An Introduction to Cloning

Presented by

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What is cloning ?

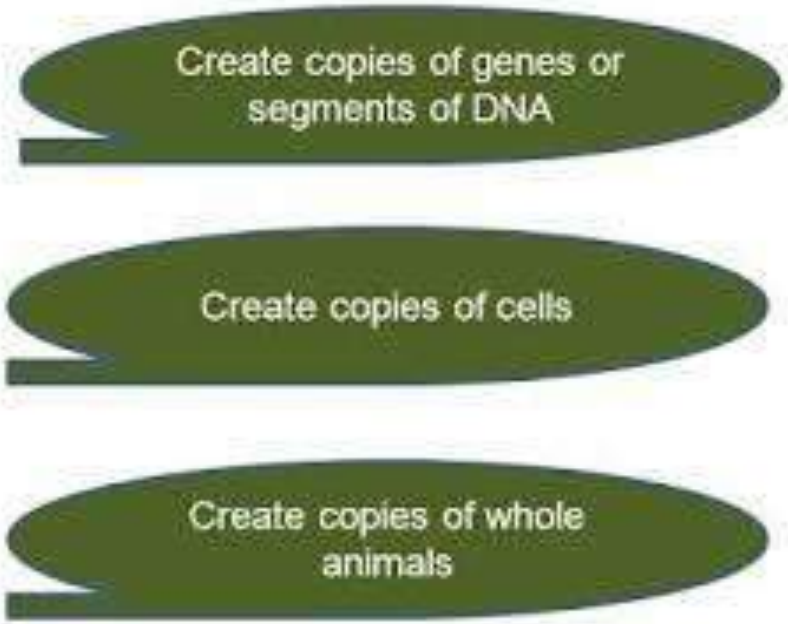
Cloning is a process that create an exact genetic replica of another cell, tissue or organism.

Copied material that has the same genetic makeup as the original is called CLONE.

Clones: Genetically identical organisms or molecules derived from a common ancestor

Types of cloning

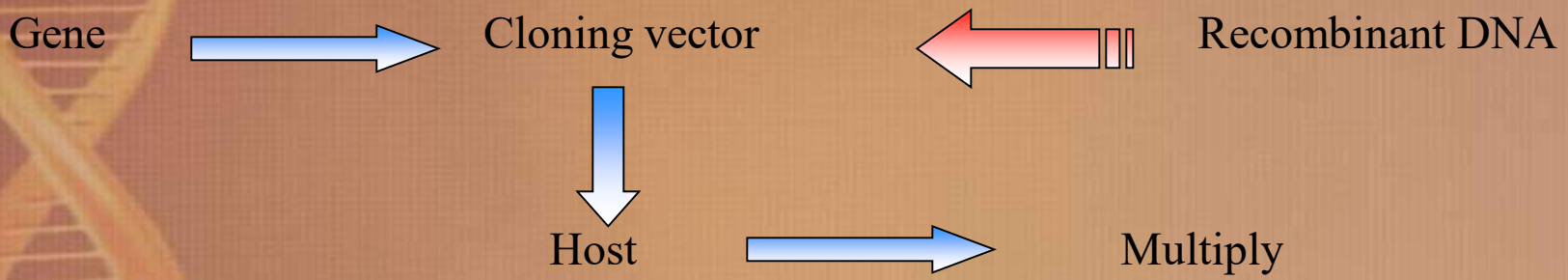
- Molecular cloning
- Cellular cloning
- Organism cloning



TYPE I: MOLECULAR CLONING

- To work directly with specific genes, scientists prepare well-defined segments of DNA in identical copies, a process called *DNA cloning.*
- DNA cloning yields multiple copies of a gene or other DNA segment.

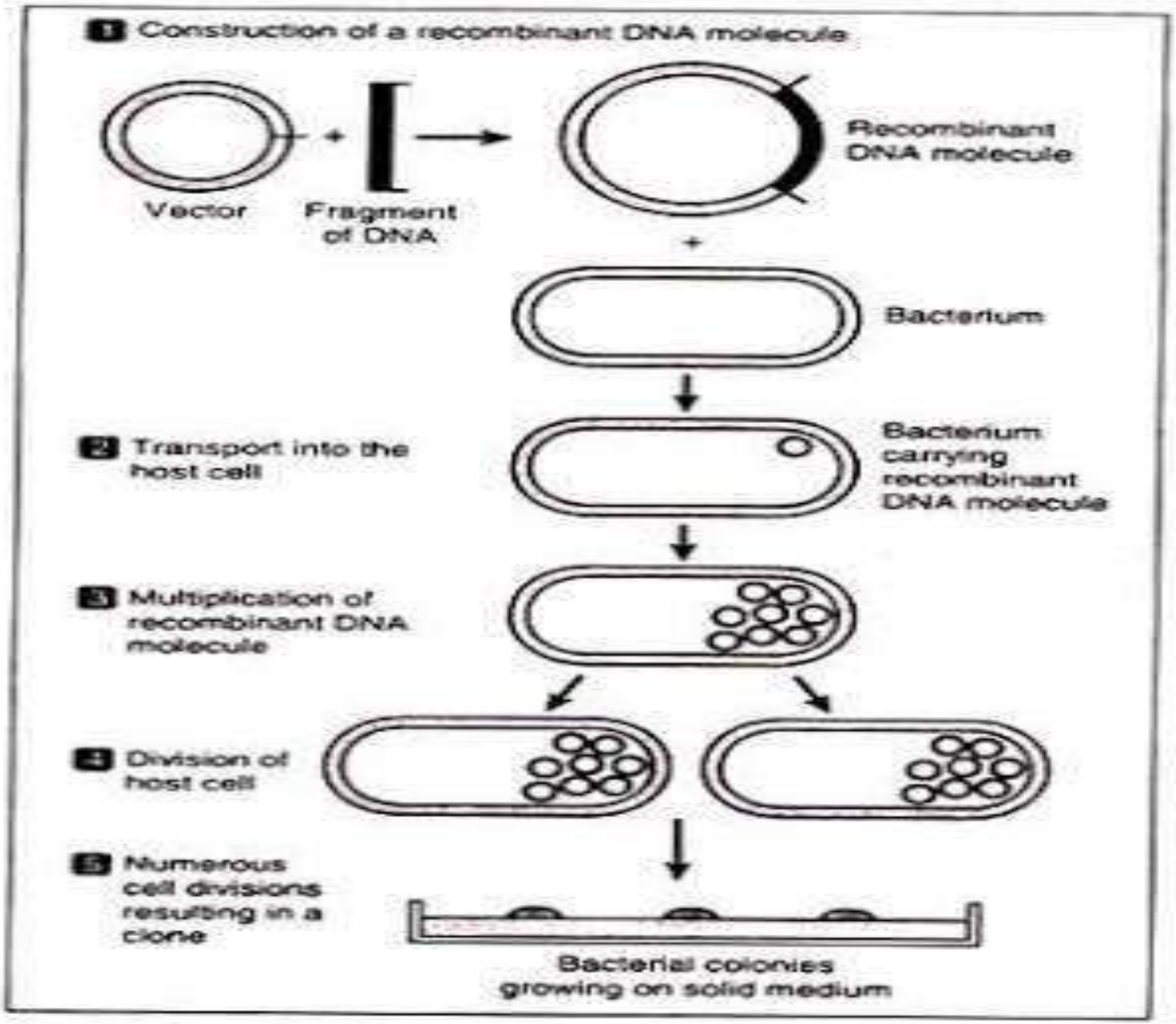
GOAL: To get enough copies of the gene to manipulate



Started with: few copies

Ended with: Many copies.

All identical to starting gene - CLONES



BASIC STEPS OF GENE CLONING

Selecting Cells with Vectors

- Vectors carry antibiotic resistance genes
- Growing antibiotic-sensitive cells on media with antibiotics ensures that all growing cells must carry the vector
- Selecting Cells with Recombinant Vectors
- While inserting the donor DNA, an existing gene in the vector is inactivated

OR

- In addition to the Donor gene a marker gene is added

Cloning Vectors

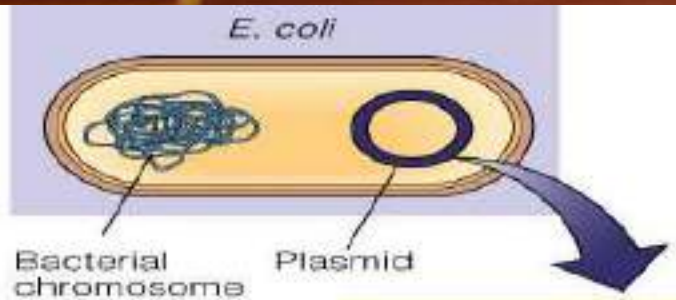
- In gene cloning, the original plasmid is called a **cloning vector**
- A cloning vector is a DNA molecule that can carry foreign DNA into a host cell and replicate there.

