March, 2021

Science of Living System

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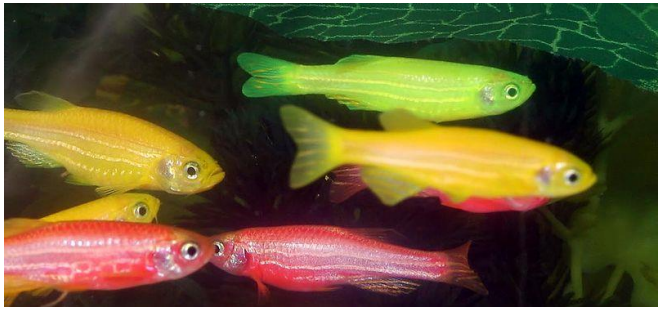

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Genetic Engineering

- Manipulation of an organism's genome
- Can range from changing one base pair (A-T or C-G), deleting a whole region of DNA, or introducing an additional copy of a gene
- Extracting DNA from an organism's genome and combining it with the DNA of another individual
- Used to enhance or modify the characteristics of an individual organism

An Overview of Genetic Engineering

Agricultural application (Bt Cotton, Genetically modified sweet corn)

Application in Dairy, Poultry Farms etc (Growth hormones to increase production of cow milk)

Environmental application (*Pseudomonas putida*, *an oil eating bacteria)*

Therapeutic application (Human insulin, many growth hormones, etc)

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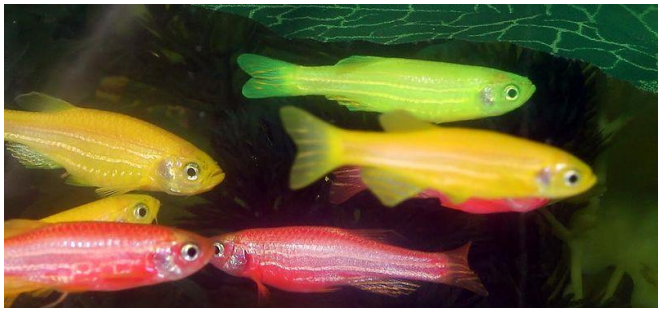
Genetically modified Product

Flavr Savr (also known as CGN-89564-2; pronounced "flavor saver"), a genetically modified tomato

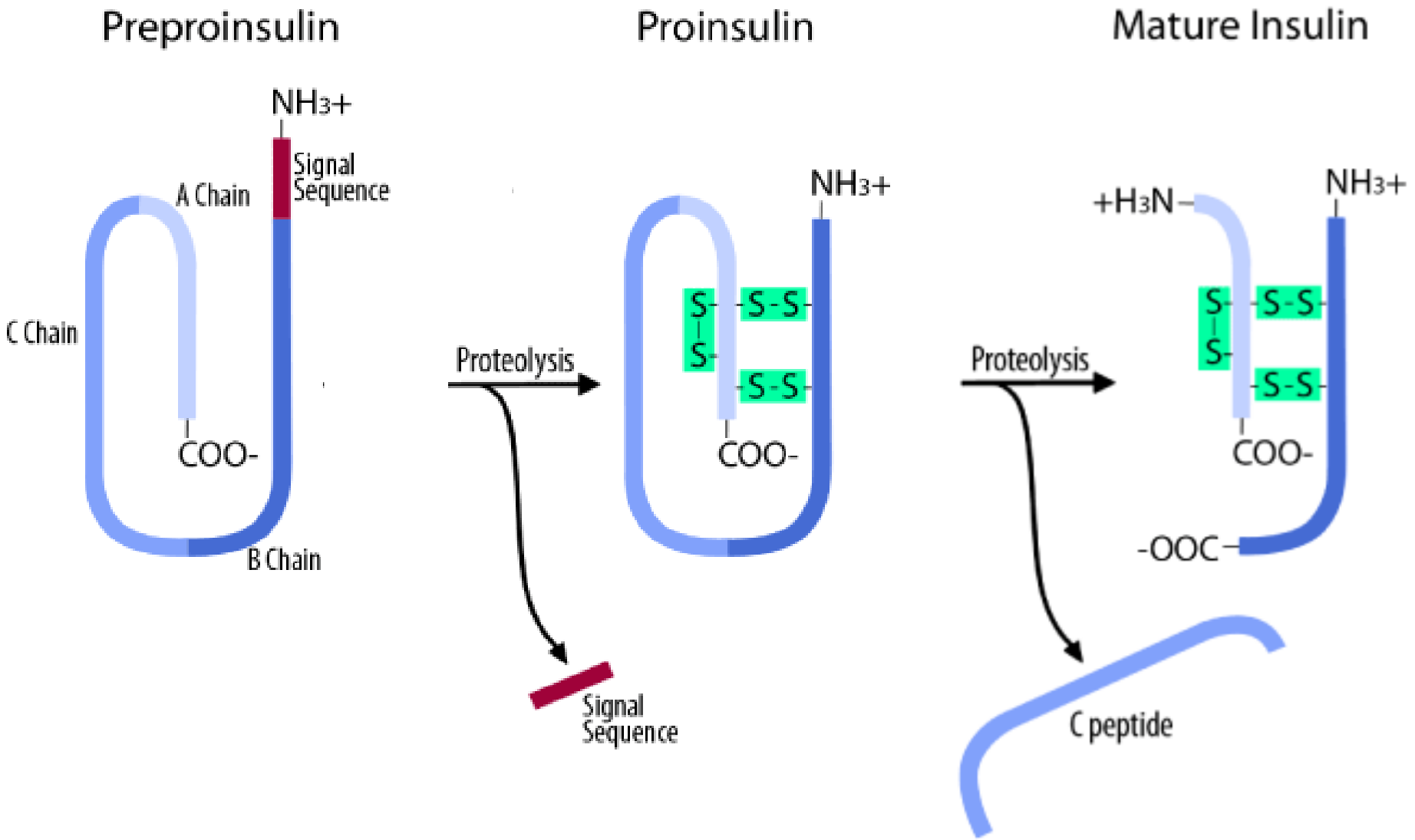
Glow Fish: Zebra fish with green fluorescent protein (GFP) and others

Wild type zebra fish



Glow fish (genetically modified fish)

Production of Human Insulin: The best example of Genetic Engineering/Recombinant DNA Technology



Insulin: an indispensable hormone in our body

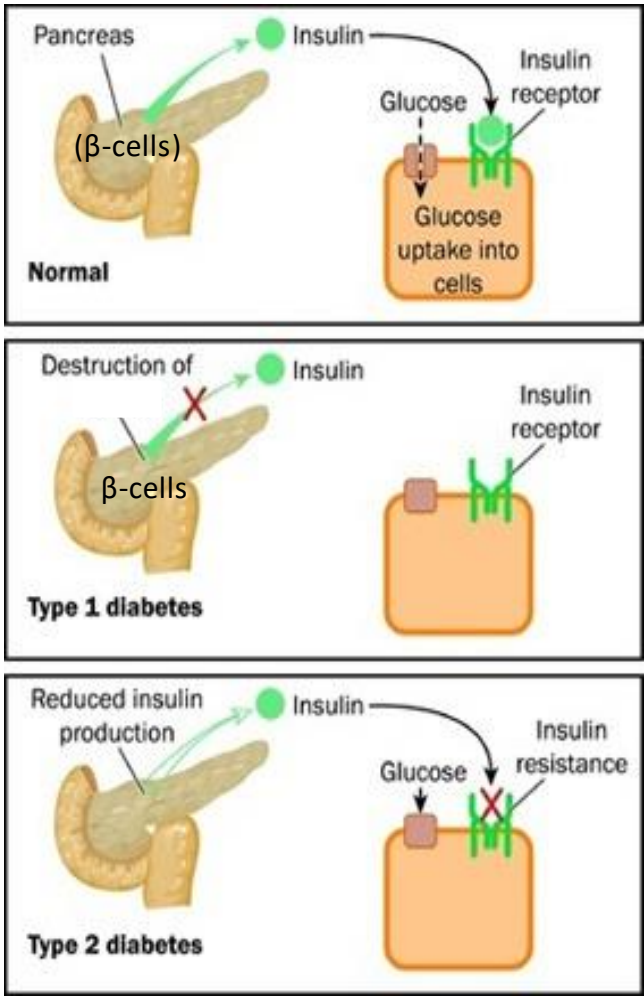
Insulin is a hormone that regulates the amount of glucose (sugar) in the blood and is required for the body to function normally.

