

# Lecture 12-13

**BT 632**

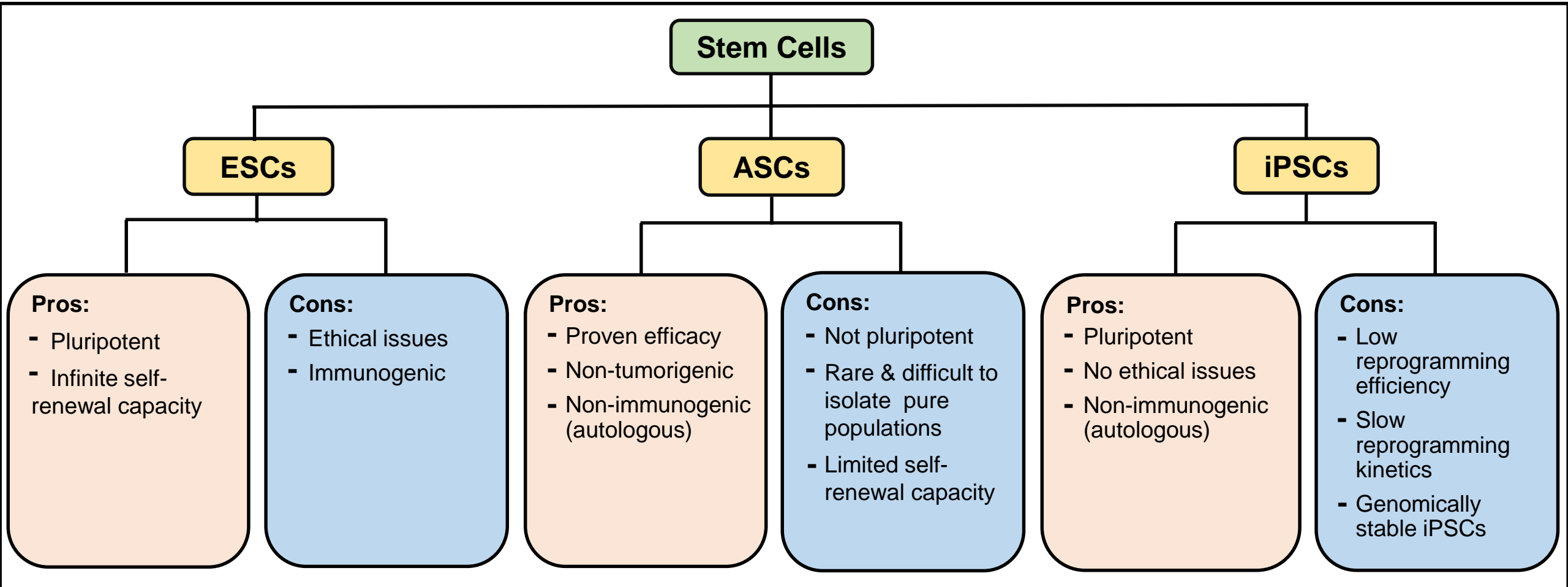
# **Stem Cells, Cancer and Therapy**

**(3-0-0-6)**

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# Types of stem cells



**What are the important things  
required in a cell culture lab?**

**Laminar Flow Cabinet**



**Refrigerated Centrifuge**



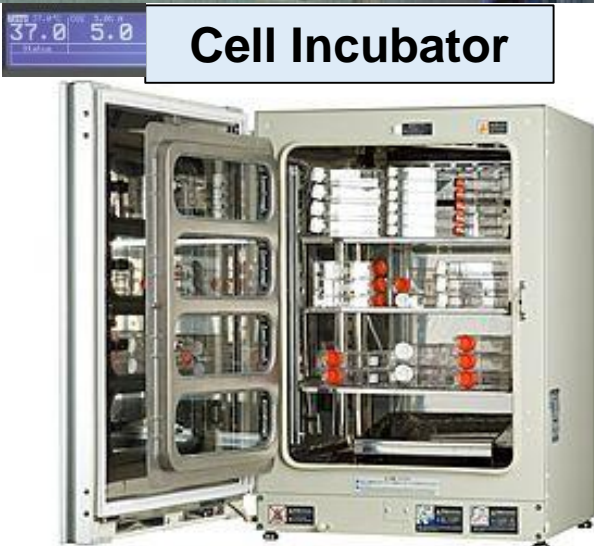
**WATER BATH SHAKER**



**Cell Culture Plastics**



**Cell Incubator**



**Microscope**



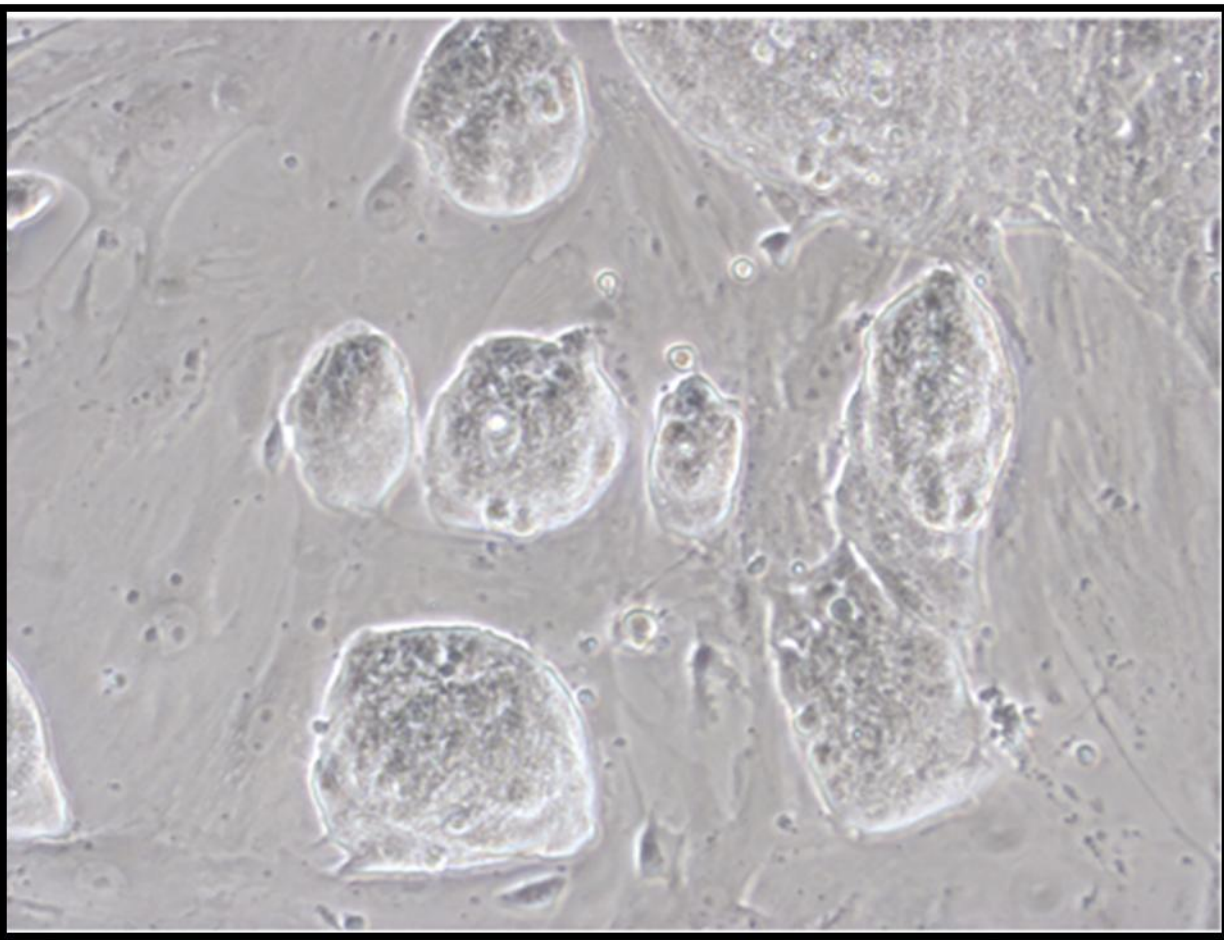
**Liq N2 tank**



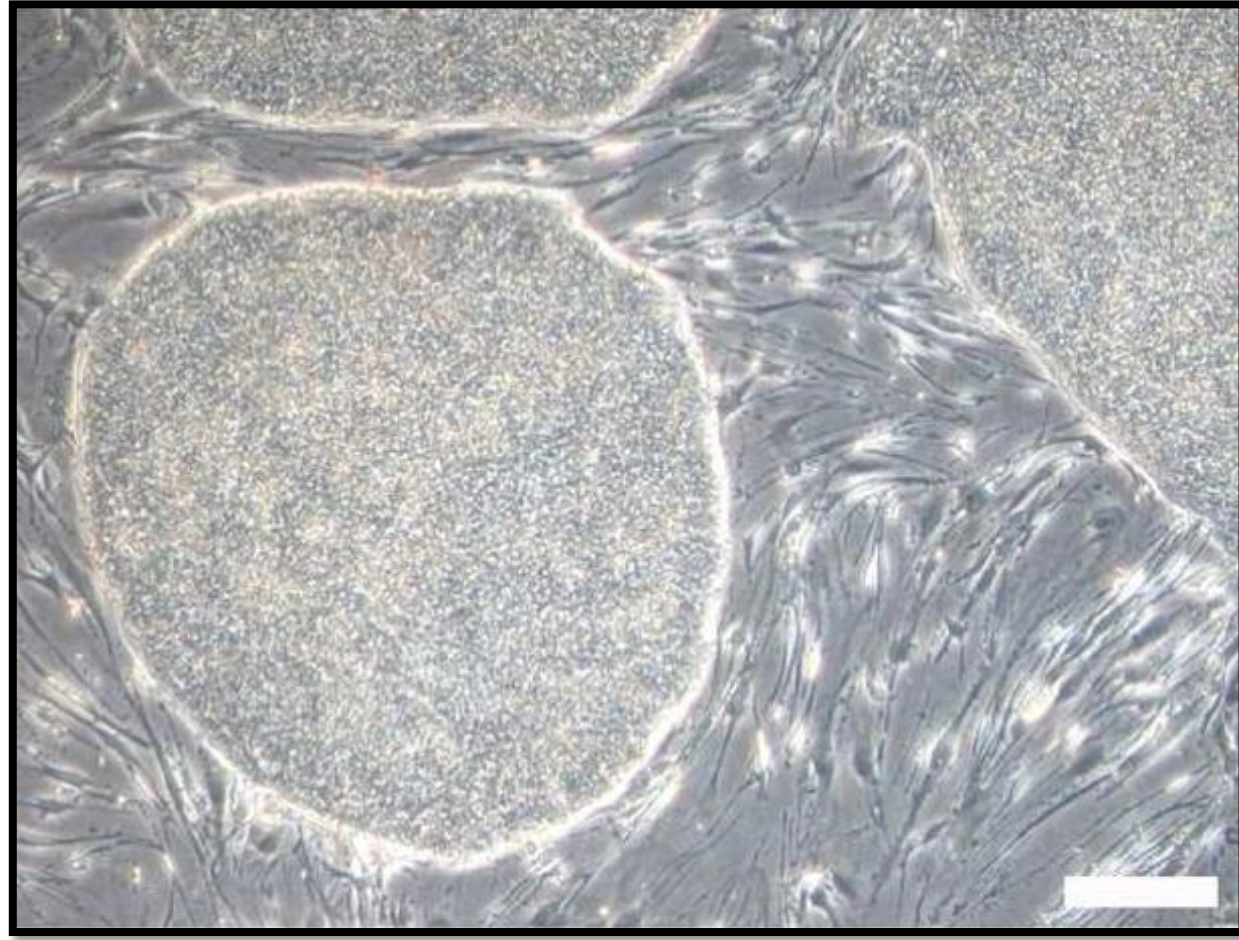
**Refrigerator**







**Mouse ES cells**



**Human ES cells**



**Mouse ES/iPS cells:**

**High Glucose DMEM** – contains glucose, inorganic salts, amino acids, vitamins, etc.

**Fetal Bovine (Calf) Serum** – contains proteins, growth factors, etc.

