Generation of induced pluripotent stem cell (iPSC) lines from urine samples.

María Romero Muñoz
Master in Health Biotecnology, University Pablo de Olavide.
Department of Regeneration and Cell Therapy,
Andalusian Center for Molecular Biology and Regenerative Medicine (CABIMER)

1.Introduction.

- 1.1 iPSC origin.
- 1.2. iPSC sources.
- 1.3. Methods to produce IPSC: Sendai Virus.
- 1.4. Advantages of this work. UDC.

2. Material and methods.

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- 2.2. Alkaline Phosphatase stainning Kit
- 2.3.Trilineage Diferentiation.
- 2.4. i009 line characterization. PCR,inmunofluorescence and Flow citometry.

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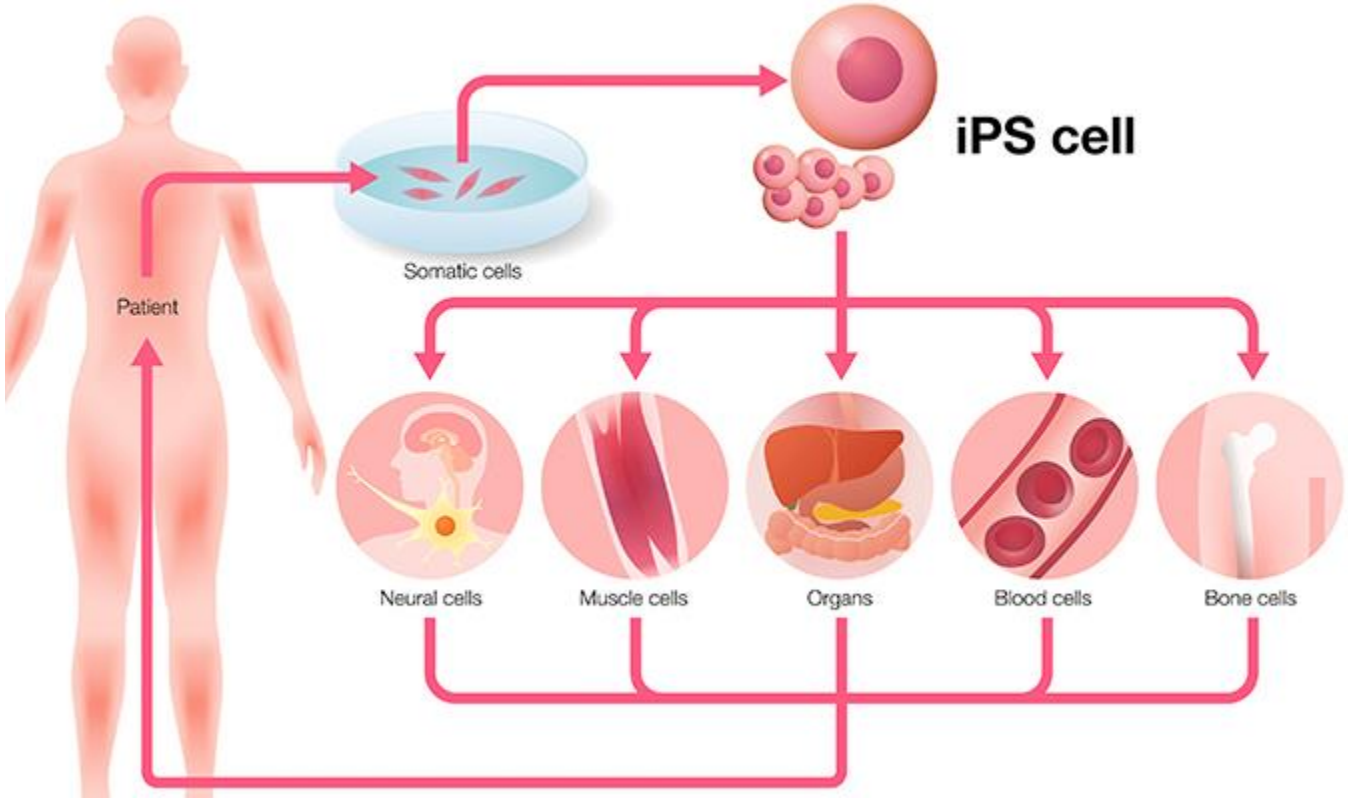
- 3.3. iPSC differentiation ability: Trilineage markers by inmunofluorescence.

4. Conclusion and remarkable points.

What are iPSCs?

iPSCs

Induced Pluripotent Stem cells



https://www.news-medical.net/

1 .Introduction.

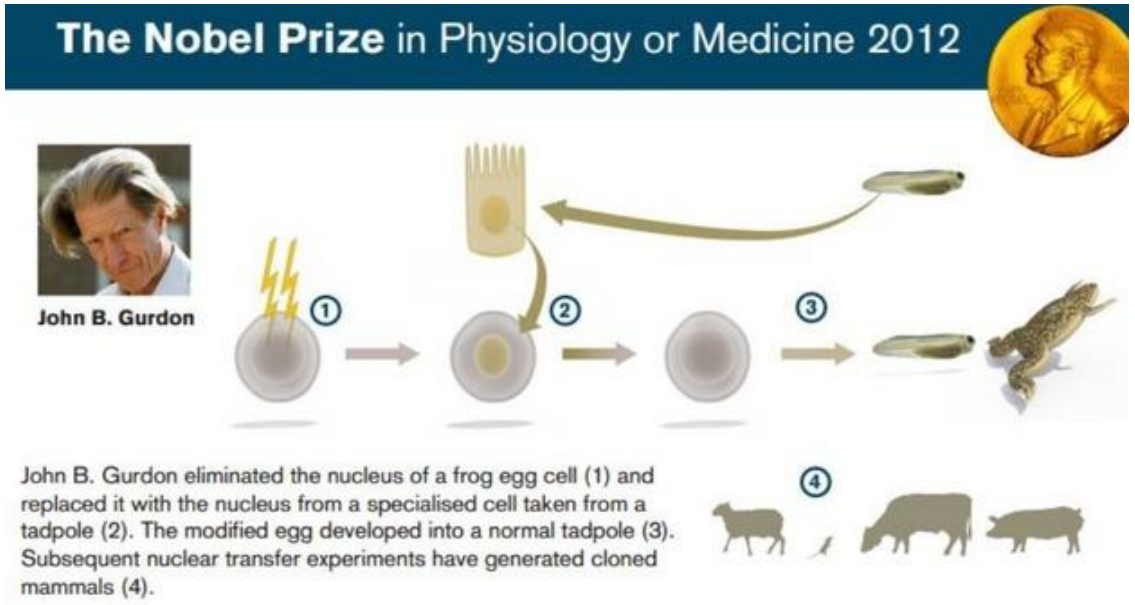
1.1 iPSC origin.

John B. Gurdon

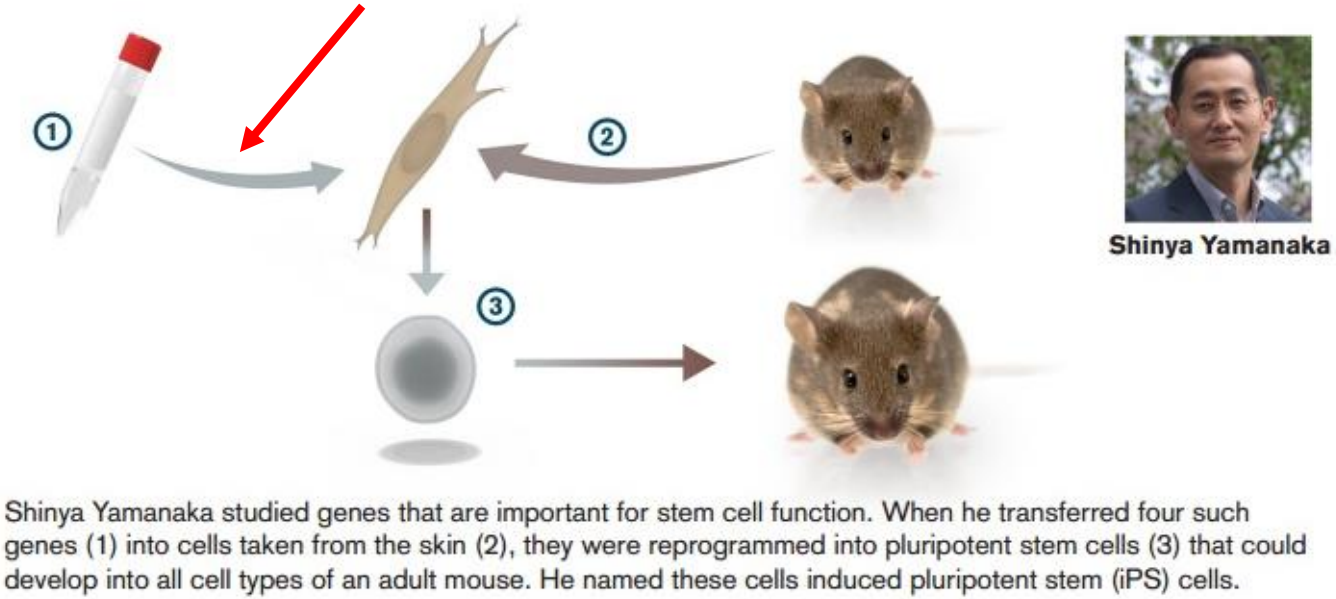
S. Yamanaka

Nobel Prizes in 2012

Yamanaka´s factors: Oct3/4, Sox2, c-Myc y Klf4



John B. Gurdon eliminated the nucleus of a frog egg cell (1) and replaced it with the nucleus from a specialised cell taken from a tadpole (2). The modified egg developed into a normal tadpole (3). Subsequent nuclear transfer experiments have generated cloned mammals (4).

