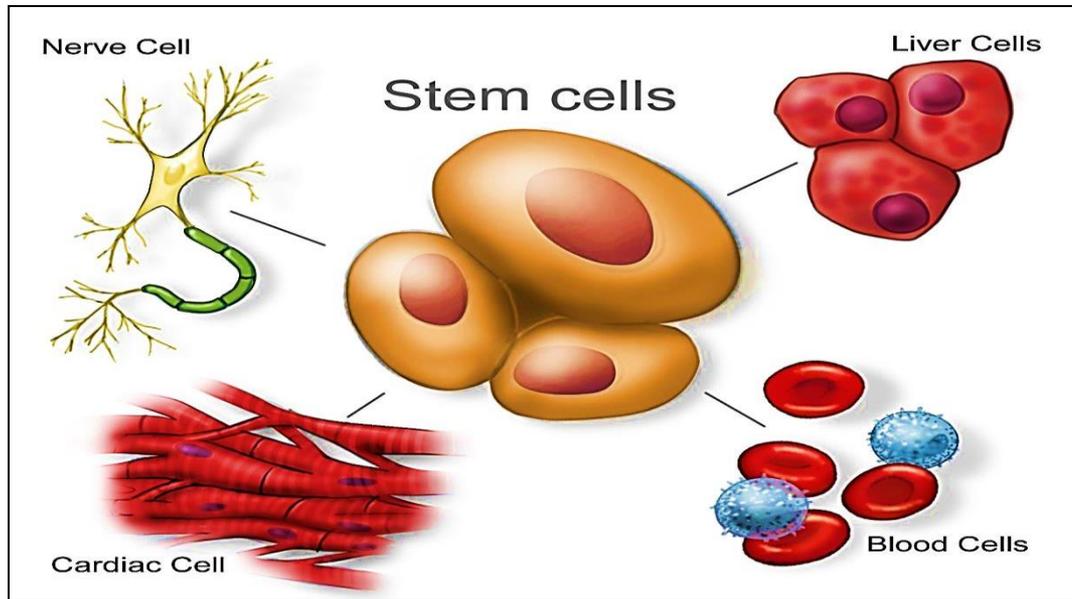


Cell Bio

Introduction to stem cell



Stem cells have the potential to change life as we know it

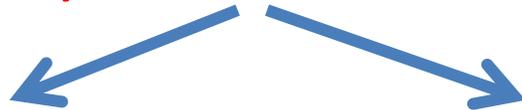
What is a stem cell?

“Any time you have healed after an injury it’s a stem cell mediated event”

Stem cells are unprogrammed cells that are:

- Capable of dividing and renewing themselves (**Self-renew**) for long periods of time i.e. Proliferation
- Have the potential to give rise to specialized cell types i.e. Differentiate
- Stem cell is **unique** because it Can do both:

Self-renew

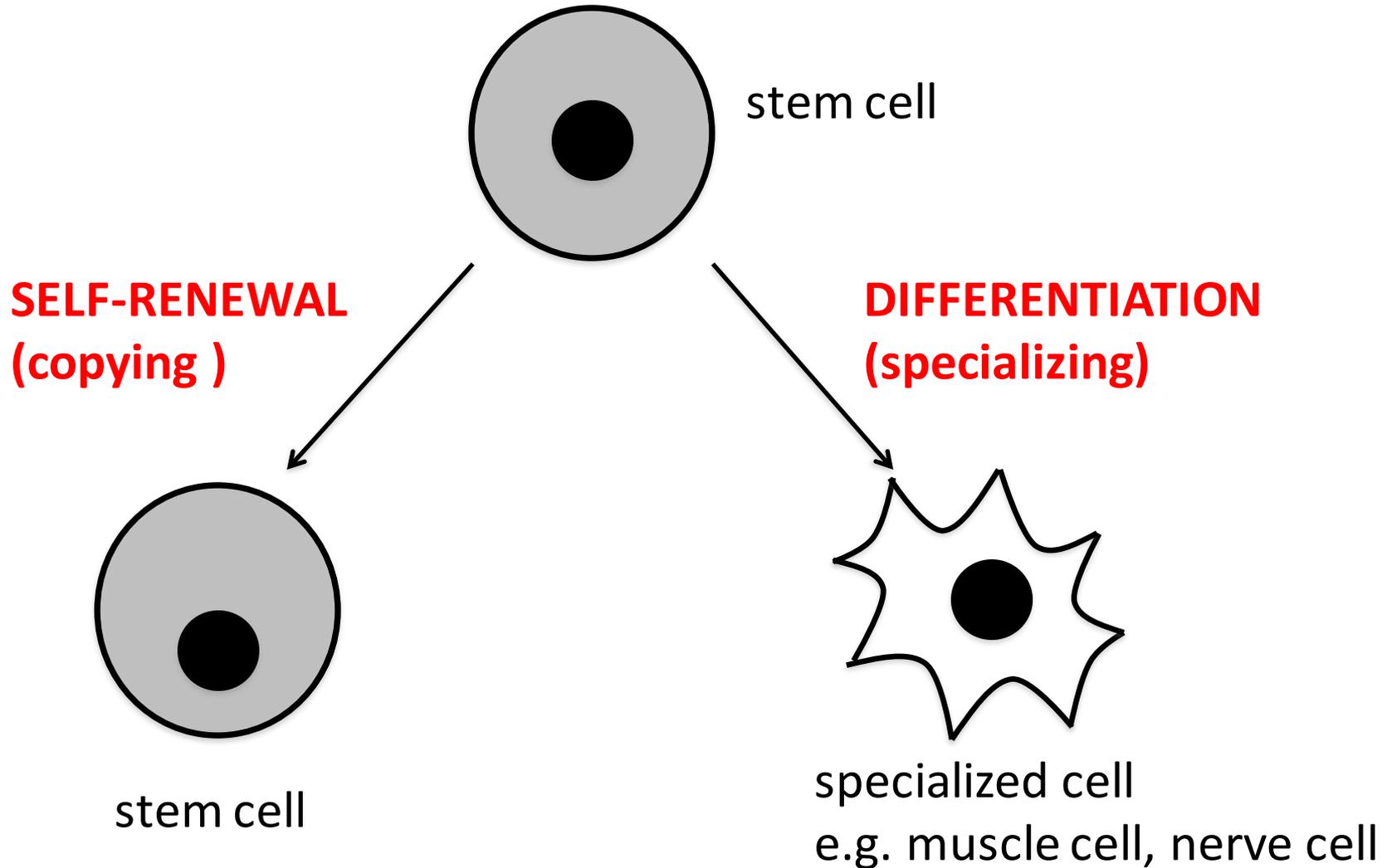


Differentiate

Make copies of itself

Make other types of cells
(specialized cells of the body)

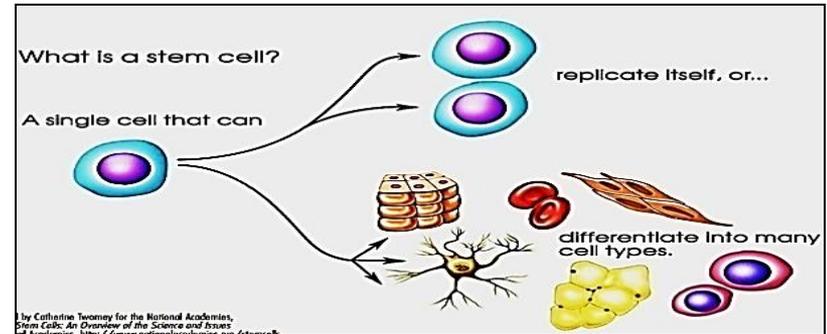
What is a stem cell?



Why self renew & Why differentiate?

A- Self renewal

- Because if they didn't copy themselves, they would **finish** quickly.
- It is important for the body to **maintain a storage** of stem cells to use throughout your life.



B- Differentiation

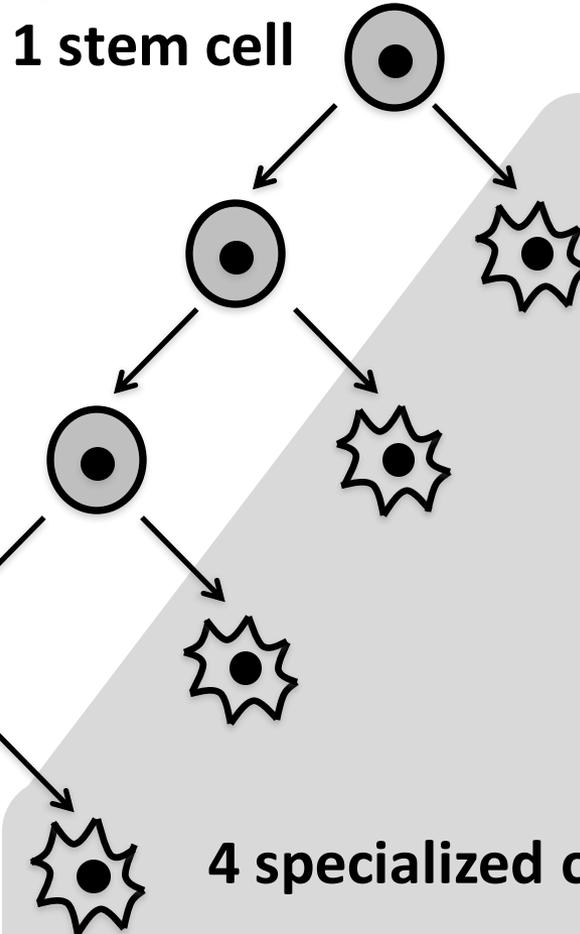
- Specialized cells are mature cells **cannot divide** or make copies of themselves, so if they damage or die they need to be replaced so the body can keep on working
- *Specialized* or *differentiated* cells have **particular roles (Functions)** in the body e.g. blood cells, nerve cells, muscle cells

- There are 2 theories for stem cell division:

1- Obligate asymmetric stem cell replication

2- Stochastic differentiation

1- Obligate asymmetric stem cell replication



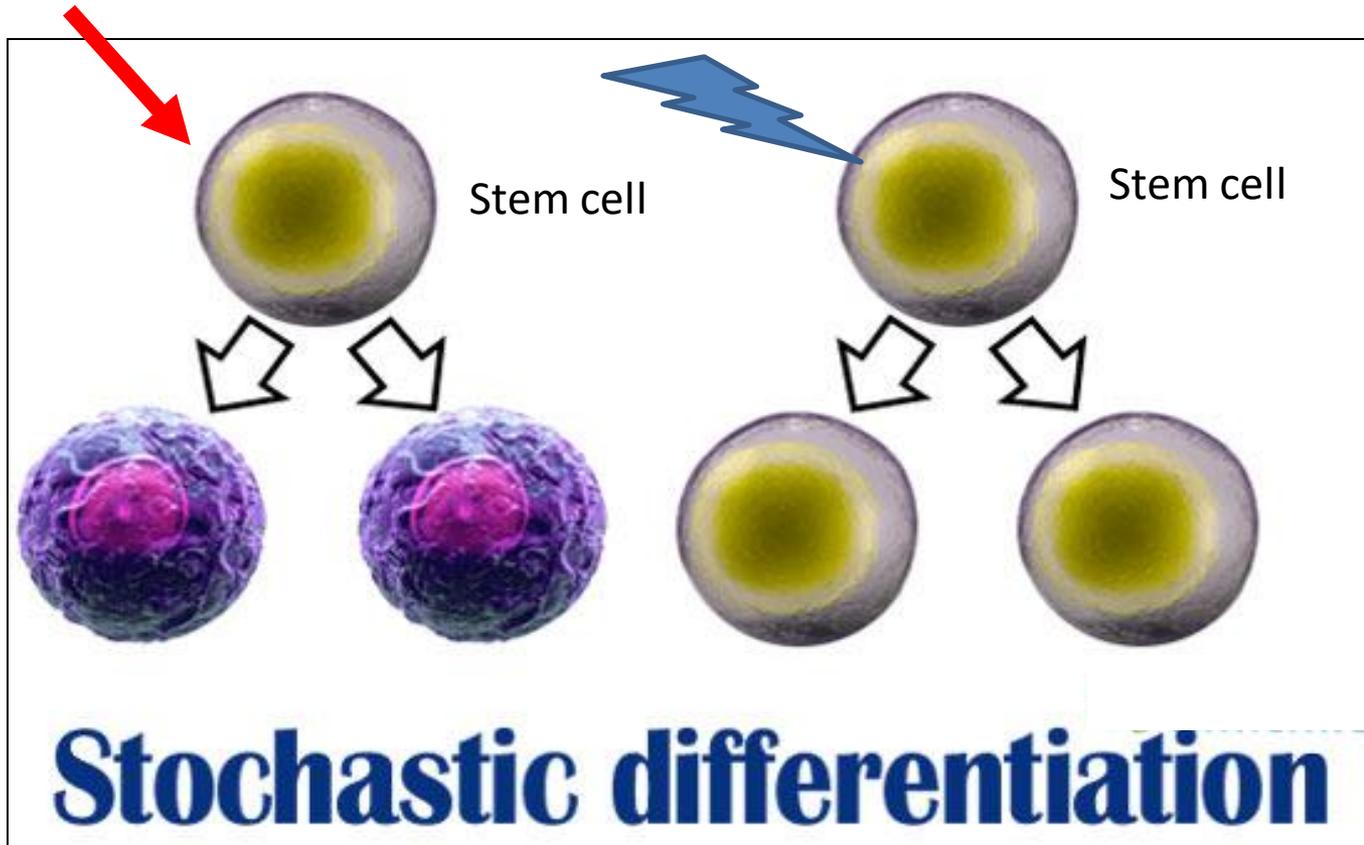
1 stem cell 

Self renewal - maintains the stem cell pool

4 specialized cells

Differentiation - replaces dead or damaged cells throughout your life

2- Stochastic differentiation

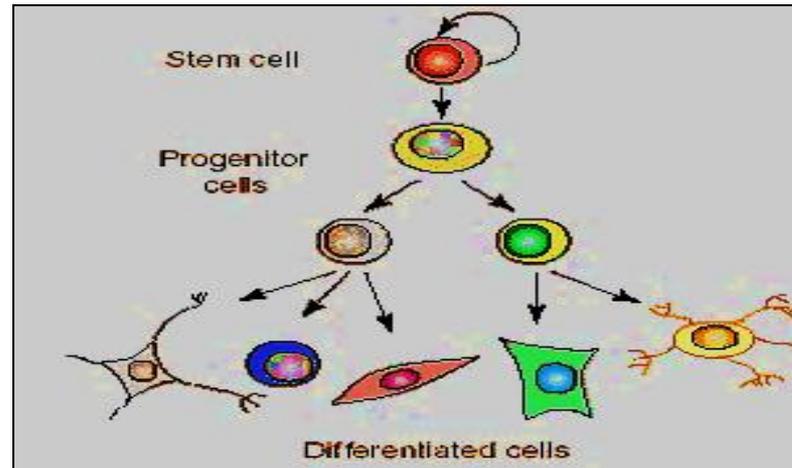


If one stem cell differentiate into 2 specialized cells another stem cell will notice that and make up for the lost stem cell and divide by mitosis to produce 2 identical stem cells

progenitor (intermediate) cells

Cells that are at a stage between stem cells and mature specialized cells.

- They are yet not fully programmed, somehow more mature than a stem cells.
- They have less capacity to self-renew themselves than a stem cells



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

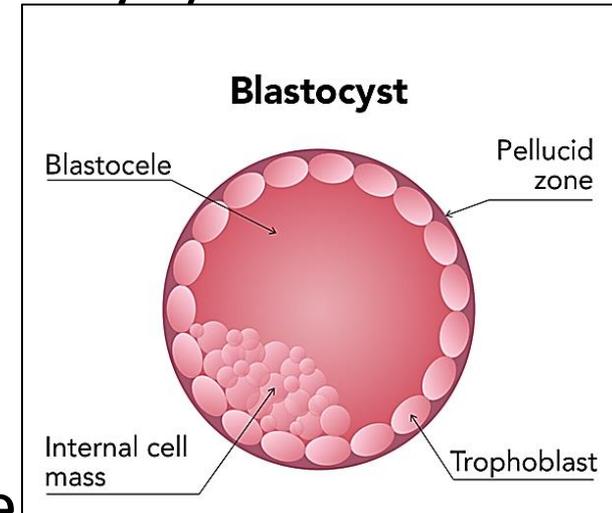
Blastocyst: a very early stage of the embryo life that has about 50 to 100 cells

Tissue (adult /somatic)stem cells

Small populations of cells that are found in the 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 build 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)

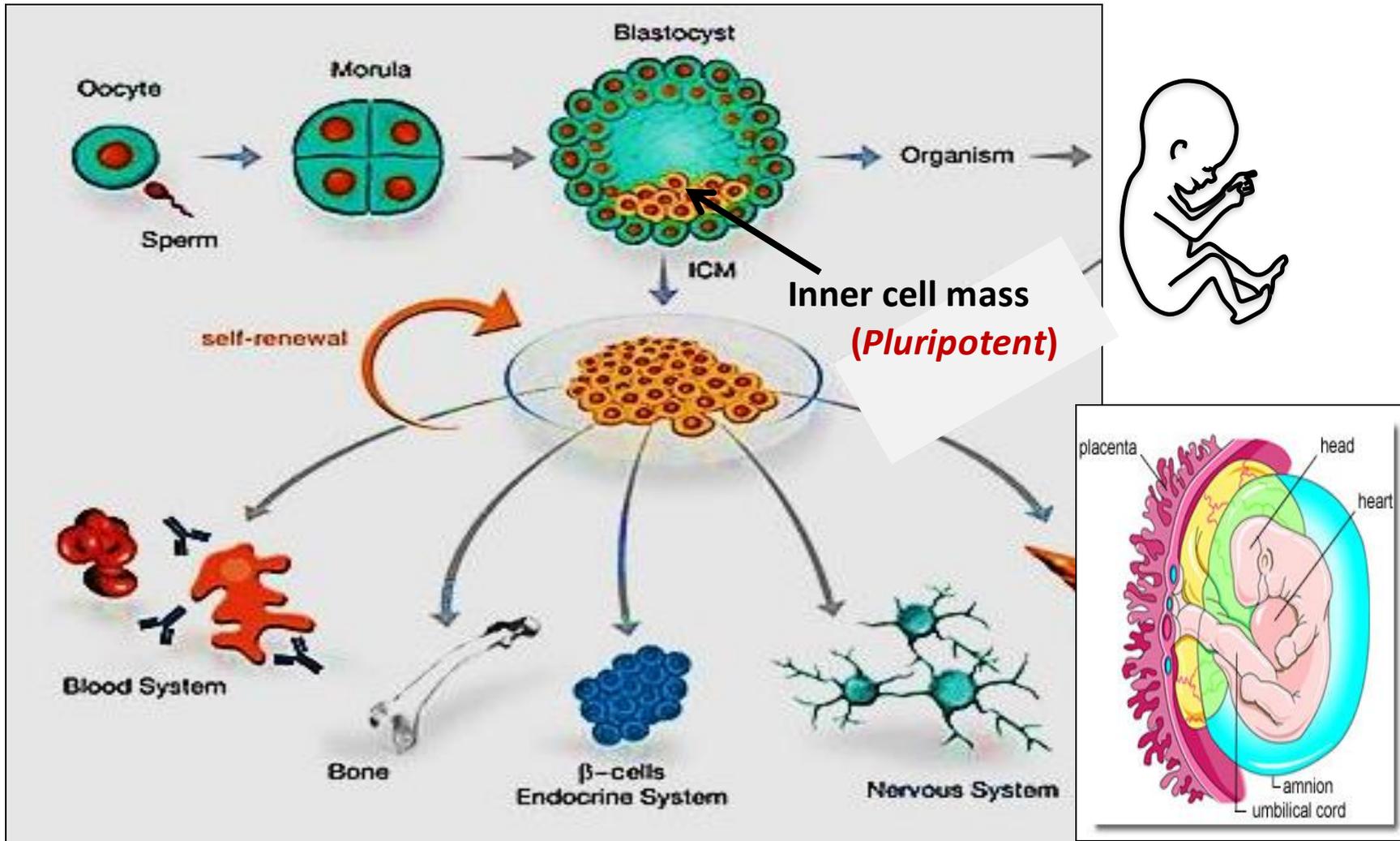


- Human embryos reach the blastocyst stage 4-5 days post fertilization (consists of **50–150 cells**) it is the stage at which implantation on the wall of the uterus occurs
- ES of the inner cell mass are pluripotent.

