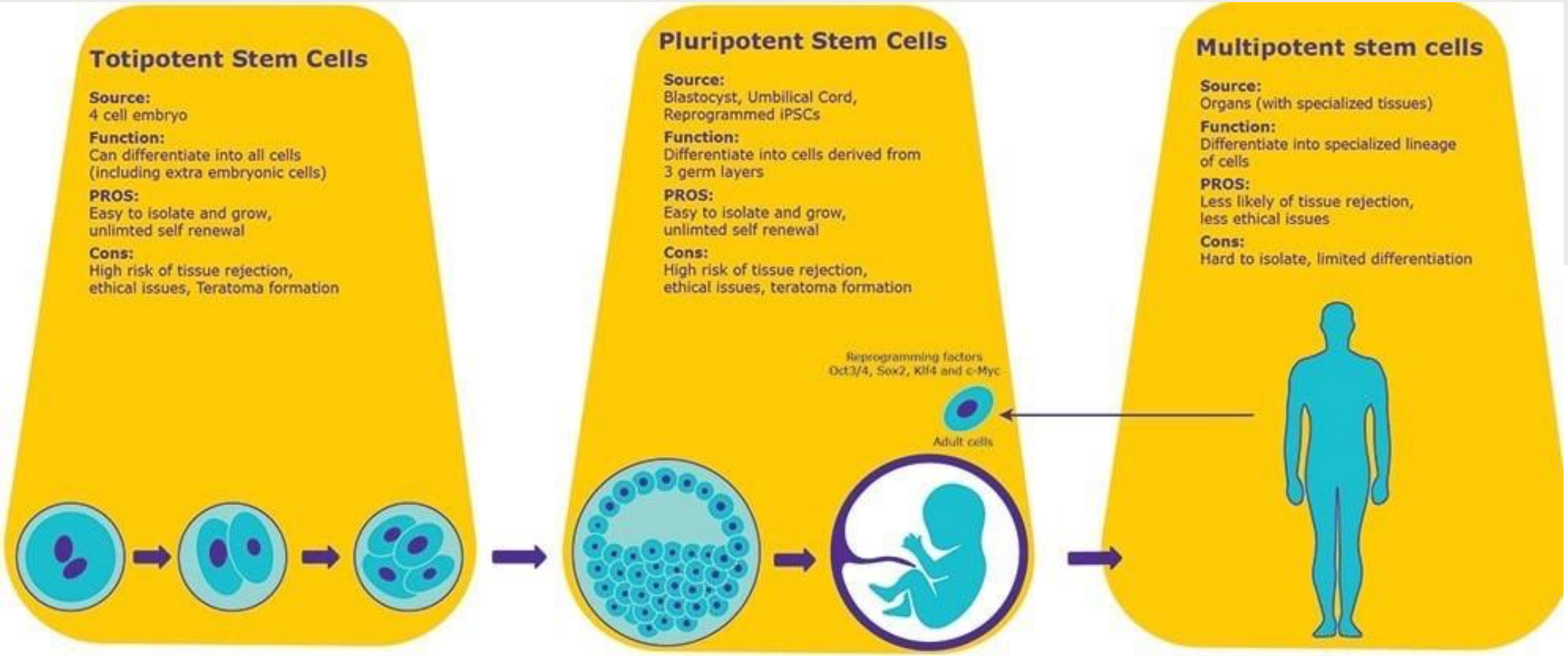
# iPSC as powerful models for translational medicine

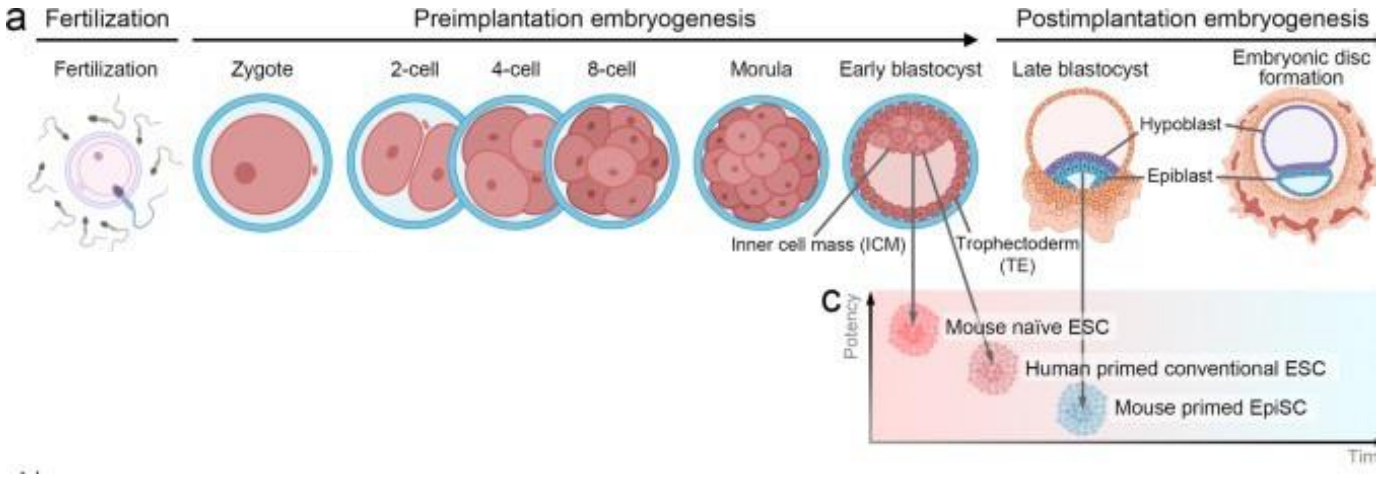
# Types of stem cells




https://www.sigmaaldrich.com/IT/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/stem-cell-culture/what-are-stem-cells

# Dynamics of stem cells development

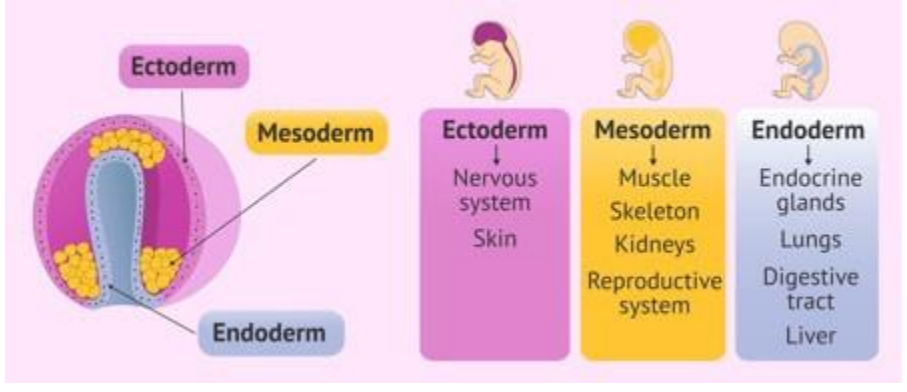
- Differences in environmental conditions applied to each cell in the mass result in the generation of the first heterogeneous population: the ICM and trophectoderm (TE)
- Trophoblasts organize surrounding structures such as the chorion, which supports embryogenesis, whereas the ICM is critical for the formation of the embryo
- After implantation of a blastocyst in the maternal endometrial epithelium, the ICM undergoes subsequent morphogenetic changes.
- The ICM of a postimplantation blastocyst contains epiblasts and hypoblasts
- The morphogenetic events include the polarization of the epiblast, which forms the central lumen that develops into the amniotic cavity; creation of the amniotic epithelium, which forms the amniotic sac membrane; and differentiation of primordial germ cells, which are precursors of eggs or sperm.
- Moreover, extraembryonic mesenchyme cells derived from the hypoblast surround the generated structure to isolate it from the outer cell membrane (OCM) formed by the trophoblast
- Thereafter, epiblasts in the ICM form a primitive streak, gastrulate and differentiate into three germ layers: The ectoderm, mesoderm, and endoderm[47] .




Adapted from Che, 2022

totipotent

pluripotent



5740–5741 | PNAS | April 9, 2013 | vol. 110 | no. 15

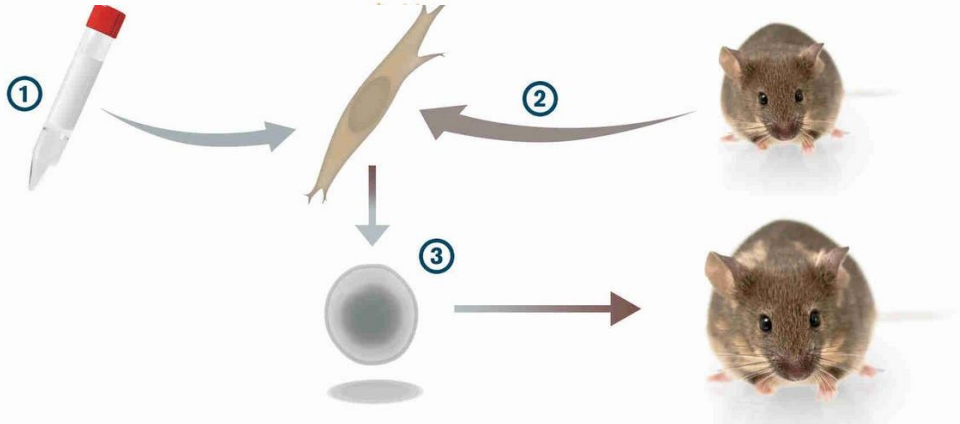
# Profile of John Gurdon and Shinya Yamanaka, 2012 Nobel Laureates in Medicine or Physiology

In 1962, by inserting the nuclei of intestinal epithelial cells into enucleated eggs, Gurdon was able to create healthy swimming tadpoles. These experiments were the first successful instances of **somatic cell nuclear transfer (SCNT)** using genetically normal cells.