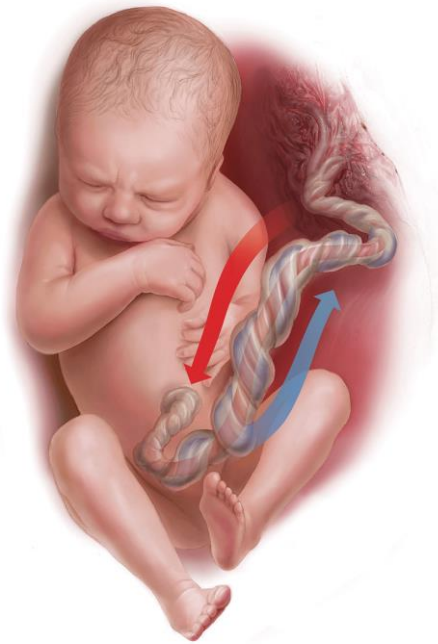


Shinya Yamanaka studied genes that are important for stem cell function. When he transferred four such genes (1) into cells taken from the skin (2), they were reprogrammed into pluripotent stem cells (3) that could develop into all cell types of an adult mouse. He named these cells induced pluripotent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

https://healthmanagement.org/c/icu/news/the-nobel-assembly-at-karolinska-institutet-awarded-the-nobel-prize-in-physiology-or-medicine-2012

1.2. iPSC sources.

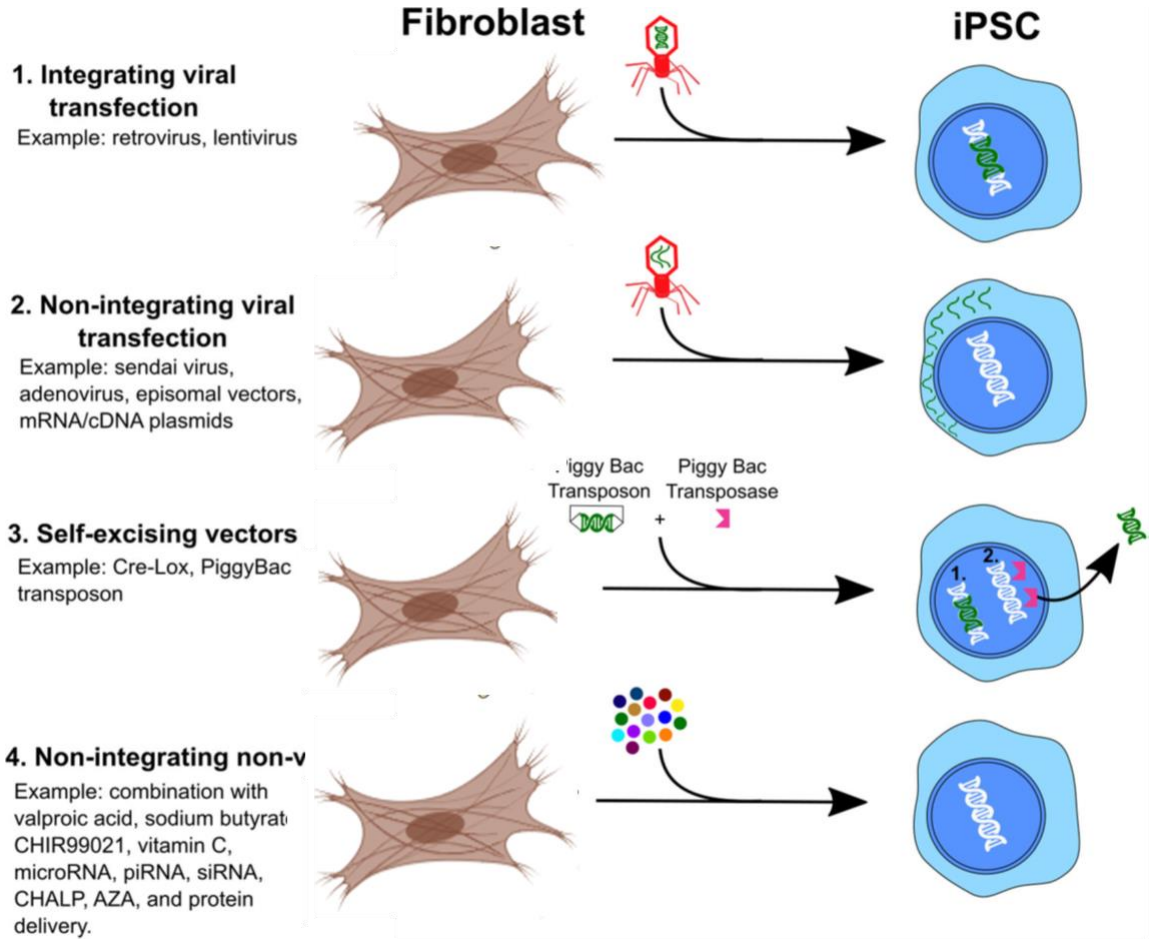
- Skin: fibroblasts and keratinocytes.
- Peripheral blood cells.
- Umbilical cord blood.
- Extraembryonic tissues.
- Adipose tissue.



https://www.wired.com/
https://www.istockphoto.com/

Figures designed by https://biorender.com/

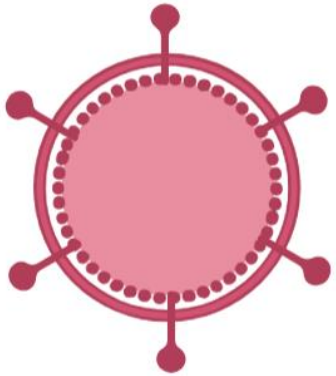
1.3. Methods to produce iPSC: Sendai Virus.

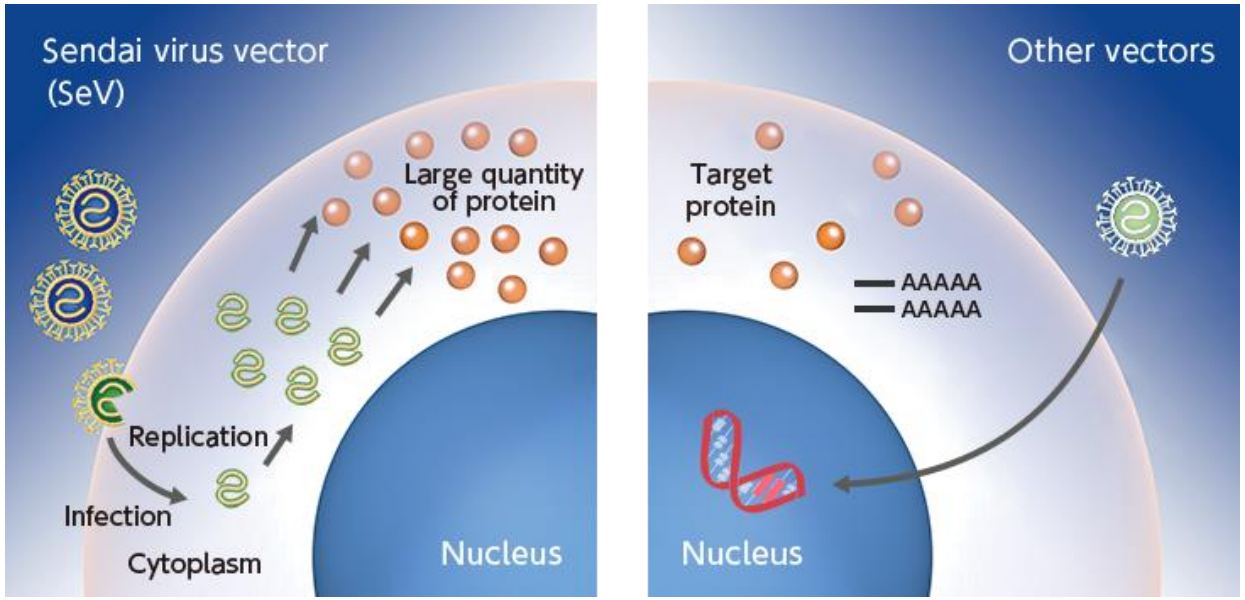


invitrogen CytoTune™-iPS 2.0 Sendai Reprogramming Kit and CytoTune™-EmGFP Sendai Fluorescence Reporter FAQs

Product	Qty	Cat. No.
CytoTune™-iPS 2.0 Sendai Reprogramming Kit	1 pack (1 vial of each vector)	A16517
CytoTune™ -iPS 2.0 Sendai Reprogramming Kit	3 pack (3 vials of each vector)	A16518

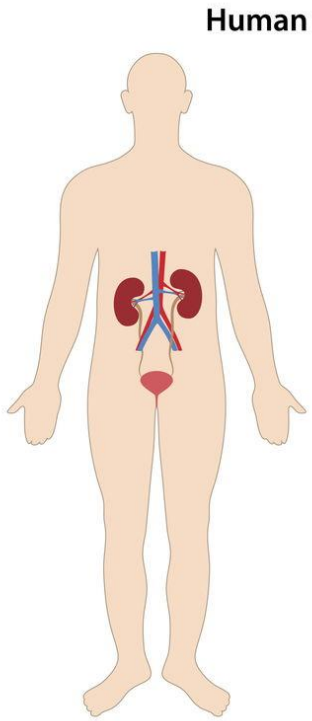
CytoTune™ 2.0 KOS
CytoTune™ 2.0 hc-Myc
CytoTune™ 2.0 hKlf4