Pluripotent:

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

Embryonic stem cell

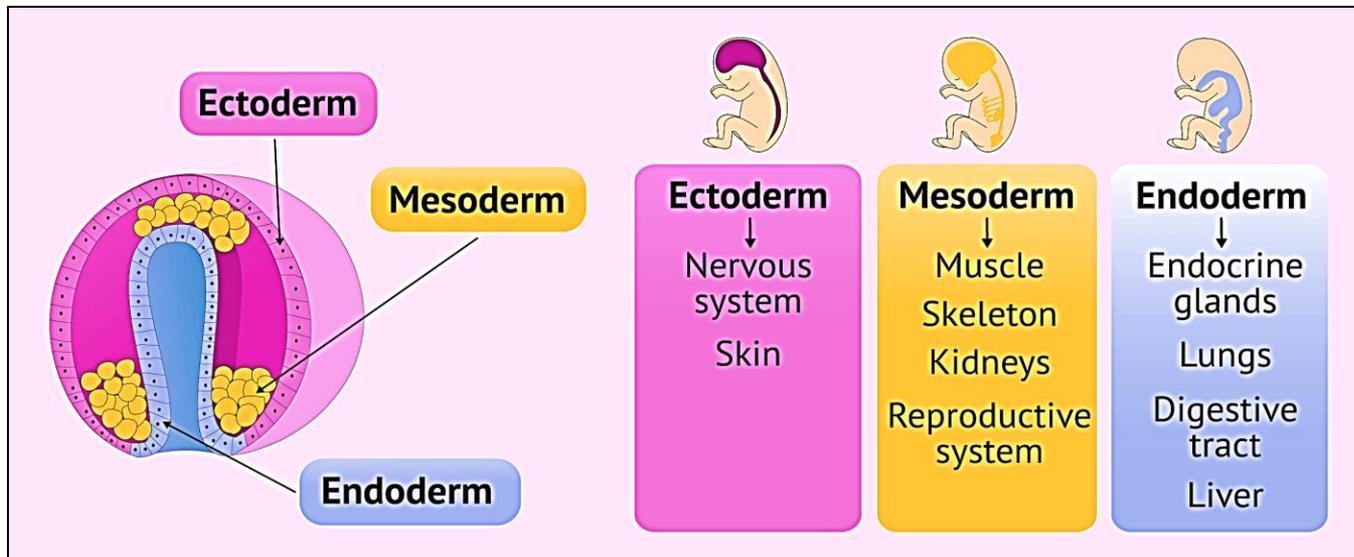


ES cells don't contribute to the extra-embryonic membranes or placenta

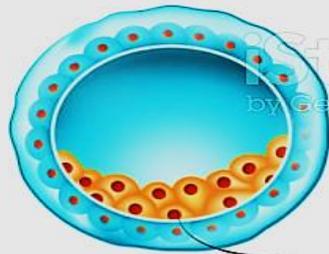
- Few weeks later after implantation the cells will organize into the 3 primary cell layers → germinal layers →

Ectoderm, Mesoderm, Endoderm

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



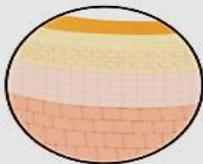
Embryonic stem cells



ECTODERM

MESODERM

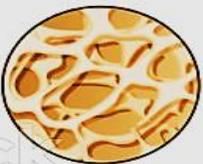
ENDODERM



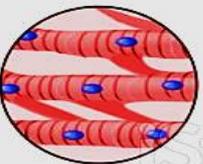
Skin



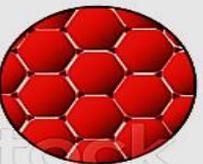
Nerves



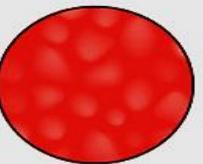
Bones



Muscles



Liver



Thyroid

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

The cells in each germ layer differentiate into tissues and organs

- **Ectoderm** → skin, nervous system, & parts of head & neck
- **Mesoderm** → muscles, blood, blood vessels, & beginning of bone & connective tissue
- **Endoderm** → digestive, respiratory tracts, pancreas & liver

Embryonic stem cell research

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

1. Destruction of embryo
2. Rejection due to different genetic background
3. Changed into tumor cells. Once they put in the body they can never be taken out

GERM LAYERS

ECTODERM

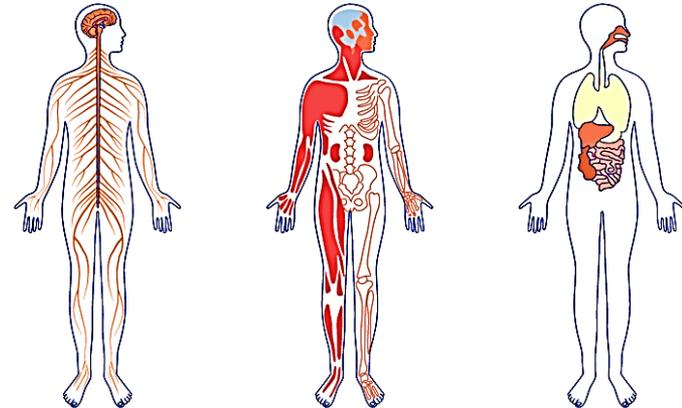
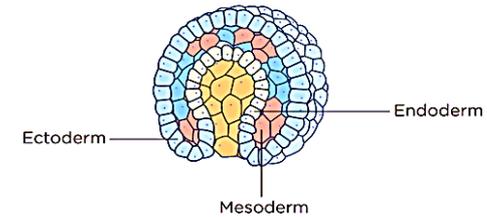
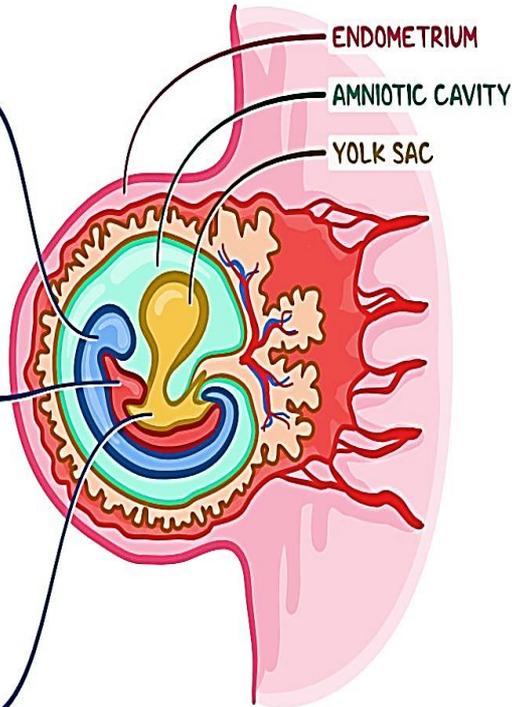
- Adrenal medulla
- Central & peripheral nervous systems
- Skin
- Appendages

MESODERM

- Kidneys & ureters
- Reproductive system
- Bone & cartilage
- Muscles
- Vasculature
- Lymphatics
- Spleen
- Adrenal cortex

ENDODERM

- GI tract
- Liver
- Endocrine system
- Respiratory tract
- Bladder
- Urethra



Ectoderm

- Epidermis of skin and its derivatives (including sweat glands, hair follicles)
- Epithelial lining of mouth and anus
- Cornea and lens of eye
- Nervous system
- Sensory receptors in epidermis
- Adrenal medulla
- Tooth enamel
- Epithelium of pineal and pituitary glands

Mesoderm

- Notochord
- Skeletal system
- Muscular layer of stomach and intestine
- Excretory system
- Circulatory and lymphatic systems
- Reproductive system (except germ cells)
- Dermis of skin
- Lining of body cavity
- Adrenal cortex

Endoderm

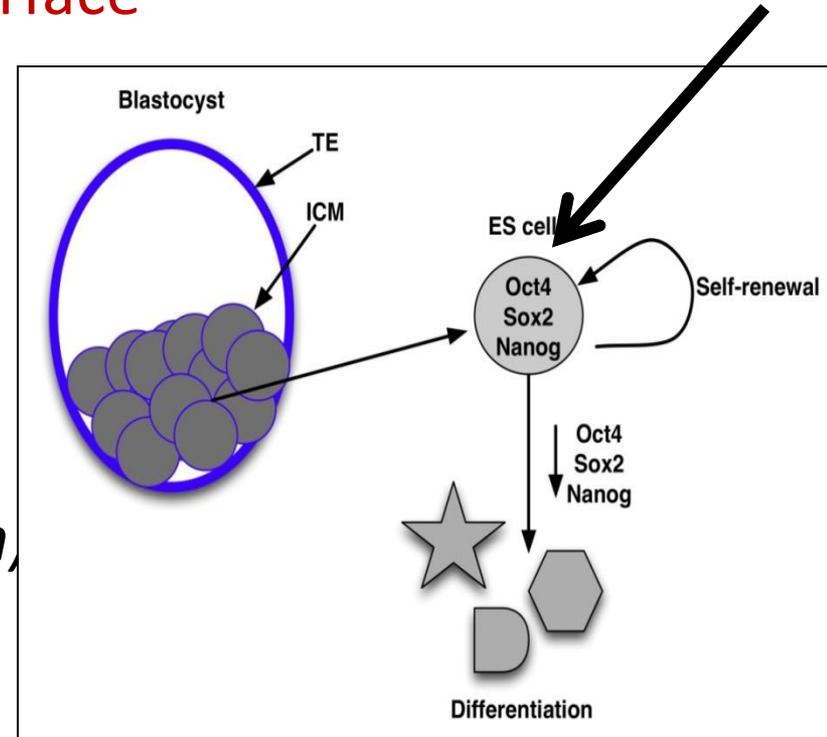
- Epithelial lining of digestive tract
- Epithelial lining of respiratory system
- Lining of urethra, urinary bladder, and reproductive system
- Liver
- Pancreas
- Thymus
- Thyroid and parathyroid glands

Jack Westin

The three primary cell layers or germinal layers

- Stem cells is surrounded by a special microenvironment **called the stem cell niche**. Niches consist of a multiple factors that can influence stem cell behavior.
- Any human ES cell is defined by the expression of **several transcription factors on its cell surface**

