Reprogramming somatic cells into rabbit and avian iPSC

Marielle Afanassieff, Bertrand Pain

Infrastructures nationales en biologie et santé
Réunion de lancement du projet CRB-Anim

Vendredi 9 novembre 202, de 9h30 à 17h00

Amphithéâtre du siège de l'INRA - 147, rue de l'Université – 75 007 Paris

9h00 – 9h30 Accueil des participants

9h30 – 9h45 Séance d'ouverture

INRA

9h45 – 10h15 L'Appel « Infrastructures nationales en biologie et santé » des projets « Investissements d'Avenir » :

ANR

CGI

Ministère de la Recherche

10h15 – 10h45 Les enjeux du CRB-Anim pour l'agriculture française et les ressources génétiques

Ministère de l'Agriculture

10h45 – 11h15 L'exemple européen du Centre for Genetic Resources, the Netherlands (CGN)

Sipke-Joost Hiemstra (Wagenigen University)

11h45 – 12h15 Présentation du projet CRB-Anim

Michèle Tixier-Boichard (INRA)

12h15 – 13h30 Buffet déjeuner

Session 1 : Les Centres de Ressources Biologiques : Rôles et missions pour la recherche

13h30 – 14h00 Le projet Investissements d'Avenir « Biobanques »

(Bruno Clément, INSERM)

14h00 – 14h30 L'exemple du European Marine Biological Resource Centre (EMBRC)

(Bernard Kloareg, UPMC)

14h30 – 15h00 Management of genetic diversity in genebanks with genomics and other tools

(Jack Windig, CGN, WUR)

15h00 – 15h30 Pause café

Session 2 : Illustration des objectifs spécifiques du CRB-Anim

15h30 – 16h00 Du centre de ressources biologiques aux résultats scientifiques et économiques : l'exemple du modèle canin

(Catherine André, CNRS)

16h00 – 16h30 L'impact des CRB sur l'innovation en biotechnologies de la reproduction

(Marielle Afanassieff , Bertrand Pain, INSERM)

16h30 – 17h00 Discussion finale

Table ronde avec les représentants des différents partenaires

Entrée libre sur inscription sur le site : https://colloque.inra.fr/kickoff-meeting-crb-anim

Contact : Olivier Ruetsch (olivier.ruetsch@paris.inra.fr, 01 42 75 93 25)

Reprogramming somatic cells into rabbit and avian iPSC

Marielle AFANASSIEFF & Bertrand PAIN

AgroBioStem - INSERM U846 - INRA USC 1361 - Bron

CRB-Anim 9/11/2012

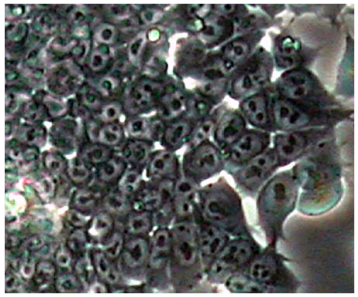
Developments for somatic samples to be used for reproductive biology (WP2.2)

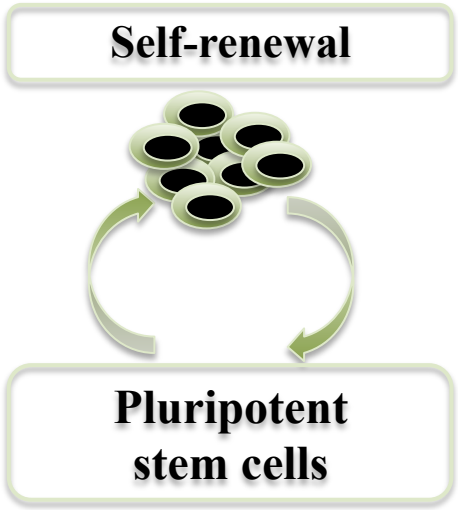
Goals: To increase the type and the number of samples for cryobanking