**Glutamax** – direct substitute for L-glutamine. L-glutamine is an essential nutrient in cell cultures for energy production as well as protein and nucleic acid synthesis.

**NEAA** – provides necessary non-essential amino acids.

**Na pyruvate** – added as an additional source of energy, but may also have protective effects against hydrogen peroxide.

**2-mercaptoethanol** – prevent toxic levels of free radicals.

**LIF** – Growth factor

**Human ES/iPS cells:**

**DMEM/F12:** high concentrations of glucose, inorganic salts, amino acids, and vitamins with F-12's wide variety of components.

**KnockOut Serum Replacement (KOSR):** serum-free defined formulation providing all the necessary proteins and growth factors.

**L-Glutamine** – L-glutamine is an essential nutrient in cell cultures for energy production as well as protein and nucleic acid synthesis.

**NEAA** – provides necessary non-essential amino acids.

**2-mercaptoethanol** – prevent toxic levels of free radicals.

**FGF** – Growth factor

# Coating of flasks – to prevent detachment



## FEEDERS:

### **Mouse embryonic fibroblasts**

The mouse cells in the bottom of the culture dish provide the cells a sticky surface to which they can attach. Also, the feeder cells release nutrients into the culture medium. Mitotic inactivation of feeder cells is done by UV or X- or  $\gamma$ -irradiation or treatment of drugs like Mitomycin C (10 $\mu$ g/ml for 2-4 hours).

**Researchers have now devised ways to grow embryonic stem cells without mouse feeder cells. This is a significant scientific advance because of the risk that viruses or other macromolecules in the mouse cells may be transmitted to the human cells.**

## Mouse ES/iPS cells:

### **Gelatin (0.1% gelatin solution)**

Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen extracted from the skin, bones, and connective tissues of animals.

## Human ES/iPS cells:

### **Matrigel:**

is the trade name for a gelatinous protein mixture secreted by Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells (a tumor rich in extracellular matrix proteins) produced and marketed by Corning Life Sciences. This material, once isolated, is approximately 60% laminin, 30% collagen IV, and 8% entactin. Entactin is a bridging molecule that interacts with laminin and collagen IV, and contributes to the structural organization of these extracellular matrix molecules. Matrigel is used as an attachment substrate for human pluripotent stem cells. When human embryonic stem cells are grown in the absence of feeder cells, extracellular matrix components are needed to maintain the pluripotent, undifferentiated state (self-renewal).