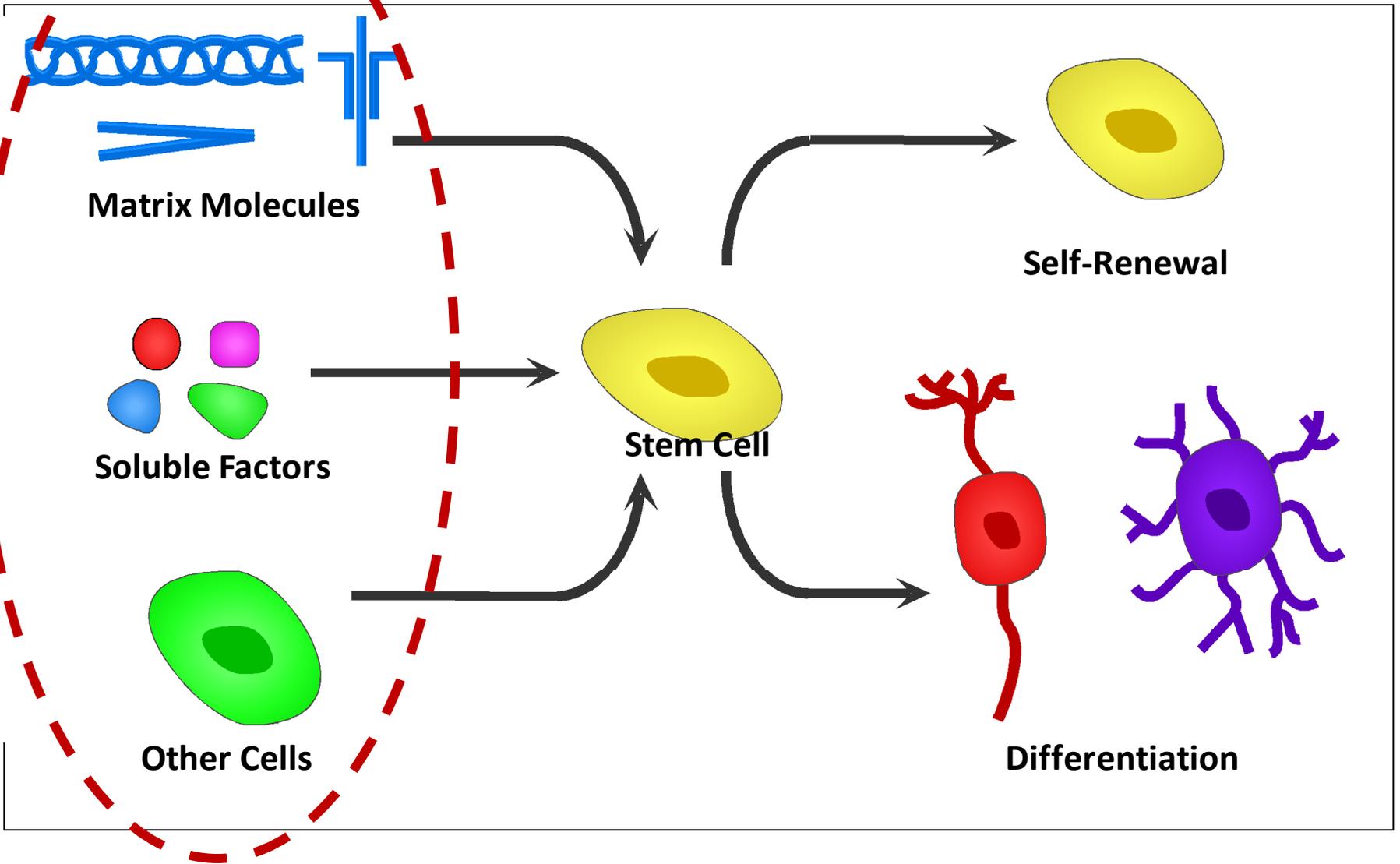
- The transcription factors **Oct-4, Nanog, Sox 2, max, Smad 1, FoxC2**
(Proteins that control rate of Transcription of genetic information,



- 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

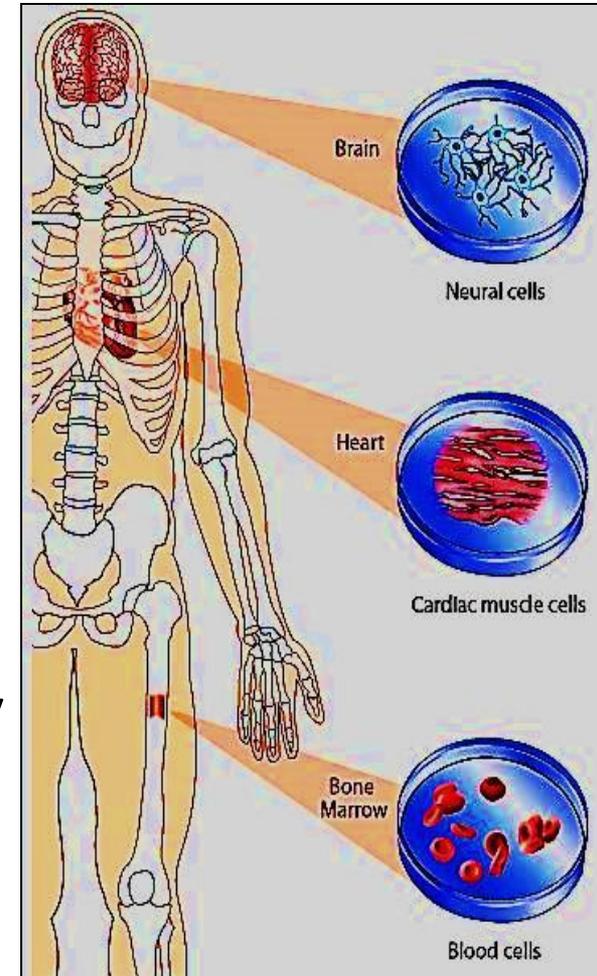
Signals to stem cell

Niches

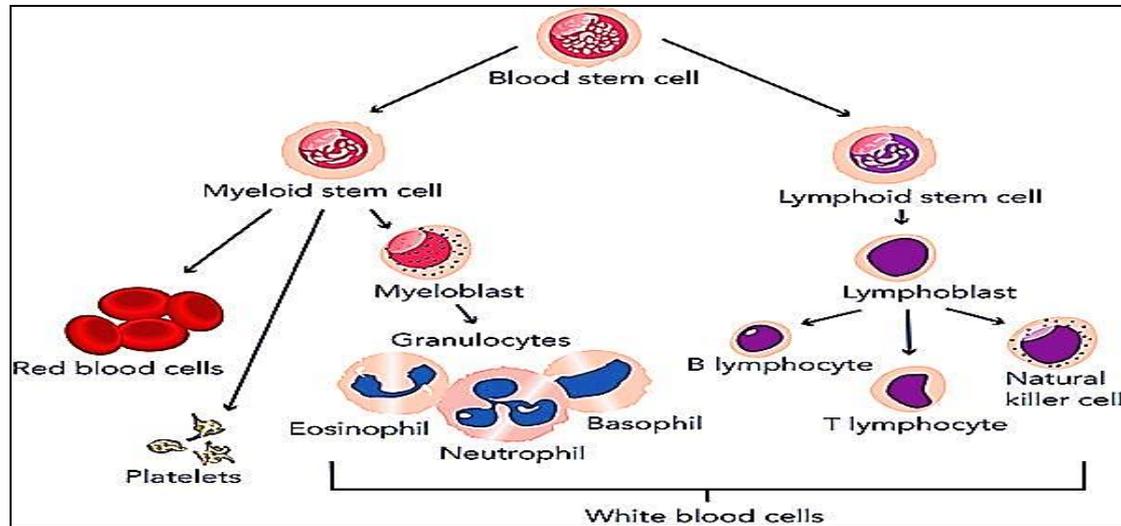


2- Tissue (adult/somatic) stem cells

- Undifferentiated cells, found among the differentiated cells in the tissues & organs . **Used as** internal repair system, regenerating to replace lost or damaged cells for the life of a person.
- They are small in number, have restricted ability to self- renew itself & to differentiate into various types of cells
- Its origin in mature tissue is unknown
- They **differentiate** only to specialized cells similar to cells of the tissue in which they are found
- They are **multipotent**

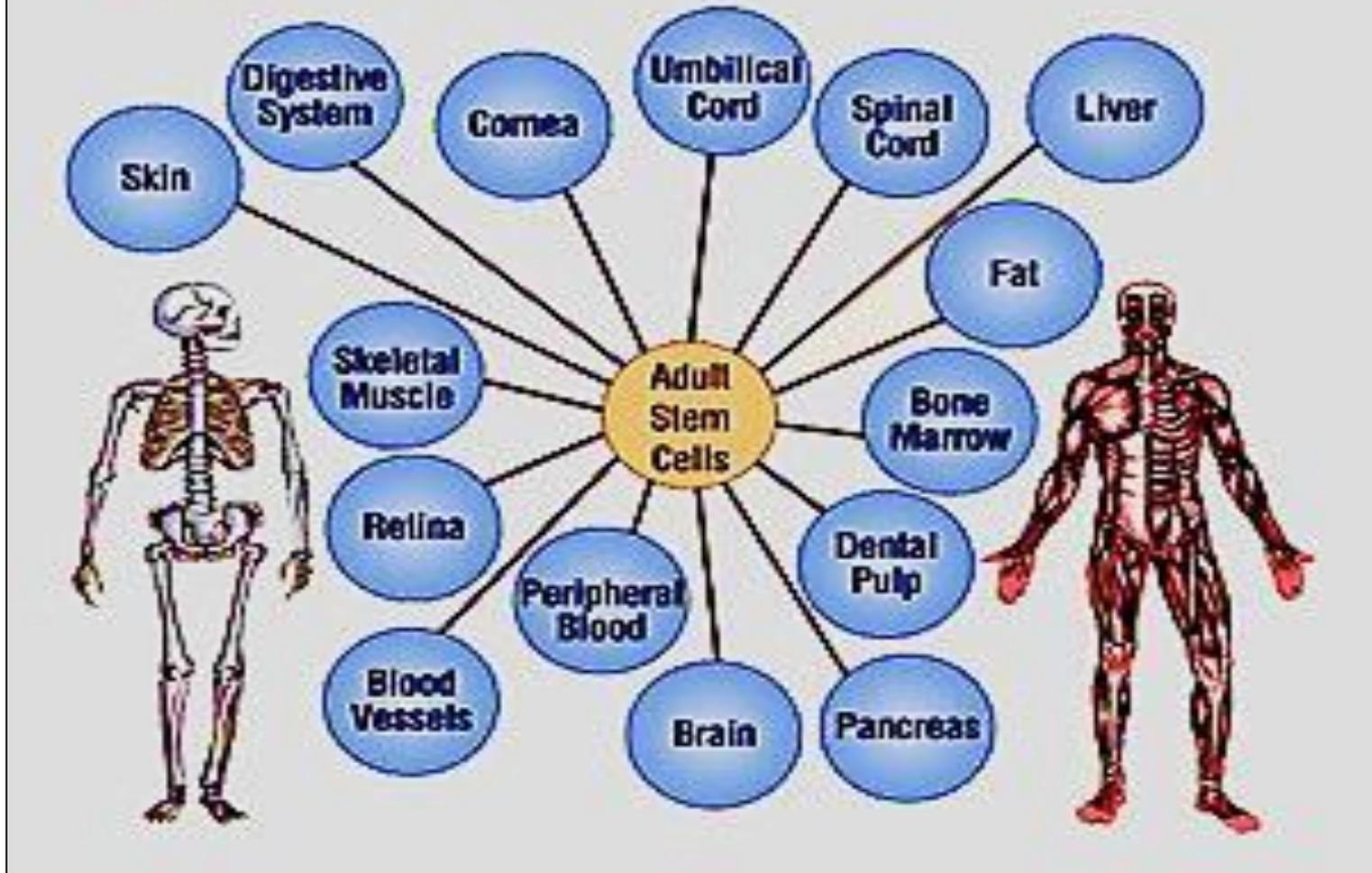


- **Adult stem cells** has been successfully used in **Therapy** for many years to treat **leukemia** and related bone/blood cancers through bone marrow transplants.