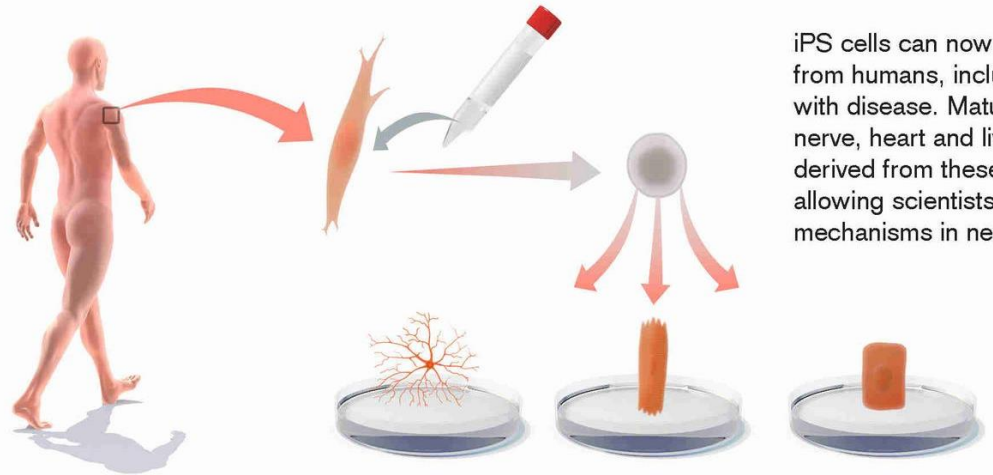
In 2006, Yamanaka with four defined transcription factors induced intact mouse somatic cells to revert to a pluripotent state without an egg or embryo as intermediary.

# **The Nobel Prize** in Physiology or Medicine 2012

**Shinya Yamanaka**




Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent stem (iPS) cells.



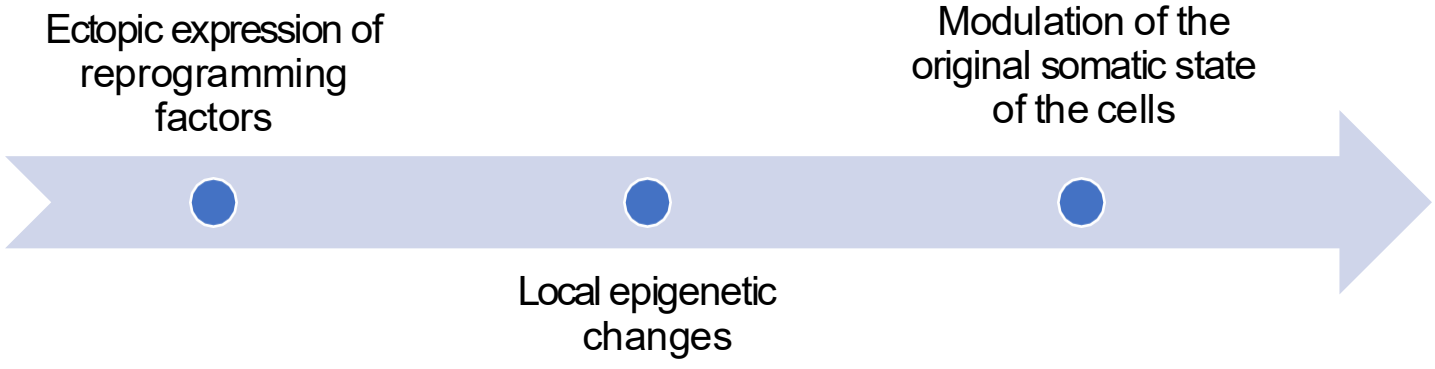
iPS cells can now be generated from humans, including patients with disease. Mature cells including nerve, heart and liver cells can be derived from these iPS cells, thereby allowing scientists to study disease mechanisms in new ways.



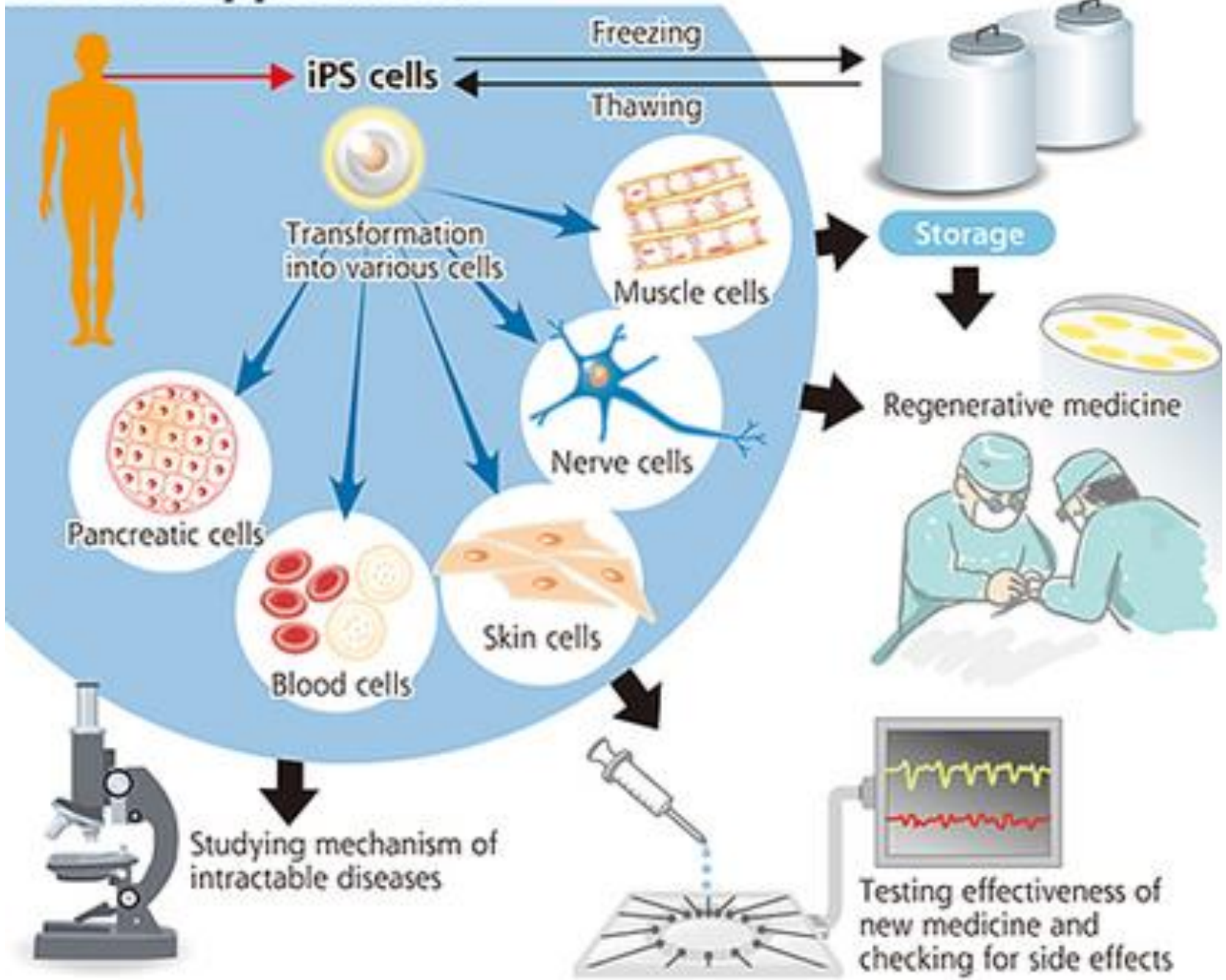
Illustration and layout: Mattias Karlén

# REPROGRAMMING iPSC

- Reprogramming is achieved over several weeks by forced expression of genes that are known to be master regulators of pluripotency.
- At the end of this process, these master regulators will remodel the expression of an entire network of genes.
- Features of differentiated cells will be replaced by those associated with the pluripotent state, essentially reversing the developmental process.
- mesenchymal genes ➔ initially repressed by OCT4 and SOX2
- Proliferation ➔ enhanced by MYC
- epithelial genes ➔ induced by KLF4
- self-sustaining regulatory network providing cells with pluripotency factors is reactivated
- ectopic expression of pluripotency factors then no longer needed
- key role of chromatin remodeling & epigenetic makeup (origin) of the cells



