# GENETIC ENGINEERING

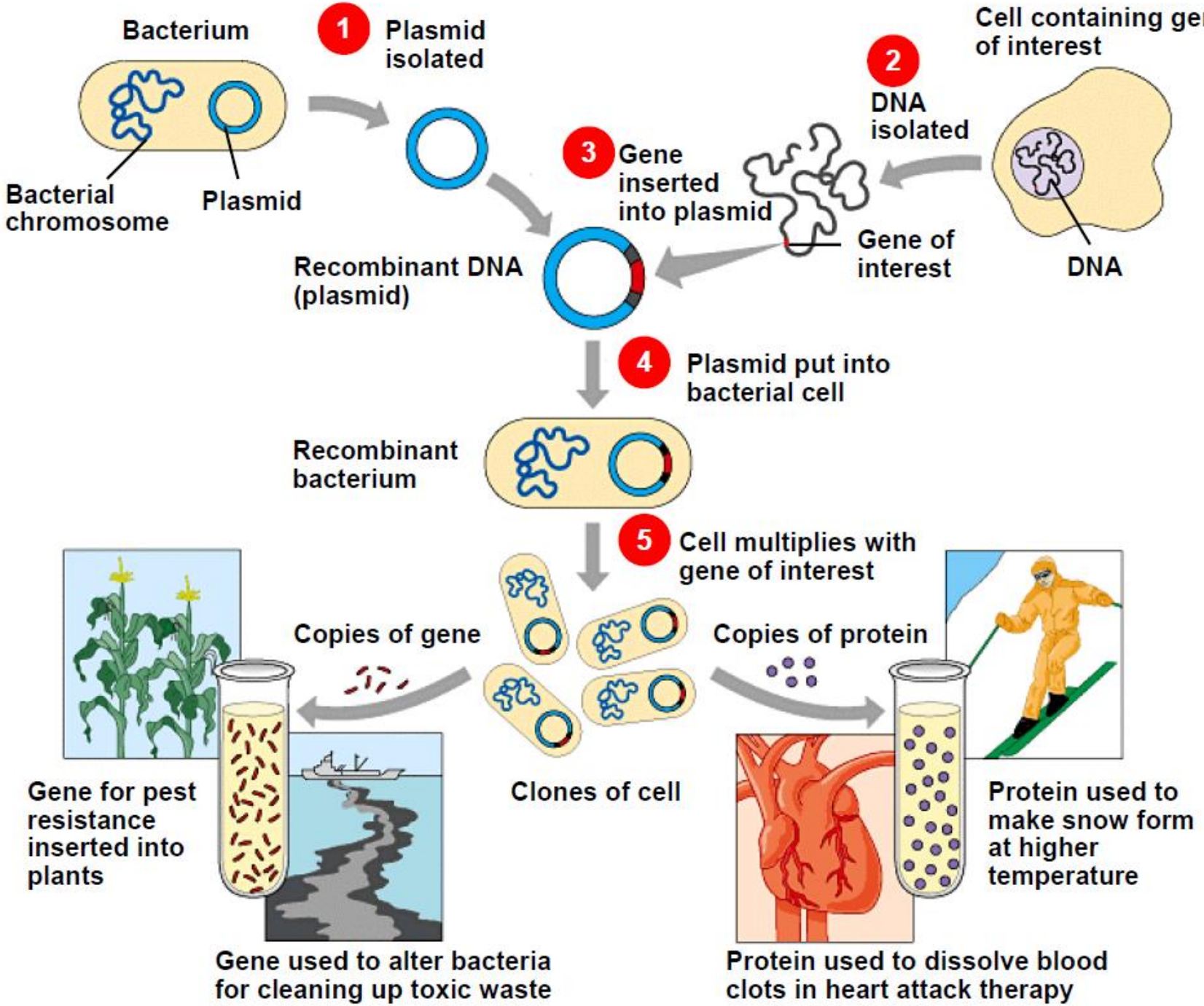
1

Dr. Manu Smriti Singh

Department of Biotechnology

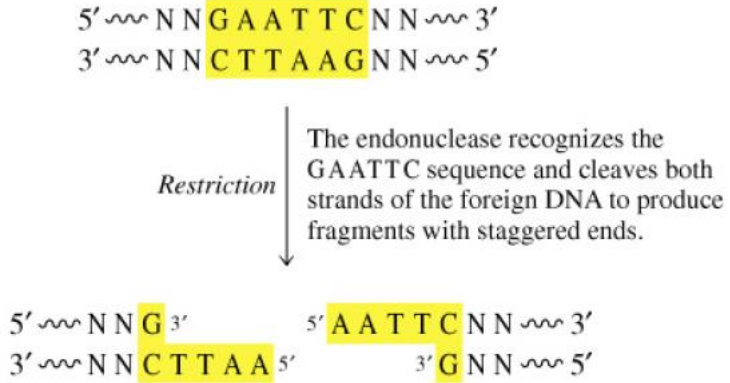
Bennett University

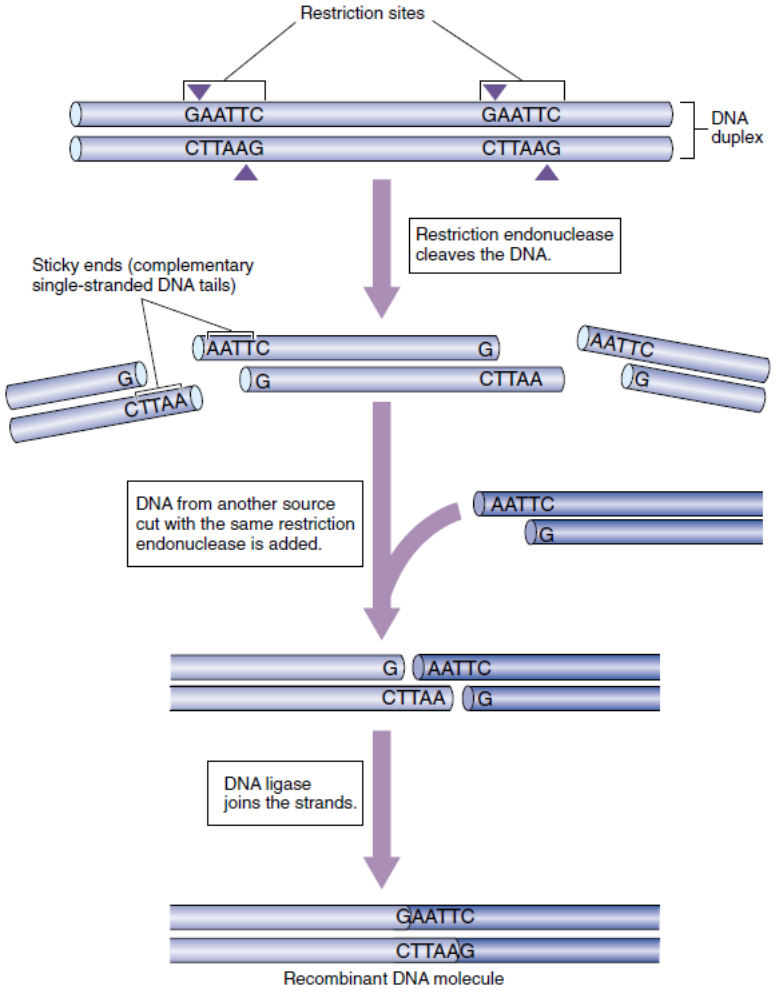
# rDNA


Bacterium
1 Plasmid isolated
Bacterial chromosome
Plasmid
Cell containing gene of interest
2 DNA isolated
3 Gene inserted into plasmid
Gene of interest
DNA
Recombinant DNA (plasmid)
4 Plasmid put into bacterial cell
Recombinant bacterium
5 Cell multiplies with gene of interest
Copies of gene
Copies of protein
Gene for pest resistance inserted into plants
Clones of cell
Protein used to make snow form at higher temperature
Gene used to alter bacteria for cleaning up toxic waste
Protein used to dissolve blood clots in heart attack therapy