- 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

Where are adult stem cells found?



- **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- One is called hematopoietic stem cells, forms all the types of blood cells in the body.

B- The second is called Mesenchymal stem cells (bone marrow stromal cells) that generates bone, cartilage, fat, and fibrous 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.

Allogeneic Stem Cell Transplantation

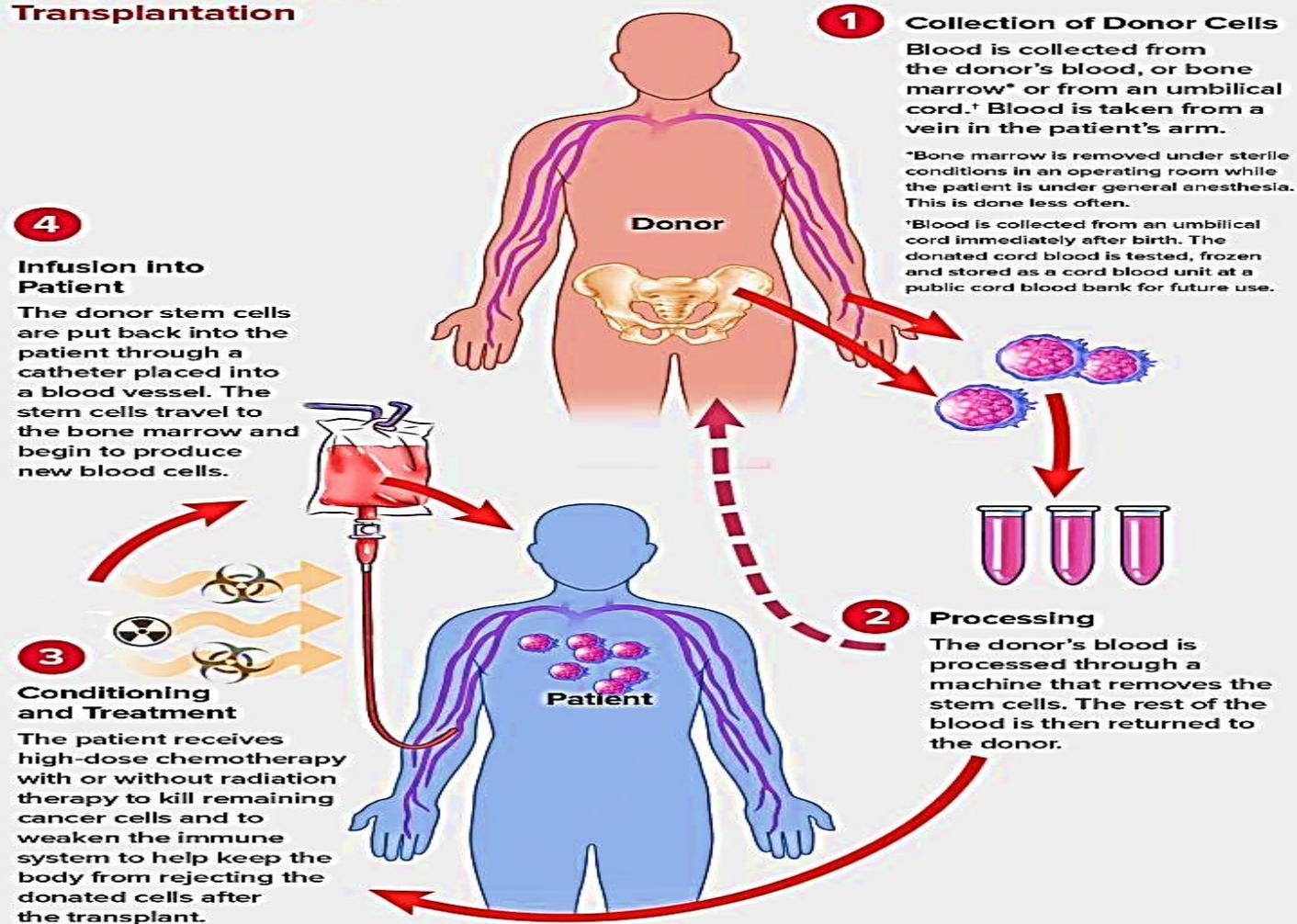


Figure 3. This illustration shows the allogeneic stem cell transplantation process. Once the stem cells are collected from the donor, the cells are mixed with a cryoprotective agent so that they can be frozen (for many years) and later once a patient is identified and the cells are needed, the cells can be thawed without injury and shipped to the patient.

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



Induced pluripotent stem cells (iPSC)

- iPSC technology is a huge discovery (2006) → NP 2012
- Concept: mature cells can be reprogrammed to become pluripotent.
- Technique : done by introduce a few specific pluripotency genes into already specialized cells (Ex: ms cells) → the cells will forget what type of cells they are & revert back & reprogrammed back into pluripotent stem 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 any immune- rejection complication

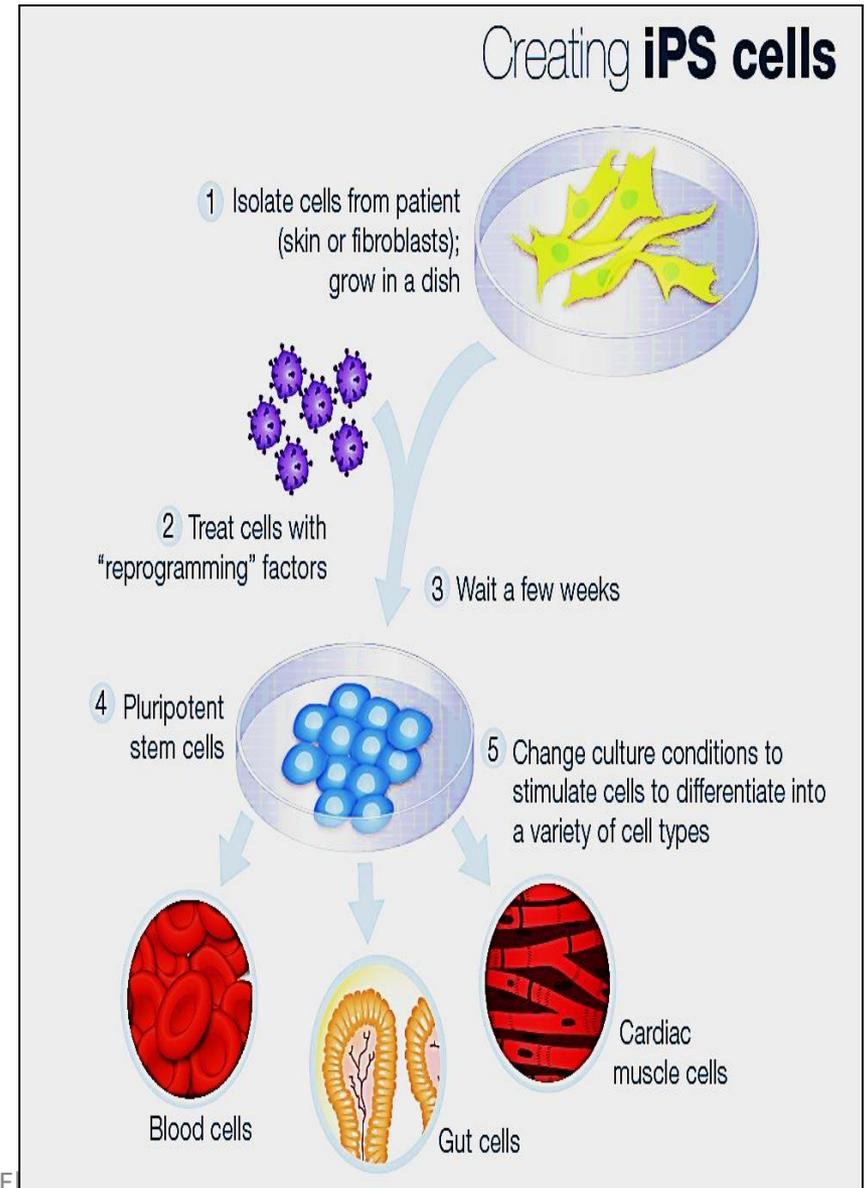
Induced pluripotent stem cells (iPSC)

1st isolate and culture skin cells from a patient.

2nd introduce three or four pluripotency genes into the skin cells by using an engineered virus carrier.

The expression of these genes regenerates the stem cell phenotype.

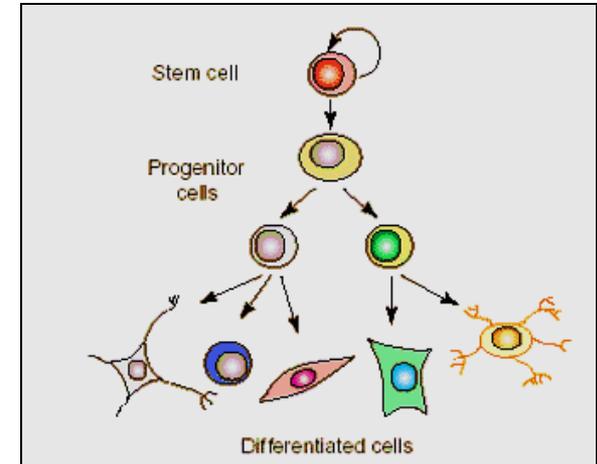
The viruses simply deliver the genes of interest and are themselves engineered not to be harmful.