Properties of pluripotent stem cells

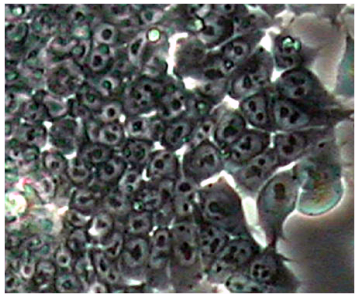
Pluripotent stem cells

Properties of pluripotent stem cells

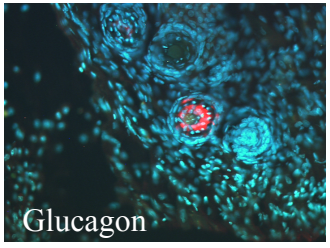




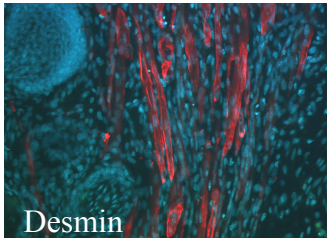
Properties of pluripotent stem cells



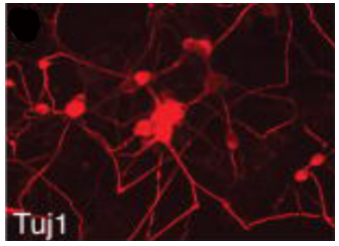
Endoderm



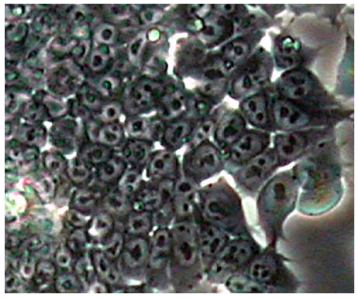
Mesoderm



Ectoderm



