THE DEVELOPMENT OF AN ORGANISM

FERTILIZATION

ZYGOTE | DIVISION INTO BLASTOMERES | BLASTOCYST | EMBRYO | FETUS | BABY | ELDERLY

TOTIPOTENT CELLS

PLURIPOTENT CELLS

MULTIPOTENT AND UNIPOTENT CELLS (PROGENITOR CELLS)

POTENTIAL OF DEVELOPMENT / POTENTIAL OF DIFFERENTIATION

Classification based on derivation/source of stem cells

Embryo-derived stem cells

↓

derived from an embryo

↓

- Fertilized Egg (or Zygote)
- Morula
- **Embryonic Stem cells (ESC)**
- Epiblast Stem Cells (EpiSCs)
- Embryonic Germ Cells (EGCs)
- Embryonic Carcinoma Cells (ECCs)

Adult stem cells

↓

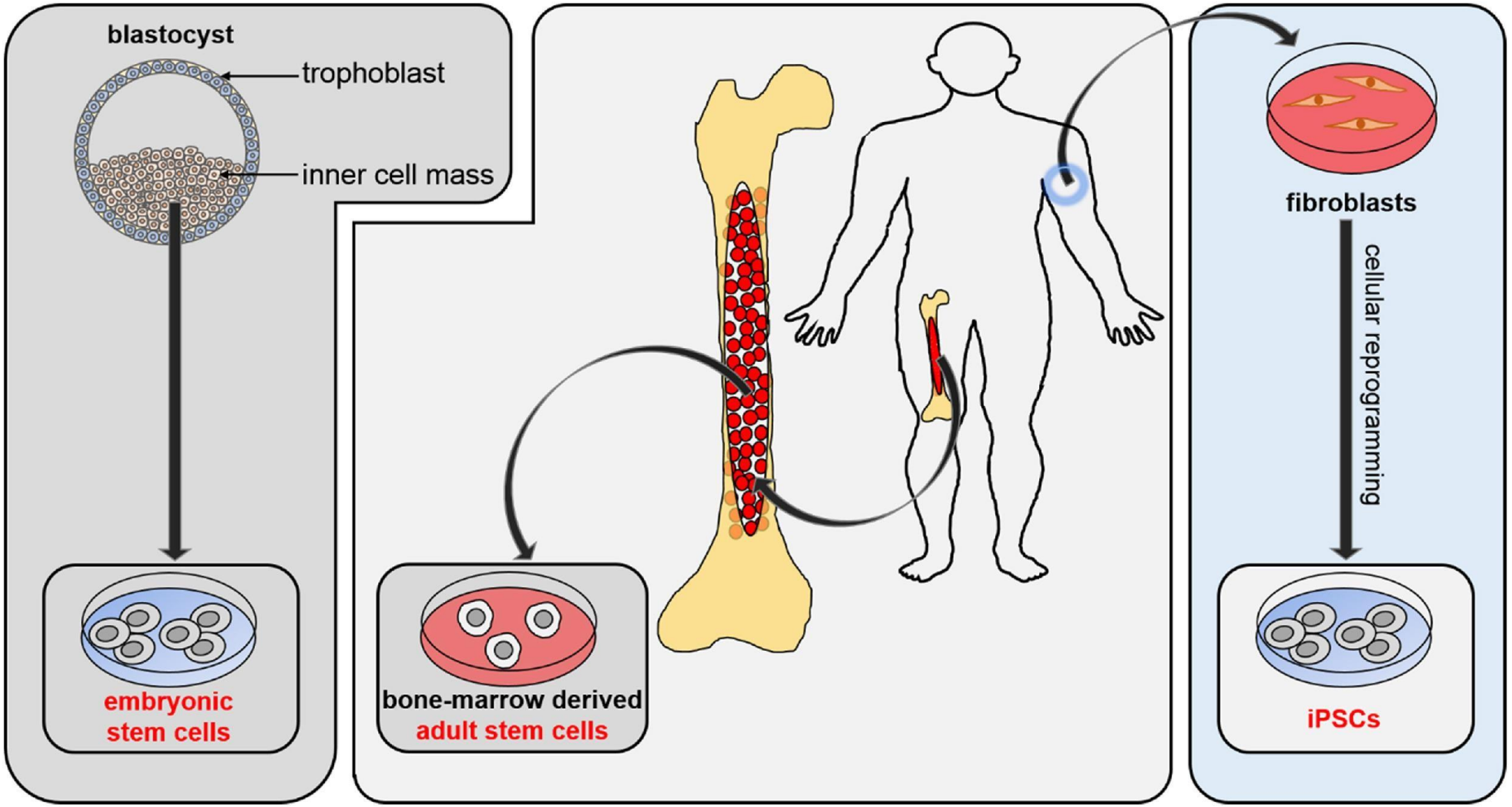
derived from an adult organism

↓

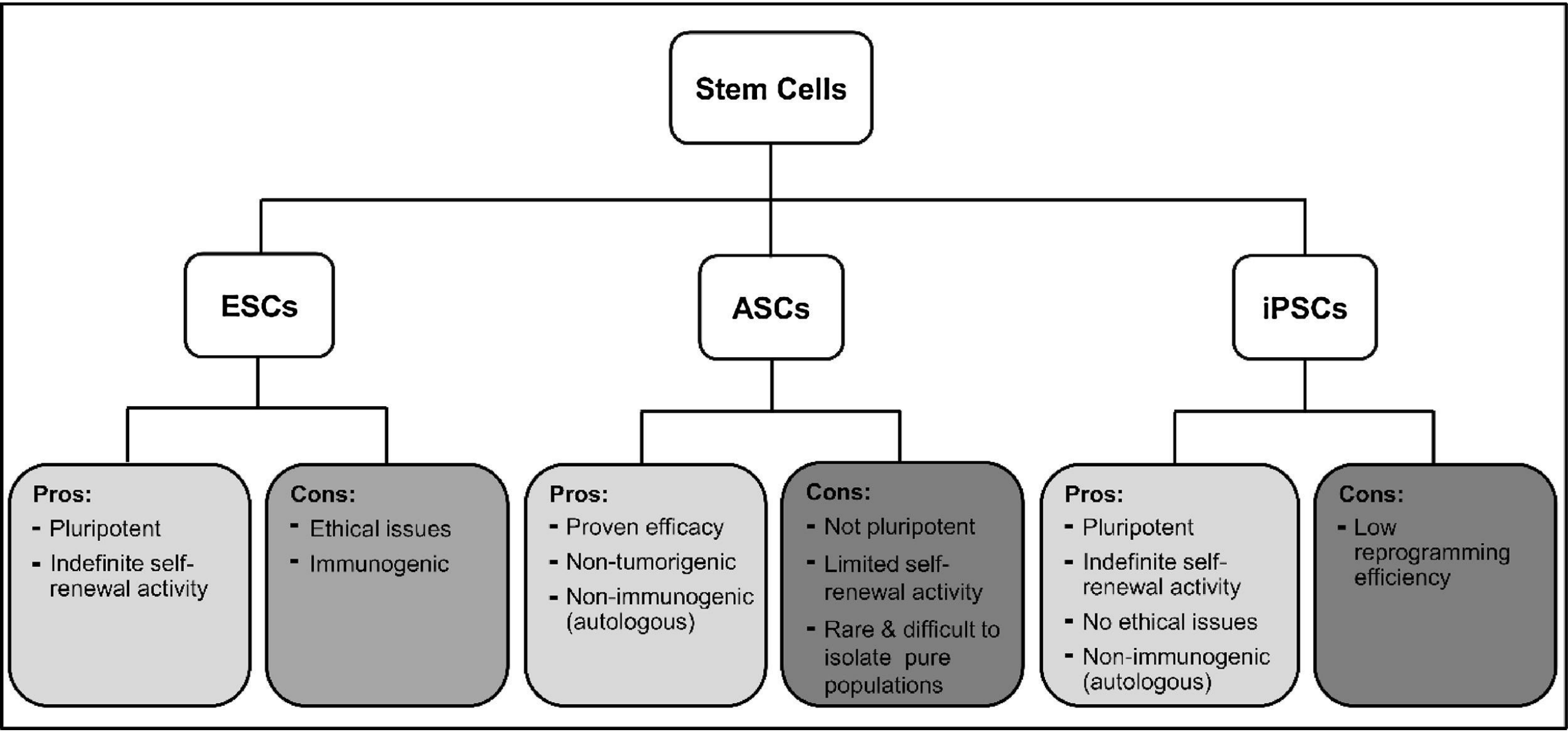
- Hematopoietic Stem Cells (HSCs) – from bone marrow and umbilical cord blood stem cells
- Mesenchymal Stem Cells (MSCs)
- Neural Stem Cells (NSCs)
- Intestinal Stem Cells
- Endothelial Stem Cells
- Spermatogonial Stem Cells
- etc. etc. etc.

Induced Pluripotent Stem Cells (iPSCs)

