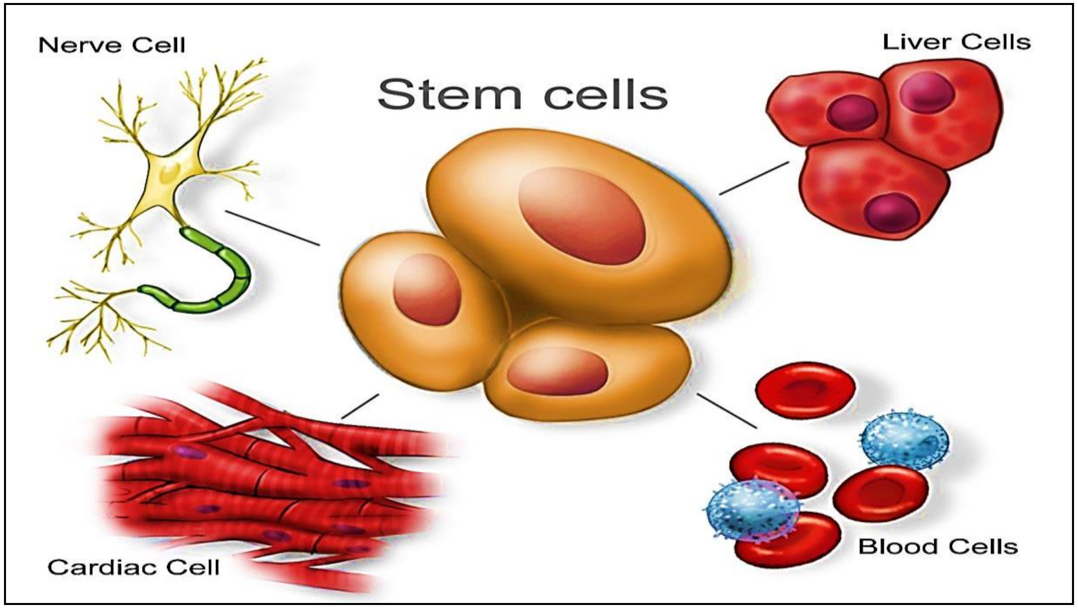
<u>Cell Bio</u>
<u>Introduction to stem cell</u>



"Any time you have healed after an injury it's a stem cell mediated event"

What is a stem cell?

Stem cells are undifferentiated cells that have the ability

1. To divide and renew themselves (Self-renew) throughout life i.e. **Proliferation**

2. To develop to specialized cell types i.e. **Differentiate**

Stem cell is unique because it Can do both

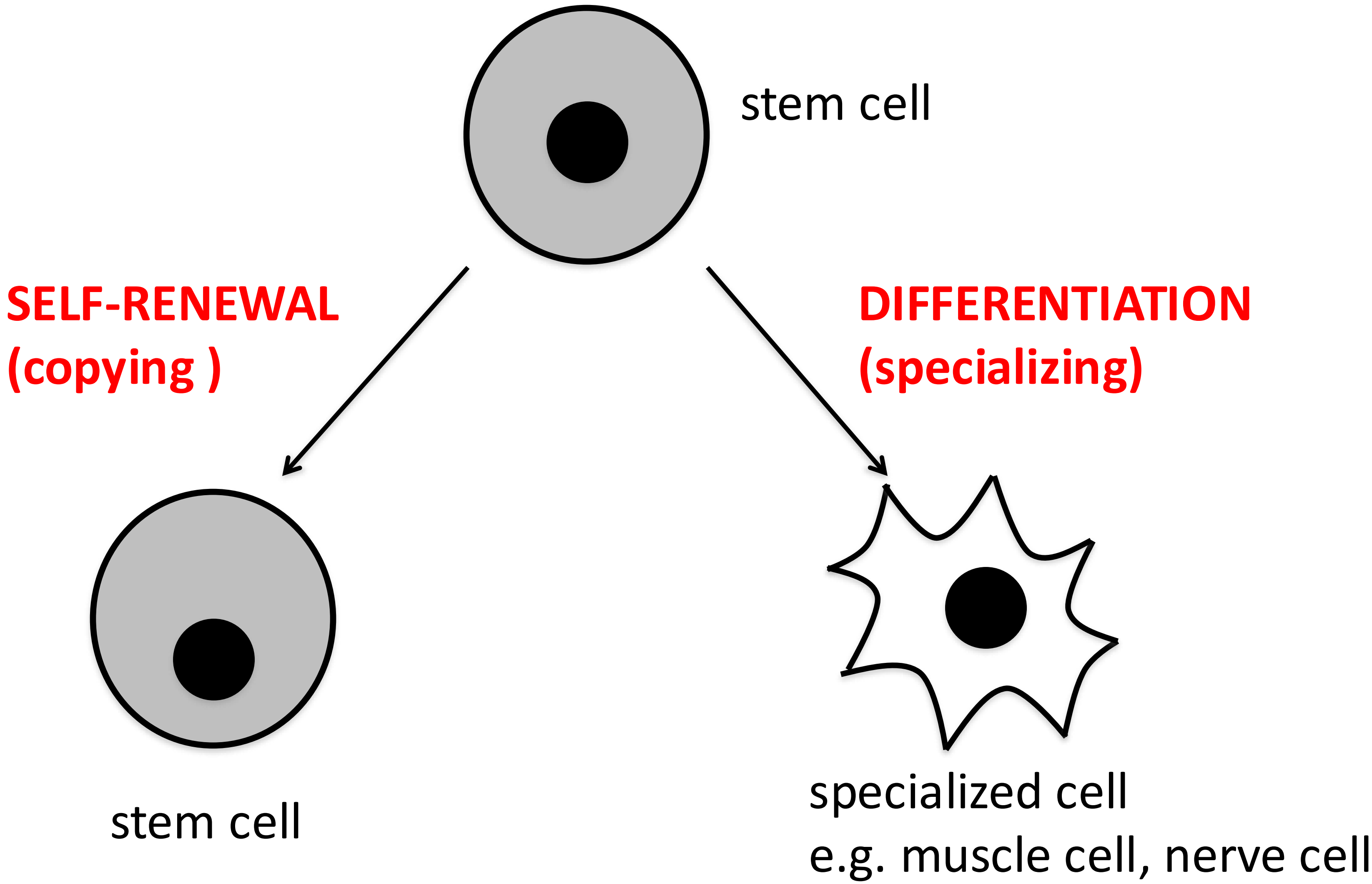
Self- renew

Differentiate

Make copies of itself

Specialized to other cells

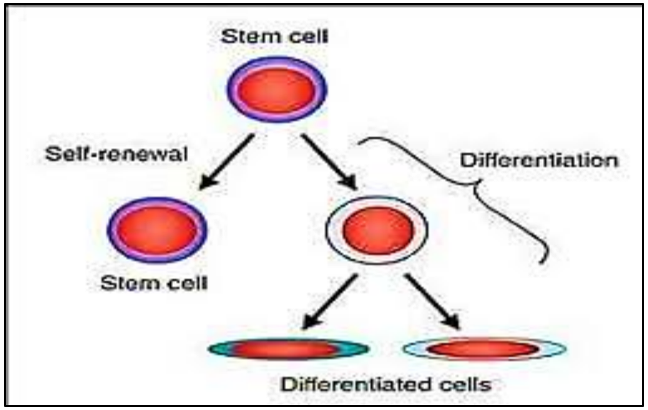
What is a stem cell?



Why self renew & Why differentiate?

A- Self renewal

- **maintain a storage** Because if they didn't copy themselves they would finish quickly. They are important for the body to use throughout our life.



B- Differentiation

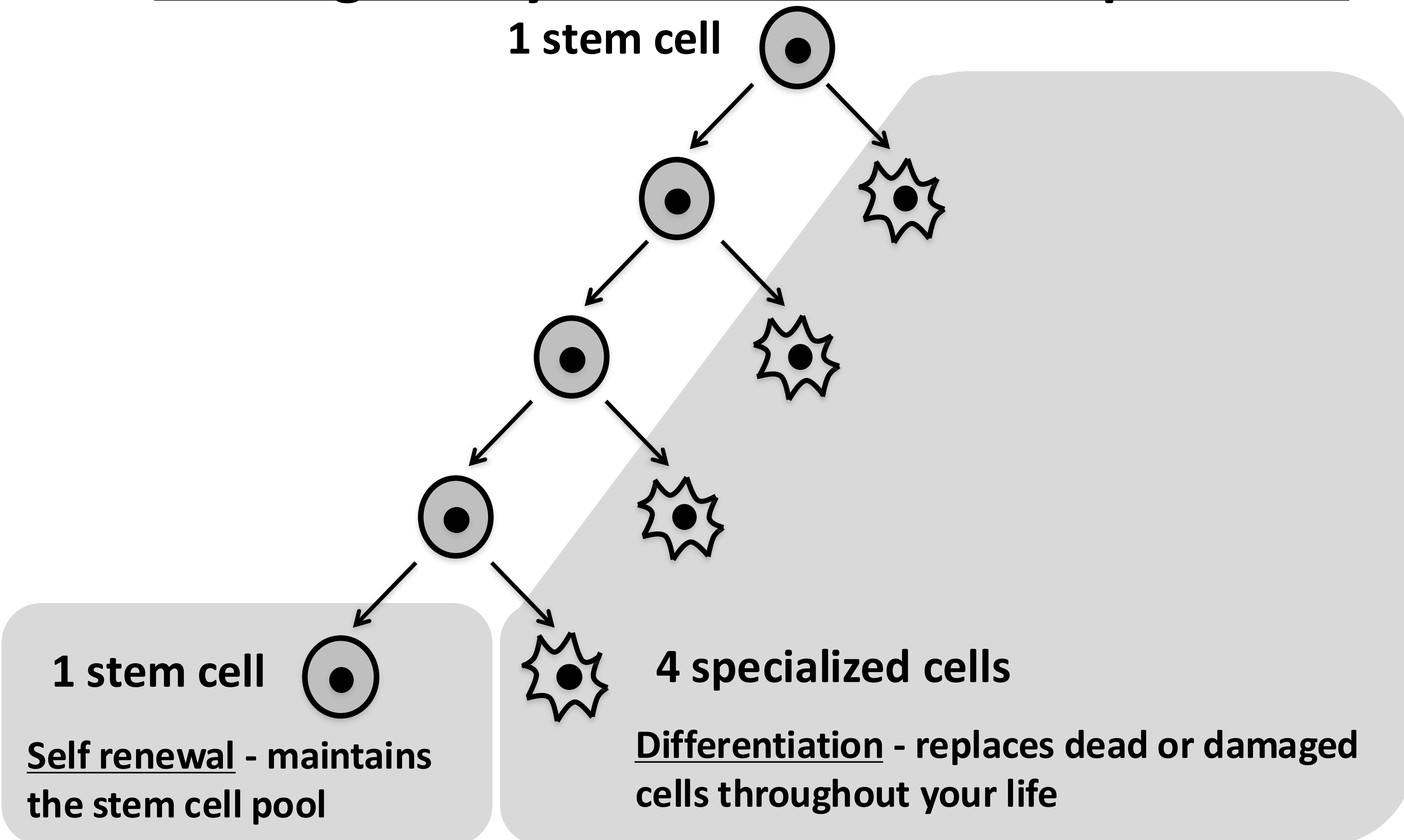
- Specialized cells are mature cells **cannot divide** or make copies of themselves, so if they damaged or die they need to be replaced so that the body can keep on working
- *Specialized*' or '*differentiated*' cells have **particular roles (Functions)** in the body e.g. blood cells, nerve cells, muscle cells

- There are 2 theories for stem cell division:

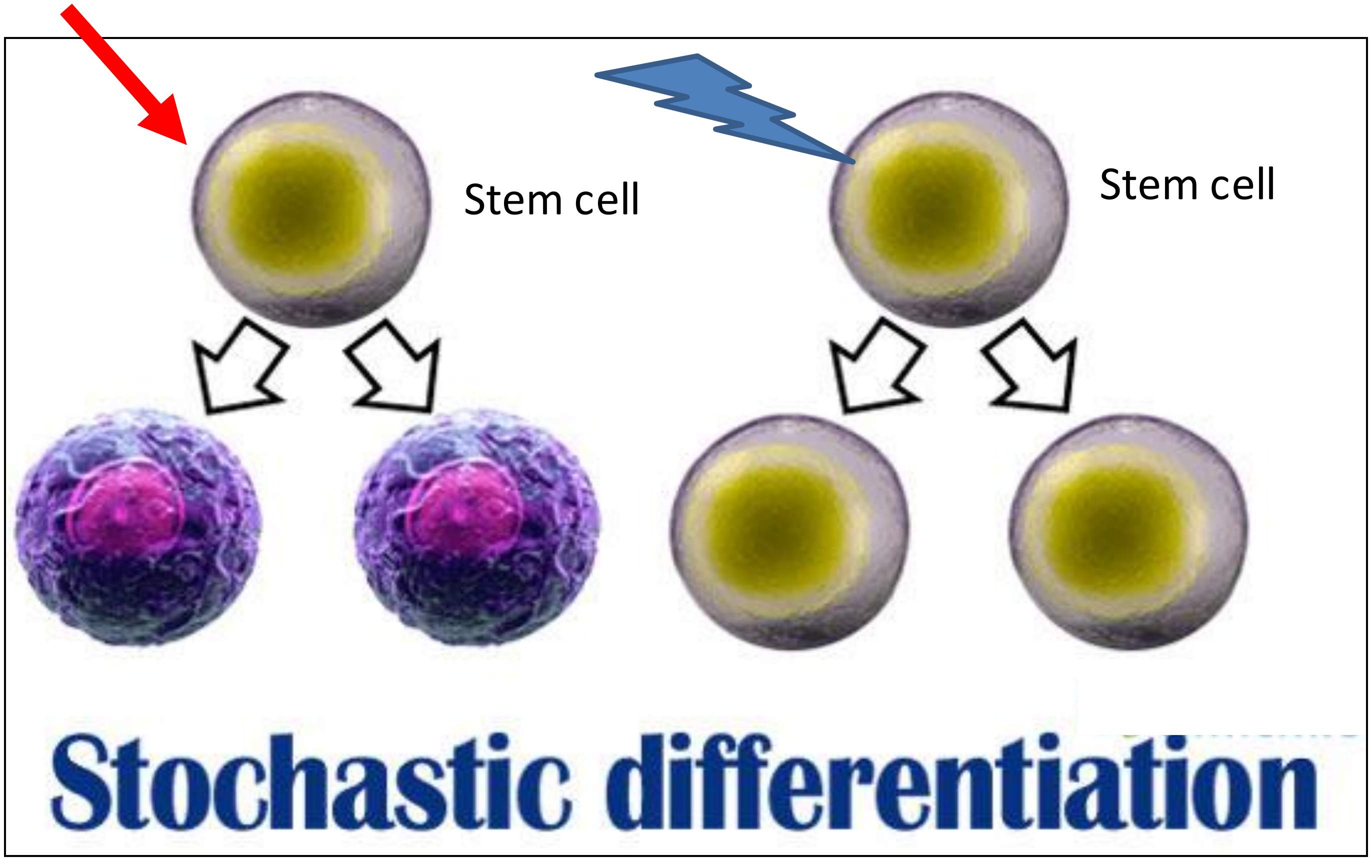
1- Obligate asymmetric stem cell replication

2- Stochastic differentiation

1- Obligate asymmetric stem cell replication



2- Stochastic differentiation

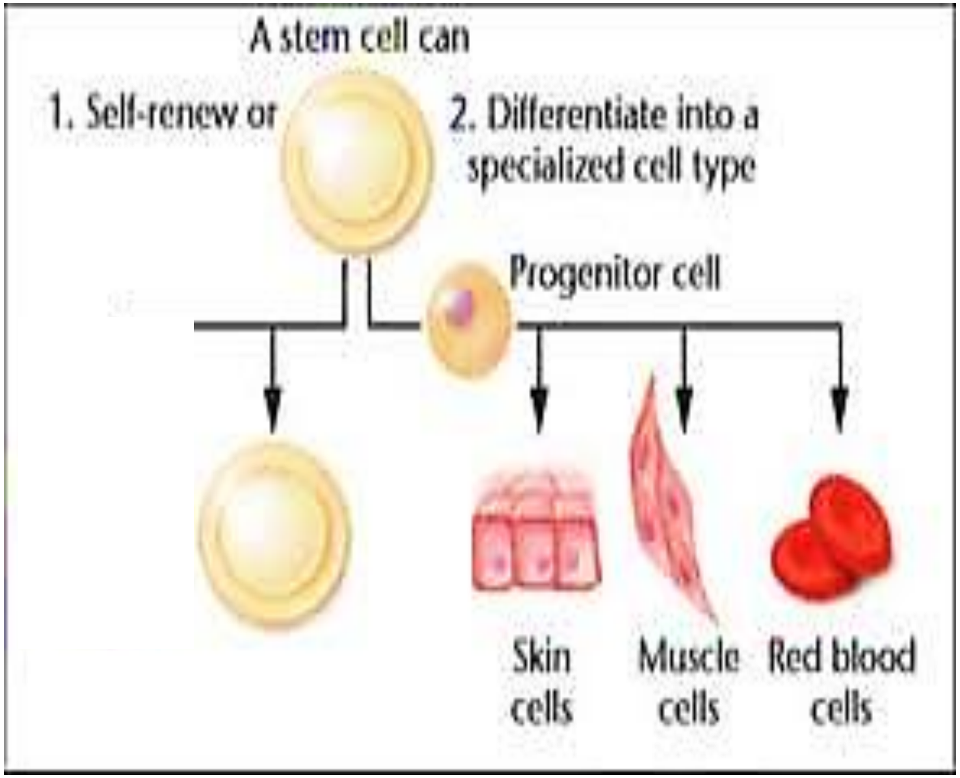


If one stem cell differentiate into 2 specialized cells another stem cell will notice that and make up for the lost stem cell and divide by mitosis to produce 2 identical stem cells

Progenitor (intermediate) cells

Cells that arise from stem cells and is more specialized than stem cells but less differentiated than fully specialized / mature

Progenitor cells have limited capacity to self-renew than stem cells & they are the precursor that capable to differentiate to specific cells
They are usually multipotent or unipotent



Potency: stem Cell's ability to differentiate into other cell types

Types of stem cells

There are 2 types of stem cells:

1. Embryonic stem cells (ES)
2. Tissue (adult /somatic) stem cells (TS)

Embryonic stem cells

Exist only briefly during early development before tissues begin to form. Found in the inner cell mass of blastocyst

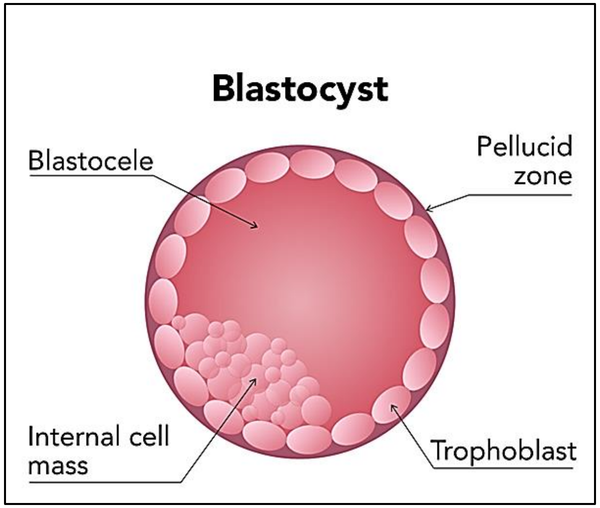
Blastocyst :a very early stage of the embryo life that has about 50 to 150 cells

Tissue (adult /somatic)stem cells

Small populations of cells that are found in tissues of the body after the tissues are formed (found in a fetus, baby, child or adult) they give rise to a limited number of mature cell types that repair the tissue in which they reside..

1- Embryonic stem cells (ES)

- Embryonic stem (ES) cells derived from the **inner cell mass of a blastocyst** (an early- stage embryo)

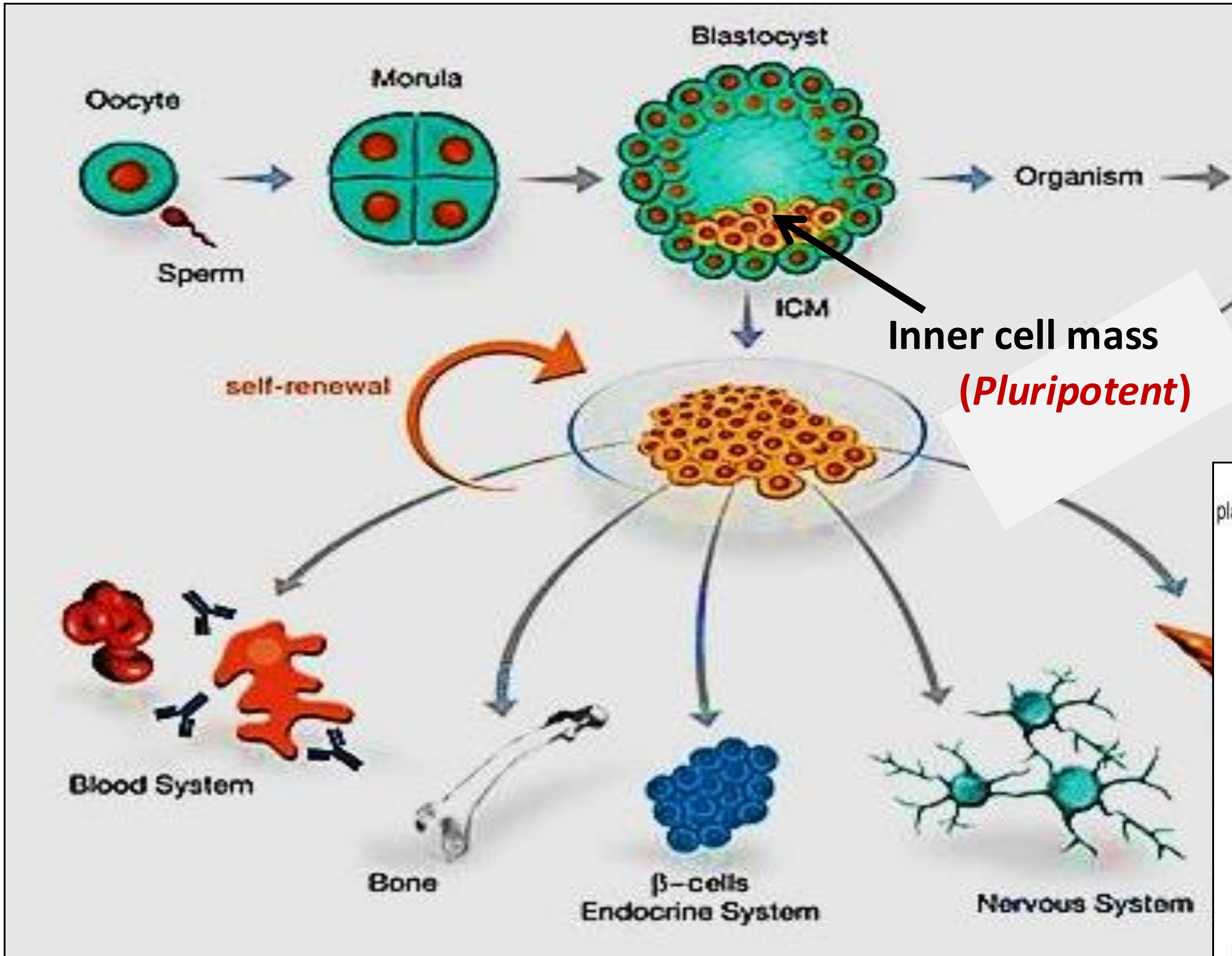


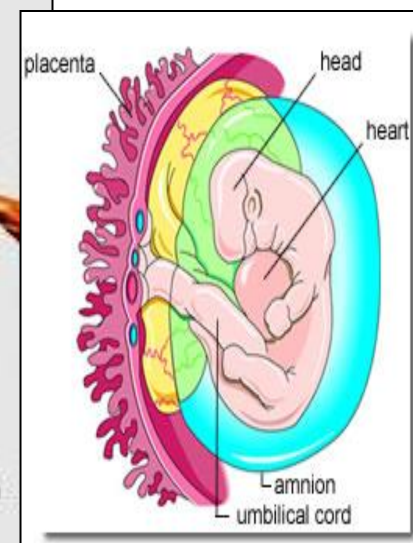
- Human embryo reaches the blastocyst (consists of 50–150 cells) stage 4-5 days after fertilization
- Blastocyst is the stage at which implantation in the wall of the uterus occurs
- ES of the inner cell mass are **pluripotent**.

