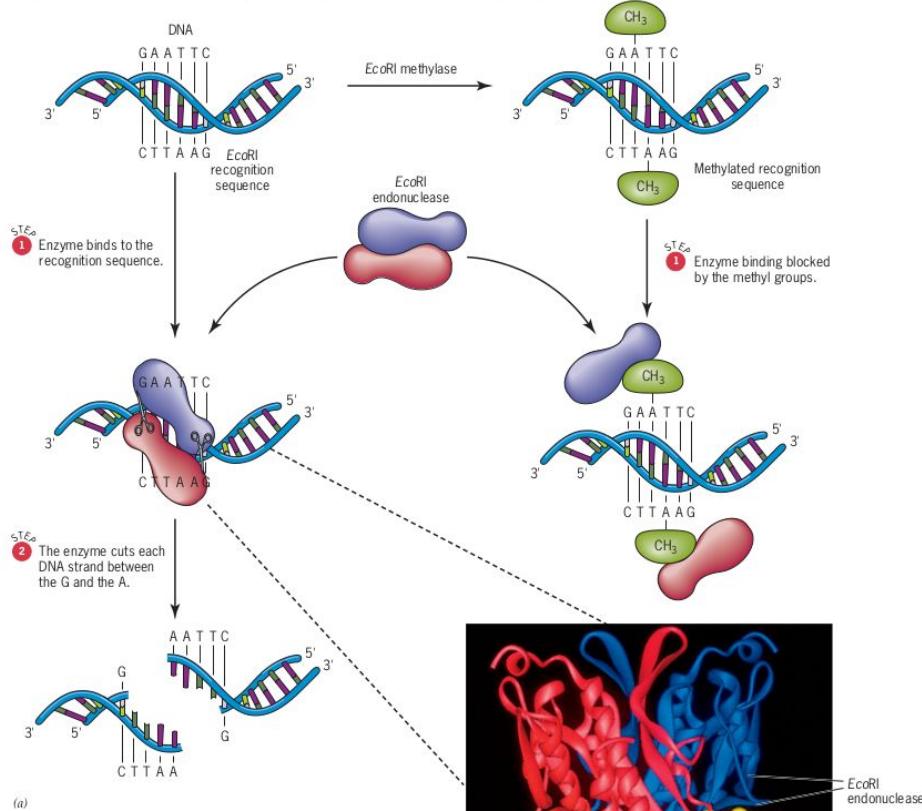
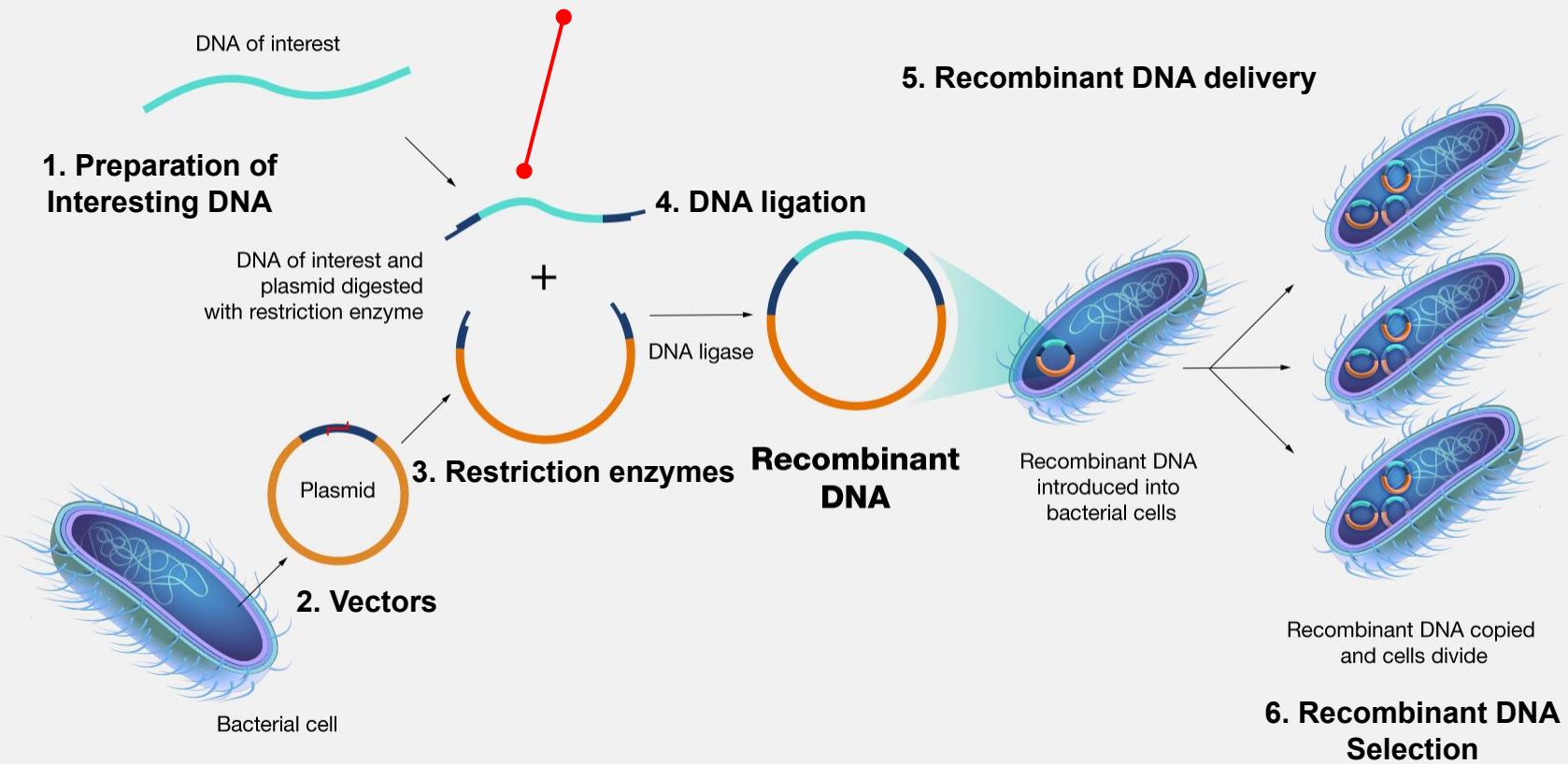


FIGURE 14.1 The EcoRI restriction-modification system. (a) Cleavage of the unmethylated EcoRI recognition sequence by EcoRI restriction endonuclease and protection of the recognition sequence from cleavage by methylation catalyzed by the EcoRI methylase. (b) Diagram of the structure of the EcoRI-DNA complex based on X-ray diffraction data. The two subunits of the EcoRI endonuclease are shown in red and blue.

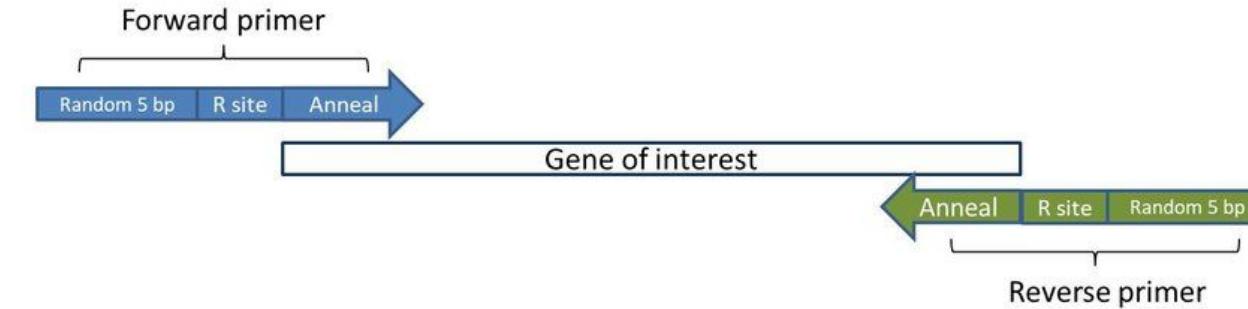
(b) Structure of an EcoRI-DNA complex based on X-ray diffraction data.

HOW?

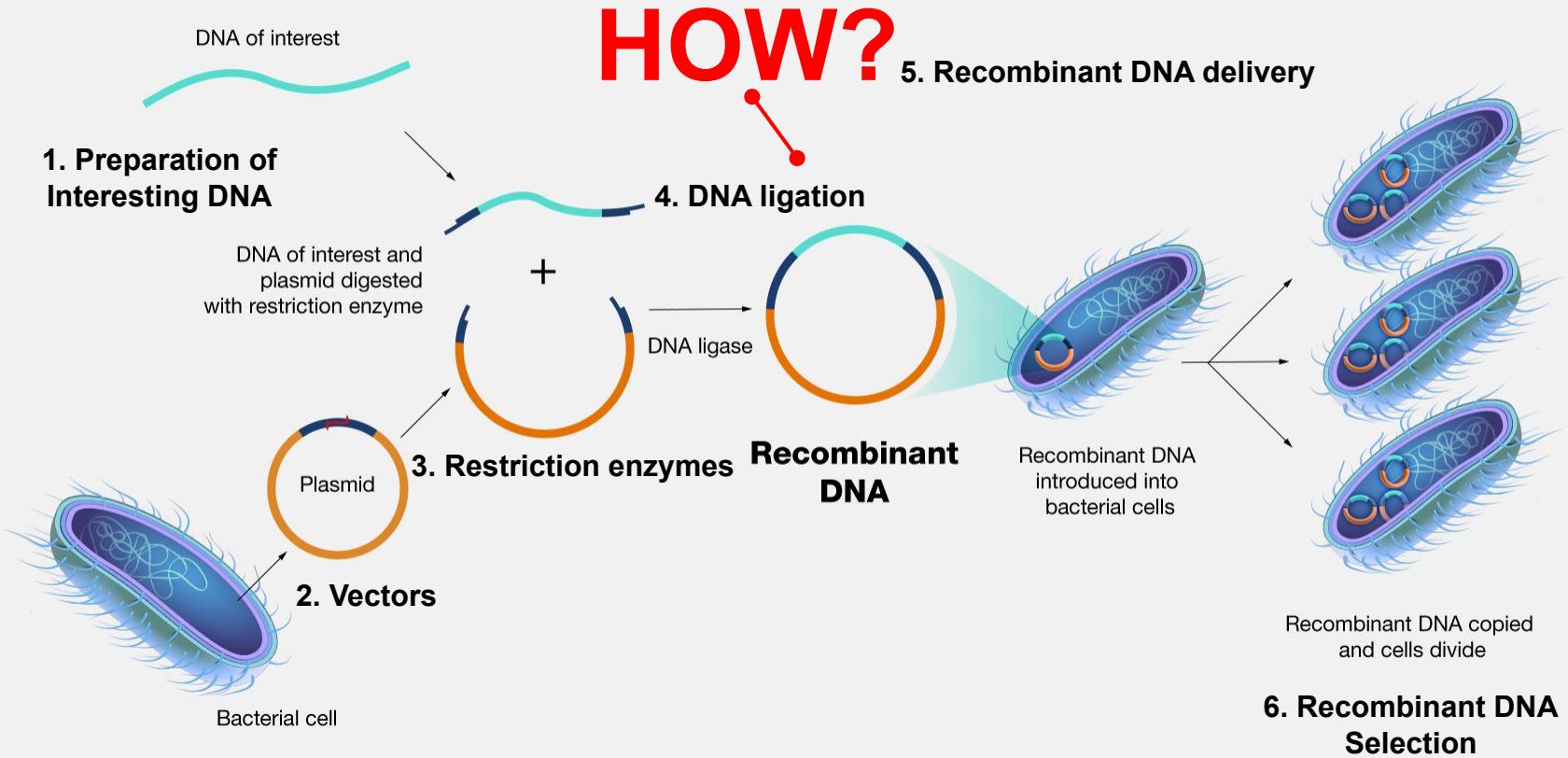


PCR-Primer Design for Recombinant DNA

- Design Forward and Reverse Primer with **recognition sites of RE (R site)**
- **Random 5 bp** for the efficient of Digestion
 - RE can bind R site easier



HOW?

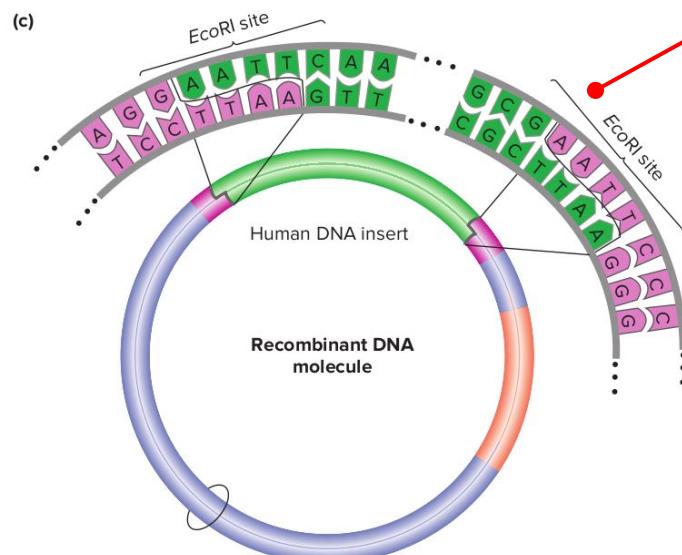
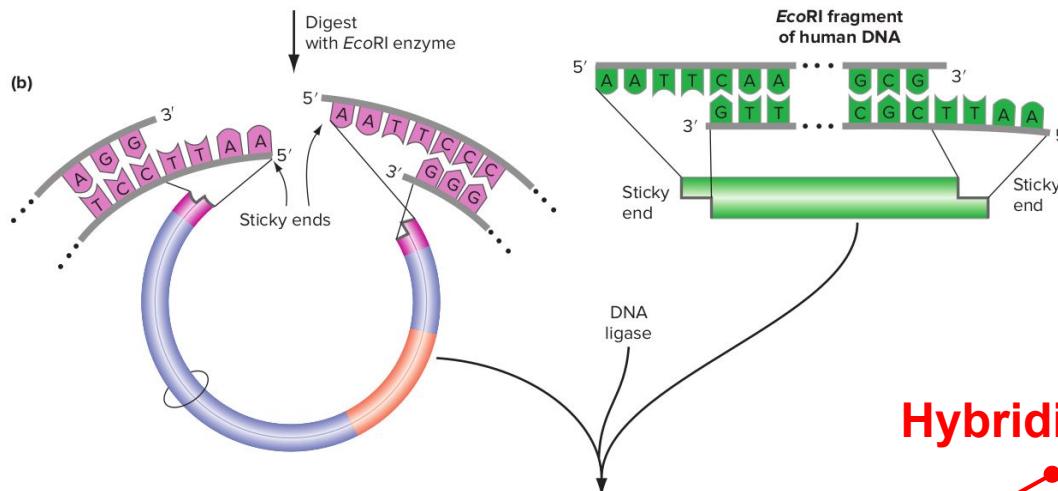


DNADNADN
ADNADNA

HOW?