# ALL BEGAN WITH AN ENZYME!

- Scissors → Sticky ends
- Palindromes → Radar/ Reviver



# RESTRICTION ENDONUCLEASES

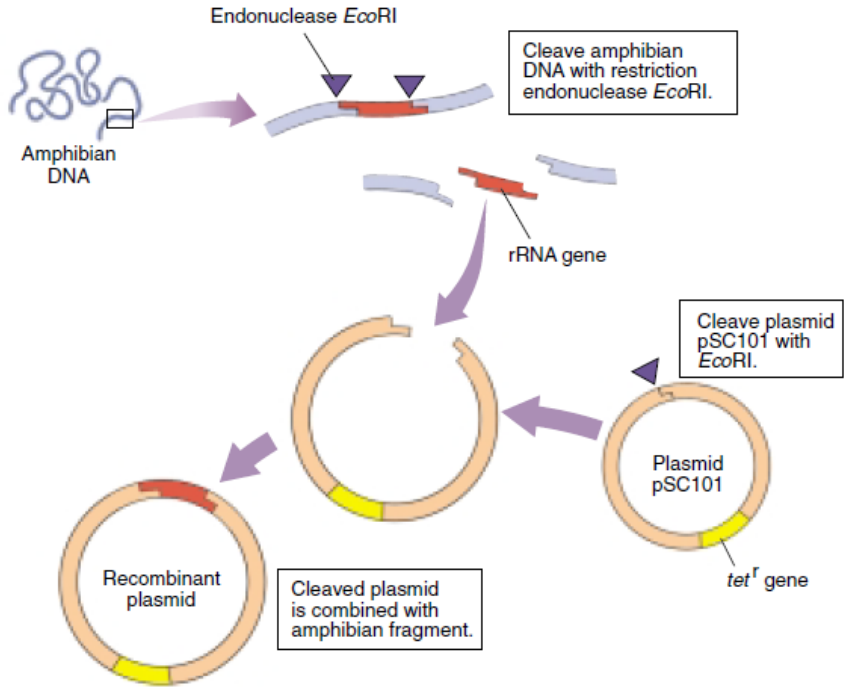



**FIGURE 19.3**
**One of the first genetic engineering experiments.** This diagram illustrates how Cohen and Boyer inserted an amphibian gene encoding rRNA into pSC101. The plasmid contains a single site cleaved by the restriction endonuclease *Eco*RI; it also contains *tet*$^r$, a gene which confers resistance to the antibiotic tetracycline. The rRNA-encoding gene was inserted into pSC101 by cleaving the amphibian DNA and the plasmid with *Eco*RI and allowing the complementary sequences to pair.

Cut → Restriction Endonuclease
Join → DNA Ligase

**Fragments of elephant and ostrich DNA cleaved by the same endonuclease can be joined to one an- other as readily as two bacterial DNA fragments.**

Genetic engineering experiments consist of four stages:

**Step 1: DNA cleavage**

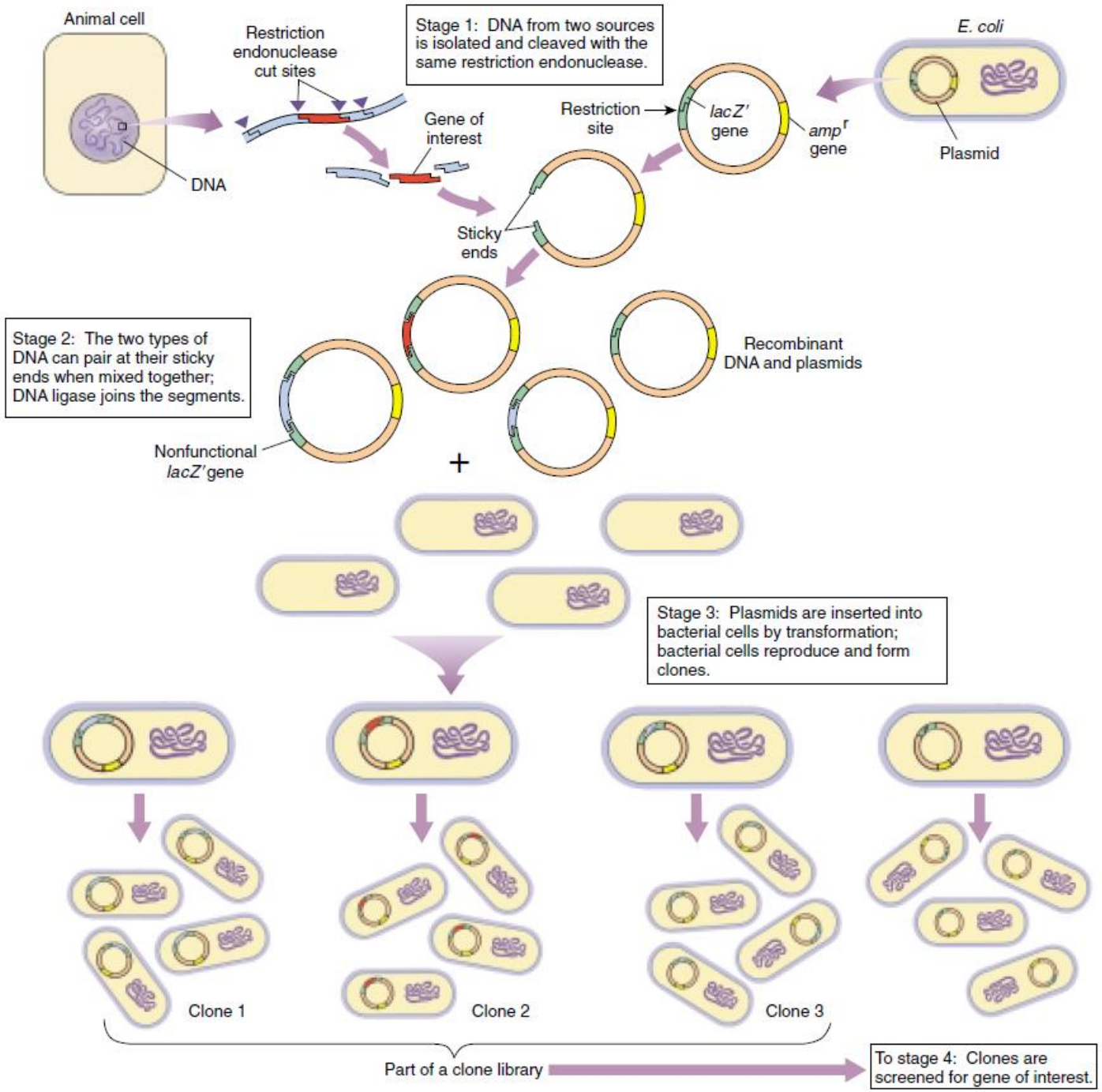
**Step 2: Production of recombinant DNA**