# iPS cell applications

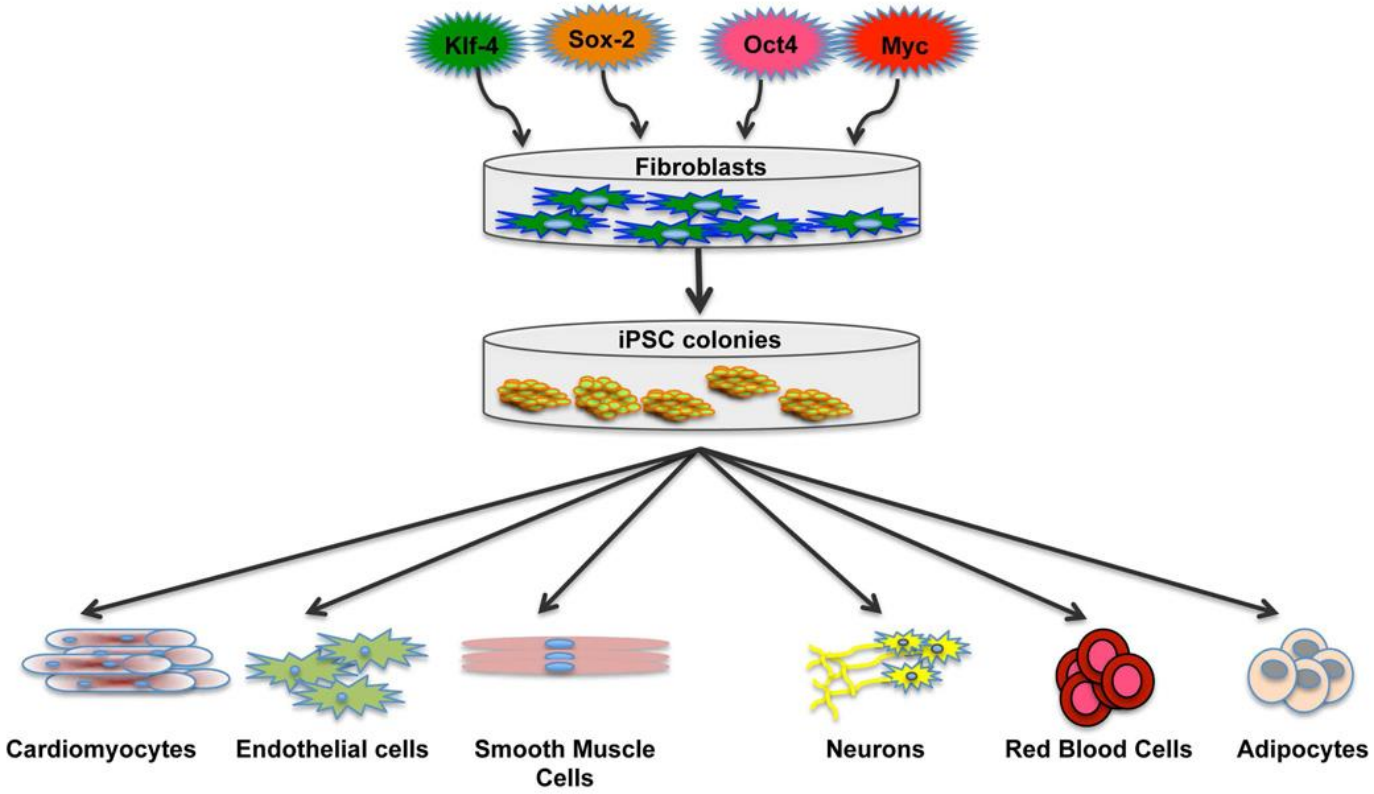


Shinya Yamanaka, Nobel Laureate in Medicine or Physiology, 2012

In 2006, Yamanaka with four defined transcription factors induced intact mouse somatic cells to revert to a pluripotent state without an egg or embryo as intermediary.

# Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024
Cell 126, 663-676, August 25, 2006 



# Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1]Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
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DOI 10.1016/j.cell.2006.07.024
Cell 126, 663-676, August 25, 2006 ©2006 Elsevier Inc.

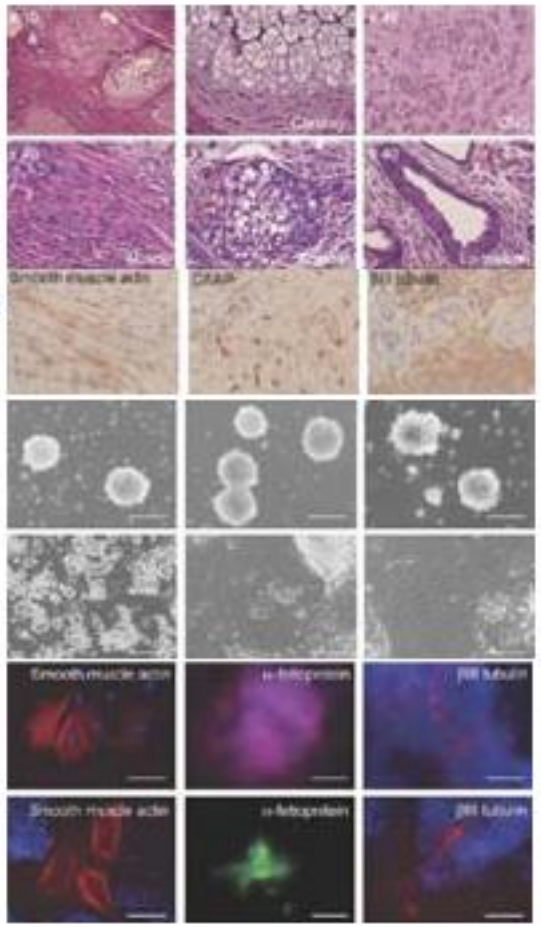
Induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, **Oct3/4, Sox2, c-Myc**, and **Klf4** in the FBX15 locus, under ES cell culture conditions.

Various tissues present in teratomas derived from iPS

Neural tissues and muscles in teratomas

In vitro embryoid body formation and differentiation

In vitro differentiation into all three germ layers.

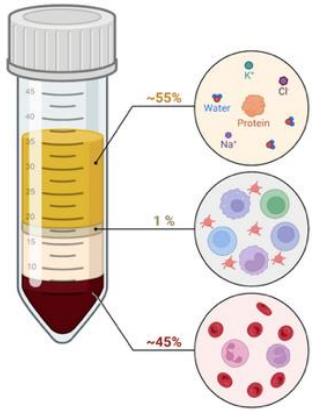


These cells, which were designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes.

1- Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers.

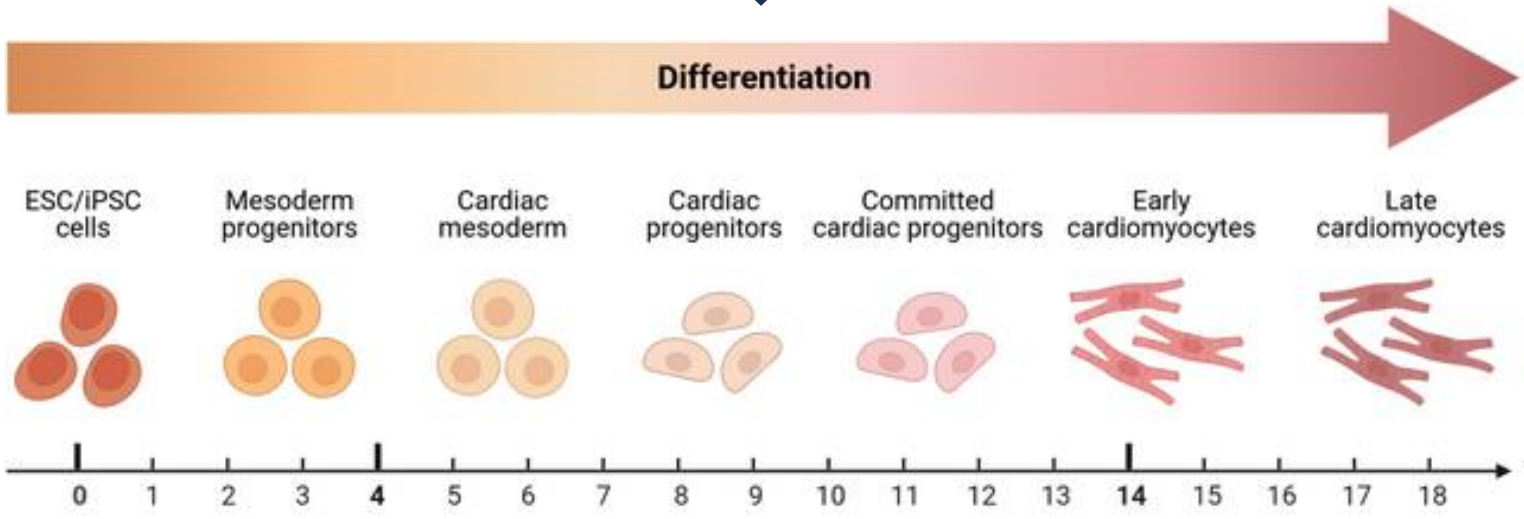
2- Following injection into blastocysts, iPS cells contributed to mouse embryonic development, but embryos failed to develop beyond mid-gestation stage.