Properties of pluripotent stem cells



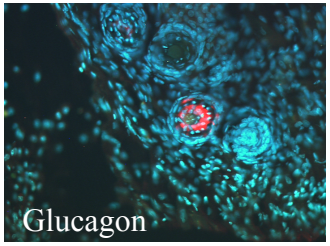
Self-renewal

Oncogenesis

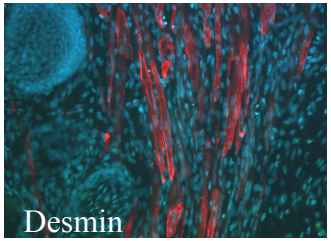
Pluripotent stem cells

Differentiation

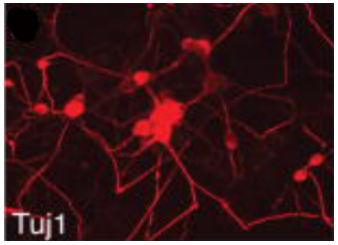
Endoderm



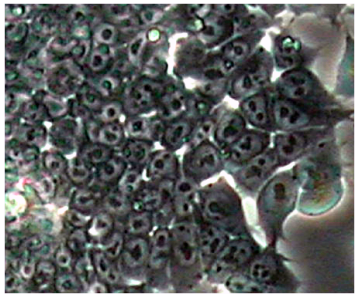
Mesoderm



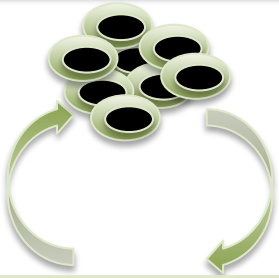
Ectoderm



Properties of pluripotent stem cells