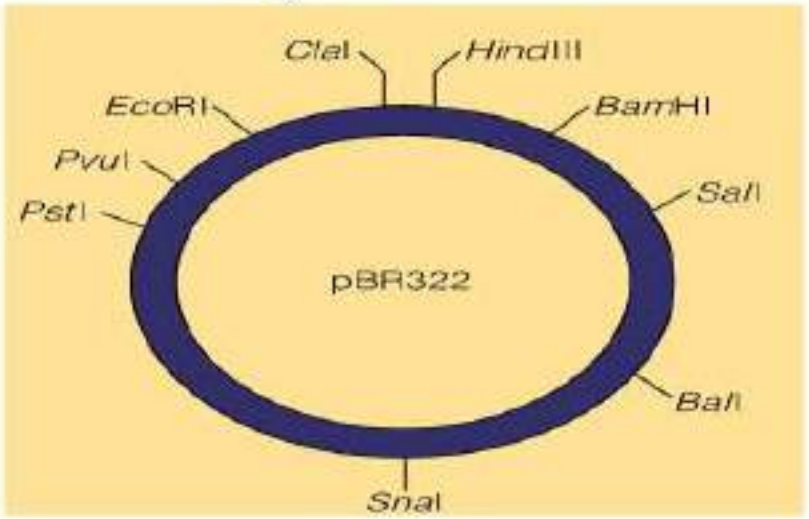
Different types of Vector

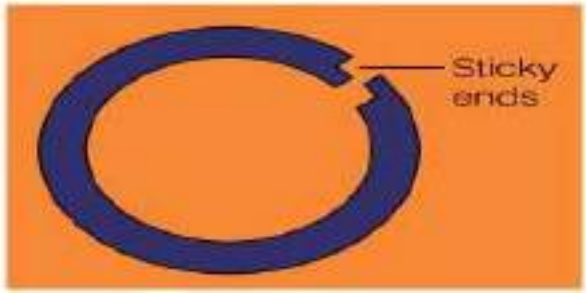
Vector	Size of Insert Accepted (kb)
Plasmid	up to 15
Bacteriophage	up to 90
Bacterial artificial chromosome (BAC)	100–500
Yeast artificial chromosome (YAC)	250–2,000

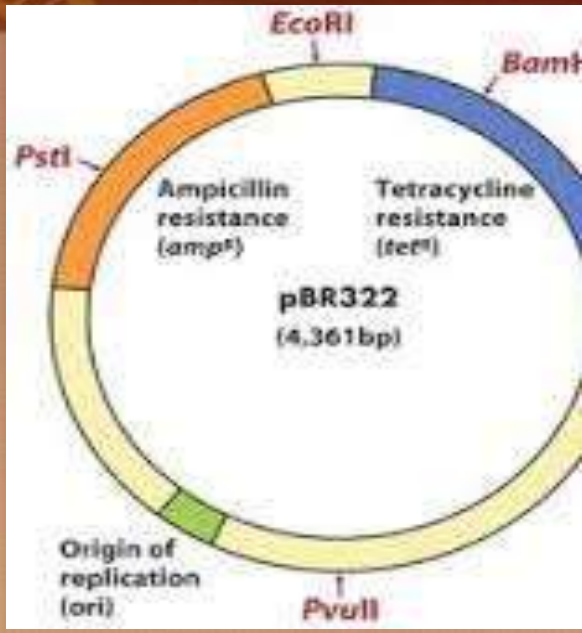
Overview of Cloning Vectors

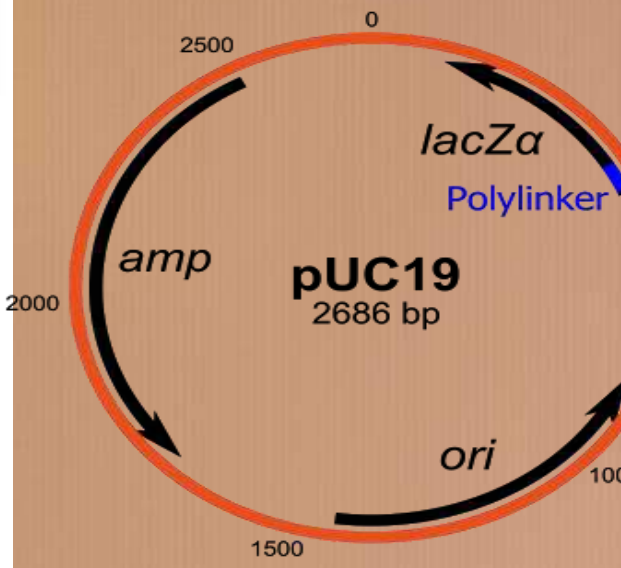
- **Plasmids** are small circular DNA molecules that replicate separately from the bacterial chromosome.
- **Cosmid vector** is a type of plasmid that contains a Lambda phage cos sequence (cos sites+plasmid=cosmids).
- **Shuttle vector** is a vector constructed so that it can propagat in two different host species. Therefore, DNA inserted into a shuttle vector can be tested or manipulated in two different cell types.
- A **bacterial artificial chromosome (BAC)** is a large plasmid th has been trimmed down and can carry a large DNA insert
- BACs are another type of vector used in DNA library construction