# Induced Pluripotent Stem Cell-Derived Cardiomyocytes



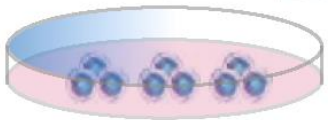

**Peripheral blood mononuclear cells**

iPSCs




## Disease modelling

Patient-derived hIPSC biobank



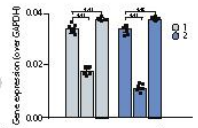
Cardiomyocytes

Cardiac fibroblasts

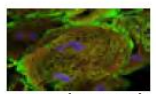
Endothelial cells

Adipocytes

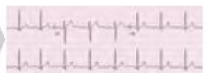
Macrophages



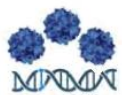
Gene expression analysis



Cytological analysis



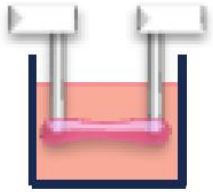
Electrophysiological analysis



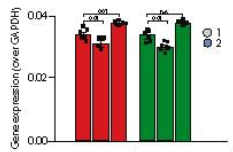
Gene addiction effect

## Heart-on-a- chip models

Patient-derived EHT biobank

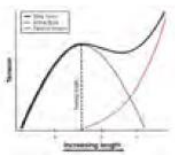


Engineered heart tissues



Gene expression analysis

Hystopathological analysis



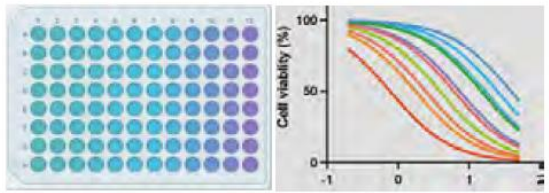
Mechanical force analysis

Gene addiction effect

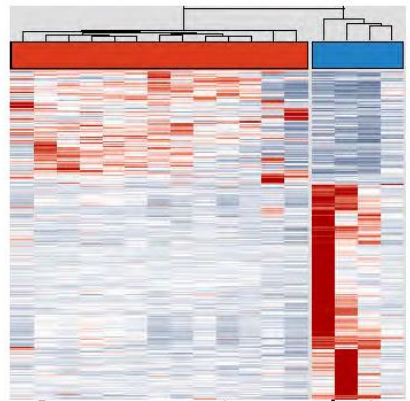
## Therapeutic development

Patient-specific drug screening

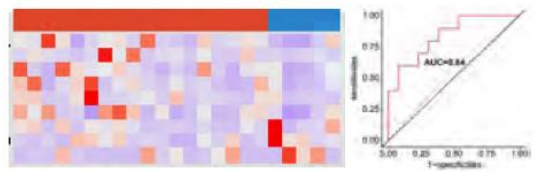
Clinical trial "in a dish"




In vitro drug screening



Gene expression analysis



Biomarker expression analysis

## Therapeutic validation

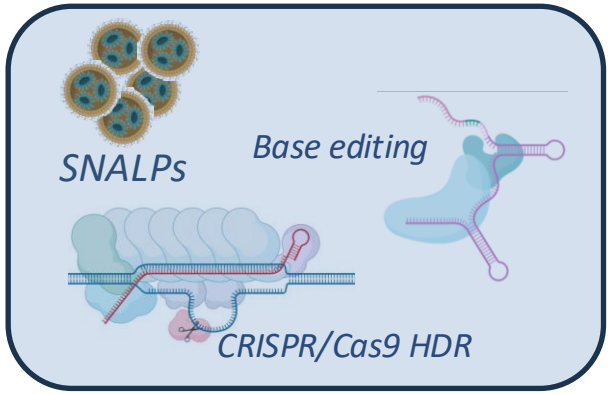
Patient-specific drug validation

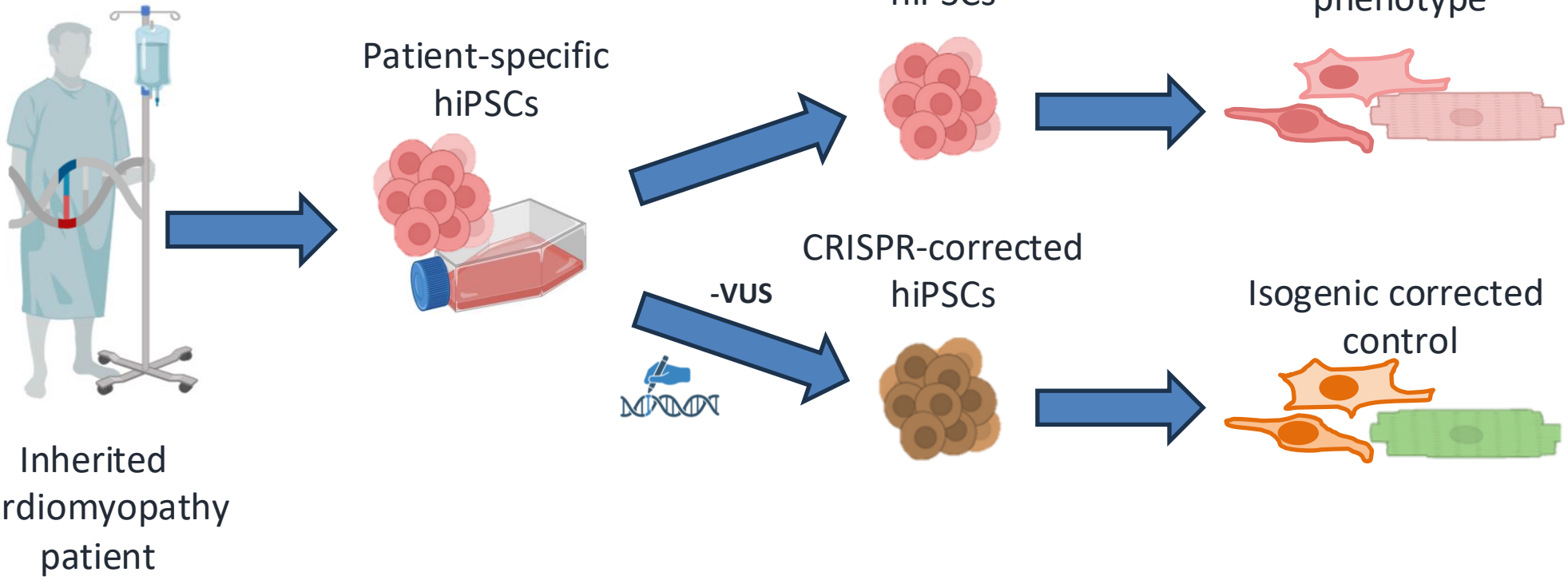
Patient-targeted therapy

Pro-active monitoring of the disease

Preventive drug treatment of family members

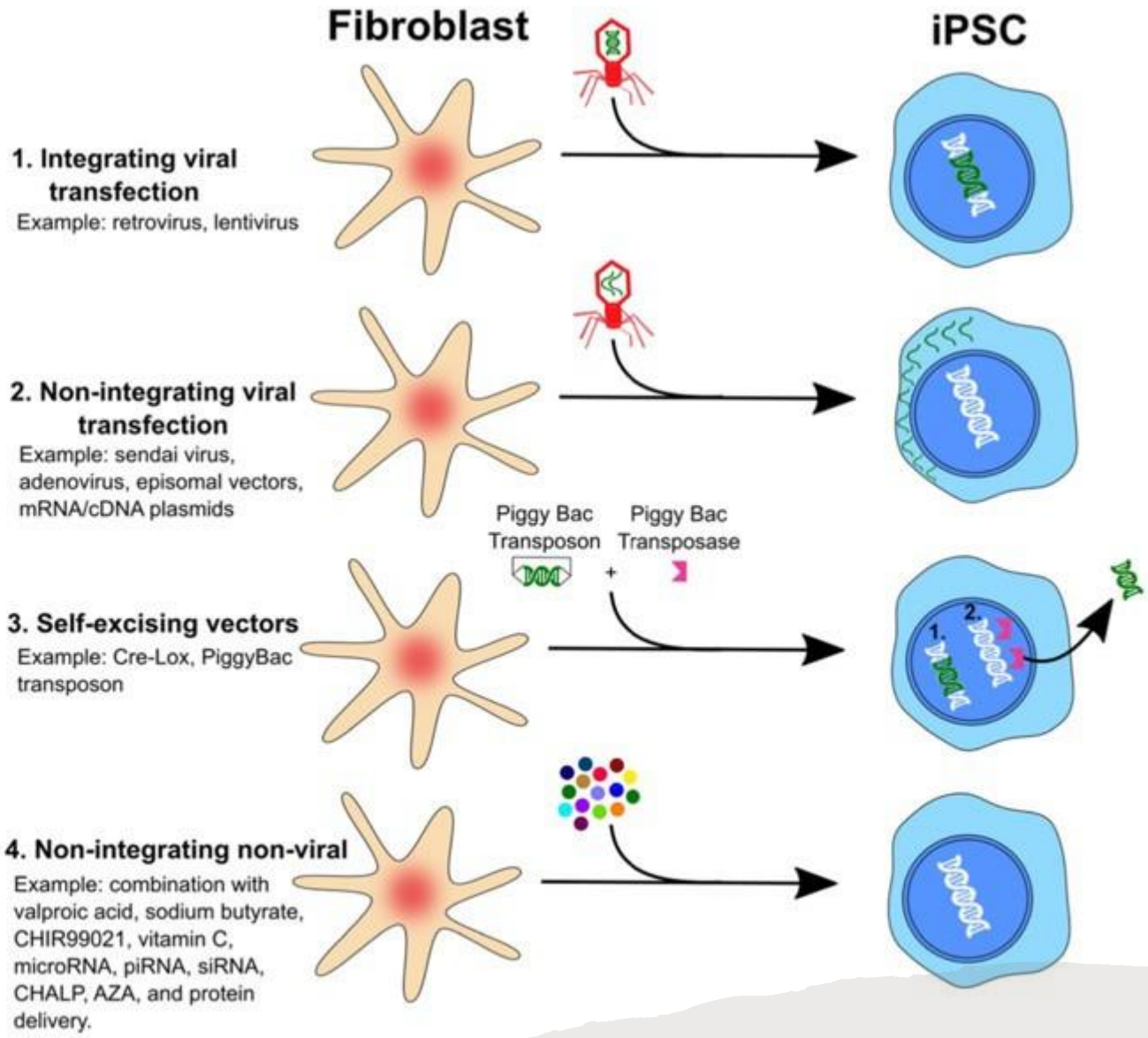
# *Isogenic Control hiPSC generation*



# REPROGRAMMING METHODS

**Fig. 2.** The four key methods for delivering reprogramming factors. Integrating viral systems were the first to be used to deliver transcription factors to generate stem cells, but they have the disadvantage of incorporating their genetic material and contributing to teratoma formation. By avoiding integration, novel methods (non-integrating vectors, self-excising vectors, and non-integrating non-viral vectors) represent iterative improvements upon this initial methodology. Such approaches provide significant advances in the safety and efficacy of iPSCs, which may then be applied for downstream scientific and clinical applications.