**Step 3: Cloning**

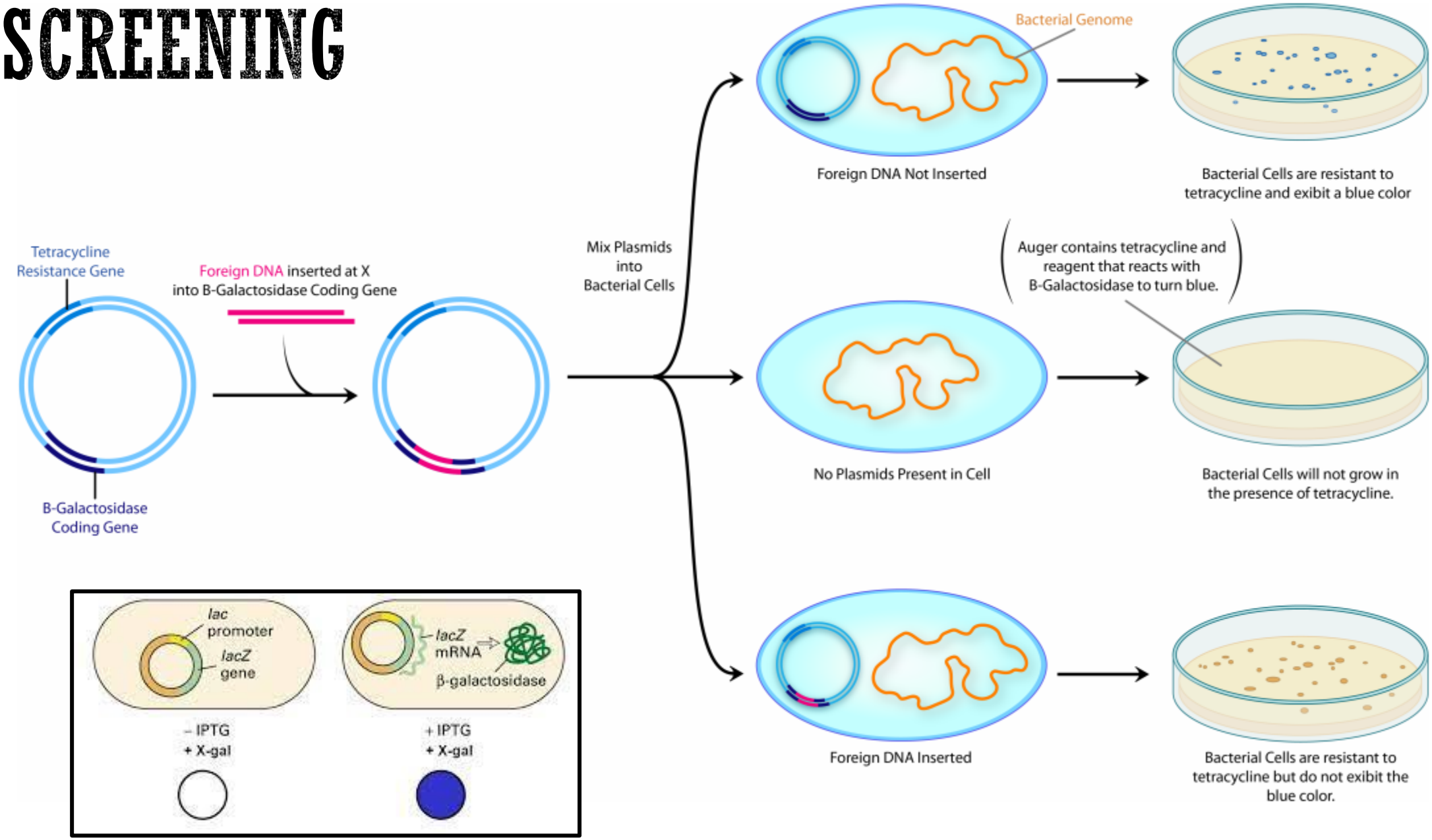
**Step 4: Screening**

**Transformation: Recombinant Plasmid+ Bacteria**

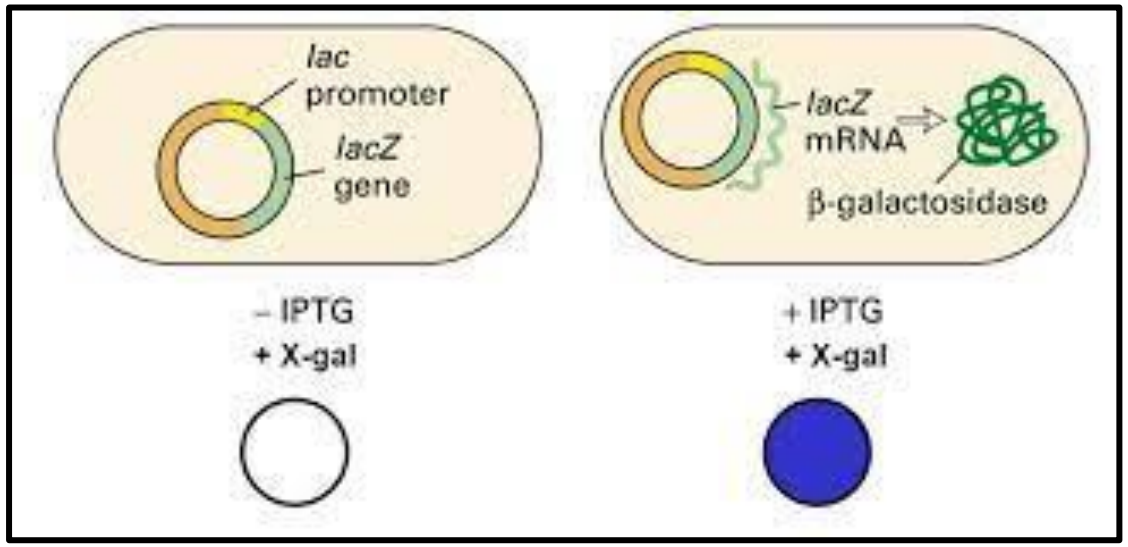
- **Calcium Chloride**
- **Electroporation**

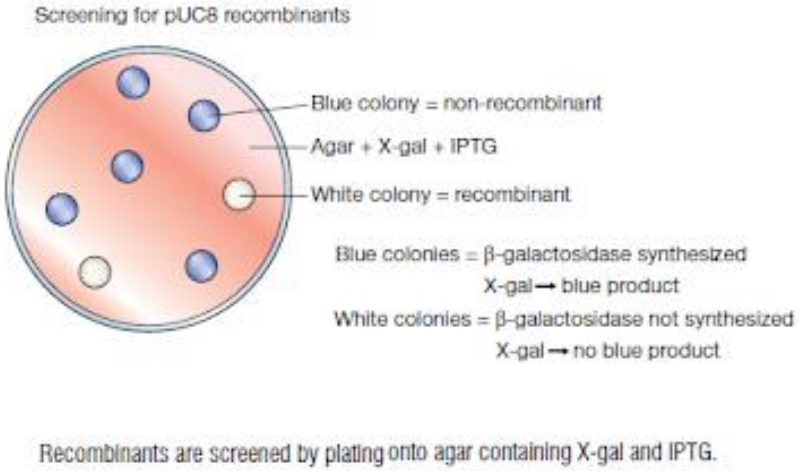


# SCREENING


Tetracycline Resistance Gene
Foreign DNA inserted at X into B-Galactosidase Coding Gene
B-Galactosidase Coding Gene
Mix Plasmids into Bacterial Cells
Bacterial Genome
Foreign DNA Not Inserted
Bacterial Cells are resistant to tetracycline and exibit a blue color
Auger contains tetracycline and reagent that reacts with B-Galactosidase to turn blue.
No Plasmids Present in Cell
Bacterial Cells will not grow in the presence of tetracycline.
Foreign DNA Inserted
Bacterial Cells are resistant to tetracycline but do not exibit the blue color.
lac promoter
lacZ gene
lacZ mRNA
β-galactosidase
– IPTG
+ X-gal
+ IPTG
+ X-gal