Insulin is produced by cells in the pancreas, called the islets of Langerhans.

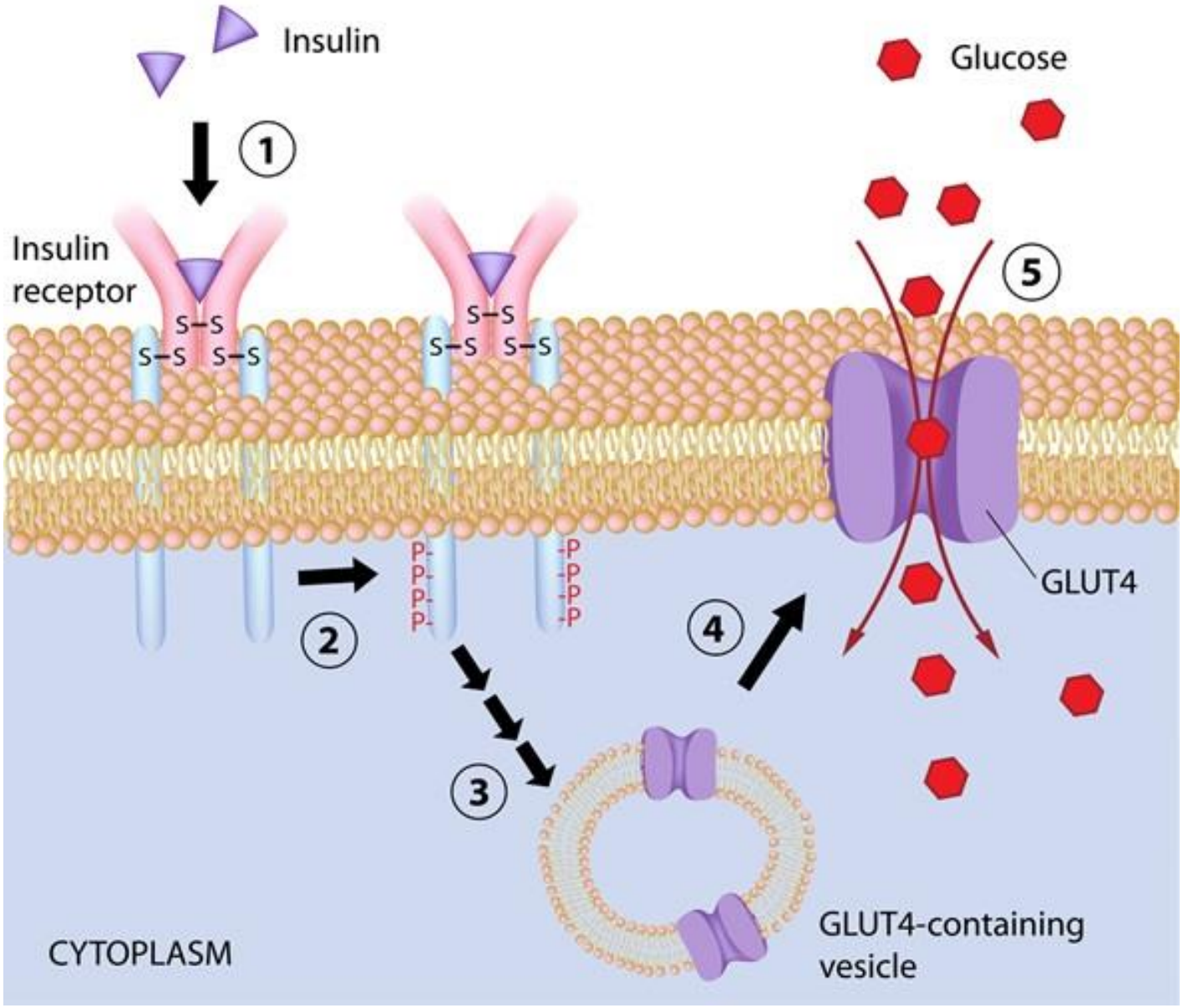
These cells continuously release a small amount of insulin into the body, but they release surges of the hormone in response to a rise in the blood glucose level.

Every time a person eats, the blood glucose rises. Raised blood glucose triggers the cells in the islets of Langerhans to release the necessary amount of insulin. Insulin allows the blood glucose to be transported from the blood into the cells.

Once transported into the cell, the blood glucose level is returned to normal within hours.



Effect of Insulin on Glucose Uptake



Production of Insulin

1921: Frederick Banting and Charles Best successfully purified insulin from a dog's pancreas. Over the years scientists made continual improvements in producing insulin.

In the 1980s, researchers used genetic engineering to manufacture a human insulin.

In 1982, the Eli Lilly Corporation produced a human insulin that became the first approved genetically engineered pharmaceutical product.

At present, researchers could produce genetically engineered insulin in unlimited supplies without depending on animals.

HOW??