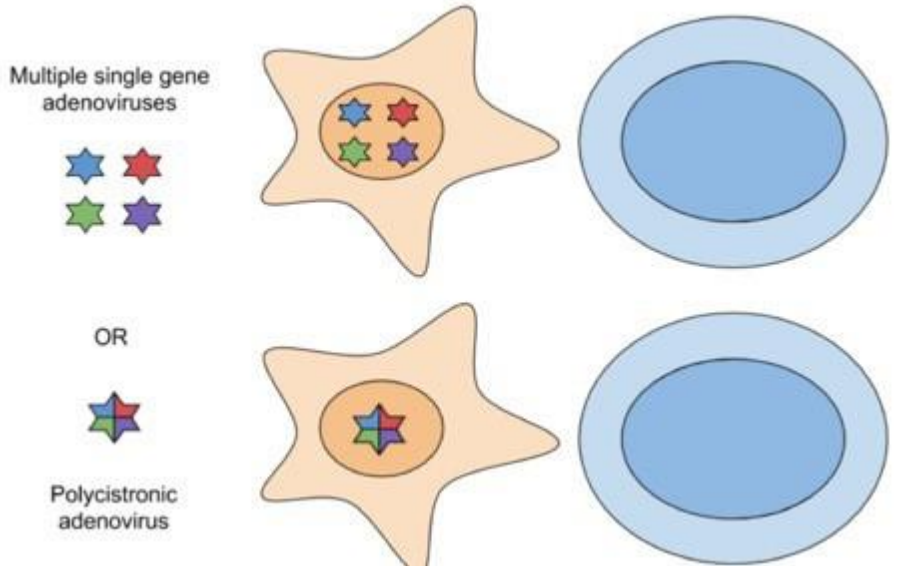

Adapted from Liu, 2020

# Non-integrating iPSC delivery methods

## Viral Vectors

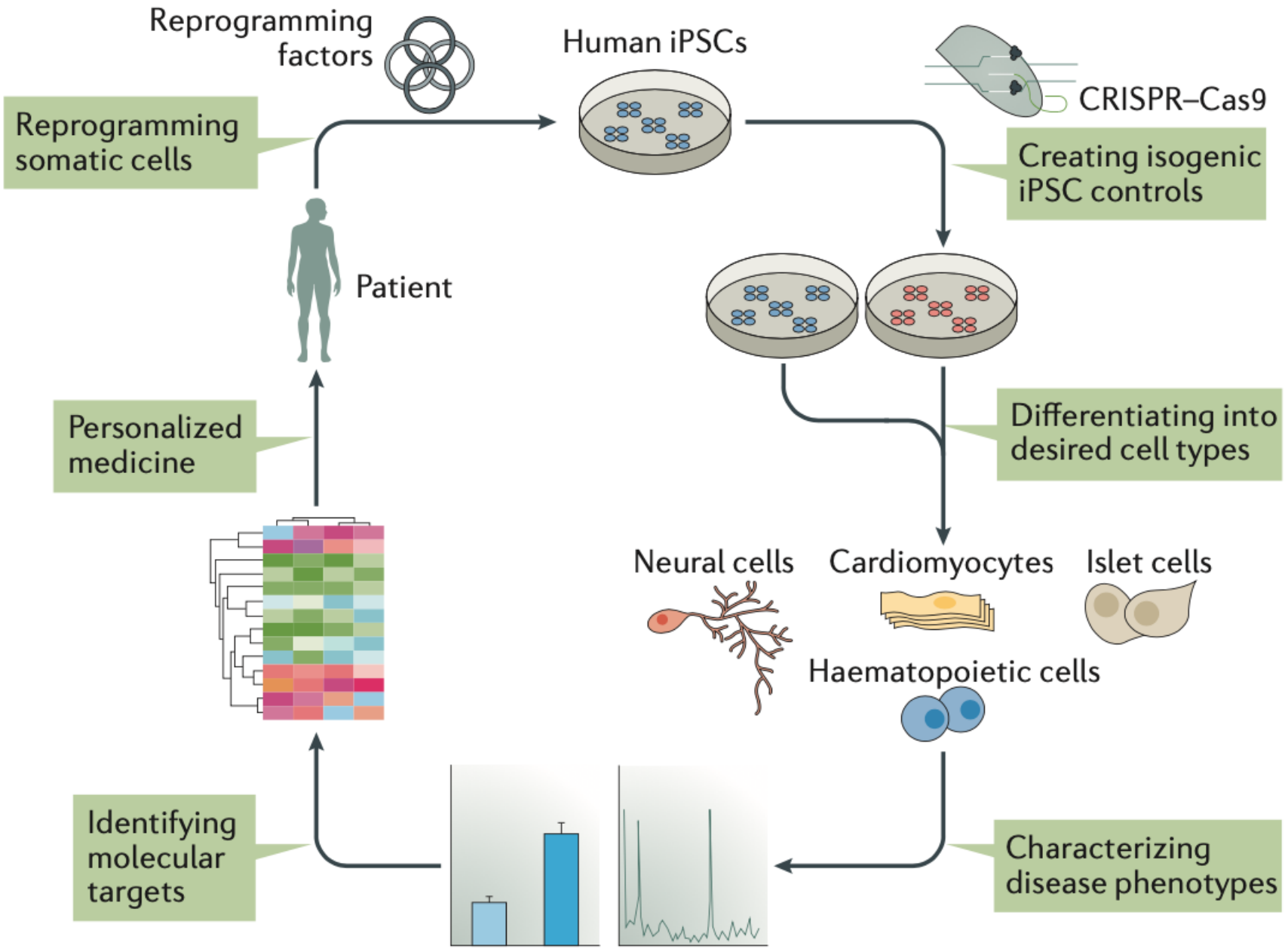
- **Adenovirus**: Adenoviral vectors infect dividing and nondividing cells and have an ~8kb packaging capacity. With this packaging capacity, reprogramming factors can be delivered either as a single polycistronic transgene or with four different adenoviruses, each expressing one factor. These vectors don't integrate into the genome and are instead lost by dilution via cell division. A drawback to this approach is it has lower levels of efficiency at generating iPSCs, usually several orders of magnitude lower than retroviruses; however, because they are less likely to cause insertional mutagenesis, adenoviral vectors are considered a safer way to express reprogramming factors for therapeutic applications.

- **Sendai viral vectors**: Sendai virus is a single stranded, negative sense RNA virus. It's a member of the *Paramyxoviridae* family of viruses, which also includes measles and mumps. Sendai transduces a wide range of cell types and replicates in the cytoplasm independent of the cell cycle. A challenge of using Sendai is that since it's replication competent, it's difficult to eliminate the virus from all cells, even after many passages. Ban et al developed a temperature sensitive Sendai virus