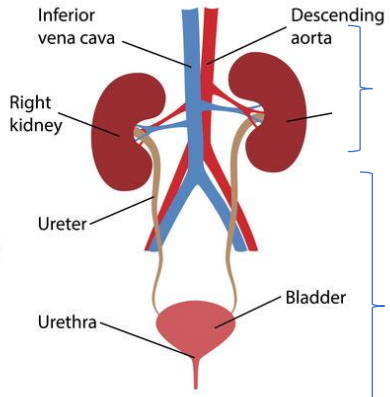




Modified . Liu, G. et al.2020. Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications. Stem Cell Reviews and Reports.
https://www.iromgroup.co.jp/en/group/advanced01.html

1.4. Advantages of this work. UDC.





upstream parts of the urinary tract

downstream parts of the urinary tract

2000 to 7000 cells downstream of the urinary tract are excreted in the urine

Urine cells are fully functional

Easy collection

Without ethical impediments

https://www.ncbi.nlm.nih.gov/books/NBK65775/figure/CDR0000304478__24/

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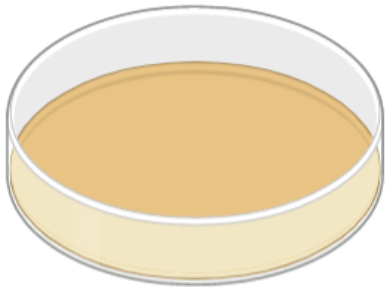
2. Material and methods

- **Isolation and cultivation UDC.**
- **Transfection with sendai**
- **iPSC line maintenance.**
- **iPSC line characterization: alkaline phosphatase PCR, Flow citometry inmunofluorescence**
- **Differentiation capacity (Trilineage diferentiation): specific culture media kit, PCR and inmunofluorescence**

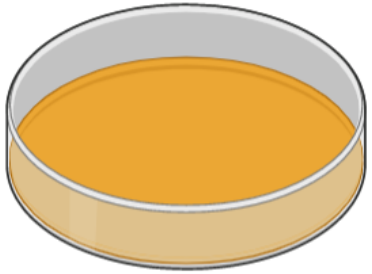
2.1. Culture media and culture conditions and transfection.

Isolation medium:
UDCI



Centrifugation and separation of the main urine waste

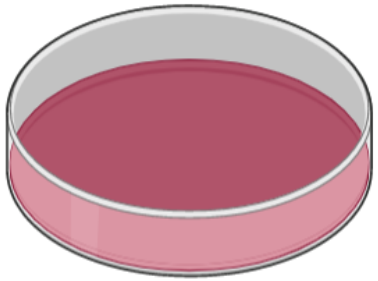
Expansion medium:
UDCE



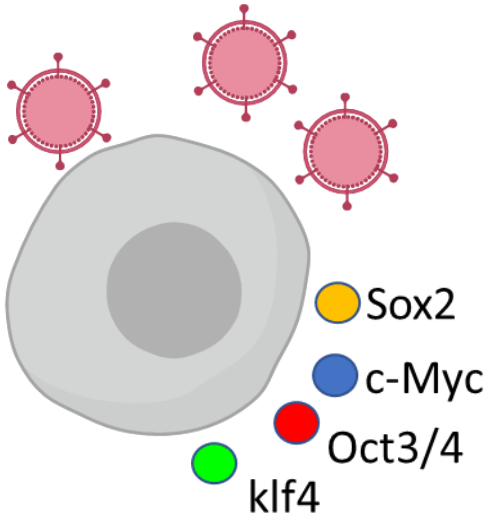
After pass 1
(20 days of the beginning of cell culture)

Cells transfection in pass 2

Maintenance medium: **TeSR™-E8™**

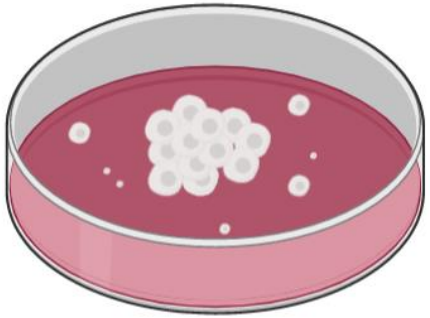


A week after transfection



Figures designed by https://biorender.com/

2.2. Alkaline Phosphatase stainning Kit.



Wash with PBS

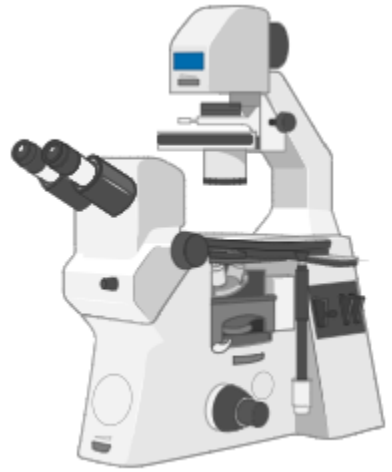
Fix the cells with 4% PFA, 5 min, Room temperature.

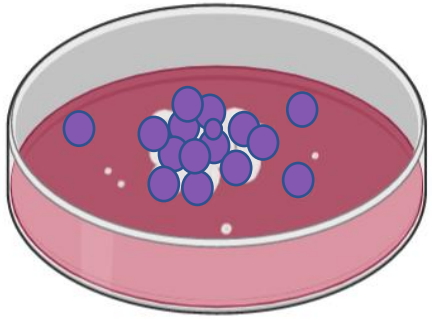
Wash PBS-Tween 2x2min

Add AP substrate solution 20 min, room temperatura, dark

Wash with PBS

Take picture of the cells





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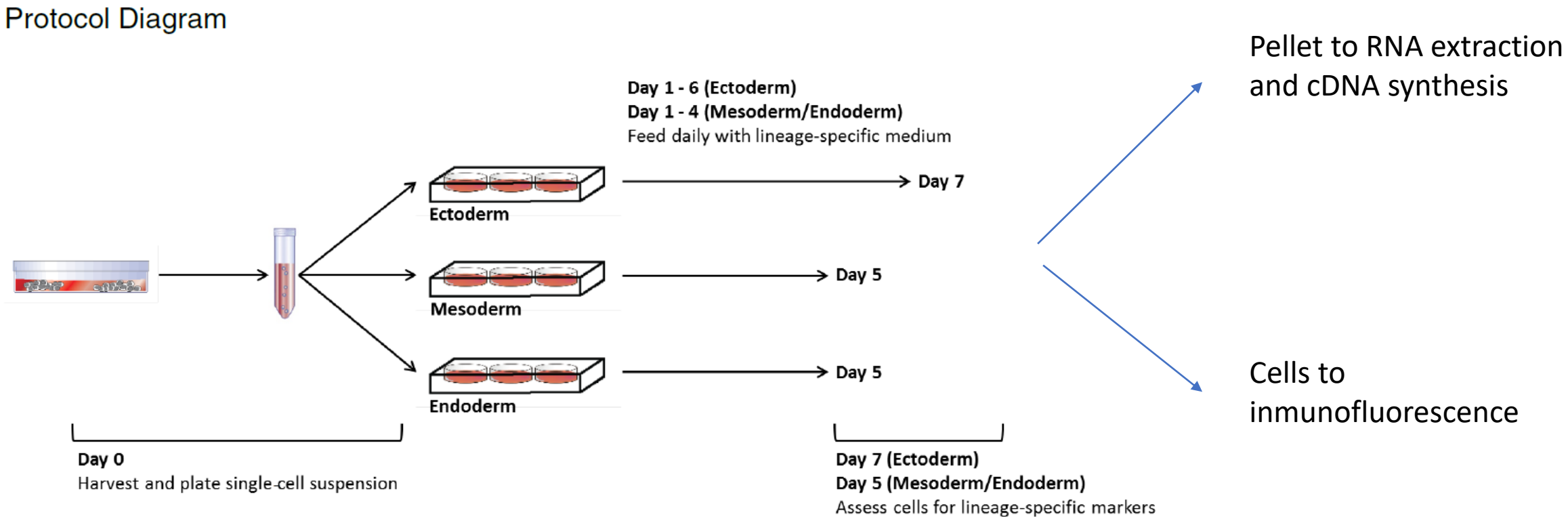
2.3. Line characterization. Trilineage diferentiation.



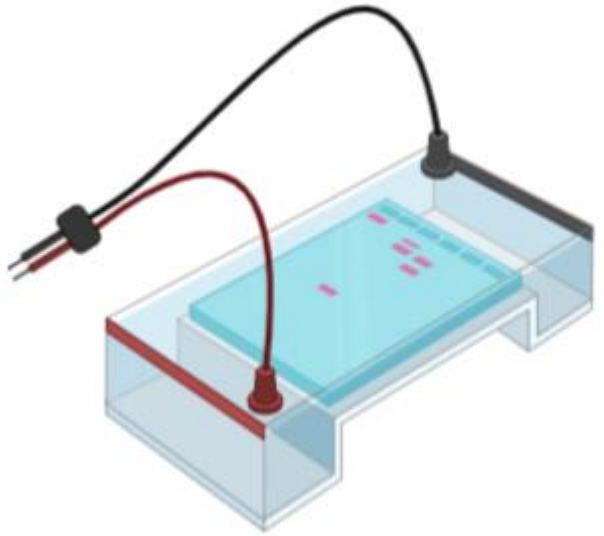
STEMdiff™ Trilineage Differentiation Kit*, Stem cells technologies*.

2.4. i009 line characterization: PCR.