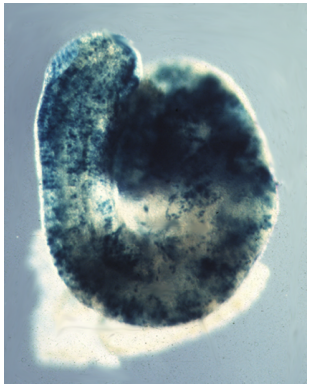
Self-renewal



Oncogenesis

Pluripotent stem cells

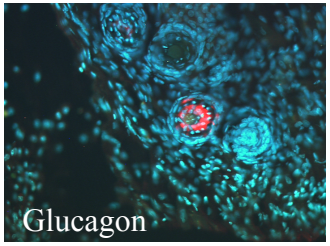
Embryo colonization



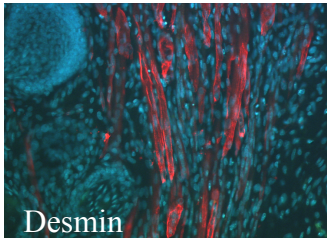
Chimeras

Differentiation

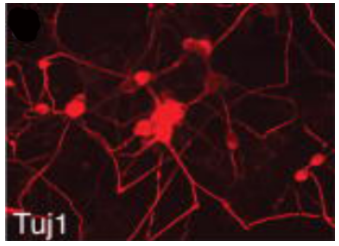
Endoderm



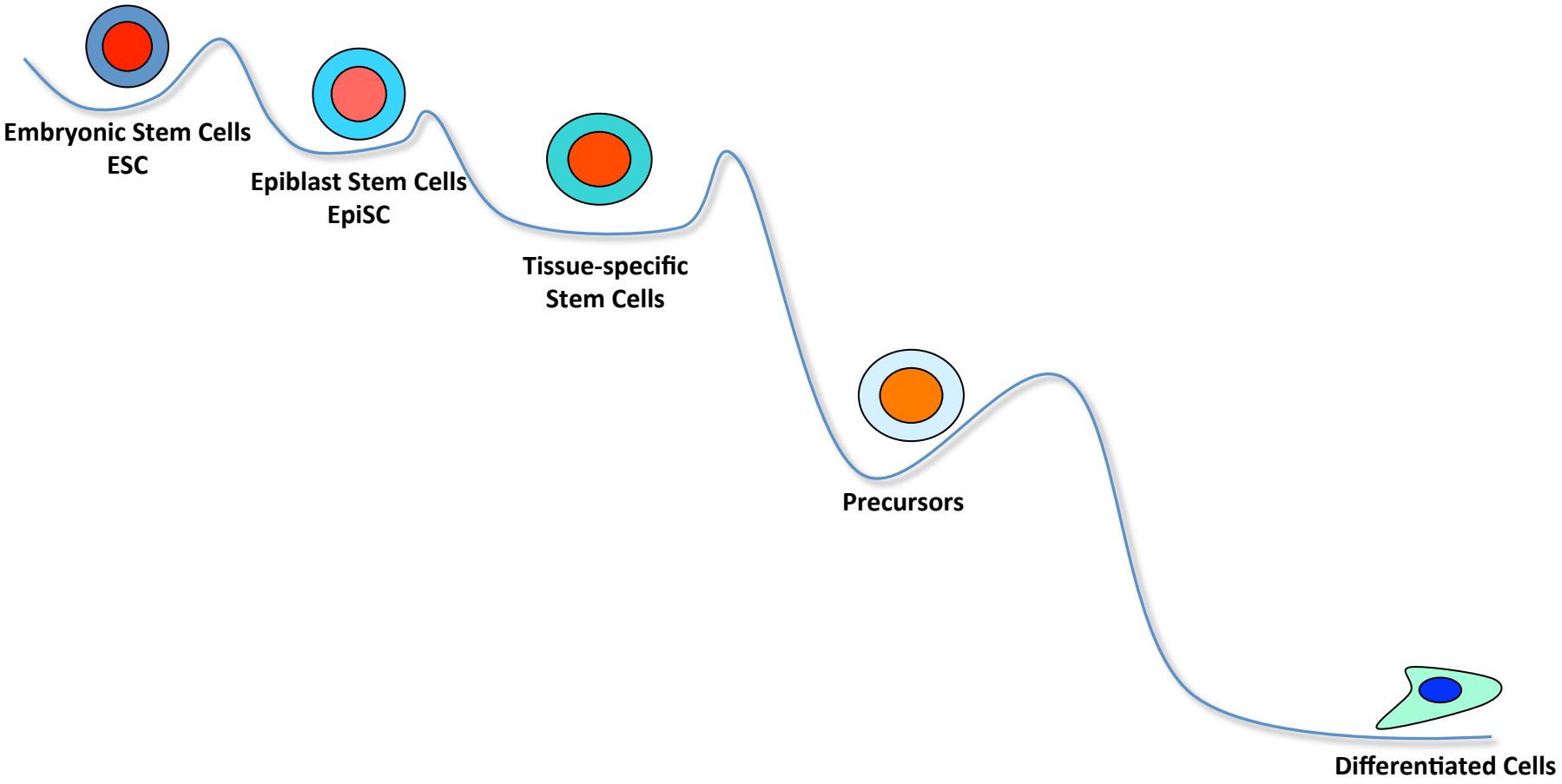
Mesoderm



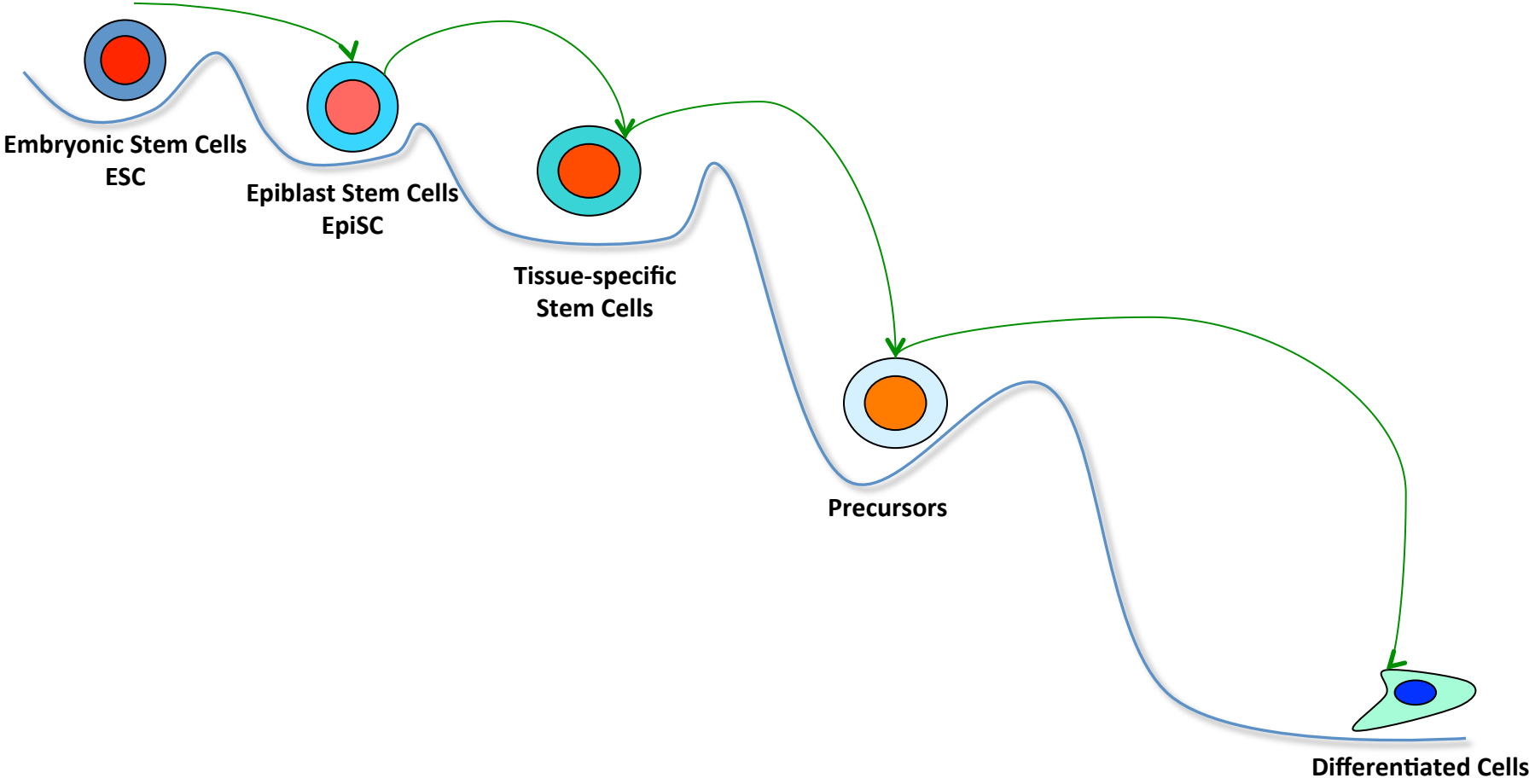
Ectoderm