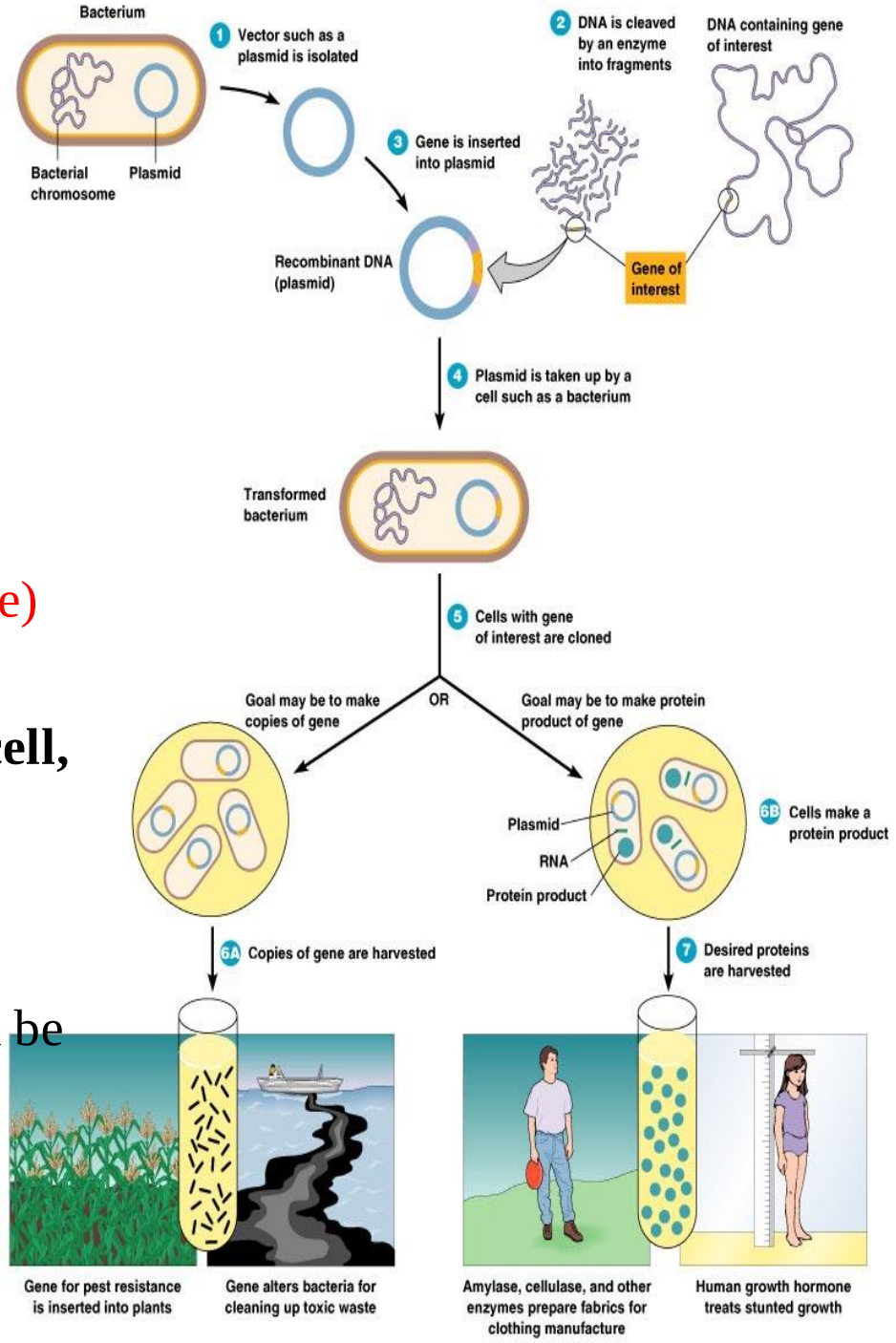
Genetic Engineering

Key Words in Genetic Engineering

- Gene of interest: DNA segment that is to be inserted or deleted
- Plasmid: a small, circular, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA (commonly found in bacteria and may provide antibiotic resistance)
- Vector: DNA molecule (such as plasmid) used as a vehicle to carry gene of interest (or foreign genetic material) into another cell where it can be expressed
- Transformation: Transfer of gene of interest in to a host cell (may be bacteria) where it can be maintained as well as expressed.
- Clone: Organisms carrying identical genes

An Overview of Genetic Engineering

1. **Gene of interest (DNA)** is isolated
   (DNA fragment)
2. **A desired gene** is inserted into a DNA molecule **- vector**
   (plasmid, bacteriophage or a viral genome)
3. **The vector** inserts the DNA into **a new cell,** which is grown to form a **clone**.
   (bacteria, yeast, plant or animal cell)
4. Large quantities of the **gene product** can be harvested from **the clone**.



Tools for making a collage

Colourful papers

Scissors

Glue

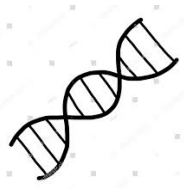
Drawing paper

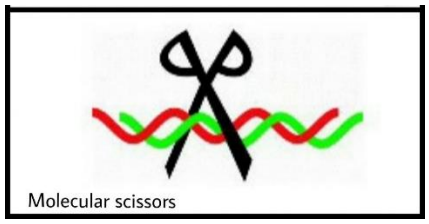
DNA of interest

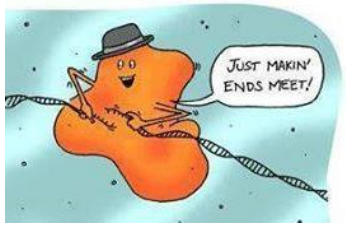
Restriction enzymes

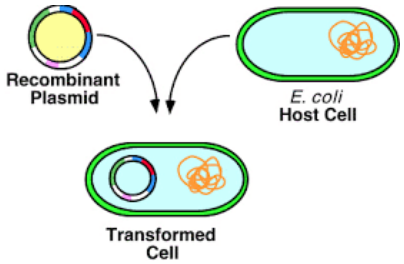
Ligase enzyme

Host organism









Tools for making a Recombinant DNA

Tools for Genetic engineering

1. Restriction Enzymes

- **Naturally produced by bacteria – restriction endonucleases**
  - **Natural function -** destroy bacteriophage DNA in bacterial cells
  - Cannot digest host DNA with methylated C (cytosine)

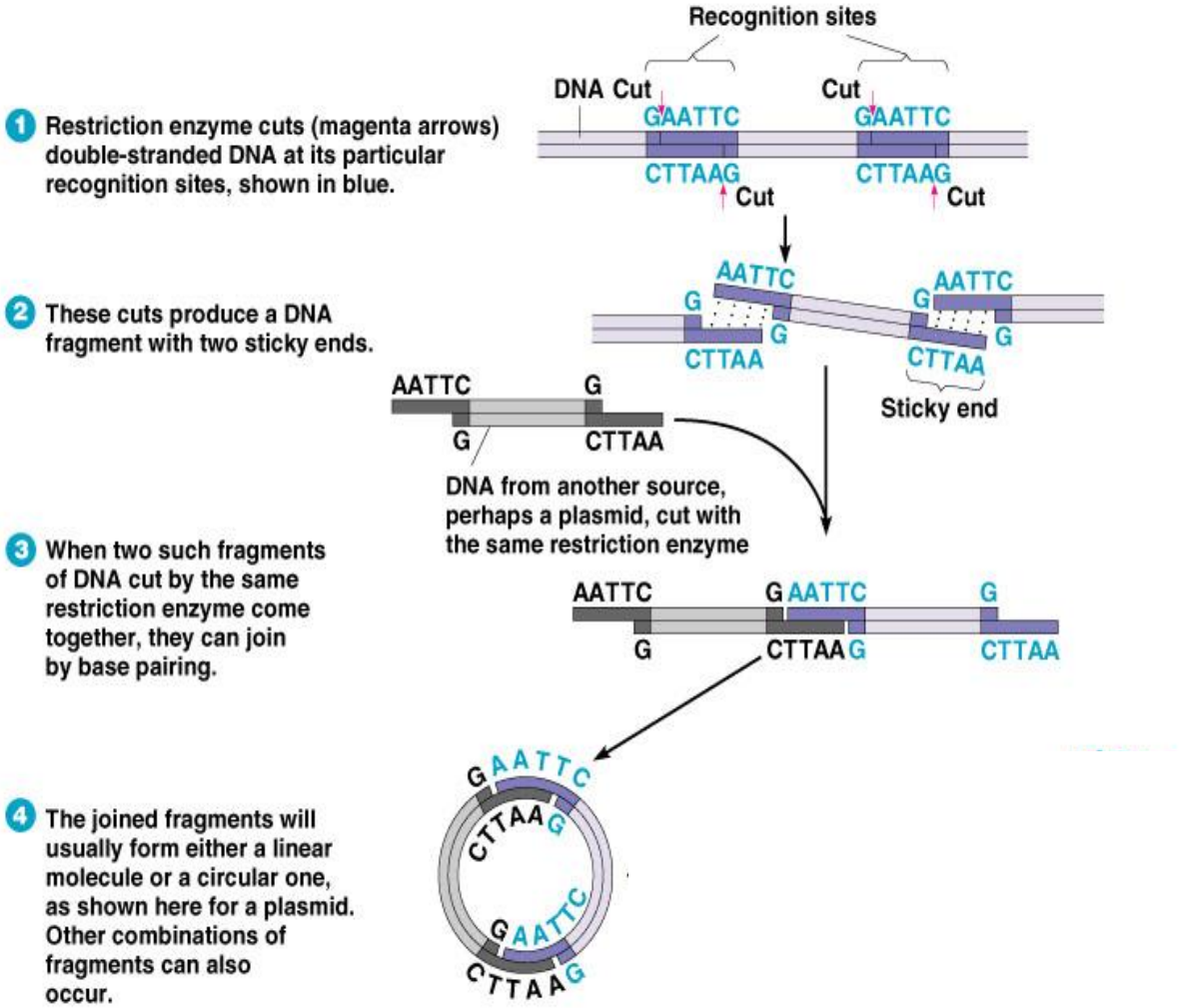
- **A restriction enzyme**
  - **Substrate –DNA -**recognizes one particular nucleotide sequence in DNA and **cuts** the DNA molecule (breaks down the bond between two nucleotides)

sticky ends **blunt ends**

- Prepackaged kits are available for rDNA techniques

Restriction Enzymes: How it Works?