Charge load viral control — **SeV, cMyc,Oct3,4, Klf4,KOS**

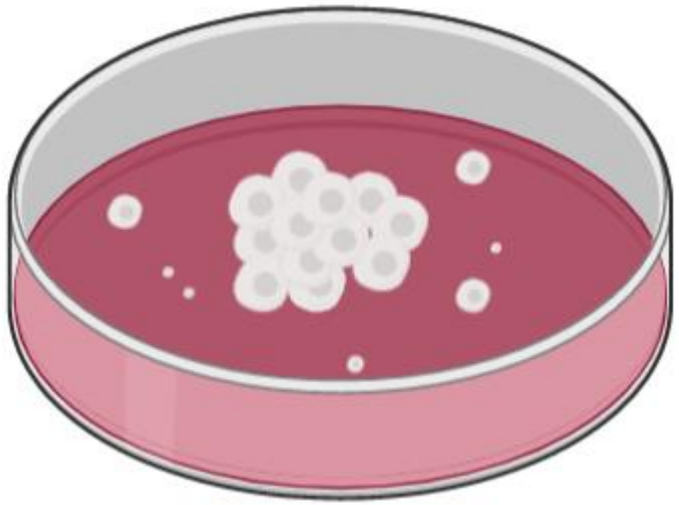
Pluripotency genes — **oct-4, sox-2, nanog, telo, HLA-A,HLA-B, HLA-C**

Trilineage differentiation genes — **Foxa2, sox17, cxcr4, brach, negn3,pax6**



Figures designed by https://biorender.com/

2.4. i009 line characterization: Inmunofluorescence.



Pluripotency markers

Nuclear markers **OCT**

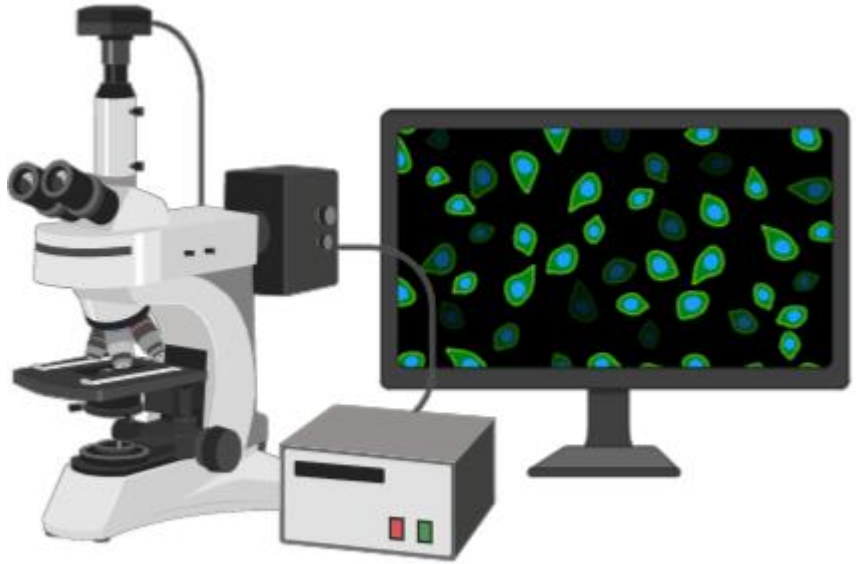
Surface markers **TRA60** **TRA81** **SSEA4**

Trilineage surface markers

Ectoderm **NESTIN**

Mesoderm **SMA**

Endoderm **SOX17**



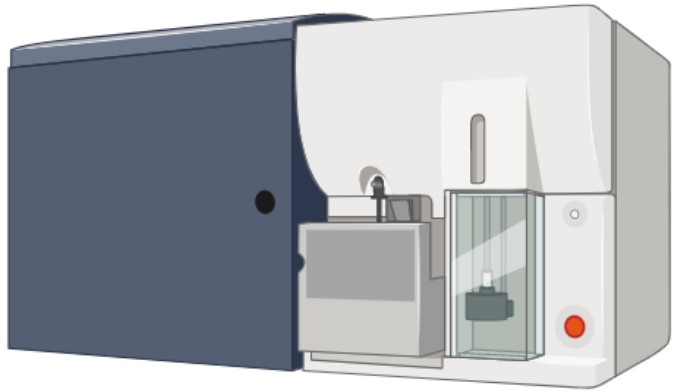
Figures designed by https://biorender.com/

2.3. i009 line characterization: Flow Citometry

Cells tripsinization

Final concentration 10^6cells/ml

5µl isotipe
20µl pluripotency markers
20’ in dark
Centrifugation and wash with PBS
Add 1ml of PBS