Pluripotent:

i.e. can give rise to all of the cell types that make the body

Embryonic stem cell



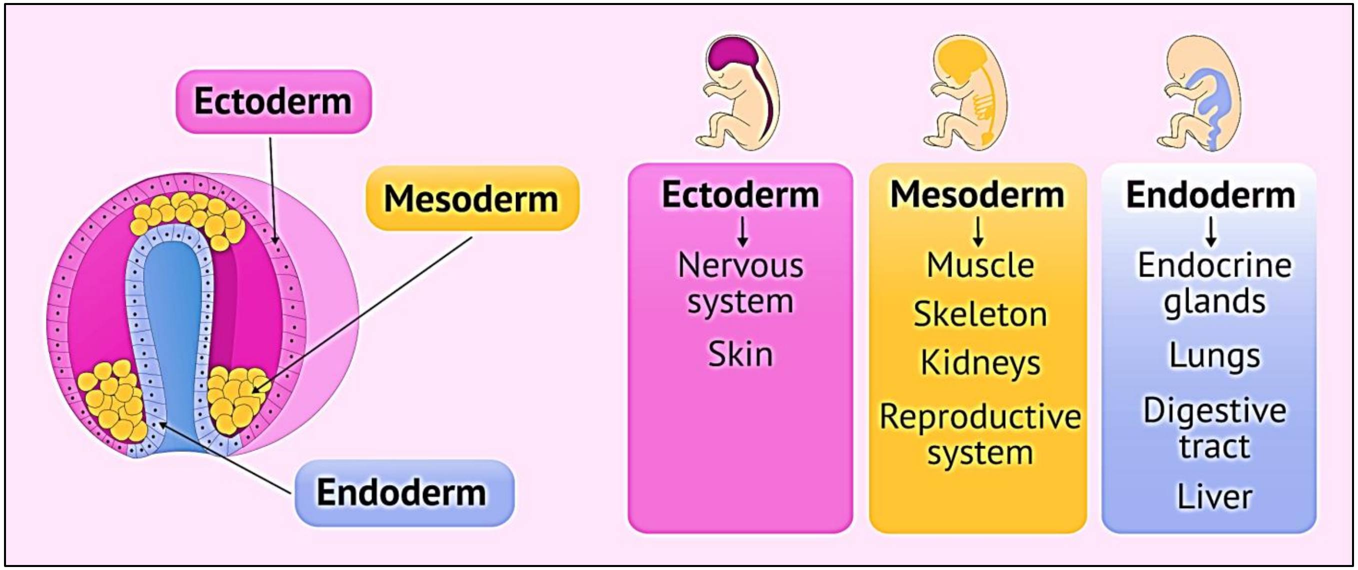


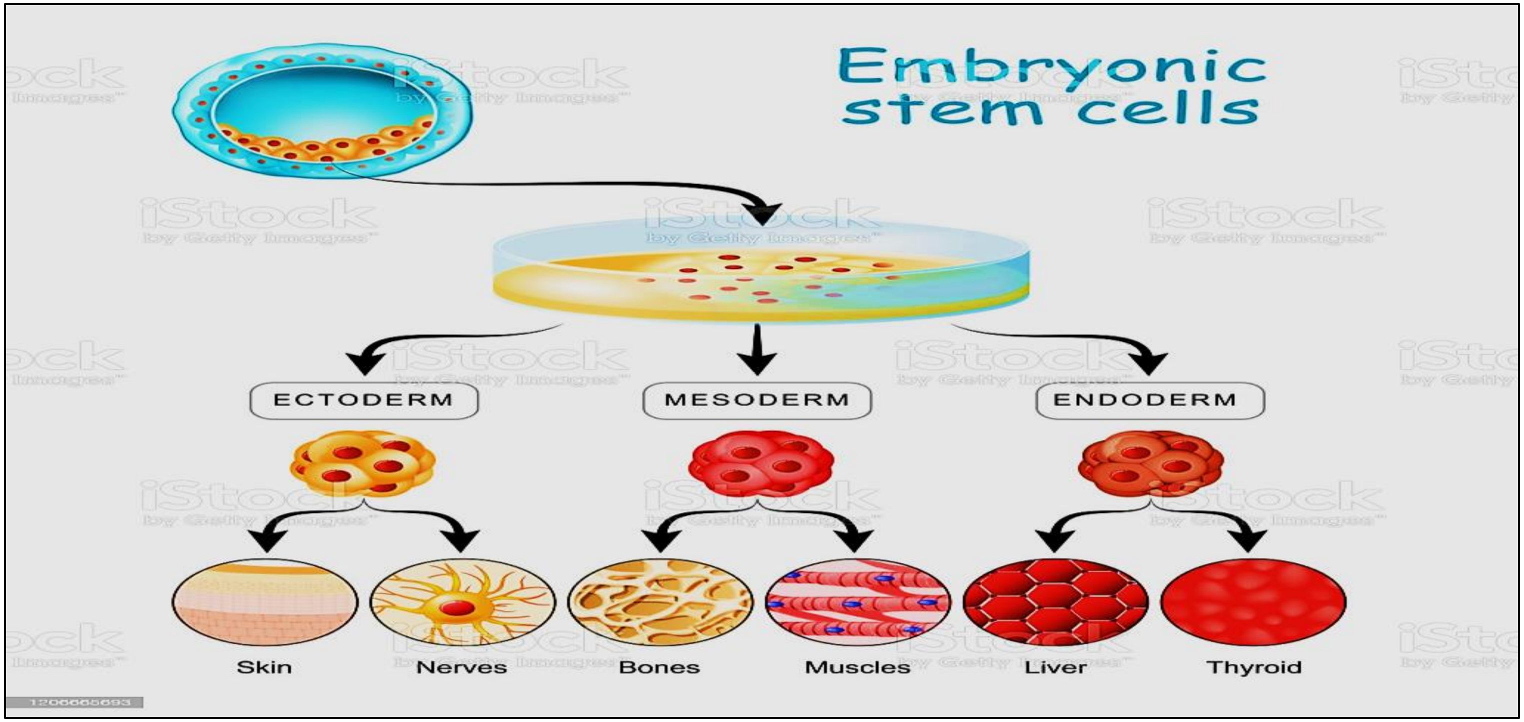
ES cells don’t contribute to the extra-embryonic membranes or placenta

- Few weeks later after implantation the cells will organize into the 3 primary cell layers = germinal layers →

Ectoderm, Mesoderm, Endoderm

- Cells in these 3 layers are **No more pluripotent**
- As development continue the cells of these layers will differentiate to form > 200 types of cells that form the body





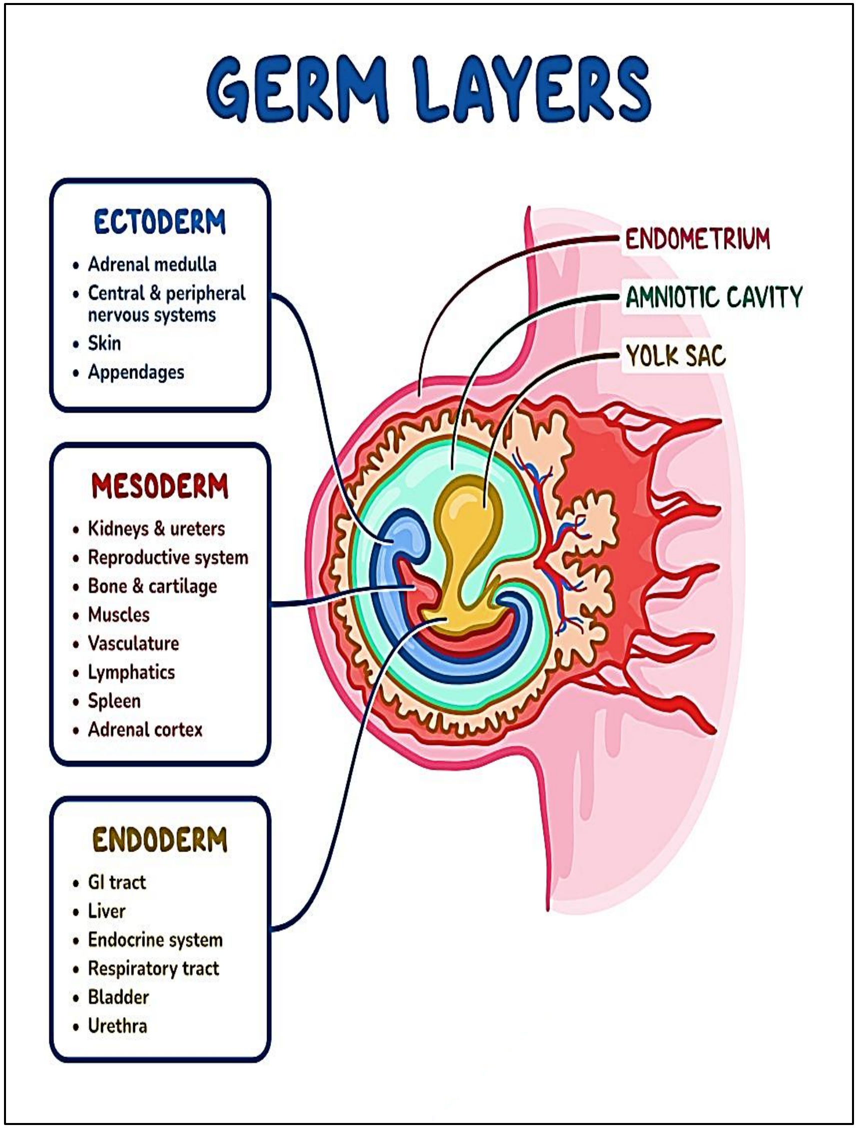
The 3 primary cell layers are formed in the earliest stages of the embryonic development

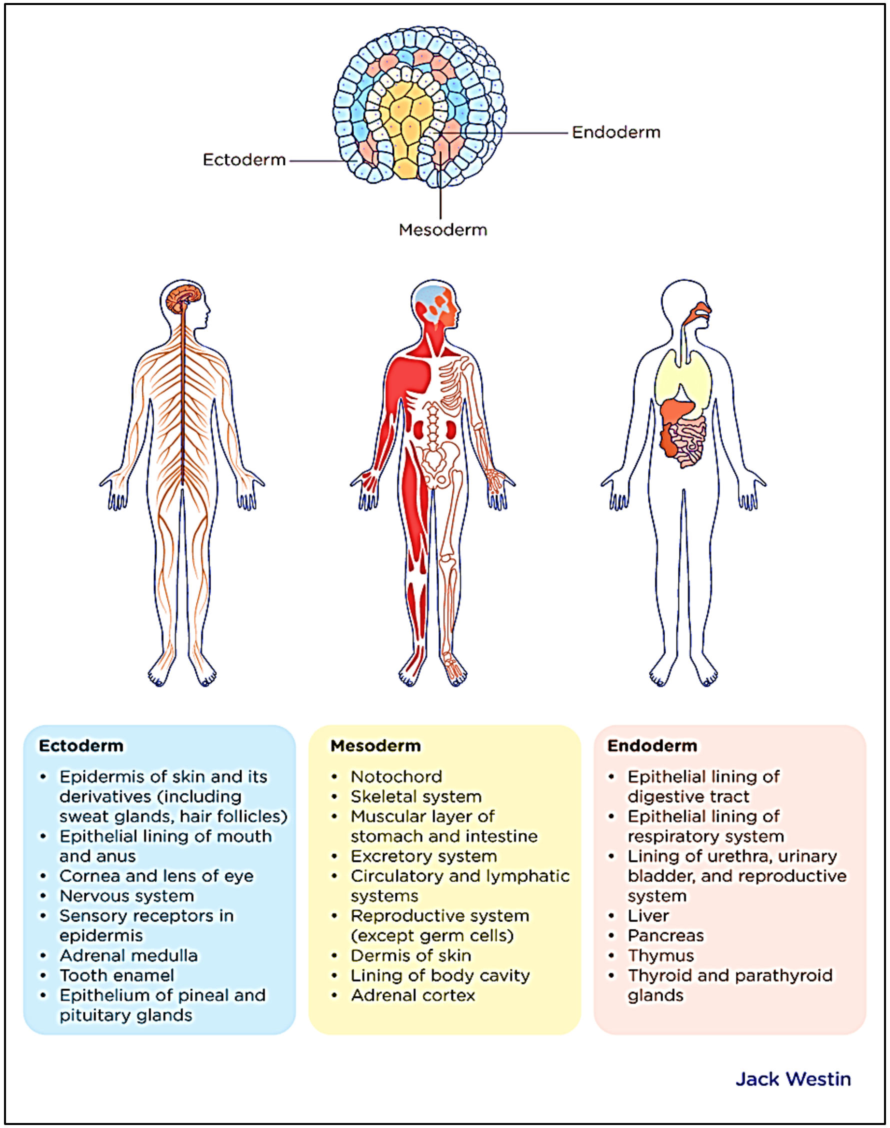
The cells in each germ layer will differentiate into tissues and organs