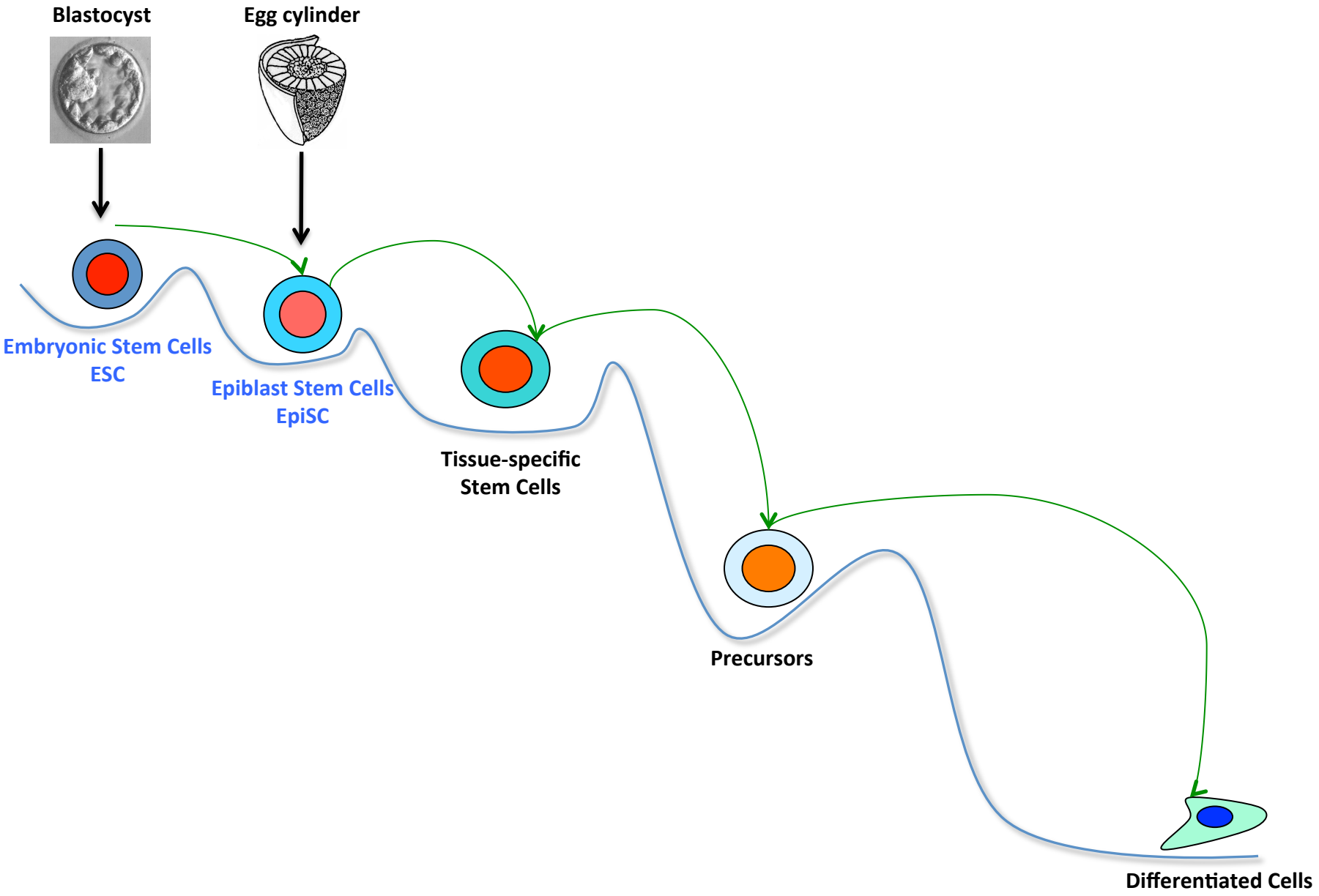
Types of pluripotent stem cell



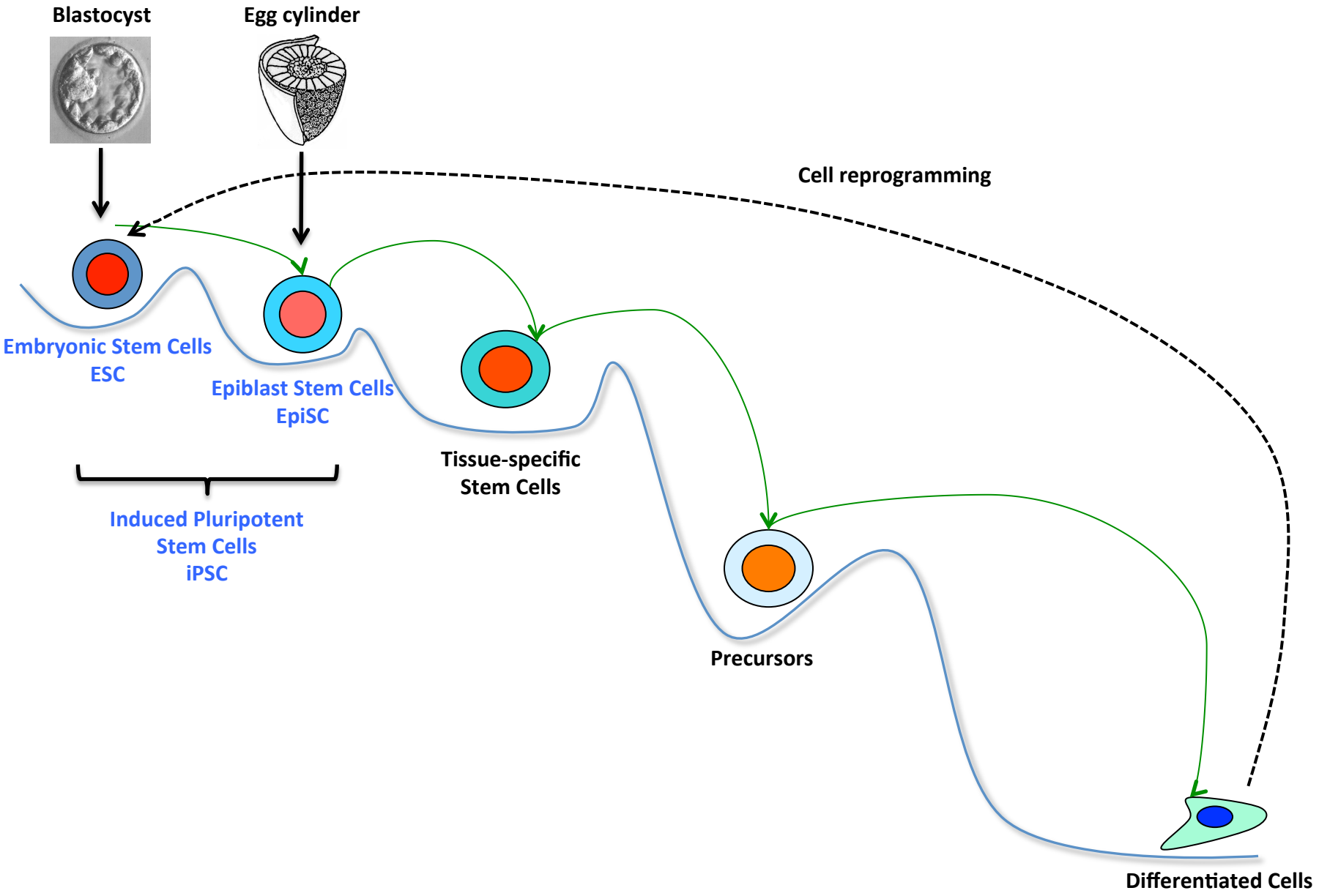
Types of pluripotent stem cell



Types of pluripotent stem cell



Types of pluripotent stem cell



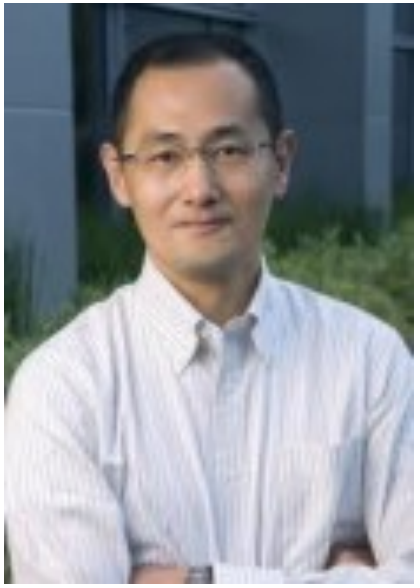
Reprogramming: the stem cell revolution – the iPSC

The Nobel Prize in Physiology or Medicine 2012

jointly to

John B. Gurdon and Shinya Yamanaka

for the discovery that mature cells can be reprogrammed to become pluripotent



The Nobel Prize recognizes two scientists who discovered that mature, specialised cells can be reprogrammed to become immature cells capable of developing into all tissues of the body. Their findings have revolutionised our understanding of how cells and organisms develop.