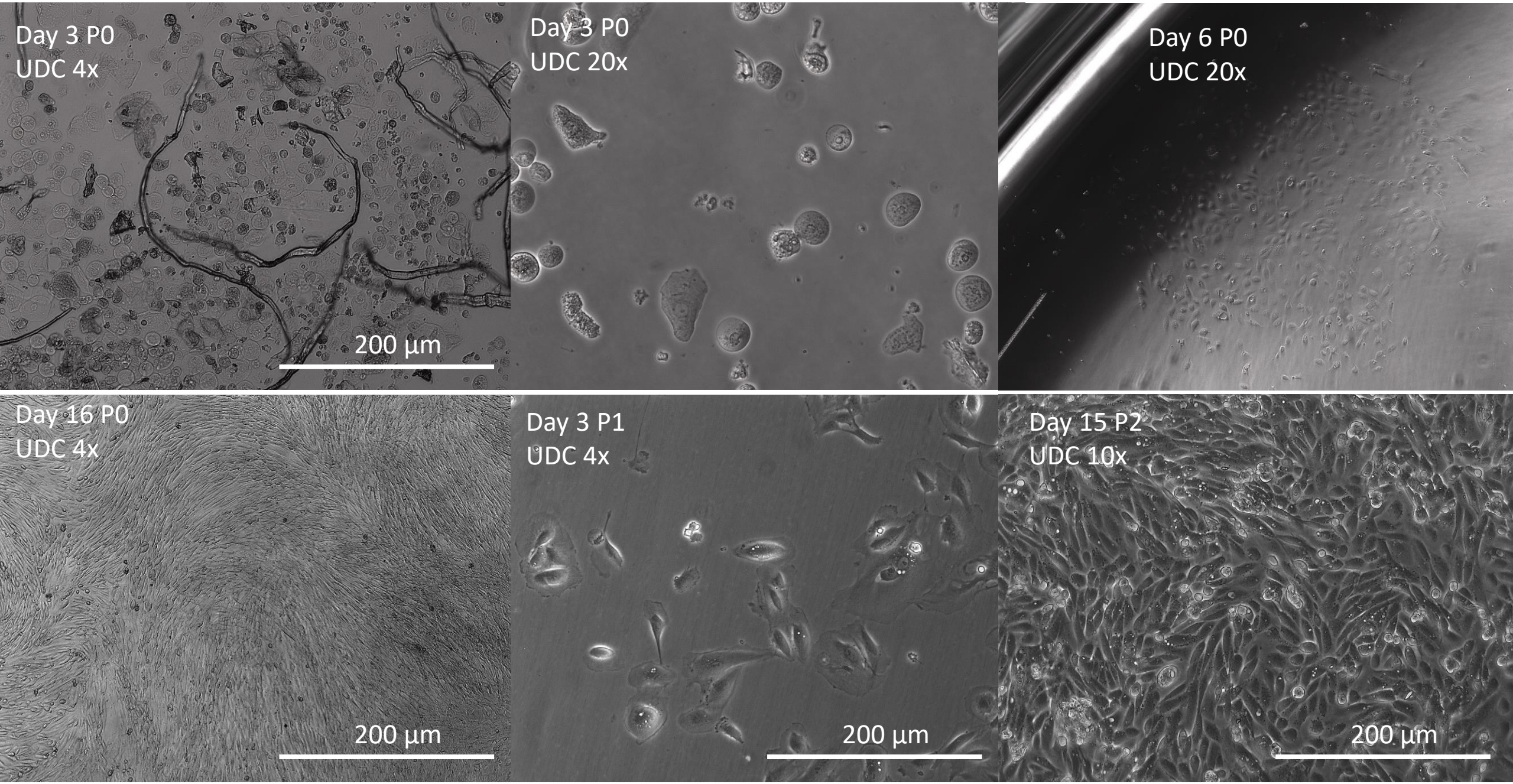


Pluripotency markers

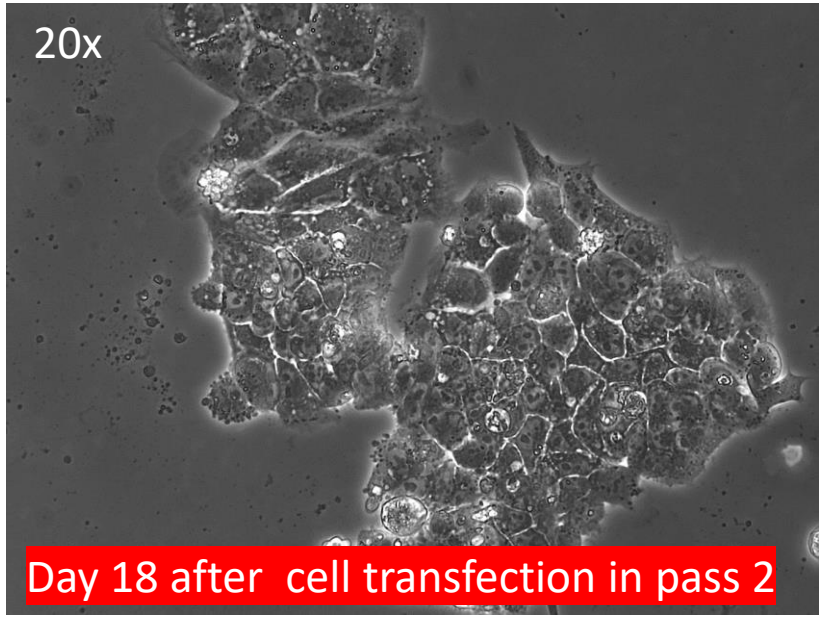
TRA-60
SSEA-4

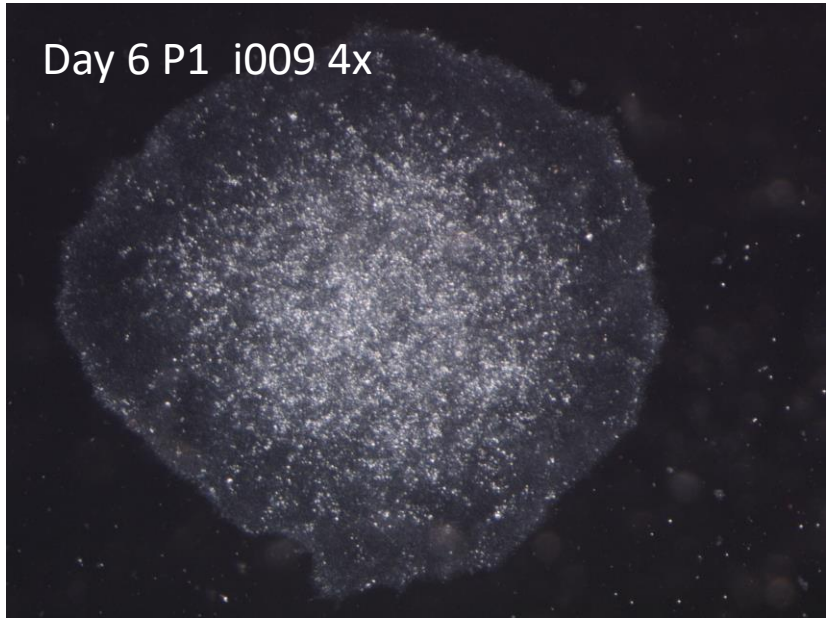
Figures designed by https://biorender.com/

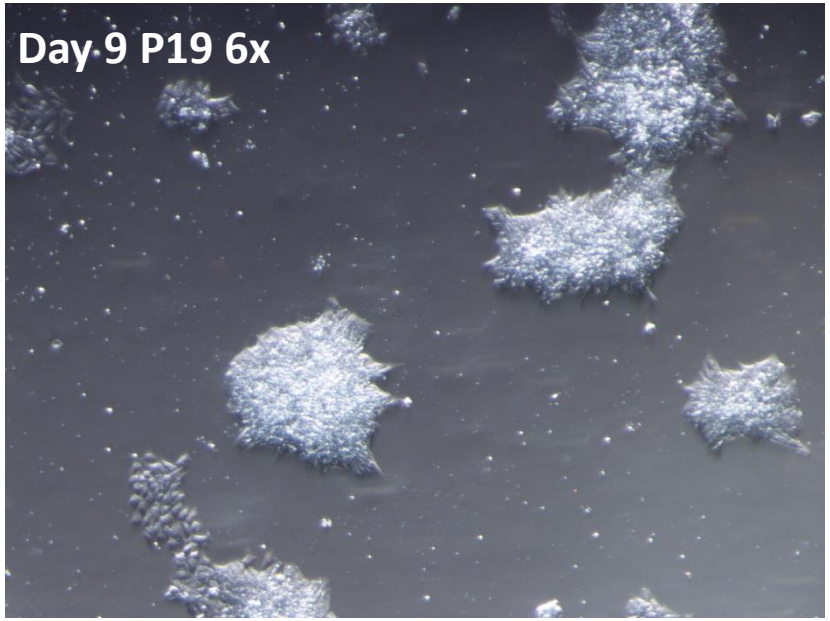
3.1 iPSC culture evolution.

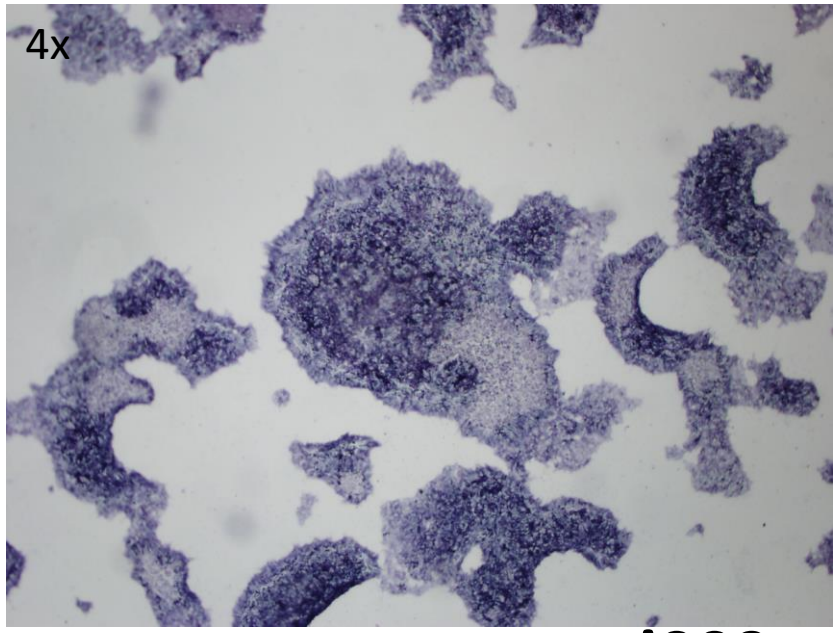


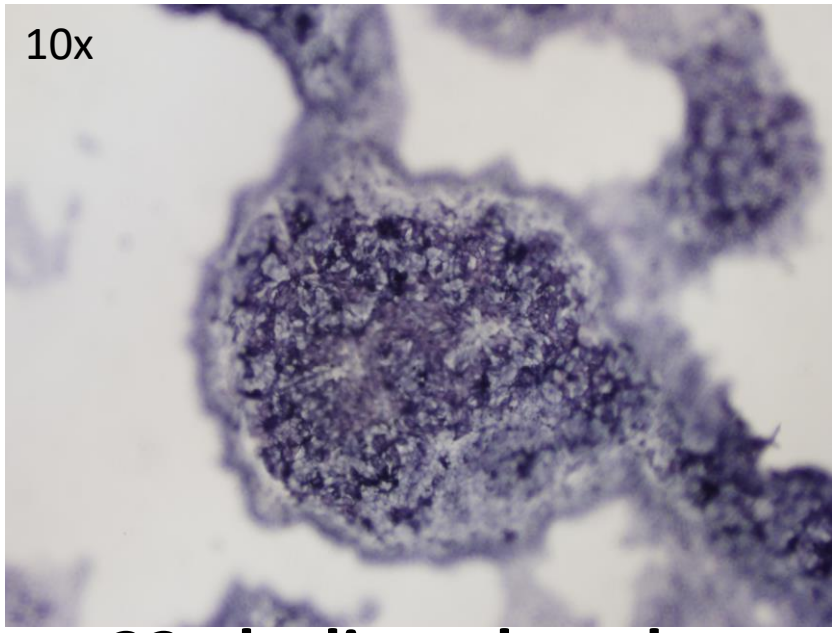
3.1 iPSC culture evolution.

