Chapter 12
Bio tech Application

Plants, bacteria, fungi and animals whose genes have been altered by manipulation are called Genetically Modified Organisms (GMO). GM plants have been useful in many ways. Genetic modification has:

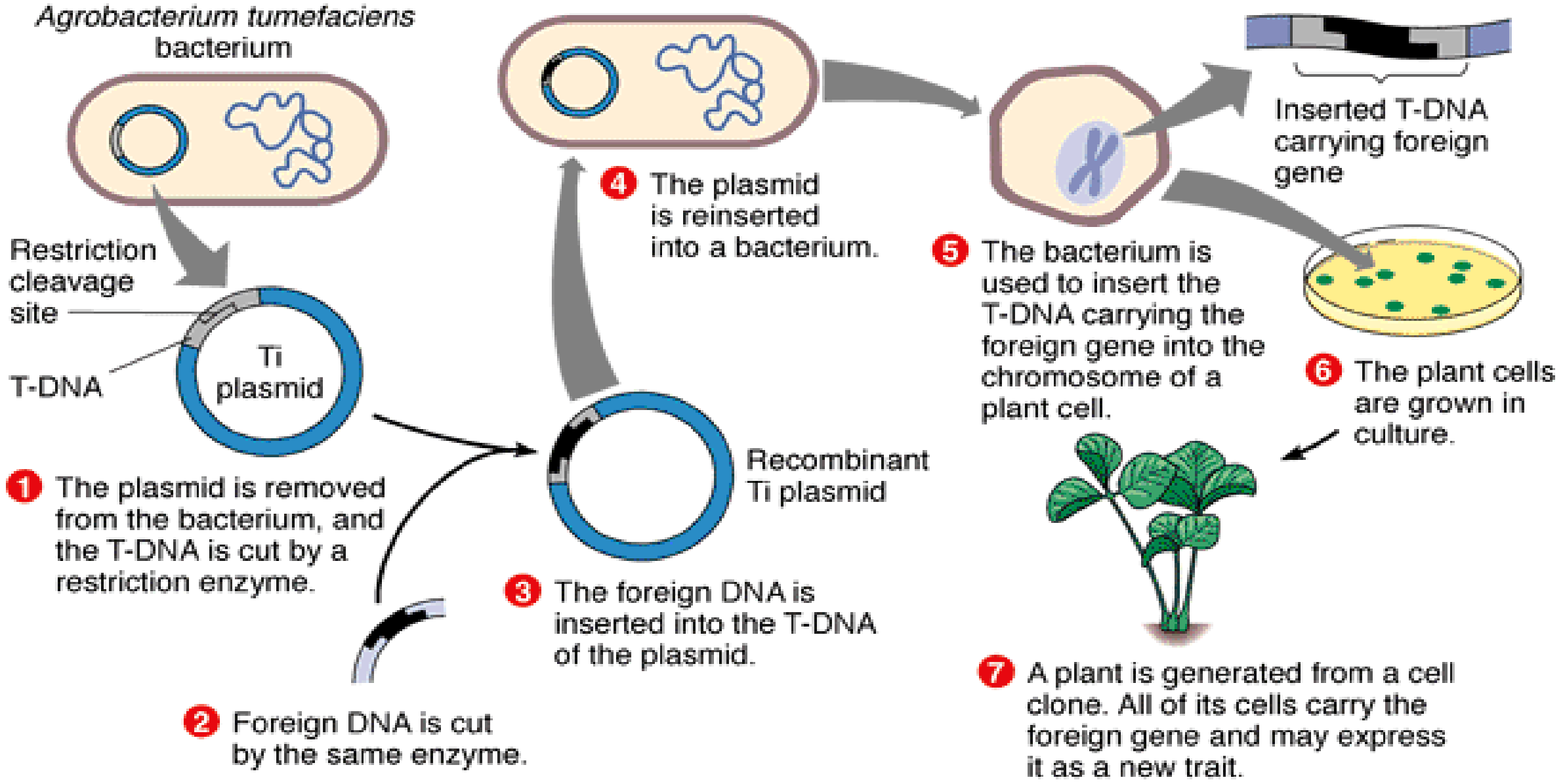
(i) made crops more tolerant to abiotic stresses (cold, drought, salt, heat).

(ii) reduced reliance on chemical pesticides (pest-resistant crops).

(iii) helped to reduce post harvest losses.

(iv) increased efficiency of mineral usage by plants (this prevents early exhaustion of fertility of soil).

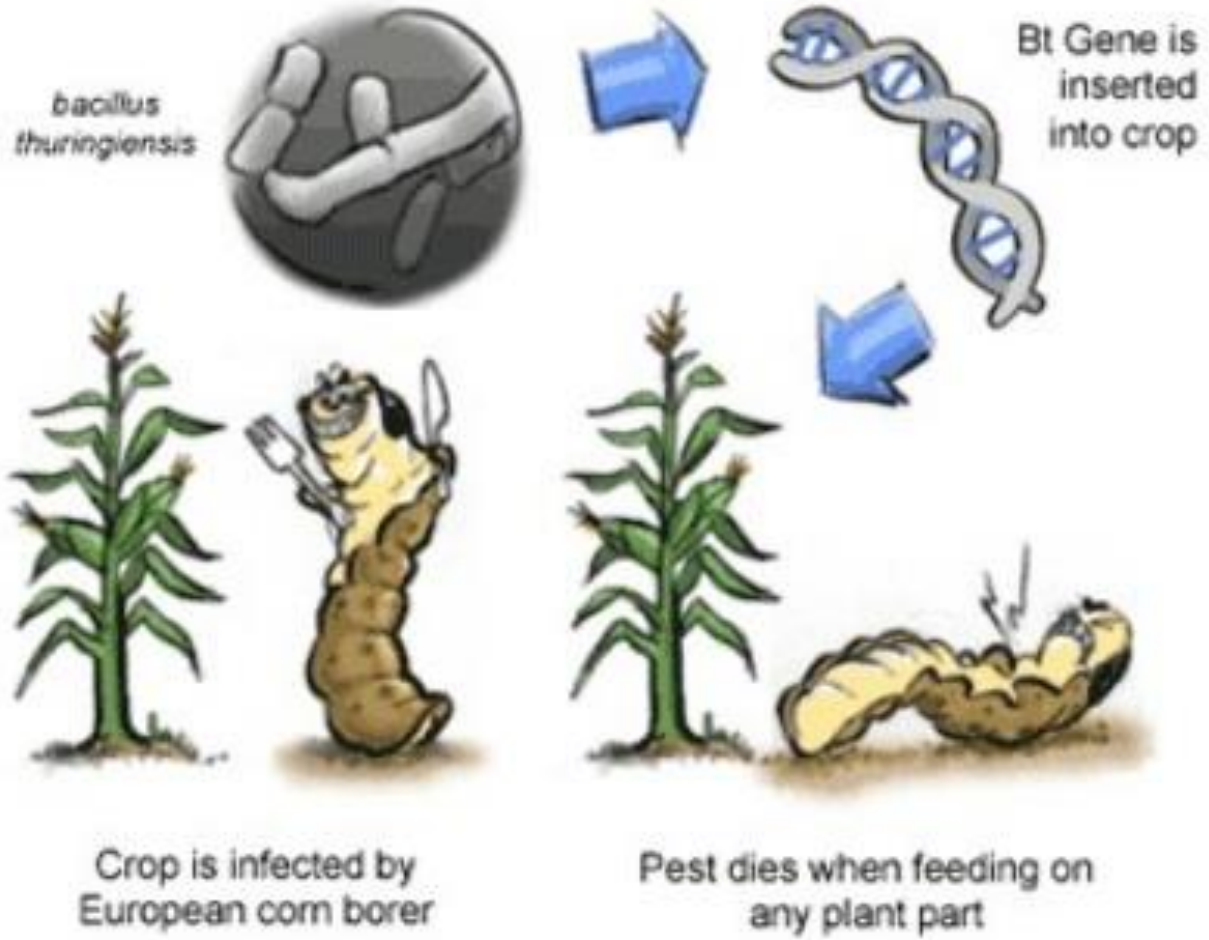
(v) enhanced nutritional value of food, e.g., Vitamin ‘A’ enriched rice.