https://blog.addgene.org/delivery-methods-for-generating-ipscs

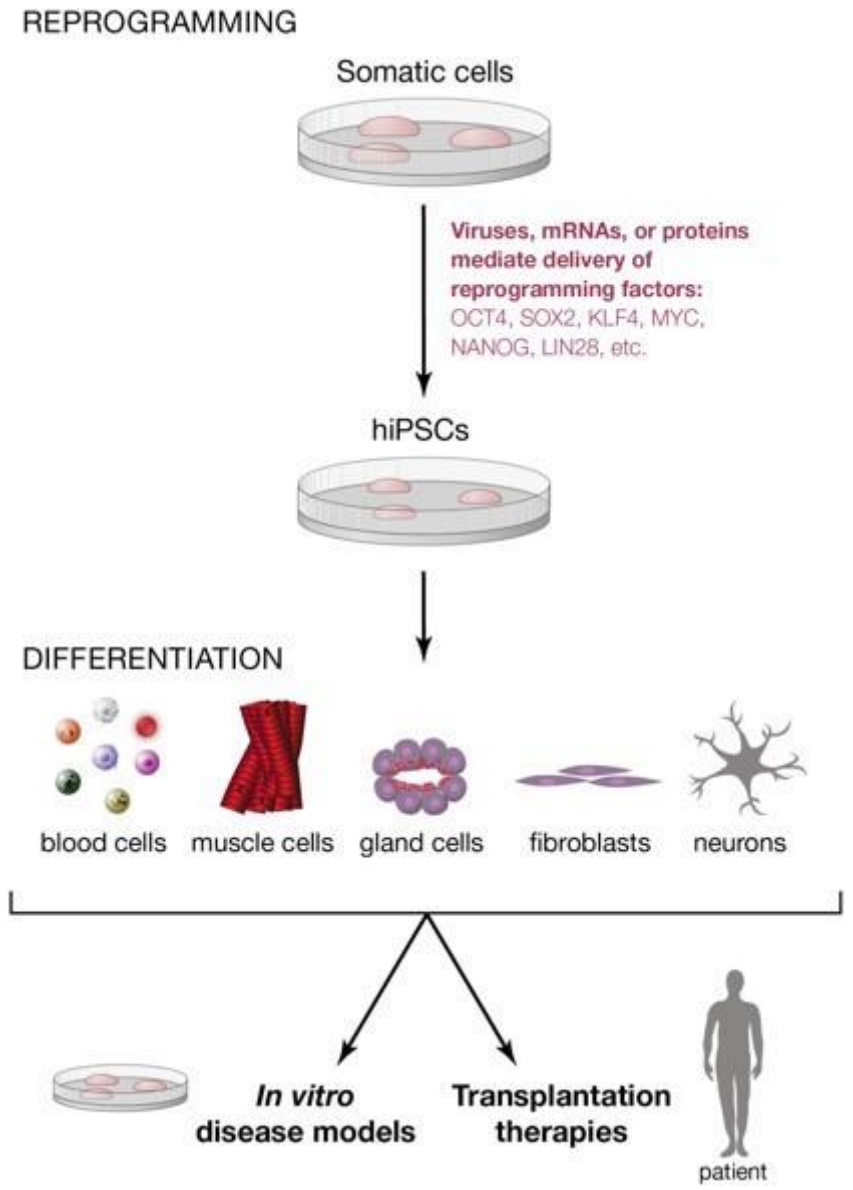
# Disease modeling through iPSC-CMs



*“Patient tailored therapy”*

# Applications of iPSCs

- Regenerative Medicine: iPSCs can be differentiated into specialized cells for transplantation
- Drug Discovery and Screening: iPSCs can be used to test the effectiveness and safety of new drugs
- Disease Modeling: iPSCs can be used to understand disease mechanisms and develop targeted therapies




Induced Pluripotent Stem Cells Meet Genome Editing

# Organoids: complex tissue in a dish

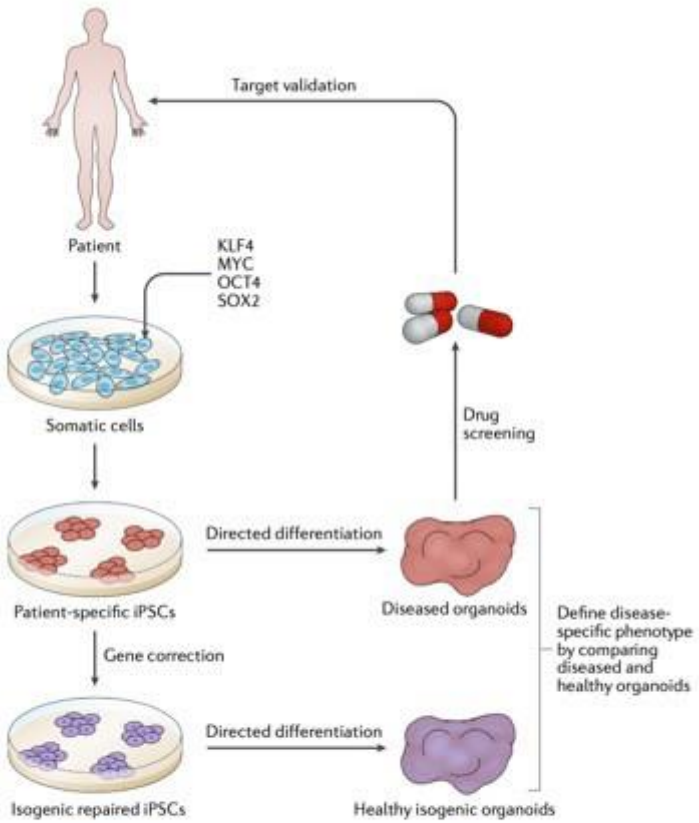



Fig. 2 | **Application of organoids derived from iPSCs to disease modelling and drug discovery.** Remarkable progress has been made in the differentiation of increasingly complex multicellular and diverse organoid systems across many tissues. We propose that parallel differentiation of organoids from patient-derived induced pluripotent stem cells (iPSCs) as well as genetically corrected, isogenic control iPSCs will allow attribution of an organoid-level disease phenotype to a specific molecular lesion. Once a clear organoid-level readout is established, diseased organoids can be used in drug screening and validation studies.

Table 1 | **Phenotypes modelled in 2D and 3D systems based on iPSCs**

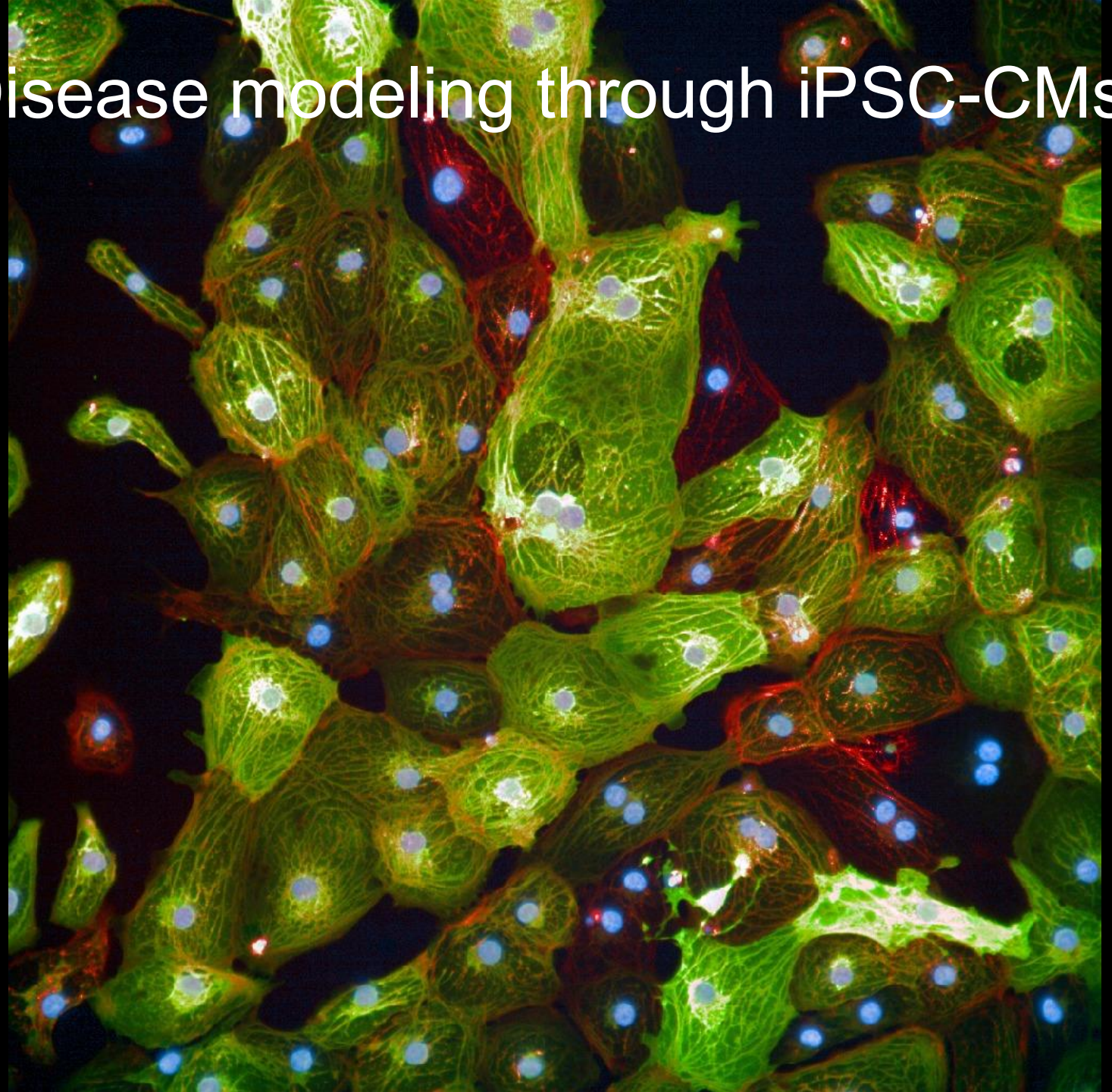
| Tissue | 2D phenotype | 3D phenotype | Refs |
|---|---|---|---|
| Blood | Oligopotent differentiation | Multipotent differentiation and engraftment | |
| Neural | Neural differentiation, gene expression and neurite formation | Cortical organization, regional specification, cell–cell interactions and neuronal migration | |
| Cardiac | Action potential and contractility | Self-organization and integration of biophysical cues | |
| Gastrointestinal | Differentiation | Bile secretion, motility and cell–cell interactions | |

iPSCs, induced pluripotent stem cells.

- An important advance in disease modelling with iPSCs occurred with the development of iPSC-derived organoids.
- Organoids are 3D multicellular aggregates derived from stem cells that differentiate and self- organize to recapitulate the structural features and cell– cell interactions of mature tissues.
- The soluble and biophysical cues used to guide organoid differentiation from PSCs have been incrementally refined to generate increasingly complex ‘tissues in a dish’.
- Although several limitations exist in current 3D technology, combining disease-specific iPSCs with 3D technology enables the examination of spatiotemporal cellular interactions that could reveal the physiological disease status, thus providing an unprecedented drug-screening platform and offering a new option for tissue-replacement therapy.
- Furthermore, investigators have achieved specification of particular regions of organs such as the brain and gastrointestinal tract.