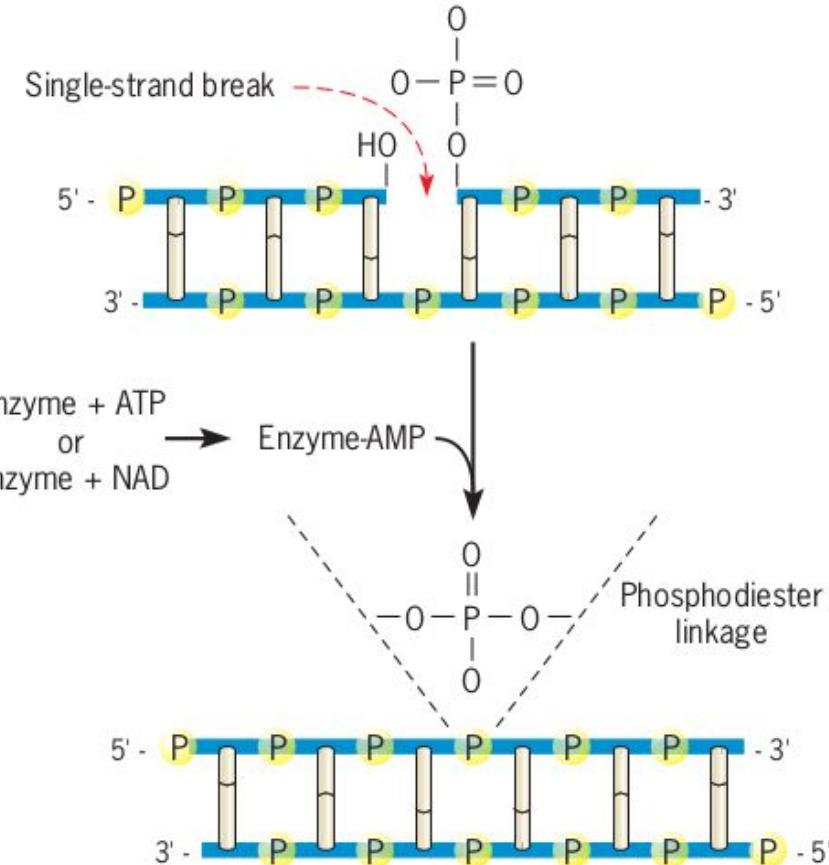


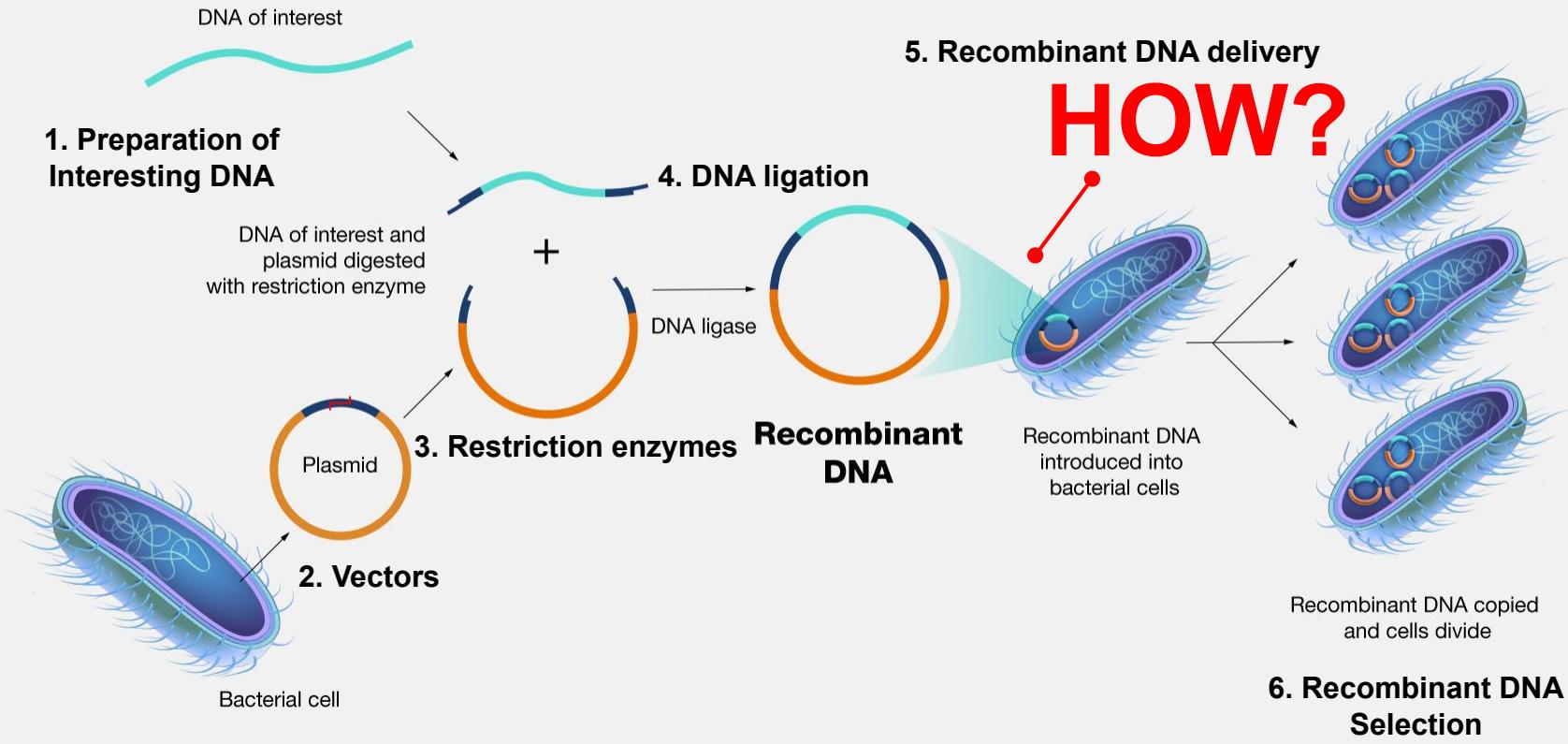
DNADNADNADNADNA



4. DNA Ligation

- **DNA ligation**
 - DNA ligase
 - link DNA Fragments
 - By Phosphodiester linkage



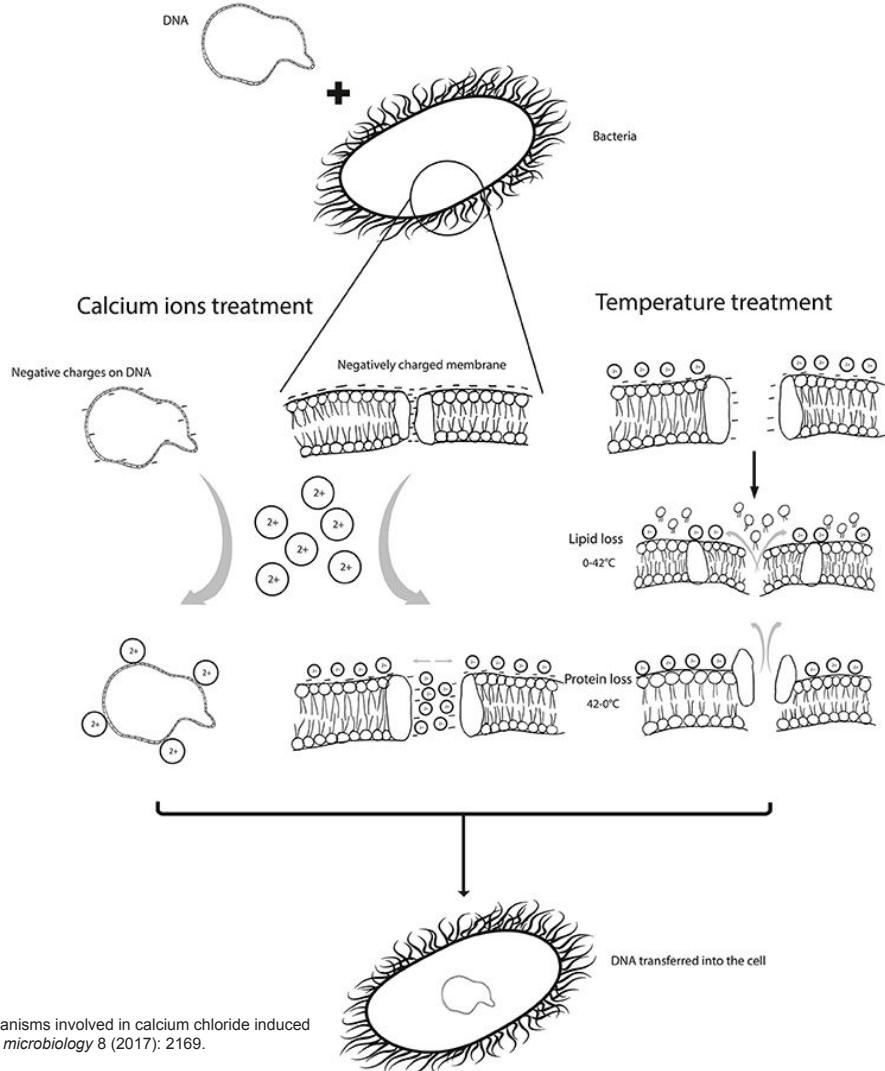


5. Recombinant DNA delivery

- Mostly use **Chemical Transformation** and **Electroporation**
- **Chemical Transformation**
 - Preparation of Competent cells by CaCl_2 : make cell membrane loss their permeability
 - Heat shock at 42 °C: enhance the fluidity of membrane to increase the chance to uptake foreign DNA
- **Electroporation**
 - Use electrical pulse to make artificial pore

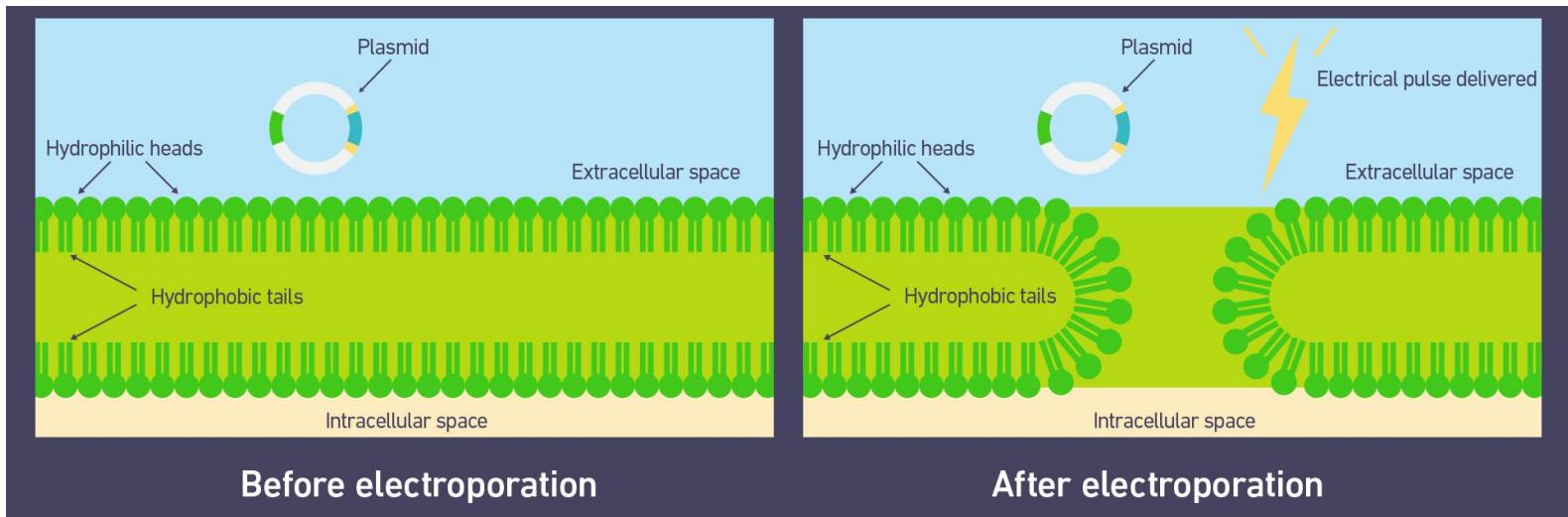
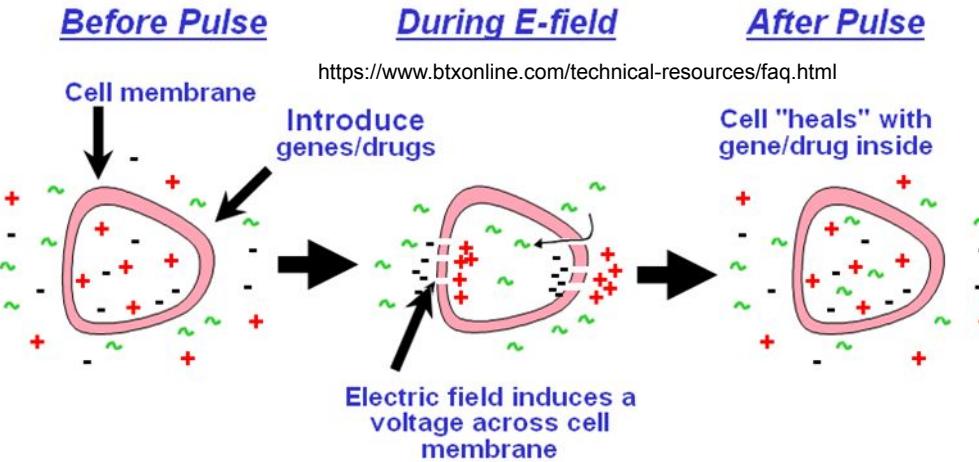
Chemical Transformation