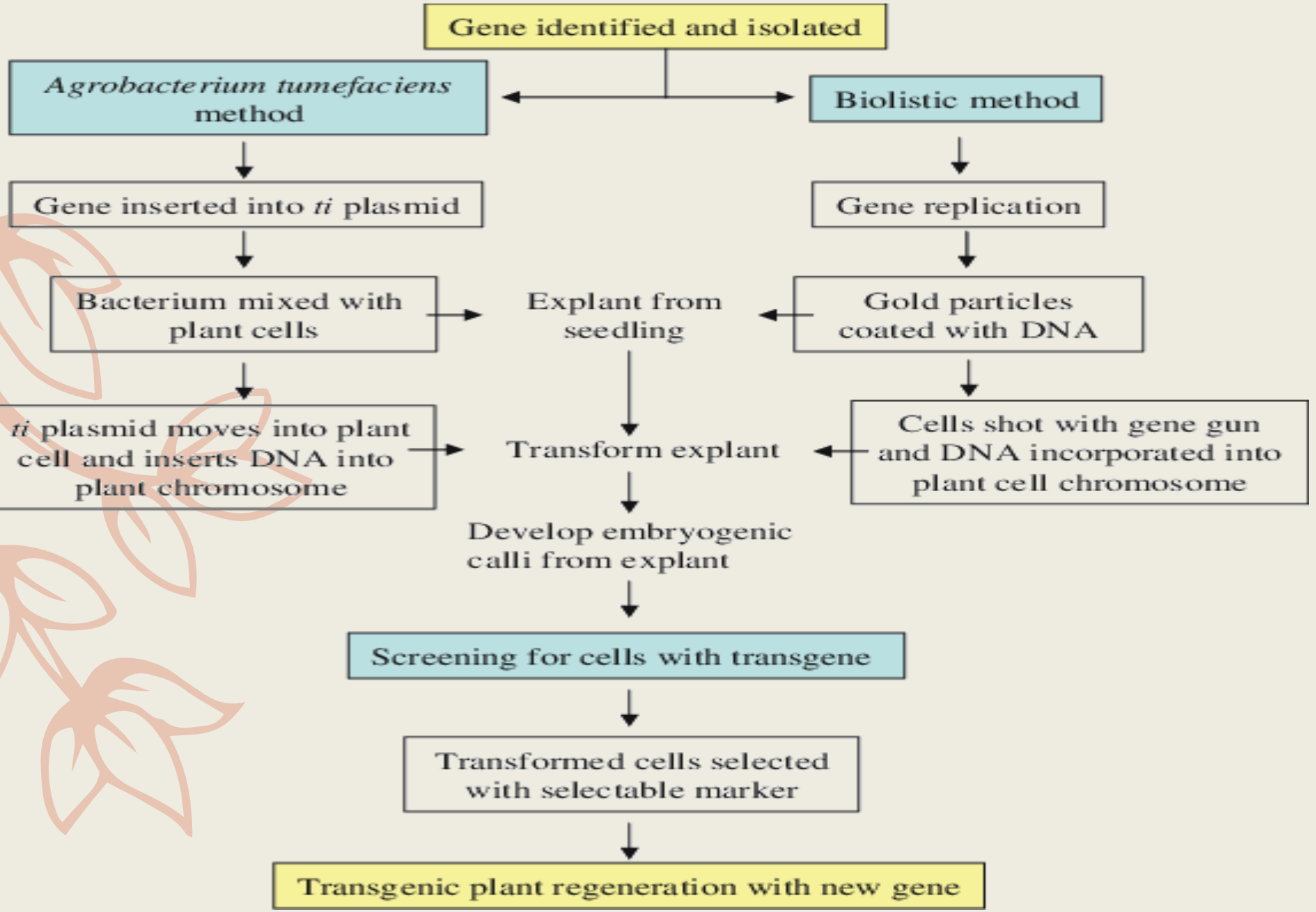

Agrobacterium tumefaciens bacterium
Restriction cleavage site
T-DNA
Ti plasmid
1 The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2 Foreign DNA is cut by the same enzyme.
3 The foreign DNA is inserted into the T-DNA of the plasmid.
Recombinant Ti plasmid
4 The plasmid is reinserted into a bacterium.
5 The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.
Inserted T-DNA carrying foreign gene
6 The plant cells are grown in culture.
7 A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.

GMO	Description	Picture
Golden Rice	Rice modified with daffodil genes to have more beta-carotene, which the body converts to Vitamin A	
Flavr Savr Tomatoes	Tomatoes modified by the removal of genes responsible for the softening of fruit, meaning the tomatoes spoil more slowly	
Bt Corn	Corn modified with a bacterial insecticide gene so that it produces insect toxins within its cells, protecting it from pest species	
Aqua Advantage Salmon	Salmon modified with growth hormone regulating genes in order to grow to market sizes in significantly less time	
Glow in the Dark Animals	Animals modified with genes for fluorescent proteins will glow in the dark – this novel feature serves no practical purpose	

Bt gene

- Bacillus thuringiensis is a bacteria found in soil.
- It produces a protein that paralyzes the insects digestive system. The insect dies of starvation.
- The gene that is responsible for coding for the protein that kills insects is inserted into the DNA of many plants such as corn and cotton.
- The plant then produces the protein that is responsible for killing insects (its own personal insecticide)




Gene identified and isolated
Agrobacterium tumefaciens method
Biolistic method
Gene inserted into ti plasmid
Gene replication
Bacterium mixed with plant cells
Explant from seedling
Gold particles coated with DNA
ti plasmid moves into plant cell and inserts DNA into plant chromosome
Transform explant
Cells shot with gene gun and DNA incorporated into plant cell chromosome
Develop embryogenic calli from explant
Screening for cells with transgene
Transformed cells selected with selectable marker
Transgenic plant regeneration with new gene

- RNA interference (RNAi) is the biological process of mRNA degradation induced by complementary sequences double-stranded (ds) small interfering RNAs (siRNA) and suppression of target gene expression.

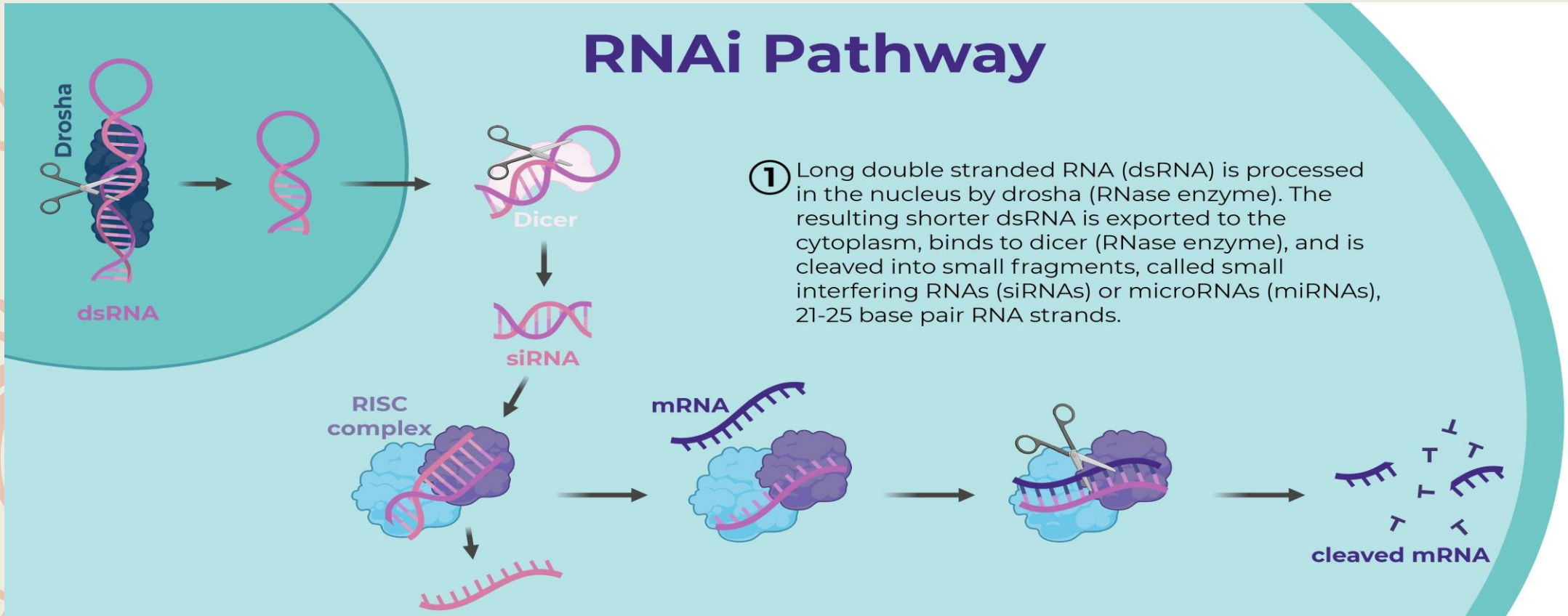
- Exogenous siRNAs (perfectly paired dsRNAs of ∼21–25 nt in length) play an important role in host defense against RNA viruses and in transcriptional and post-transcriptional gene regulation in plants and other eukaryotes.