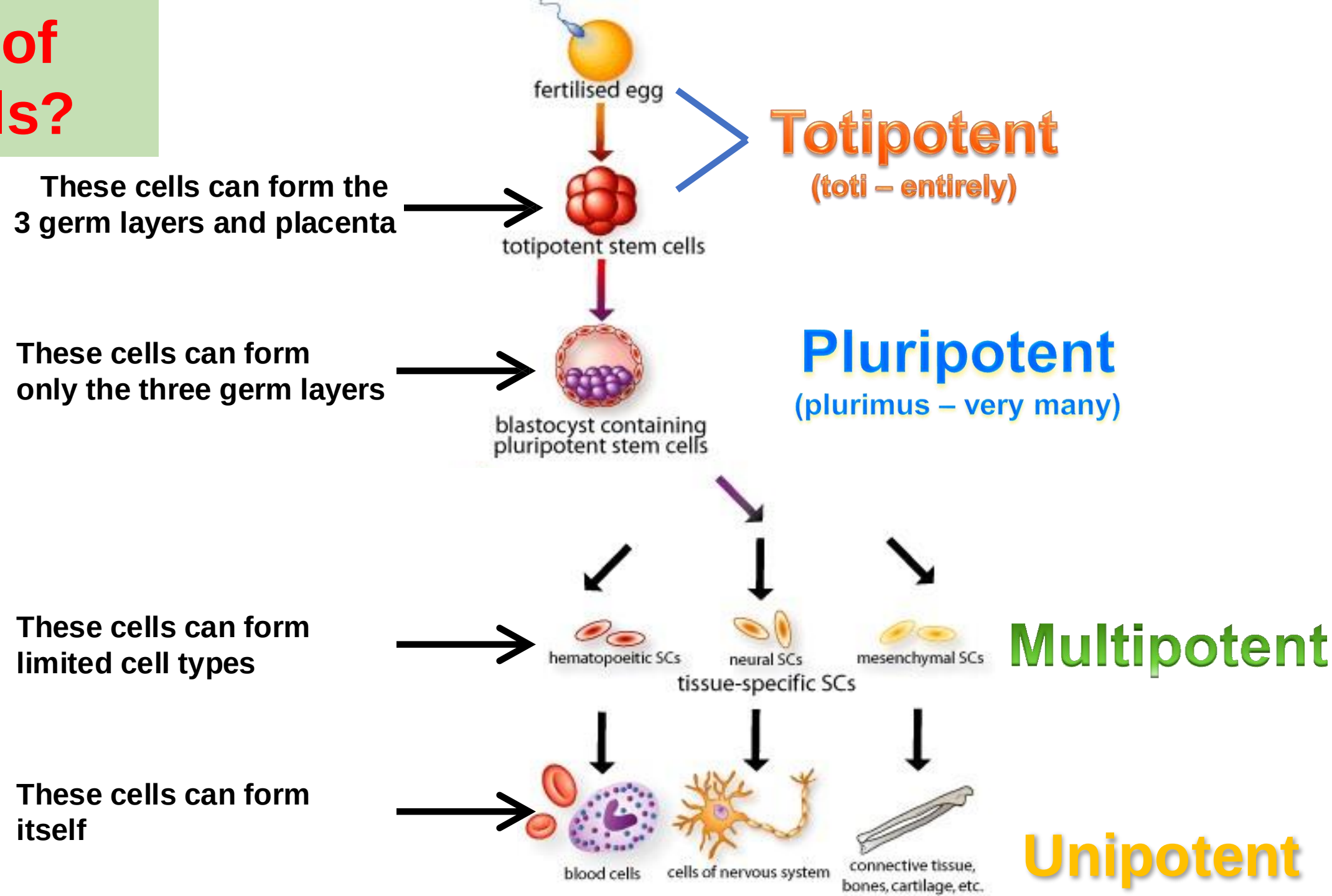
Types of Stem Cells



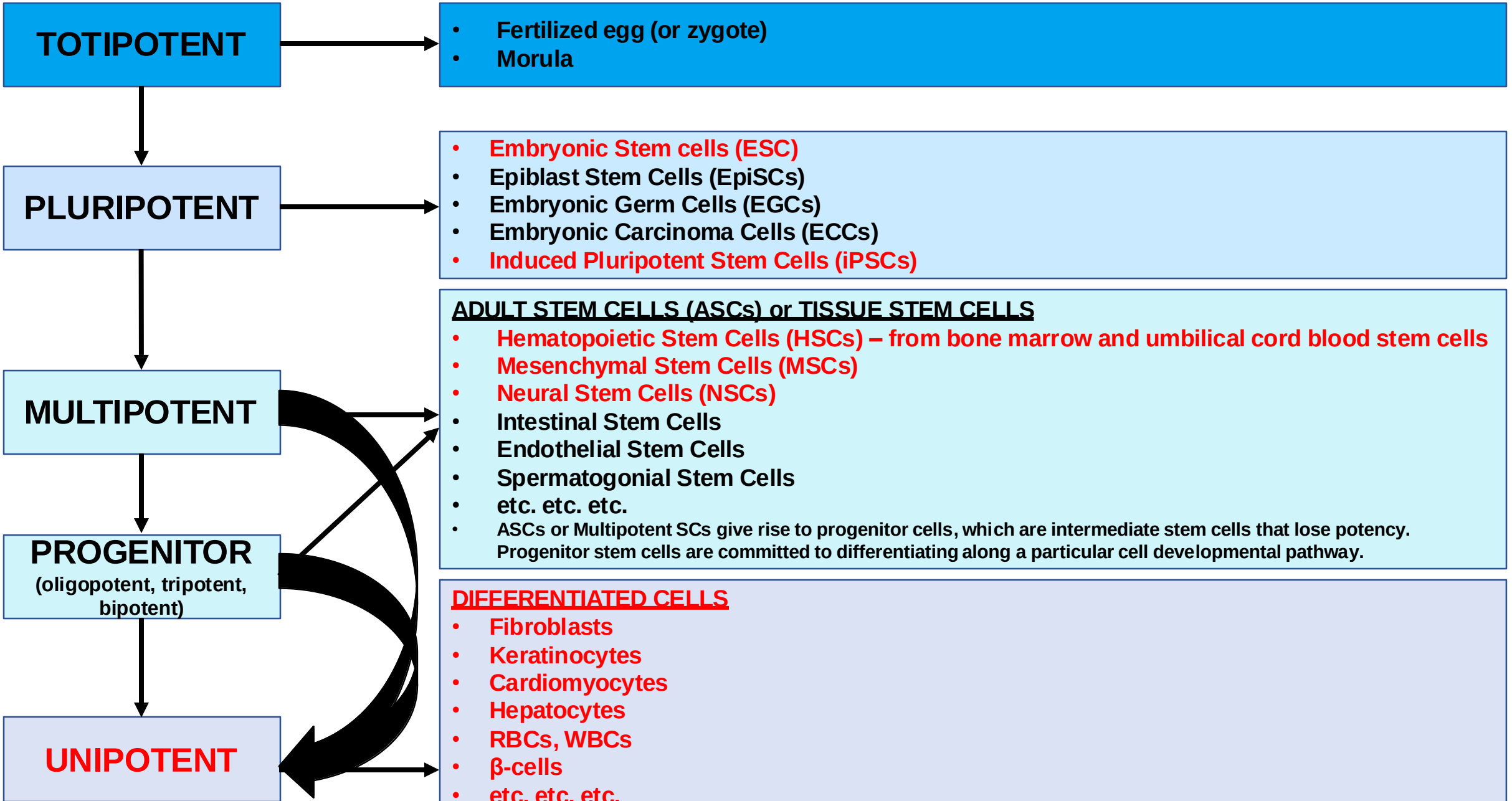
Saha B, …, **Thummer RP** (**2018**) Prospective applications of Induced Pluripotent Stem Cells in Military Medicine. Med J Armed Forces India. 74(4): 313-320.

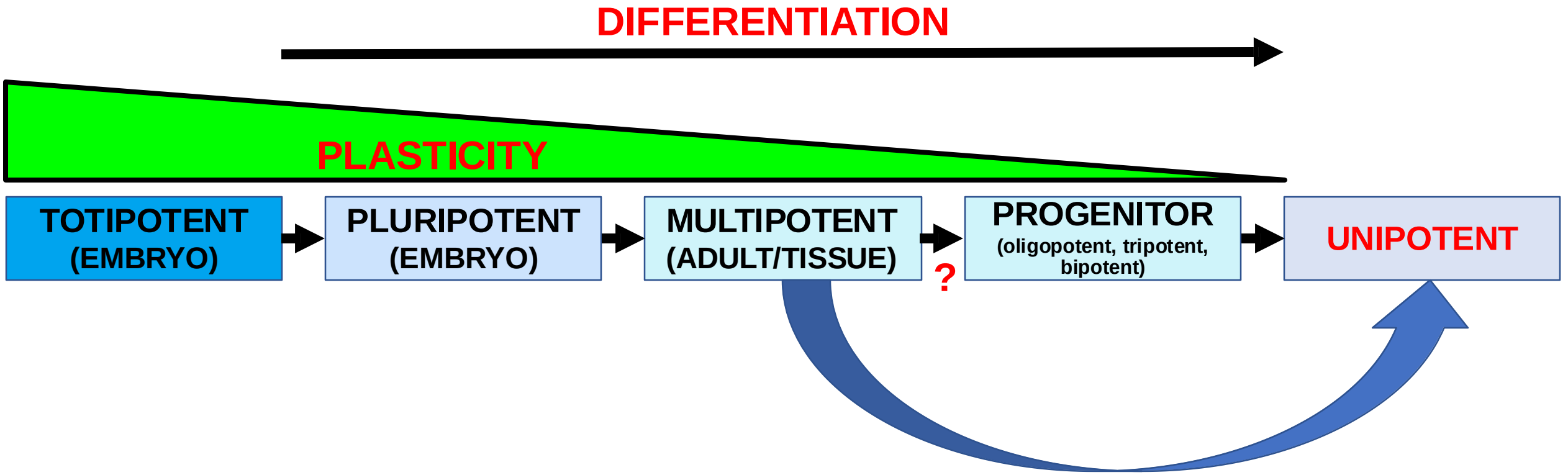

Stem Cells
ESCs
ASCs
iPSCs
Pros:
- Pluripotent
- Indefinite self-renewal activity
Cons:
- Ethical issues
- Immunogenic
Pros:
- Proven efficacy
- Non-tumorigenic
- Non-immunogenic (autologous)
Cons:
- Not pluripotent
- Limited self-renewal activity
- Rare & difficult to isolate pure populations
Pros:
- Pluripotent
- Indefinite self-renewal activity
- No ethical issues
- Non-immunogenic (autologous)
Cons:
- Low reprogramming efficiency

Potency of stem cells?



Classification based on Potency of cells





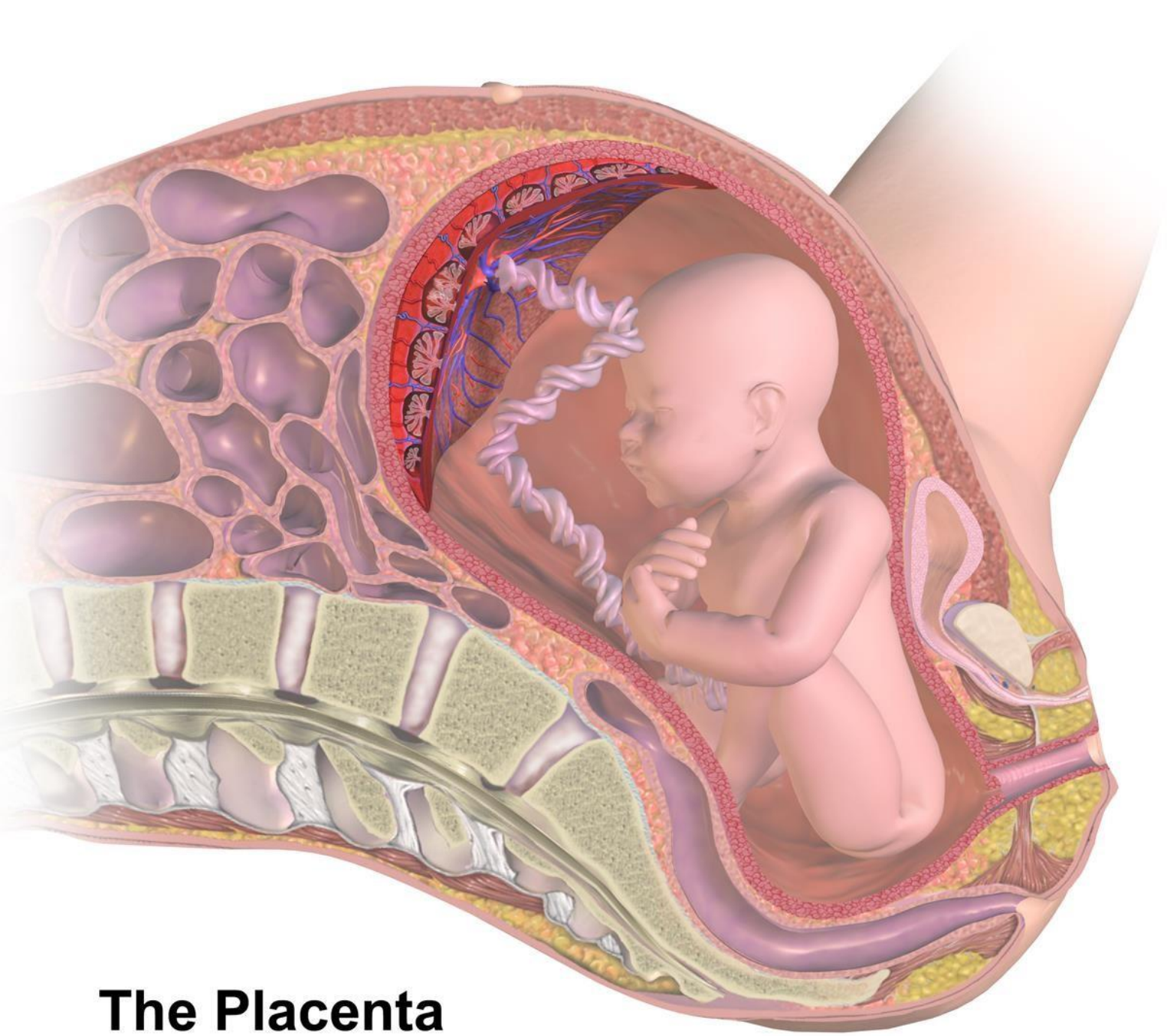
http://thenode.biologists.com/stem-cells-versus-progenitors/discussion/

Progenitor Cell

- A **progenitor cell** is a biological cell that, like a stem cell, has a tendency to differentiate into a specific type of cells, but is already more specific than a pluripotent stem cell and is pushed to differentiate into its "target" cell.

- The most important difference between pluripotent stem cells and progenitor cells is that stem cells can replicate indefinitely, whereas progenitor cells can divide only a limited number of times.

- http://thenode.biologists.com/stem-cells-versus-progenitors/discussion/



The **placenta** is a temporary organ that connects the developing fetus via the **umbilical cord** to the uterine wall to:

- Allow nutrient uptake
- Thermo-regulation
- Waste elimination
- Gas exchange via the mother's blood supply
- To fight against internal infection
- Produce hormones which support pregnancy.

Definition

TOTIPOTENT:

An undifferentiated cell that can self-renew (???), and has the capacity to differentiate into all cell types of a human body, including placental cells (extraembryonic) tissues.

PLURIPOTENT:

An undifferentiated cell that can self-renew indefinitely *in vitro*, and has the capacity to differentiate into all cell types of a human body, except placenta (extramembryonic) tissues.

MULTIPOTENT (ADULT/TISSUE) STEM CELLS:

An undifferentiated cell that can self-renew a number of times but not indefinitely, and has the capacity to differentiate into multiple cell types or a number of related cell types but not all cell types of a human body; and replenish lost or dying or non-functional cells and regenerate these damaged tissues.

Quiescence

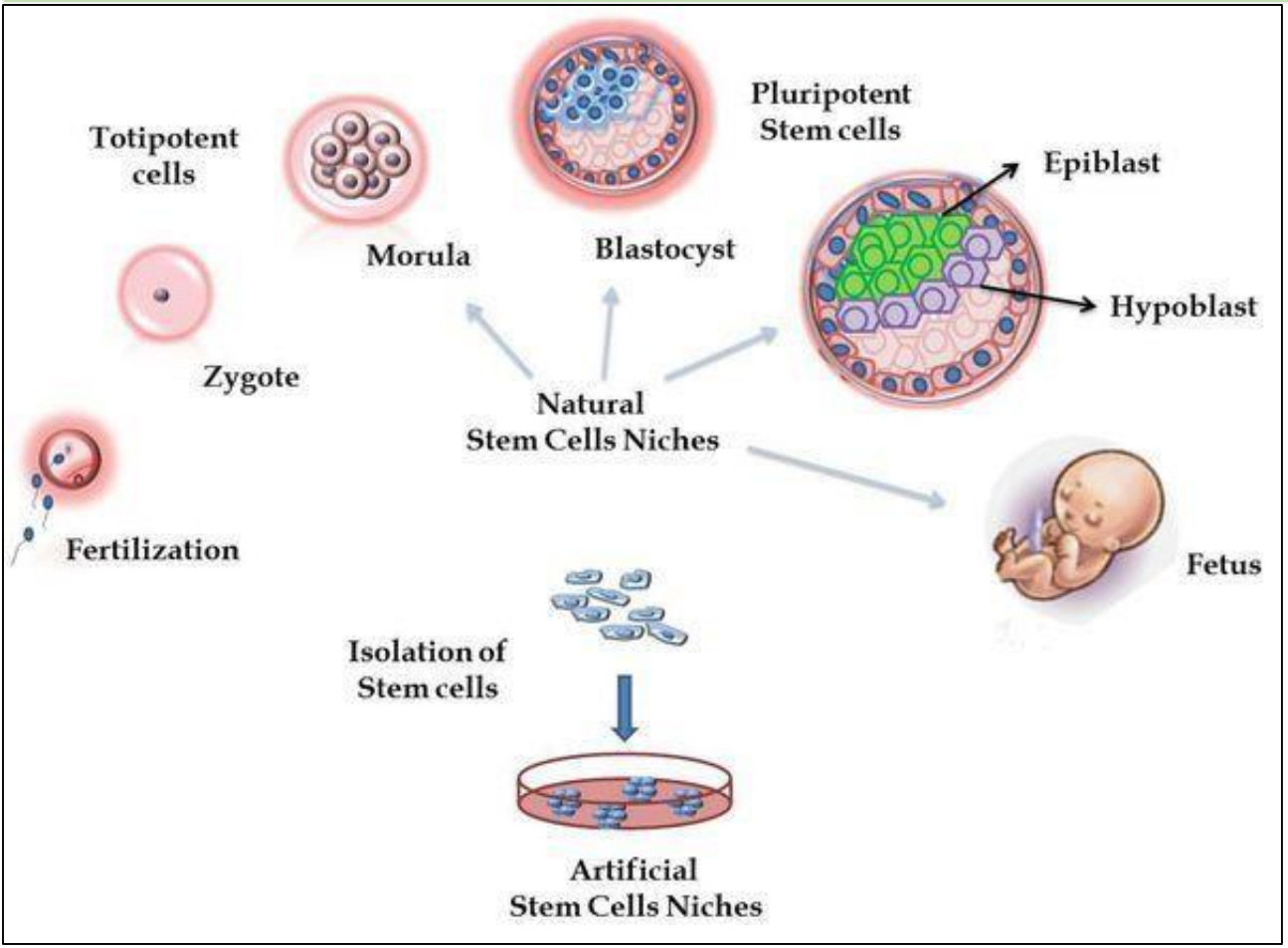
PROGENITOR or PROGENITOR STEM CELL (Oligopotent/tripotent/bipotent):

An undifferentiated cell which has very limited self-renewal potential and can form very few cell types of a specific lineage only and replenish lost or dying or non-functional cells and regenerate these damaged tissues.