Ectoderm → skin, nervous system,& parts of head & neck

Mesoderm → muscles, blood, blood vessels, & begging of bone & connective tissue

Endoderm→ digestive ,respiratory tracts, pancreas &liver



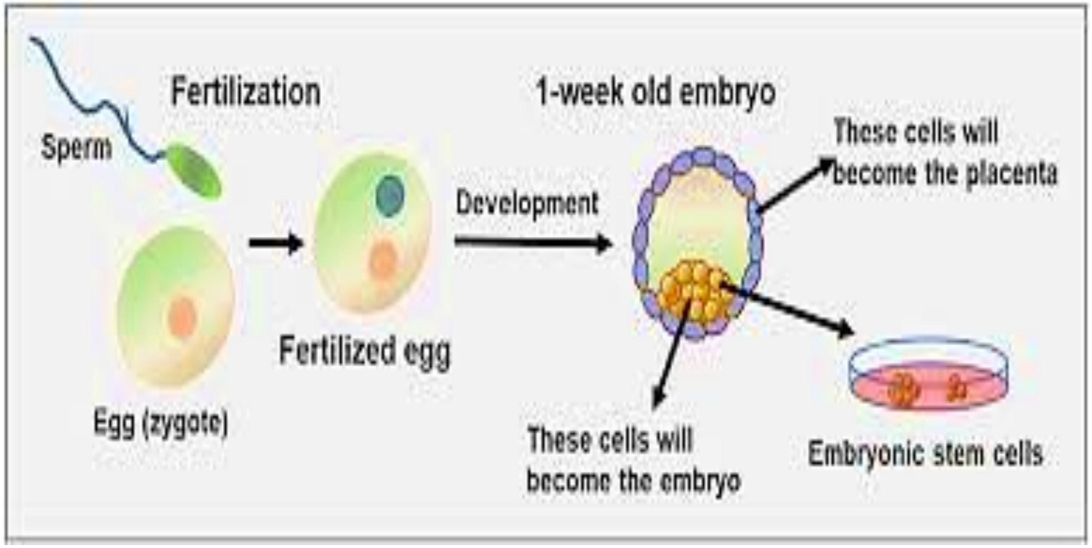


The three primary cell layers or germinal layers

Embryonic stem cell research

ES cells offer hope for new therapies, but their use in research has been **strongly debated** because:

1. Ethical & religious concerns = Destruction of embryo
2. Immune rejection due to different genetic background
3. Tumor formation, changed into tumor cells. Once they put in the body they can never be taken out



Stem cell Niches

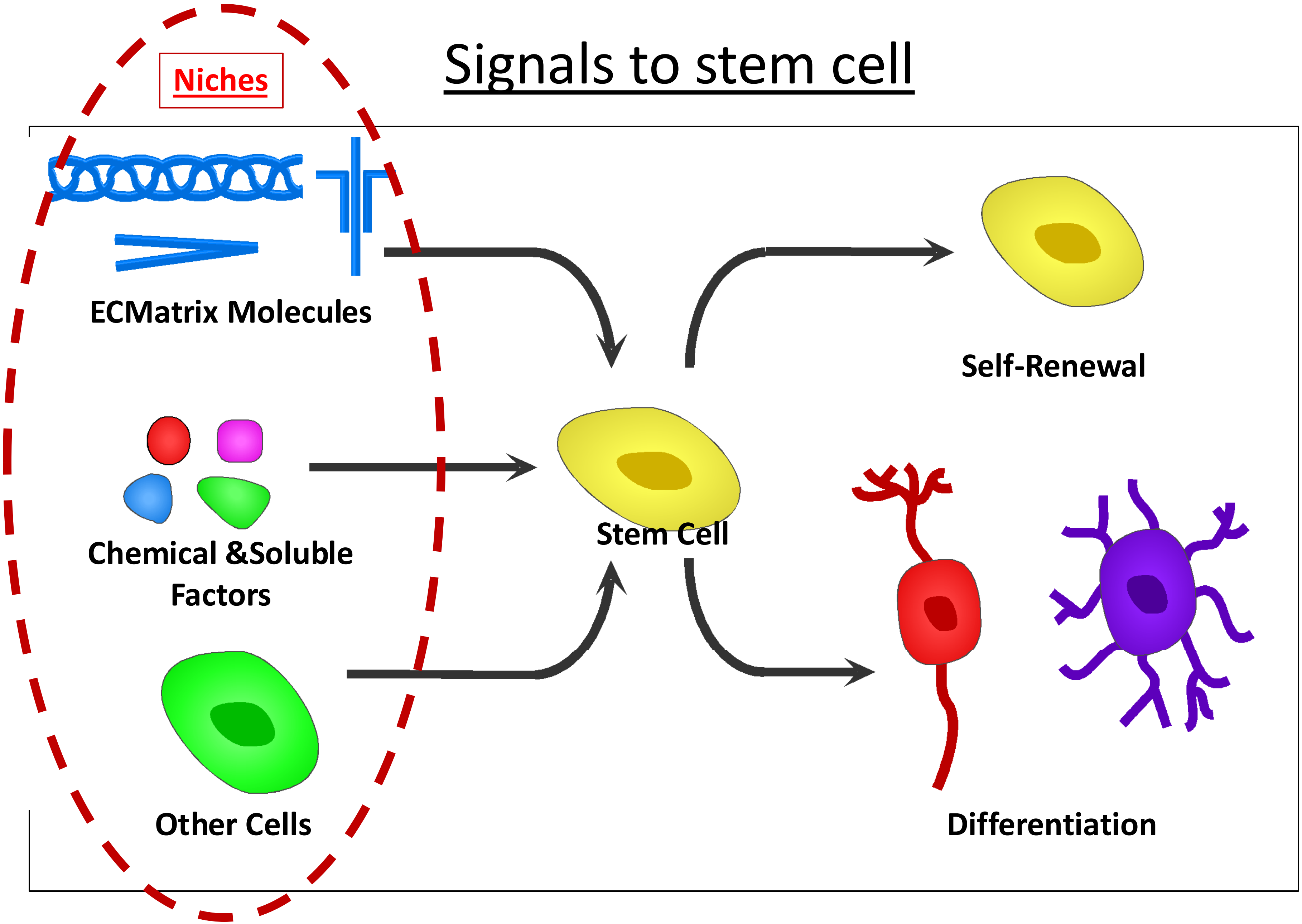
- Stem cells is surrounded by a special microenvironment **called the stem cell niche is** where the stem cells reside, interact & receive signals

- These niches play important role in maintaining the balance between stem cell self-renewal and differentiation→ homeostasis

- This niches composed of cellular & non cellular components & function to :

1. Provide physical support
2. Regulate stem cell activity through signals & cell-cell interactions
3. Protect stem cells from damage & maintain their undifferentiated state

Signals to stem cell



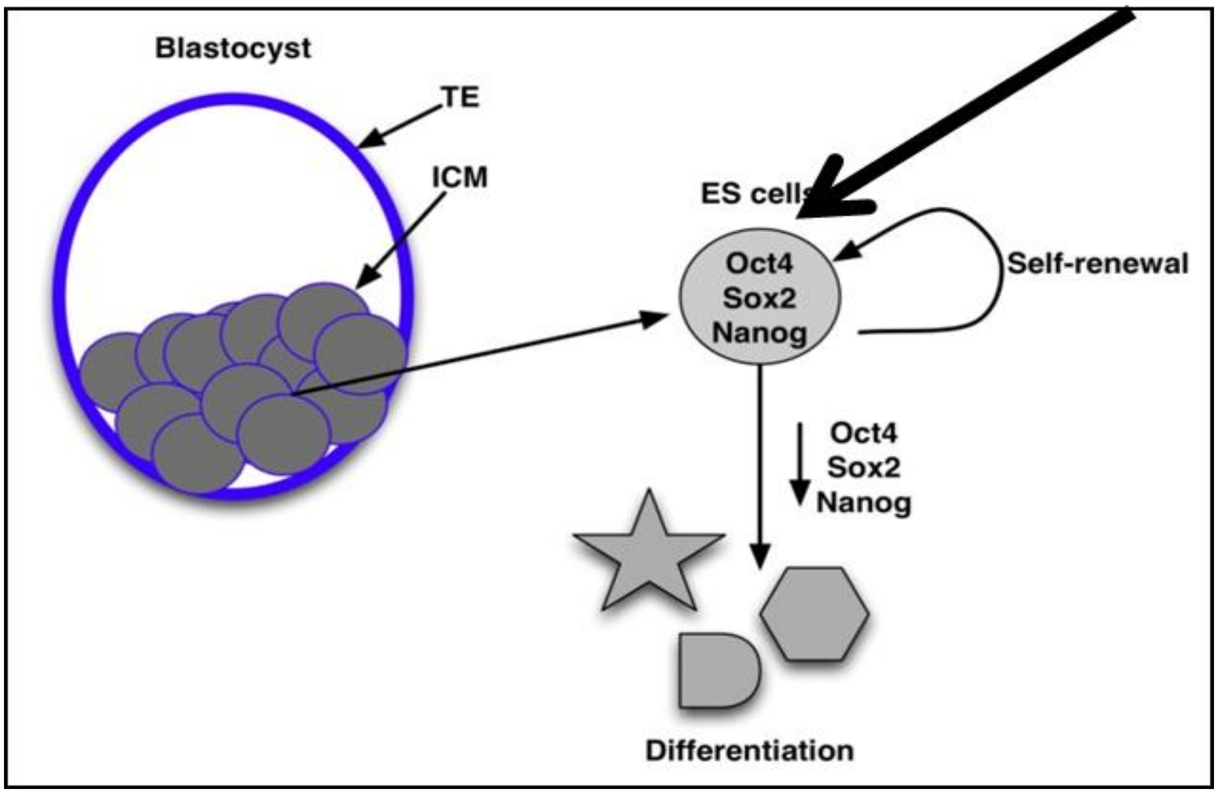
- Any human **ES cell** is defined by the expression of several transcription factors on its cell surface

- They are proteins which play imp role in balancing & regulating the rate of self renewal, pluripotency & differentiation of ES

- They control gene expression by binding to specific regions of DNA affecting the expression or suppression of certain genes

- The transcription factors are Oct-4, Nanog, Sox 2, max, Smad 1, FoxC2

These factors control the expression of **genes** that either maintain ES pluripotency or induce ES differentiation into progenitors of any of the 3 germ layers



2- Tissue (adult/somatic) stem cells

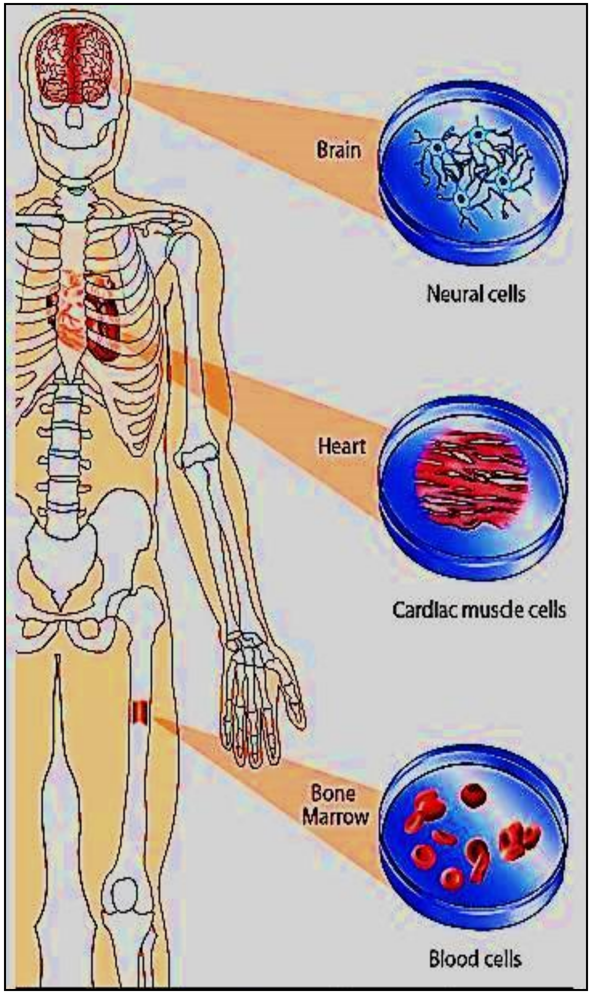
- Specialized stem cells, found among the differentiated cells in the tissues & organs . Responsible for maintaining & repairing lost or damaged cells in the body throughout life of a person.