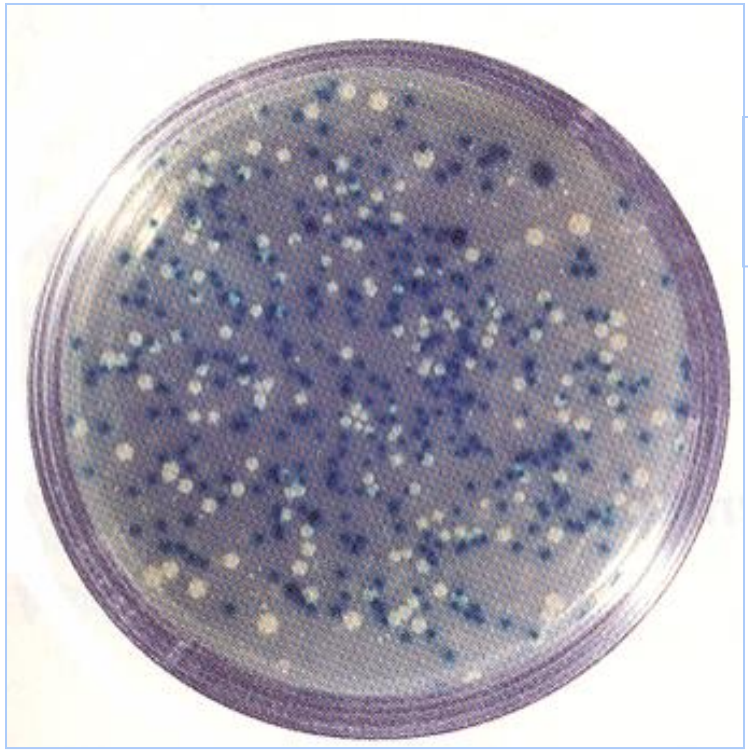
# COLONY PICKING




# Analysis of Recombinant DNA Technology Results

## Selection for Recombinant



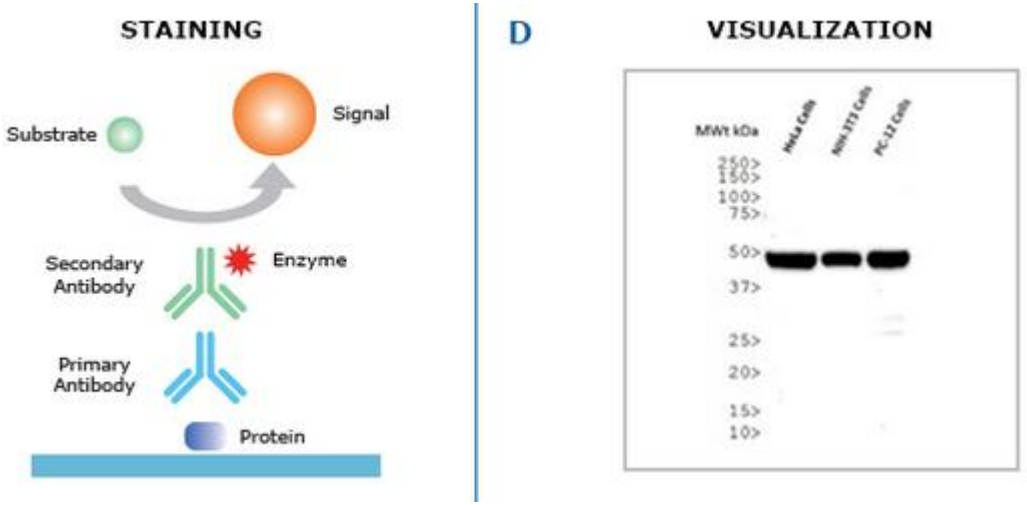
- Agar dish after rec DNA Technology shows **both blue and white** (clear) colonies.

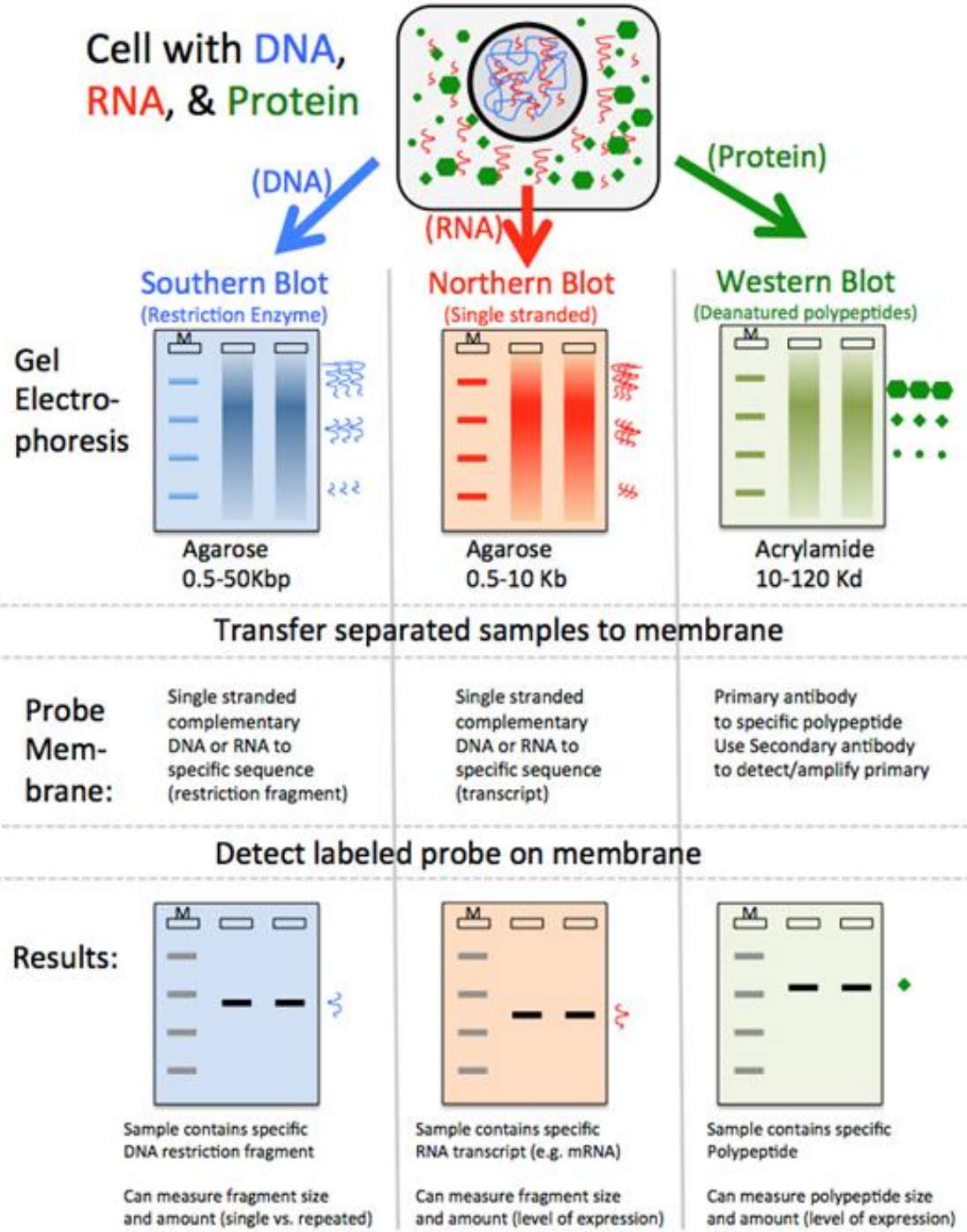
WHY **both blue and white** (clear) colonies?

Which of the colonies is the color we want?

If you forgot to add antibiotics in the plate, What results would you expect?

# Detection Techniques



# POLYMERASE CHAIN REACTION (PCR)

**What is PCR?:**

Use of DNA polymerase to selectively amplify a segment of DNA from a much larger sample.

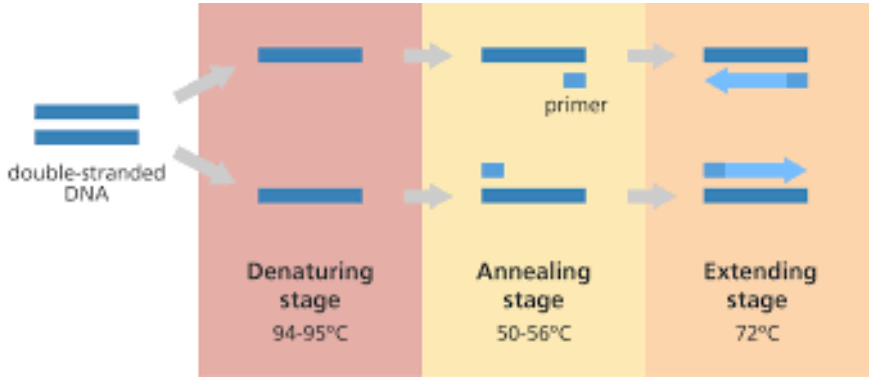
Xeroxing DNA, start with one page and get many.