- Using RNAi technology by transfecting synthetic siRNAs into eukaryotic cells to silence genes has become an indispensable tool to investigate gene functions, and siRNA-based therapy is being developed to knockdown genes implicated in diseases.

- Other examples of RNAi technology include method of producing highly potent and purified siRNAs directly from Escherichia coli cells, based on an unexpected discovery that ectopic expression of p19, a plant viral siRNA-binding protein, stabilizes a cryptic siRNA-like RNA species in bacteria.

- Those siRNAs, named as pro-siRNA for “prokaryotic siRNA”, are bacterial RNase III products that have chemical and functional properties that like eukaryotic siRNAs.

RNAi Pathway

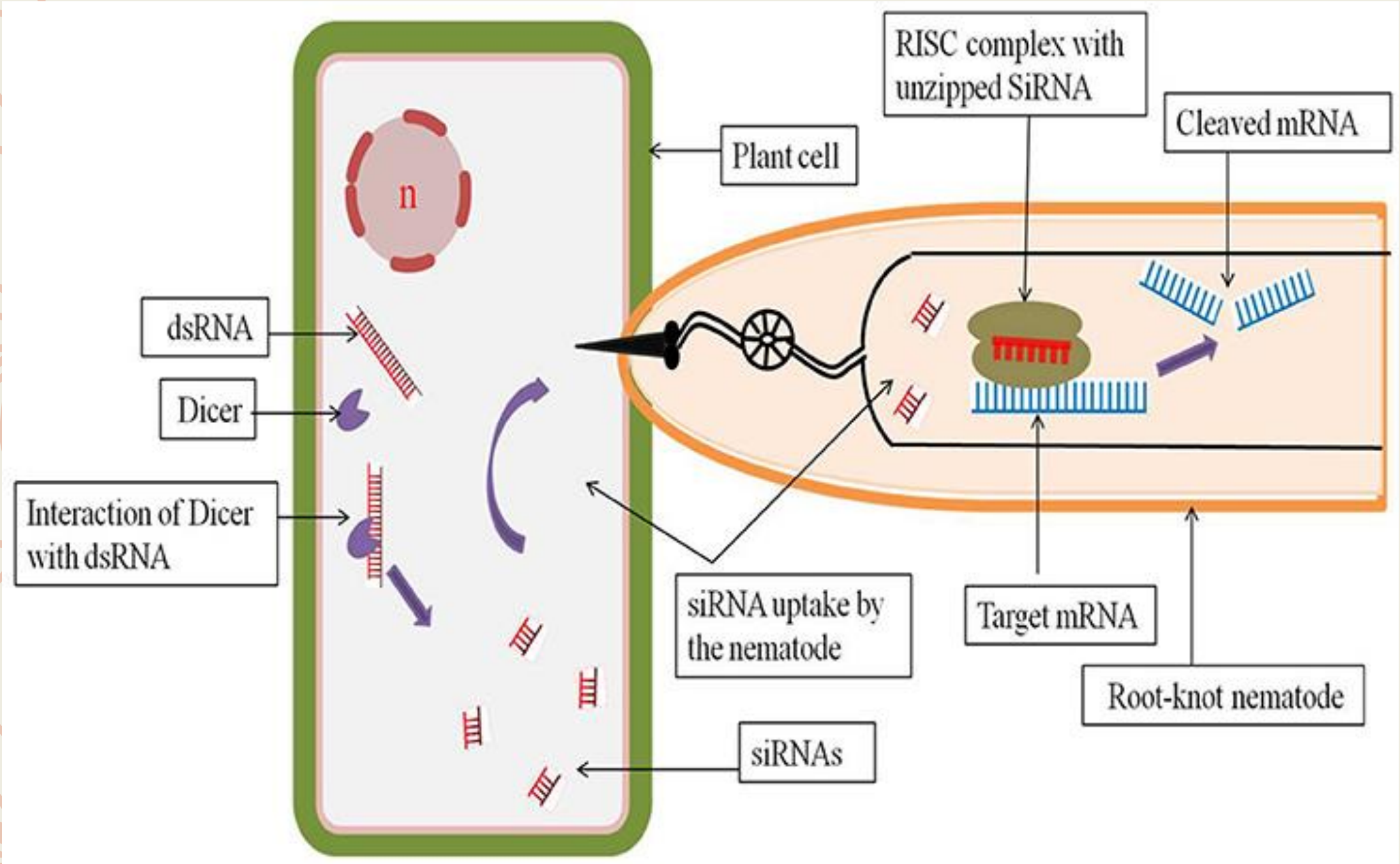


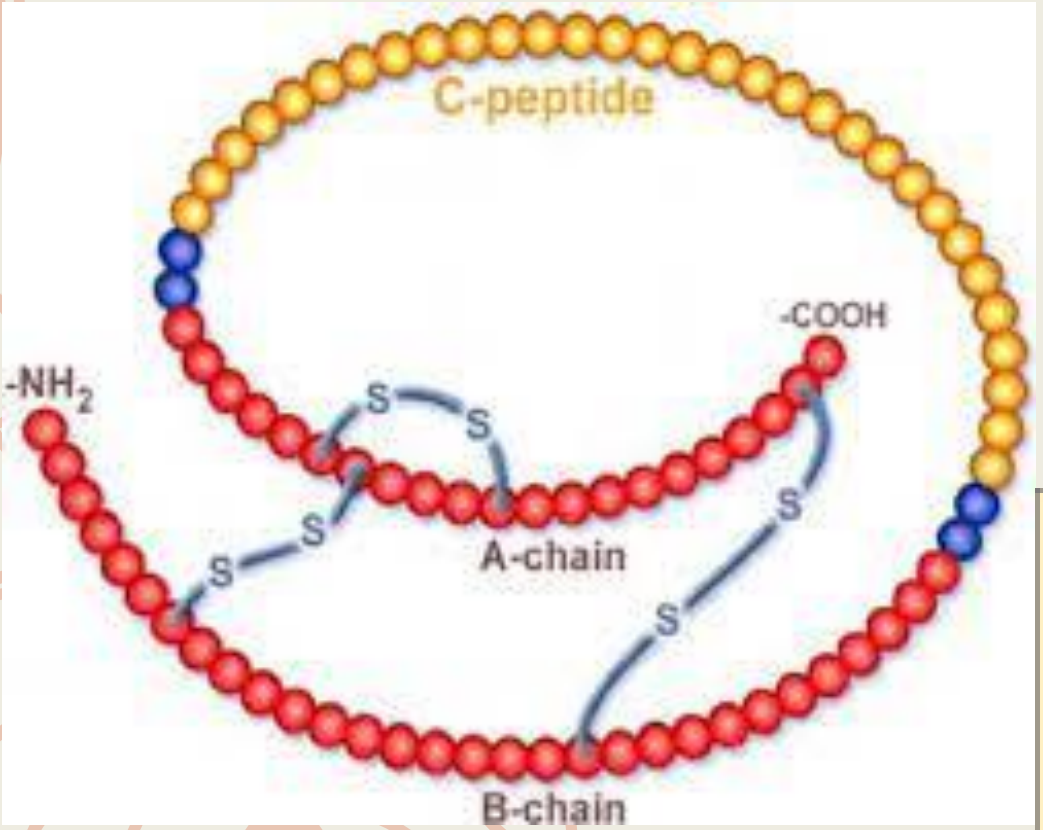
1. Long double stranded RNA (dsRNA) is processed in the nucleus by drosha (RNase enzyme). The resulting shorter dsRNA is exported to the cytoplasm, binds to dicer (RNase enzyme), and is cleaved into small fragments, called small interfering RNAs (siRNAs) or microRNAs (miRNAs), 21-25 base pair RNA strands.