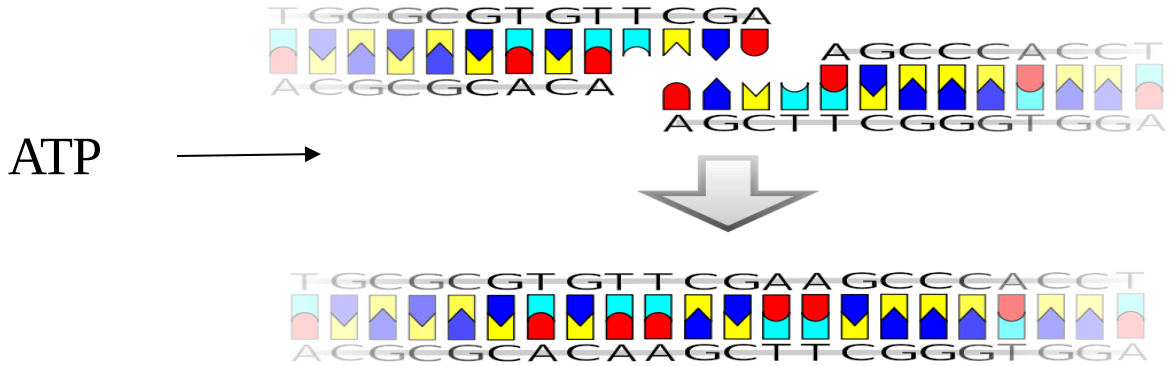
- Fragments of DNA produced by the same restriction enzyme will spontaneously join by **base pairing**.
- Each of the DNA strands will have a break



Tools for Genetic engineering

2. Ligase

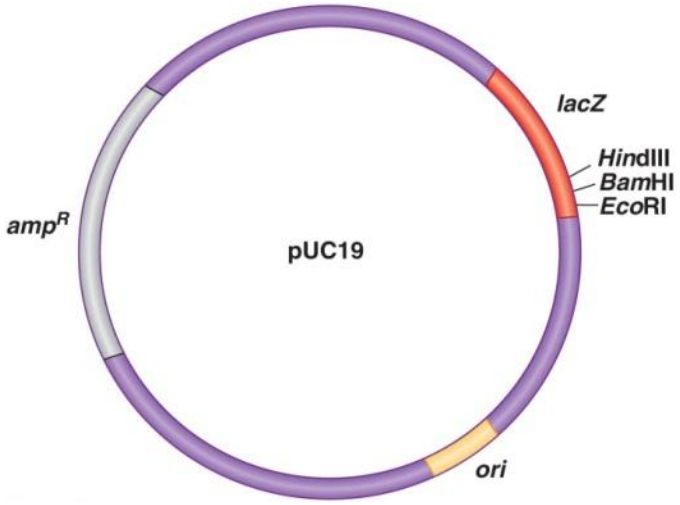
- **DNA ligase** is a enzyme that can link together DNA strands that have double-strand breaks (a break in both complementary strands of DNA).
  - Naturally DNA ligase has applications in both **DNA replication** and **DNA repair .**
  - Needs ATP
- DNA ligase has extensive use in molecular biology laboratories for **genetic recombination experiments**



Tools for Genetic engineering

3. Plasmids

Vectors - **Small pieces of circular DNA used for cloning**



Requirements of the Vector

1. Self-replication - able to replicate in the host (independent origin of replication)

2. Cloning site -(region containing multiple restriction sites)

3. Promoter (and operator) - to support the expression of insert DNA (i.e. gene of interest) in the host.

4. Selectable marker – antibiotic resistance (Ampicillin resistant)

5. Proper size- for easy handling

Hosts for Recombinant DNA Technology

1. Bacteria

- ***E. coli*** - used because is easily grown and its genomics are well understood.
  - Gene product is purified from host cells

2. Yeasts - *Saccharomyces cerevisiae*

- Used because it is easily grown and its genomics are known
- May express eukaryotic genes easily
- Continuously **secrete** the gene product.
- Easily collected and purified