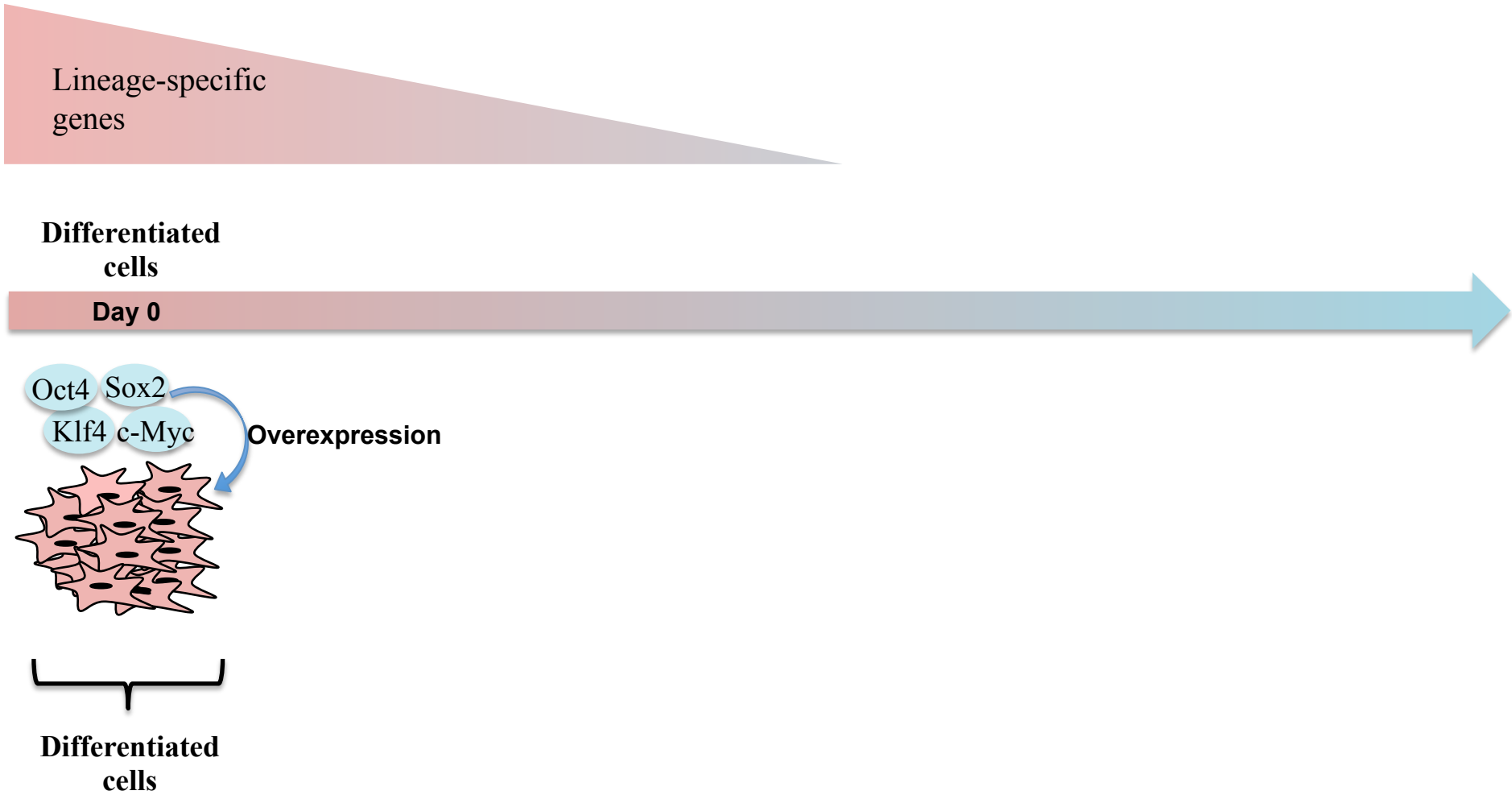
John B. Gurdon discovered in 1962 that the specialisation of cells is reversible. In a classic experiment, he replaced the immature cell nucleus in an egg cell of a frog with the nucleus from a mature intestinal cell. This modified egg cell developed into a normal tadpole. The DNA of the mature cell still had all the information needed to develop all cells in the frog.

Shinya Yamanaka discovered more than 40 years later, in 2006, how intact mature cells in mice could be reprogrammed to become immature stem cells. Surprisingly, by introducing only a few genes, he could reprogram mature cells to become pluripotent stem cells, i.e. immature cells that are able to develop into all types of cells in the body.