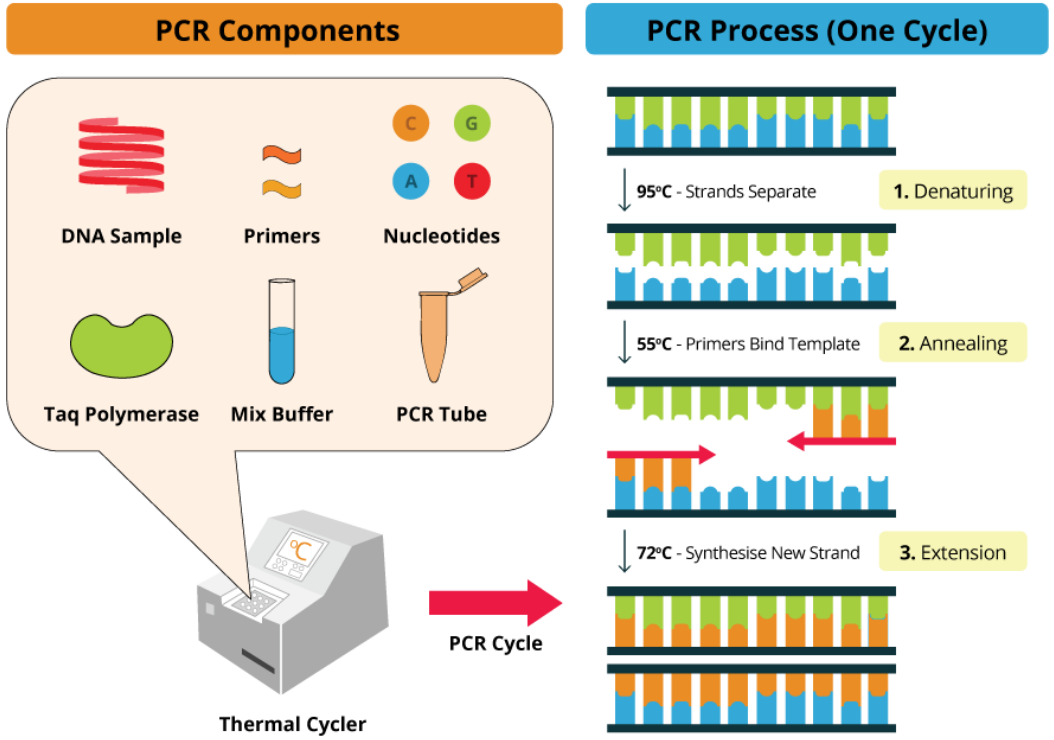
**Examples of what PCR is used for:**

→Forensics, DNA typing from very small samples

→Clinical diagnostics e.g. detection of HIV, detection of some microbial infections, detection of whether an individual carries a mutation predisposing them to some sort of cancer or genetic disease.

→Research, mapping and sequencing of genomes, cloning, basic research



# REAL TIME PCR (RT-PCR)

## Coronavirus Testing

"Gold Standard" RT-PCR Test

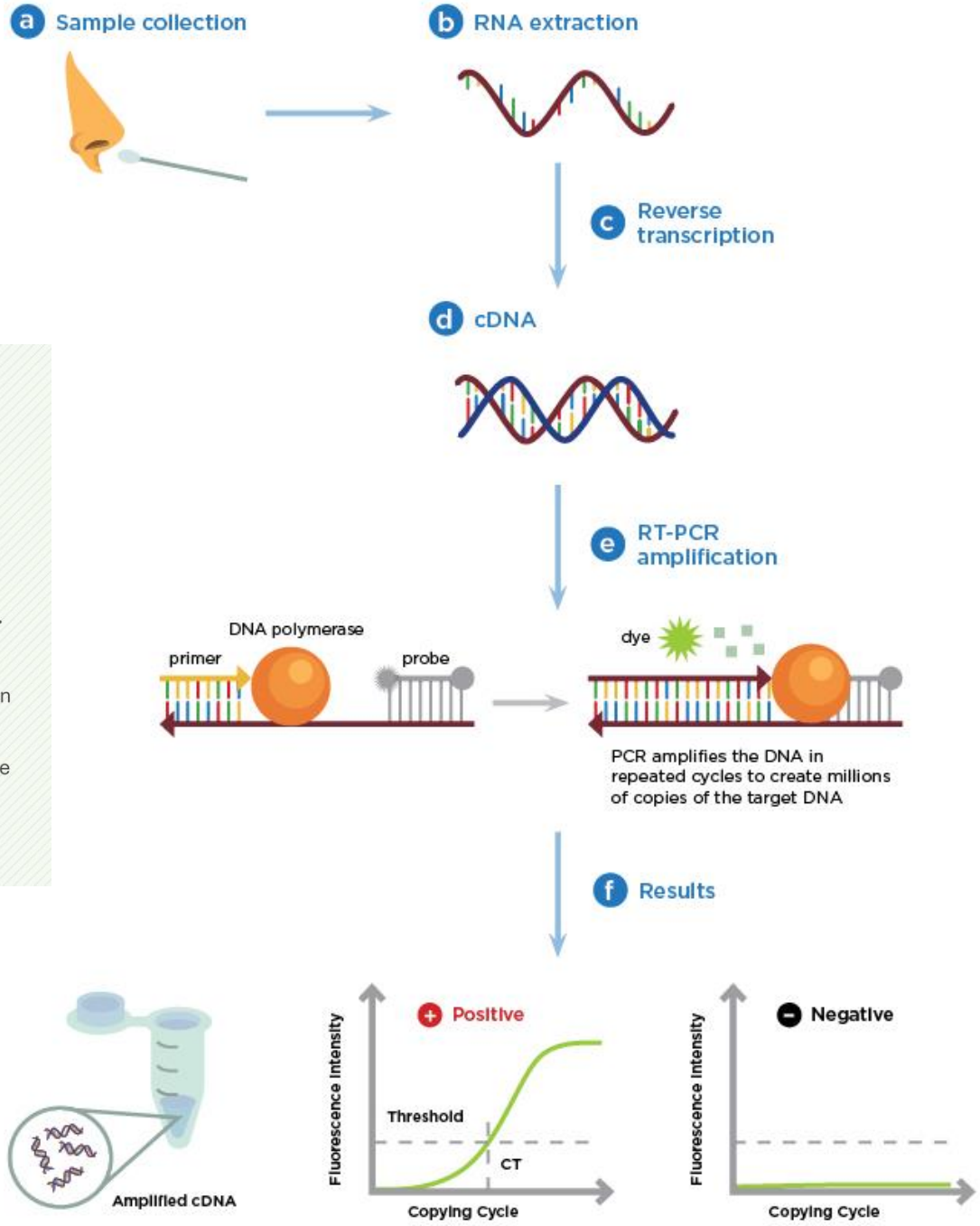
The virus is collected via a saliva sample or nose or throat swab.

Researchers break the virus, releasing its genetic material —RNA.

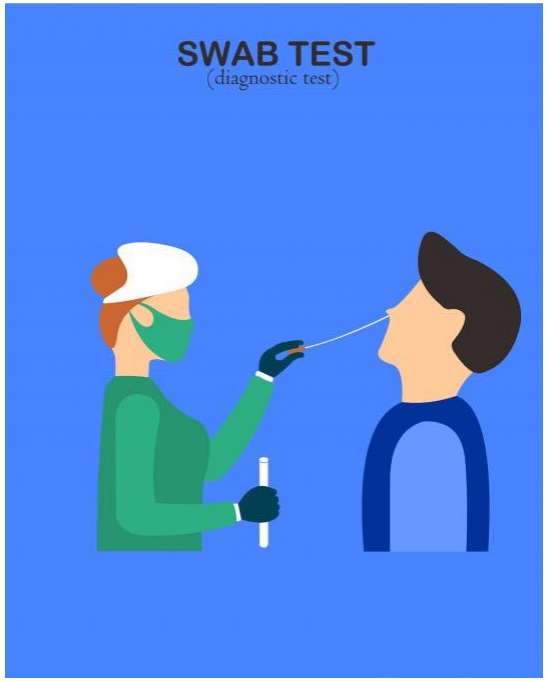
An enzyme called reverse transcriptase (RT) converts viral RNA to double-stranded DNA.

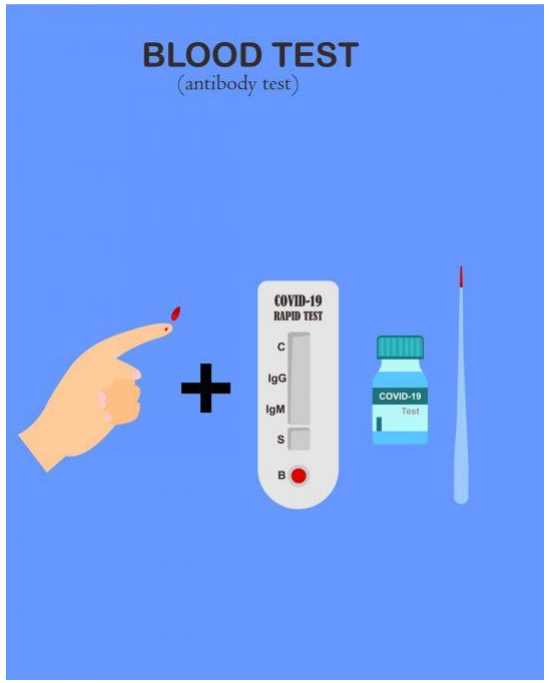
Researchers use a technique called polymerase chain reaction (PCR) to create millions of copies of the converted viral DNA, making it easier to detect.