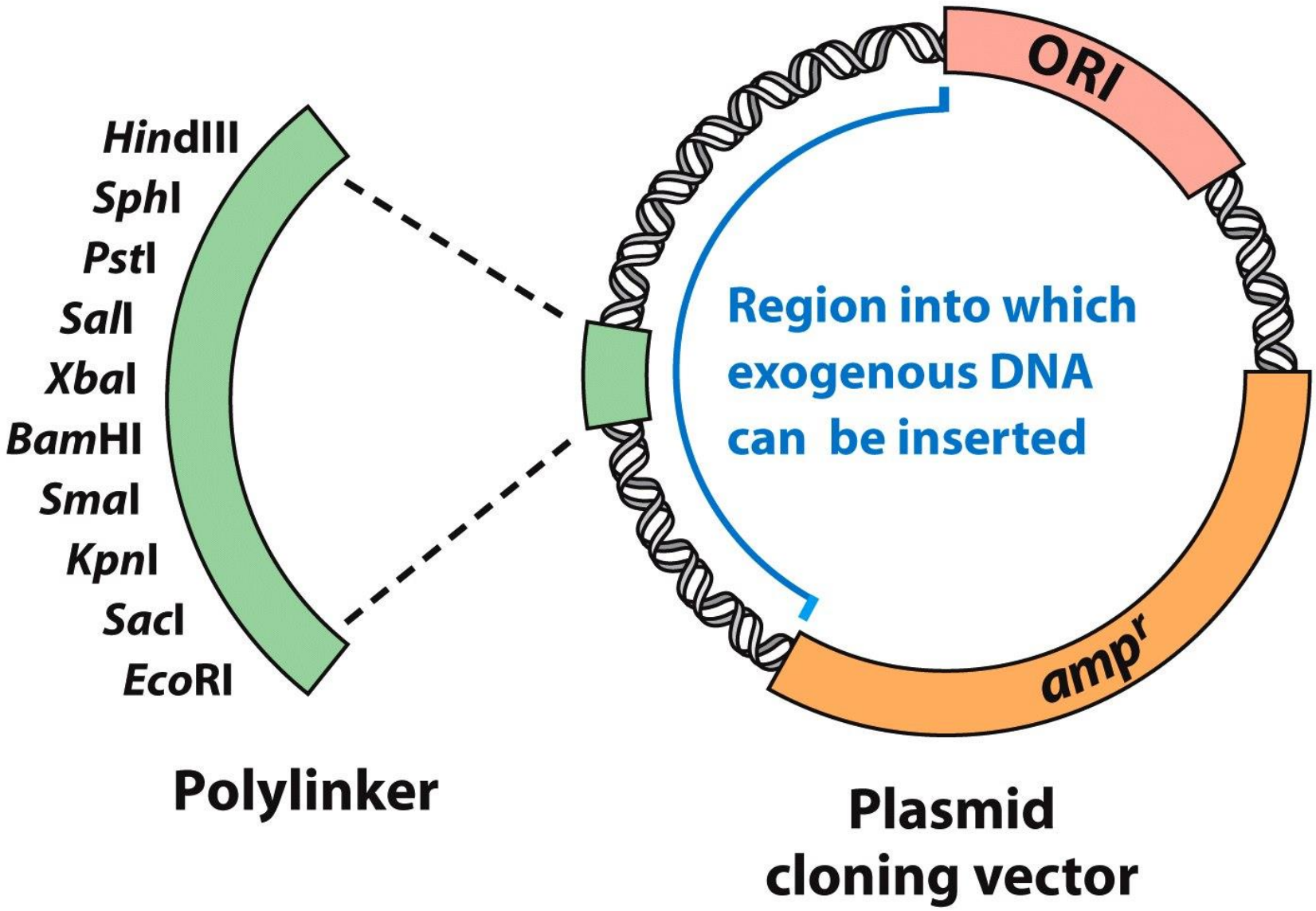
3. Plant cells and whole plants

- May express eukaryotic genes easily
- Plants are easily grown - produce plants with new properties.

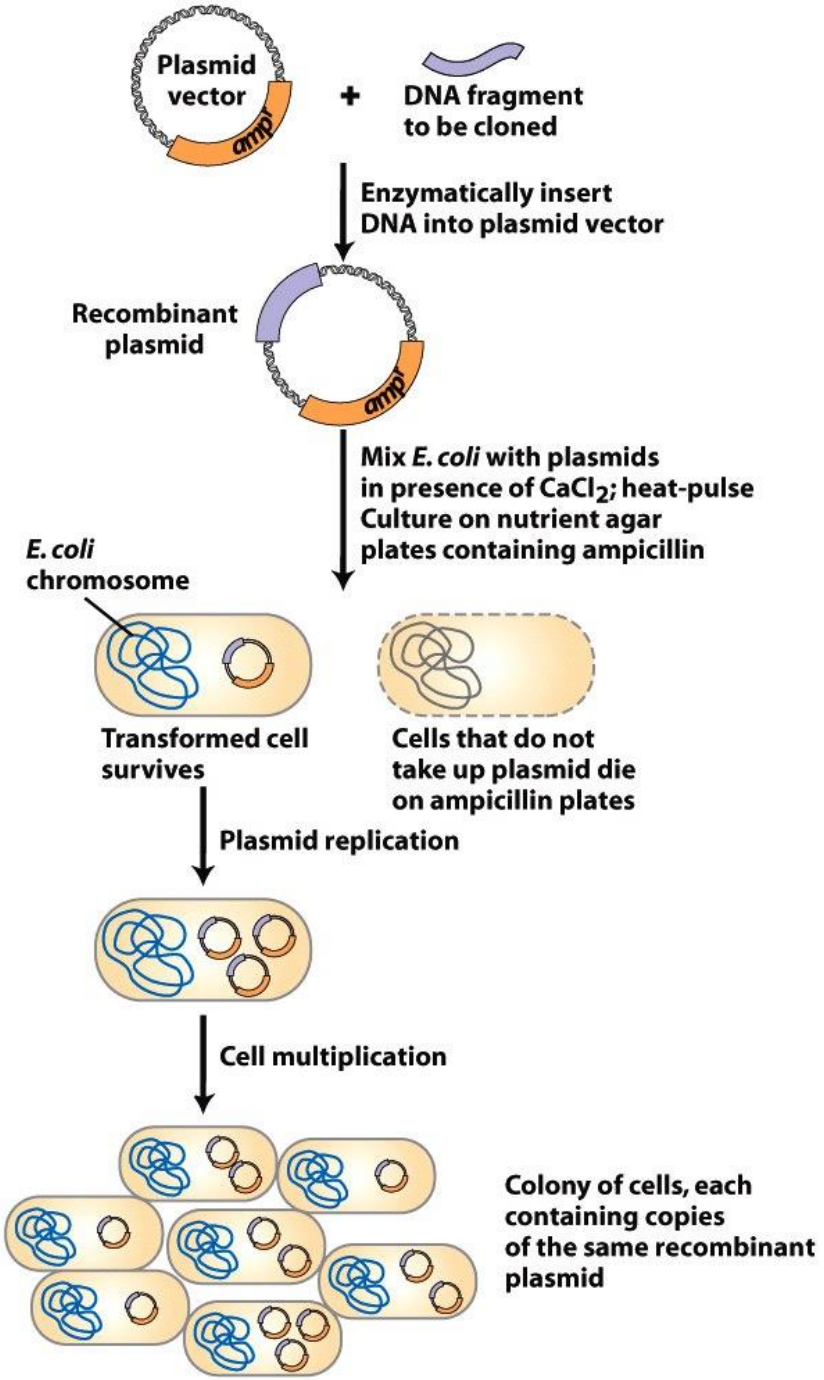
4. Mammalian cells

- May express eukaryotic genes easily
- Harder to grow
- Medical use.