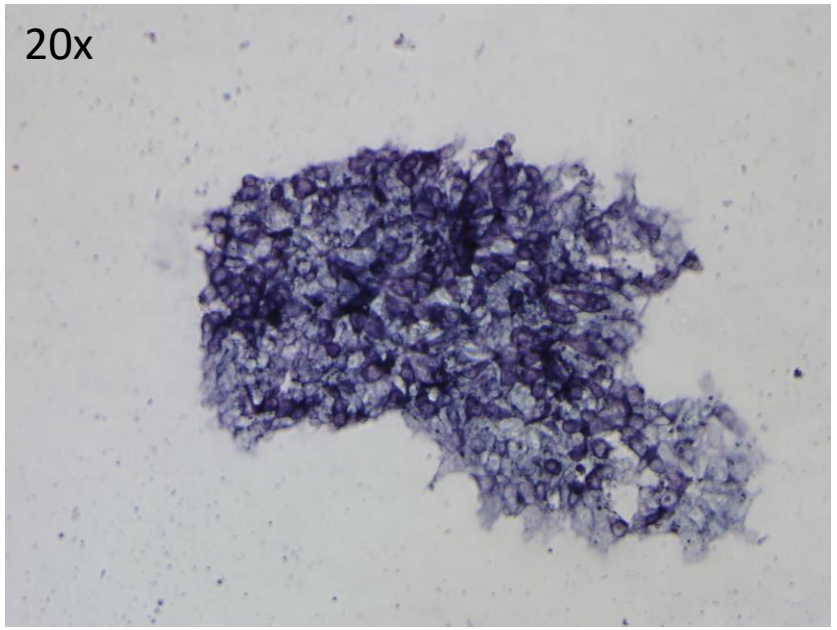






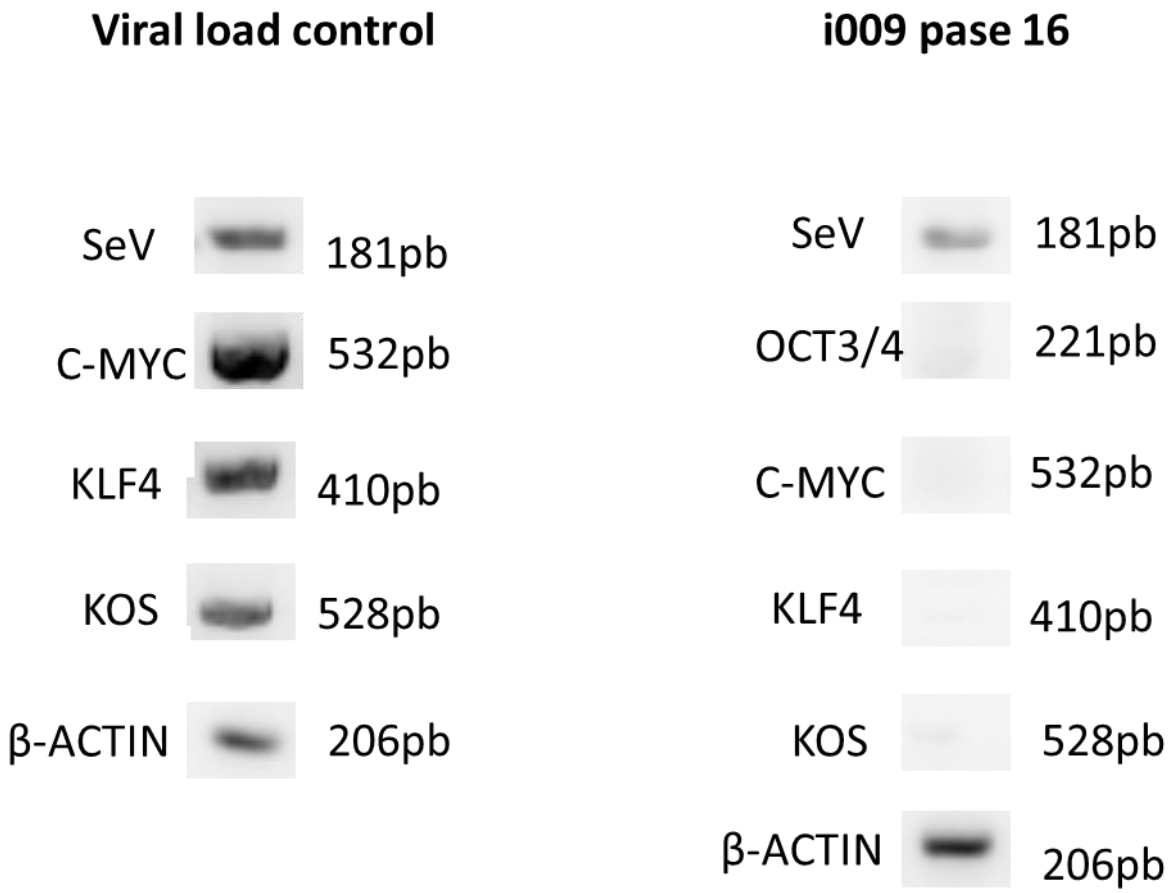




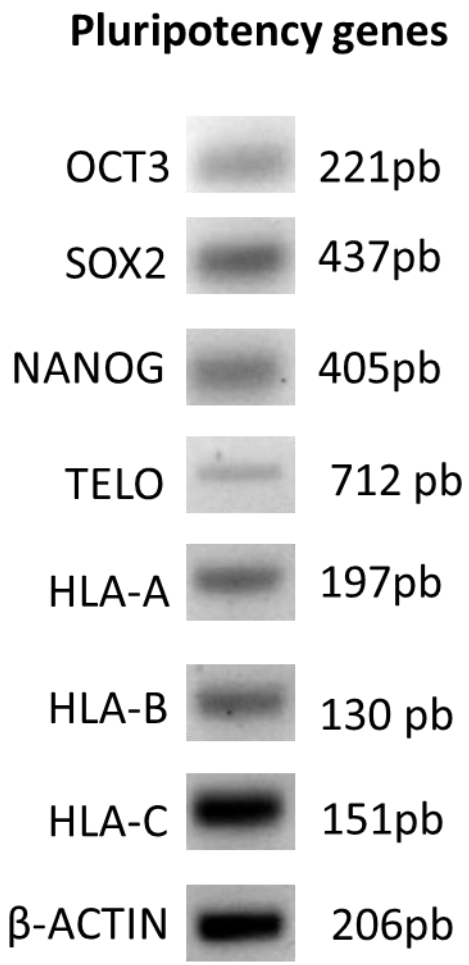


i009 pass 22 alcaline phosphatase assay

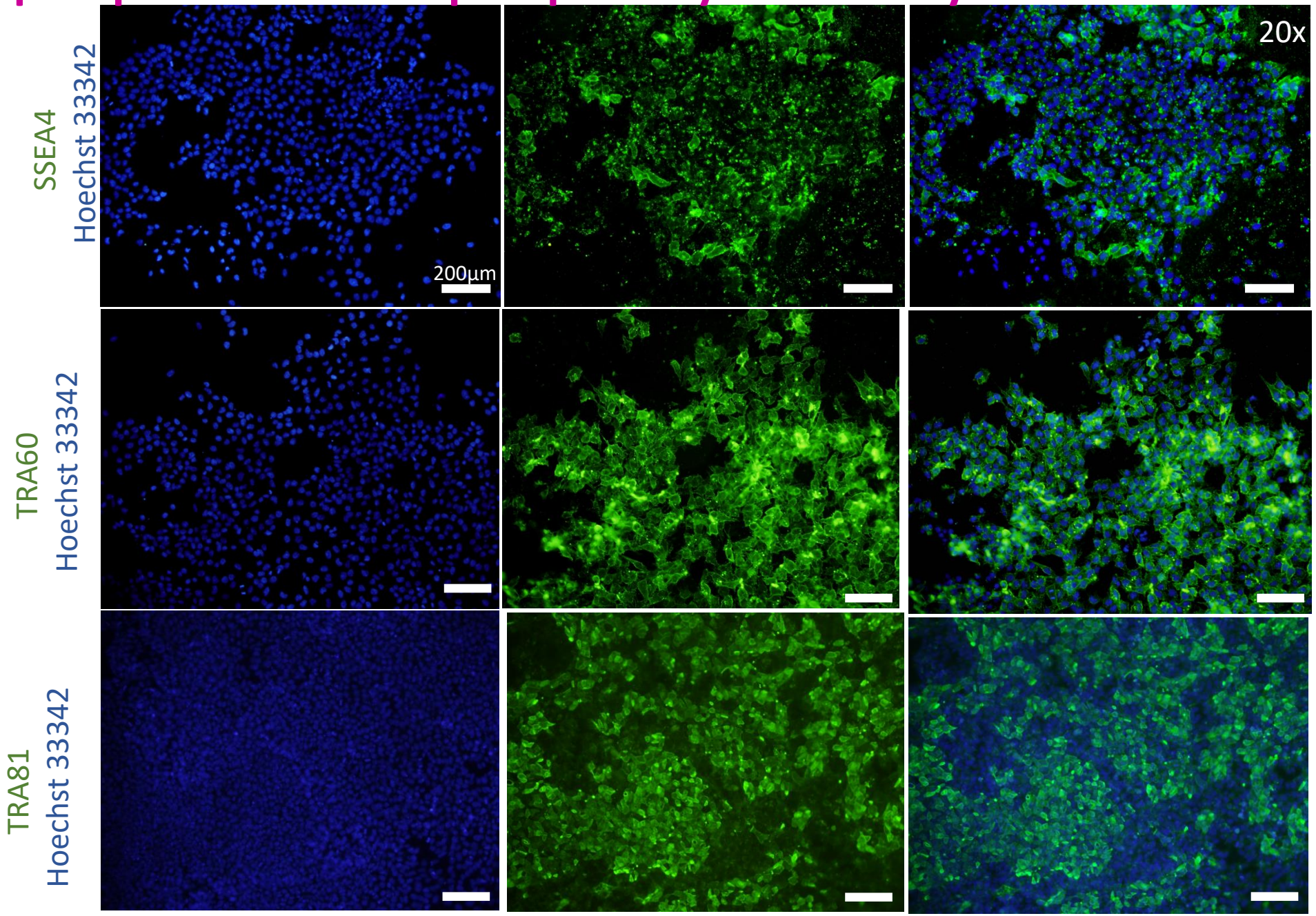
3.2 Viral vector absence.



3.2 iPSC pluripotence results: PCR



3.2 iPSC pluripotent results: pluripotency markers by immunifluorescence.