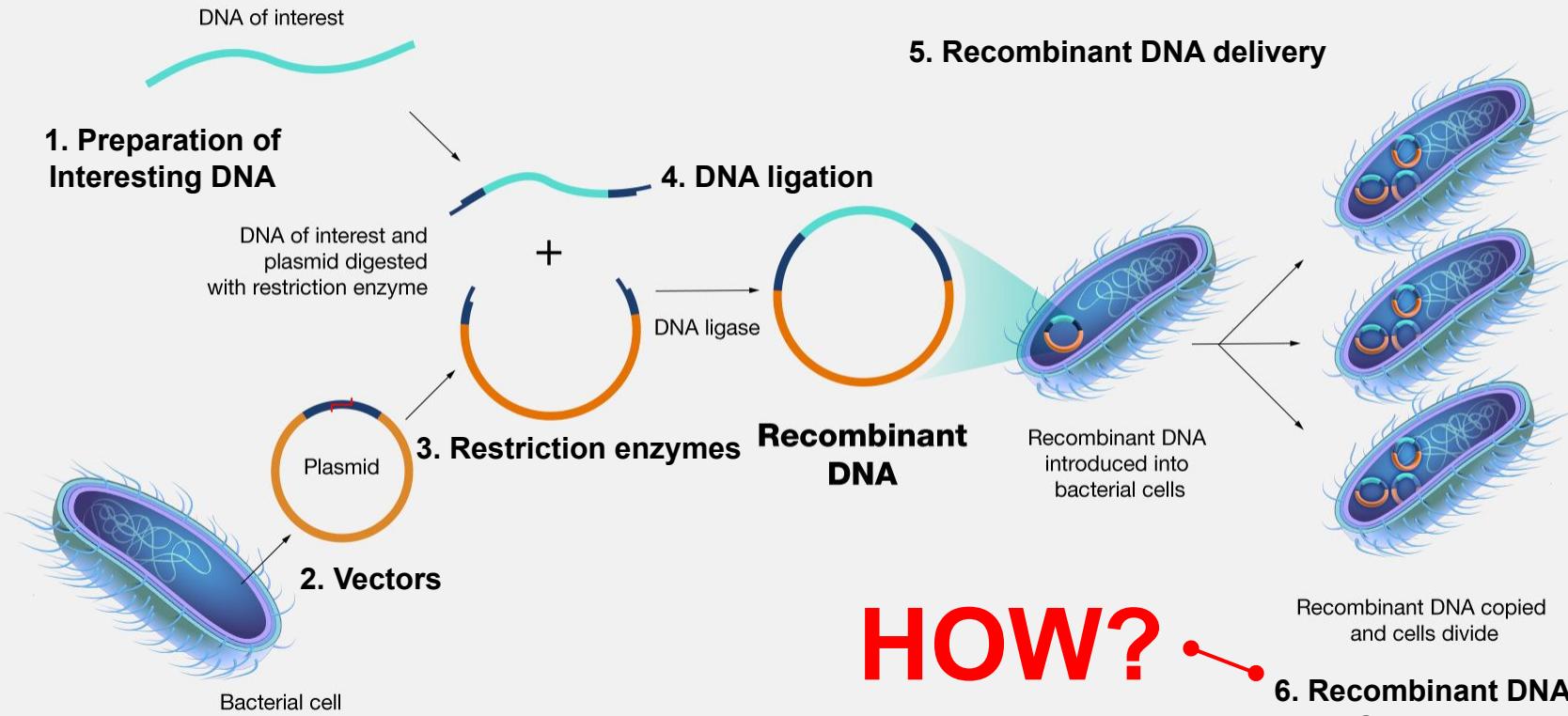
- **CaCl₂ treatment to produce competent cell**
- **Heat shock** by rapidly increase temperature to 42 °C and rapidly decrease temperature to 0 °C, **enhance** the entering of foreign DNA to competent cells



Electroporation

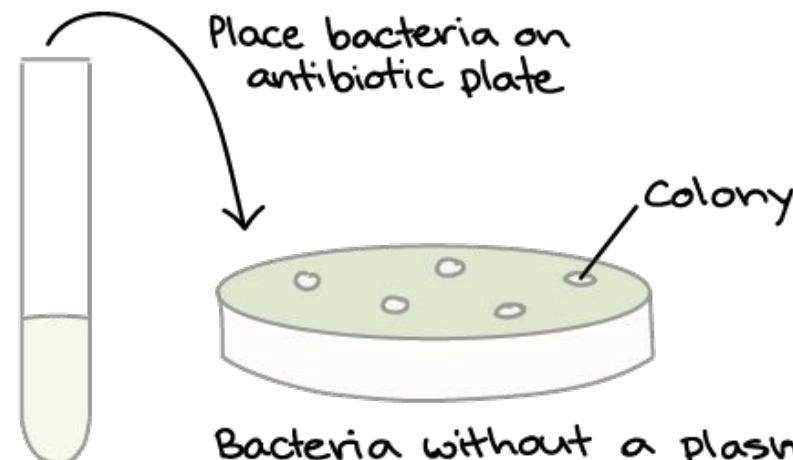
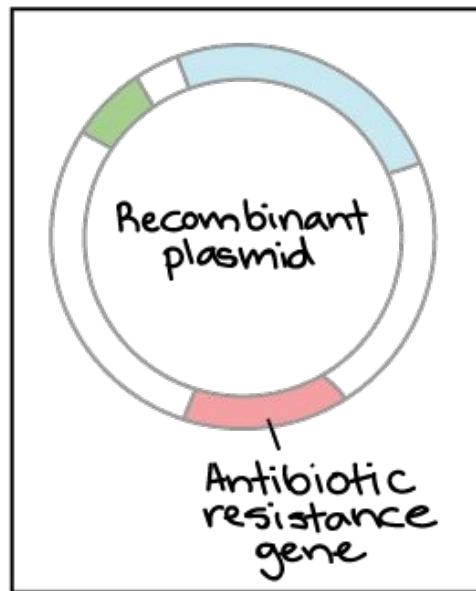
- Use electrical pulse to make pore
- The foreign DNA can entering via transient pore





Selection of Bacteria/Yeast contained Vector

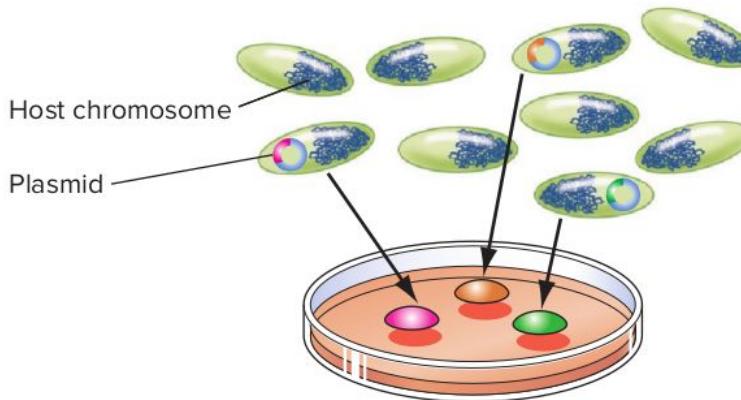
- Not all bacteria/yeast cell contained Vectors after transformation, we have to select and pick the bacteria/yeast that contained Vectors



Bacteria without a plasmid die.
Each bacterium with a plasmid makes a colony.

Selection in Bacteria/Yeast system

- Selection by **Antibiotic Resistance**
 - Growth transformed bacteria in media supplemented antibiotic
- Selection by **Auxotrophy**
 - Growth transformed yeast in minimal media lacked essential amino-acid supplement

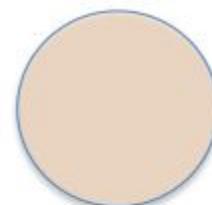


E. coli plated onto medium containing ampicillin. Only cells containing plasmids are able to grow.

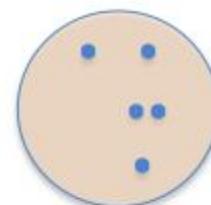
Auxotroph & Prototroph

Simple comparison of an Auxotroph and Prototroph

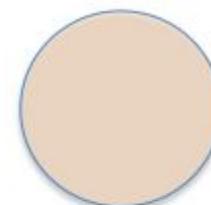
**Arginine
Auxotroph**
(Needs Arginine to grow)



Minimal media (MM)

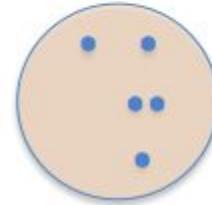


MM + Arginine

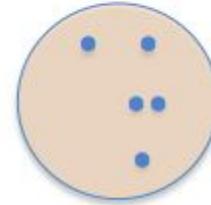


MM + Lysine

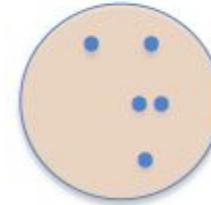
Prototroph



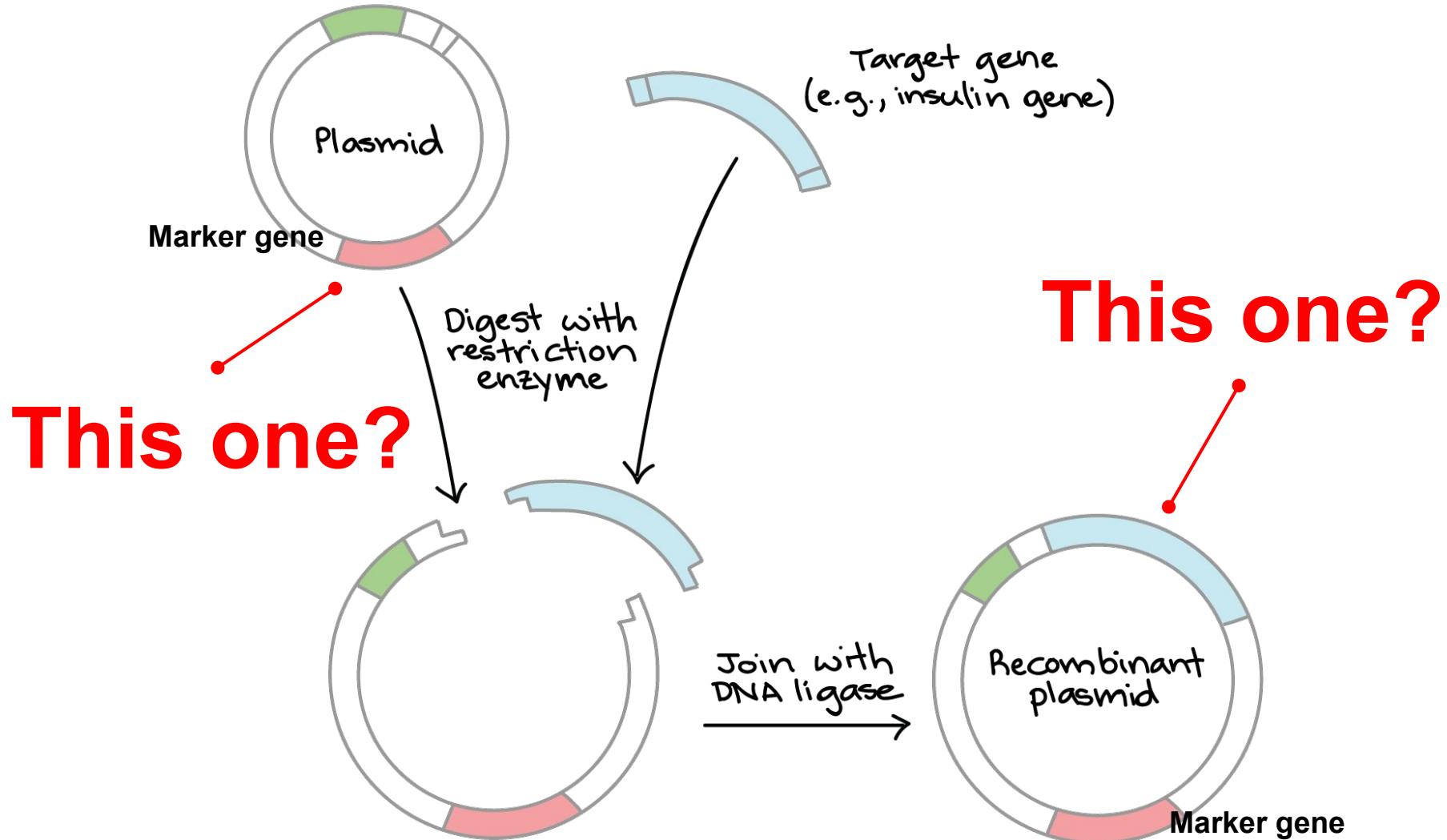
Minimal media (MM)



MM + Arginine

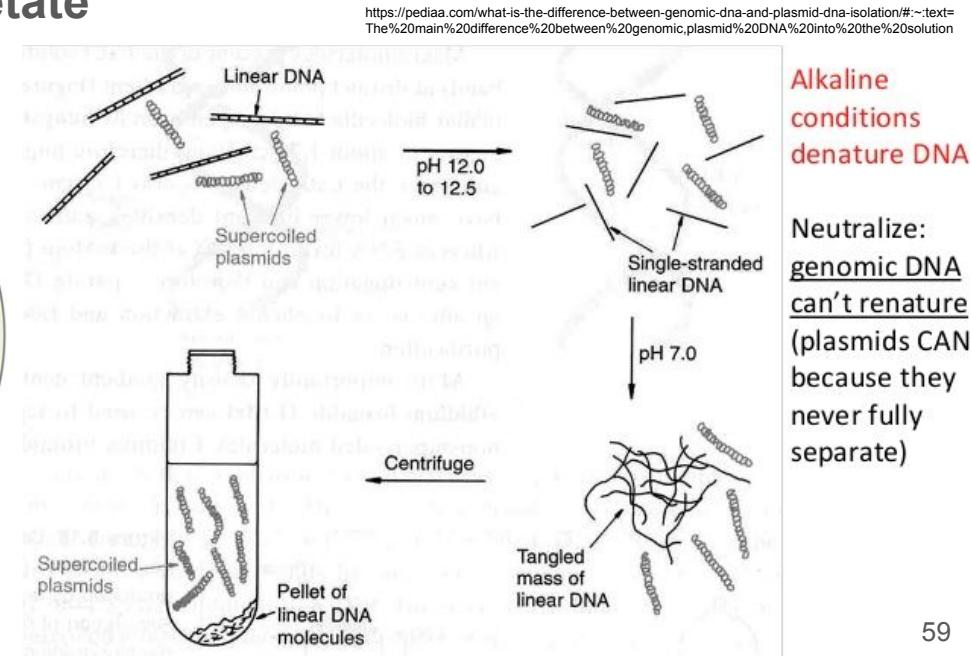
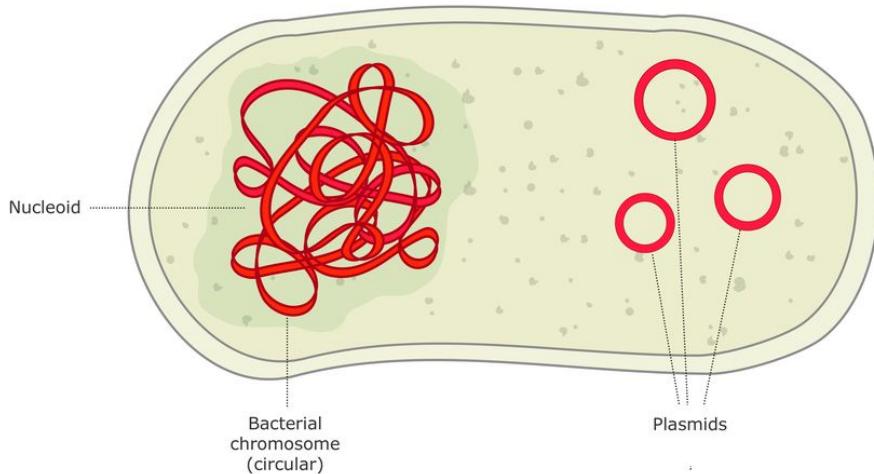