- They are small in number, have restricted ability to self- renew itself & can only differentiate to limited number of cells or single type of cells

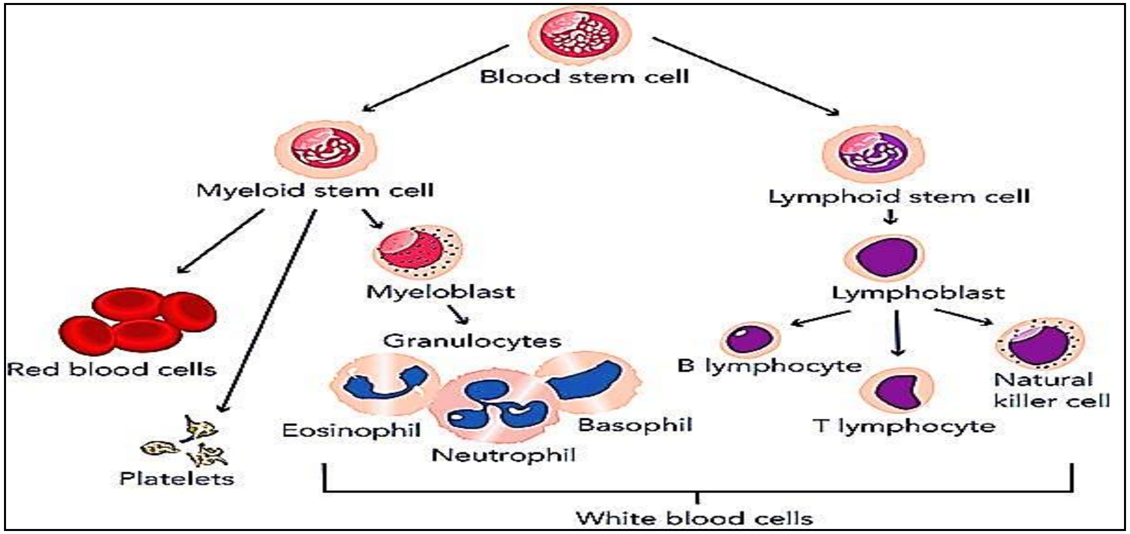
- Its origin during the early embryonic life

- They **differentiate** only to specialized cells similar to cells of the tissue in which they are found

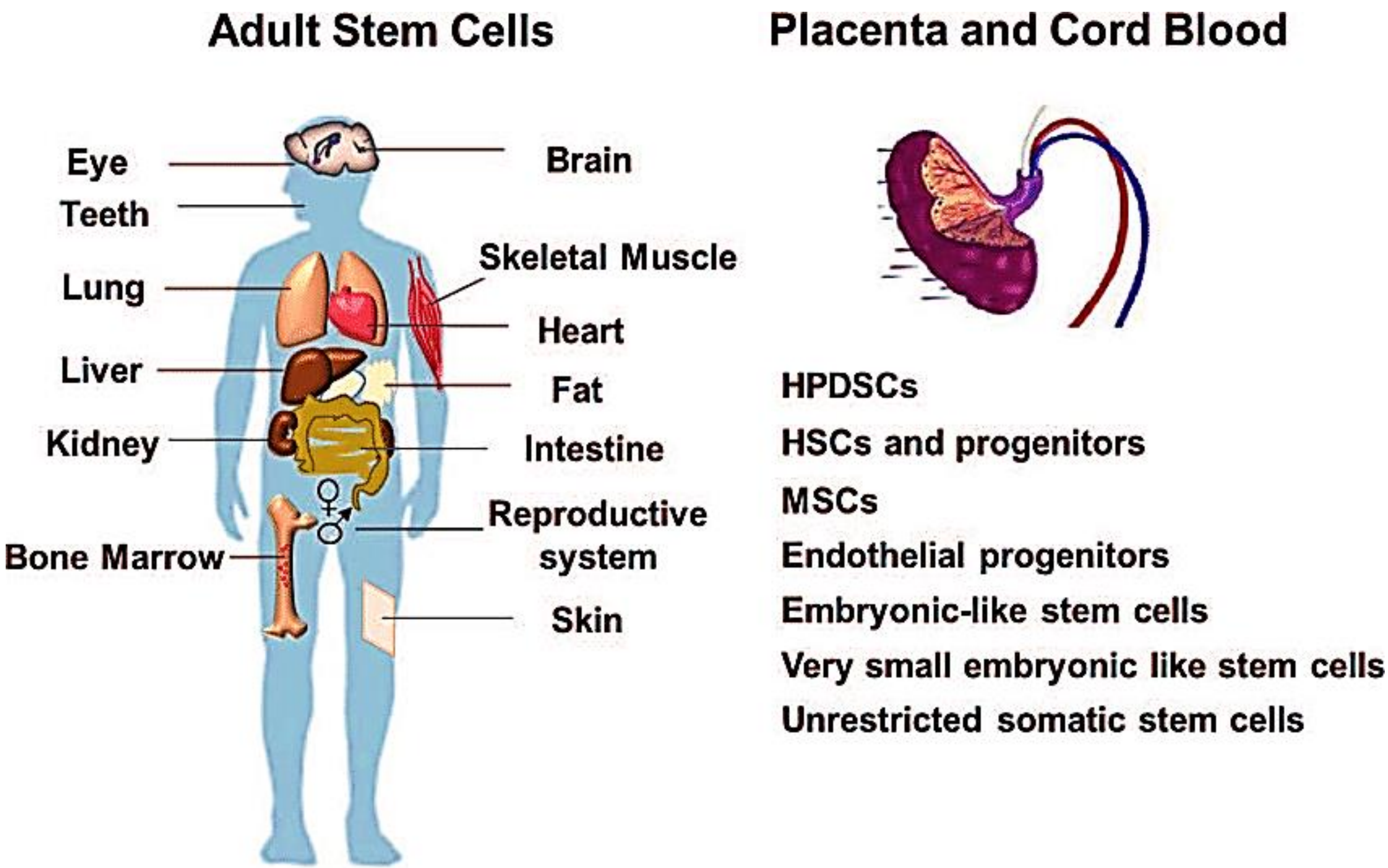
- They are **multipotent or unipotent**



- **Adult stem cells** has been successfully used **in Therapy** for many years to treat **leukemia** and related bone/blood cancers through bone marrow transplants.



- The use of adult stem cells in research & therapy **is not as controversial as** the use of ES cells, because the production of adult stem cells does not require the destruction of an embryo



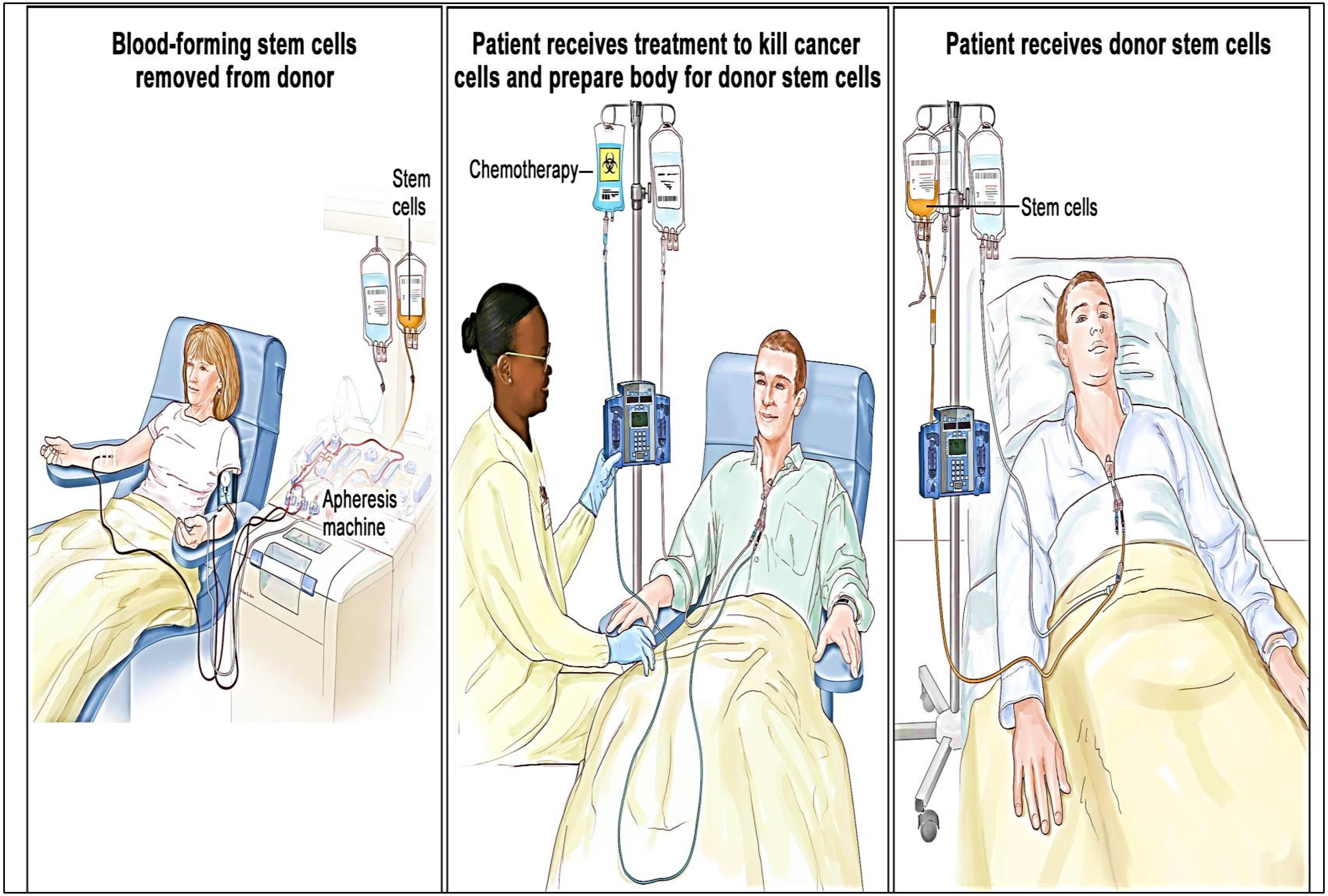
- **Adult stem cells exist throughout the body from The time an embryo develops**
- **Adult stem cells replace cells that are damaged or used up.**

The bone marrow contains at least two kinds of stem cells:

A- **Hematopoietic stem cells (HSCs)**, forms all the types of blood cells (erythrocytes, leukocytes, platelets)

B- **Mesenchymal stem cells (MSCs)** (bone marrow stromal cells) that generates bone cells , cartilage cells, fat cells, muscle cells, connective tissue cells.

- Stem cells are thought to reside in a specific areas of each tissue where they may remain quiescent (non-dividing) for many years until they are activated by **signals** e.g. disease or tissue injury.


Blood-forming stem cells removed from donor
Stem cells
Apheresis machine
Patient receives treatment to kill cancer cells and prepare body for donor stem cells
Chemotherapy
Patient receives donor stem cells
Stem cells

Cord blood stem cells

- Umbilical cord blood was once discarded as waste material but is now known to be a useful source of blood stem cells.

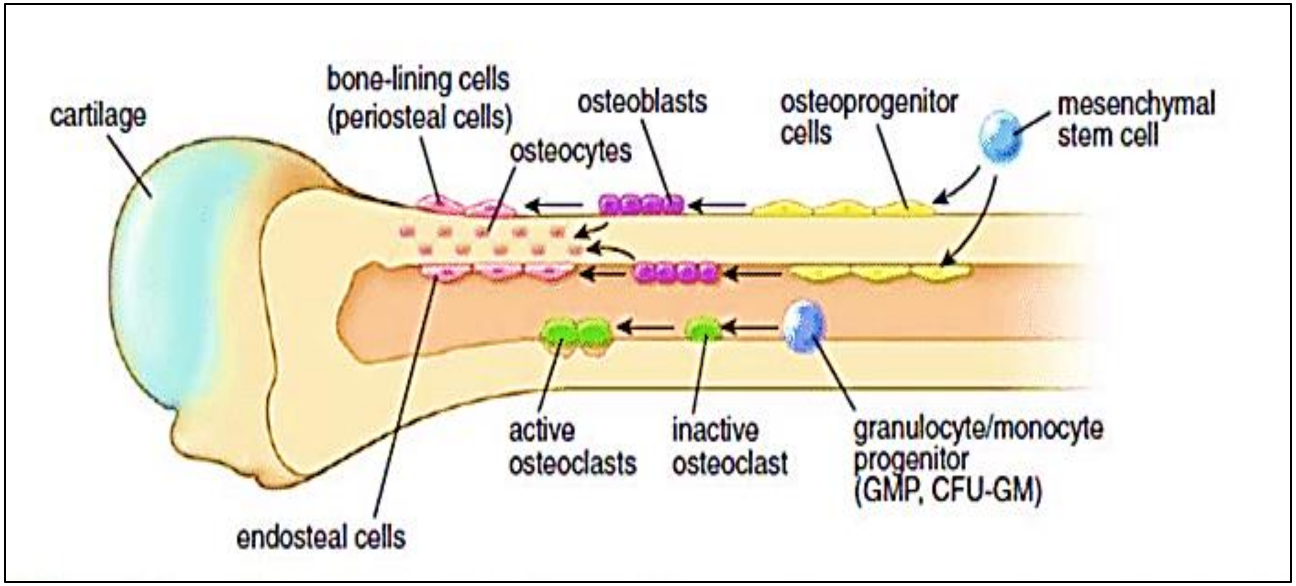
- After a baby is born, cord blood in the umbilical cord & placenta is relatively easy to collect it contains **Hematopoietic (blood) stem cells** (will give rise to red cells, white cells, platelets)

- is used to reconstruct bone marrow following radiation treatment in various blood cancers, and for various forms of anemia