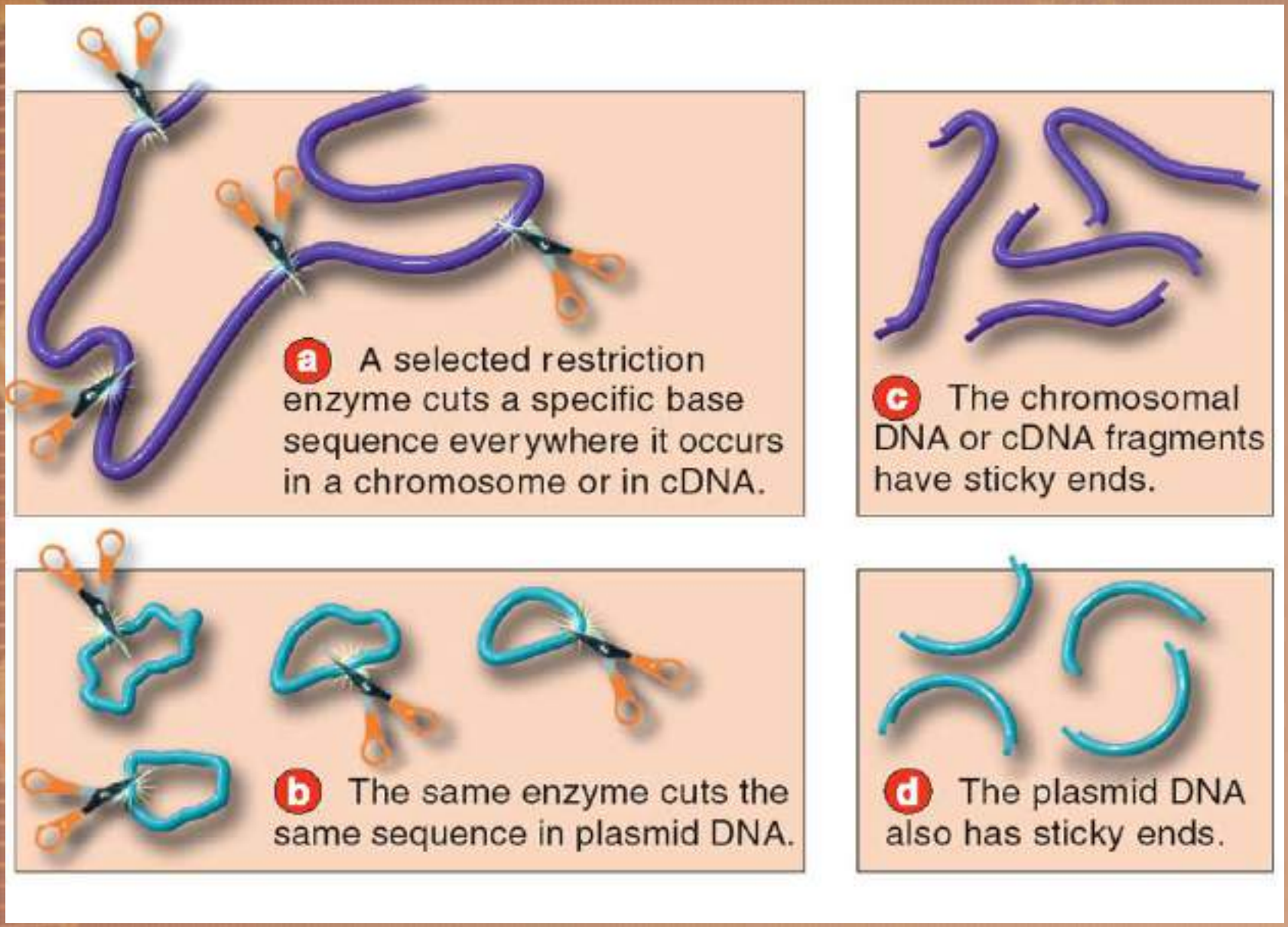






PLASMID VECTORS

Cloning DNA in Plasmid Vectors



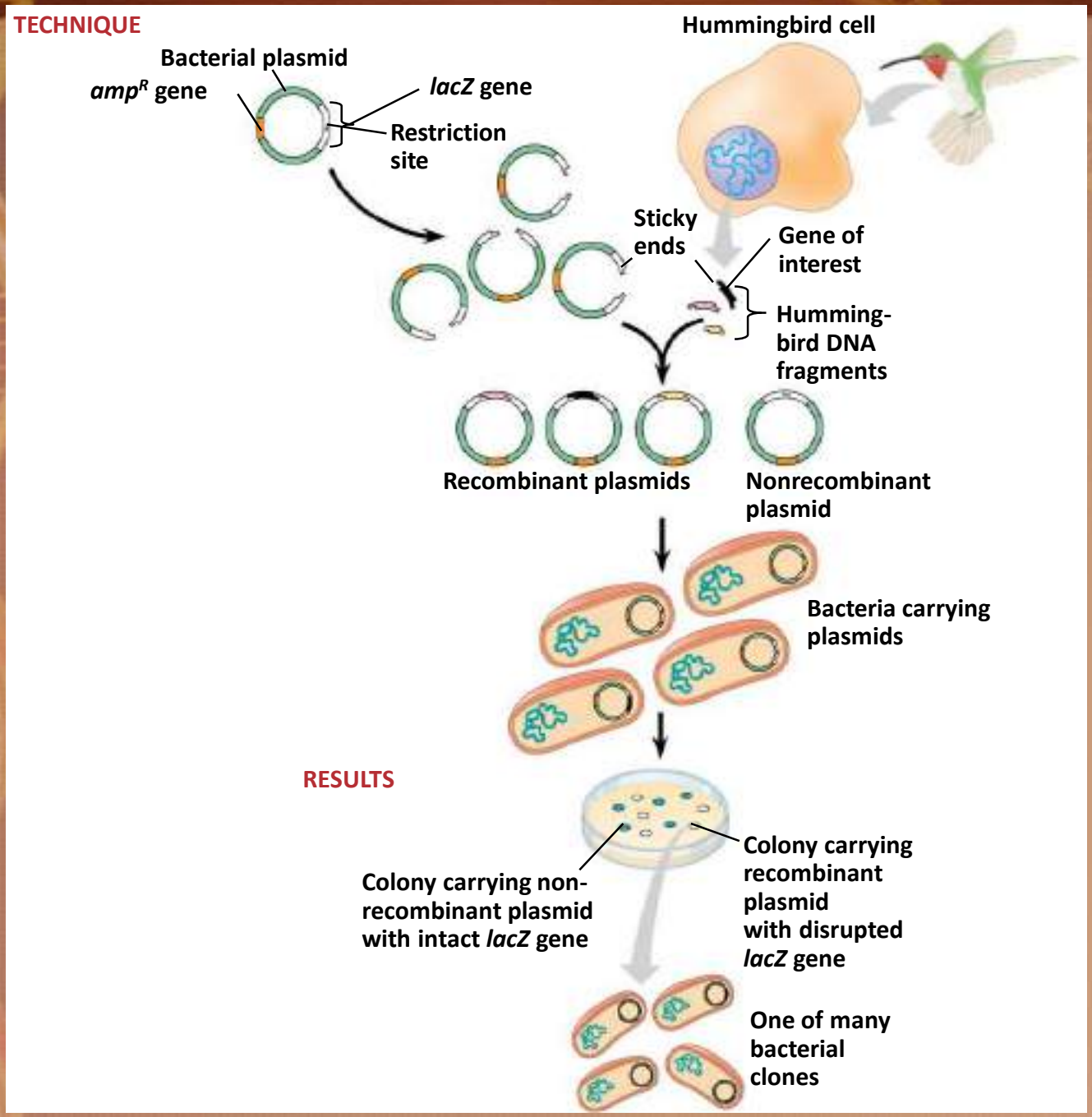
e The plasmid DNA and the foreign DNA are mixed in a solution with other enzymes that can seal them together.

f The result? A collection of recombinant plasmids that incorporate foreign DNA fragments.

g Host cells that can divide rapidly take up the recombinant plasmids.

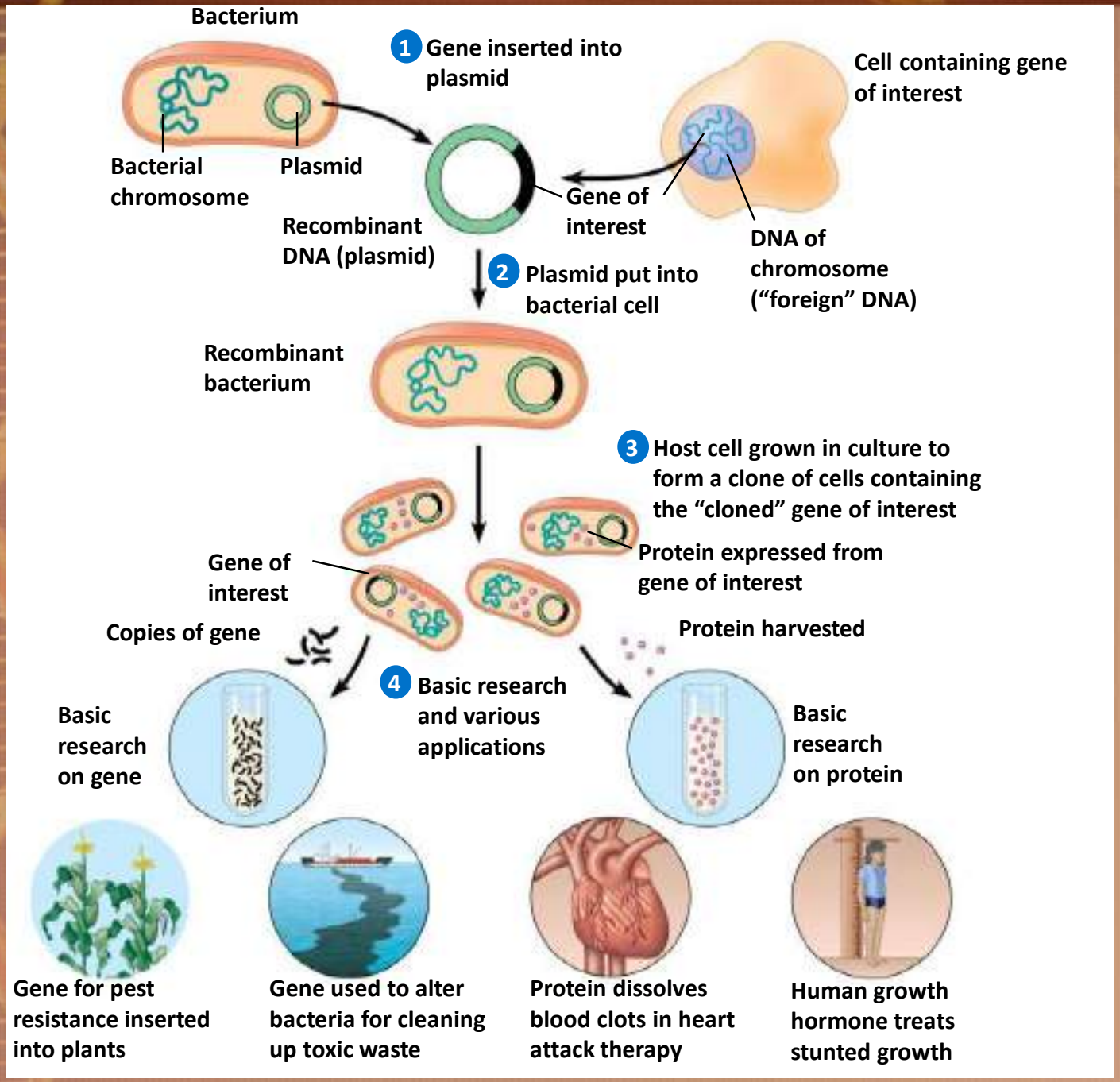
Producing Clones of Cells Carrying Recombinant Plasmids

- Several steps are required to clone the hummingbird *β*-globin gene in a bacterial plasmid
  - The hummingbird genomic DNA and a bacterial plasmid are isolated
  - Both are cut with the same restriction enzyme
  - The fragments are mixed, and DNA ligase is added to bond the fragment sticky ends