MM + Lysine



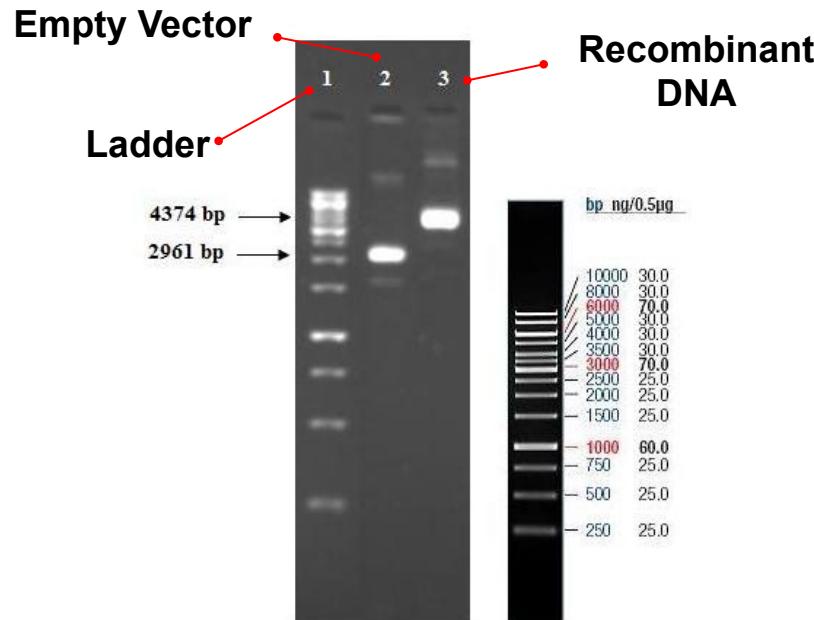
Vector isolation

- Different size of genomic DNA and plasmid DNA
- Alkaline lysis used to extract plasmid from genomics
- Neutralization by Potassium Acetate

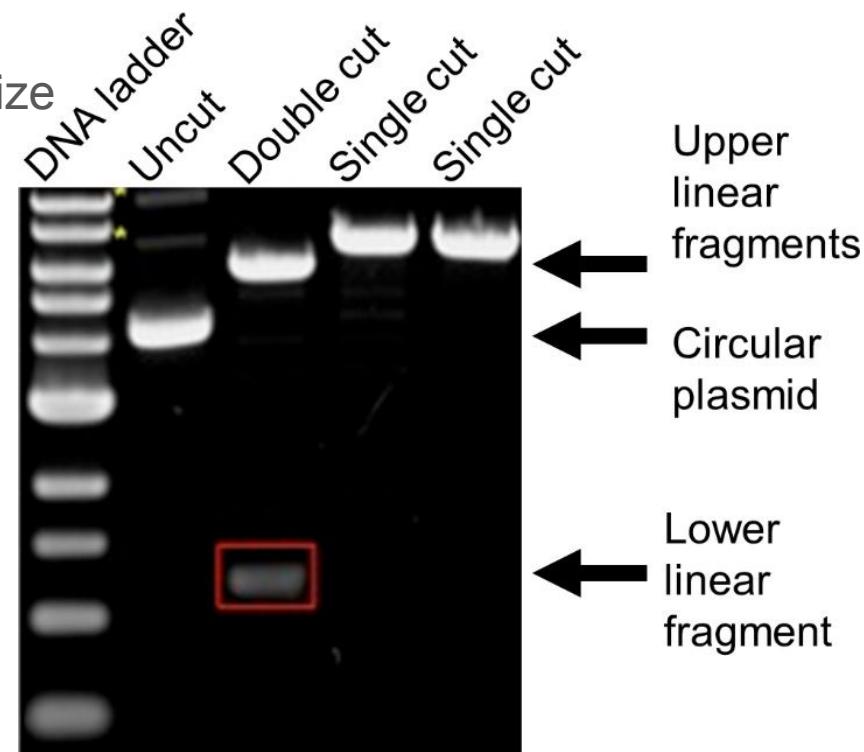


RE Digestion and Electrophoresis

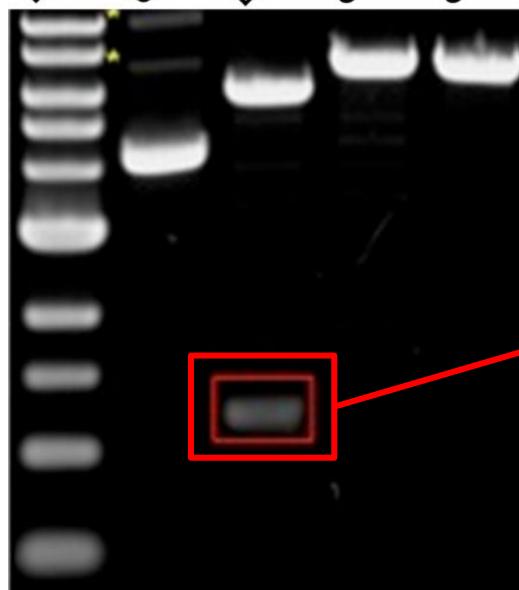
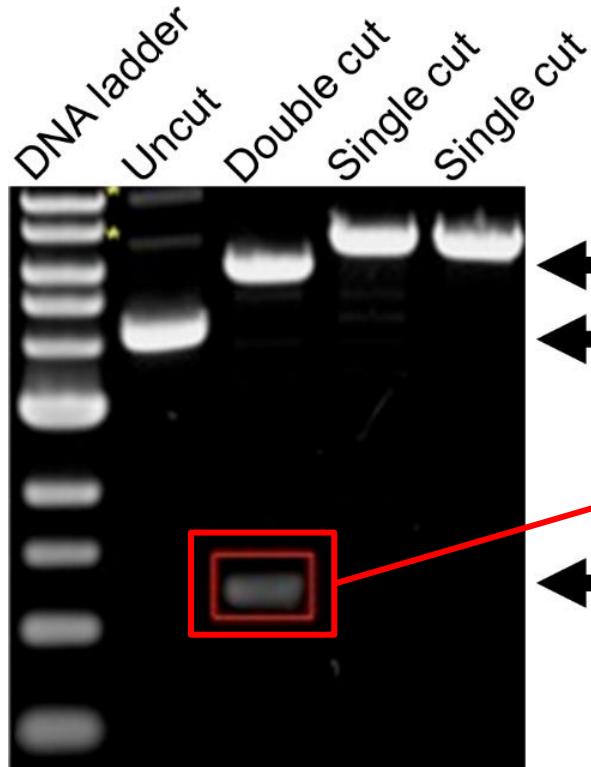
- Digest recombinant DNA with same RE
- Run electrophoresis to check fragment size



bp ng/0.5μg	
10000	30.0
8000	30.0
6000	70.0
5000	30.0
4000	30.0
3500	30.0
3000	70.0
2500	25.0
2000	25.0
1500	25.0
1000	60.0
750	25.0
500	25.0
250	25.0

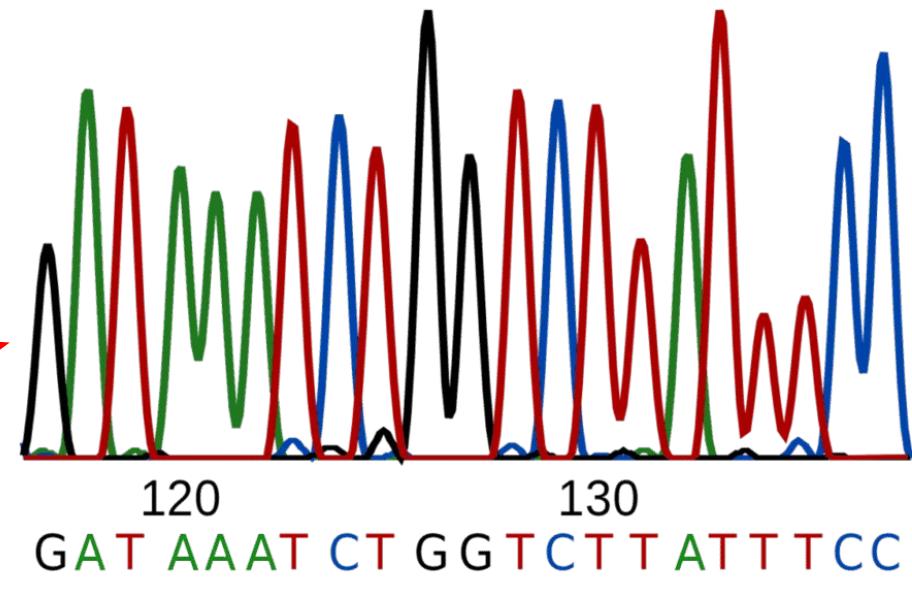


Gel Cutting and Sequencing

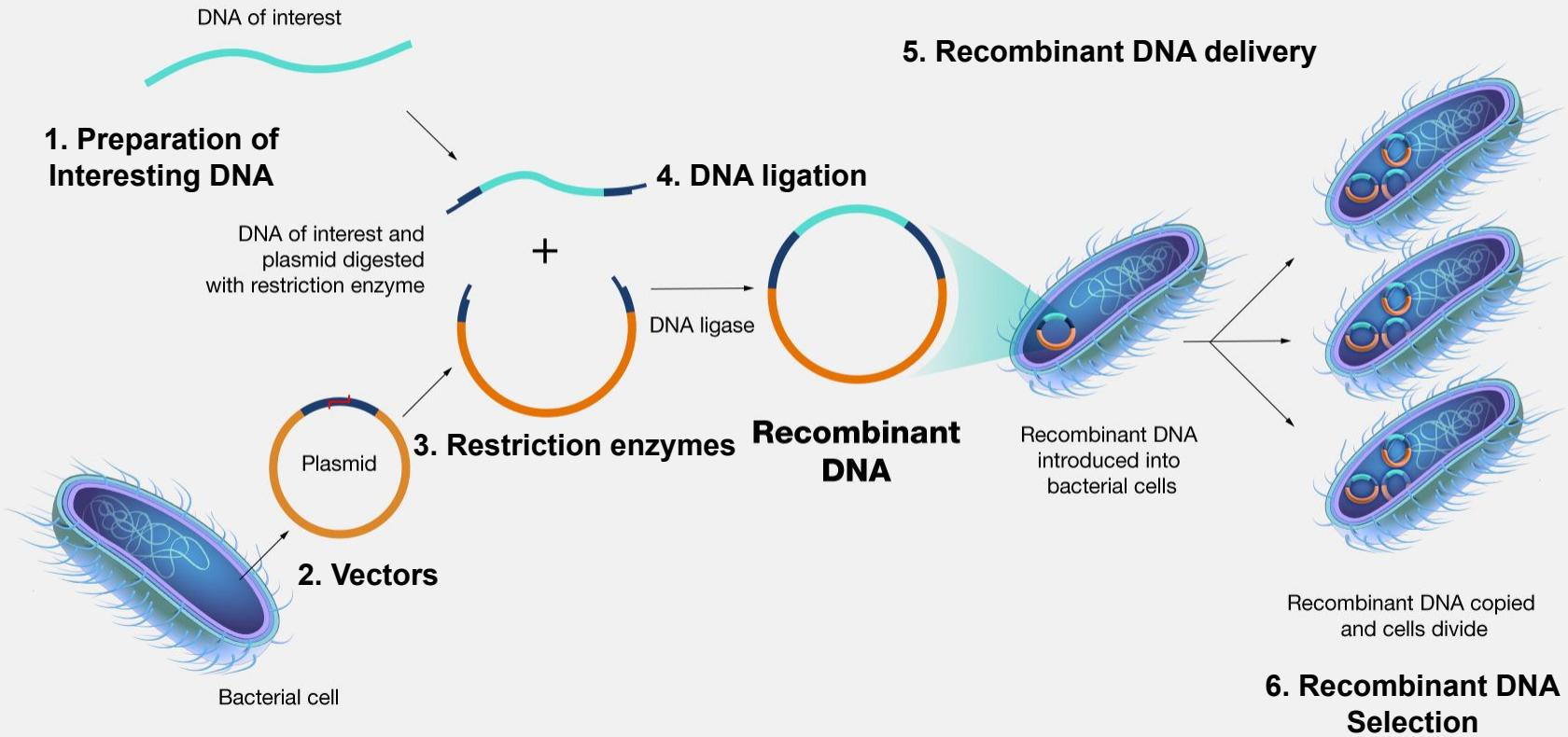


Upper linear fragments
Circular plasmid
Lower linear fragment

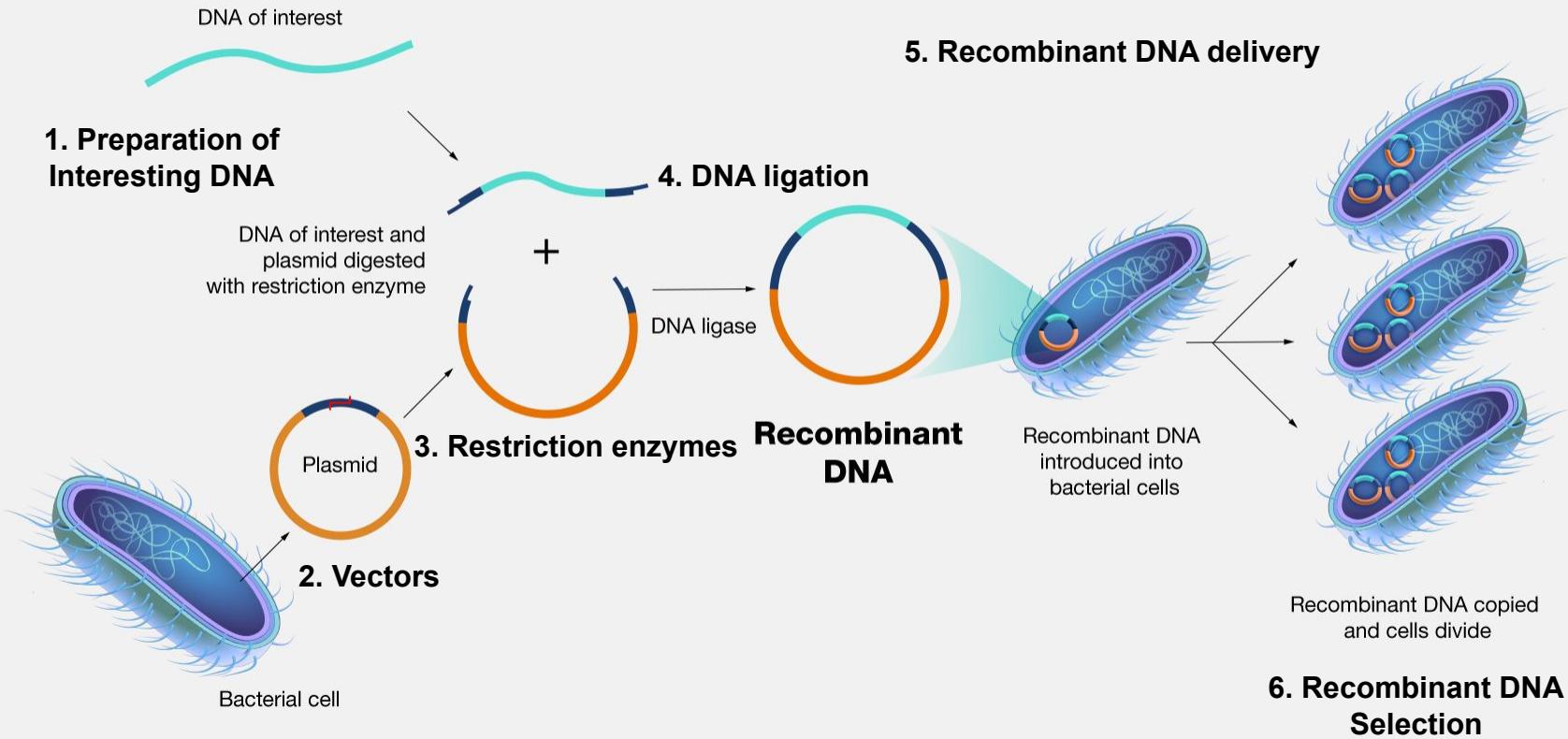
DNA sequencing



Finish



But Not “END”