UNIPOTENT:

A differentiated cell which can give rise to only one cell type/lineage.

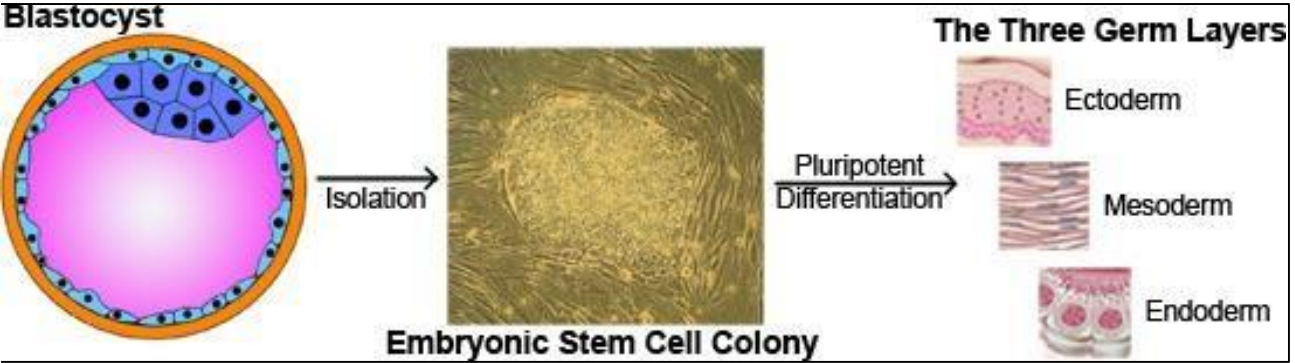
Embryonic Stem Cells (ESCs)



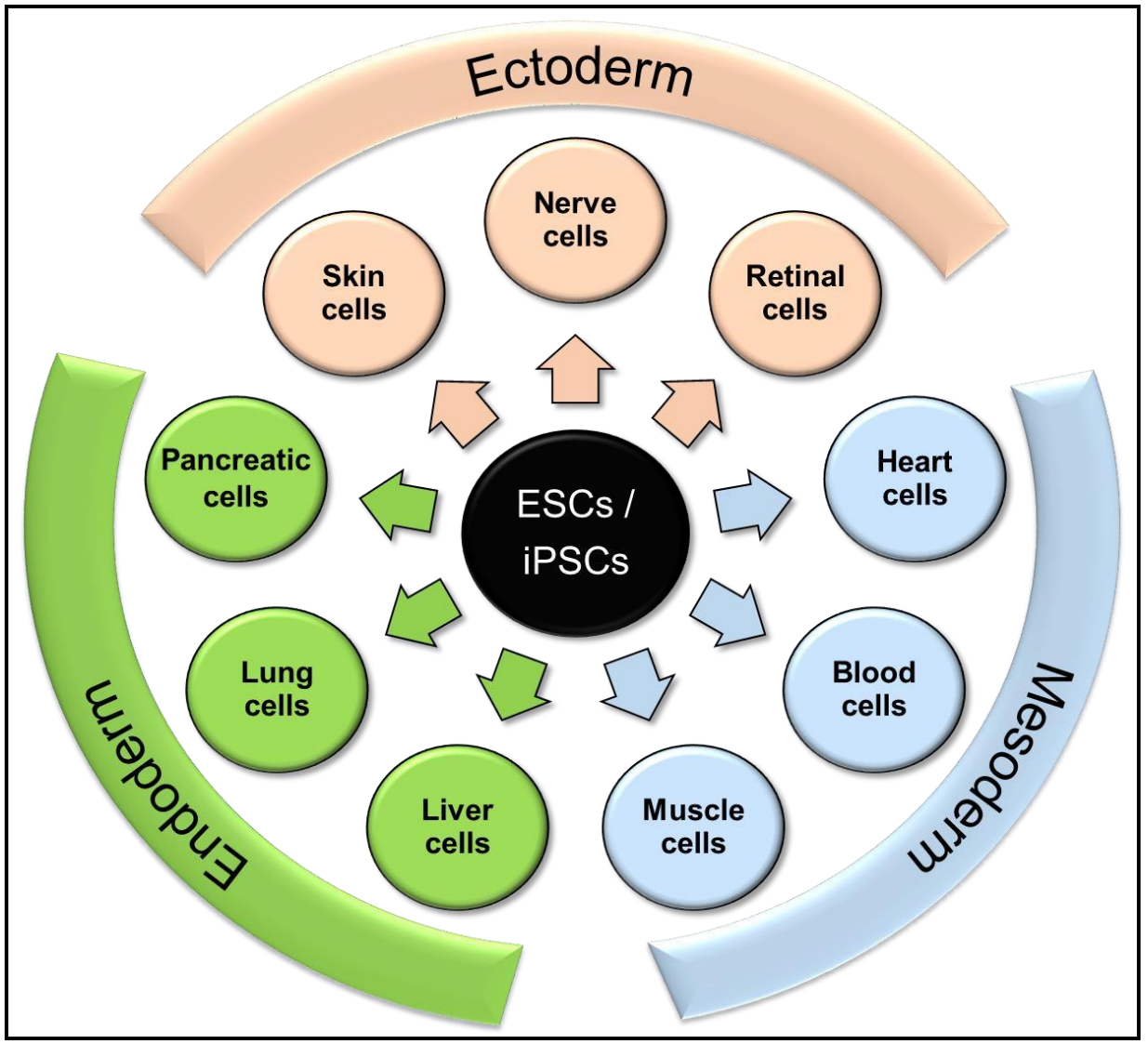
Undifferentiated cells

Two special characteristics:

- Self-renewal
- Pluripotency



Pluripotent Stem Cells (ESCs)



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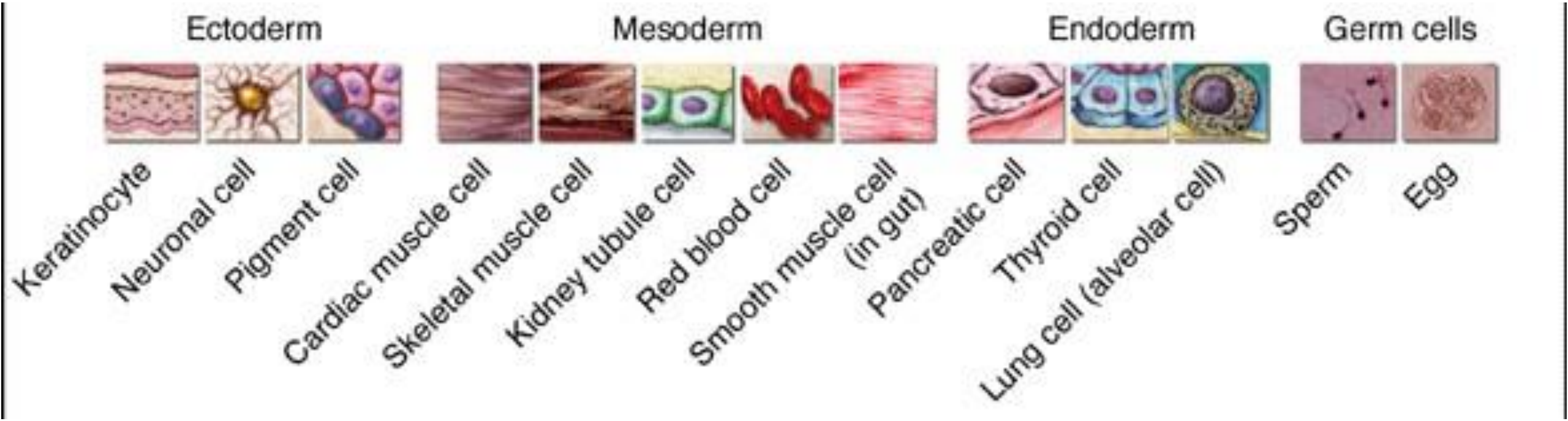
Ectoderm, Mesoderm and Endoderrm



Ectoderm, Mesoderm and Endoderrm

- **Ectoderm (outer layer):**
- Nervous system (spine, peripheral nerves and brain), tooth enamel and the epidermis of skin. It also forms the lining of mouth, anus, nostrils, sweat glands, hair and nails.

- **Mesoderm (middle layer):**
- Bone, cartilage, muscle, connective tissue (including that of the dermis), blood vascular, reproductive, excretory and urinogenital systems and contributes to some glands.

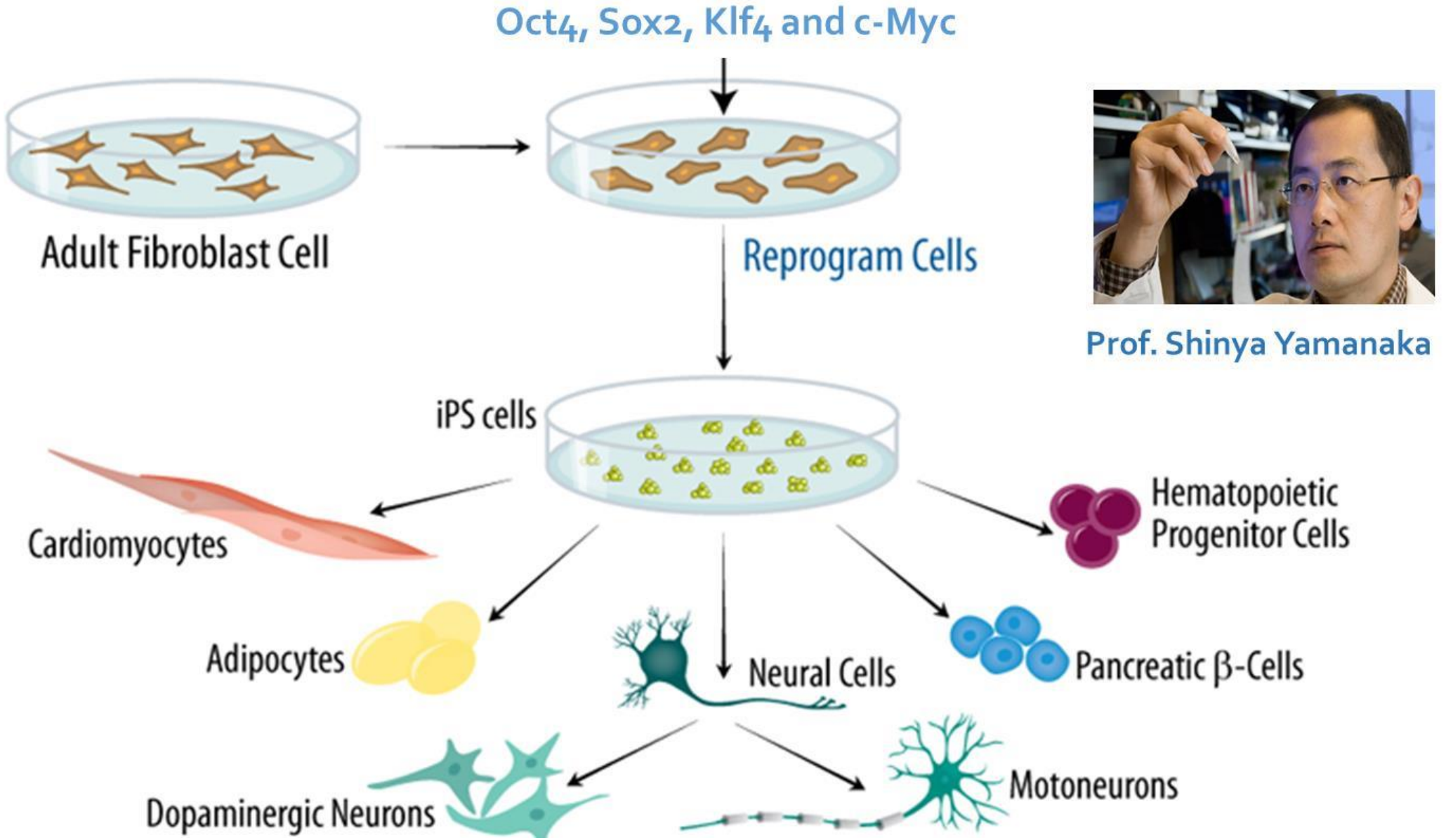
- **Endoderm (inner layer):**
- Lung cell, trachea, bronchi, thyroid cell, pancreatic cell, the epithelium of the auditory tube and tympanic cavity, the urinary bladder and part of the urethra

Limitations of Embryonic Stem Cells

- **Ethical issues** (involves destruction of embryo so controversial)
- **Tumor formation** (unlimited self-renewal potential)
- **Immune rejection**

iPS technology seems to be the answer but still a long way to go

Induced Pluripotent Stem Cells (iPSCs)