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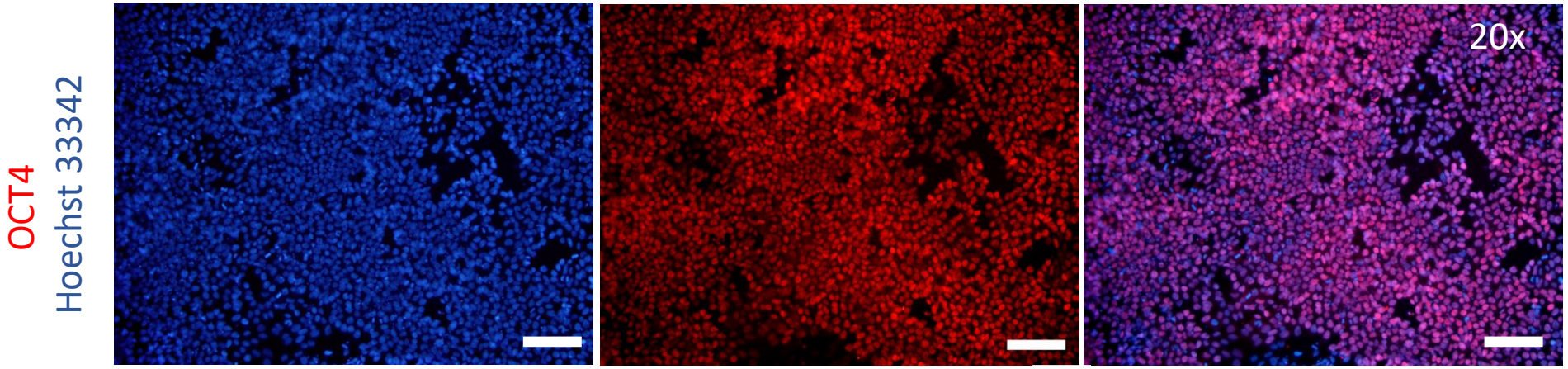
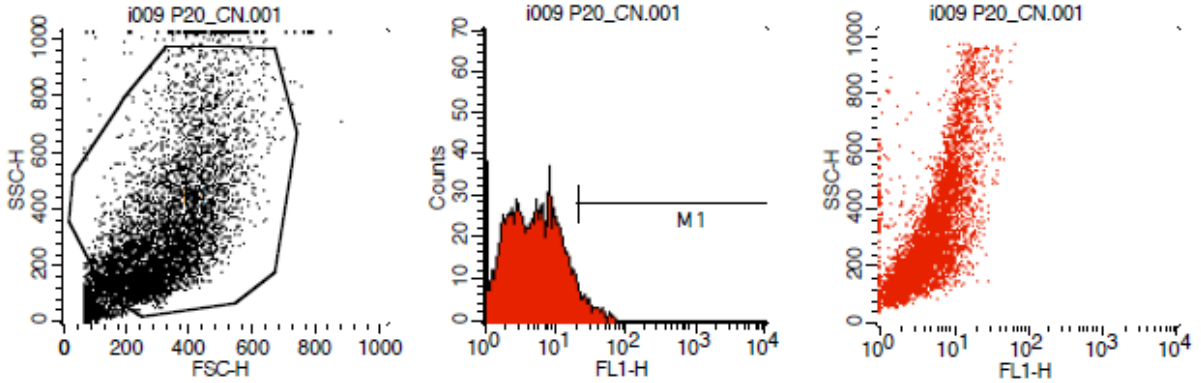


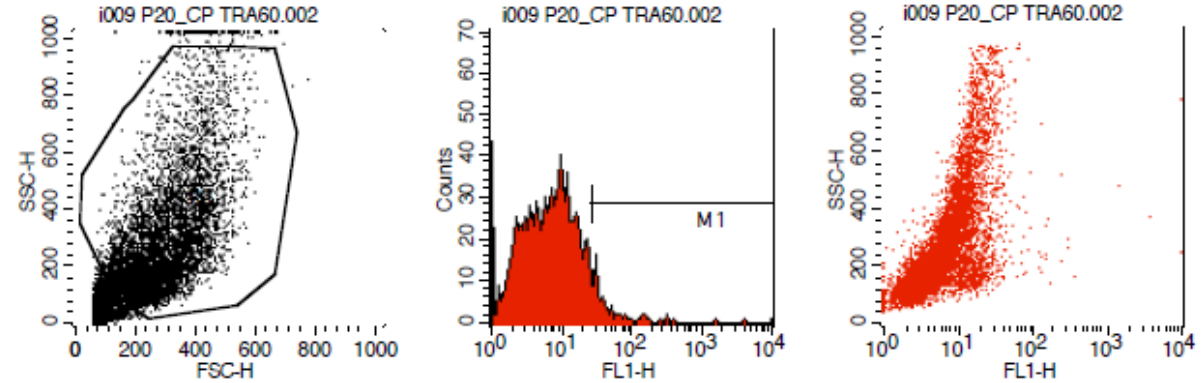
3.2 iPSC pluripotent results: pluripotency markers by Flow citometry.



Histogram Statistics

File: i009 P20_CN.001
Acquisition Date: 10-Feb-20
Gated Events: 5420
Total Events: 6645

Marker	Events	% Gated	% Total
All	5420	100.00	81.57
M1	177	3.27	2.66

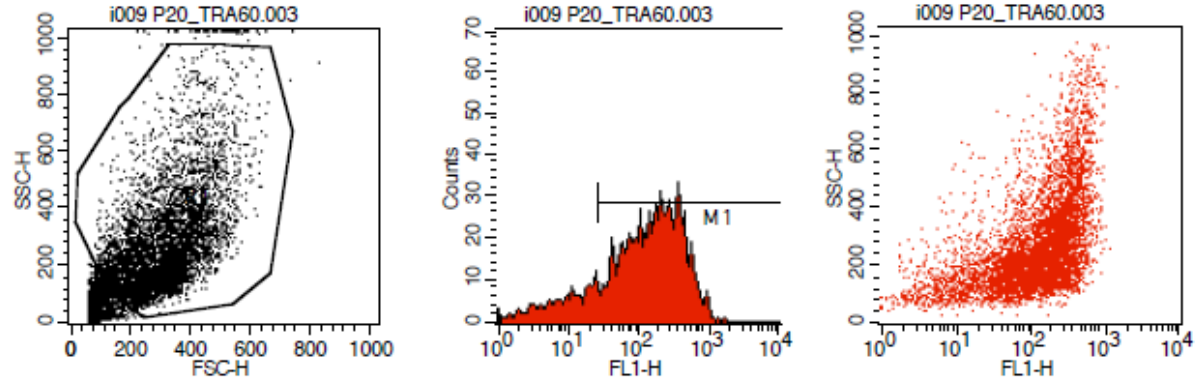


Histogram Statistics

File: i009 P20_CP TRA60.002
Acquisition Date: 10-Feb-20
Gated Events: 6084
Total Events: 7912

Marker	Events	% Gated	% Total
All	6084	100.00	76.90
M1	276	4.54	3.49

TRA 60

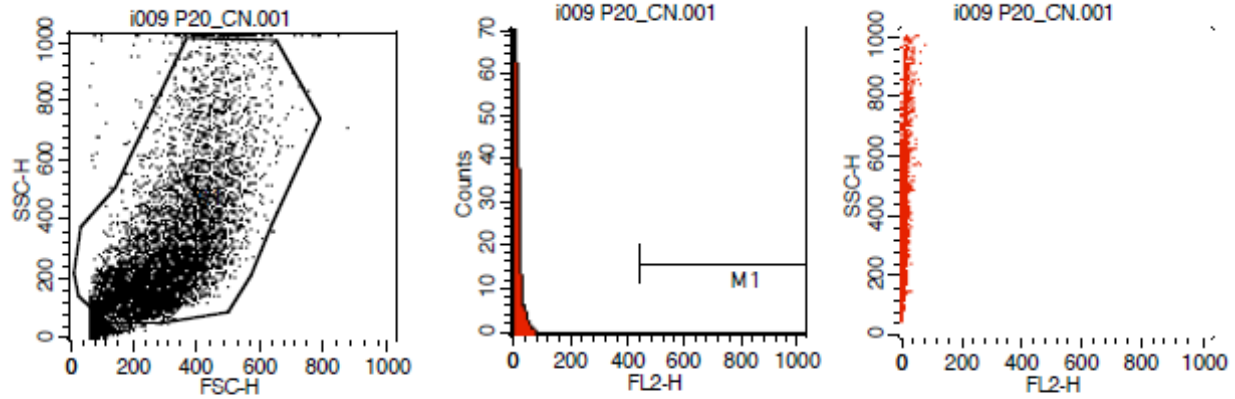


Histogram Statistics

File: i009 P20_TRA60.003
Acquisition Date: 10-Feb-20
Gated Events: 5982
Total Events: 7604

Marker	Events	% Gated	% Total
All	5982	100.00	78.67
M1	5210	87.09	68.52

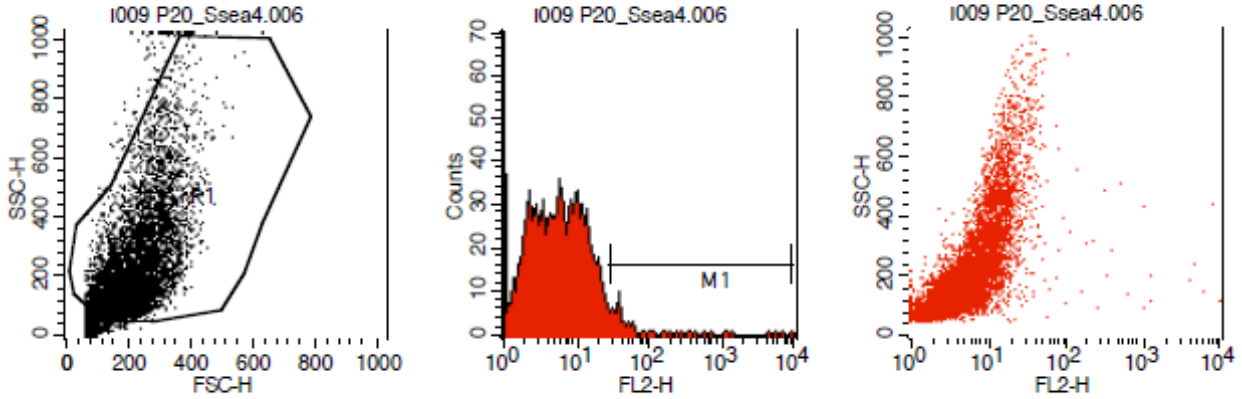
3.2 iPSC pluripotent results: pluripotency markers by Flow citometry.