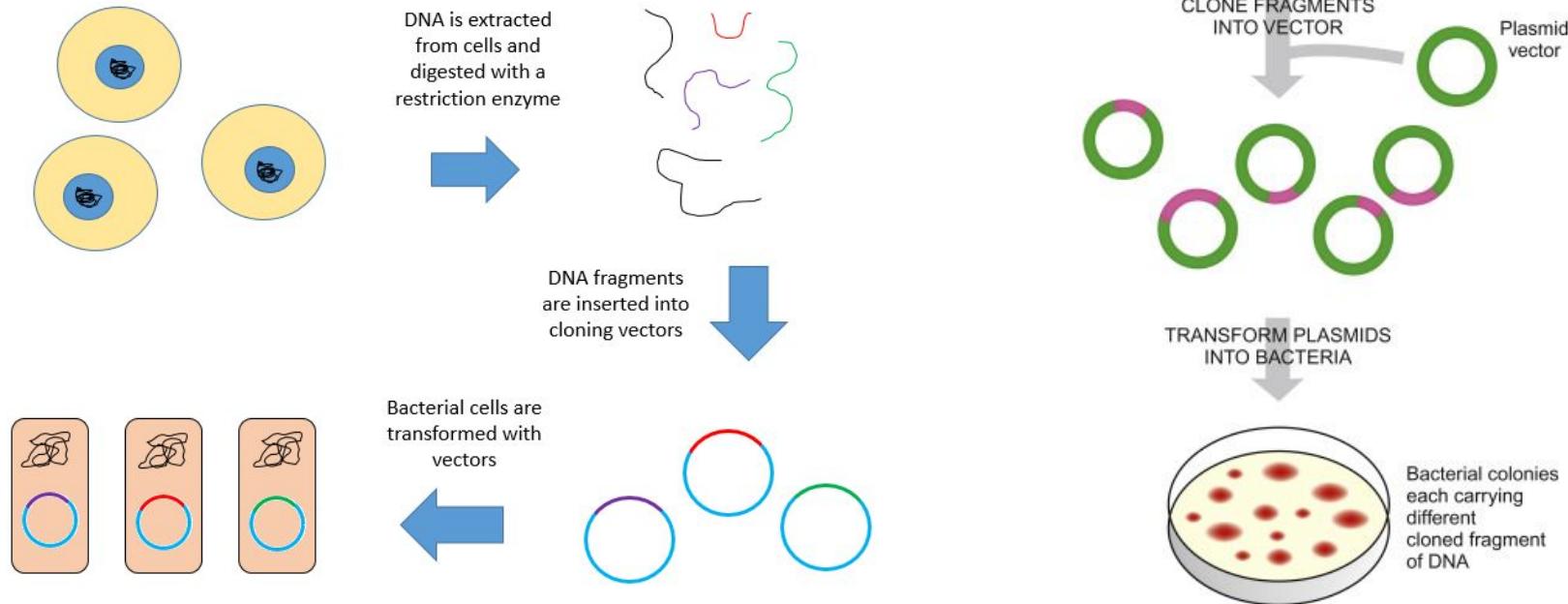


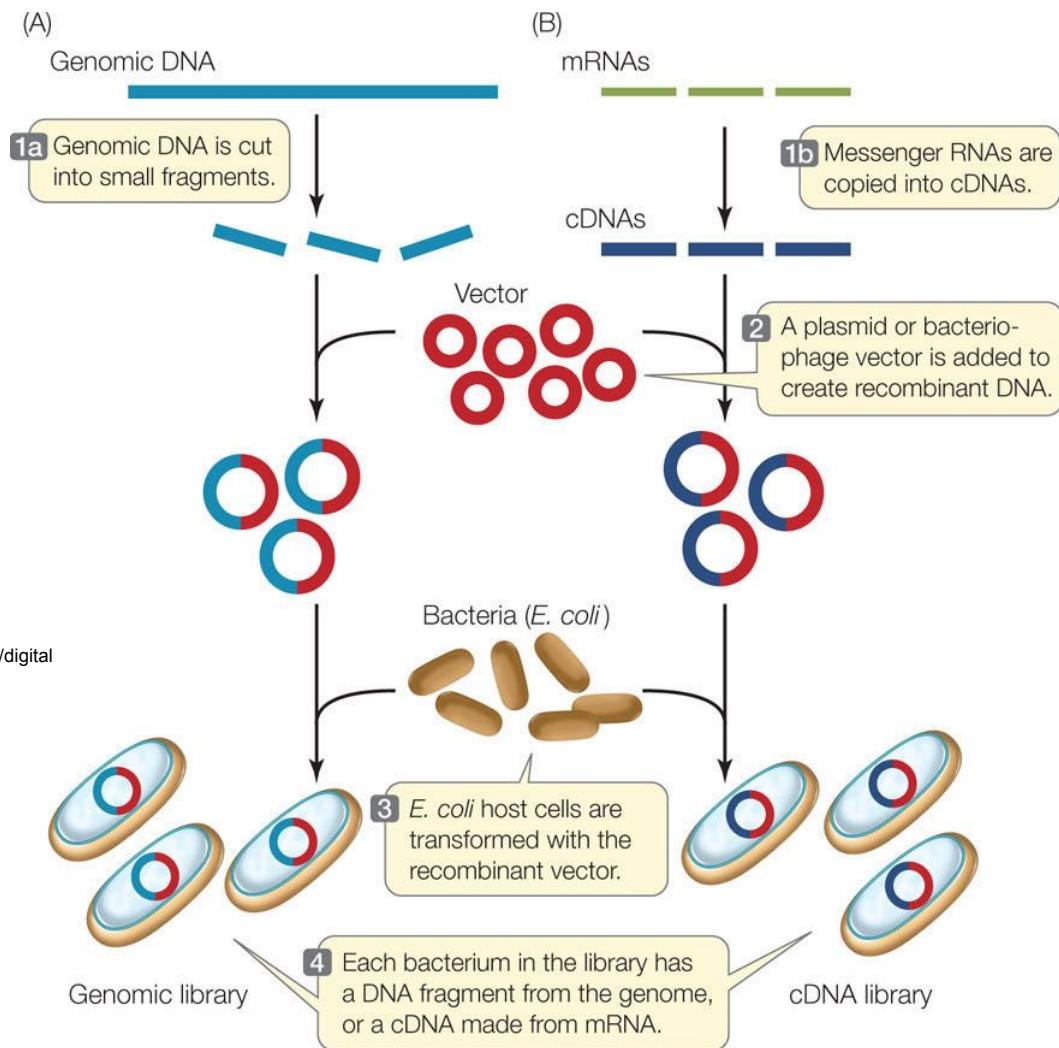
Applications of Recombinant DNA Technology

- **Genomic Library**
 - Clone genome of organism
- **Cell Factories and Metabolic engineering**
 - Produce Recombinant Proteins
 - Produce Important Metabolites
 - Produce Biofuel
- **Gene Therapy**
 - Viral vector for human
 - DNA Vaccine
 - AAV

Genomic Library/ cDNA Library

- A long-life collection of the genomic DNA from a single organism.

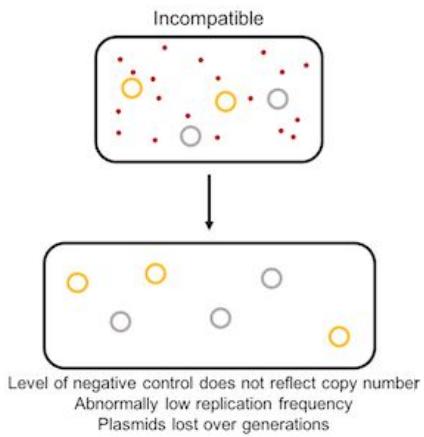
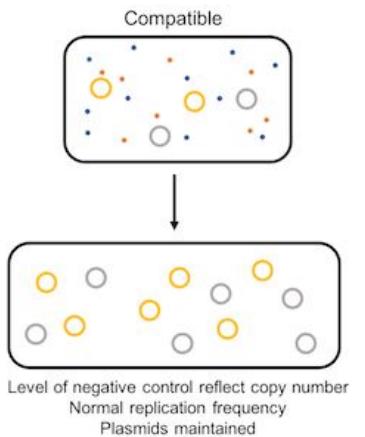




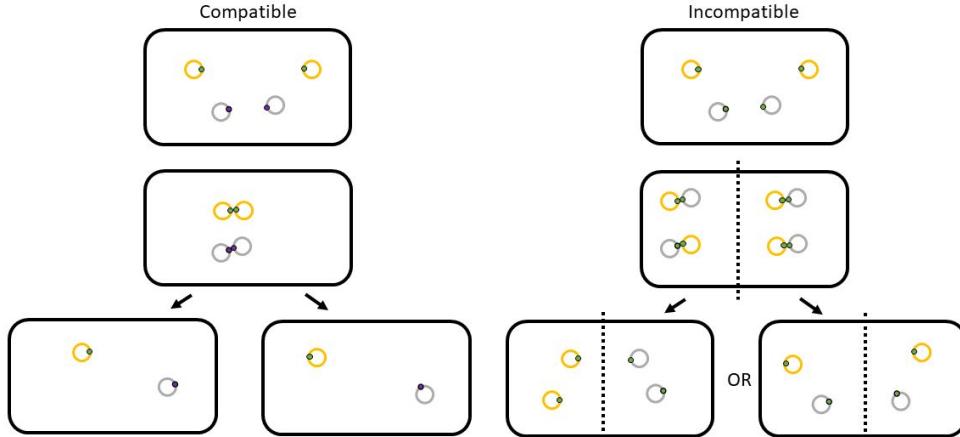
Plasmid incompatibility

- The inability of **different plasmids** to be maintained in **one bacterial cell**.
- Multiple plasmids within one cell have **the same replicon** and/or **partitioning system**.

Replication problem

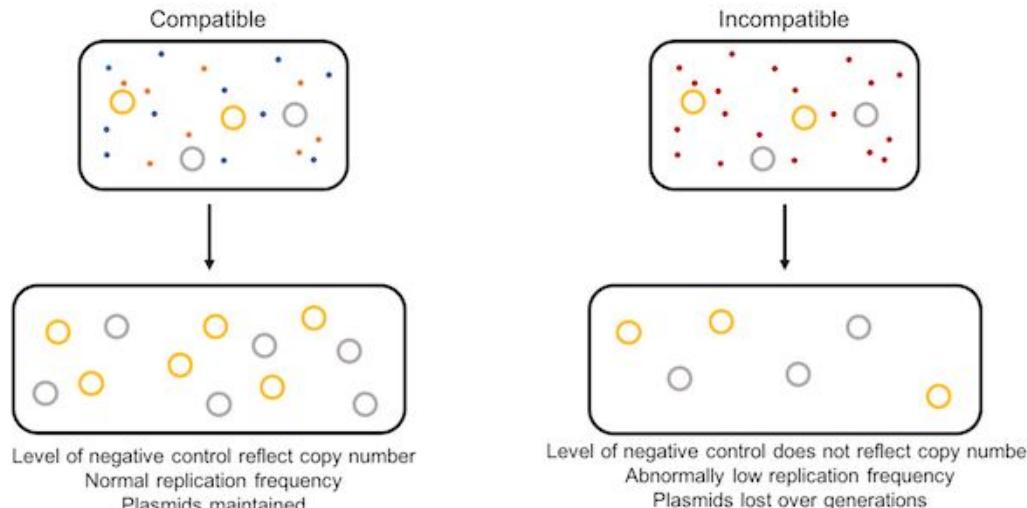


Partitioning problem



Replication Problem

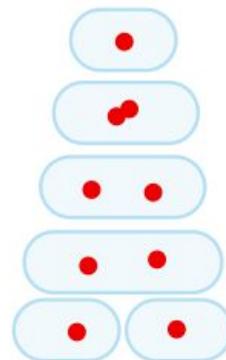
- Two plasmids with same Origin of Replication.
- *ori* could produce negative control for plasmid replication
- More plasmids -> More *ori* -> More negative control -> Inhibit plasmid replication



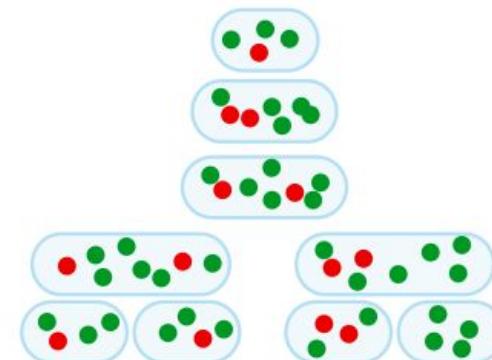
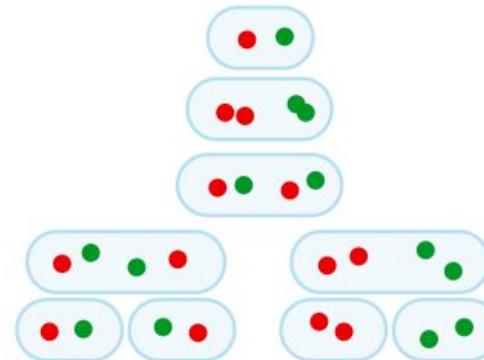
Partitioning Problem

- Mostly find in low-copy number plasmids

A Par-mediated inheritance

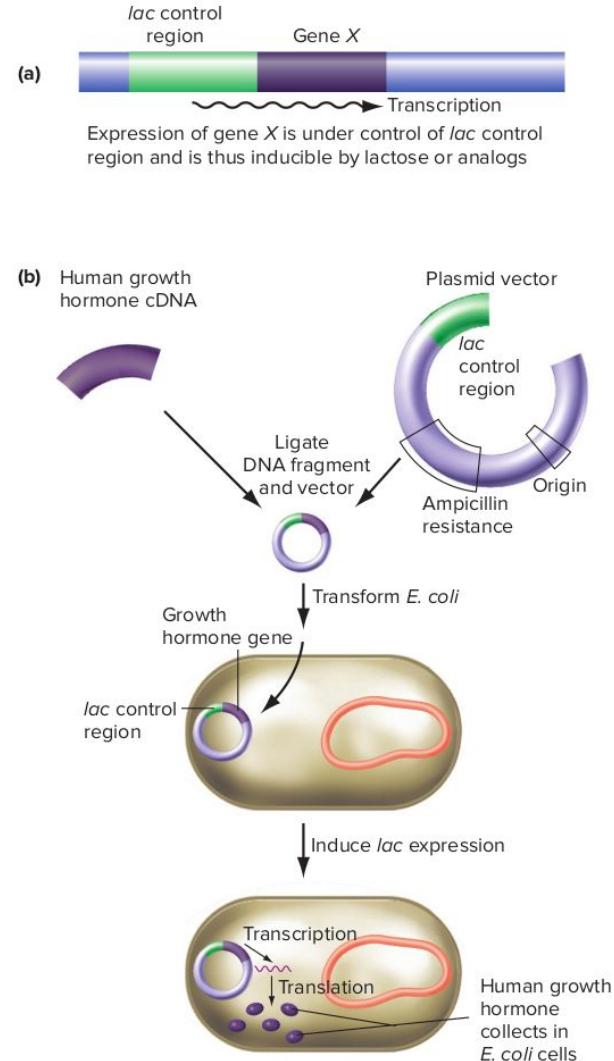


B Centromere-based incompatibility



Cell Factories

- Use Cell to produce the recombinant protein or specific metabolite (Drug, Growth hormone, etc.)
- The expression of the recombinant DNA to produce Recombinant Protein
- **Expression Vector**
 - Expression Plasmids
 - **Constitutive Expression Vector**
 - **Inducible Expression Vector**
 - Induce Expression by Inducer (Lactose, Galactose)