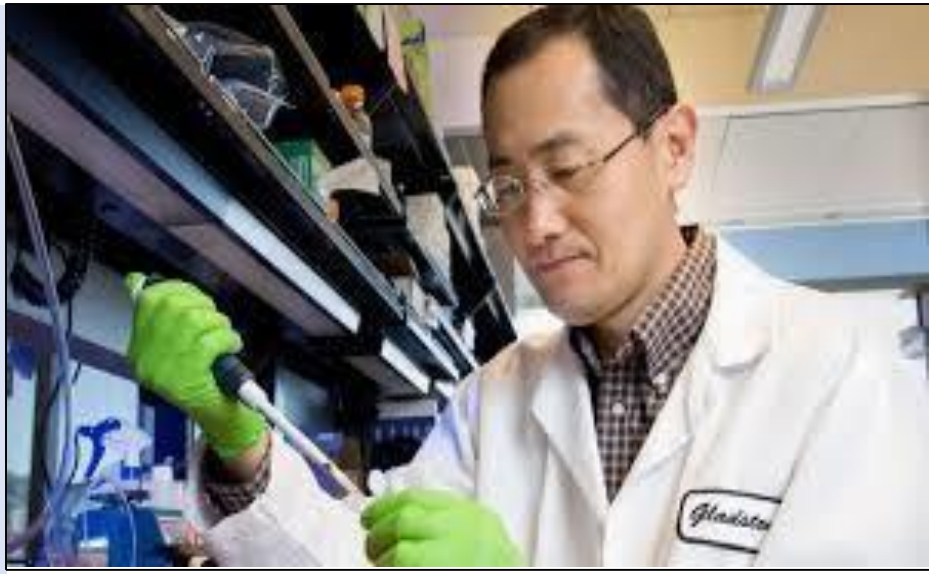


Prof. Shinya Yamanaka

induced Pluripotent Stem Cells (iPSCs)

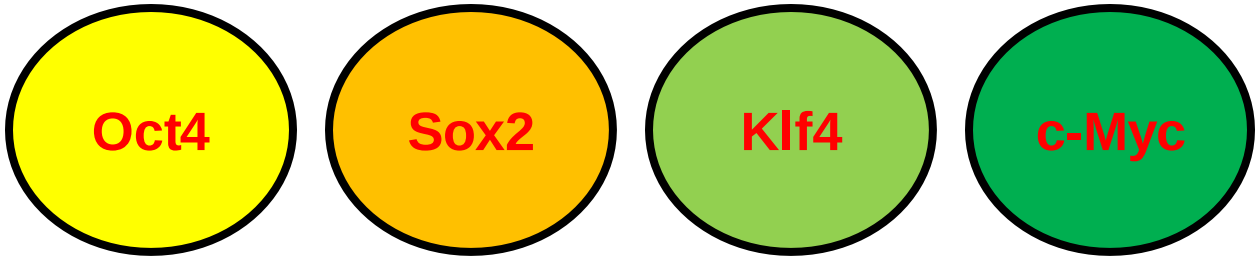
- ❑ undifferentiated cell
- ❑ **self-renew indefinitely *in vitro***, and
- ❑ differentiate into **all cell types of all the three germ layers**, except placental (extra embryonic) tissues.

- **Any somatic cell can be reprogrammed to a pluripotent state.**
- **Source of autologous cells**



Prof. Shinya Yamanaka
(2006)

Transcription factors (*Yamanaka factors*)

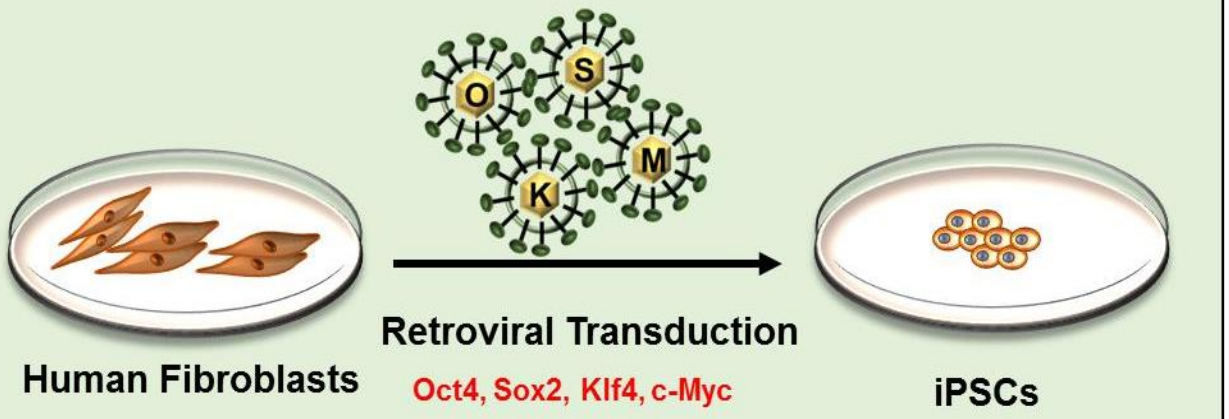


Nobel Prize (Medicine) in the year 2012
Mouse iPSCs – 2006
Gene delivery approach – Retroviral

The first human iPSC studies

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

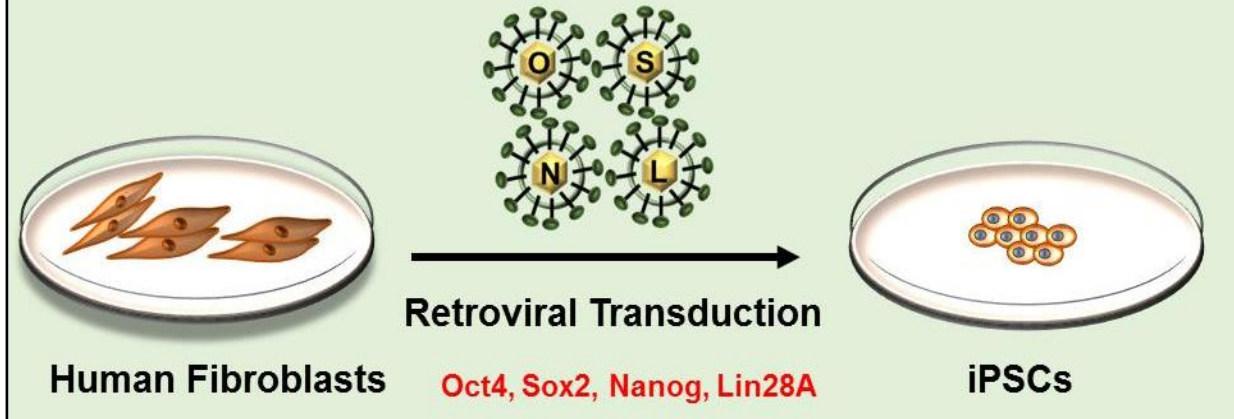


Transcription factors (*Yamanaka factors*)

Oct4 Sox2 Klf4 c-Myc

Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,[1,2*] Maxim A. Vodyanik,[2] Kim Smuga-Otto,[1,2] Jessica Antosiewicz-Bourget,[1,2] Jennifer L. Frane,[1] Shulan Tian,[3] Jeff Nie,[3] Gudrun A. Jonsdottir,[3] Victor Ruotti,[3] Ron Stewart,[3] Igor I. Slukvin,[2,4] James A. Thomson[1,2,5*]



Transcription factors (*Thomson factors*)

Oct4 Sox2 Nanog Lin28 A

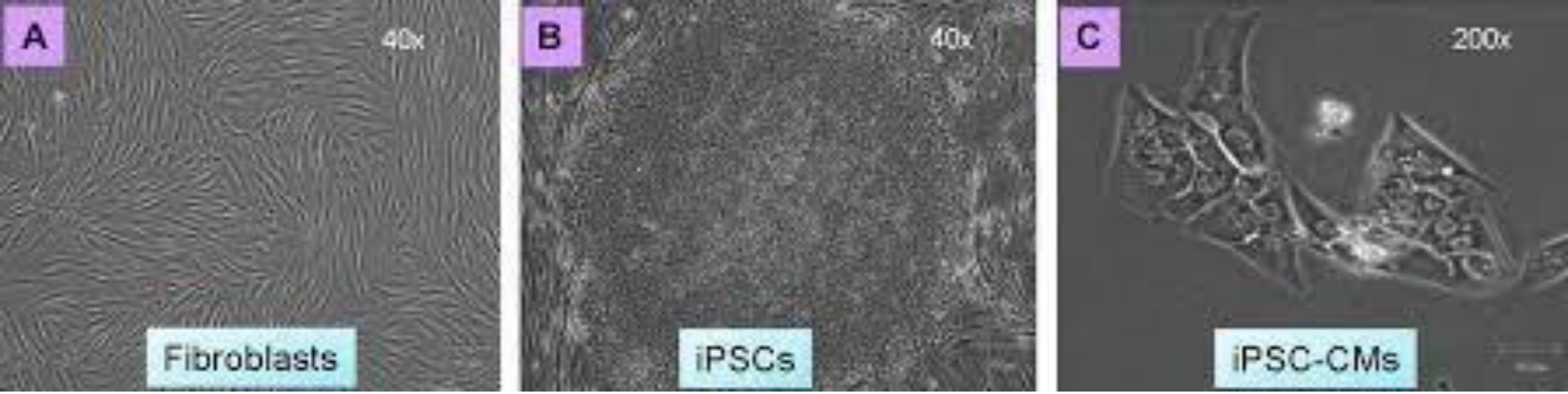
Advantages of iPSCs

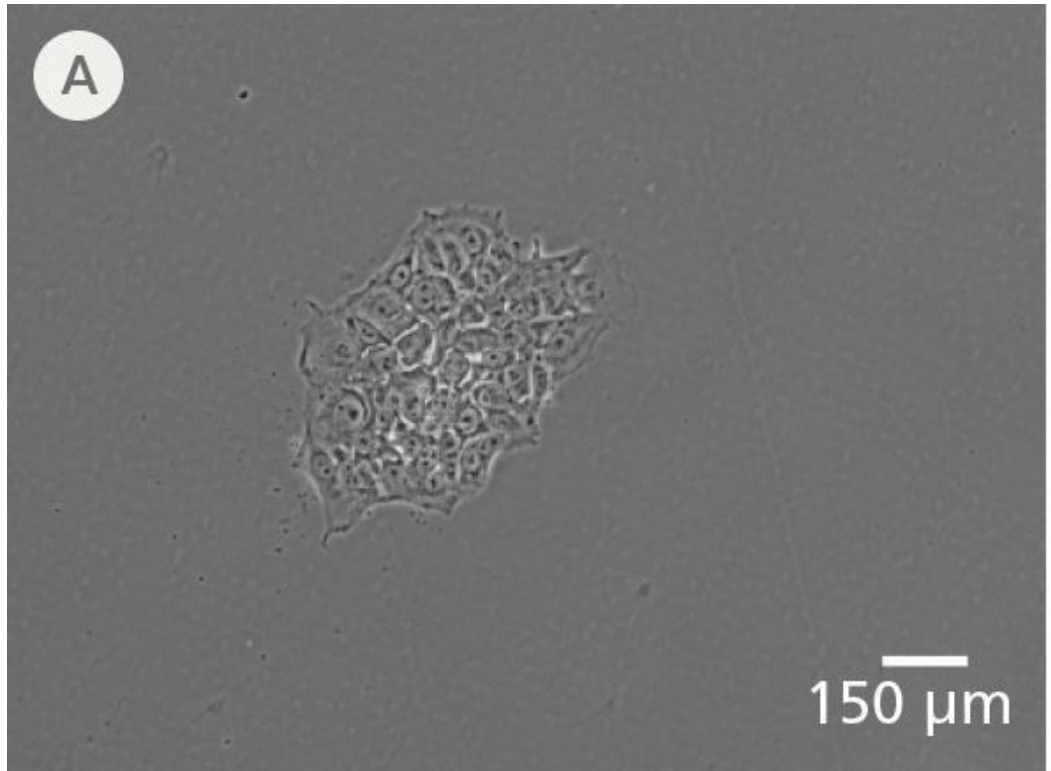
- No destruction of embryo
- Source of pluripotent cells
- Unlimited self-renewal
- Generates autologous cells (Patient-specific iPSCs)
- Non-invasive source

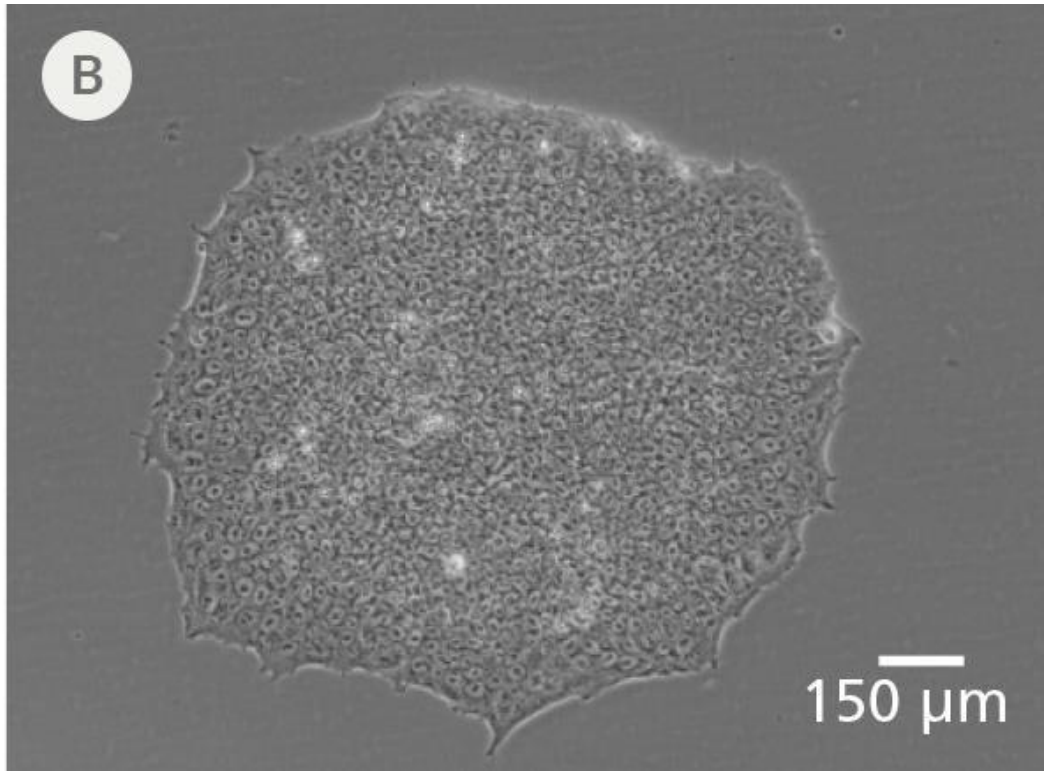
Challenges in the field of iPSCs

- **Tumorigenic potential**
- **Low reprogramming efficiency (0.01-1%)**
- **Slow reprogramming kinetics**
  - **(10-14 days in mouse and 21-28 days in human)**
- **Which cells to use?**
- **Which method to use?**
  - **Safe approach (clinical-grade or biomedically competent iPSCs)**
- **Genomic instability**

Genome instability (also genetic instability or genomic instability) refers to a high frequency of mutations within the genome of a cellular lineage. These mutations can include changes in nucleic acid sequences, chromosomal rearrangements or aneuploidy.