Committed progenitor cells

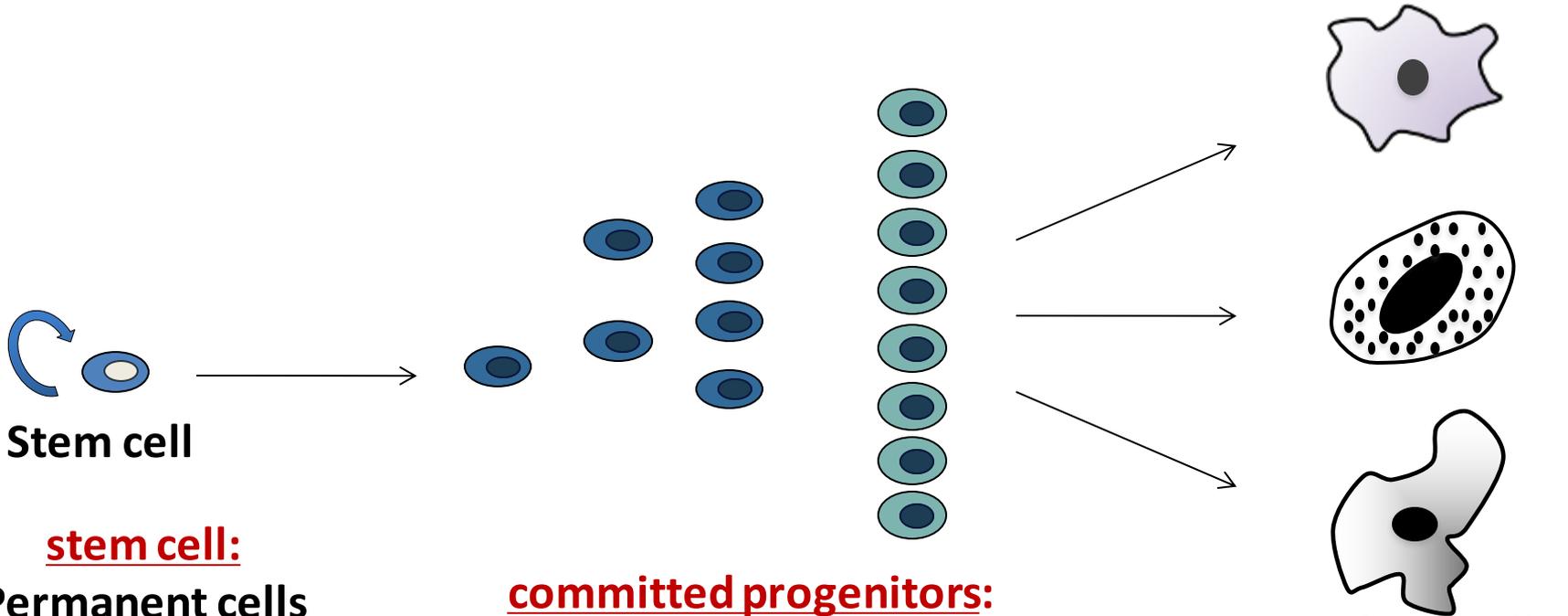
- They are descendants of stem cells but they are more differentiated and specific than stem cells

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



- 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

Example:

Hematopoietic stem cell

committed progenitors:

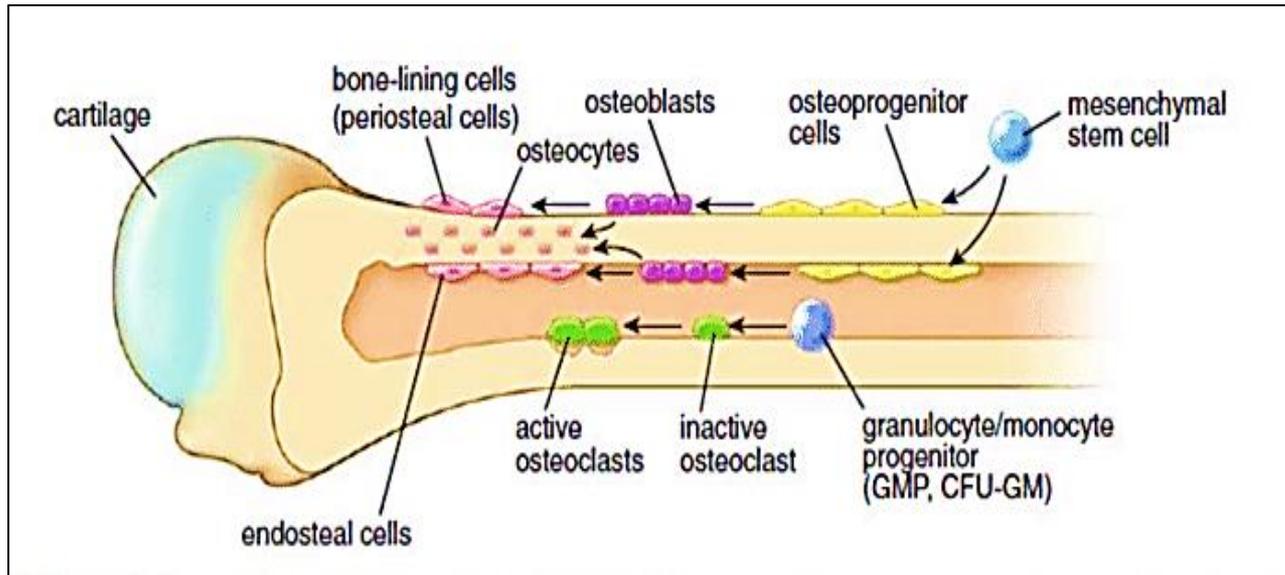
- Temporary cells
- Divide rapidly
- limited potency (multipotent)
- limited self-renewal

specialized cells

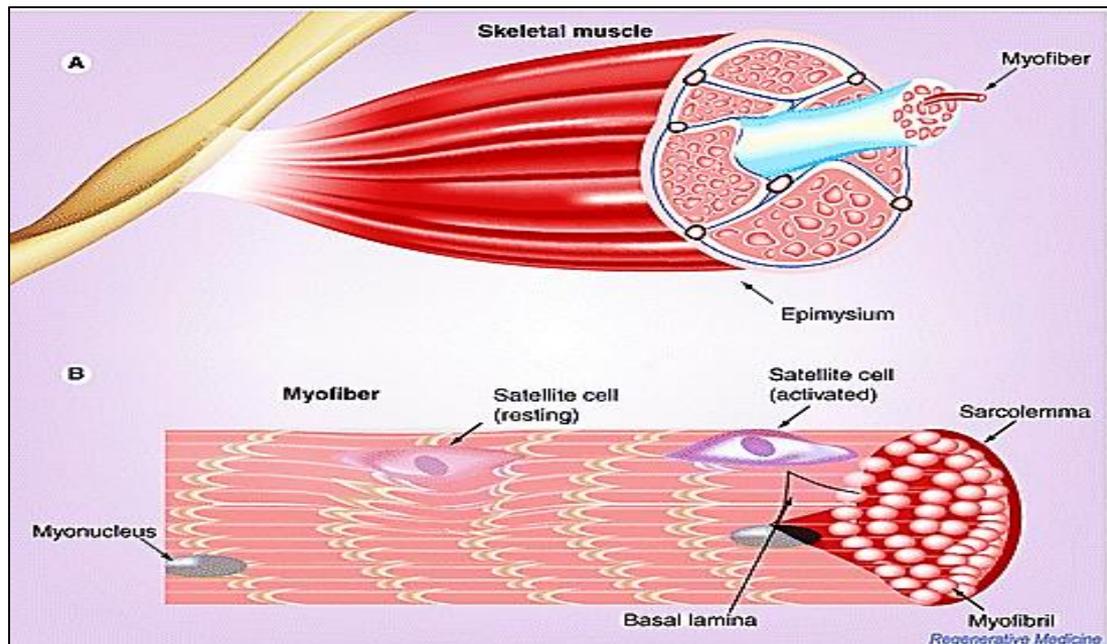
- Function
- No self renewal

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

- **Genetic & environmental factors (niches)** determine the pathway of differentiation that the progenitor cells will take it to form a specific lineage . The committed progenitor remain **dormant** in the tissue till need.
- Adult stem cells main role is to replace cells in case of tissue injury, damage or dead cells
- Example of adult stem cells:
 - ✓ **Satellite cells** found in muscle → myogenic progenitor cells → skeletal muscle cells (Myofibers)
 - ✓ **Periosteum** contains progenitor cells that develop into osteoblasts.



Stem cell in periosteum of bone



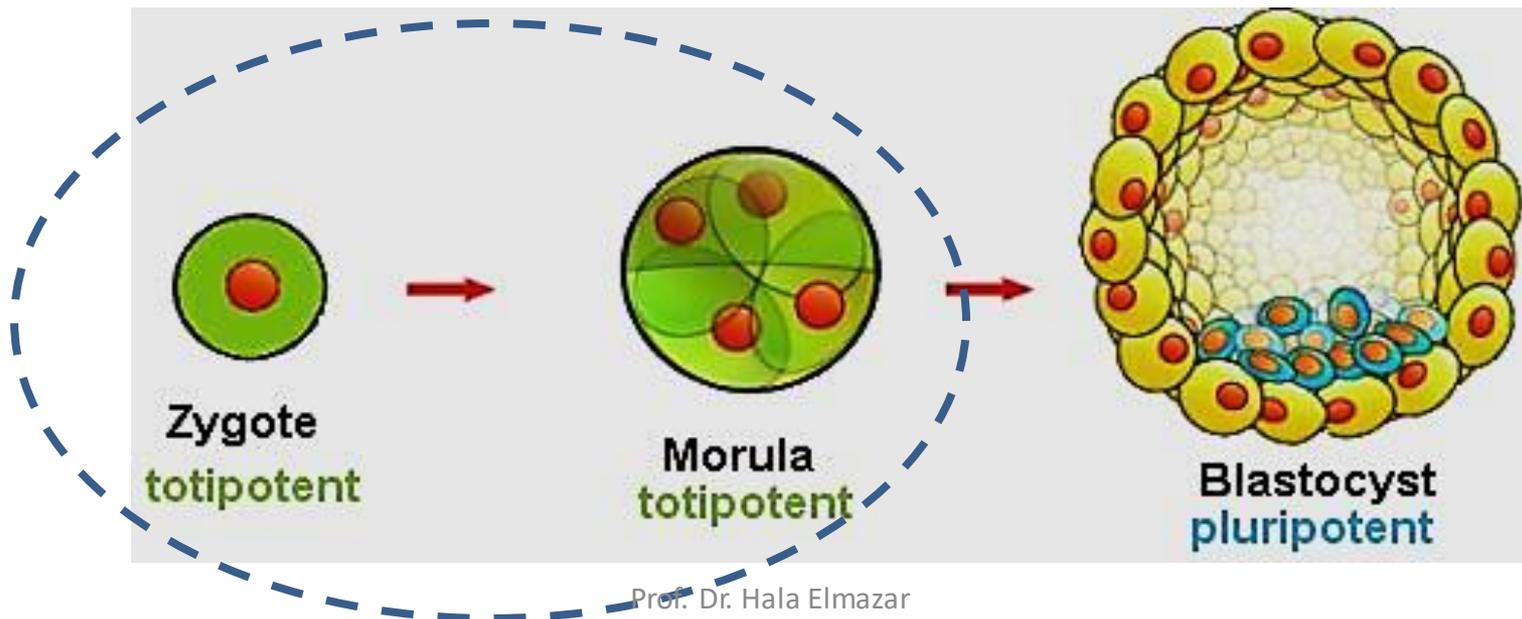
Satellite cells in perimysium of muscles

Potency:

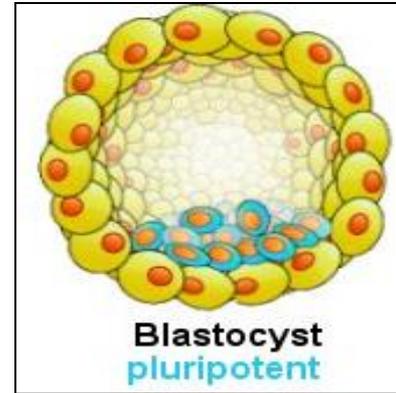
Cell's ability to differentiate into other cell types