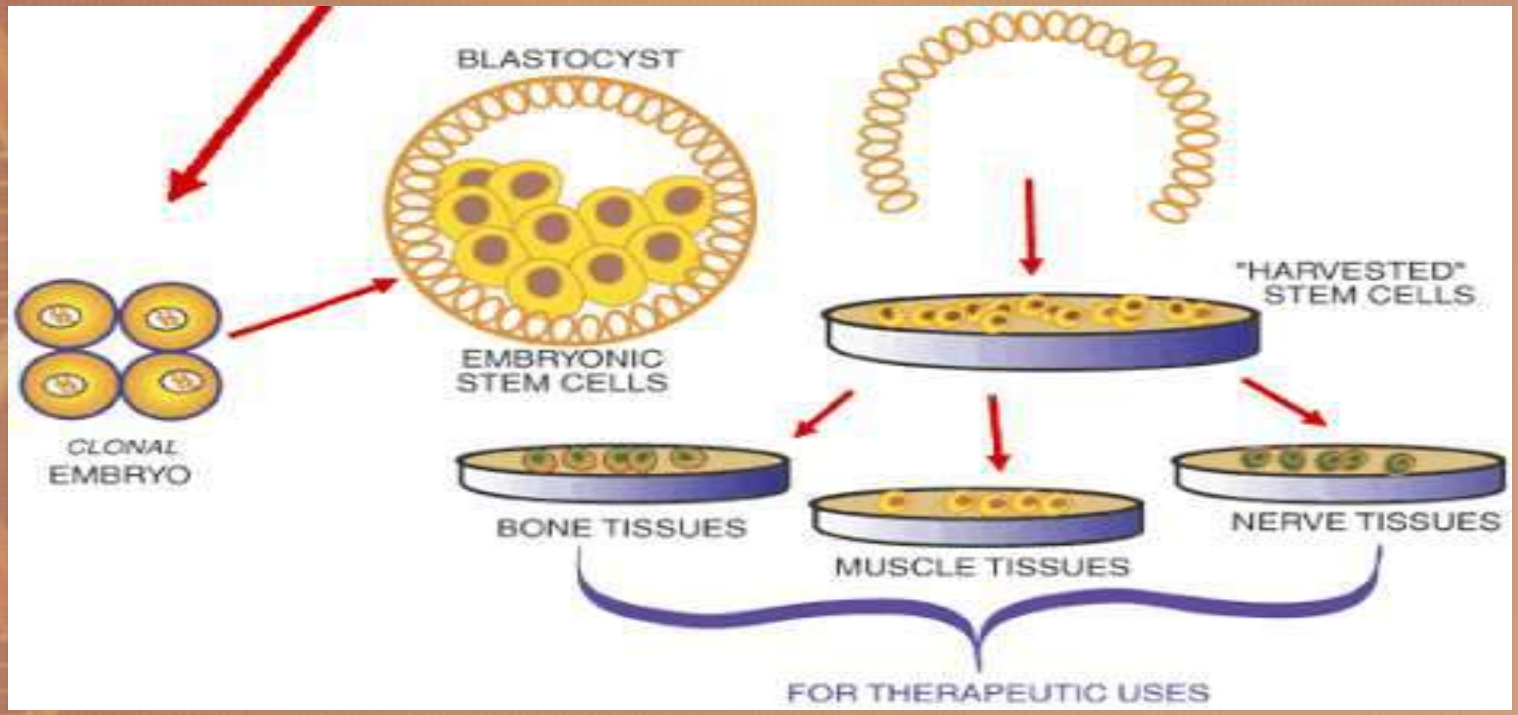
CLONING GENES IN BACTERIAL PLASMIDS

APPLICATIONS OF GENE CLONING: SOME USES OF CLONED GENES



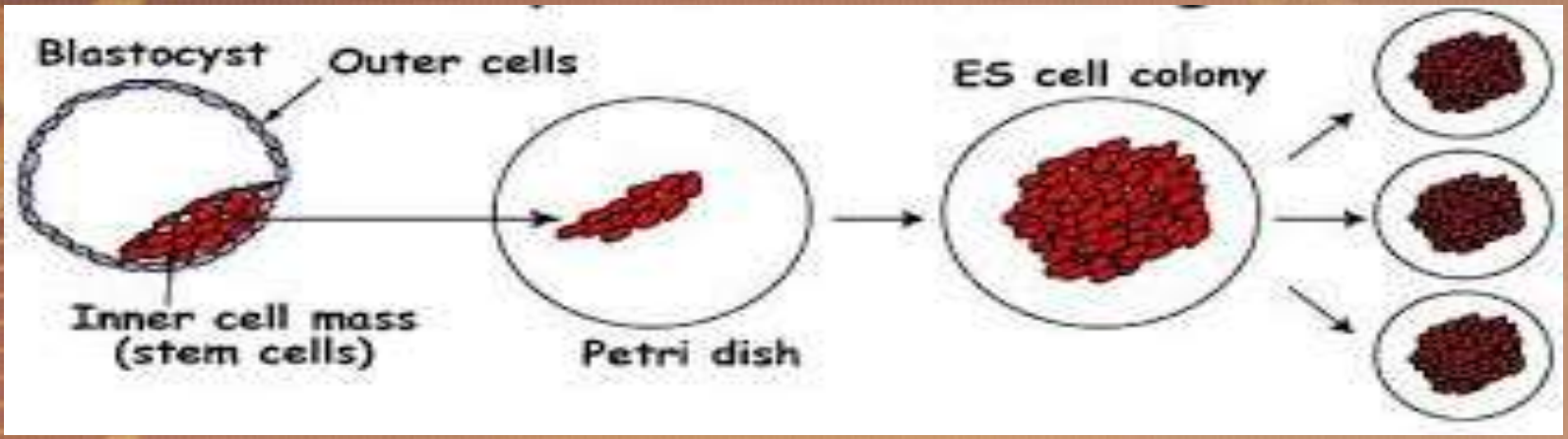
TYPE II: CELLULAR CLONING

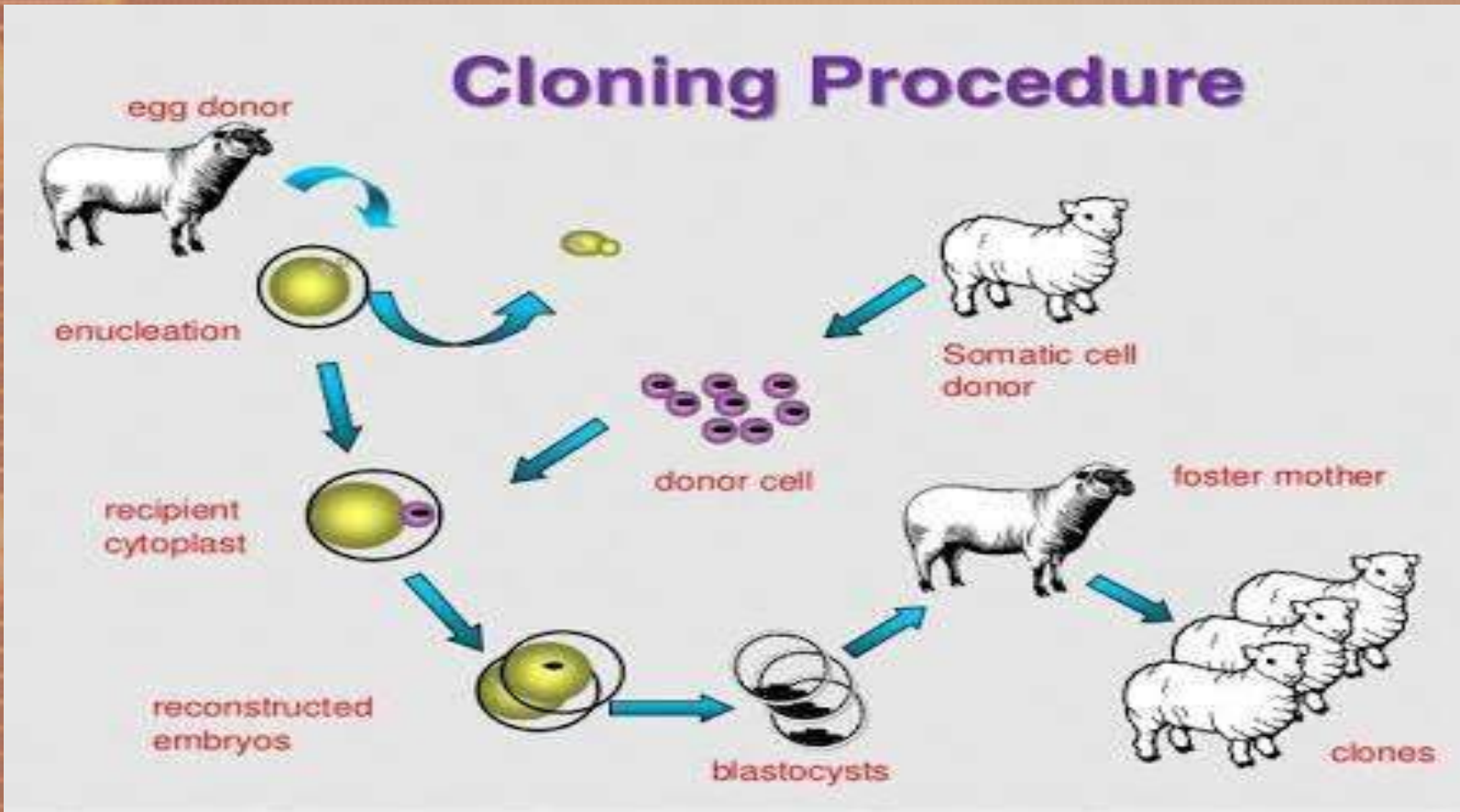
- **Cellular cloning** means to derive a population of cells from a single cell.
- Unicellular organisms, such as bacteria and yeast, naturally produce clones of themselves when they replicate asexually by binary fission; this is known as Cellular cloning.
- The nuclear DNA duplicates by the process of mitosis, which creeates an exact replica of the genetic material.



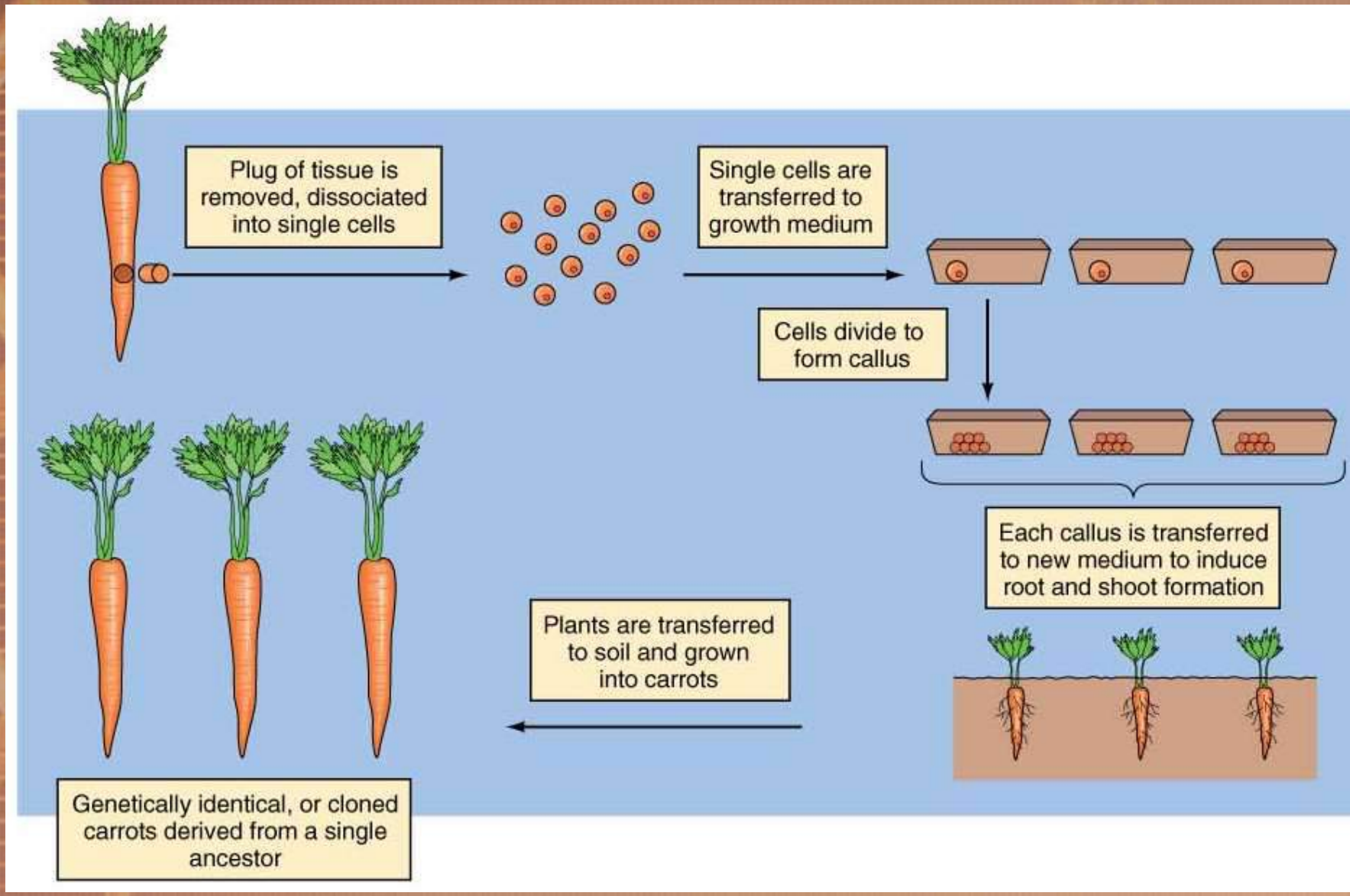
Type III: Organism Cloning

- **Organism cloning is a method used to make a clone or an identical copy of an entire multicellular organism.**