A
40x
Fibroblasts
B
40x
iPSCs
C
200x
iPSC-CMs


A
150 µm


B
150 µm

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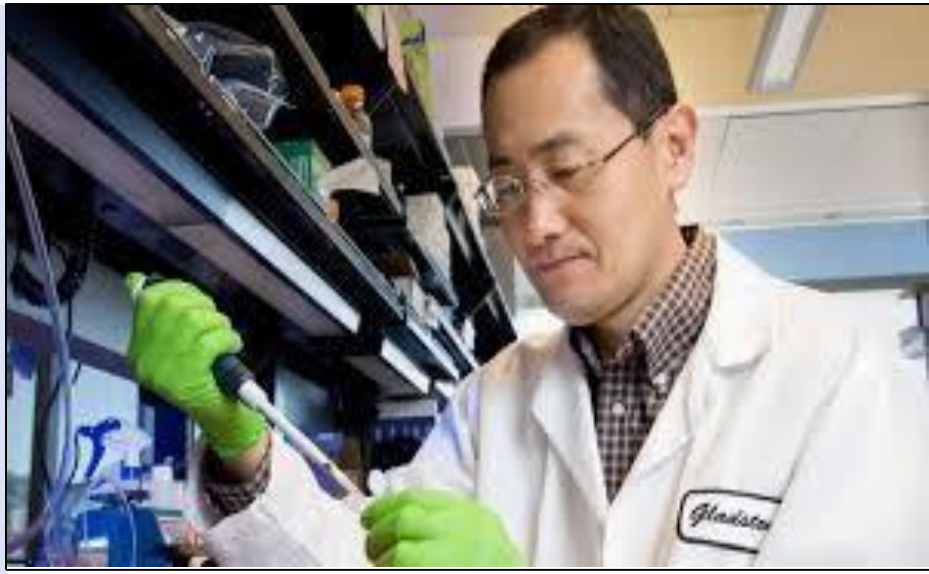

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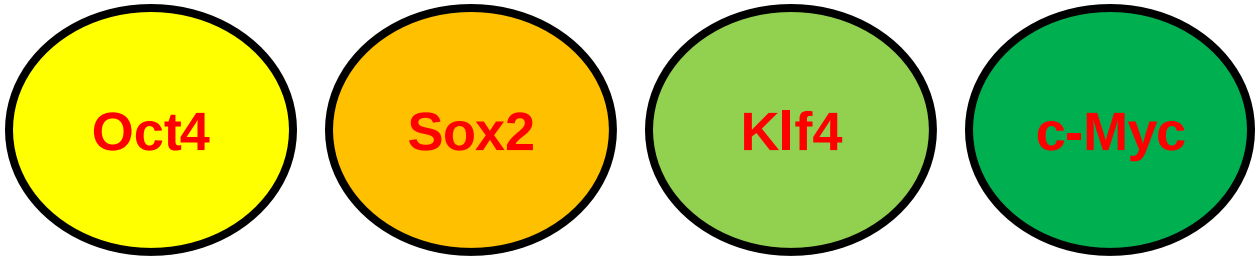

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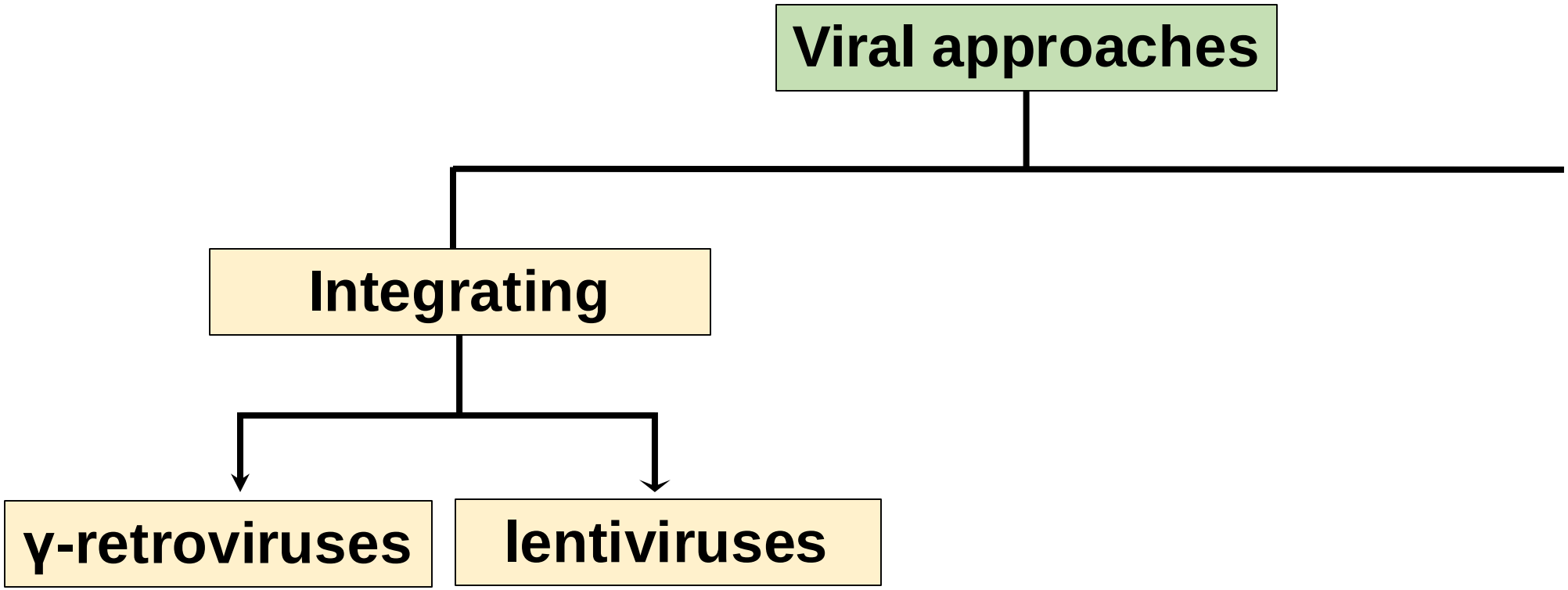
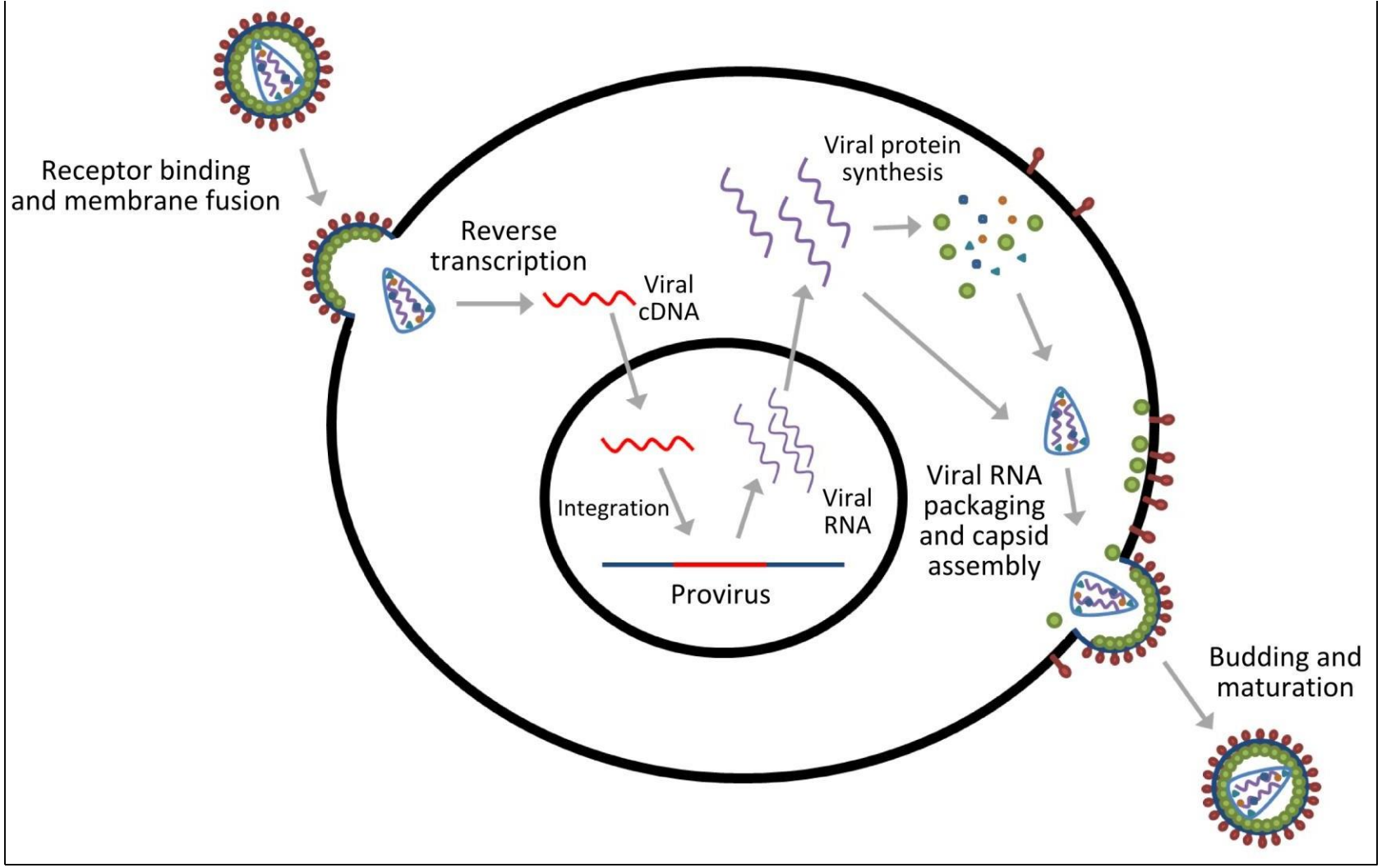
Gene delivery reprogramming approaches



- **Random integration of transgenes**
- **Induces tumorigenecity**

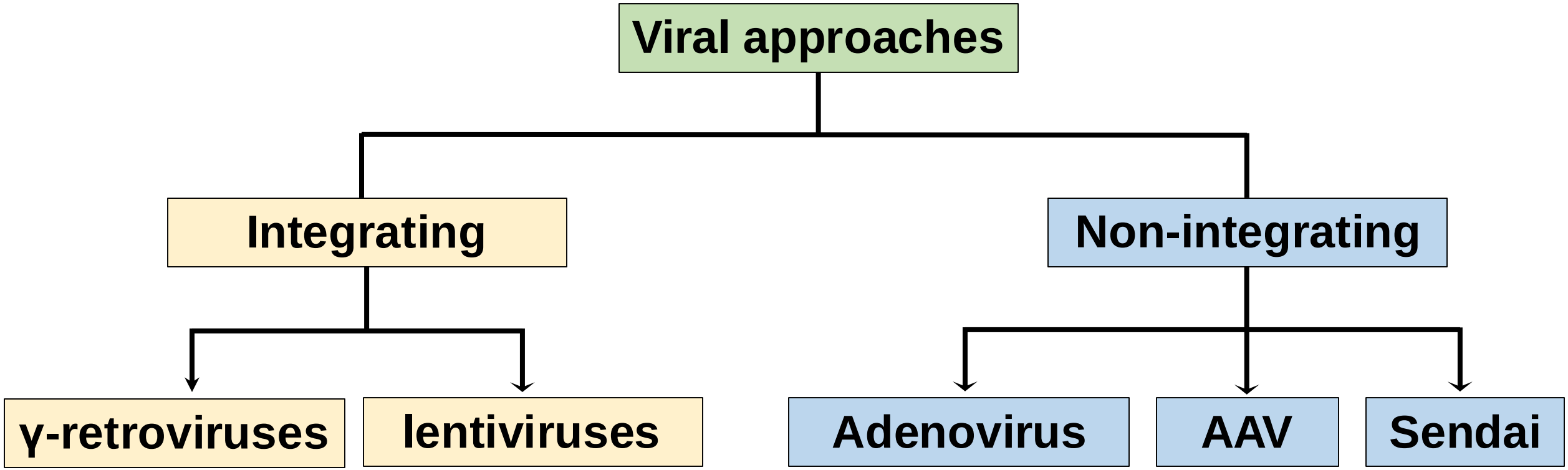
Haridhasapavalan K*, Borgohain MP*, …, **Thummer RP** (**2019**) An insight into non-integrative gene delivery approaches to generate transgene-free induced pluripotent stem cells. Gene. 686:146-159.