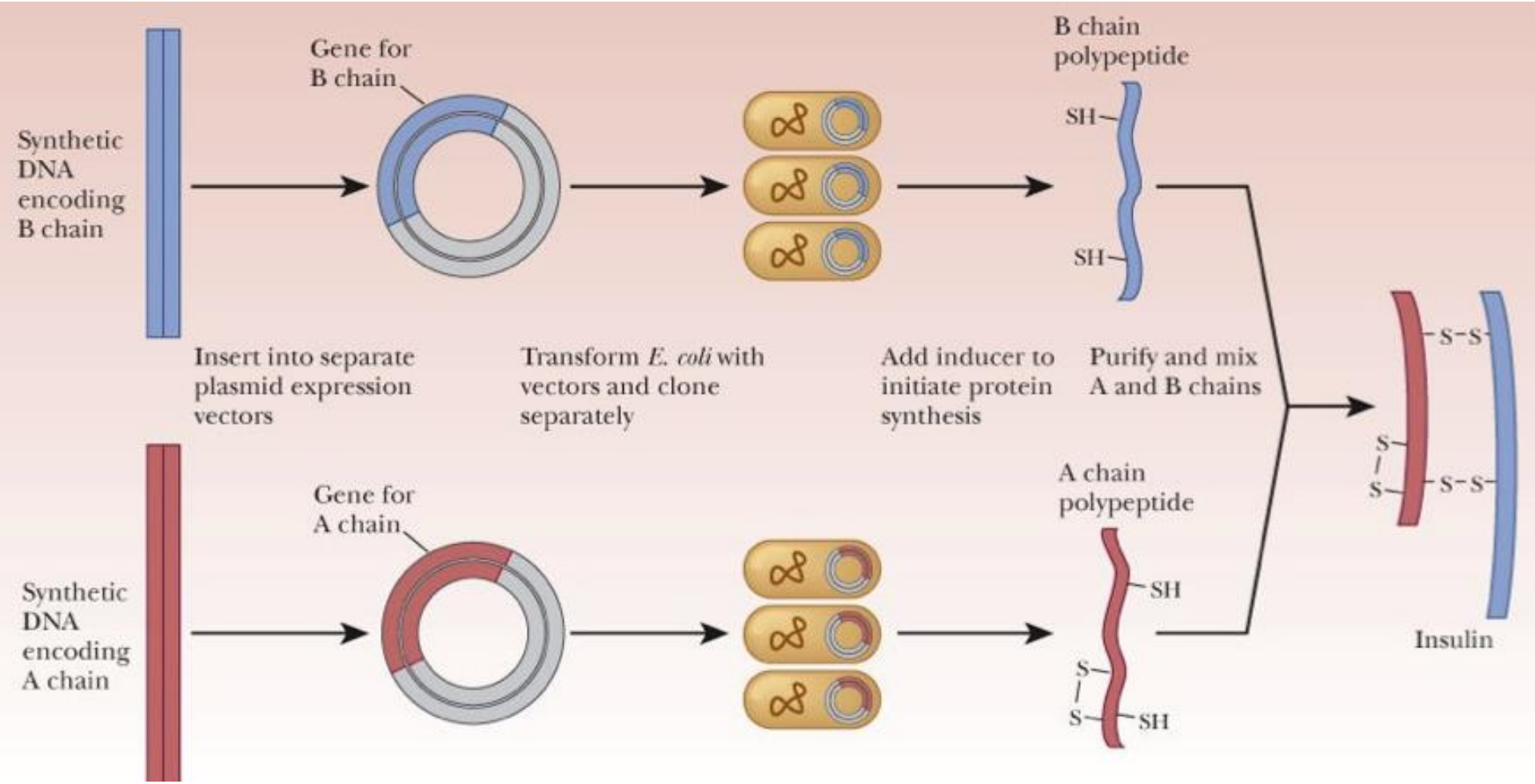
Cloning of a Gene



Cloning and Transformation of a Gene



Production of Insulin through Genetic Engineering Approach

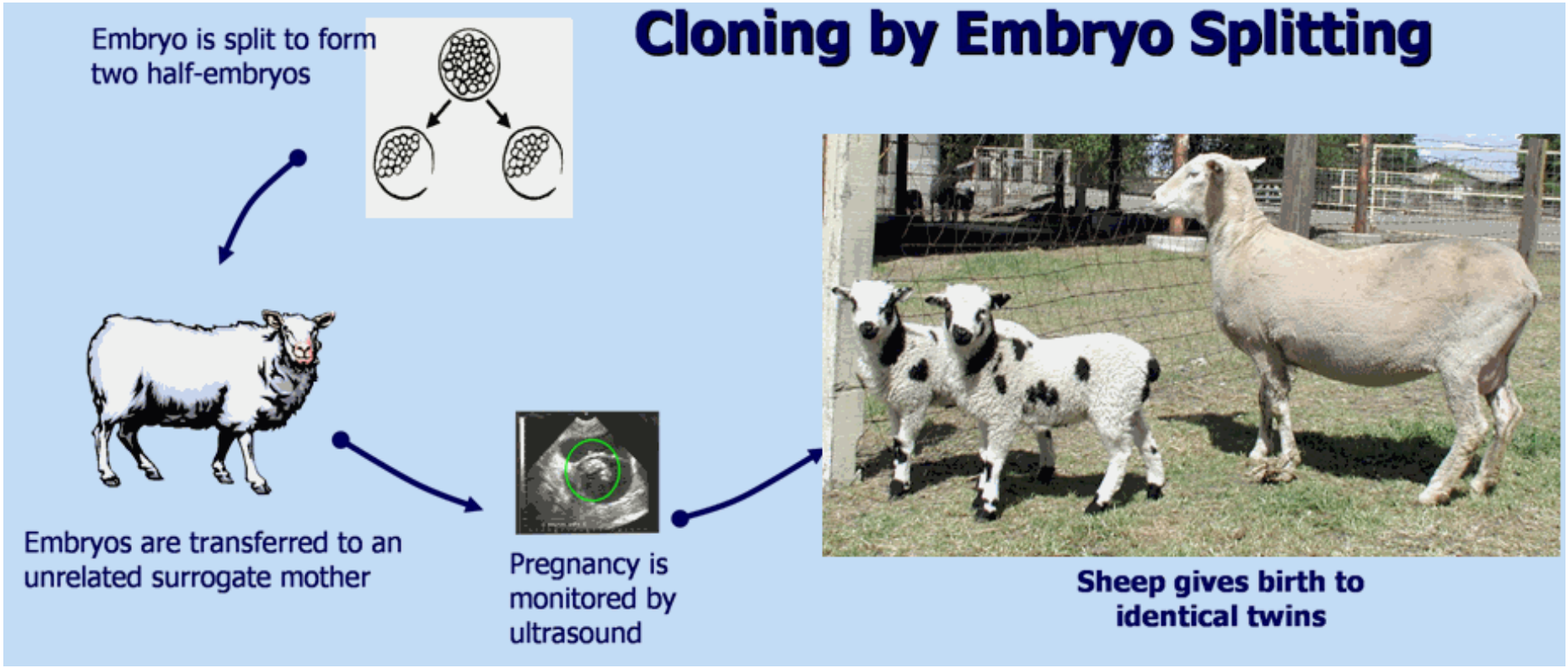


Cloning Whole Animals

Two techniques

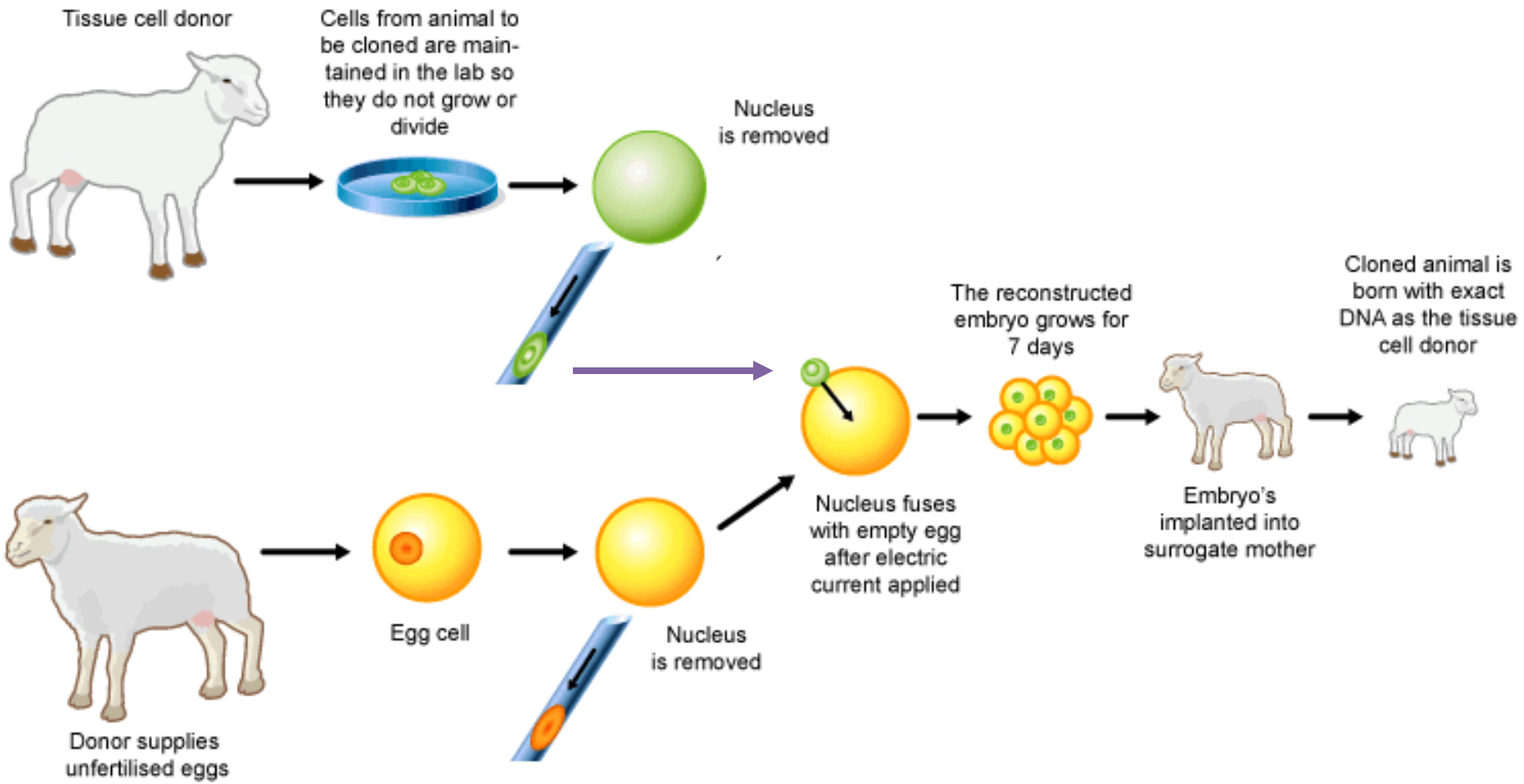
- **Embryo splitting**
- **Nuclear transfer**