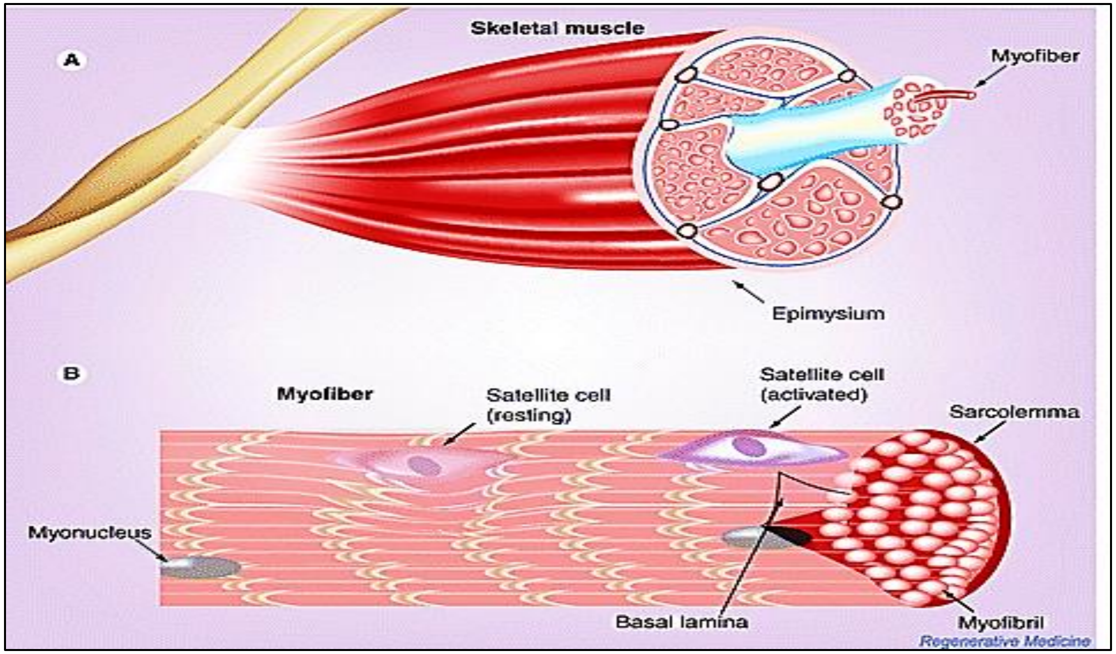
- Adult stem cells main role is to replace cells in case of tissue injury, damage or dead cells

- Other examples of adult stem cells:

- ✓ **Satellite cells** found in muscle→ myogenic progenitor cells → skeletal muscle cells (Myofibers)
- ✓ **Periosteum** contains progenitor cells that develop into osteoblasts (bone cells)



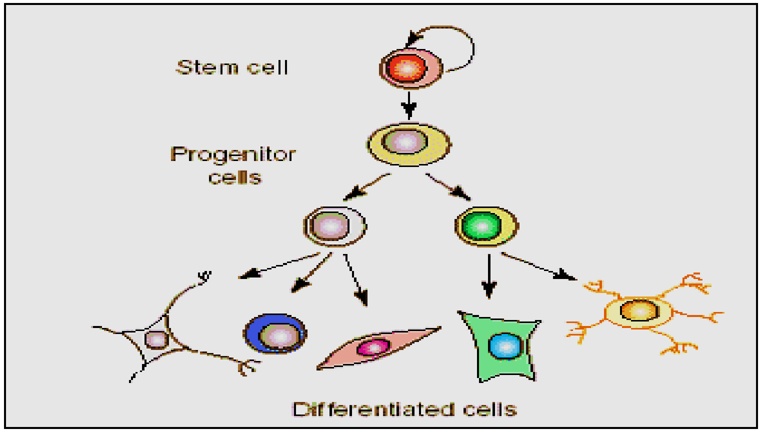
Stem cell in periosteum of bone



Satellite cells in perimysium of muscles

Committed progenitor cells

They arise from stem cells but they are more differentiated and specific than stem cells

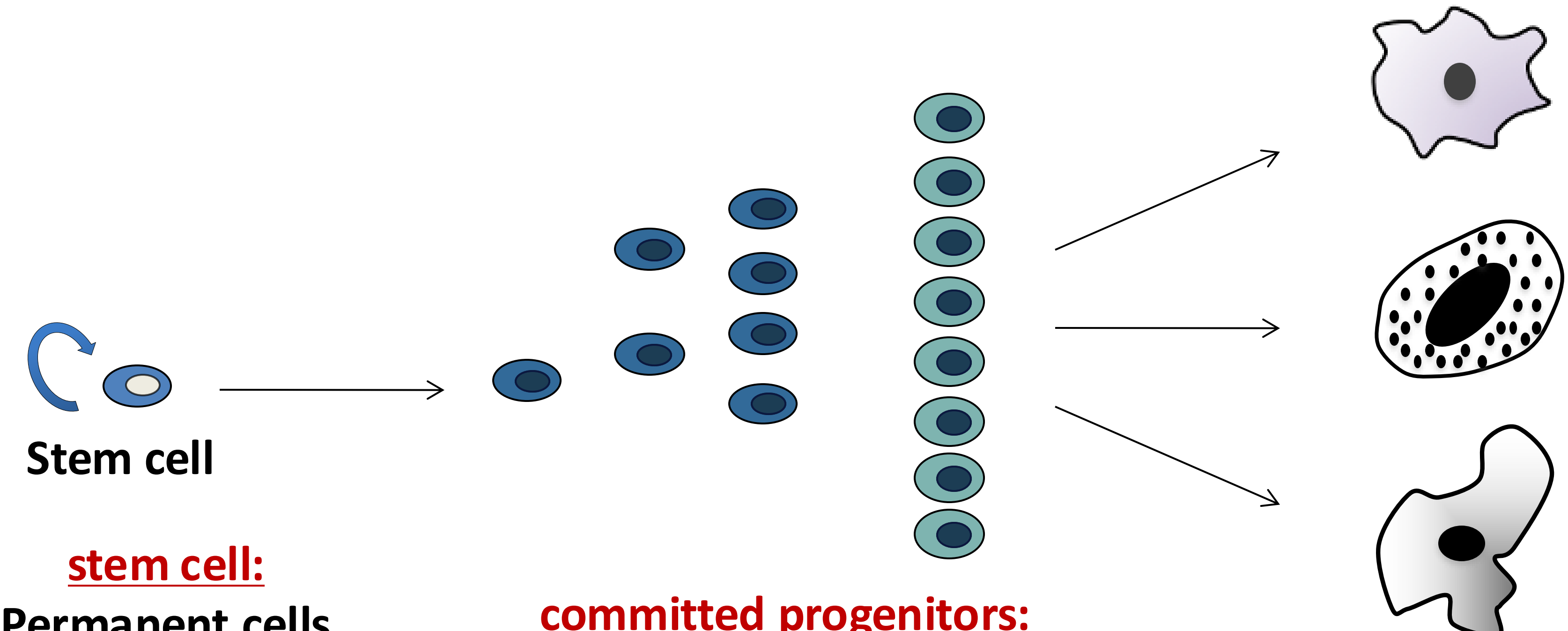


Each progenitor cell is only capable of differentiating into cells that belong to the same tissue or organ.

Genetic & environmental factors (niches) determine the pathway of differentiation that the progenitor cells will take it to form a specific linage. The committed progenitor remain **dormant** in the tissue till need.

- The most important difference between **stem cells** and progenitor cells is that:
  - They are already more specific than stem cells
  - can only be pushed to differentiate into its "target" cell
  - they can divide only for limited number of times

Committed progenitor cells



Stem cell

stem cell:
Permanent cells
high potency
Indefinite self renew

committed progenitors:
- Temporary cells
-Divide rapidly
- limited potency (multipotent)
-limited self-renewal

specialized cells
- Function
- No self renewal

Example:

Hematopoietic stem cell → form populations of progenitor cells which are committed to the main marrow cell lines:
erythroid, granulocytic and monocytic, megakaryocytic, and lymphocytic.

Induced pluripotent stem cells (iPSC)

- iPSC technology is a huge discovery (2006) → NP 2012
- *Concept*: mature cells can be reprogrammed to become pluripotent e out need for embryo
- *Technique* : done by introduce a few specific pluripotency genes into already specialized somatic cells (Ex: ms cells) → the cells will be reprogramed into an embryonic –like state (reset the somatic cell)
- *Goal*: **regenerative medicine** ….. To replace damage tissue in a given person by using pluripotent stem cells from his own body, not only the patient will **get the new tissue** he needs but also with **NO immune- rejection complications**

Induced pluripotent stem cells (iPSC)

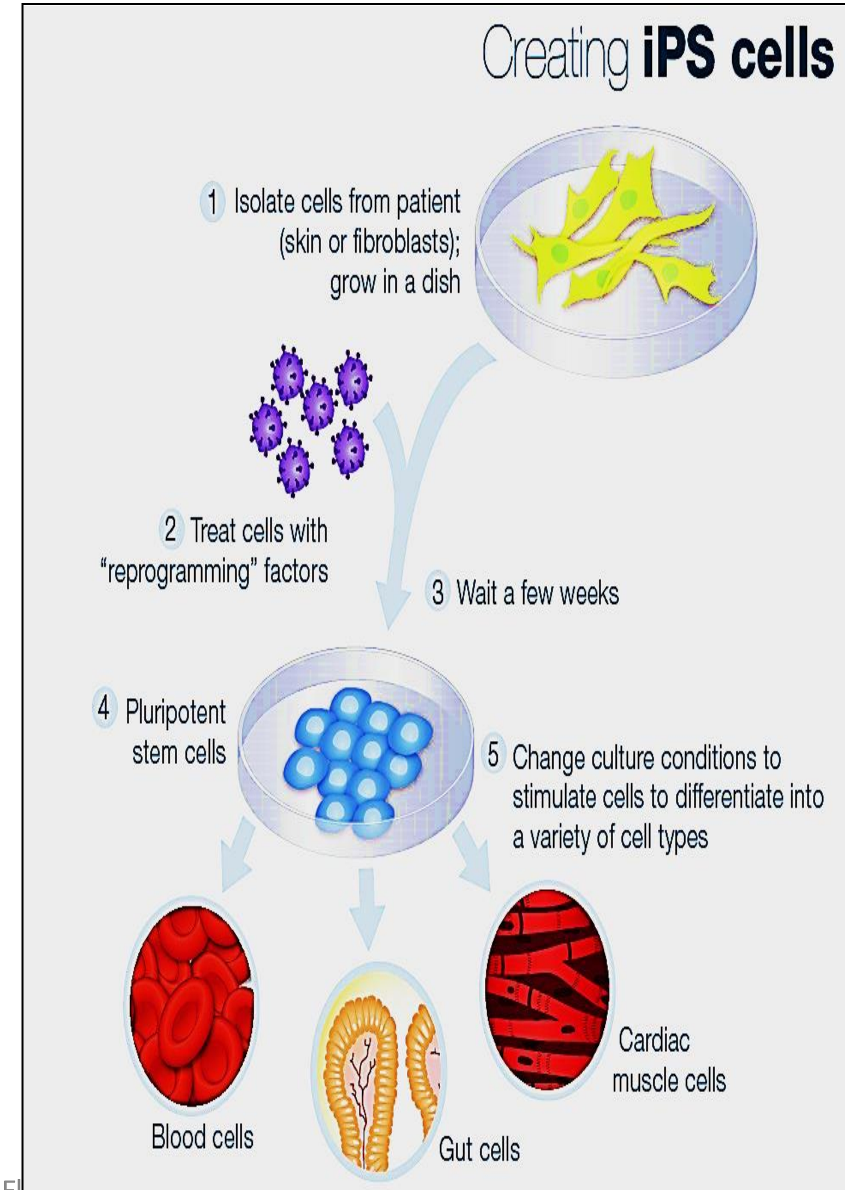
1st isolate and culture skin cells from a patient.

2nd introduce a group of pluripotency genes into the skin cells by using an engineered virus carrier.

The expression of these genes regenerates the stem cell phenotype.