Retro- and Lenti-virus Replication. Infection begins when the viral Env protein interacts with the cellular receptor and enters the cell. The RNA genome is reverse-transcribed by RT into dsDNA which then enters the nucleus and is integrated into the host genome through the activity of IN. After the accumulation of newly synthesized viral proteins and viral genomic RNA, the components are packaged and bud from the cell, acquiring a cellularly-derived membrane. The particle matures when the Gag and Gag-Pol polyproteins are cleaved by the viral protease.

https://www.labome.com/method/Nucleic-Acid-Delivery-Lentiviral-and-Retroviral-Vectors.html

Gene delivery reprogramming approaches



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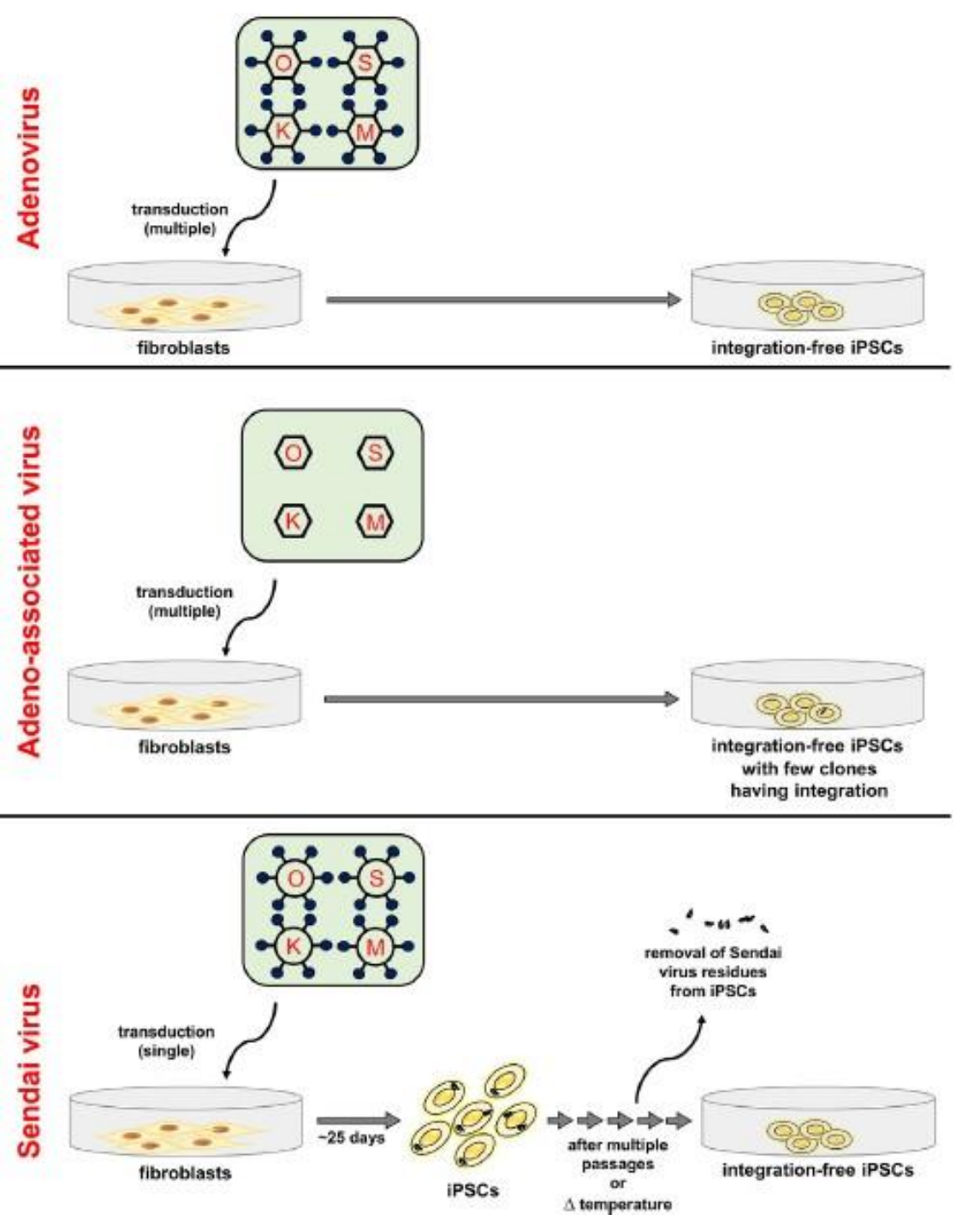


Non-integrating viral approaches to derive transgene-free iPSCs by reprogramming somatic cells.

Nonintegrating viral methods such as adenoviral, AAV and SeV vectors are important gene delivery vehicles for the ectopic expression of key reprogramming factors in the target somatic cells (e.g., fibroblasts) to reprogram them to iPSCs.

While Adenoviral and AAV vectors requiremultiple transductions, SeV requires only single transduction to derive iPSCs. Both adenoviral and SeV vectors can accommodate all the essential transgenes in a polycistronic manner, which is the limiting factor for AAV due to its restricted transgene-carrying capacity (< 5 kb) (Hirsch et al., 2016; Lee et al., 2017). Notably, AAV has very low immunogenicity whereas adenovirus and SeV are immunogenic. Though recombinant AAV genomes are largely episomal, a minimal chance of integration into the target cells has been reported which is not the case with adenoviral and SeV vectors (Lee et al., 2017).

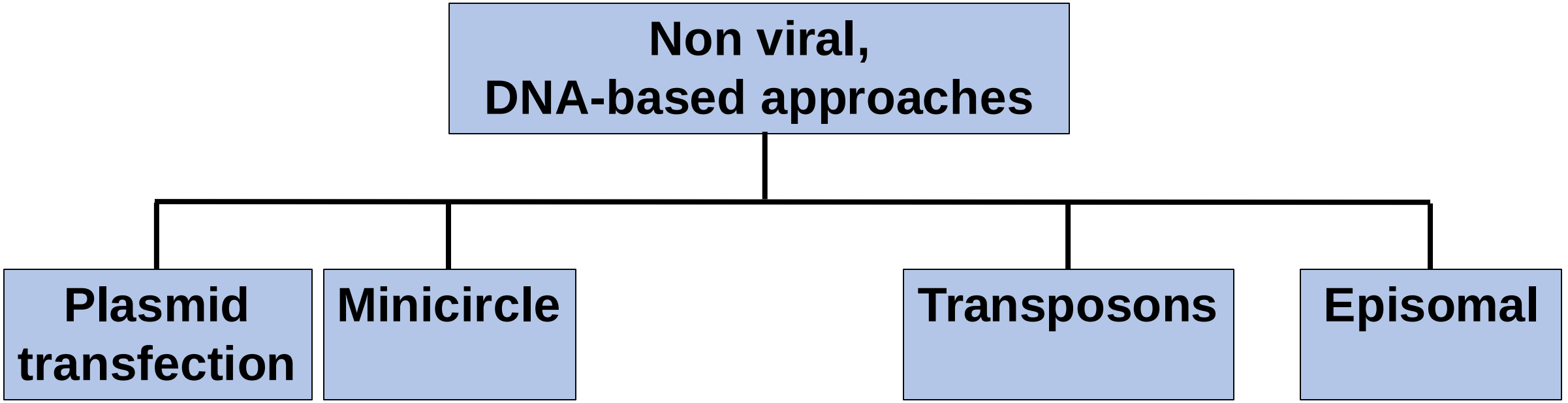
SeV and AAV vectors have a stable transgene expression for a prolonged duration compared to adenoviral vectors. However, once iPSCs are formed, removal of SeV vectors from the iPSC genome is reported to be complicated. This removal is achieved by long-term passaging of iPSCs (> 10 passages) or by using other alternative techniques (antibody-based negative selection, siRNA, temperature shift, etc.). To date, the reprogramming efficiency of SeV vectors is reported to be higher compared to adenoviral and AAV vectors.
Importantly, all the three vectors have a broad tropism and infect both replicating and non-replicating cells.

Table 1
Pros and cons of non-integrating viral-based approaches to derive transgene-free iPSCs.

Vectors	Pros	Cons
Adenovirus	☐ Replication-incompetent or replication-competent ☐ Broad cellular tropism ☐ Infects both dividing and non-dividing cells ☐ No genomic integration ☐ Large cargo capacity	☐ Transient expression. Rapid clearance from dividing cells, therefore, requires multiple rounds of infection ☐ Very low reprogramming efficiency ☐ Immunogenic ☐ Thorough screening of iPSC clones is required to confirm the absence of integration
Adeno-associated virus (AAV)	☐ Replication-incompetent or replication-competent ☐ Broad cellular tropism ☐ Infects both dividing and non-dividing cells ☐ High transduction efficiency ☐ Stable transgene expression ☐ Very low immunogenicity	☐ More than 90% cells show episomal expression. But, site-specific integration is also observed in < 10% cells. Therefore, rare chances of genomic integration can occur. Thorough screening of iPSC clones is required to confirm the absence of integration ☐ Requires multiple rounds of transduction ☐ Limited transgene capacity, therefore, requires multiple viruses containing one factor each ☐ Requires helper virus for replication ☐ Very low reprogramming efficiency ☐ Low titer production ☐ Difficult to produce pure viral stocks
Sendai virus (SeV)	☐ Replication-incompetent or replication-competent ☐ Broad cellular tropism ☐ Infects both dividing and non-dividing cells ☐ No genomic integration ☐ Cytoplasmic, devoid of DNA phase ☐ High transduction efficiency ☐ Stable transgene expression ☐ Single transduction ☐ High reprogramming efficiency	☐ Requires > 10 passages for complete removal of the viral genome. But, the introduction of mutations to generate novel temperature-sensitive vectors and other techniques are developed for easy removal of the viral genome after iPSC formation ☐ Thorough screening of iPSC clones is required to confirm absence of integration ☐ Requires multiple viruses containing one factor each ☐ Immunogenic

Very low reprogramming efficiency - < 0.01%; Low reprogramming efficiency - 0.01–0.1%; High reprogramming efficiency - > 0.1%.

Gene delivery reprogramming approaches



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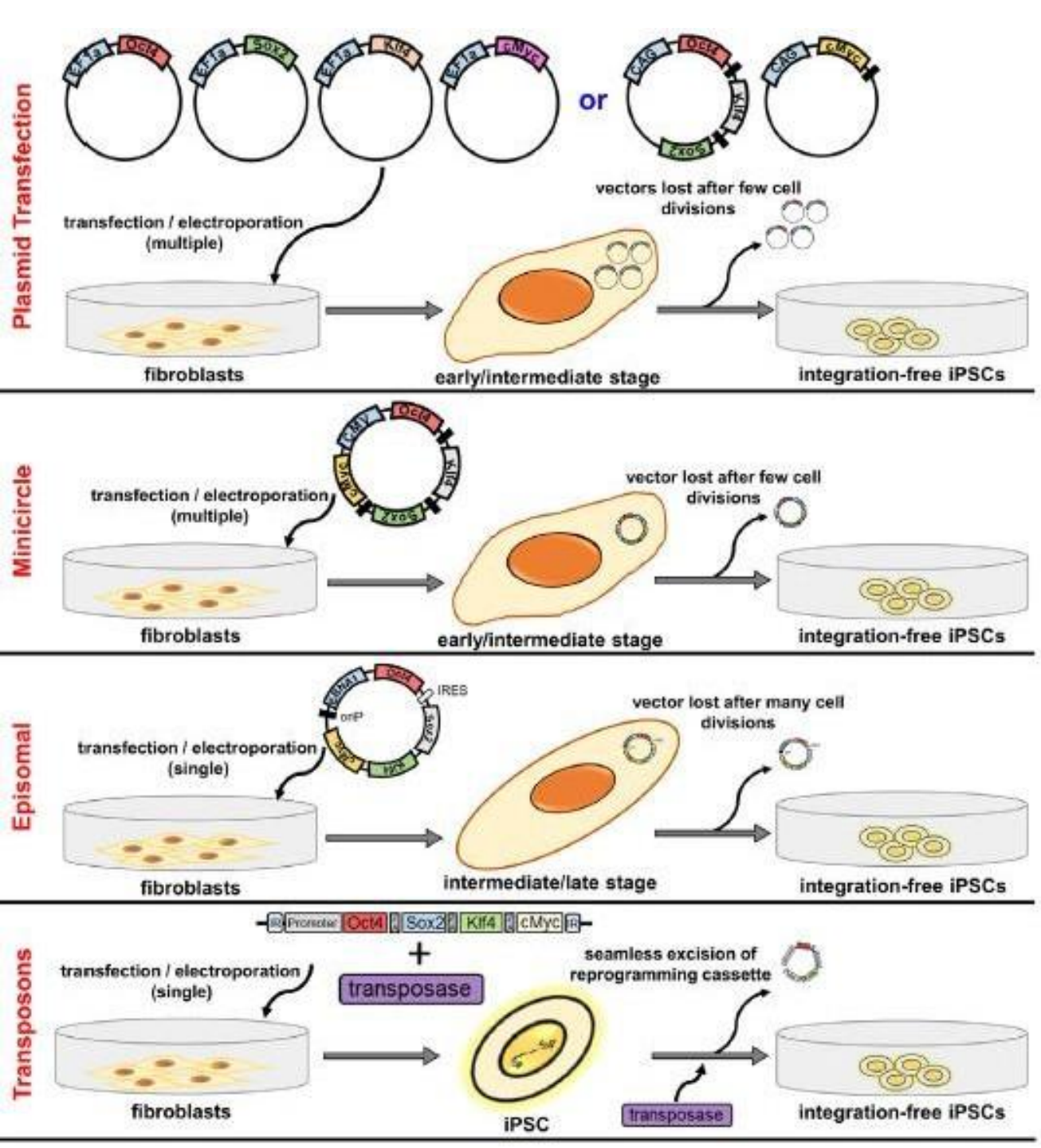


Non-integrating non-viral, DNA-based approaches to derive transgene-free iPSCs.