Embryo Splitting



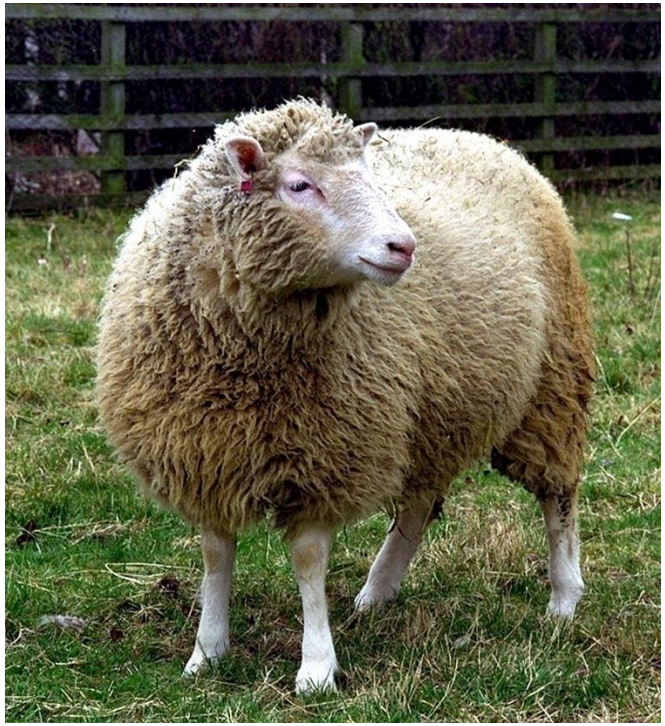
- Egg collected
- Fertilized by *in vitro* fertilization (IVF)
- Embryo is grown to 8–16 cells
- Cells are separated
- Separated cells grown into separate embryos
- Embryos transplanted into surrogate mothers

Cloning Whole Animals

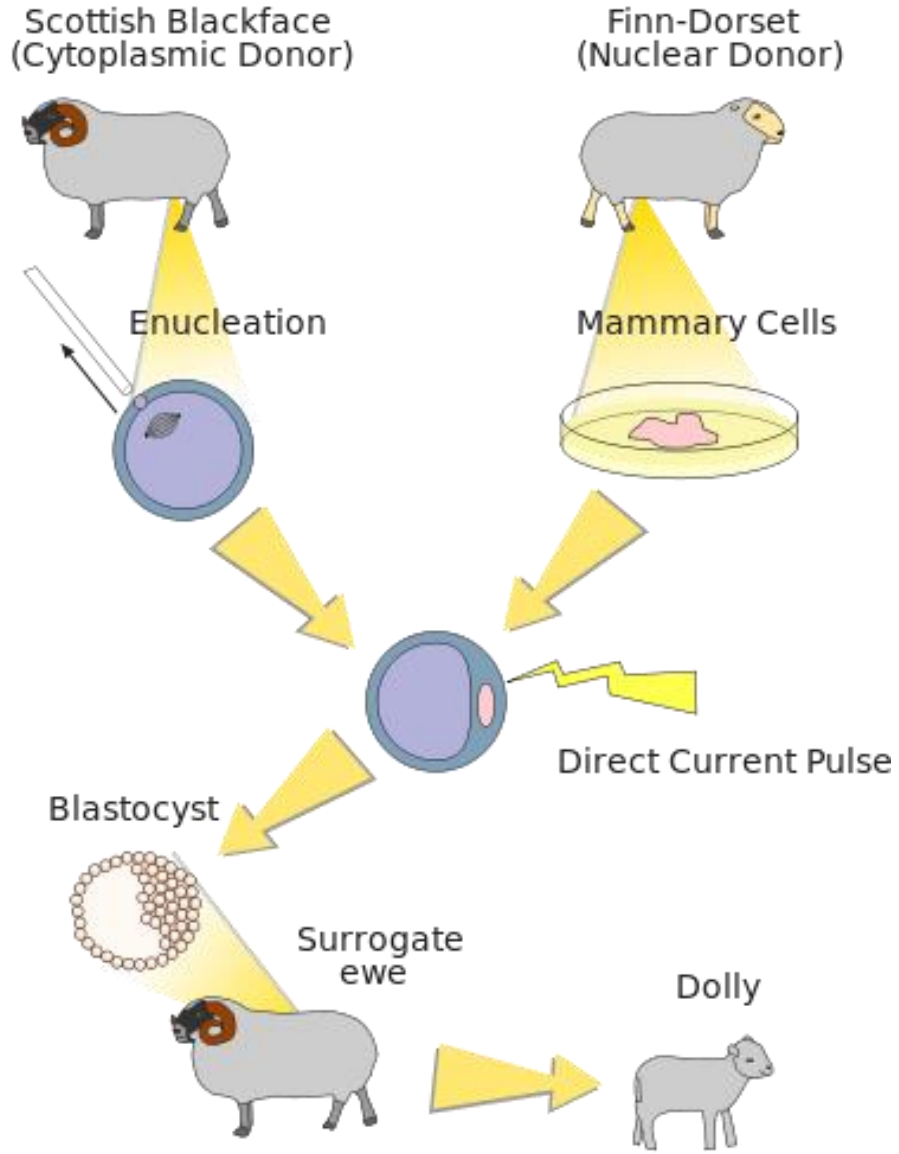


Nuclear Transfer

The First Cloned Mammal



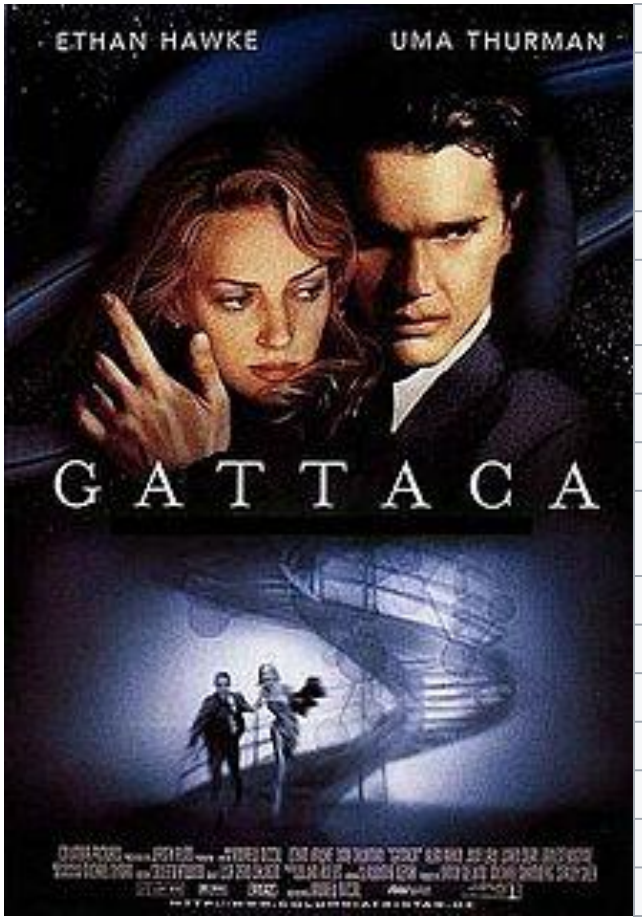
Born on 5th July, 1996
at Roslin Institute, Edinburgh, Scotland