Rowe 2017

# Disease modeling through iPSC-CMs

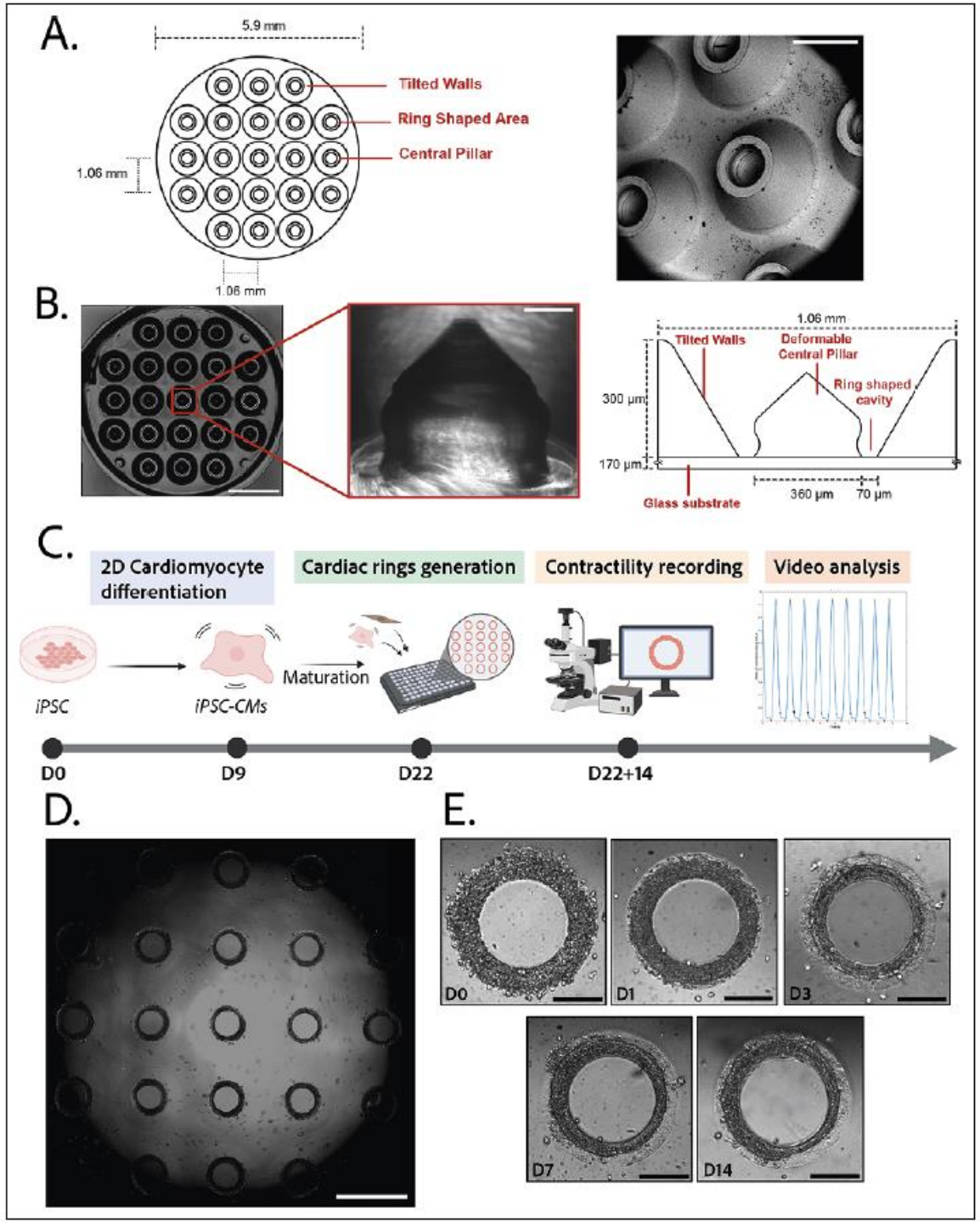


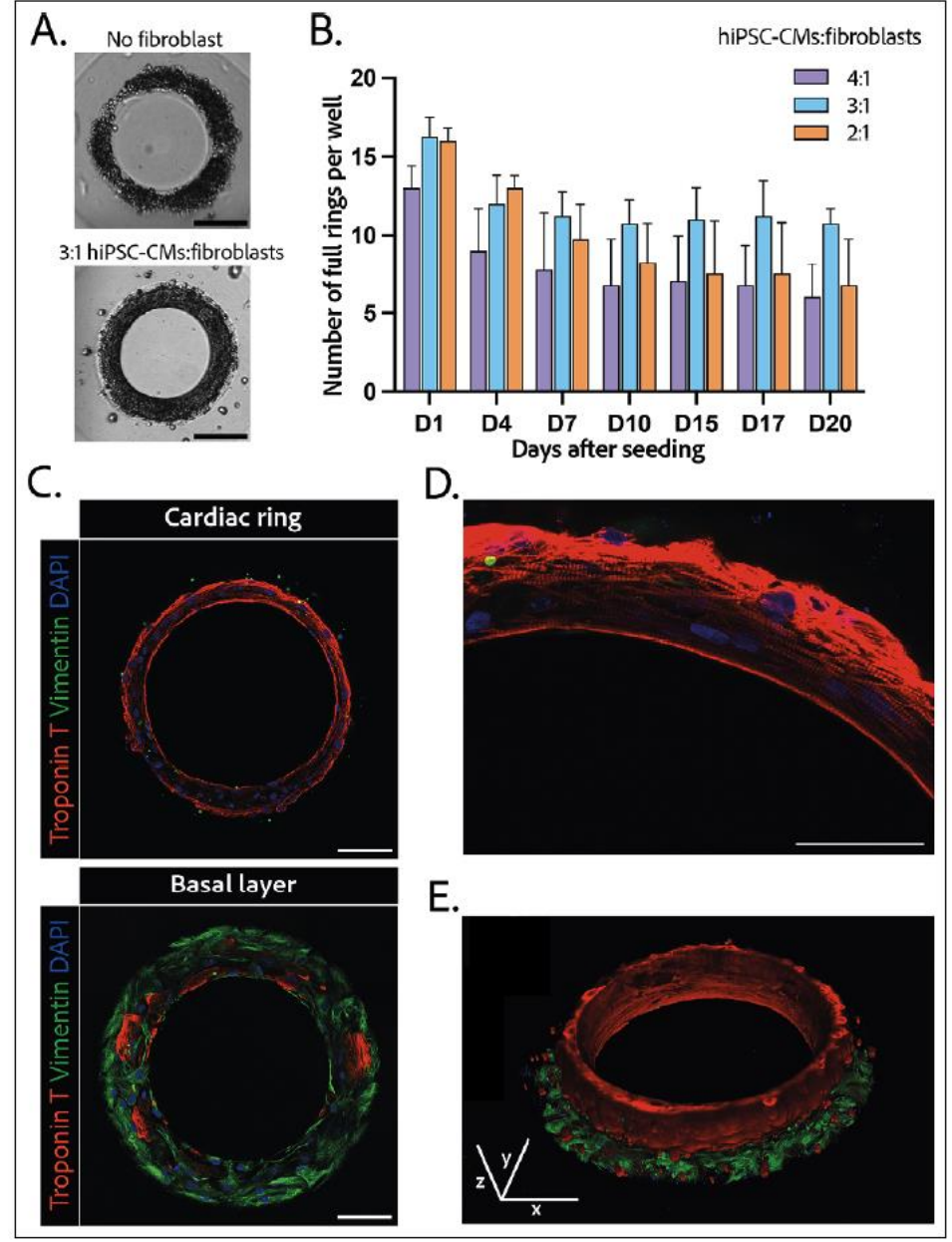
Seguret *et al*. eLife 2023;12:RP87739. DOI: https://doi.org/10.7554/eLife.87739

# A versatile high-throughput assay based on 3D ring-shaped cardiac tissues generated from human induced pluripotent stem cell-derived cardiomyocytes