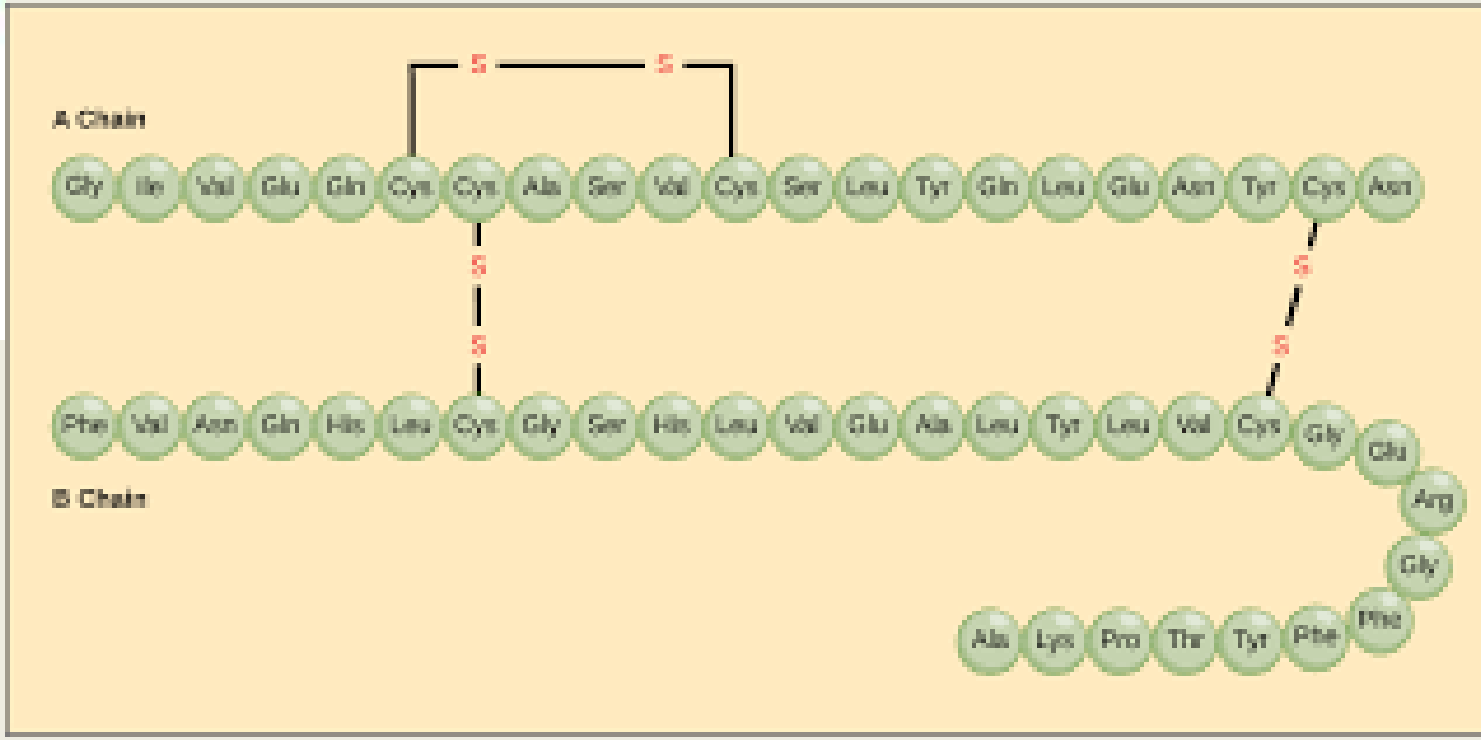
2. The siRNA or miRNA is recruited by the RISC complex, unwinds, and incorporates into a protein-RNA complex.

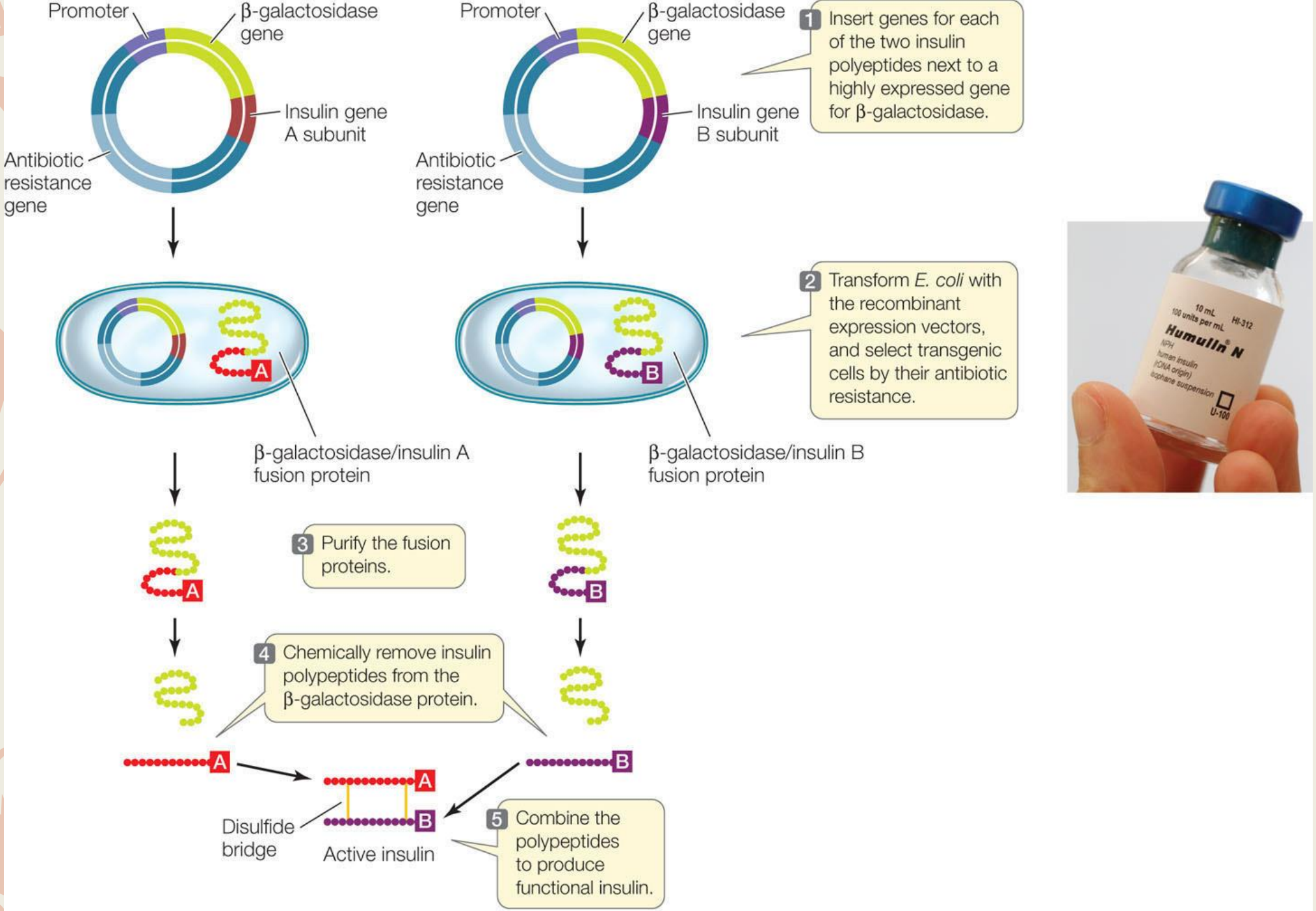
3. RISC + siRNA or miRNA bind to a complementary, targeted messenger RNA (mRNA).

4. mRNA is cleaved in a specific site and then degraded in the cell, thereby disrupting protein synthesis of the target gene.