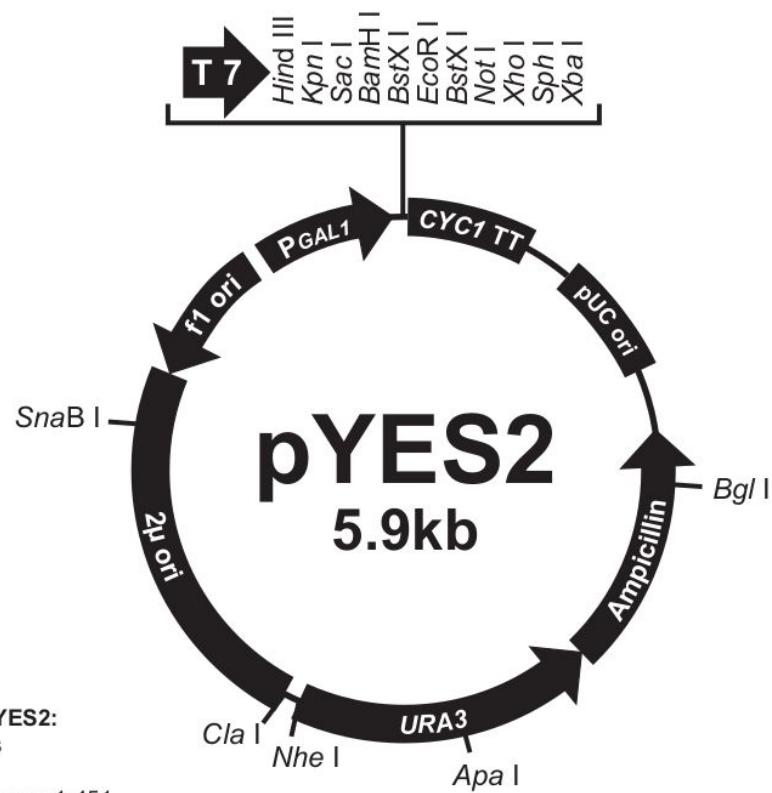


Expression Vector in Yeast

- Inducible Expression Vectors
- *GAL1* promoter (Inducer: Galactose)
- *CYC1* transcription terminator
- Selective Marker (2 systems)
 - Amp. resistance
 - Gene required for Uracil synthesis
- 2 Origins of replication

Comments for pYES2:
5856 nucleotides

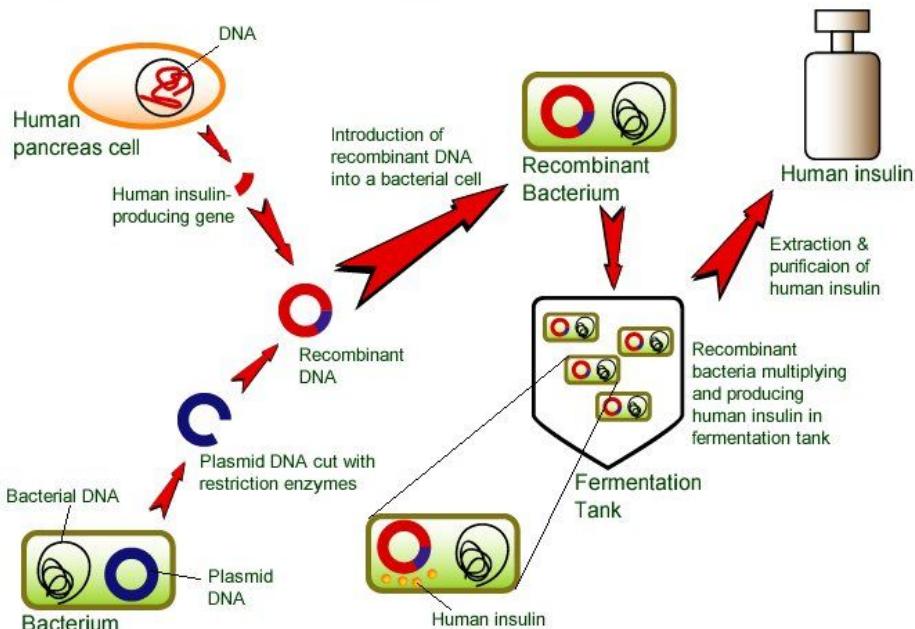
GAL1 promoter: bases 1-451
T7 promoter/priming site: bases 475-494
Multiple cloning site: bases 501-600
CYC1 transcription terminator: bases 608-856
pUC origin: bases 1038-1711
Ampicillin resistance gene: bases 1856-2716 (C)
URA3 gene: bases 2734-3841 (C)
2 micron (μ) origin: bases 3845-5316
f1 origin: bases 5384-5839 (C)
(C) = complementary strand



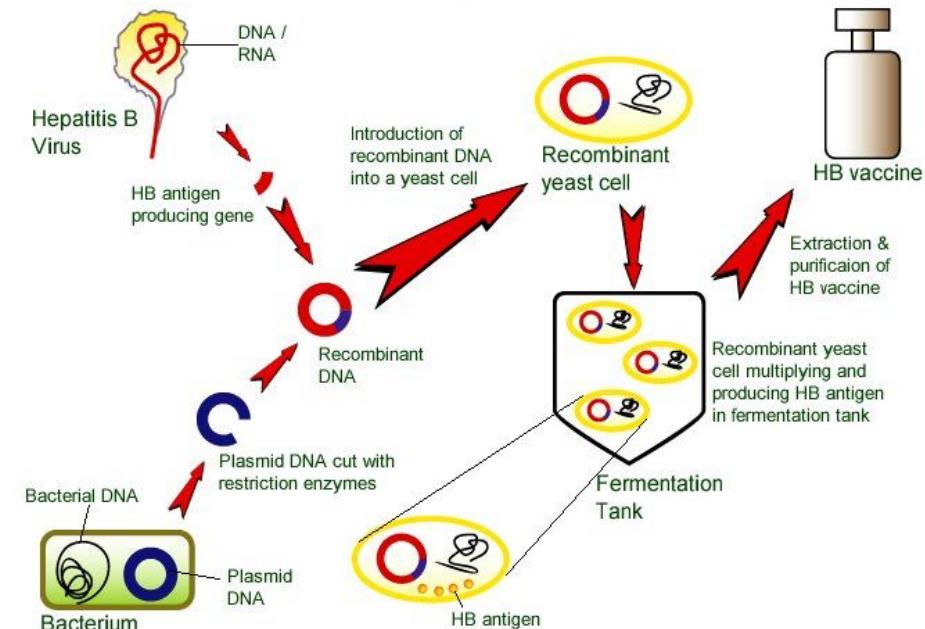
5' end of *GAL1* promoter
 1 ACGGATTAGA AGCCGCCGAG CGGGTACAG CCCTCCGAAG GAAGACTCTC CTCCGTGCCT
 GAL4 binding site GAL4 binding site GAL4 binding site
 61 CCTCGTCTTC ACCGGTCGCG TTCCTGAAAC GCAGATGTGC CTCGCGCCGC ACTGCTCCGA
 GAL4 binding site
 121 ACAATAAAAGA TTCTACAATA CTAGCTTTA TGGTTATGAA GAGGAAAAAT TGGCAGTAAC
 181 CTGGCCCCAC AACCTTCAA ATGAACGAAT CAAATTAACA ACCATAGGAT GATAATGCGA
 241 TTAGTTTTT AGCCTTATTCTGGGTAAT TAATCAGCGA AGCGATGATT TTTGATCTAT
 TATA box
 301 TAACAGATAT ATAAATGCAA AAACTGCATA ACCACTTTAA CTAATACTTT CAACATTTCA
 → start of transcription
 361 GGTTTGATT ACTTCTTATT CAAATGTAAT AAAAGTATCA ACAAAAAATT GTTAATATAC
 3' end of *GAL1* promoter
 421 CTCTATACTT TAACGTCAAG GAGAAAAAAC CCCGGATCGG ACTACTAGCA GCTGTAATAC
 T7 promoter/priming site Hind III Kpn I Sac I BamH I
 481 GACTCACTAT AGGAAATATT AAGCTTGGTA CCGAGCTCGG ATCCACTAGT AACGGCCGCC
 BstX I* Eco RI BsaB I BstX I* Not I Xho I Sph I Xba I
 541 AGTGTGCTGG AATTCTGCAG ATATCCATCA CACTGGCGGC CGCTCGAGCA TGCATCTAGA
 5' end of CYC1 transcription terminator
 601 GGGCCGGCATC ATGTAATTAG TTATGTCACG CTTACATTCA CGCCCTCCCC CCACATCCGC

Cell Factories

Human Insulin Production



Production of Recombinant HB Vaccine



Complete biosynthesis of cannabinoids and their unnatural analogues in yeast

Xiaozhou Luo^{1,15}, Michael A. Reiter^{1,2,15}, Leo d'Espaux^{3,12}, Jeff Wong^{3,12}, Charles M. Denby^{1,13}, Anna Lechner^{4,5,14}, Yunfeng Zhang^{1,6}, Adrian T. Grzybowski¹, Simon Harth³, Weiyin Lin³, Hyunsu Lee^{3,7}, Changhua Yu^{3,5}, John Shin^{3,4}, Kai Deng^{8,9}, Veronica T. Benites³, George Wang³, Edward E. K. Baidoo³, Yan Chen³, Ishaan Dev^{3,4}, Christopher J. Petzold³, Jay D. Keasling^{1,3,4,5,10,11*}

- Metabolic Engineering
- Make Yeast to produce Cannabinoids
 - Recombinant DNA technology (Insert foreign genes)
 - CRISPR-Cas9 technology (Edit own genes)

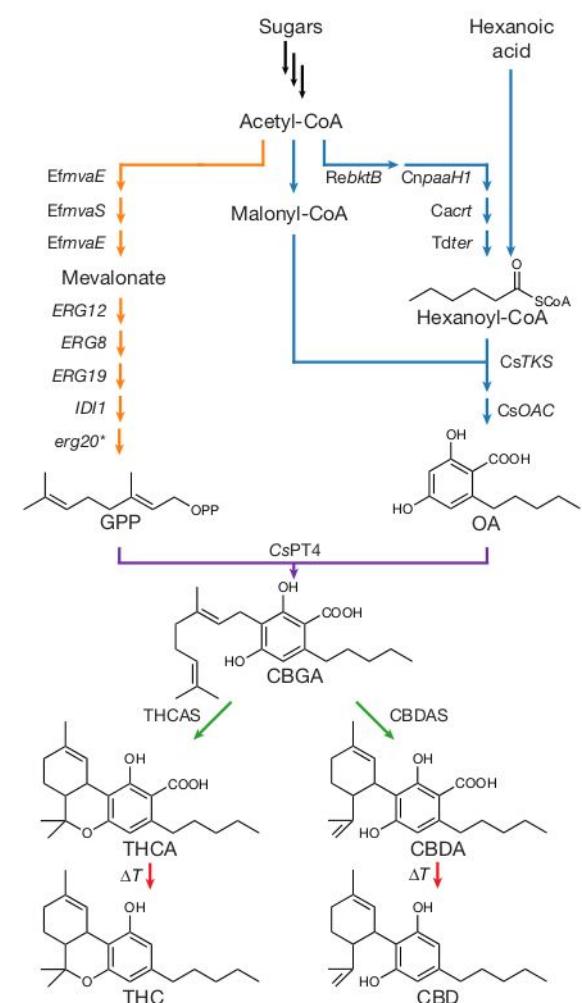
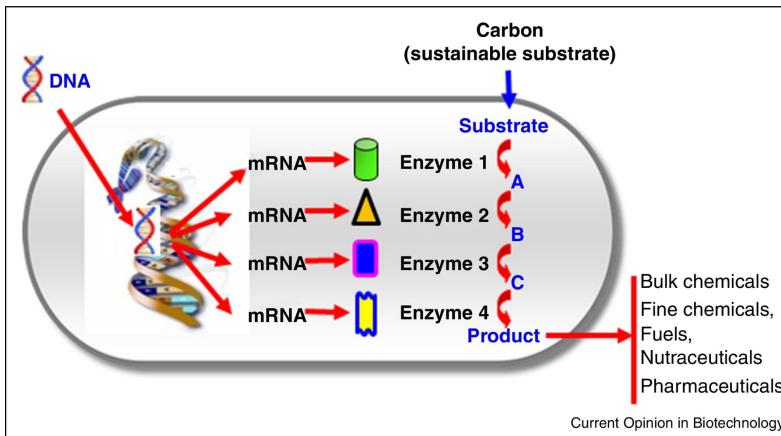
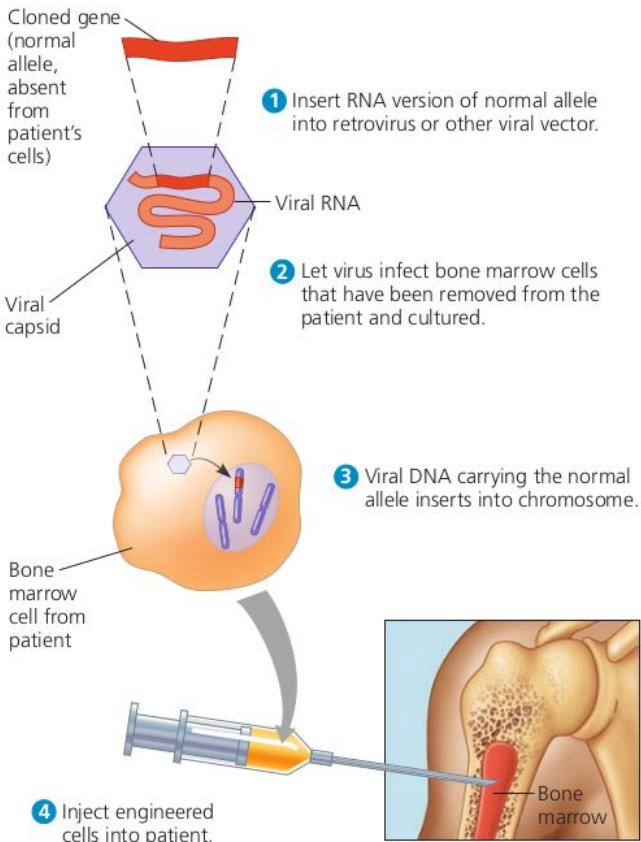


Fig. 1 | Engineered biosynthetic pathway for synthesis of cannabinoids in *S. cerevisiae*. GPP was produced by introducing the *Enterococcus*

Gene Therapy

- Use DNA to Cure Disease
- Introduced **therapeutic gene** into patient's somatic cells by Viral Vector
- **Therapeutic gene**
 - a gene whose expression will fight disease
- Two mains viral vectors
 - **Retroviral vectors**
 - **Adeno-associated viral (AAV) vectors**

▼ **Figure 20.22 Gene therapy using a retroviral vector.**
A retrovirus that has been rendered harmless is used as a vector in this procedure, which exploits the ability of a retrovirus to insert a DNA transcript of its RNA genome into the chromosomal DNA of its host cell (see Figure 19.9). If the foreign gene carried by the retroviral vector is expressed, the cell and its descendants will possess the gene product. Cells that reproduce throughout life, such as bone marrow cells, are ideal candidates for gene therapy.