Histogram Statistics

File: i009 P20_CN.001
Acquisition Date: 10-Feb-20
Gated Events: 5845
Total Events: 6645

Marker	Events	% Gated	% Total
All	5845	100.00	87.96
M1	0	0.00	0.00

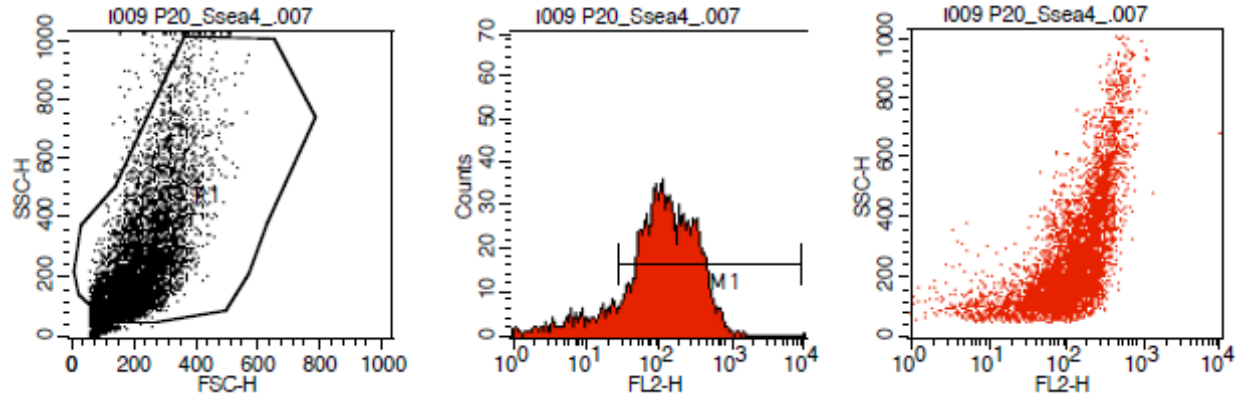


Histogram Statistics

File: I009 P20_Ssea4.006
Acquisition Date: 10-Feb-20
Gated Events: 6207
Total Events: 7071

Marker	Events	% Gated	% Total
All	6207	100.00	87.78
M1	174	2.80	2.46

SSEA-4

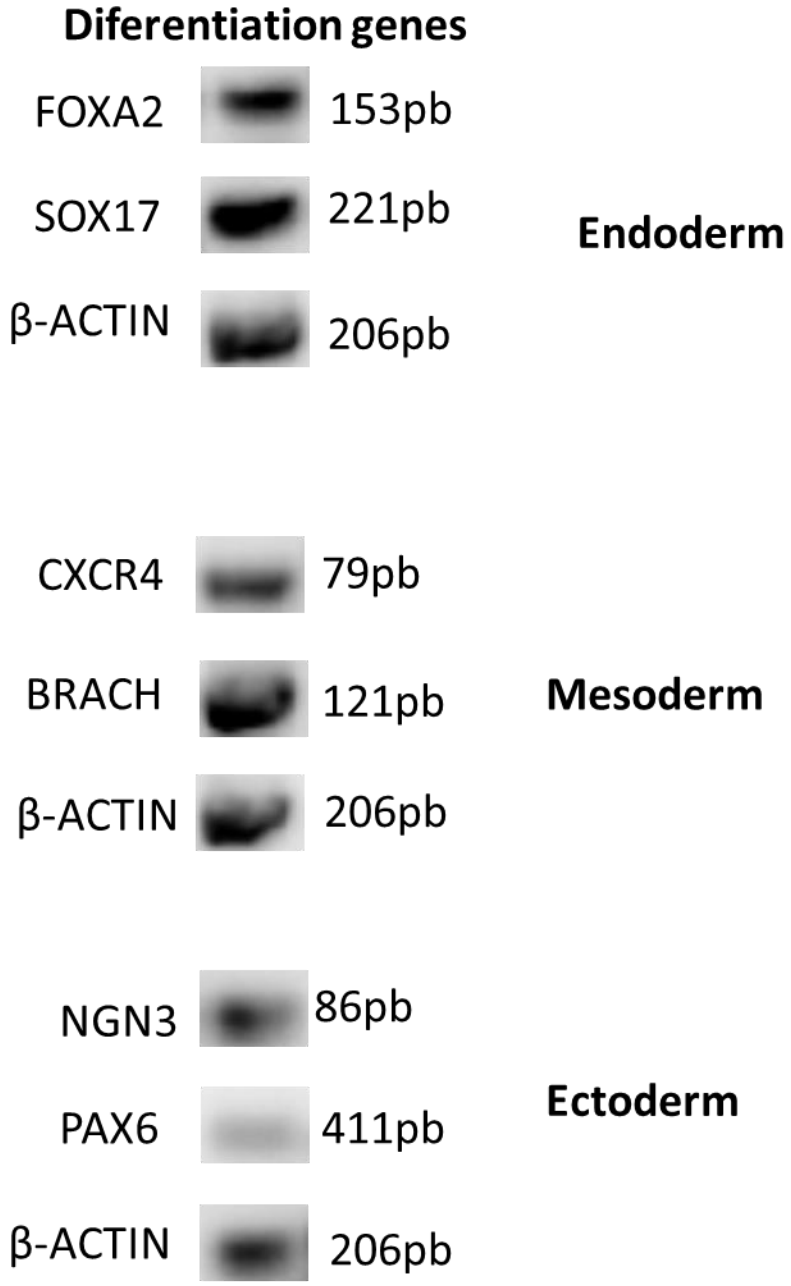


Histogram Statistics

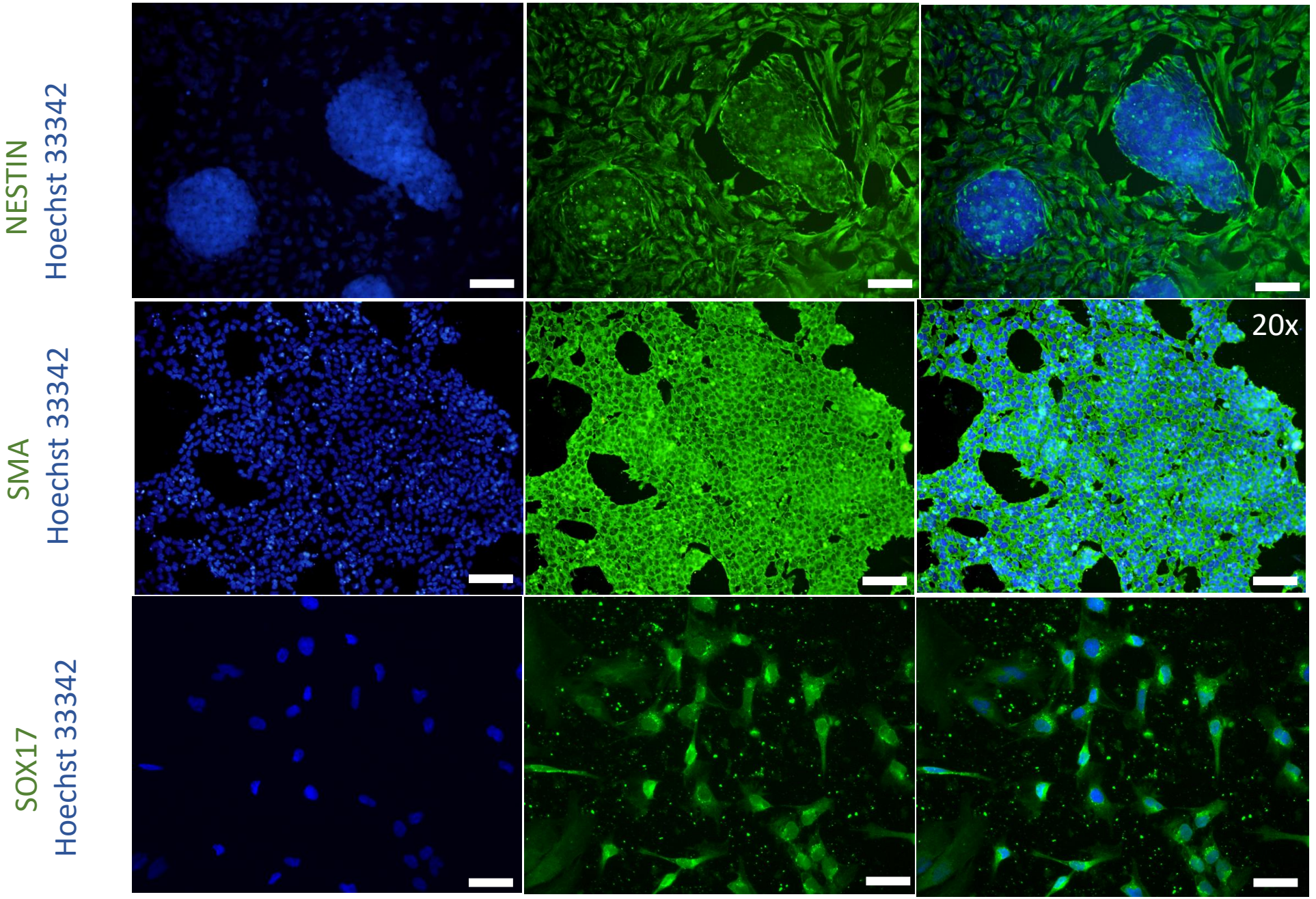
File: I009 P20_Ssea4_.007
Acquisition Date: 10-Feb-20
Gated Events: 6031
Total Events: 6788

Marker	Events	% Gated	% Total
All	6031	100.00	88.85
M1	5524	91.59	81.38

3.3. iPSC differentiation ability: Trilineage markers by PCR.



3.3. iPSC differentiation ability: Trilineage markers by inmunofluorescence.



4. Conclusions and remarkable points.

- It has been possible to isolate, cultivate and transform UDCs into iPSCs as a non invasive method.
- The generation of iPSC lines represents a great advance for cell therapy and tissues bioengineering.

- We need to further characterize our iPSC line, by studying its karyotype and testing its ability to form teratomas in vivo.
  Also we have to study possible anomalies in the culture and its karyotype.
- To apply this procedure in cell therapy, we should repeat all culture processes under Good Manufacturing Practices (GMP).

Thanks!

Any questions?