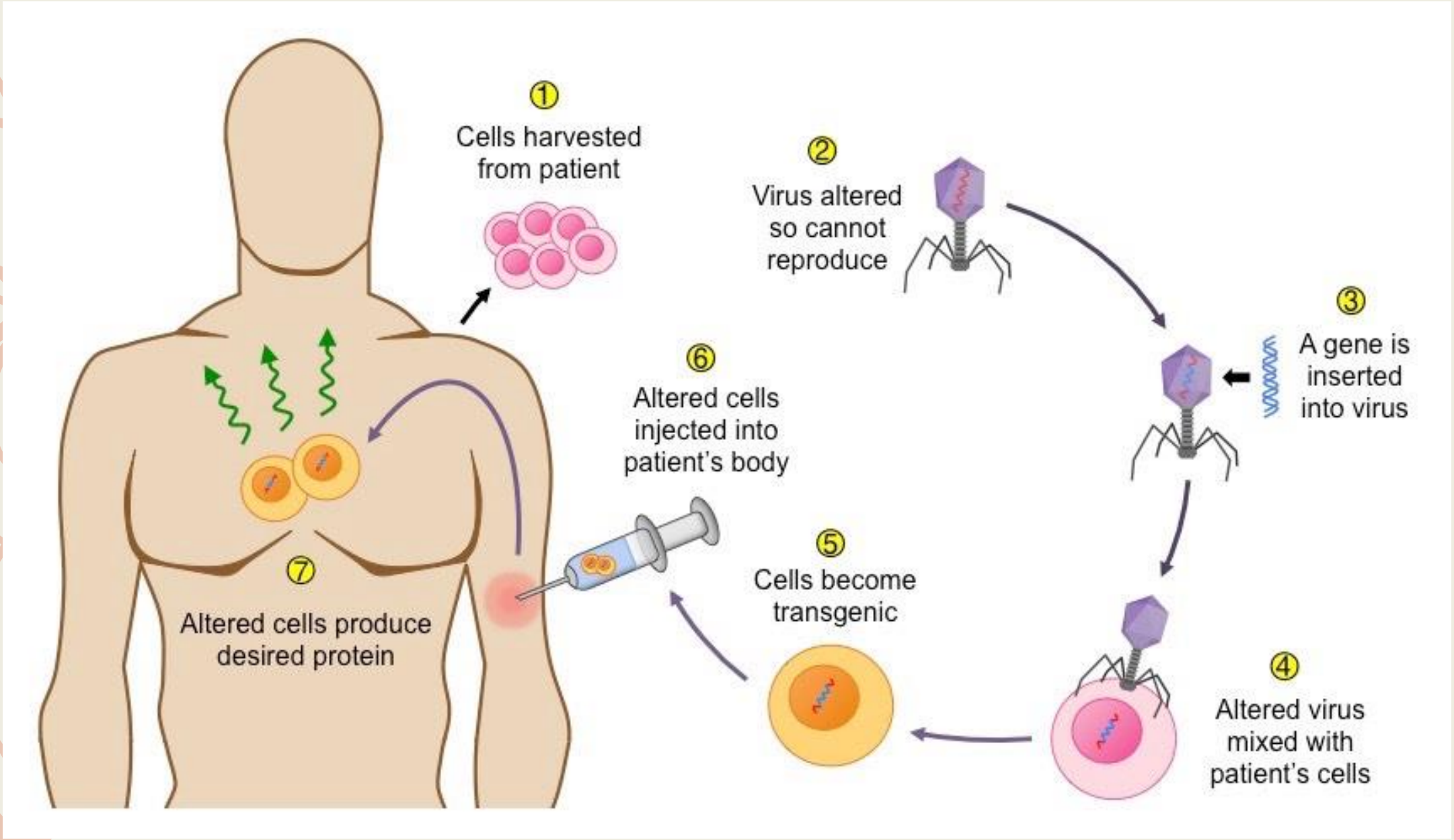

RISC complex with unzipped SiRNA
Cleaved mRNA
Plant cell
n
dsRNA
Dicer
Interaction of Dicer with dsRNA
siRNA uptake by the nematode
Target mRNA
Root-knot nematode
siRNAs