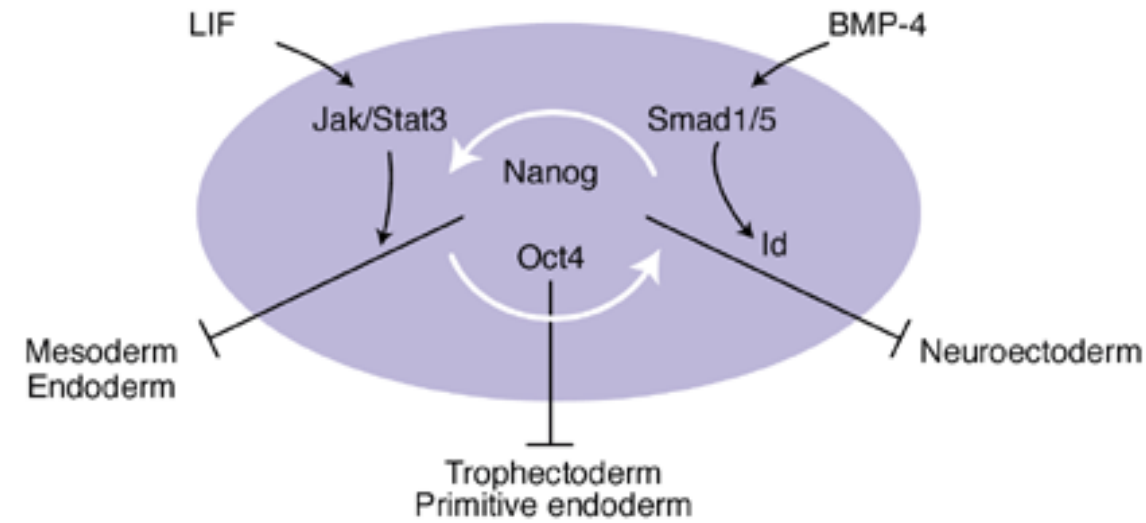
### **Vitronectin:**

is a defined, recombinant fusion protein containing the full length human vitronectin sequence, expressed in human cells under chemically defined, xeno-free conditions.

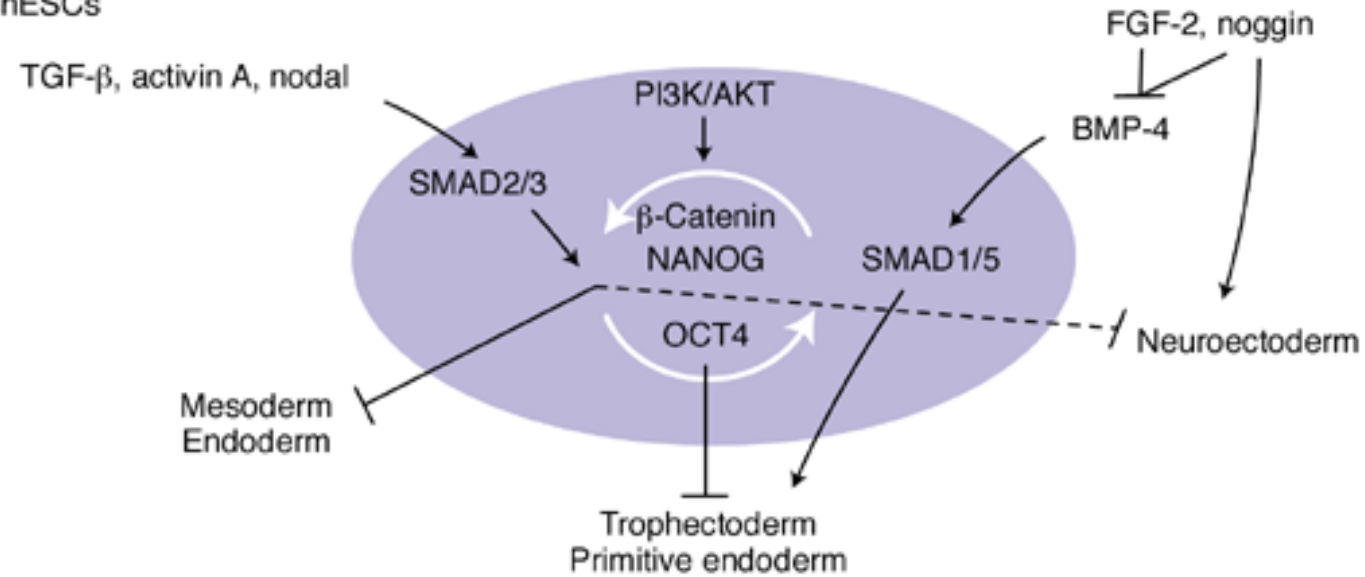
**How do ESCs/iPSCs maintain self-renewal potential?**