Non-integrating DNA-based, non-viral approaches such as plasmid transfection, minicircle vectors, transposon vectors, episomal vectors and liposomal magnetofection are employed to derive transgene-free iPSCs by reprogramming somatic cells (e.g. fibroblasts). Plasmid transfection, minicircle vectors, episomal vectors and liposomal transfection act as delivery agents for ectopic expression of reprogramming factors in the somatic cells. These techniques do not require integration into the genome whereas transposon vectors integrate into the genome and the reprogramming cassette is excised upon iPSC formation without leaving any trace in the genome. Plasmid transfection and minicircle vectors require multiple transfections to reprogram somatic cells to iPSCs whereas the other DNA-based approaches require only a single transfection. Episomal vectors can replicate extrachromosomally while autonomous replication in a cell does not take place in case of plasmid transfection and minicircle vectors. Transposon vectors integrate into the genome and result in continuous expression of reprogramming factors. Till date, plasmid transfection and minicircle vectors have been reported to generate iPSCs with very low reprogramming efficiency compared to the other three DNA-based methods. Even though these DNA-based, non-viral

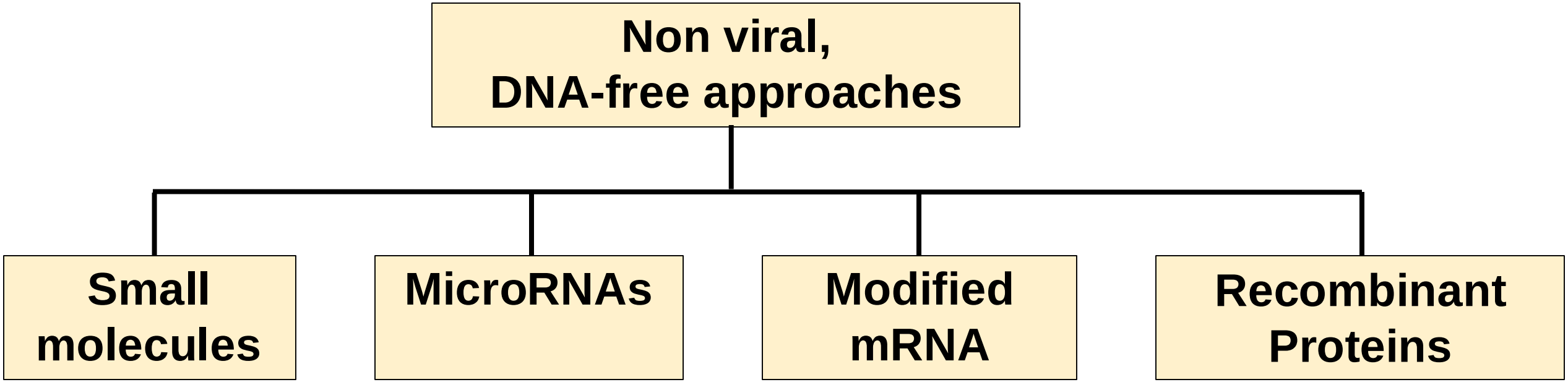
approaches are largely considered to be non-integrative, integration in few iPSC clones in some of these approaches was also observed. Hence, thorough analysis using standard and highthroughput sequencing technologies to confirm the absence of integration and genomic alterations is needed. All the above techniques are reported to successfully generate transgene-free iPSCs. However, further modifications are required to efficiently deliver large polycistronic vectors, enhance reprogramming efficiency and improve the ability to reprogram any cell type with minimal transfections.

Table 2
Pros and cons of non-integrating DNA-based, non-viral approaches to derive transgene-free iPSCs.

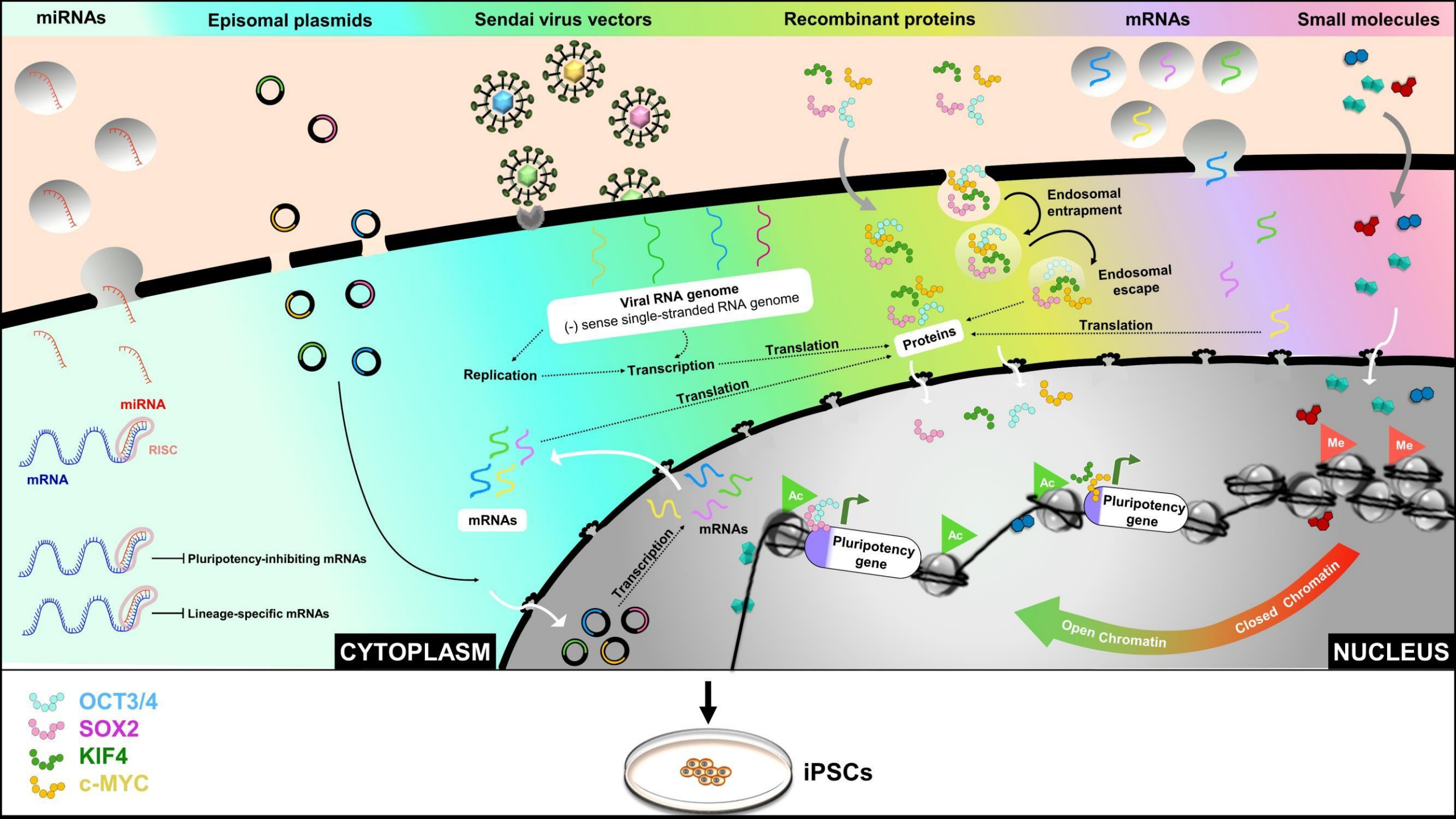
Vectors	Pros	Cons
Plasmid transfection	☐ Technically simple procedure ☐ Low immunogenicity	☐ Though rare chances of genomic integration, thorough screening of iPSC clones is required to confirm the absence of integration ☐ Cannot self-replicate ☐ Requires multiple rounds of transfection, stressful to cells ☐ Very low reprogramming efficiency
Minicircle	☐ Technically simple procedure ☐ Small constructs, easy to deliver, superior transfection efficiency ☐ Very low immunogenicity ☐ Plasmid lacks a bacterial backbone	☐ Though rare chances of genomic integration, thorough screening of iPSC clones is required to confirm the absence of integration ☐ Cannot self-replicate ☐ Requires multiple rounds of transfections, stressful to cells ☐ Very low reprogramming efficiency
Episomal	☐ Technically simple procedure ☐ Self-replicates, therefore, stable transgene expression ☐ Requires only a single transfection ☐ Low immunogenicity	☐ Though rare chances of genomic integration, thorough screening of iPSC clones is required to confirm the absence of integration ☐ Low reprogramming efficiency
Transposons	☐ Integrative, but precisely excisable ☐ Requires only a single transfection ☐ Can transport large cargo ☐ Low immunogenicity	☐ Risk of reintegration may lead to insertional mutagenesis and chromosomal rearrangements, therefore, thorough screening of iPSC clones is required to confirm the absence of integration ☐ Time consuming iPSC clone analysis ☐ Low reprogramming efficiency ☐ Extra excision step using a transposase

Very low reprogramming efficiency - < 0.01%; Low reprogramming efficiency - 0.01–0.1%; High reprogramming efficiency - > 0.1%.

Gene delivery reprogramming approaches



Borgohain MP*, Haridhasapavalan K*, …, **Thummer RP** (**2019**) An Insight into DNA-free Reprogramming Approaches to Generate Integration-free Induced Pluripotent Stem Cells for Prospective Biomedical Applications. Stem Cell Rev and Rep.

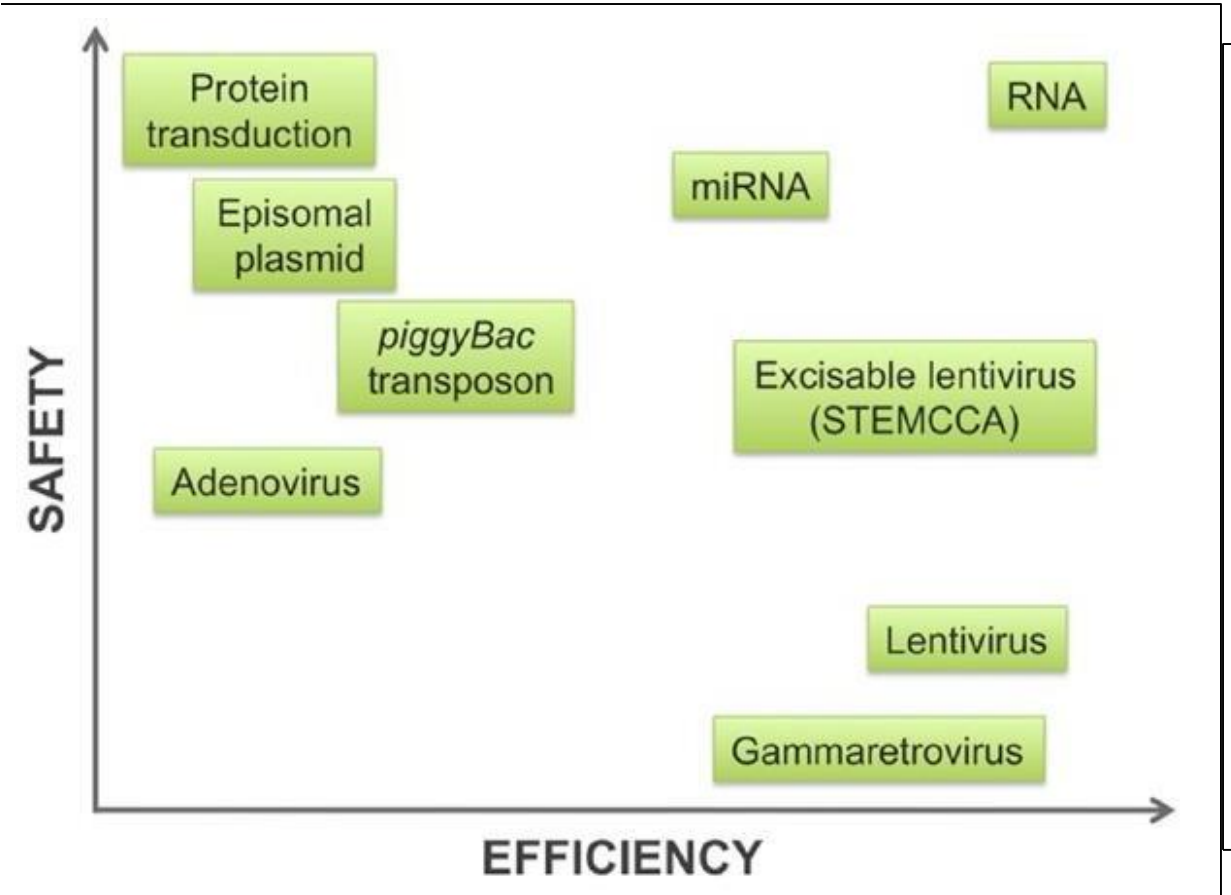

miRNAs
Episomal plasmids
Sendai virus vectors
Recombinant proteins
mRNAs
Small molecules
Endosomal entrapment
Endosomal escape
Viral RNA genome
(-) sense single-stranded RNA genome
Replication
Transcription
Translation
Translation
Translation
Proteins
miRNA
RISC
mRNA
mRNAs
Transcription
mRNAs
Pluripotency-inhibiting mRNAs
Lineage-specific mRNAs
Ac
Pluripotency gene
Ac
Ac
Pluripotency gene
Me
Me
Open Chromatin
Closed Chromatin
CYTOPLASM
NUCLEUS
OCT3/4
SOX2
KIF4
c-MYC
iPSCs

Gene delivery reprogramming approaches

The Evolving Field of Induced Pluripotency: Recent Progress and Future Challenges