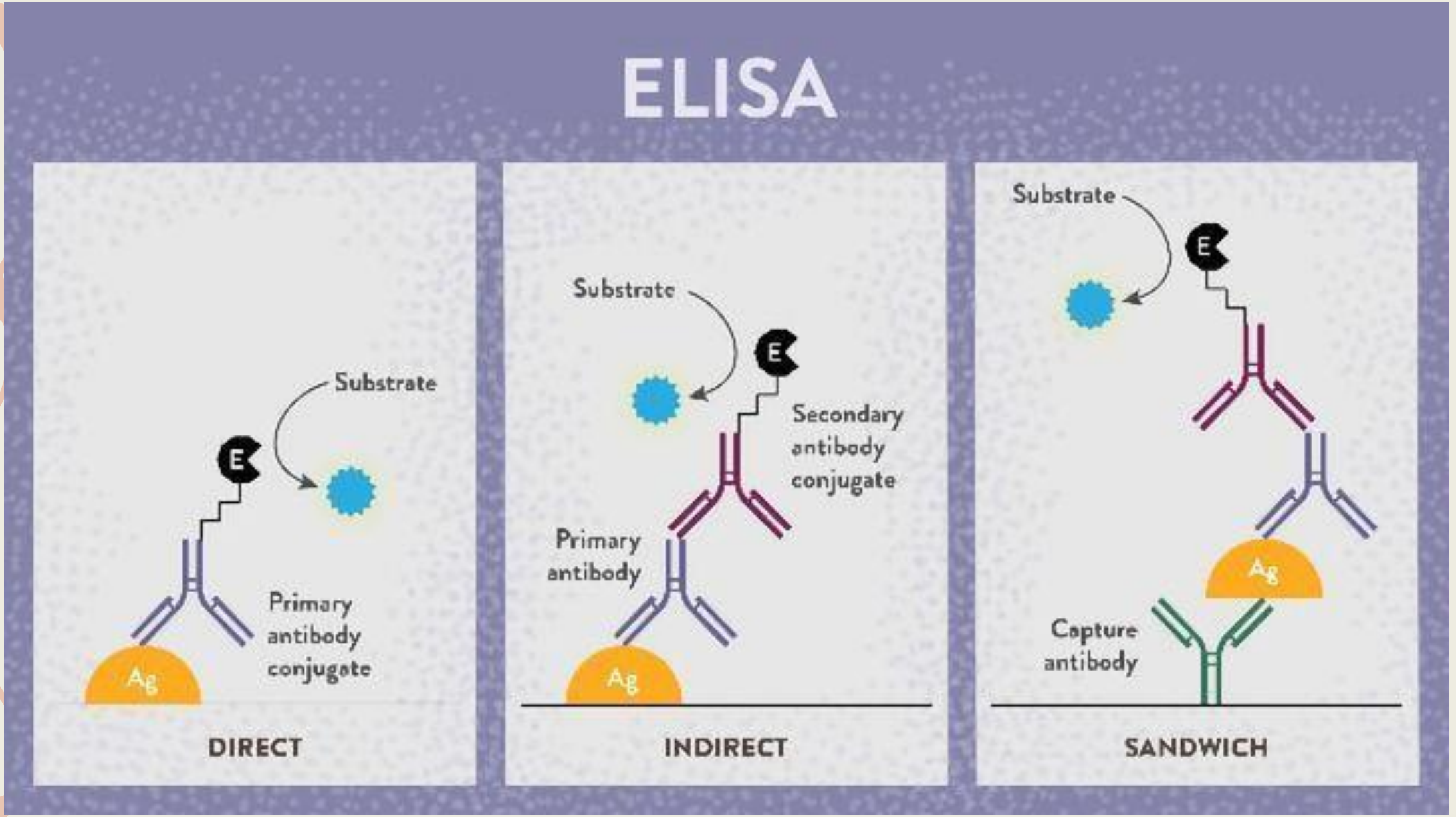

C-peptide
-COOH
-NH2
S
S
S
S
S
S
A-chain
B-chain

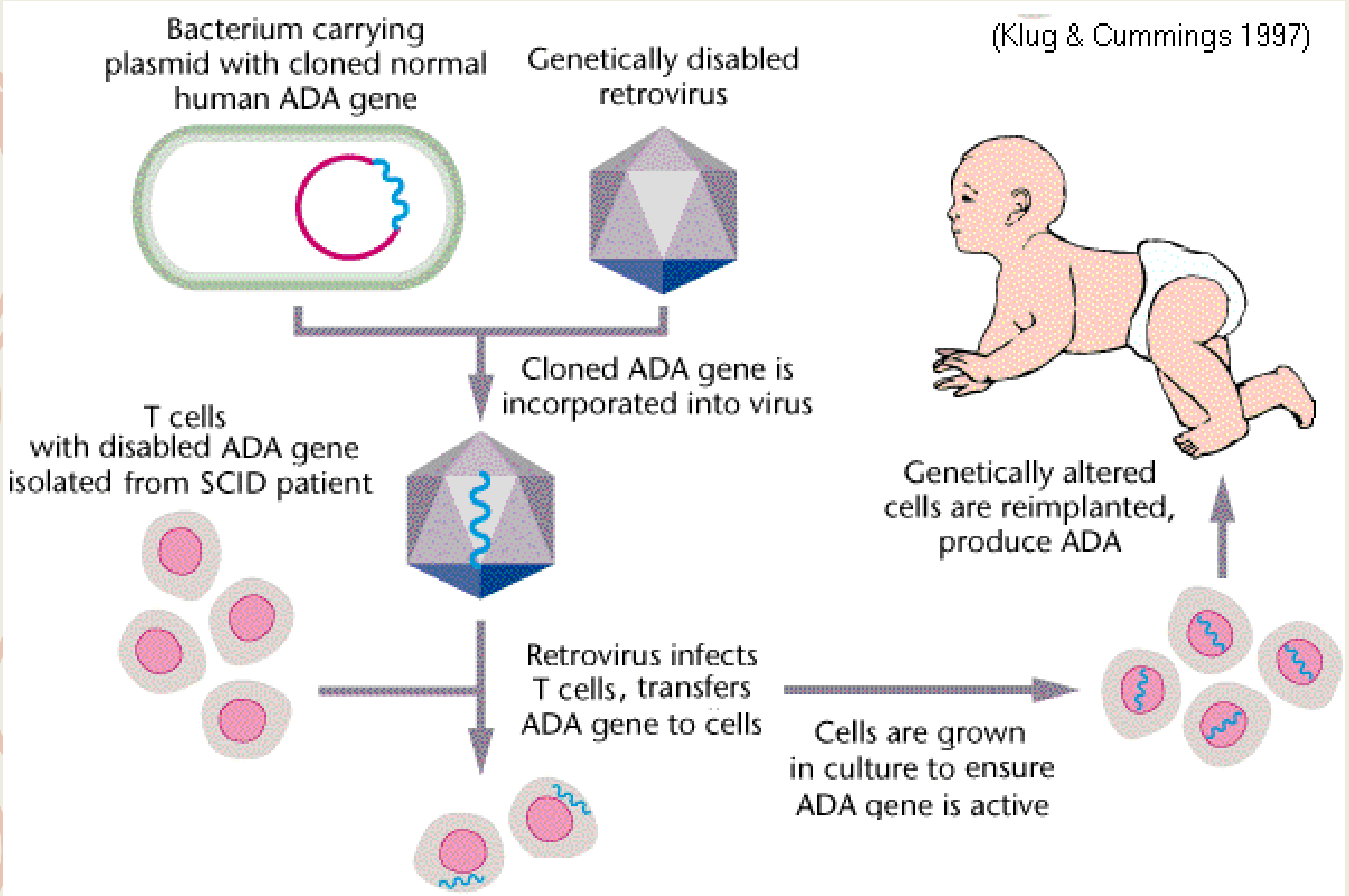

A Chain
Gly Ile Val Glu Gln Cys Cys Ala Ser Val Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
S
S
S
S
S
S
Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu
Arg
Gly
Phe
Ala Lys Pro Thr Tyr Phe
B Chain

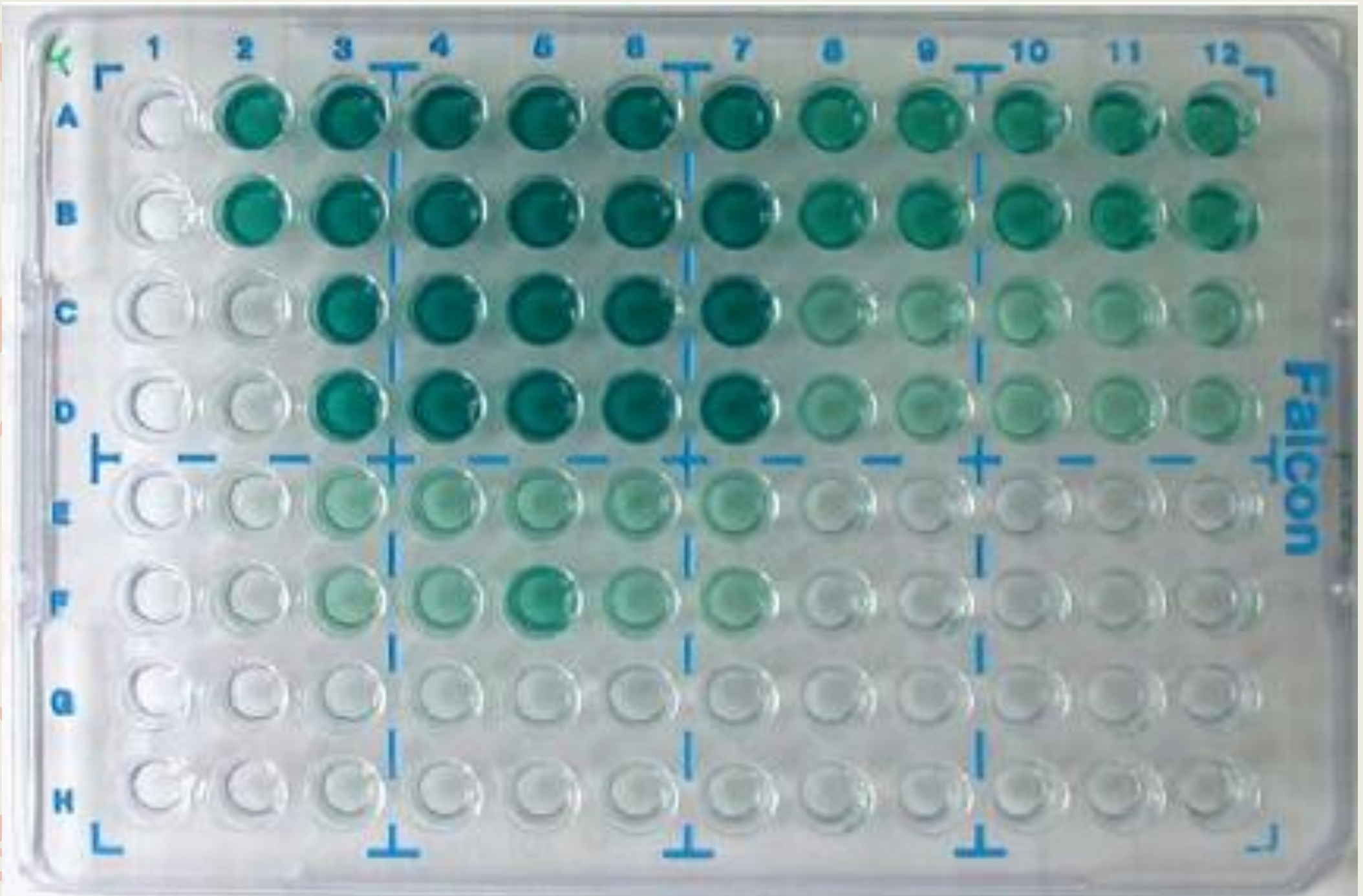

Promoter
β-galactosidase gene
Insulin gene A subunit
Antibiotic resistance gene
Promoter
β-galactosidase gene
Insulin gene B subunit
Antibiotic resistance gene
1 Insert genes for each of the two insulin polyeptides next to a highly expressed gene for β-galactosidase.
A
B
2 Transform E. coli with the recombinant expression vectors, and select transgenic cells by their antibiotic resistance.
β-galactosidase/insulin A fusion protein
β-galactosidase/insulin B fusion protein
3 Purify the fusion proteins.
4 Chemically remove insulin polypeptides from the β-galactosidase protein.
Disulfide bridge
Active insulin
5 Combine the polypeptides to produce functional insulin.

10 mL
100 units per mL
HI-312
Humulin® N
NPH
human insulin
(rDNA origin)
isophane suspension
U-100


1
Cells harvested from patient
2
Virus altered so cannot reproduce
3
A gene is inserted into virus
4
Altered virus mixed with patient's cells
5
Cells become transgenic
6
Altered cells injected into patient's body
7
Altered cells produce desired protein


ELISA
Substrate
E
Primary antibody conjugate
Ag
DIRECT
Substrate
E
Secondary antibody conjugate
Primary antibody
Ag
INDIRECT
Substrate
E
Ag
Capture antibody
SANDWICH


Bacterium carrying plasmid with cloned normal human ADA gene
Genetically disabled retrovirus
(Klug & Cummings 1997)
Cloned ADA gene is incorporated into virus
T cells with disabled ADA gene isolated from SCID patient
Retrovirus infects T cells, transfers ADA gene to cells
Cells are grown in culture to ensure ADA gene is active
Genetically altered cells are reimplanted, produce ADA