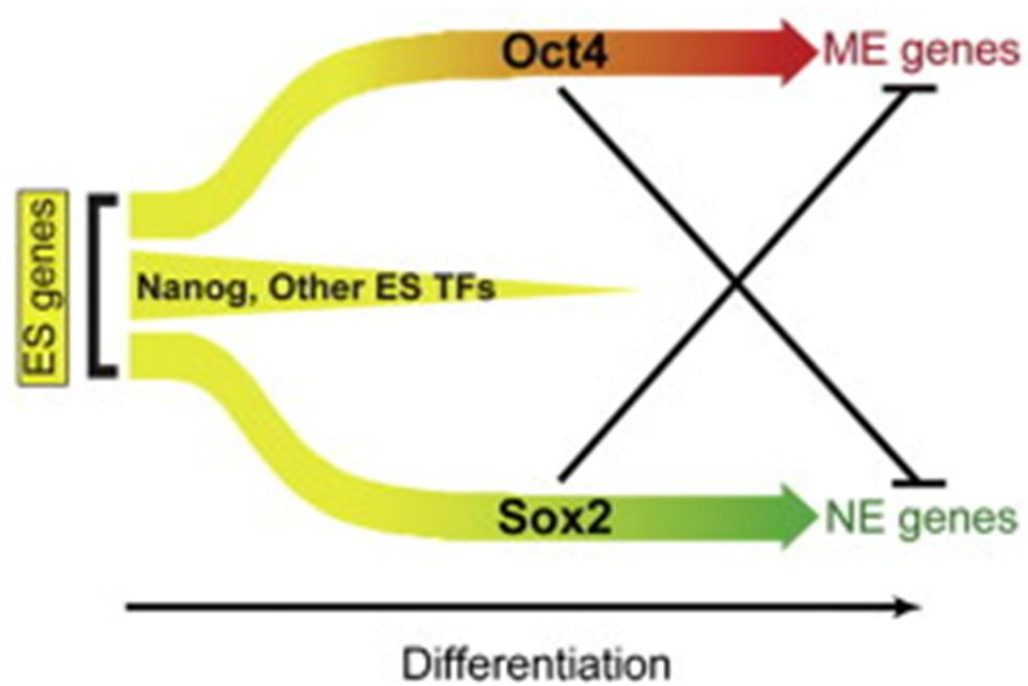
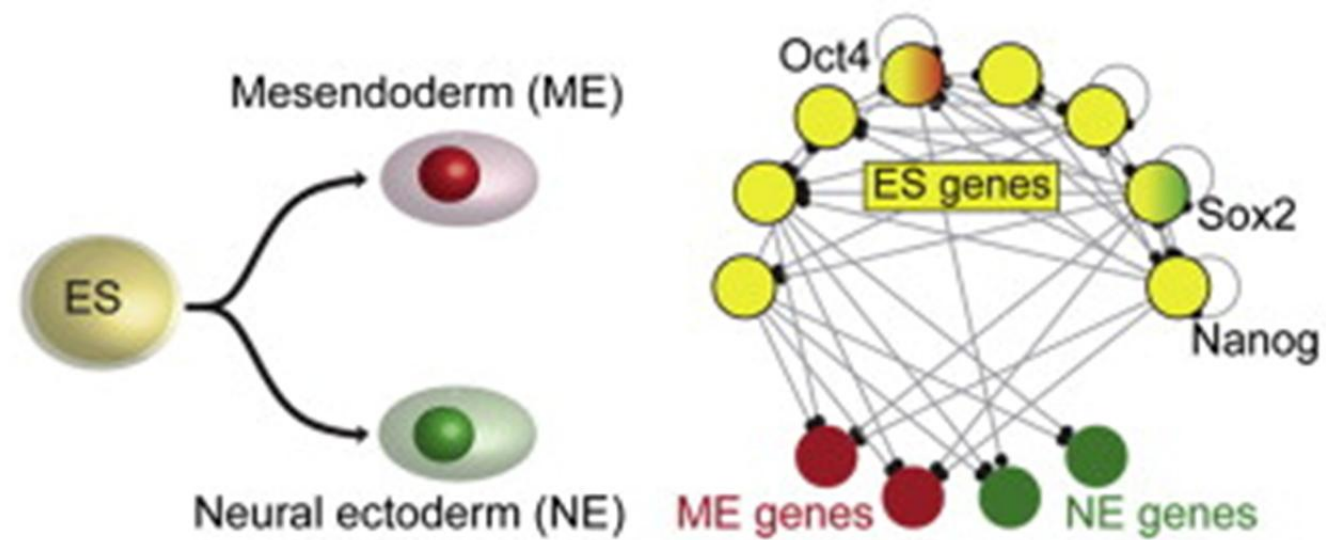
**a mESCs**