The First Cloned Cat

In 2001, scientists at Texas A&M University created the first cloned cat known as Copy Cat/Cloned Cat (CC).

Selecting Best Possible Features from Parents: Science Fiction Film



Directed by	Andrew Niccol
Produced by	Danny DeVito Michael Shamberg Stacey Sher Gail Lyon
Written by	Andrew Niccol
Starring	•Ethan Hawke •Uma Thurman
Music by	Michael Nyman
Cinematography	Sławomir Idziak
Edited by	Lisa Zeno Churgin
Production company	Jersey Films
Distributed by	Columbia Pictures
Release date	•October 24, 1997
Running time	106 minutes
Country	United States
Language	English
Budget	$36 million
Box office	$12.5 million[1]

The first cloned primate

Zhong Zhong and Hua Hua born November 2017

“Lulu and Nana”

TECH

Experiments to gene-edit babies are ‘criminally reckless,’ says Stanford bio-ethicist

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Gene-editing babies: Call to pause humanity-altering research

By James Gallagher
Health and science correspondent, BBC News

13 March 2019

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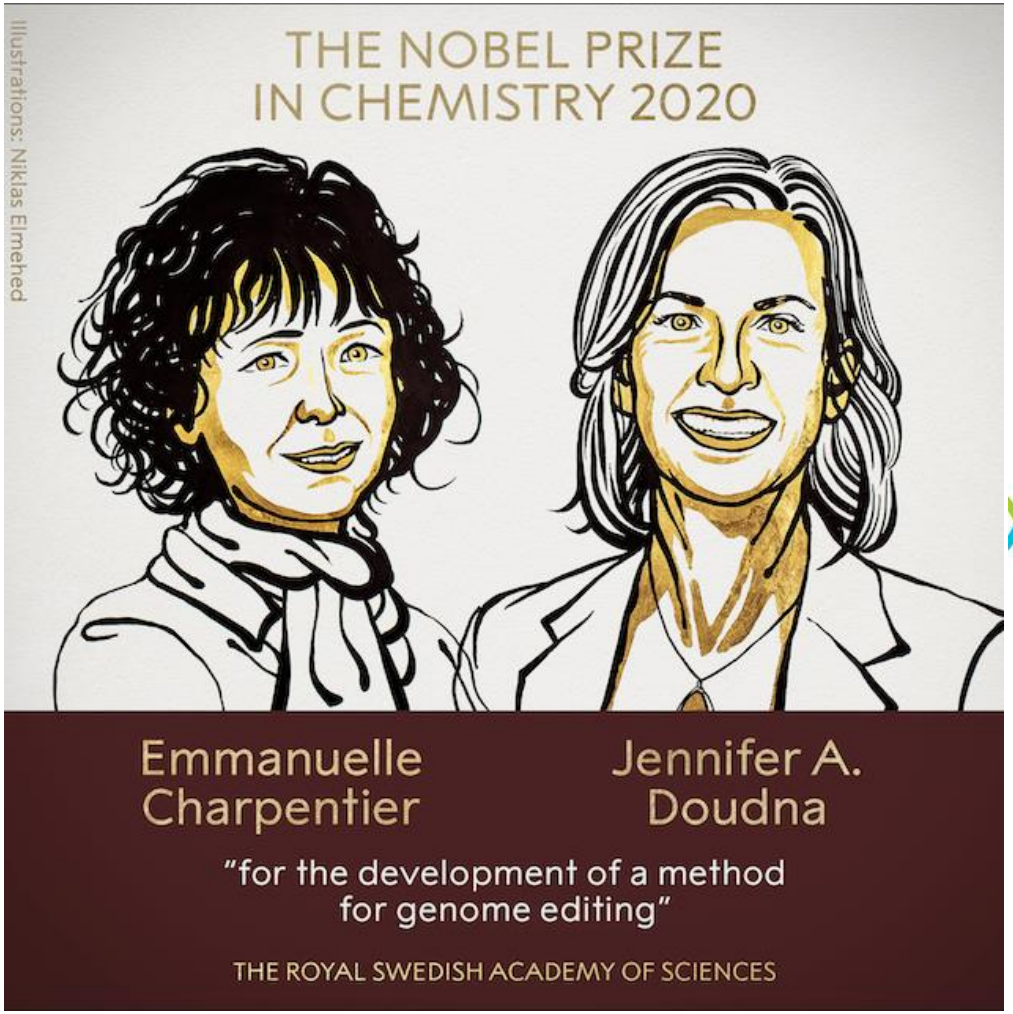
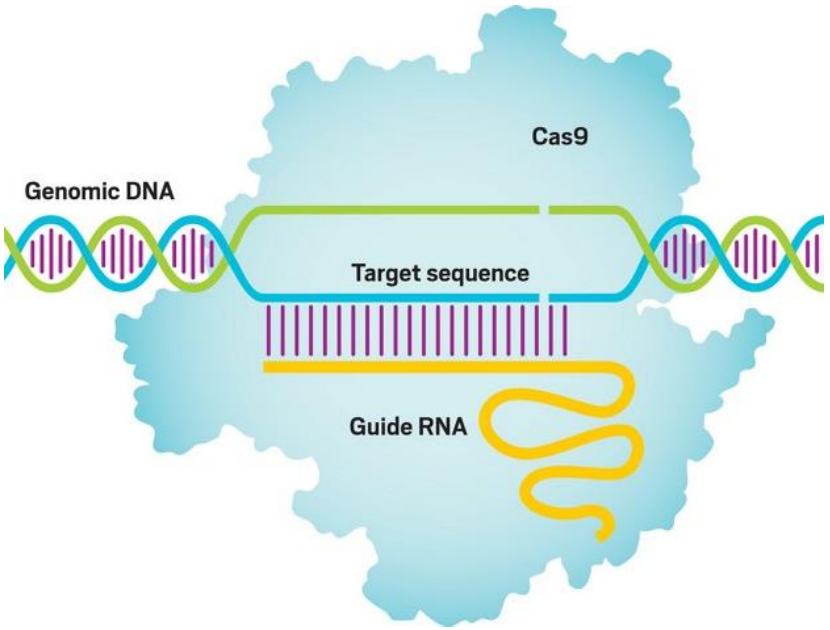
Nobel Prize for CRISPR-based Genome Editing Method





Thank You

Frequency of occurrence of restriction sites

If DNA sequence has equal amounts of each base

If bases are distributed randomly

6 base cutter $(1/4)^6 = 1$ site in ~4000 bp

4 base cutter $(1/4)^4 = 1$ site in 256 bp