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H
Falcon

Basis of DNA fingerprinting

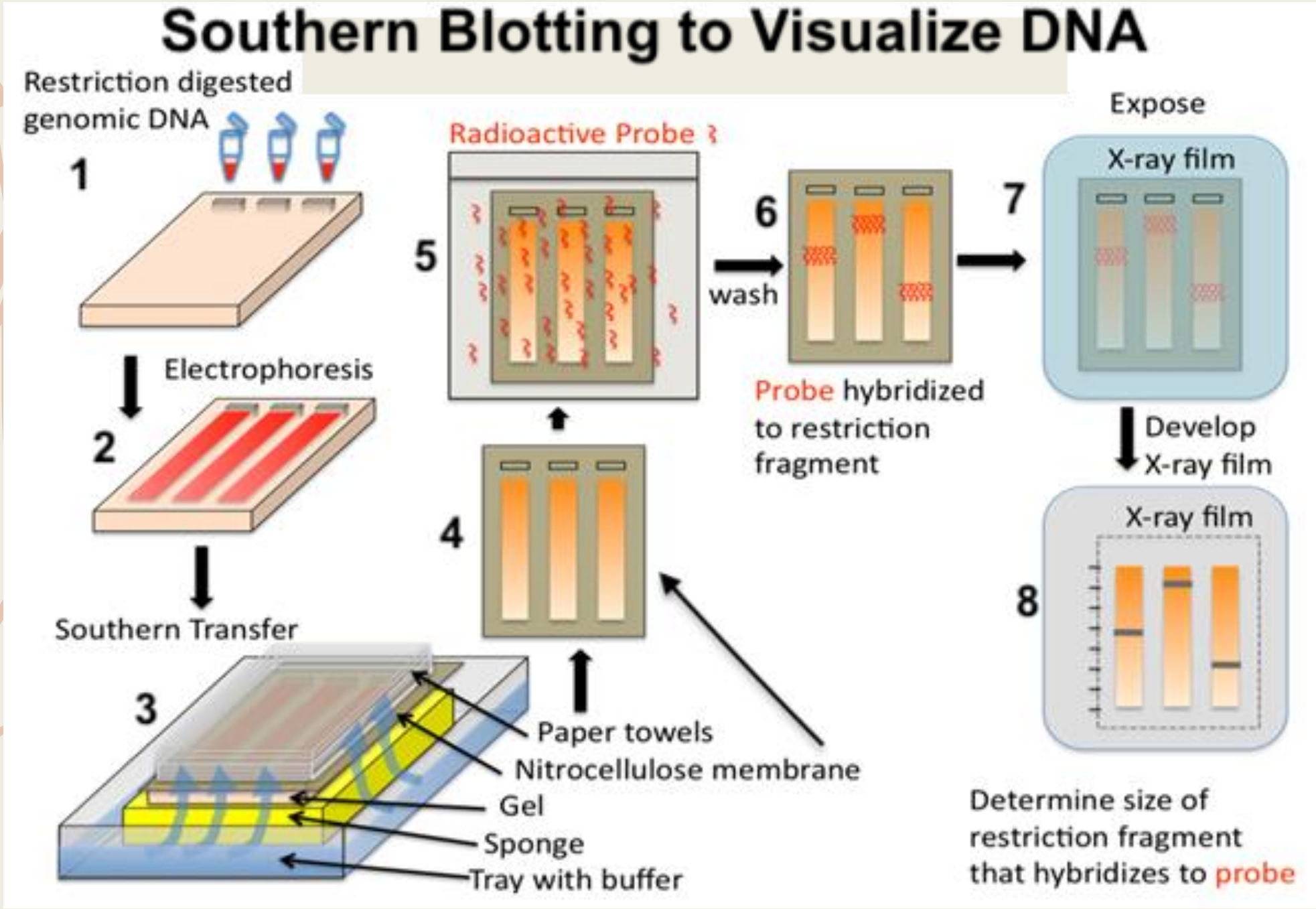
- DNA carries some **non-coding repetitive sequences.**
- Repetitive DNA can be separated from bulk genomic DNA as different peaks during **density gradient centrifugation.**
- The bulk DNA forms a **major peak** and the small peaks are called **satellite DNA.**
- Satellite DNA is classified as **micro-satellites, mini-satellites** etc. based on base composition (A:T rich or G:C rich), length of segment and number of repetitive units.
- A DNA sequence which is tandemly repeated in many copy numbers is called **variable number tandem repeats (VNTR).** It belongs to **mini-satellite DNA.**
- In a person, copy number varies in each chromosome.
- The two alleles (paternal and maternal) of a chromosome also contain different copy numbers of VNTR.
- VNTR is specific from person to person.
- The size of VNTR varies from **0.1 to 20 kb.**

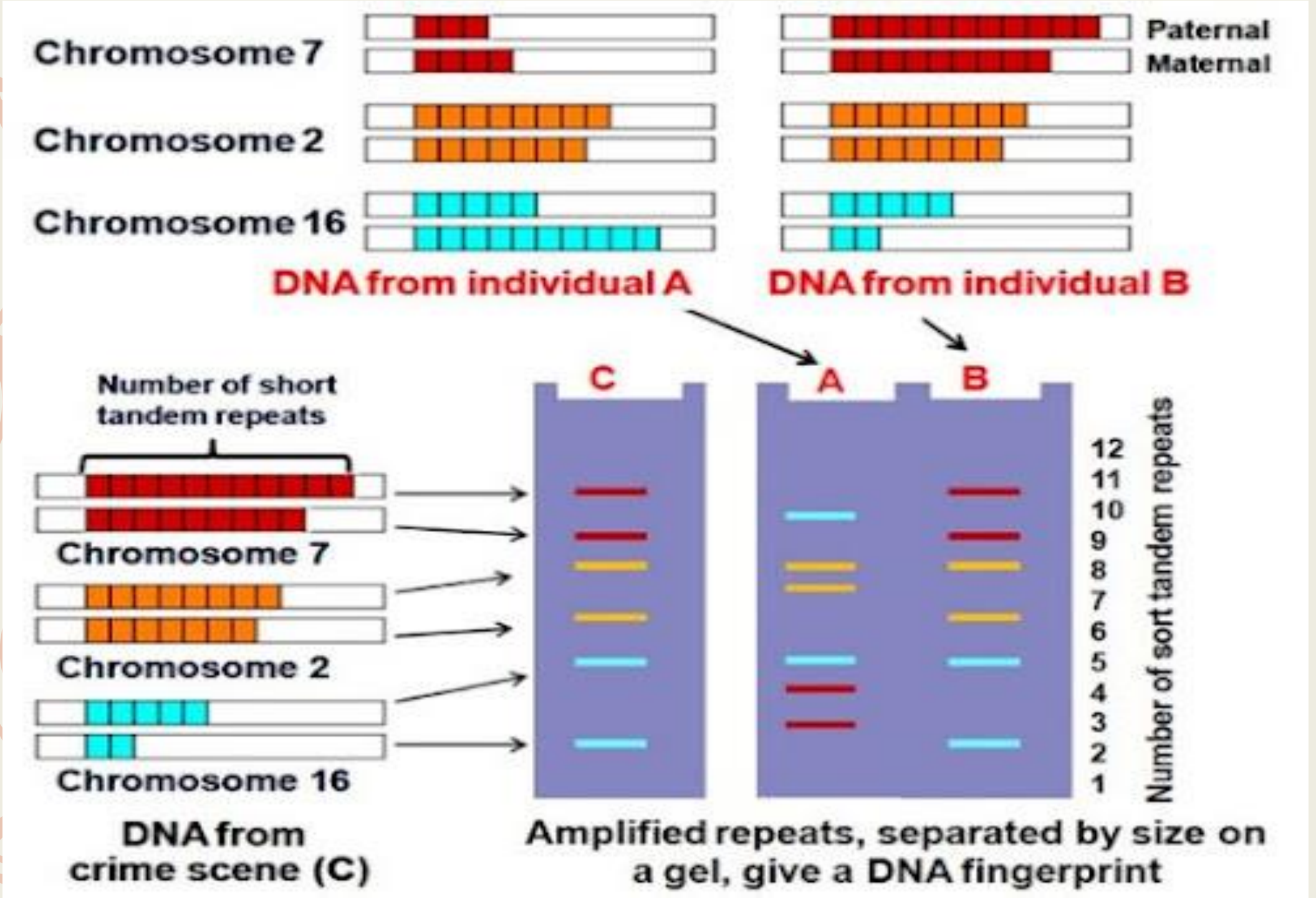
- Any difference in the nucleotide sequence (inheritable mutation) observed in a population is called **DNA polymorphism** (variation at genetic level).
- Polymorphism is higher in non-coding DNA sequence because mutations in these sequences may not affect an individual's reproductive ability. These mutations accumulate generation to generation causing polymorphism.
- Polymorphisms have great role in evolution & speciation.

Steps of DNA fingerprinting (Southern Blotting Technique)

1. **Isolation of DNA** (from any cells or blood stains, semen stains, saliva, hair roots, bone, skin etc.).
2. **Digestion of DNA** by restriction endonucleases.
3. **Separation of DNA** fragments by gel electrophoresis.
4. **Transferring (blotting) DNA fragments** to synthetic membranes such as nitrocellulose or nylon.
5. **Hybridization** using radioactive labelled VNTR probe.
6. **Detection of hybridized DNA** by autoradiography.