**b hESCs**



Signalling pathways required for the maintenance of pluripotency  
in embryonic stem cells



**hESC**

**mESC**

FGF2 Activin A  
Wnt

LIF BMP4  
Wnt

Smad2/3  
PI3K

STAT3, cMyc  
Smad1/5/8

*core transcriptional network*

Oct4 - Nanog - Sox2

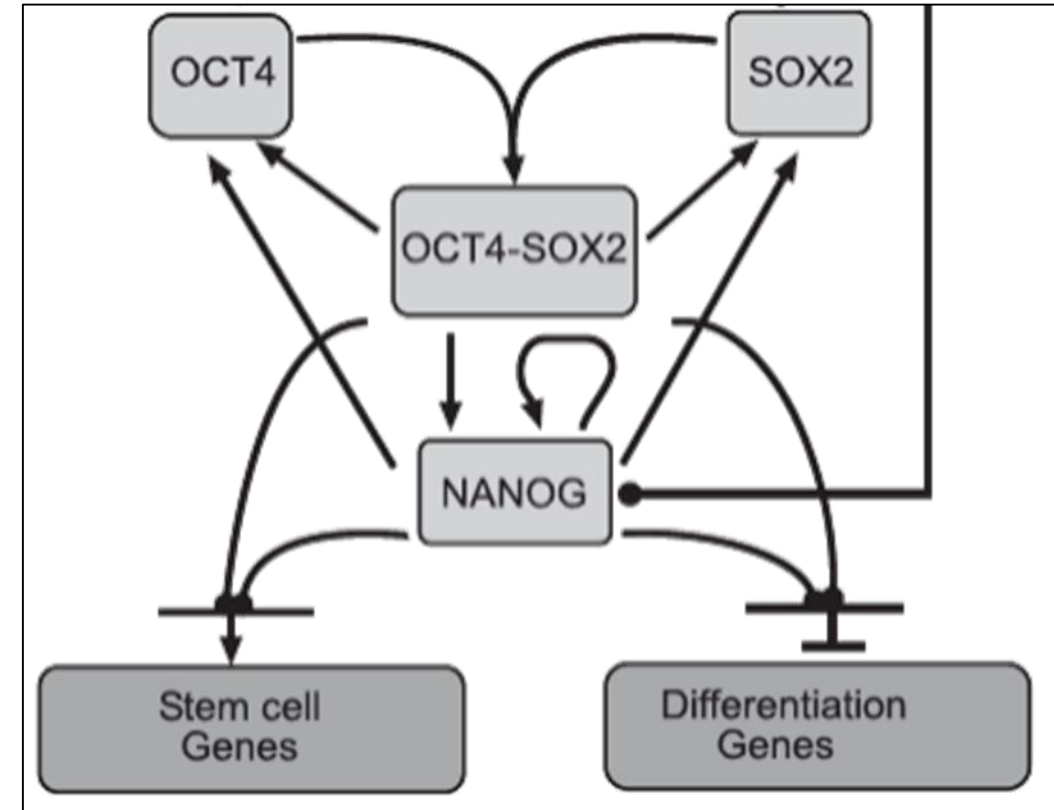
**self renewal**

**pluripotency**

ES cell ES cells

endoderm mesoderm

ectoderm



**C**