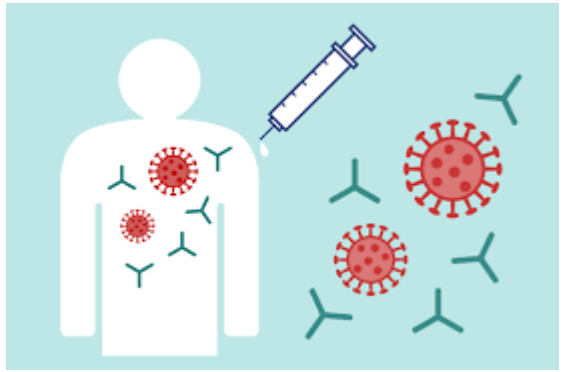
A fluorescent probe glows when it spots telltale virus DNA. This signifies a positive test.

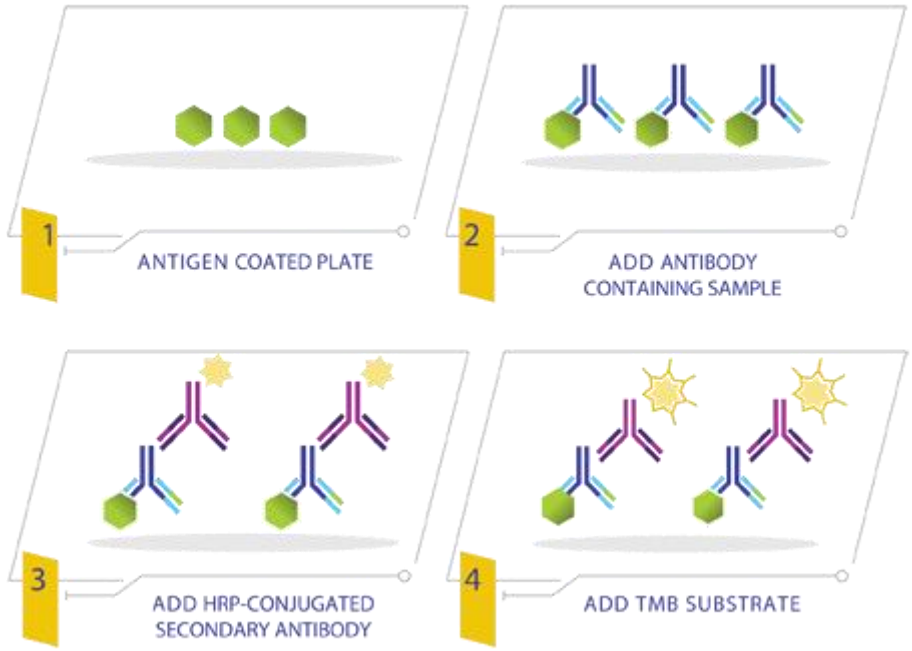


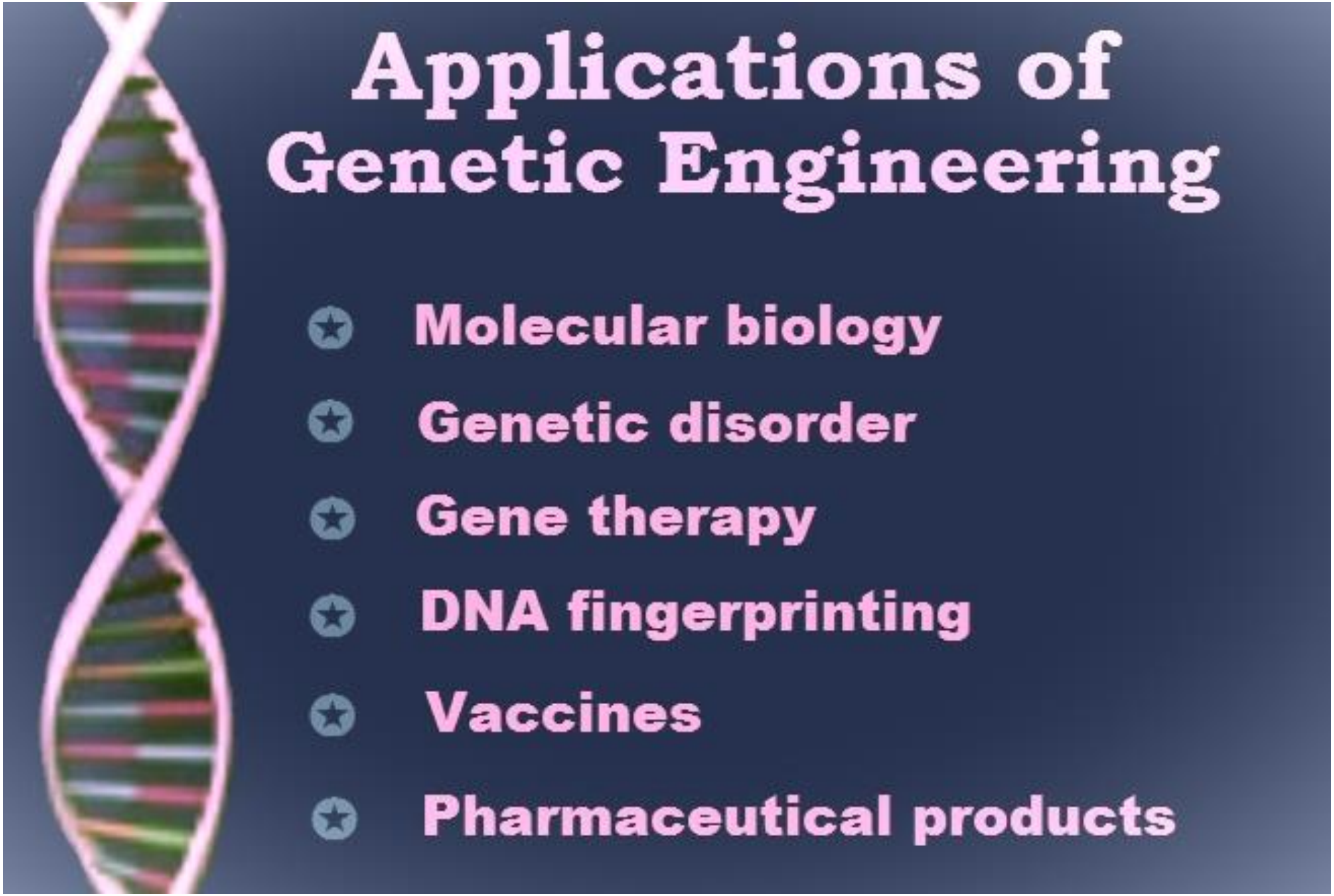
# ANTIBODY DETECTION



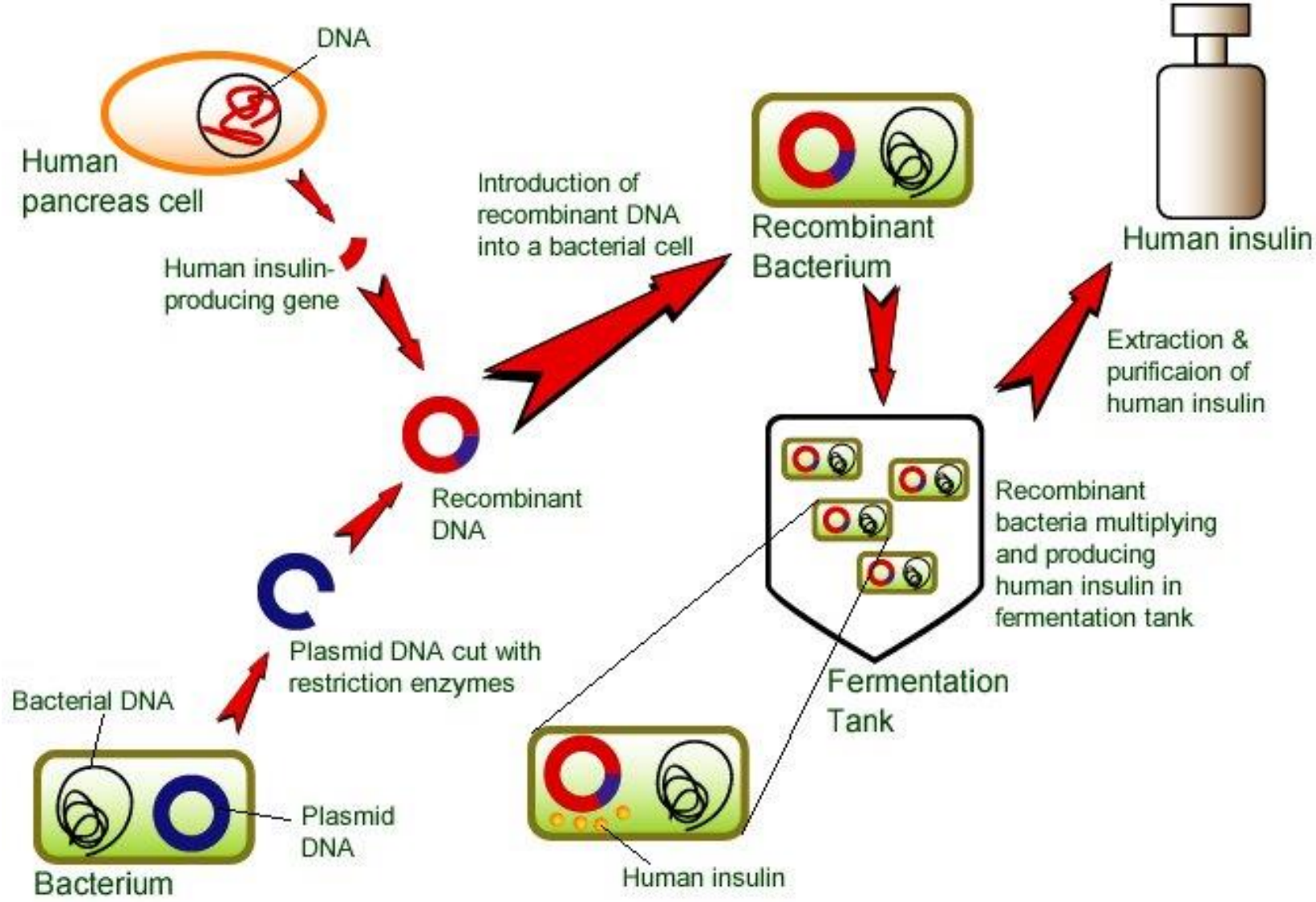



Applications of Genetic Engineering
Molecular biology
Genetic disorder
Gene therapy
DNA fingerprinting
Vaccines
Pharmaceutical products

# Human Insulin Production

1951:
10,000 POUNDS OF PIG PANCREASES MAKE 1 POUND OF INSULIN

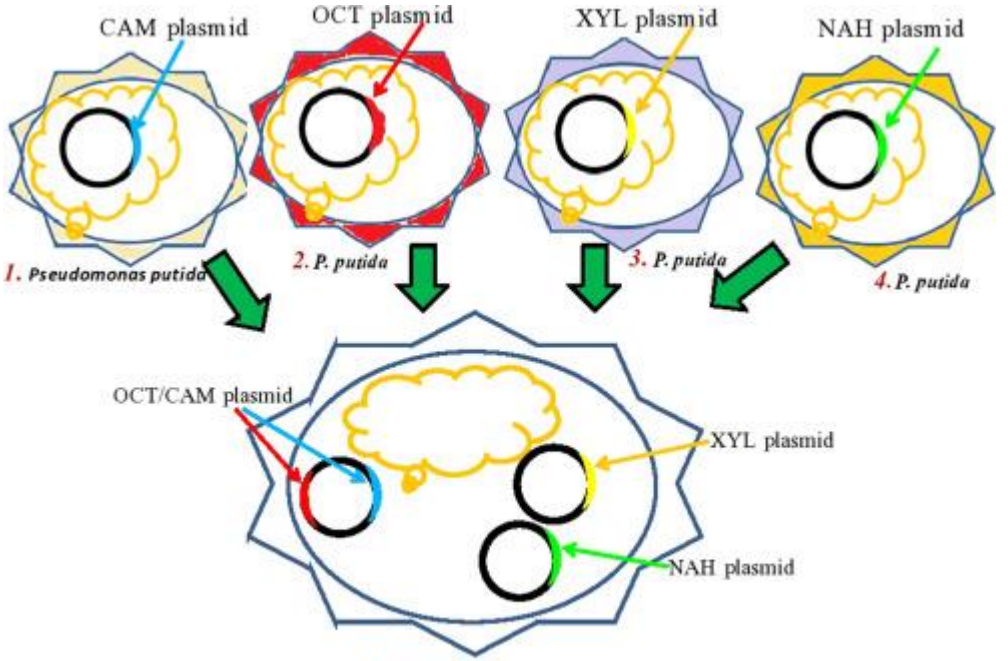
TODAY:
GENETICALLY ENGINEERED BACTERIA PRODUCE ANIMAL-FREE INSULIN



# SUPERBUG TO CLEAR OIL SPILLS

- Super bug was developed by **Anand Chakrabarty** et al. in 1979.
- It is used to treat oil spills as a measure to control oil pollution.