Southern Blotting to Visualize DNA

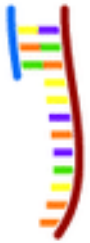



Chromosome 7
Chromosome 2
Chromosome 16
Paternal
Maternal
DNA from individual A
DNA from individual B
Number of short tandem repeats
Chromosome 7
Chromosome 2
Chromosome 16
C
A
B
12
11
10
9
8
7
6
5
4
3
2
1
Number of sort tandem repeats
DNA from crime scene (C)
Amplified repeats, separated by size on a gel, give a DNA fingerprint

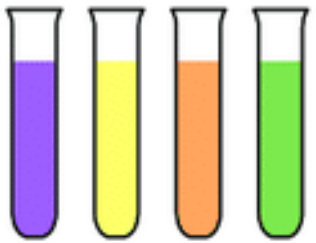
1. denature dsDNA using heat

2. make multiple copies of a segment

3. attach a primer



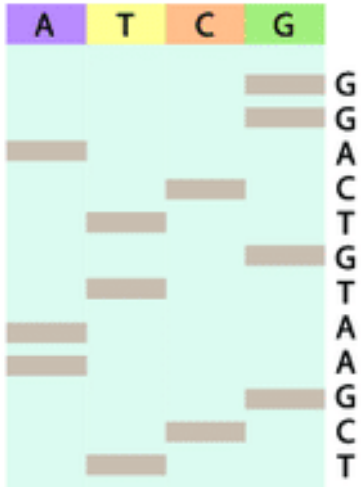
4. add to four polymerase solutions

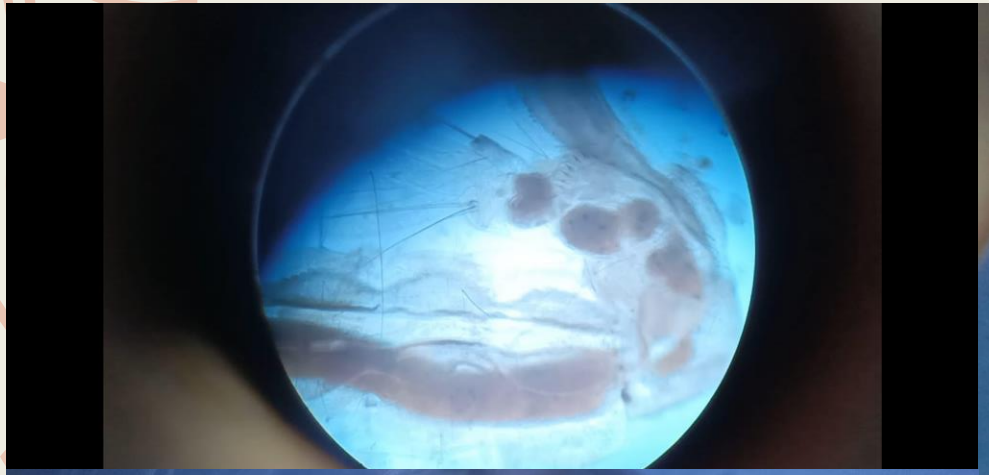
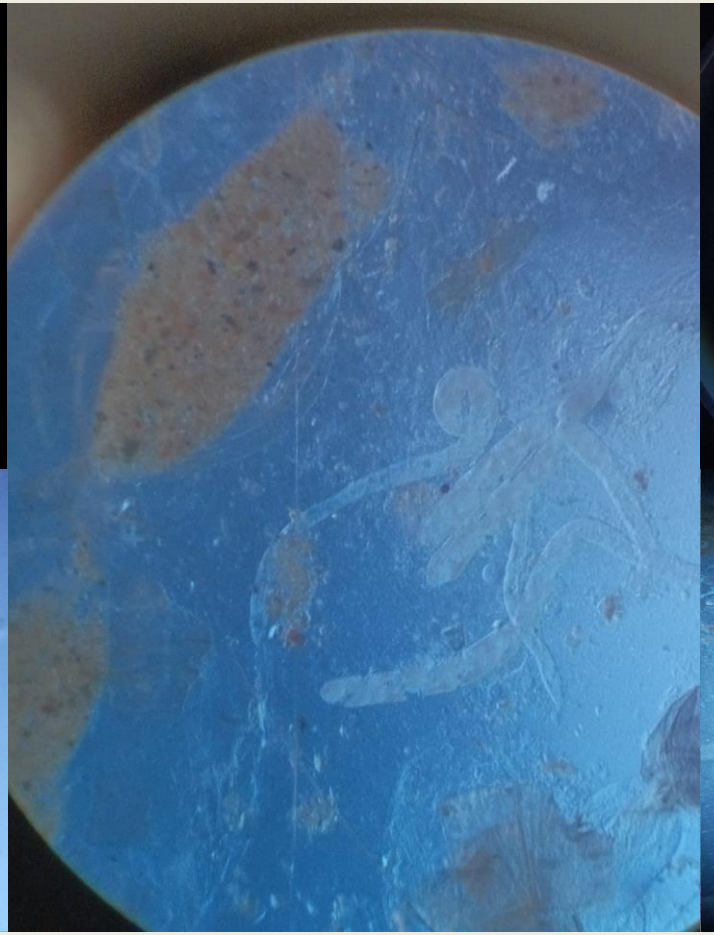
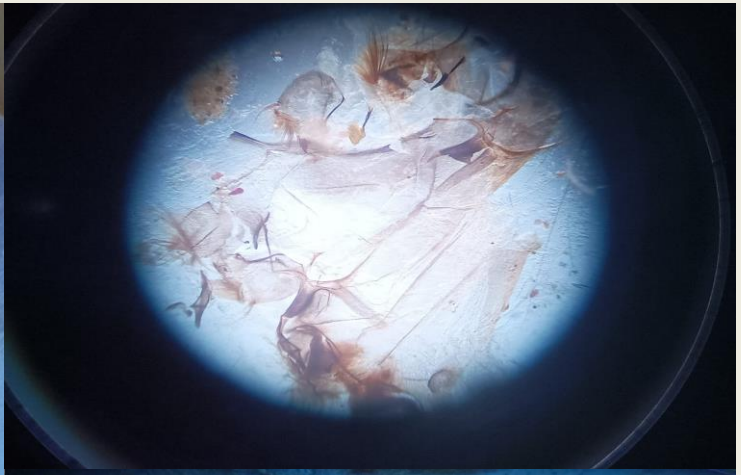
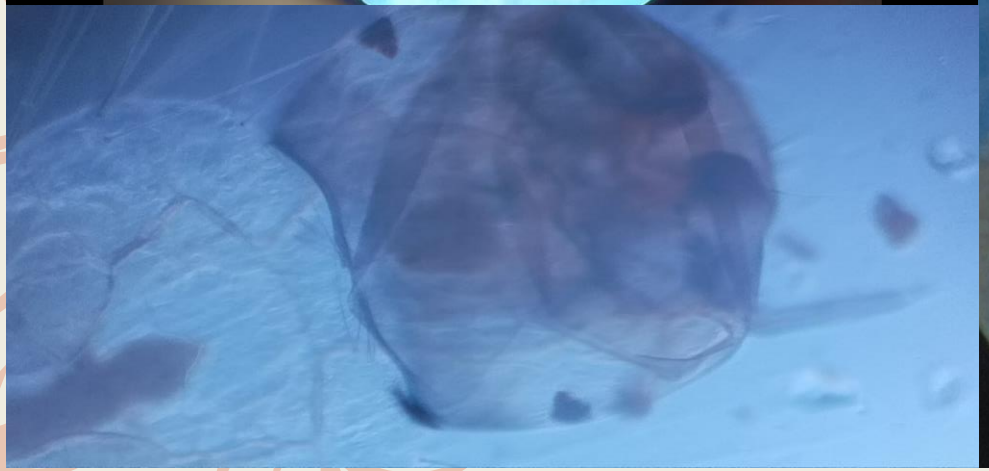


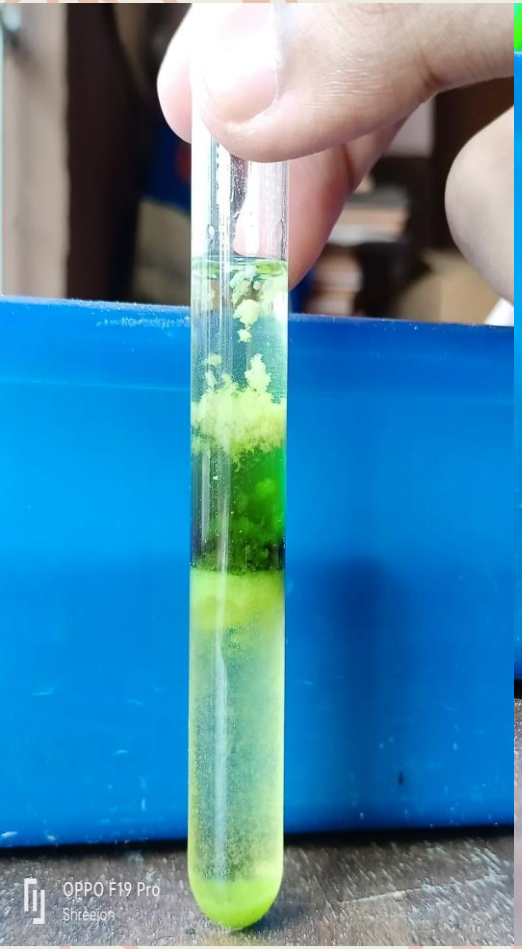
5. grow complementary chains until termination dye

6. denaturate the grown chains

7. electrophorese the four solutions





OPPO F19 Pro
Shreejon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