Blastocyst
Outer cells
Inner cell mass
(stem cells)
Petri dish
ES cell colony


Cloning Procedure
egg donor
enucleation
Somatic cell
donor
donor cell
recipient
cytoplast
foster mother
reconstructed
embryos
blastocysts
clones

Cloning Plants from Single Cell

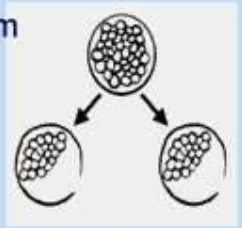


Cloning Animals from Single Cell

- Two techniques
  - Embryo splitting
  - Nuclear transfer

Cloning by Embryo Splitting

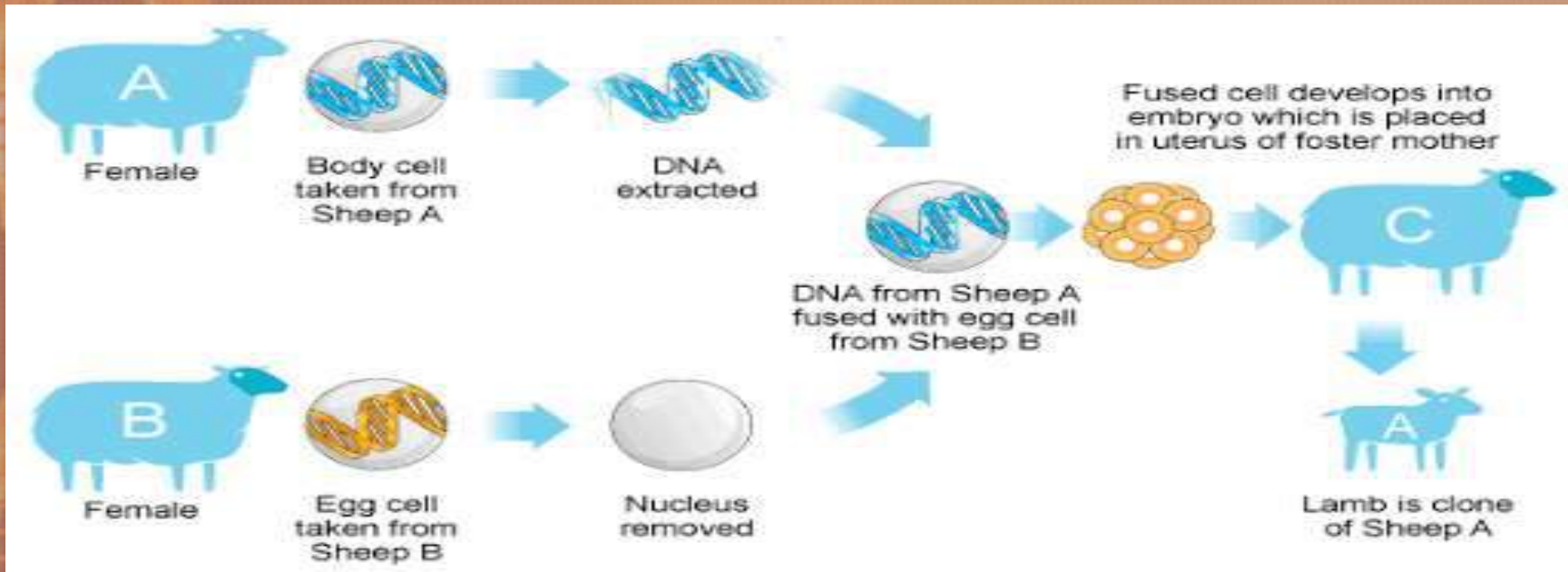
Embryo is split to form two half-embryos