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)

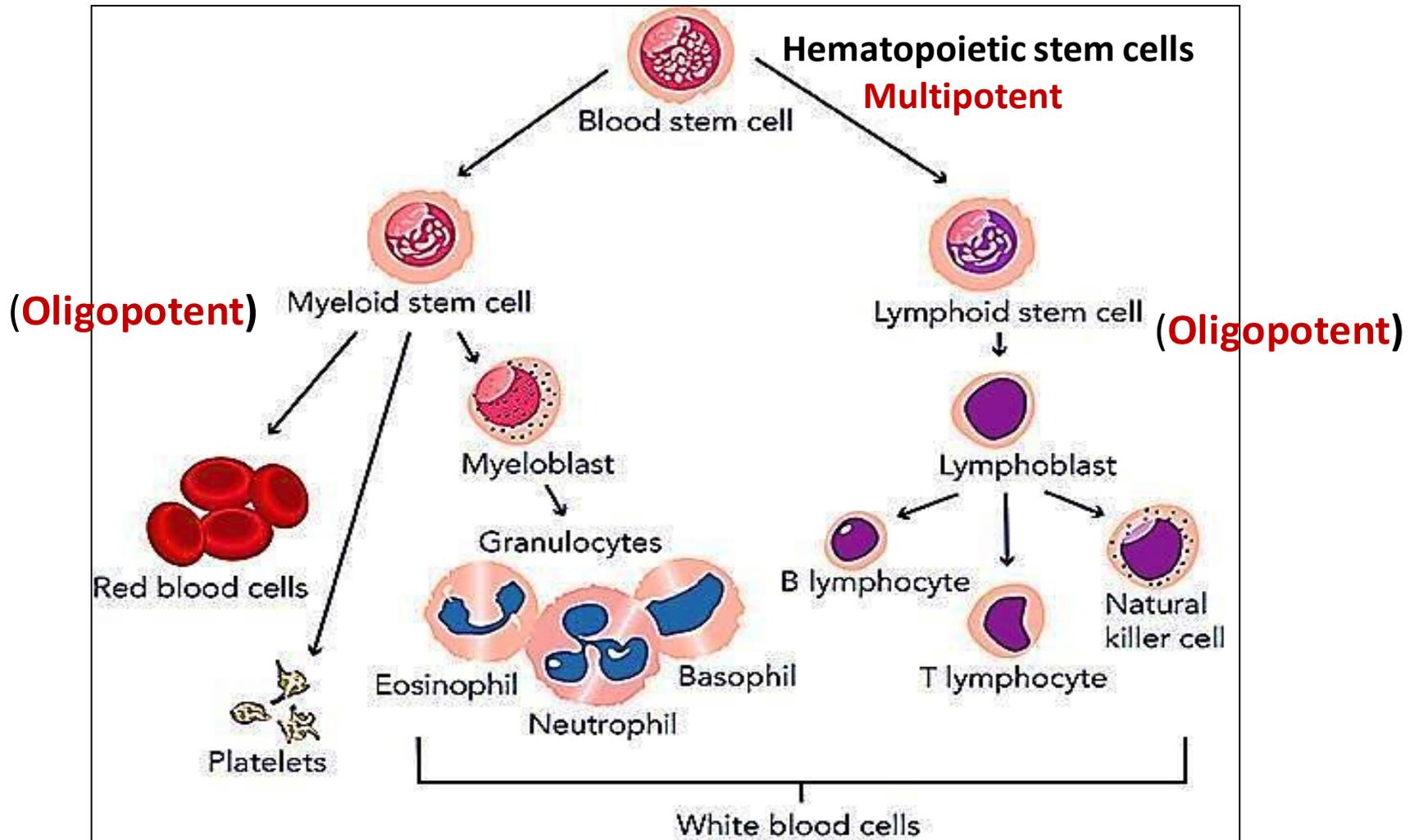


Multipotent: can give rise to **multiple** types of specialized cells, but not all cell types
(Adult SC in tissues & hematopoietic SC in cord blood)

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

Nullipotent: 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.



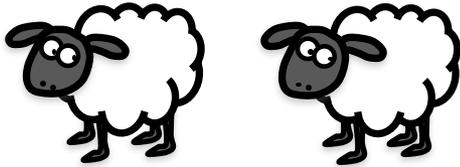
Molecular cloning:

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

Cloning

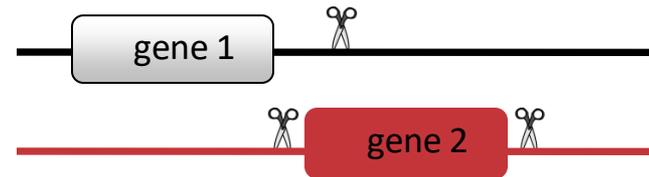
There are two VERY different types of cloning:

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

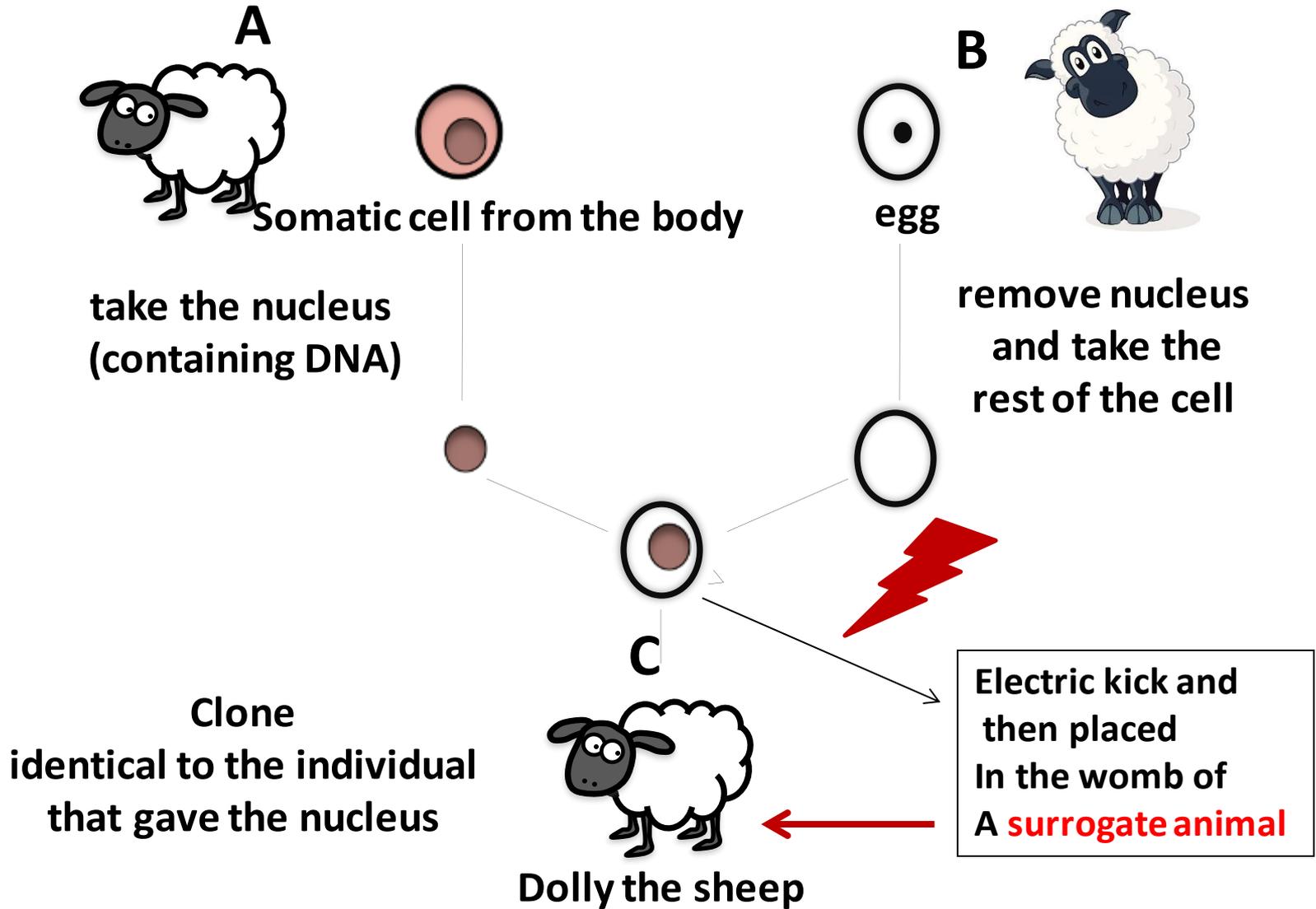
Transfer: moving an object from one place to another