Viral Vector

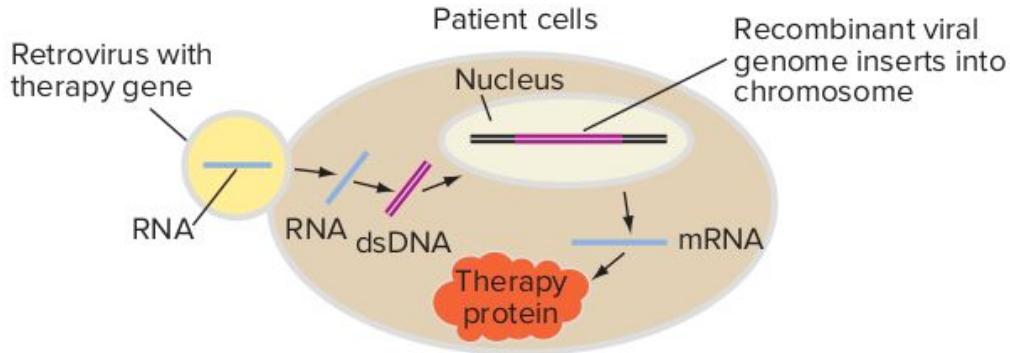
- **Retrovirus**

- Insert into host genome
- Might cause mutation

- **Adeno-associated virus**

- Extrachromosomal

(a) Gene therapy with retroviral vector



(b) Gene therapy with AAV vector

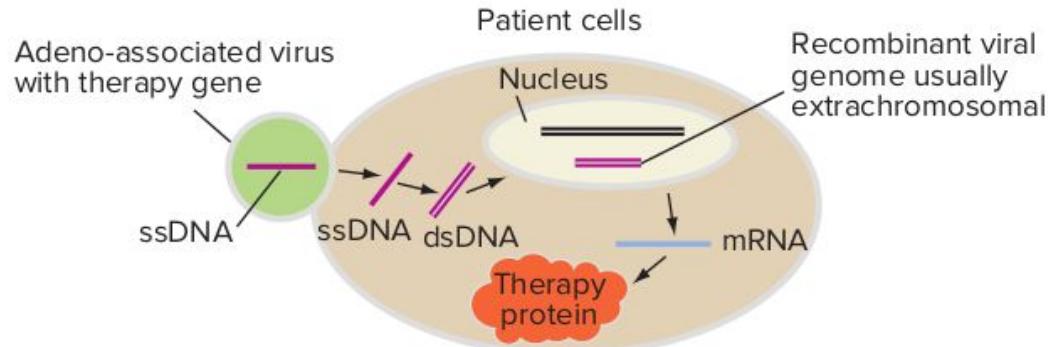
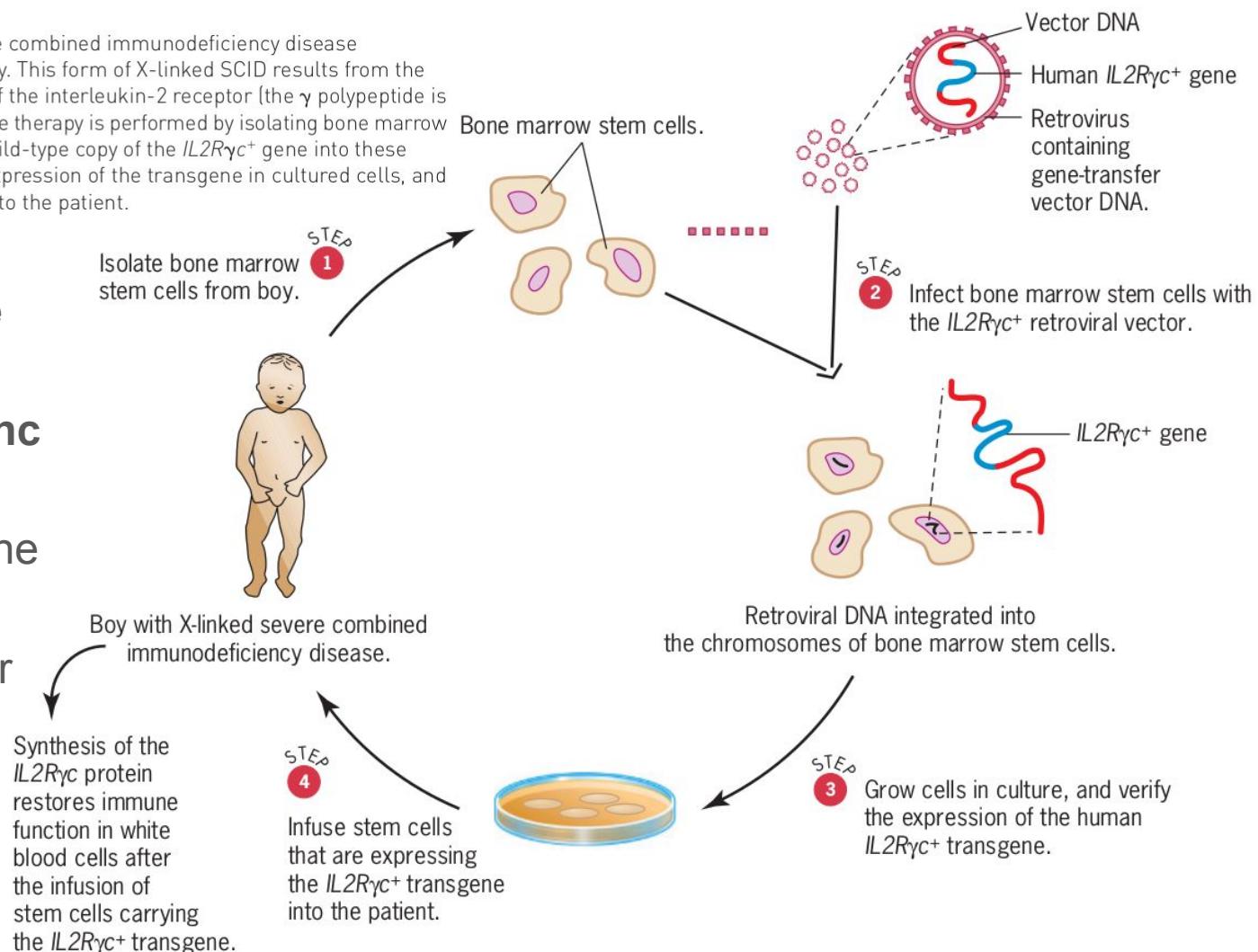


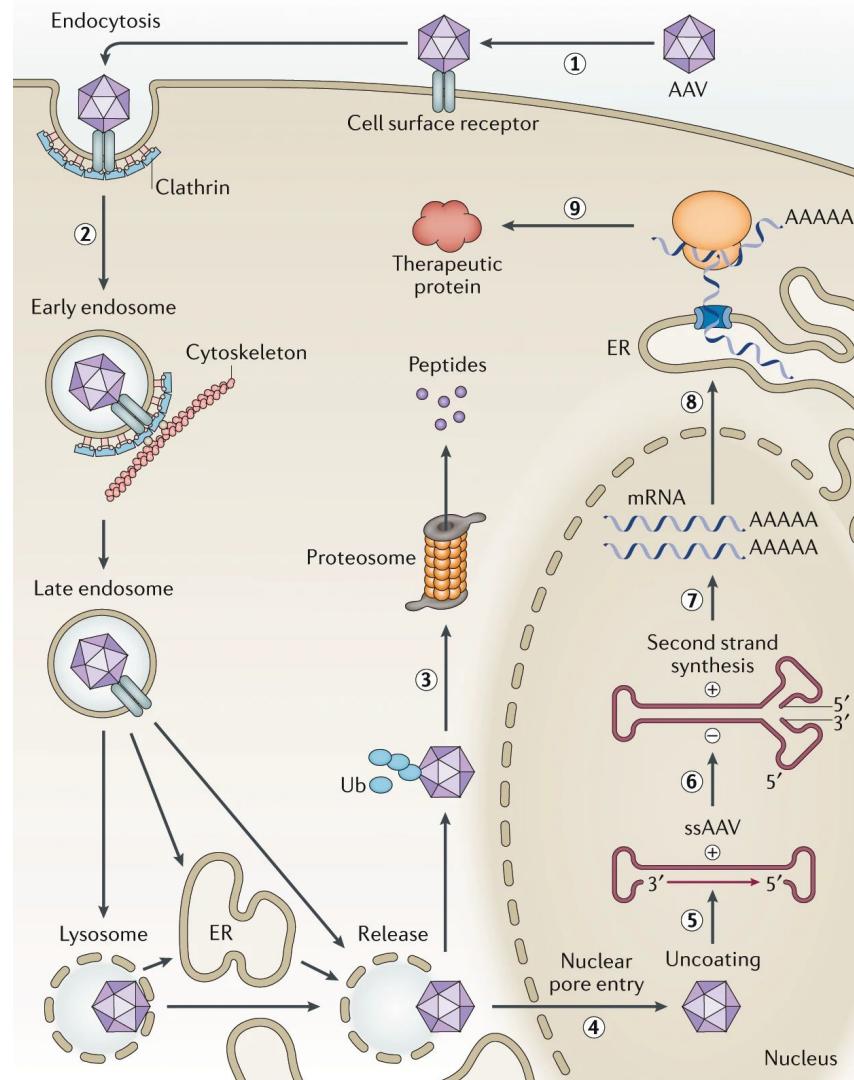
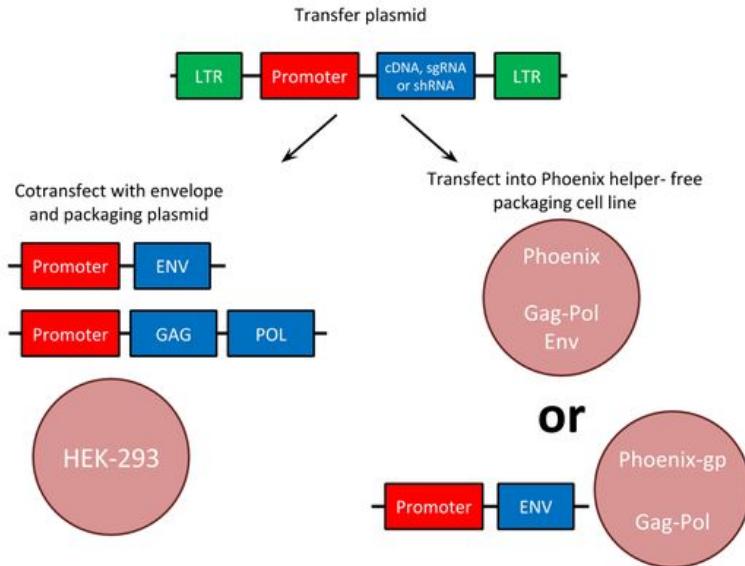
FIGURE 16.7 Treatment of X-linked severe combined immunodeficiency disease ($IL2R\gamma c^-$ SCID) by somatic-cell gene therapy. This form of X-linked SCID results from the loss or lack of activity of the γ polypeptide of the interleukin-2 receptor (the γ polypeptide is also a component of other interleukins). Gene therapy is performed by isolating bone marrow stem cells from the patient, introducing a wild-type copy of the $IL2R\gamma c^+$ gene into these cells with a retroviral vector, verifying the expression of the transgene in cultured cells, and infusing the transformed stem cells back into the patient.

- **X-linked severe combined immunodeficiency (SCID)**
- Somatic-Cell gene therapy
- Retrovirus vector