Embryos are transferred to an unrelated surrogate mother

Pregnancy is monitored by ultrasound

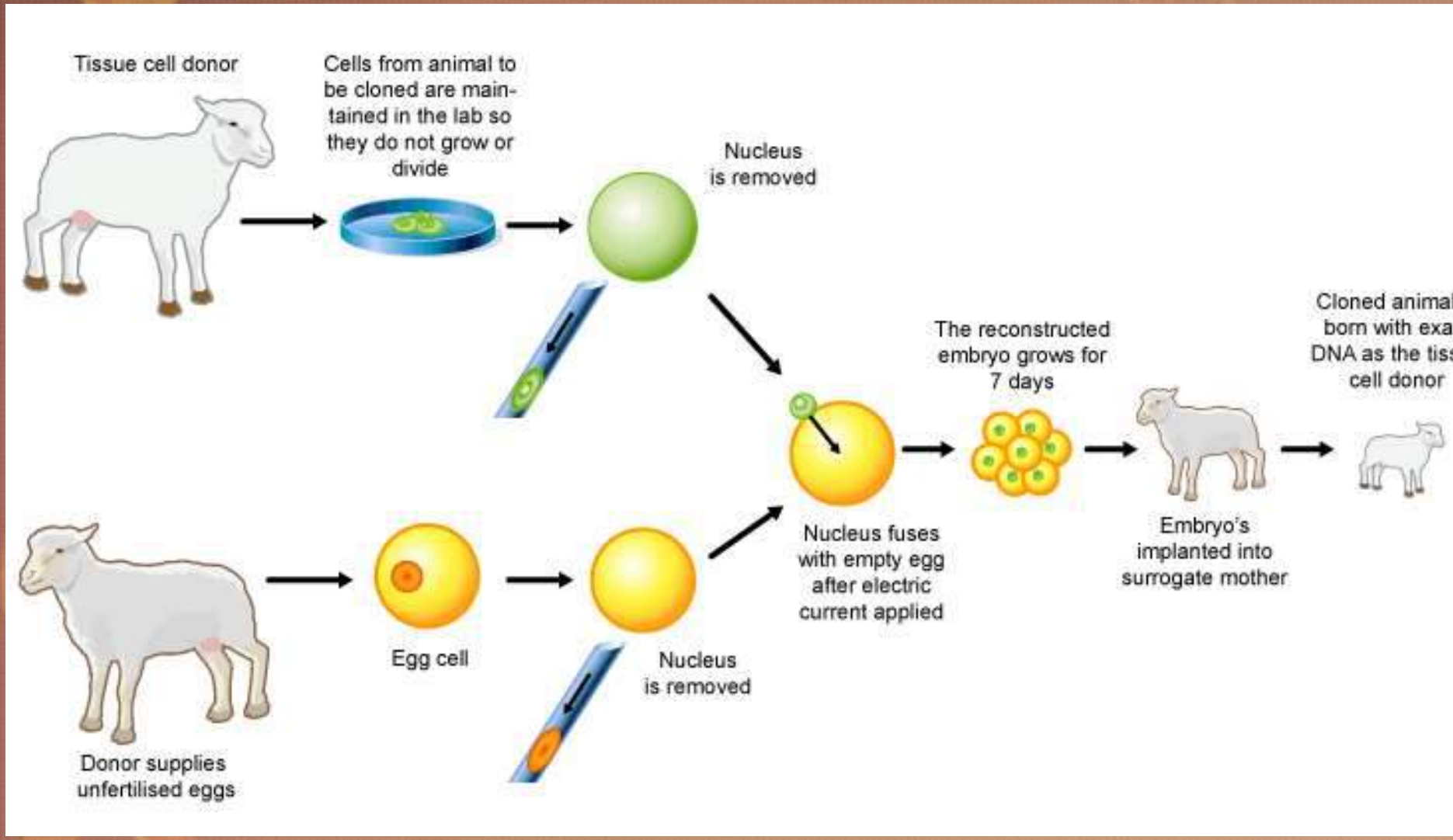
Sheep gives birth to identical twins

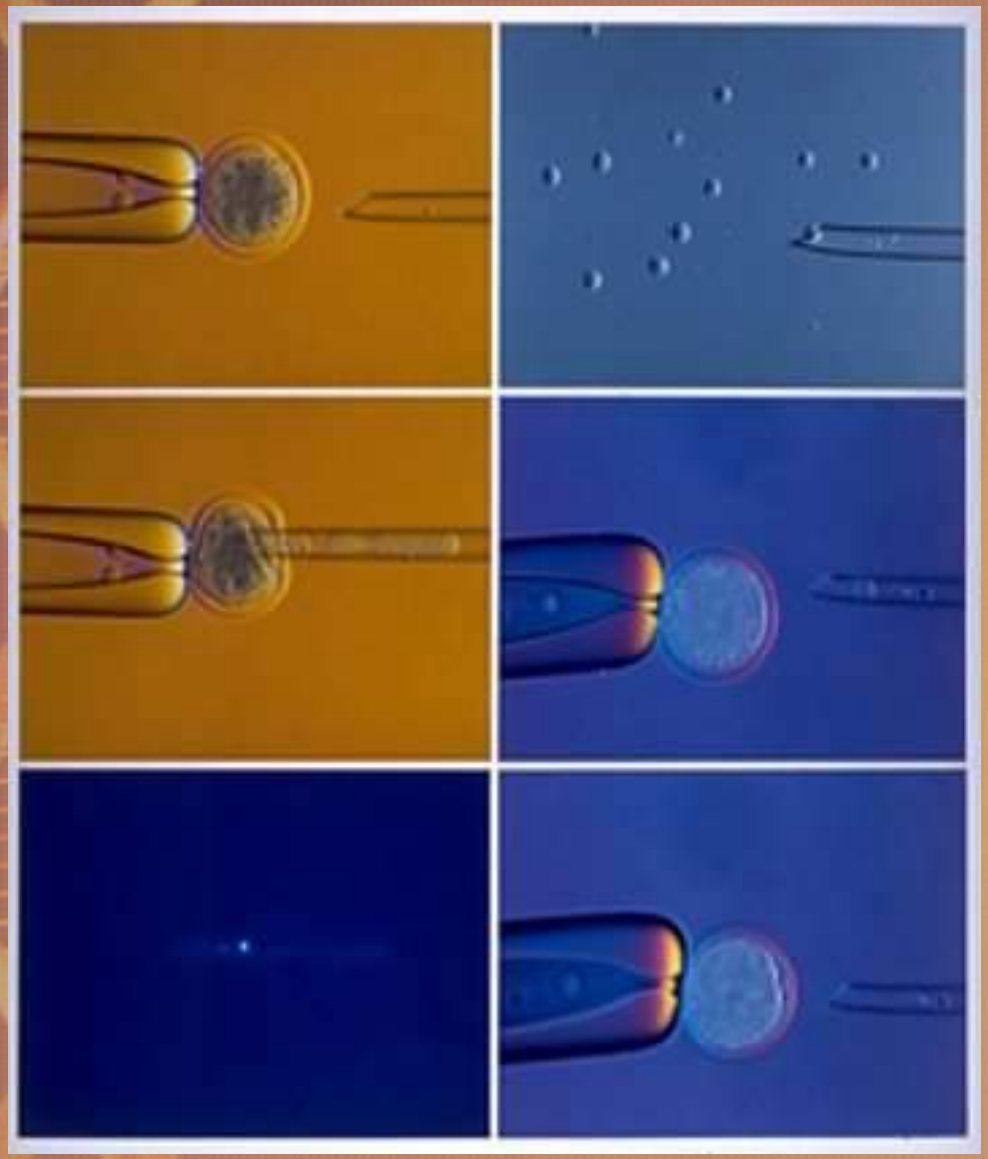


Nuclear Transfer

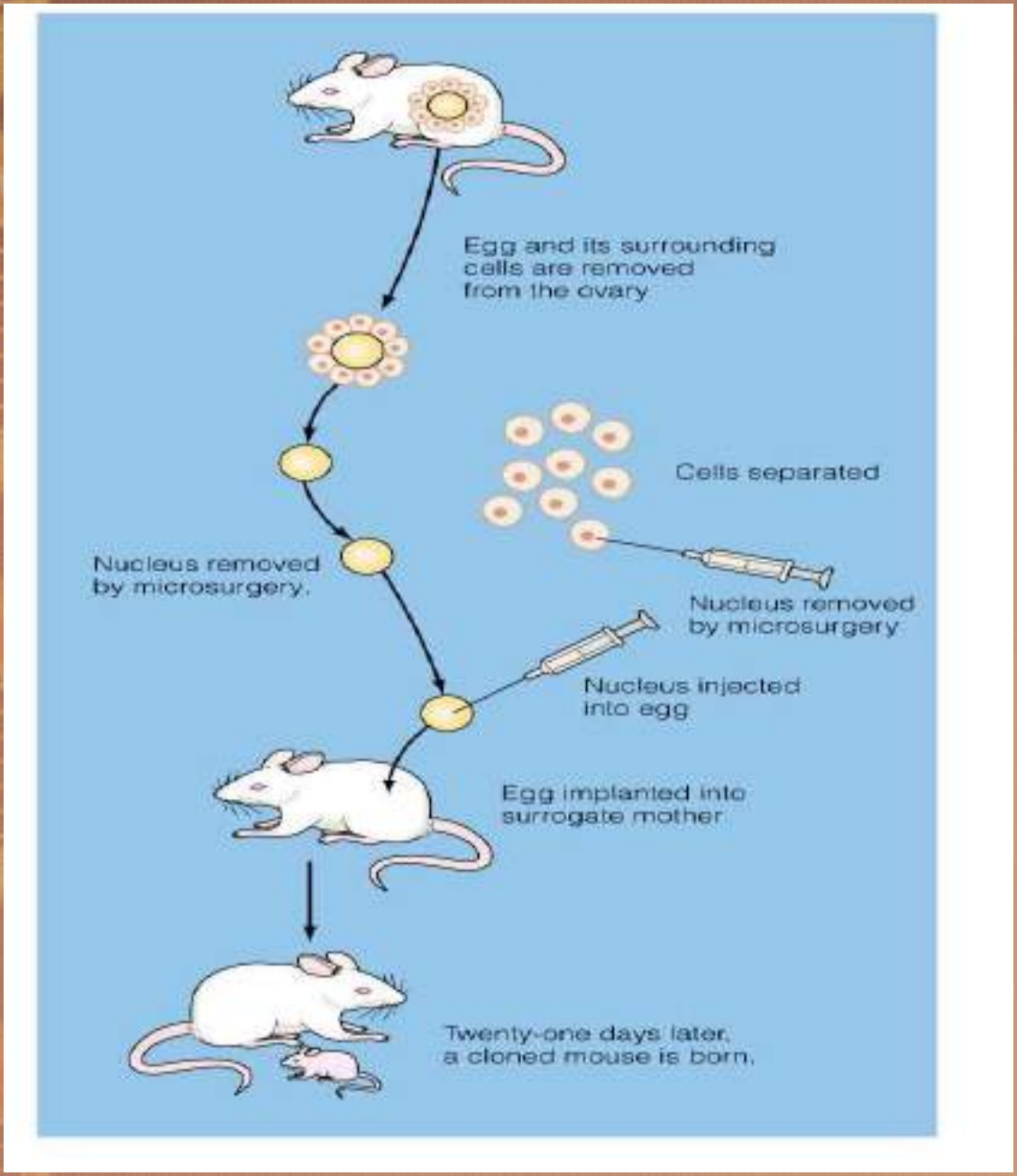
- First done in 1986
- More difficult
- Nucleus is removed from an egg
- Enucleated eggs are fused with other cells
- Embryos are transplanted into a surrogate mother
- In 1997, **Dolly the sheep** was the first mammalian clone from an adult donor cell

Cloning by nuclear transfer





Process of microinjection in Cloning by nuclear transfer



Cloning Mice by Injection of Nuclei from Adult Cells

Cloned Animals

Second addition

Second chance

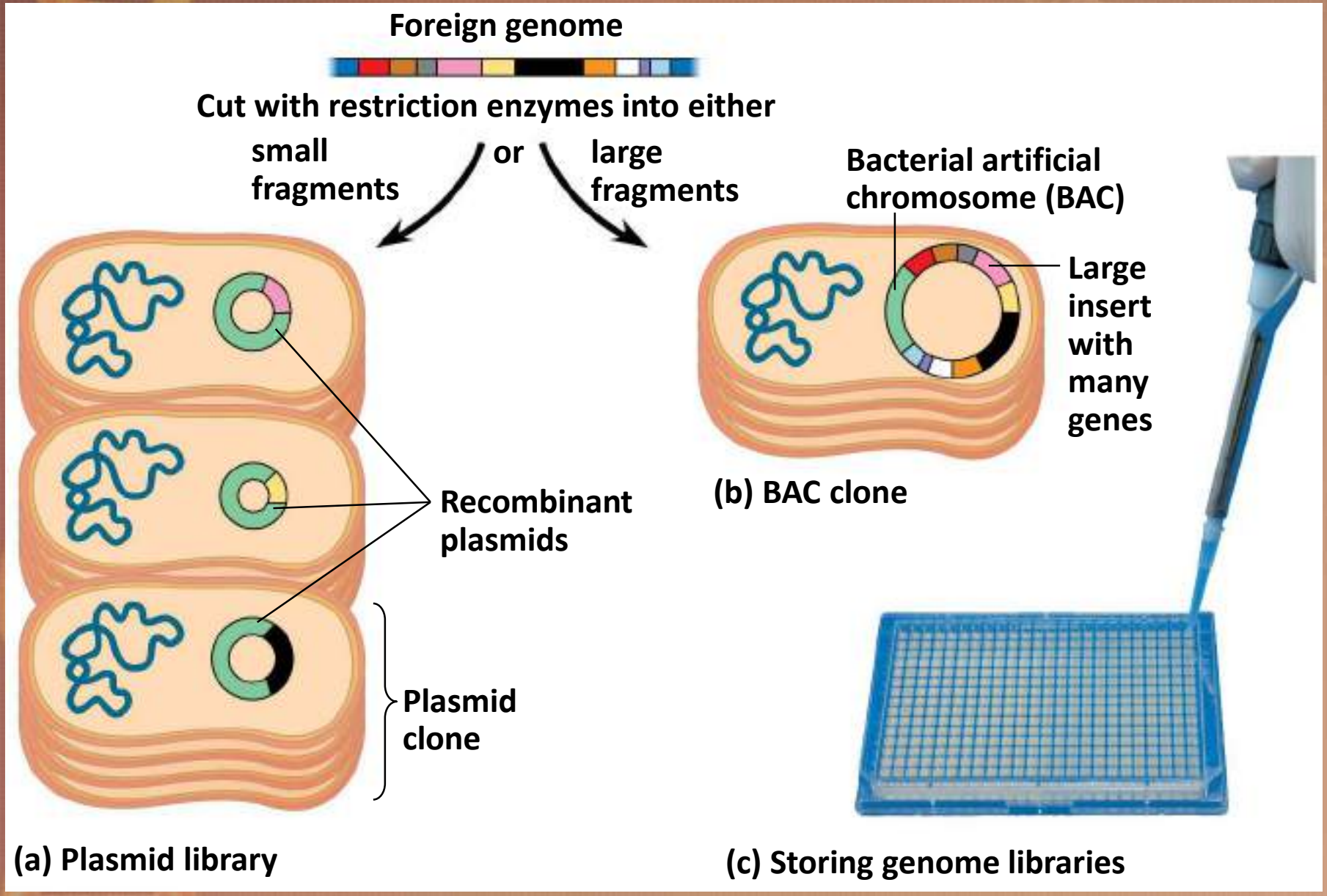
- **Also cloned animals about to go extinct - gaur etc**