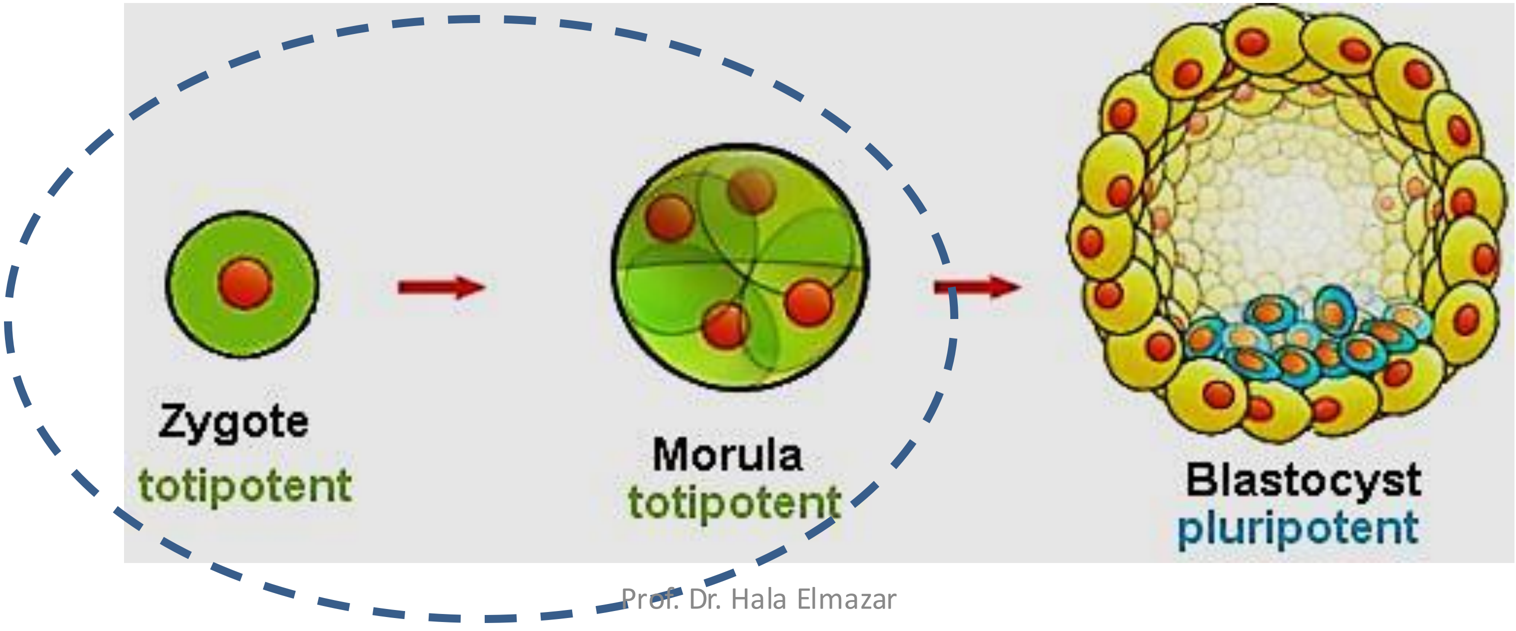
i.e. self renew & differentiate

The viruses simply deliver the genes of interest and are themselves engineered not to be harmful.

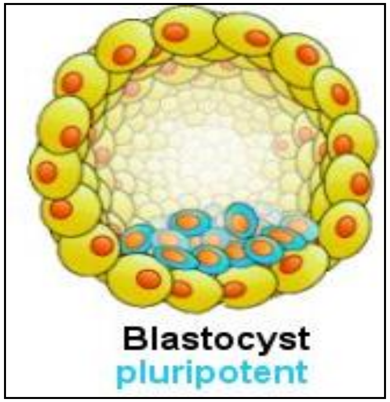


Potency: Cell's ability to differentiate into other cell types

Totipotent: can give rise to an entire functional organism {cells from early embryo (1-3 days)}



Pluripotent: can give rise to all types of specialized cells in the body (ESC: 5- 14 days) (form > 200 cell types)

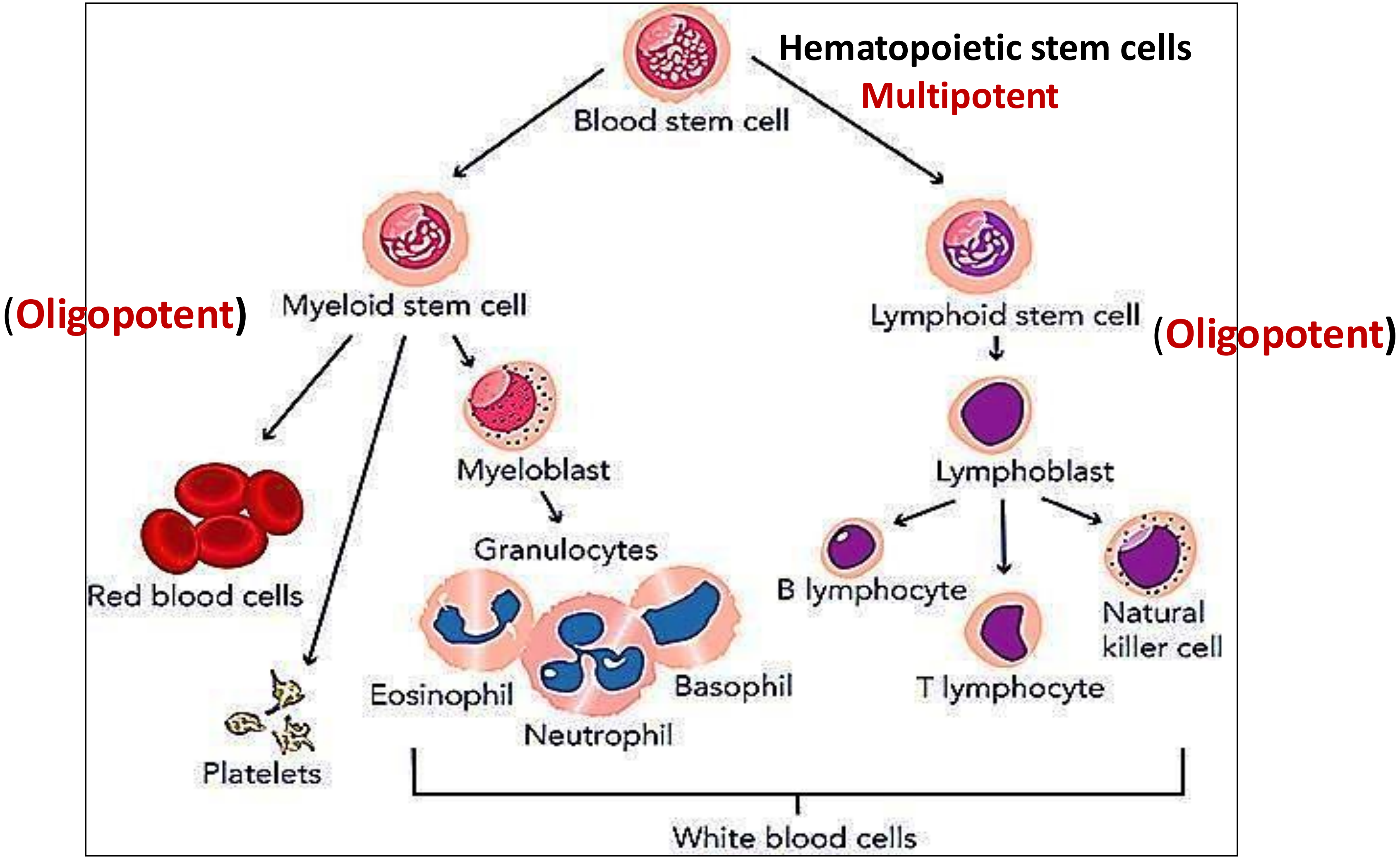


Multipotent: can give rise to multiple types of specialized cells, but not all cell types (Adult SC in tissues & hematopoietic SC in cord blood)

Oligopotent: can differentiate into few cell types e.g. lymphoid or myeloid stem cells

Unipotent: can give rise to only one type of cells.
e.g. B-lymphocyte → plasma cells
Monocyte → macrophages

Nulipotent: Terminal cells



Myeloid and lymphoid stem cells

Cloning

- The process of producing a population of genetically identical individuals (exact genetic copies)

- There are 2 types:

Reproductive cloning:

➢ Hit the headlines in the late 1990s when **'Dolly the sheep'** was cloned.

➢ It was the first mammal ever to be cloned.

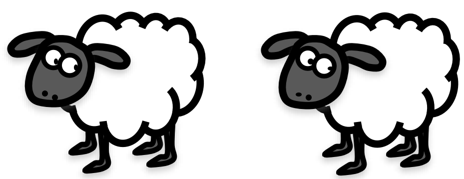
Molecular cloning:

➢ A technique used to help scientists investigate what particular genes do and how they work.

Cloning

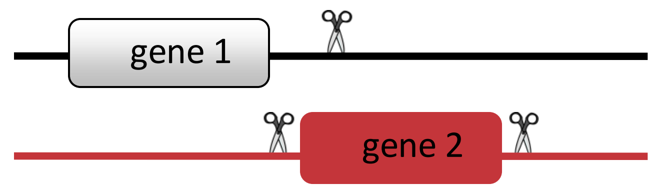
There are two VERY different types of cloning:

Reproductive cloning



- Use to make two identical individuals
- Very difficult to do
- Illegal to be done on humans

Molecular cloning



- Use to study what a gene does
- Routine in the biology labs

Reproductive cloning

To make Dolly, scientists done what so called **somatic cell nuclear transfer (SCNT)**

Somatic cell: somatic cell is any cell in the body **other than** sperm & egg. Somatic cell has the 2 complete sets of chromosomes (46 = 23pairs)

Nuclear : nucleus holds DNA which contains all the information needed to form an organism

Transfer: moving an object from one place to another

Technique

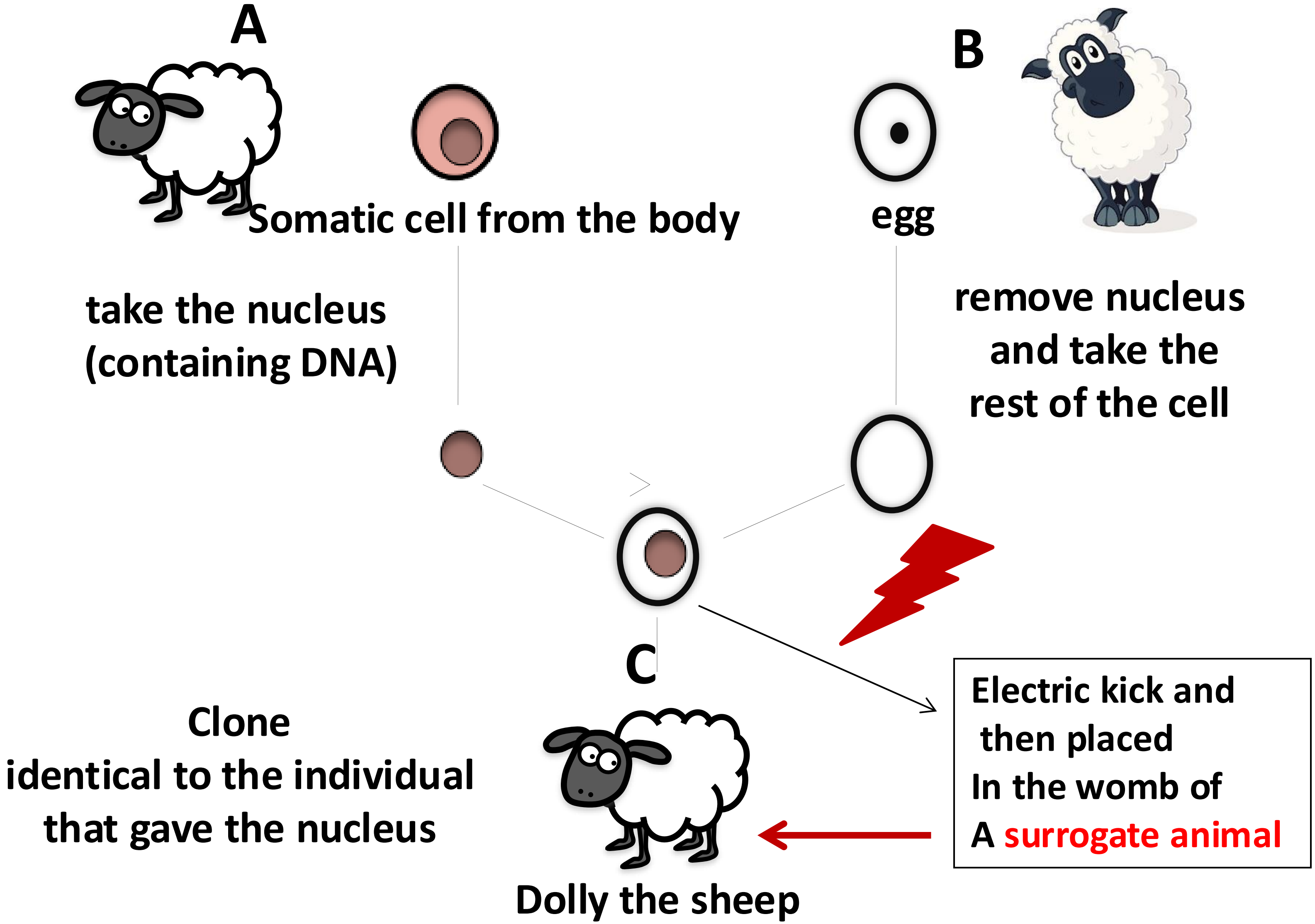
- They took the nucleus out of a normal somatic cell from a sheep (original).
- They put that nucleus into an egg cell of another sheep that had no nucleus.
- They then had a new cell.
- To make the new cell start to divide and grow, they gave it an electric shock.

- Then it started to divide and develop into an embryo. When it had grown into a very early stage embryo called a blastocyst

- it then was implanted into the womb of another sheep so that it could grow into a lamb and be born.