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

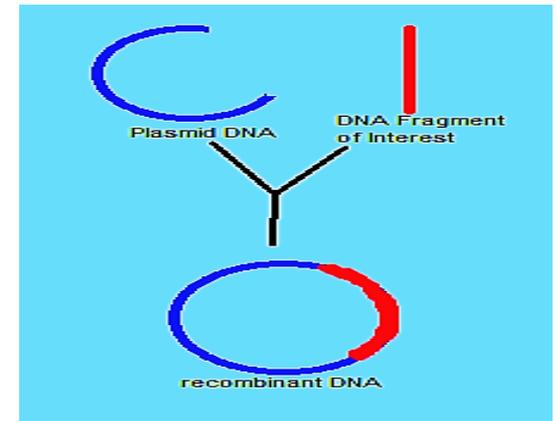
Reproductive cloning



Molecular cloning

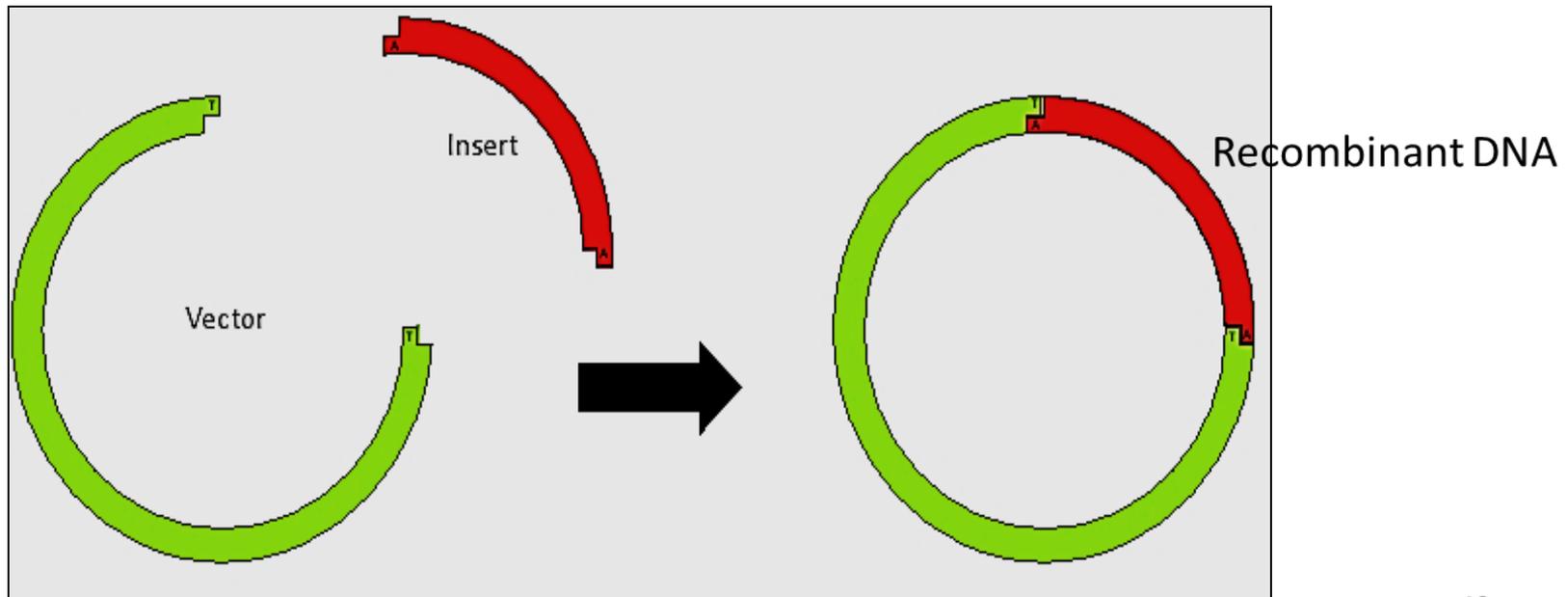
- A process used by scientists to make **copies of a specific gene or genes**.
- Isolation of a DNA sequence (gene) from any species, and its insertion into a vector for propagation, without alteration of the original DNA sequence of the vector
- Also called **recombinant DNA cloning**

Recombinant DNA

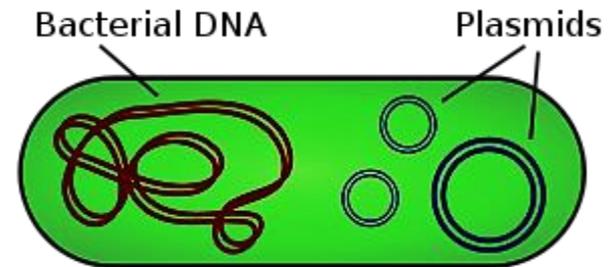


DNA molecules formed by laboratory methods of genetic recombination to bring together genetic material from multiple sources → creating new DNA sequences

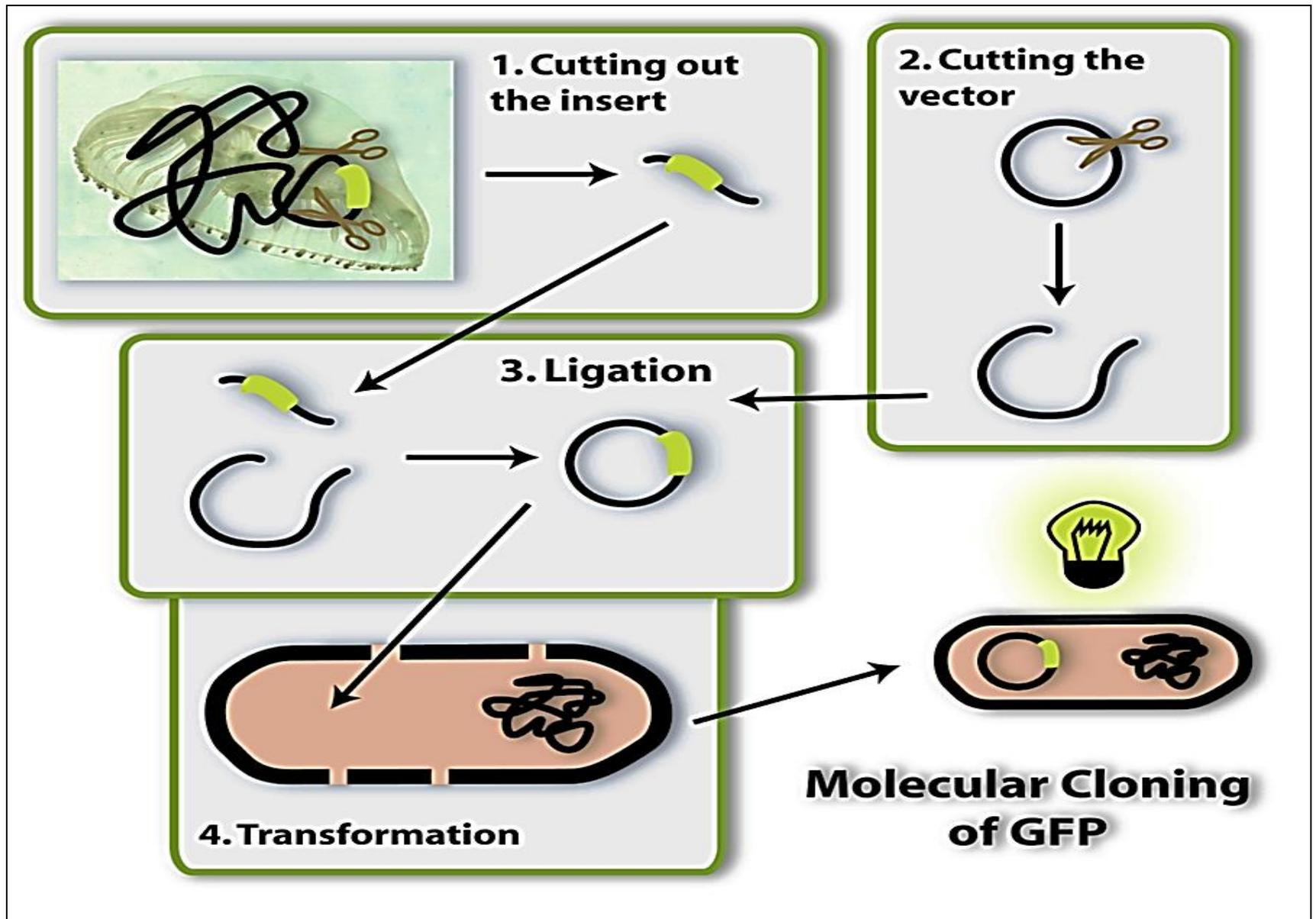
- Recombinant DNA is possible because DNA molecules from all organisms share the same chemical structure (the genetic code is universal) .
- Construction of recombinant DNA, involves insertion of a foreign DNA fragment into a plasmid vector.



- **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

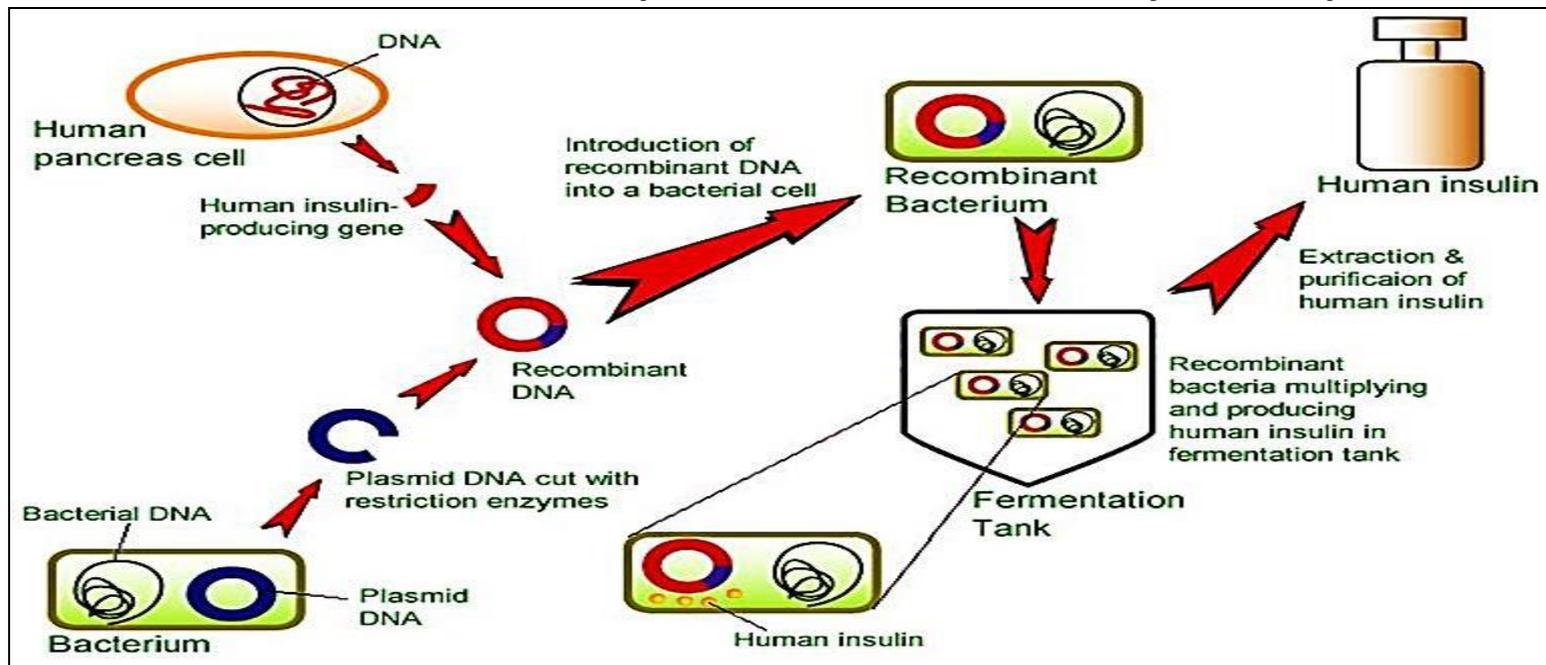
- 1- Select the **DNA molecule to be cloned** (insert)
- 2- Select **DNA molecule** that will serve as a **vector (virus / plasmid)**
- 3- cleave the vector DNA strand with **Restriction endonuclease**, then insert foreign DNA → recombinant DNA molecule
- 4- Introduction of recombinant DNA in host cells → 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- Transgenic organisms :generating genetically modified organisms (GMOs), most GMOs are generated for purposes of basic biological research e.g. transgenic mouse

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