Problems

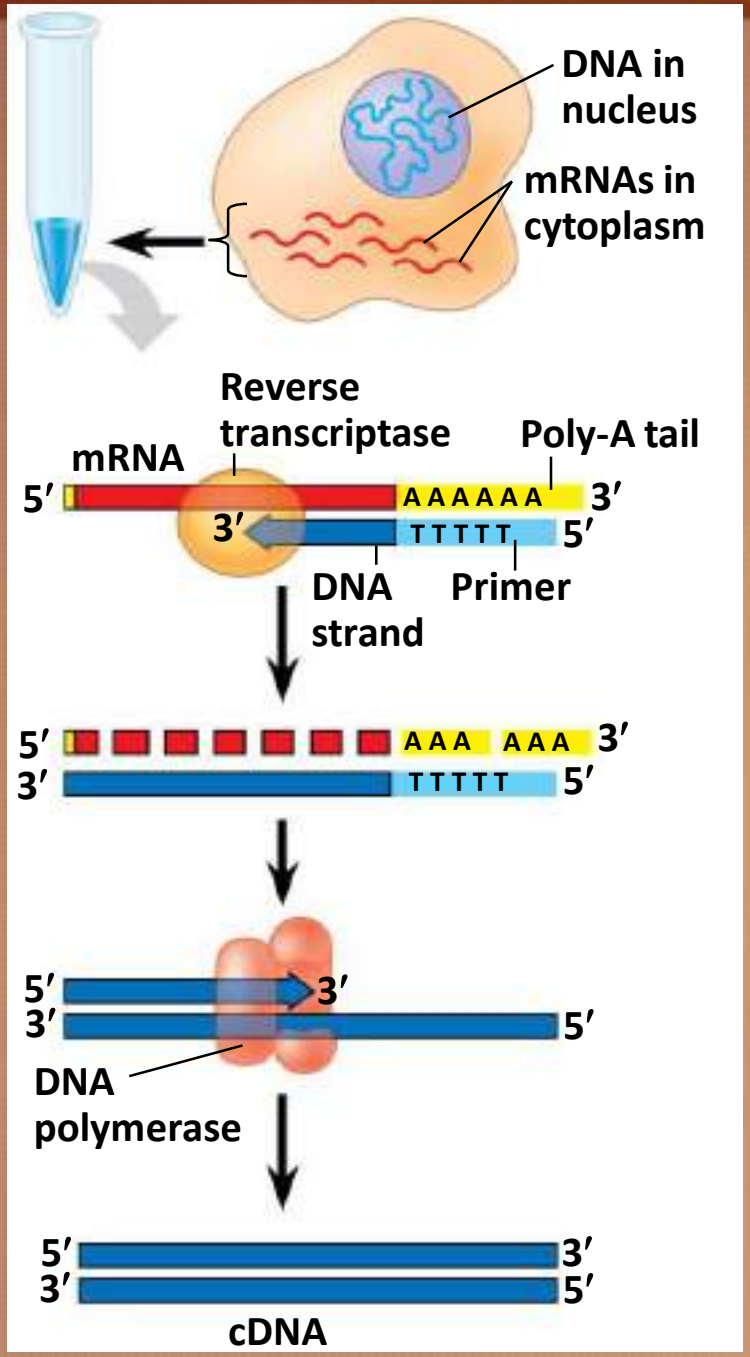
- **don’t live as long.**
- **not carbon copies/identical.**
- **develop diseases early.**
- **very low success rate - 0.1 - 3%.**
- **Dedifferentiation/reprogramming may not be complete or accurate**