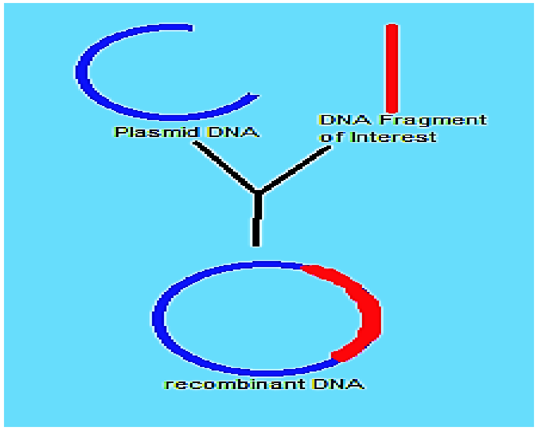
- The new sheep is a clone of the sheep that donated the somatic cell. Both sheep have the same DNA

Reproductive cloning



Molecular cloning

- A process used by scientists to make copies of a specific gene or genes.
- Isolation of a DNA sequence (gene) from any species, and its insertion into a vector for propagation, without alteration of the original DNA sequence of the vector
- Also called **recombinant DNA cloning**

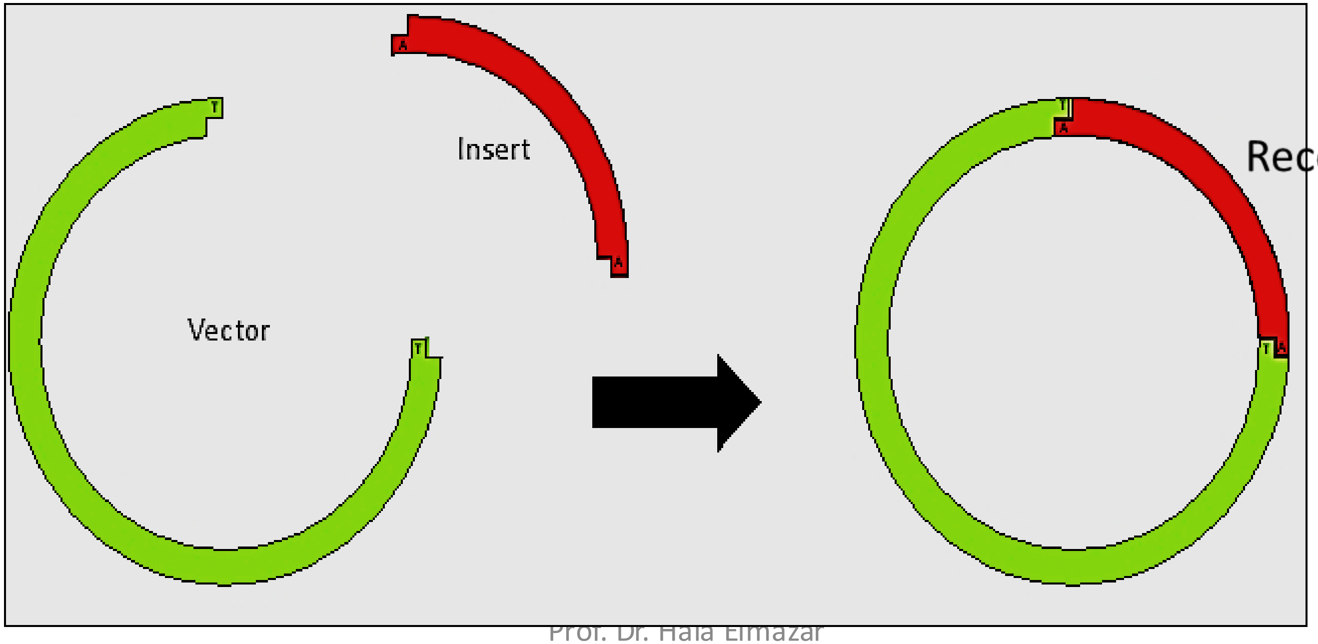


Recombinant DNA

DNA molecules formed by laboratory methods of genetic recombination to bring together genetic material from multiple sources →creating new DNA sequences

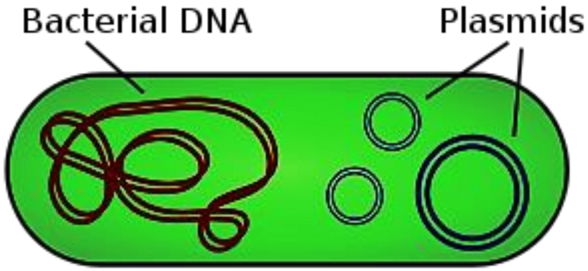
- Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure (the genetic code is universal) .

- Construction of recombinant DNA, involves insertion of a foreign DNA fragment into a plasmid vector.

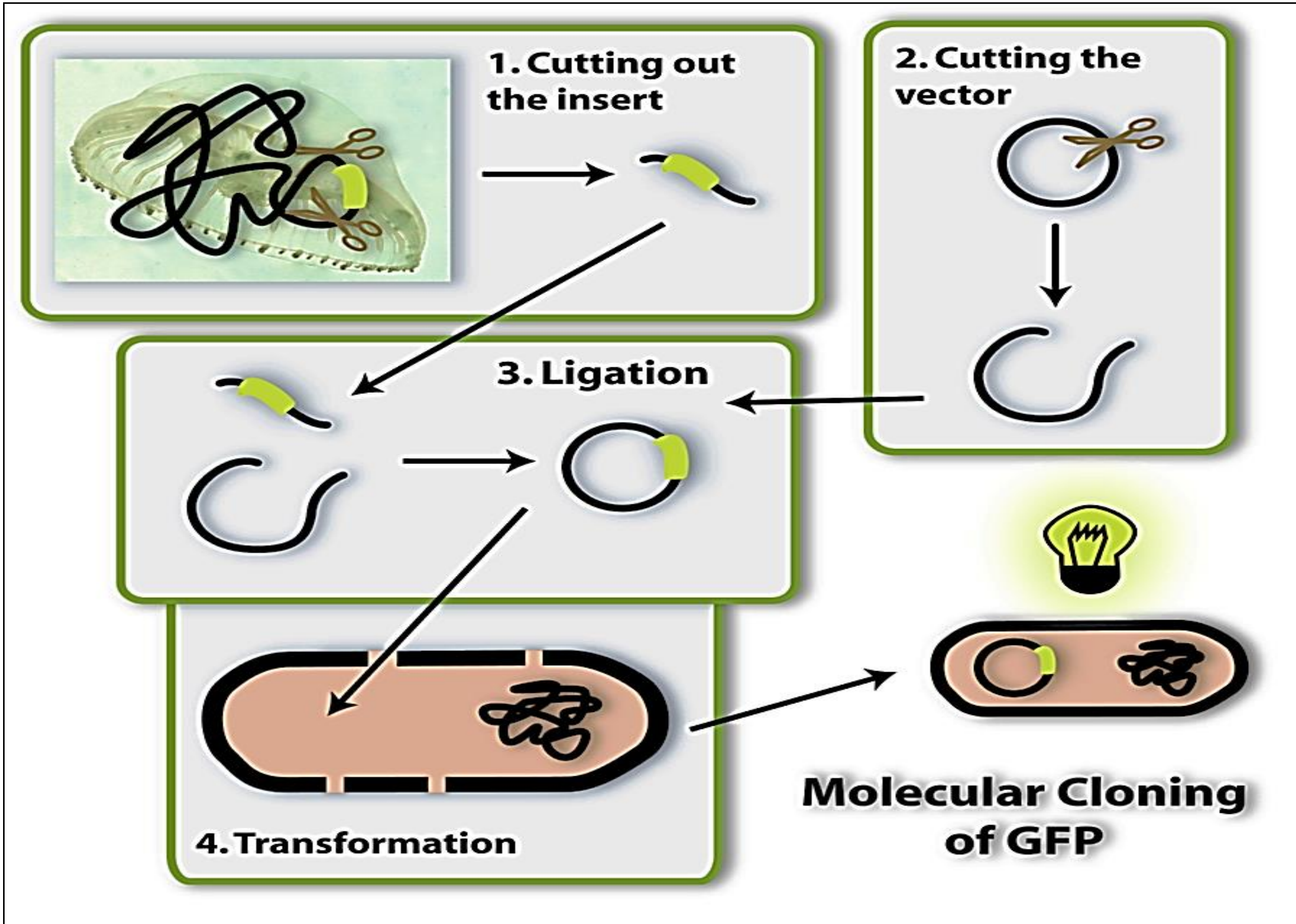


- **Cloning vector**: A cloning vector is a small piece of DNA (taken from a virus, a plasmid) into which a foreign DNA fragment (insert) can be inserted for cloning purposes

- Ligation of inserts into an appropriate cloning vector, will create recombinant molecules (e.g., plasmids)



- **Plasmid**: circular double stranded DNA molecule within a bacterial cell (physically separated from chromosomal DNA) & can replicate independently



Molecular Cloning

Technique

1- Select the DNA molecule to be cloned (insert)

2- Select DNA molecule that will serve as a vector (virus /plasmid)

3- cleave the vector DNA strand with **Restriction endonuclease**, then insert foreign DNA → recombinant DNA molecule

4- Introduction of recombinant DNA in host cells → transformation

5- When the cell divides, it makes copies of itself. Each new daughter cell contains an exact copy of the new DNA (cloned DNA)

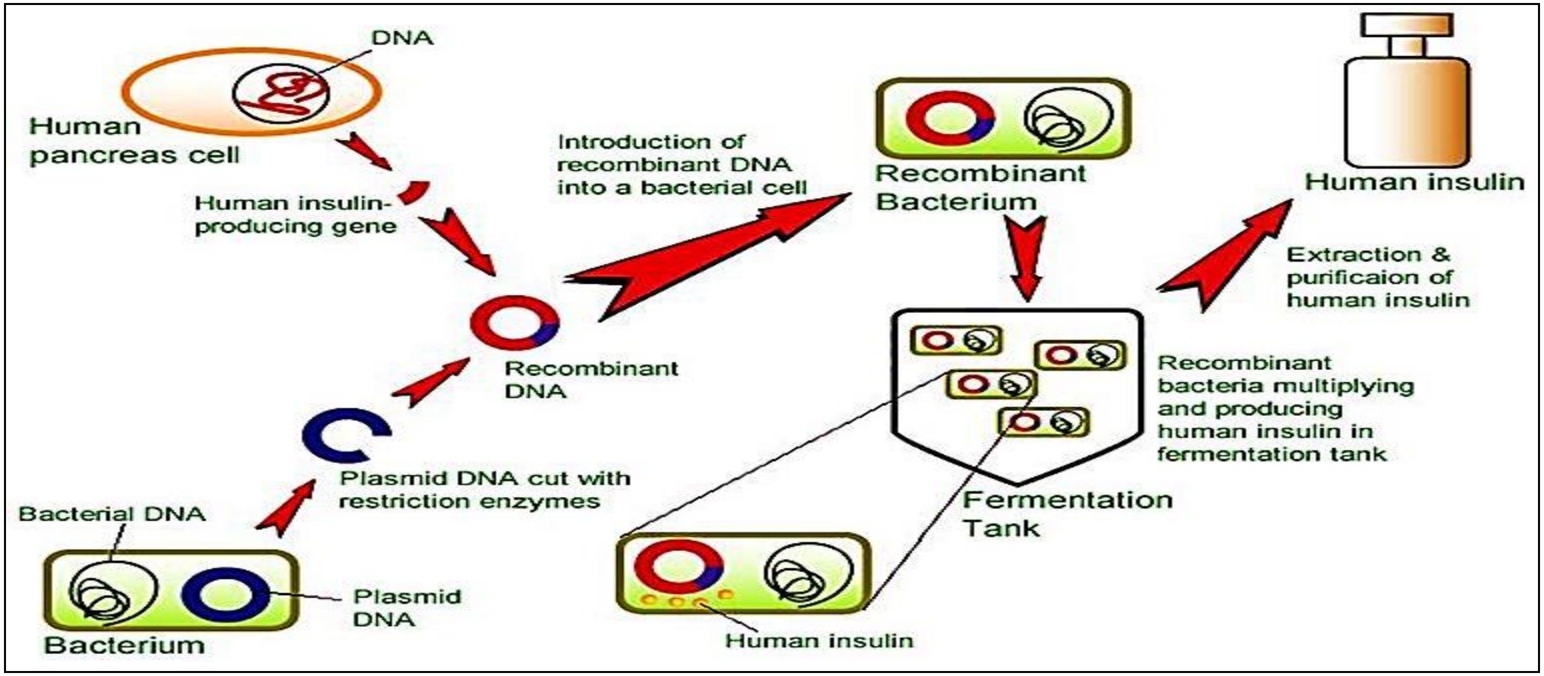
6- Selection & screening of colonies with desired DNA

Applications of molecular cloning:

1- Insulin production: we use the bacteria to be human insulin factories

Why bacteria?

- Contain plasmids
- Are unicellular and reproduce asexually → quick clones



2- Study genome organization and gene expression

A- Loss of function (gene knockout):

A genetic technique in which a gene is removed or blocked so that *it does not work*, used in learning about a gene that has been sequenced but has an unknown or incompletely known function

B- Drug development : utilized to screen drugs for efficacy & toxicity

C- Gene therapy: involves supplying a functional gene to cells lacking that function, with the aim of correcting a genetic disorder or acquired disease

Thank you