- Petroleum products contain **cycloalkenes(octane), napthenes, xylene, tolune and aromatic hydrocarbons**. Since these compounds are not easily biodegradable, oil wastes become a major pollutant on the soil and water.
- **Chakrabarty** et al. took attempts to degrade oil wastes using micro organisms.
- They developed superbug to control oil pollution.

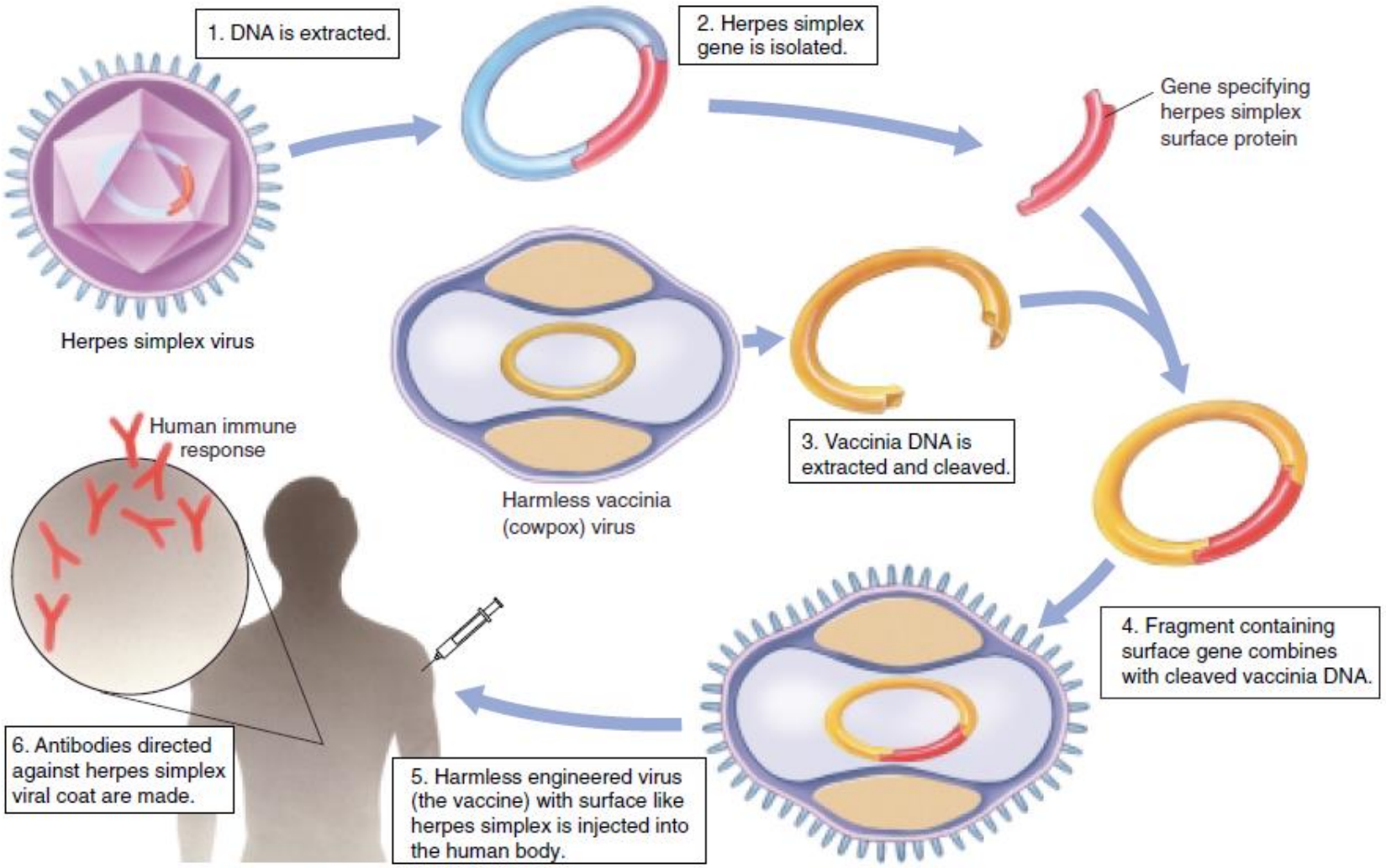
Anand Chakrabarty




Superbug

(*Pseudomonas putida* with four different plasmid's DNA

# VACCINES


1. DNA is extracted.
2. Herpes simplex gene is isolated.
Gene specifying herpes simplex surface protein
Herpes simplex virus
Harmless vaccinia (cowpox) virus
3. Vaccinia DNA is extracted and cleaved.
4. Fragment containing surface gene combines with cleaved vaccinia DNA.
Human immune response
6. Antibodies directed against herpes simplex viral coat are made.
5. Harmless engineered virus (the vaccine) with surface like herpes simplex is injected into the human body.