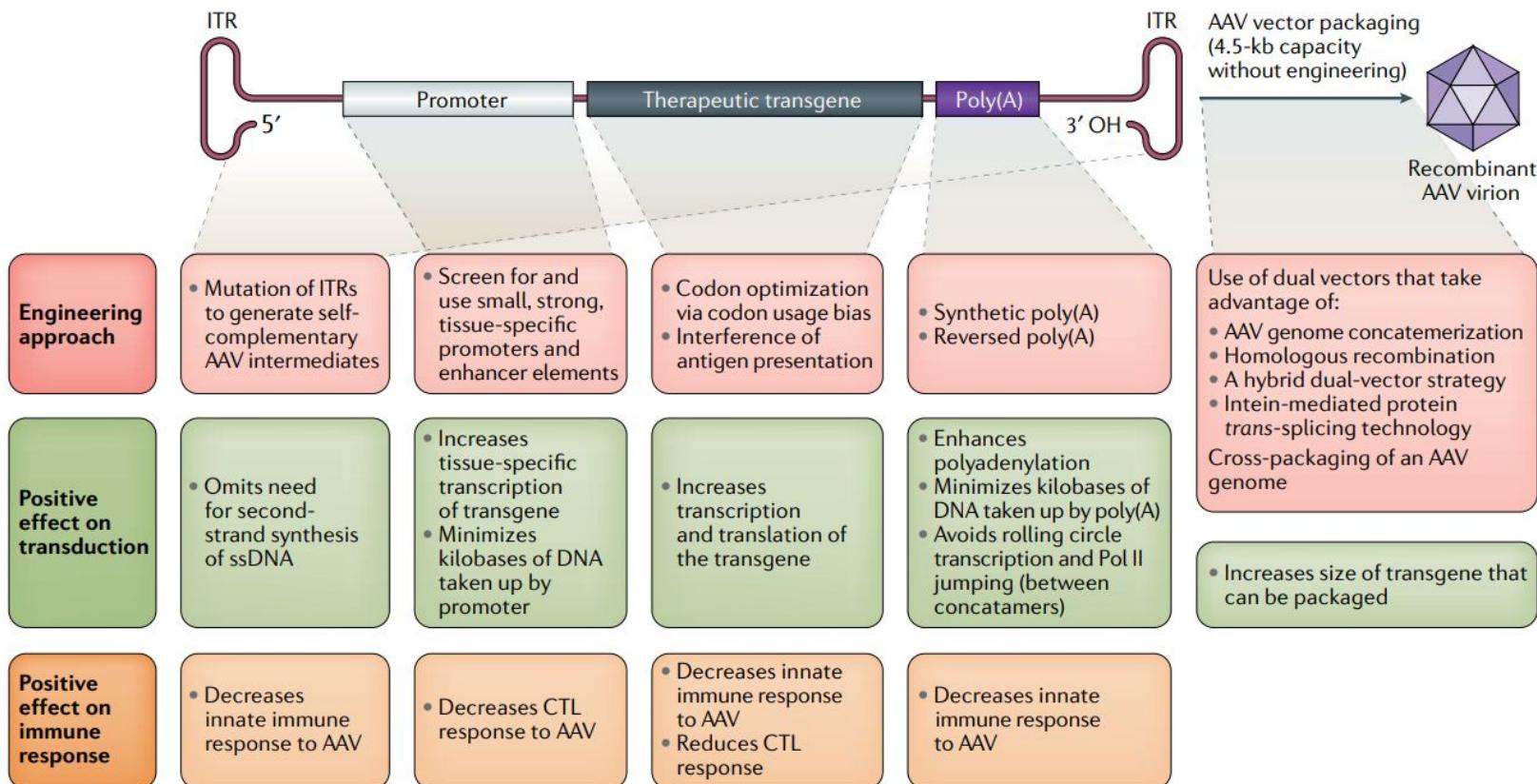


Application of AAV in Covid-19 vaccine

- AAV vector transduction pathway

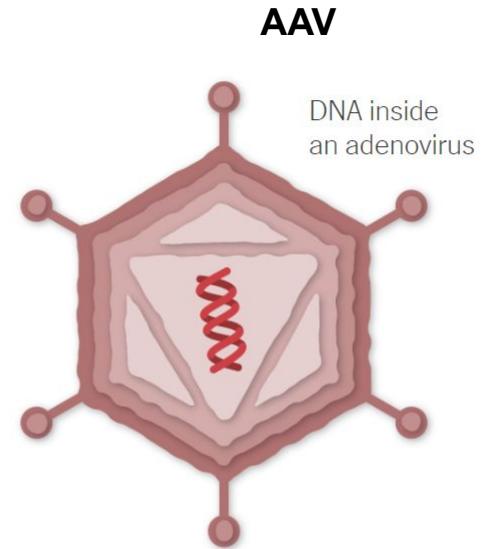
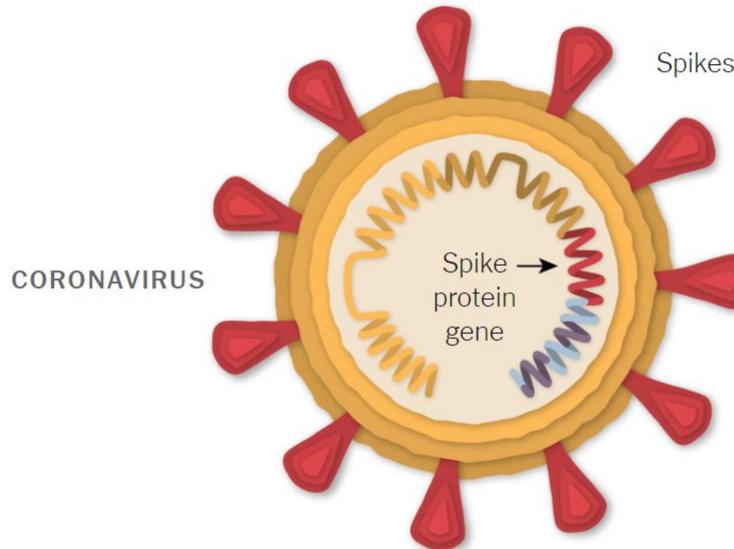


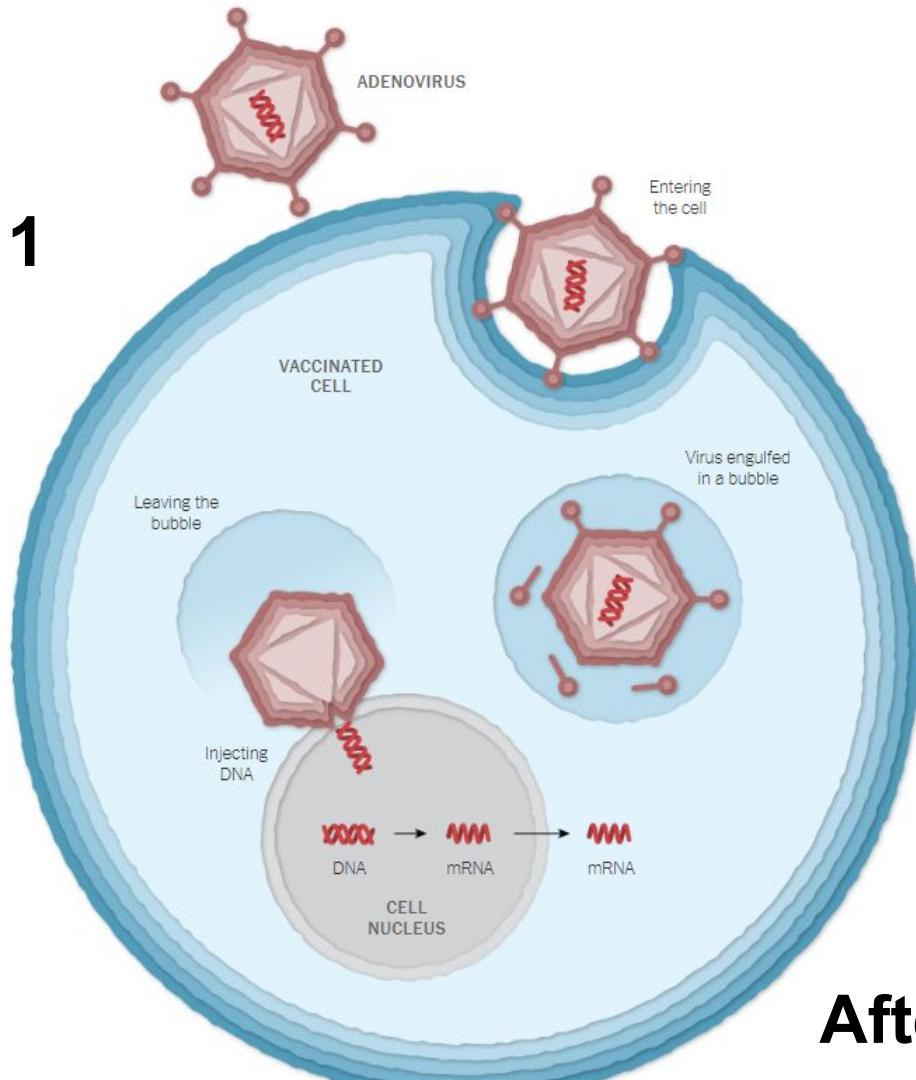
AAV vector



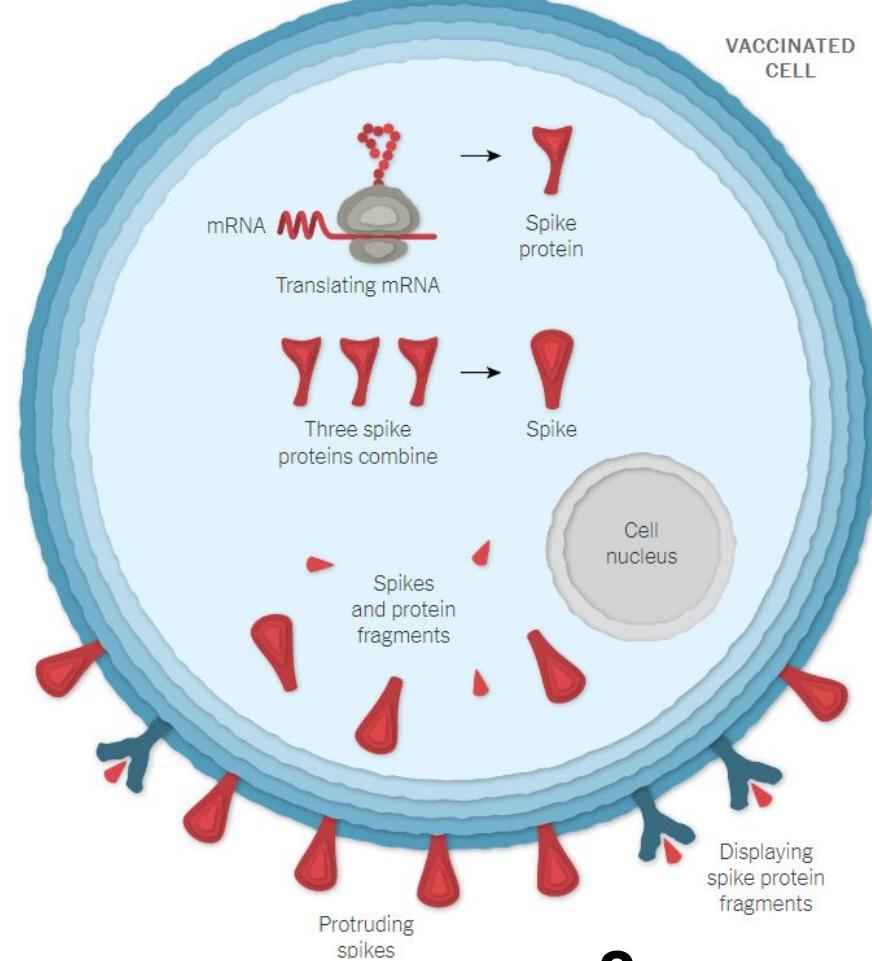
Coronavirus and Adeno-Associated Virus

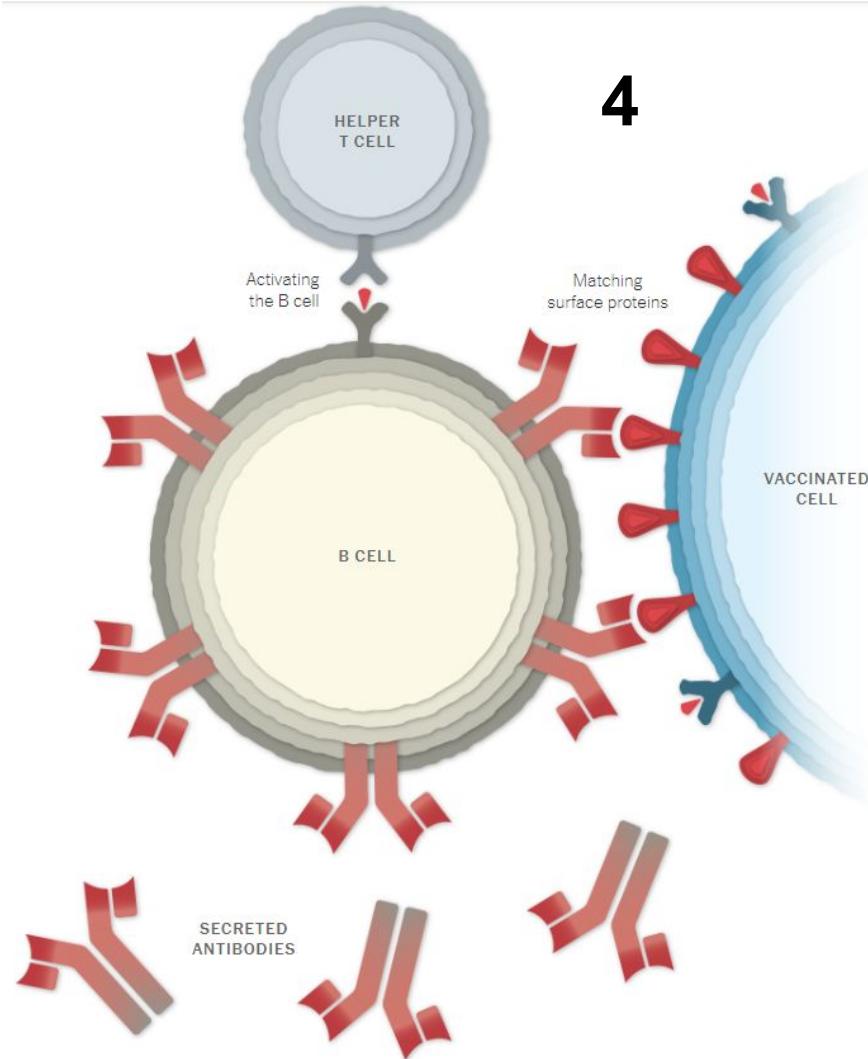
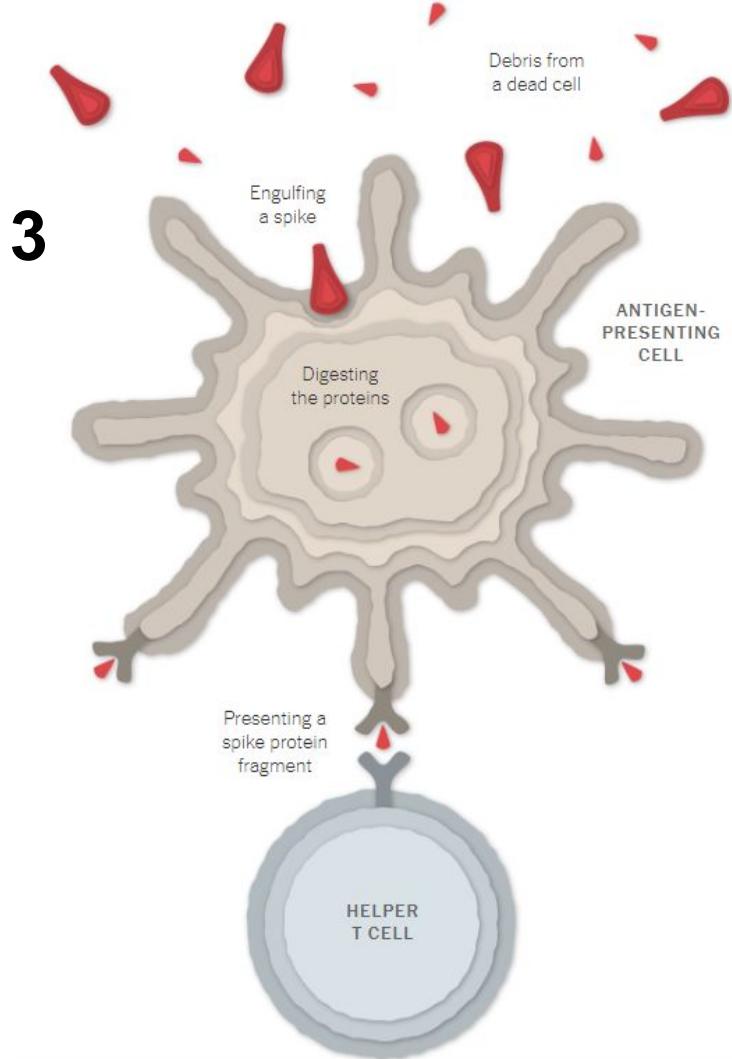
- Oxford-astrazeneca covid-19 vaccine
- AAV vector and Spike protein form Coronavirus



1**VACCINATED
CELL**

After Vaccination

**2**



After infection by Coronavirus