APPLICATIONS

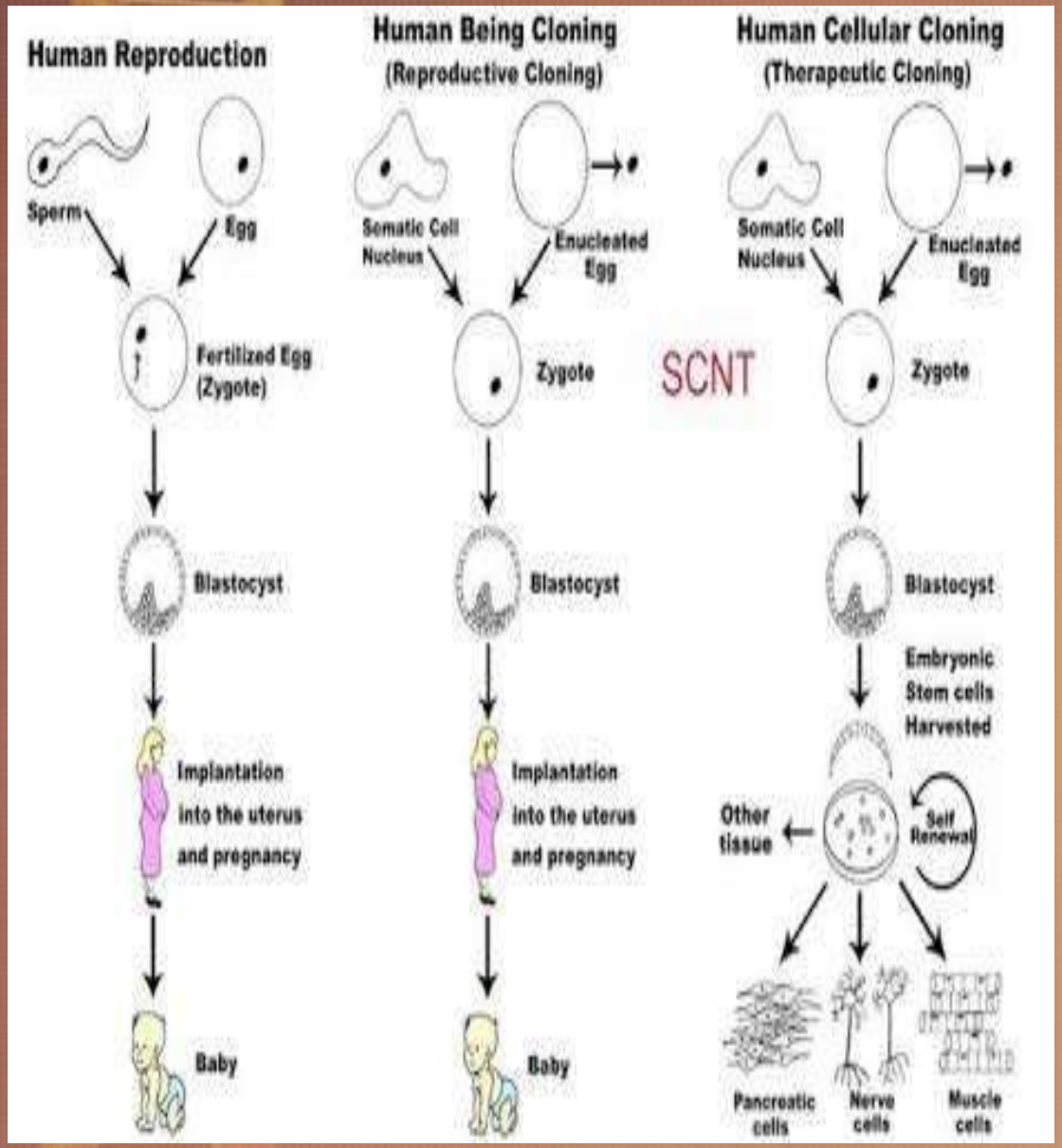
GENOMIC LIBRARIES

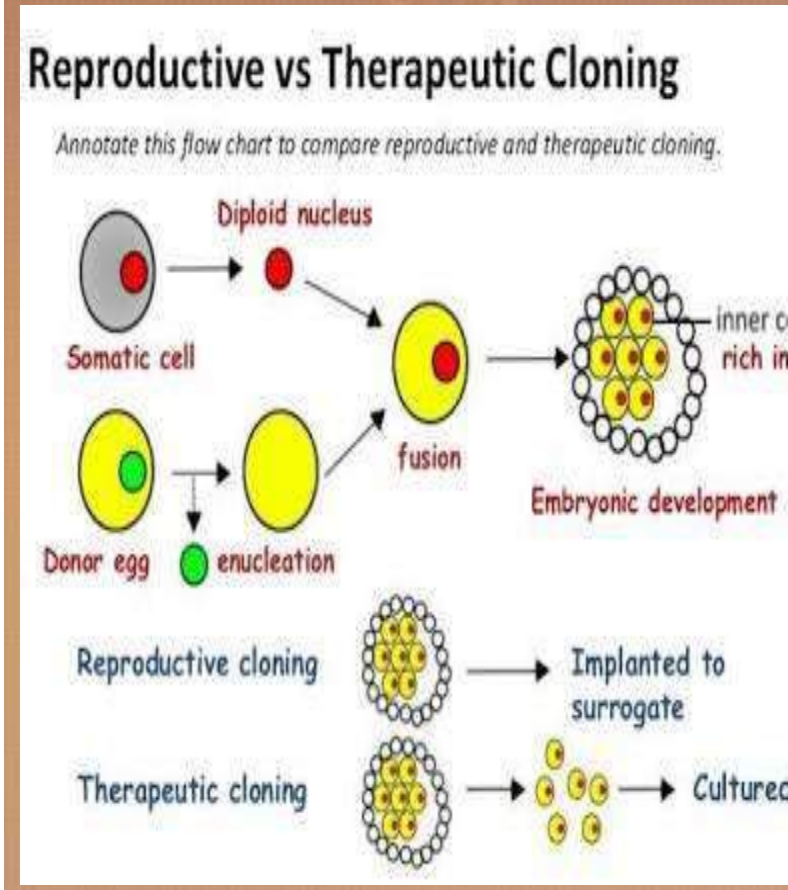


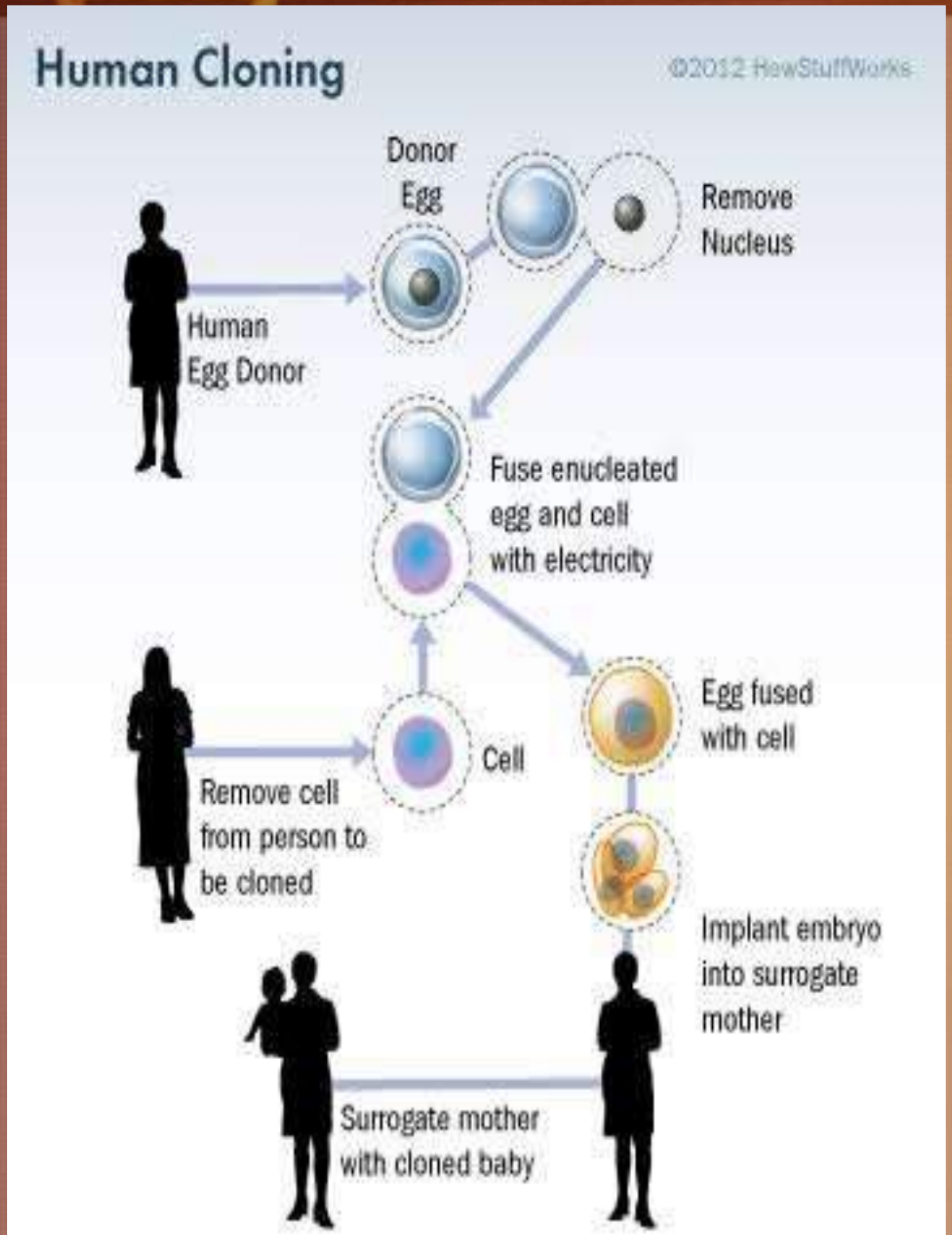
- A **complementary DNA (cDNA)** library is made by cloning DNA made *in vitro* by reverse transcription of all the mRNA produced by a particular cell
- A **cDNA library** represents only part of the genome—only the subset of genes transcribed into mRNA in the original cells



Making complementary DNA (cDNA) from eukaryotic genes.


Human Reproduction
Sperm
Egg
Fertilized Egg (Zygote)
Blastocyst
Implantation into the uterus and pregnancy
Baby
Human Being Cloning (Reproductive Cloning)
Somatic Cell Nucleus
Enucleated Egg
Zygote
Blastocyst
Implantation into the uterus and pregnancy
Baby
SCNT
Human Cellular Cloning (Therapeutic Cloning)
Somatic Cell Nucleus
Enucleated Egg
Zygote
Blastocyst
Embryonic Stem cells Harvested
Other tissue
Self Renewal
Pancreatic cells
Nerve cells
Muscle cells


Reproductive vs Therapeutic Cloning
Annotate this flow chart to compare reproductive and therapeutic cloning.
Diploid nucleus
Somatic cell
fusion
Embryonic development
Donor egg
enucleation
Reproductive cloning
Implanted to surrogate
Therapeutic cloning


Human Cloning
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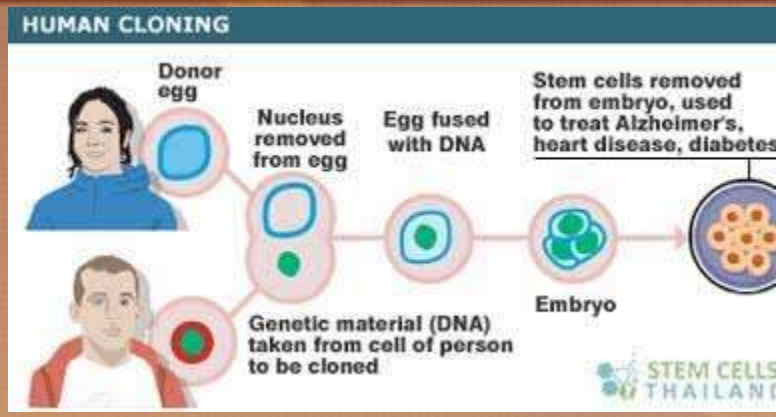
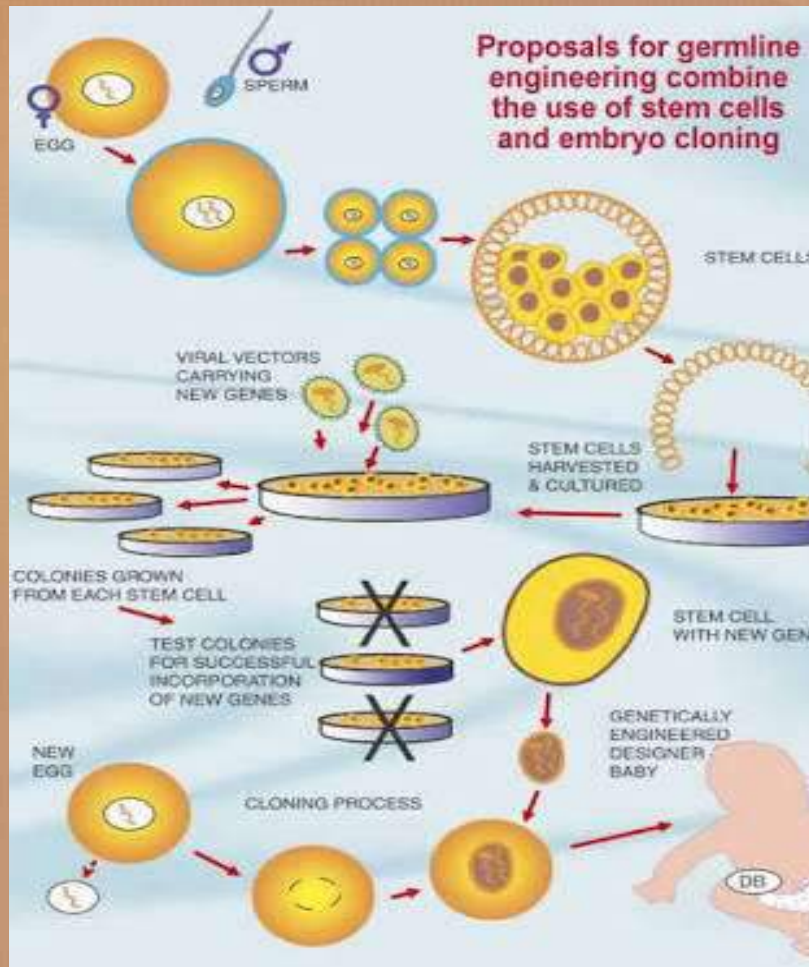
Donor Egg
Remove Nucleus
Human Egg Donor
Fuse enucleated egg and cell with electricity
Egg fused with cell
Cell
Remove cell from person to be cloned
Implant embryo into surrogate mother
Surrogate mother with cloned baby


HUMAN CLONING
Donor egg
Nucleus removed from egg
Egg fused with DNA
Stem cells removed from embryo, used to treat Alzheimer's, heart disease, diabetes
Embryo
Genetic material (DNA) taken from cell of person to be cloned
STEM CELLS THAILAND


Proposals for germline engineering combine the use of stem cells and embryo cloning
EGG
SPERM
STEM CELLS
VIRAL VECTORS CARRYING NEW GENES
STEM CELLS HARVESTED & CULTURED
COLONIES GROWN FROM EACH STEM CELL
TEST COLONIES FOR SUCCESSFUL INCORPORATION OF NEW GENES
STEM CELL WITH NEW GEN
GENETICALLY ENGINEERED DESIGNER BABY
NEW EGG
CLONING PROCESS
DB

Thank you