# FARMING

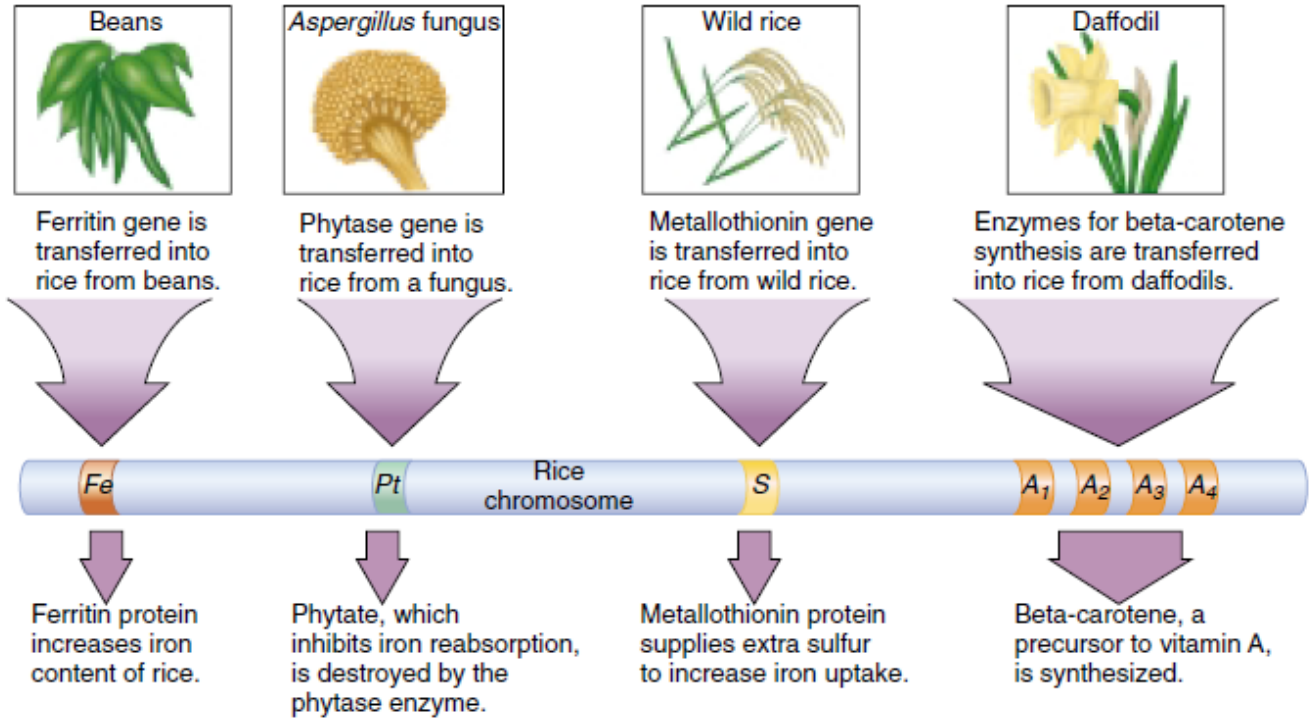
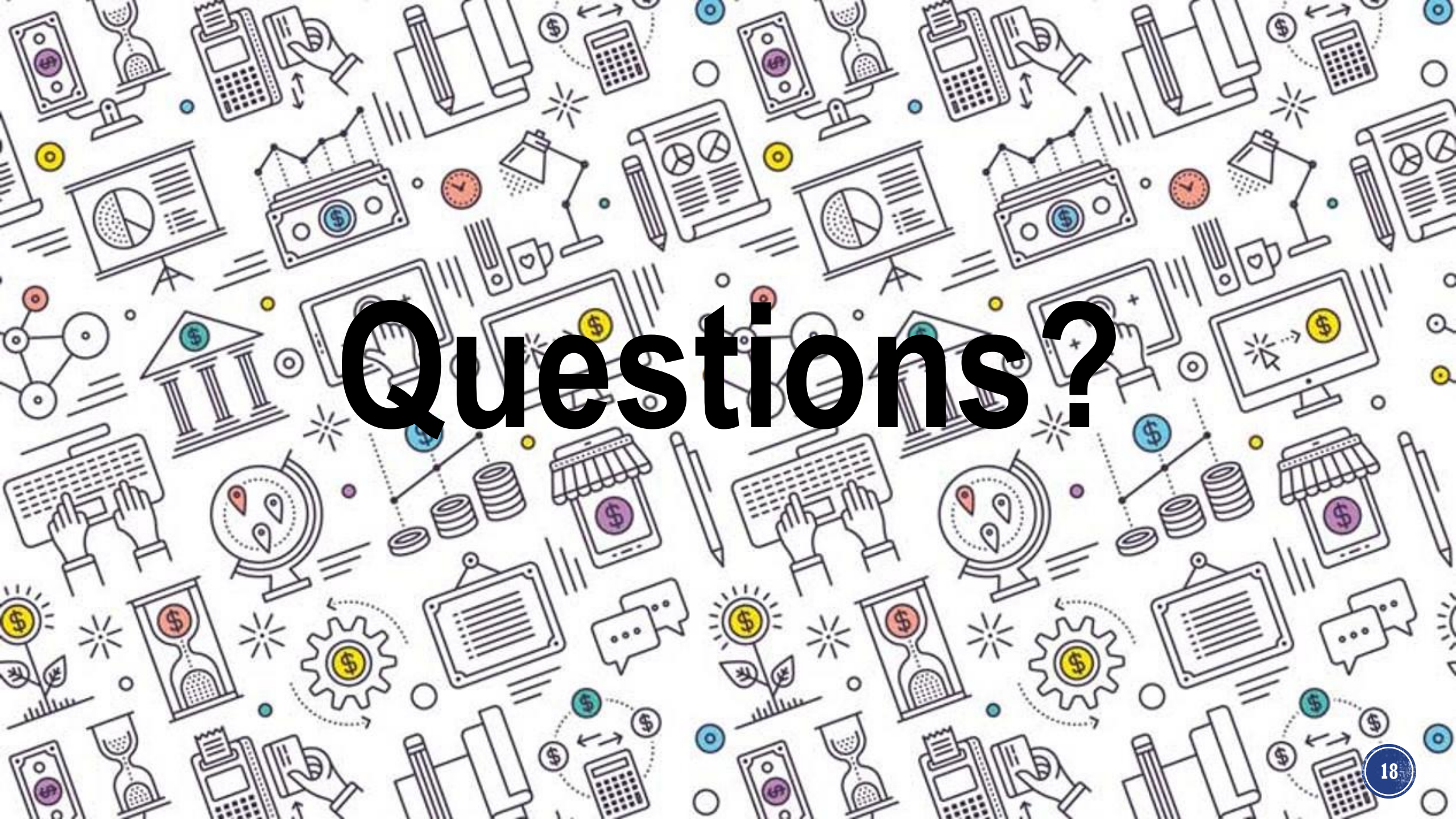



**FIGURE 19.21**
**Transgenic rice.** Developed by Swiss bioengineer Ingo Potrykus, transgenic rice offers the promise of improving the diets of people in rice-consuming developing countries, where iron and vitamin A deficiencies are a serious problem.


Questions?