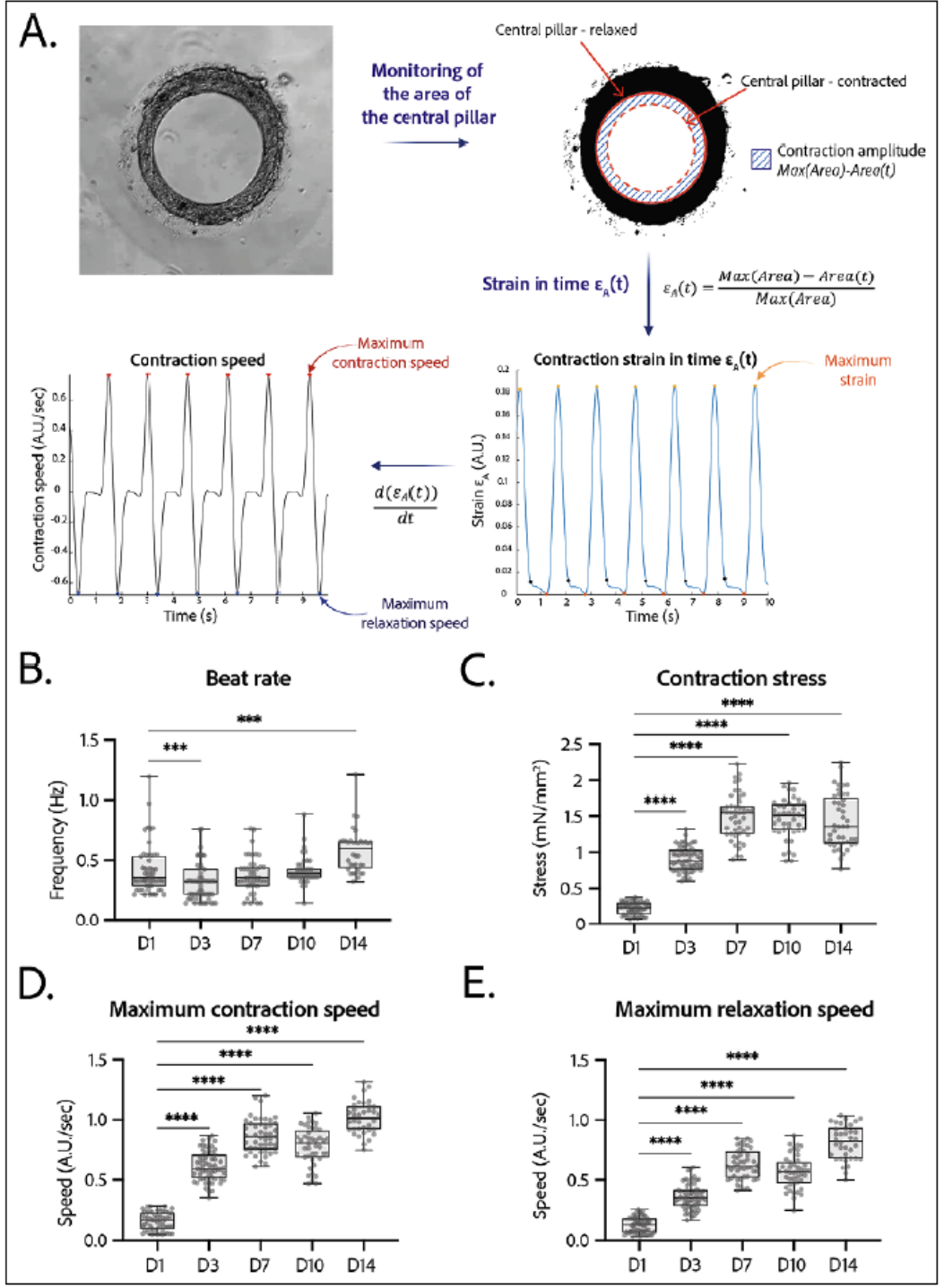
**Magali Seguret[1†], Patricia Davidson[2], Stijn Robben[2], Charlène Jouve[1], Celine Pereira[1], Quitterie Lelong[1], Lucille Deshayes[1], Cyril Cerveau[2], Maël Le Berre[2], Rita S Rodrigues Ribeiro[2*], Jean-Sébastien Hulot[1*]**

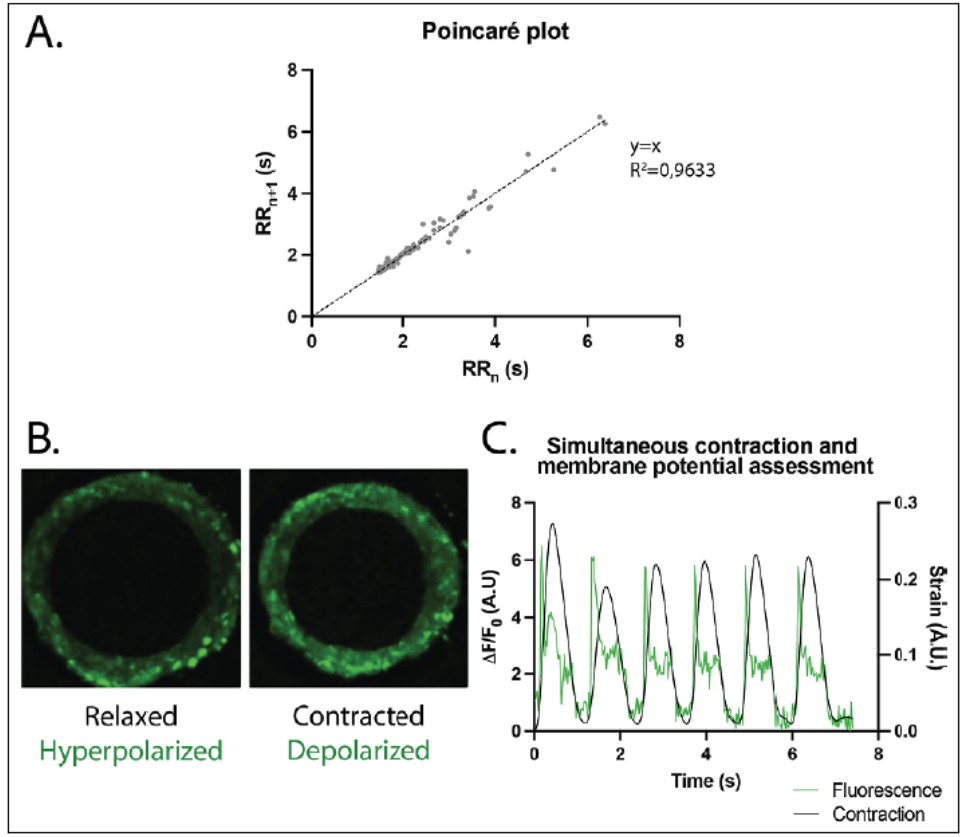
[1]Université de Paris Cité, PARCC, INSERM, Paris, France; [2]4Dcell, Montreuil, France



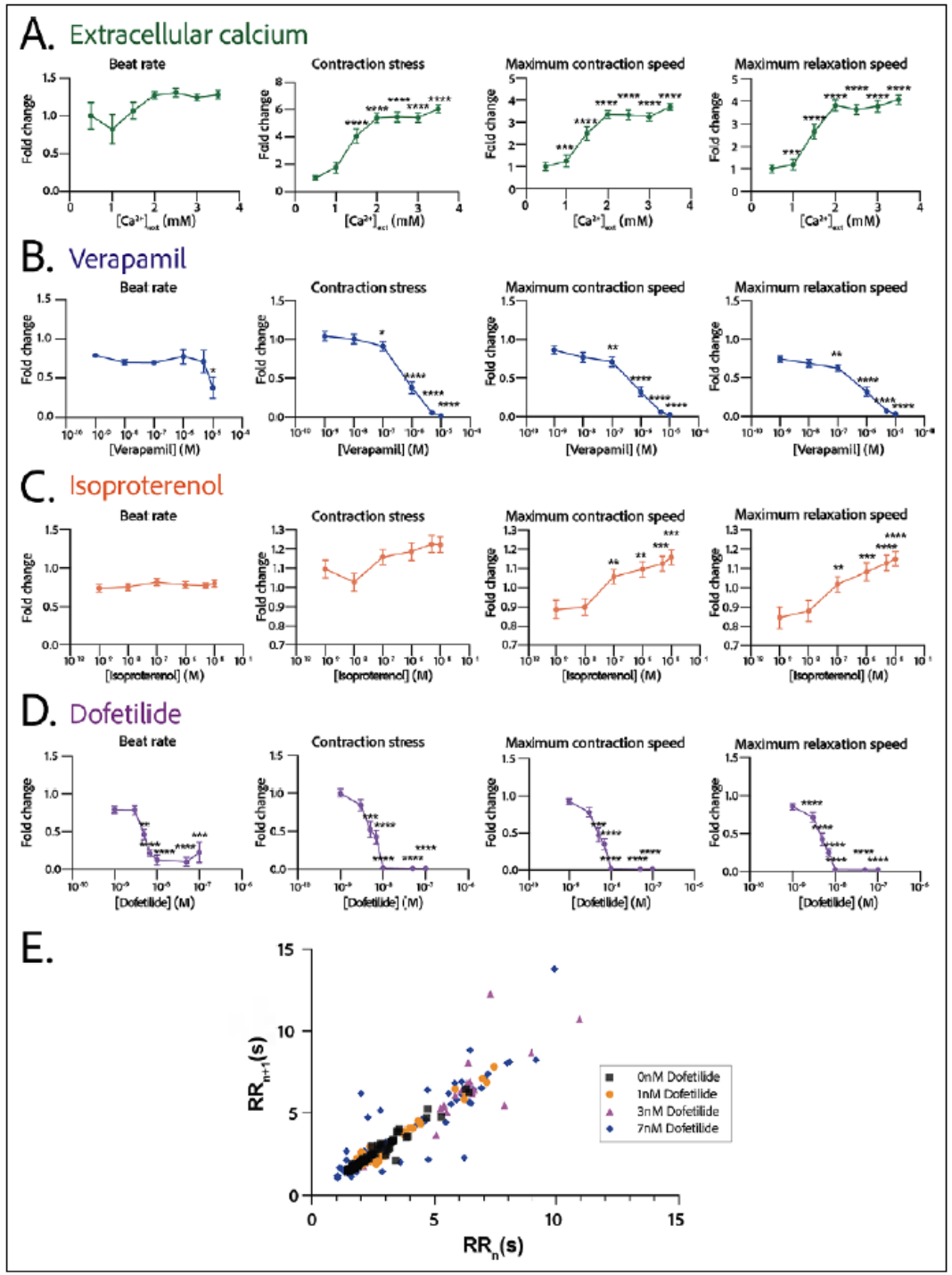

*Vimentin, stained in green, corresponds to fibroblasts, troponin T, in red, is specific to cardiomyocytes, and DAPI is in blue*




*Contractility analysis of the tissues.*

*Study of arrhythmia in ring-shaped cardiac tissues.*




*Physiological and drug testing on the cardiac tissues.*