CESAR A. SOMMER AND GUSTAVO MOSTOSLAVSKY*

2012 WILEY PERIODICALS



Methods for making induced pluripotent stem cells: reprogramming à la carte

Federico González, Stéphanie Boué* and Juan Carlos Izpisúa Belmonte*‡*

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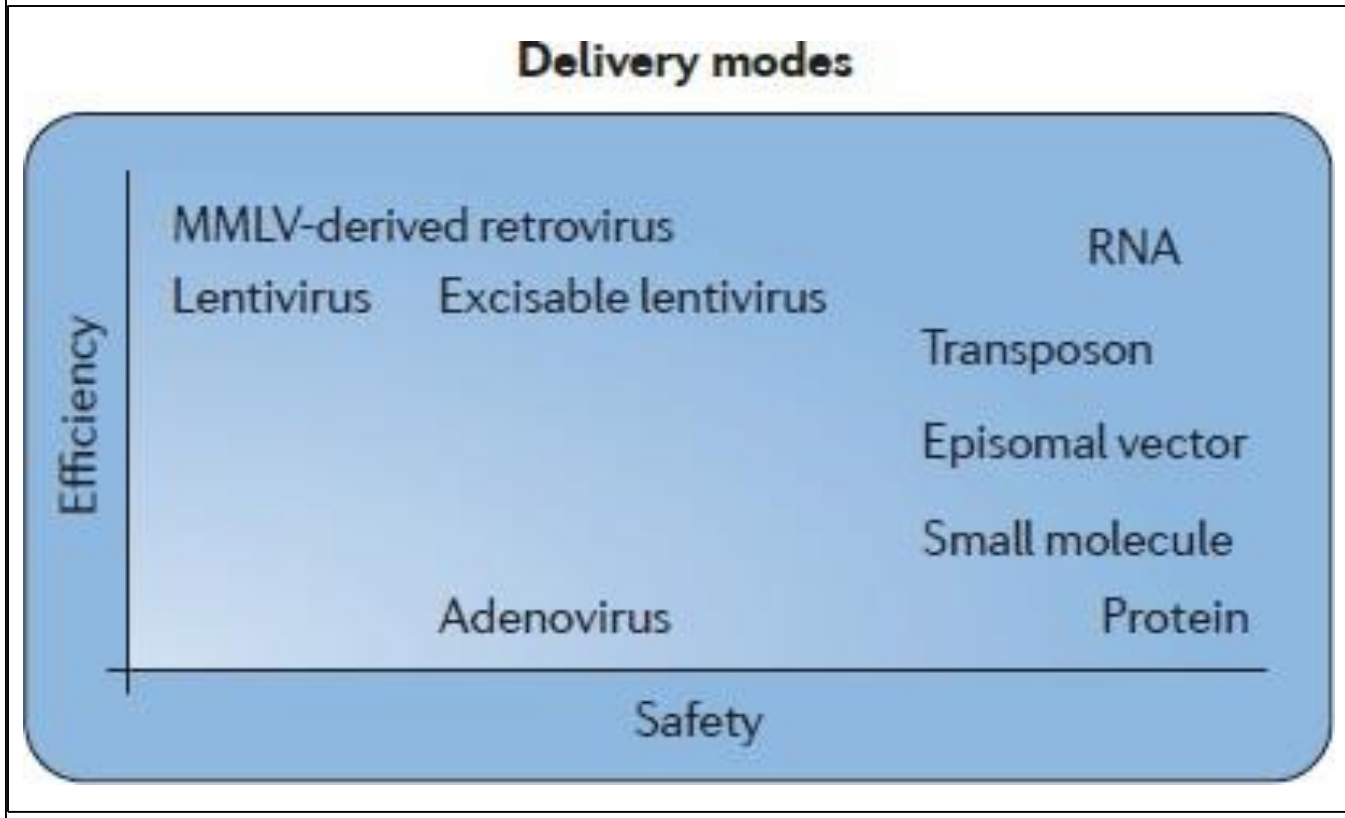
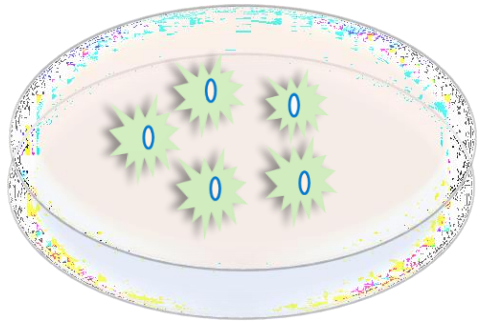
VOLUME 12 | APRIL 2011 | 231

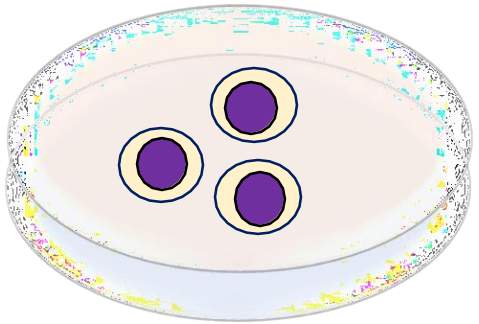


Applications of iPSCs



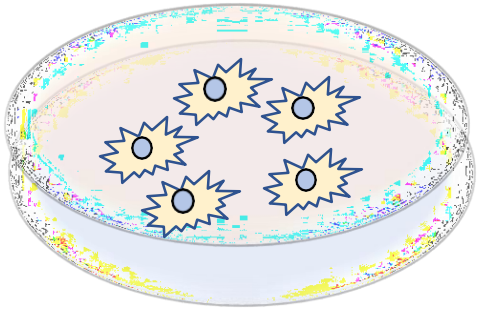
Somatic cells

Reprogramming



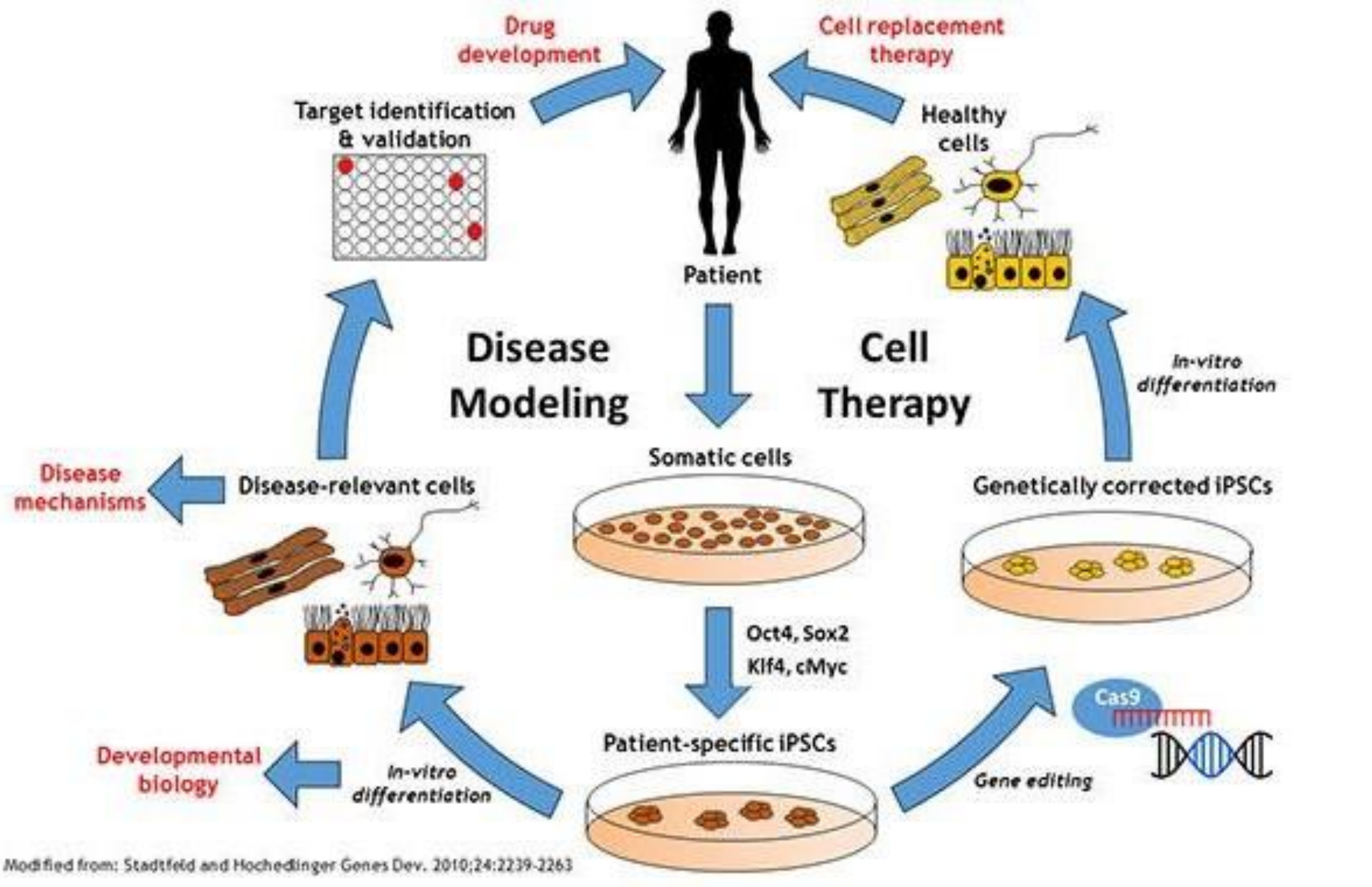
iPSCs

Differentiation



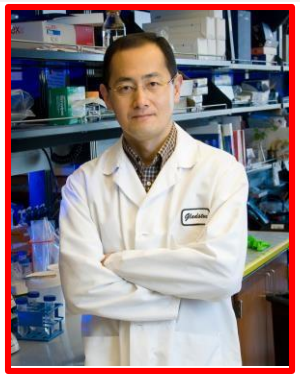
Target cells

- Regenerative therapy / tissue engineering / cell based therapy
- Drug screening
- Disease modeling
- Developmental biology

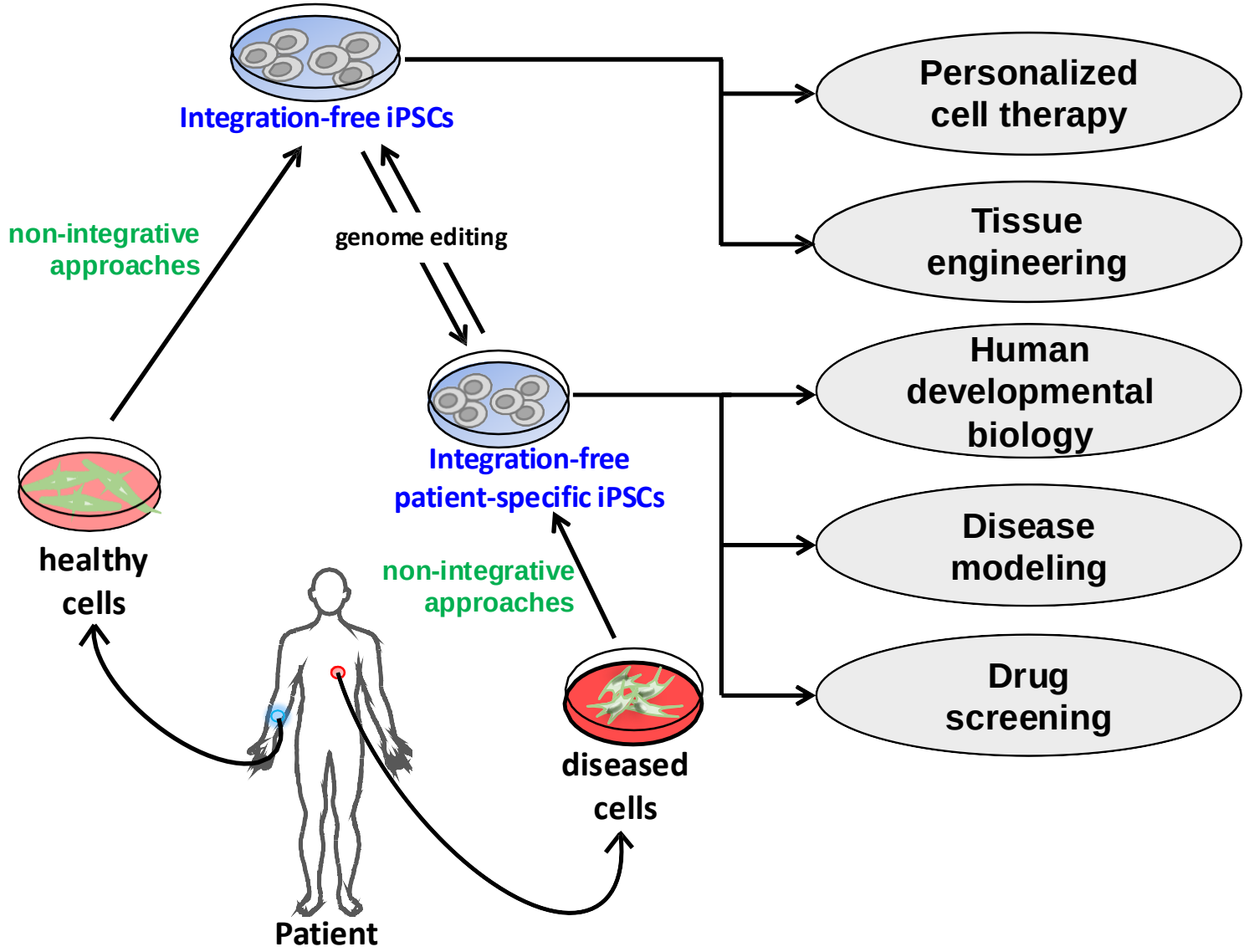


Modified from: Stadtfeld and Hochedlinger Genes Dev. 2010;24:2239-2263

Applications of iPSCs



Prof. Shinya Yamanaka



Thank you