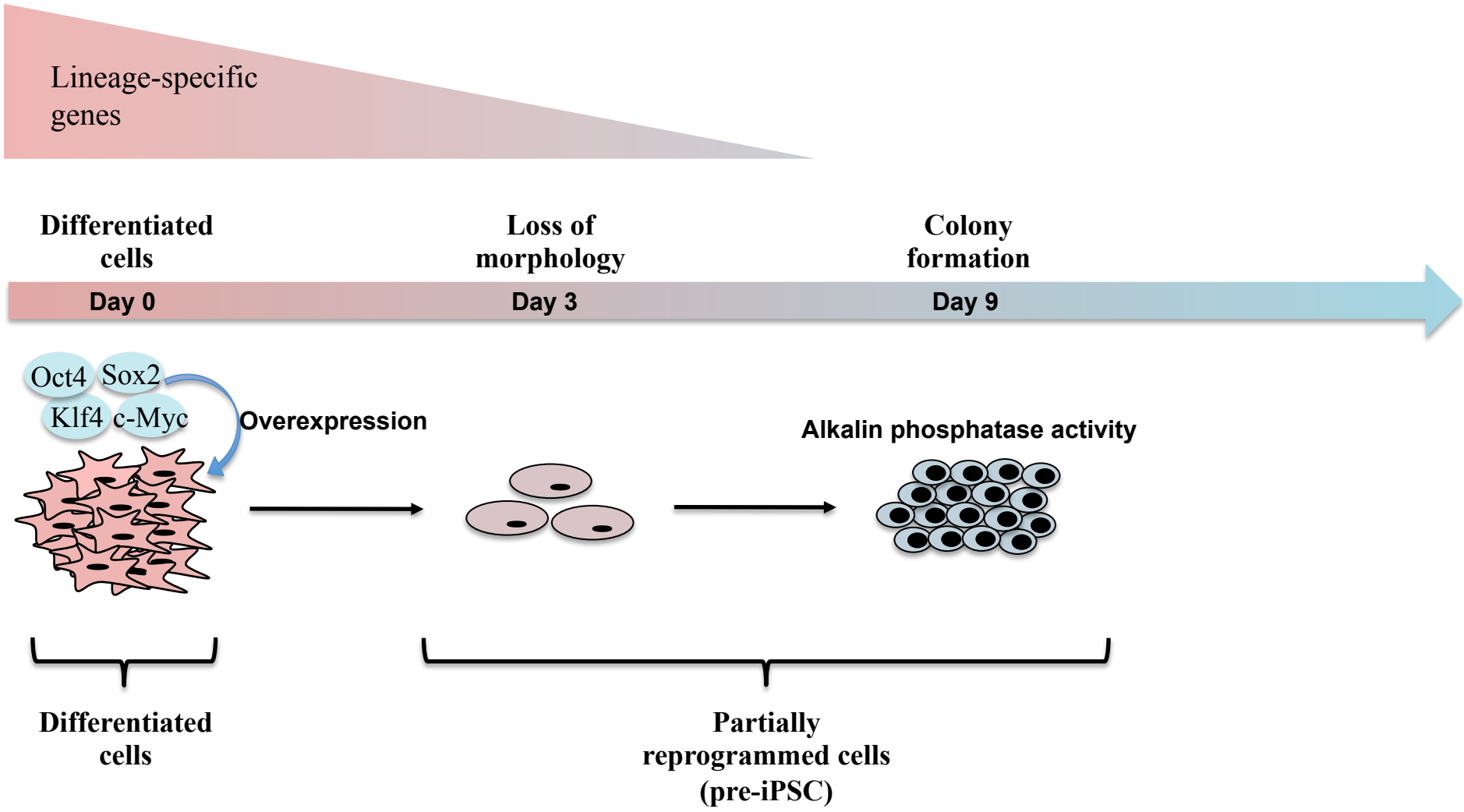
These groundbreaking discoveries have completely changed our view of the development and cellular specialisation. We now understand that the mature cell does not have to be confined forever to its specialised state. Textbooks have been rewritten and new research fields have been established. By reprogramming human cells, scientists have created new opportunities to study diseases and develop methods for diagnosis and therapy.

http://www.nobelprize.org/nobel_prizes/medicine/laureates/2012/press.html

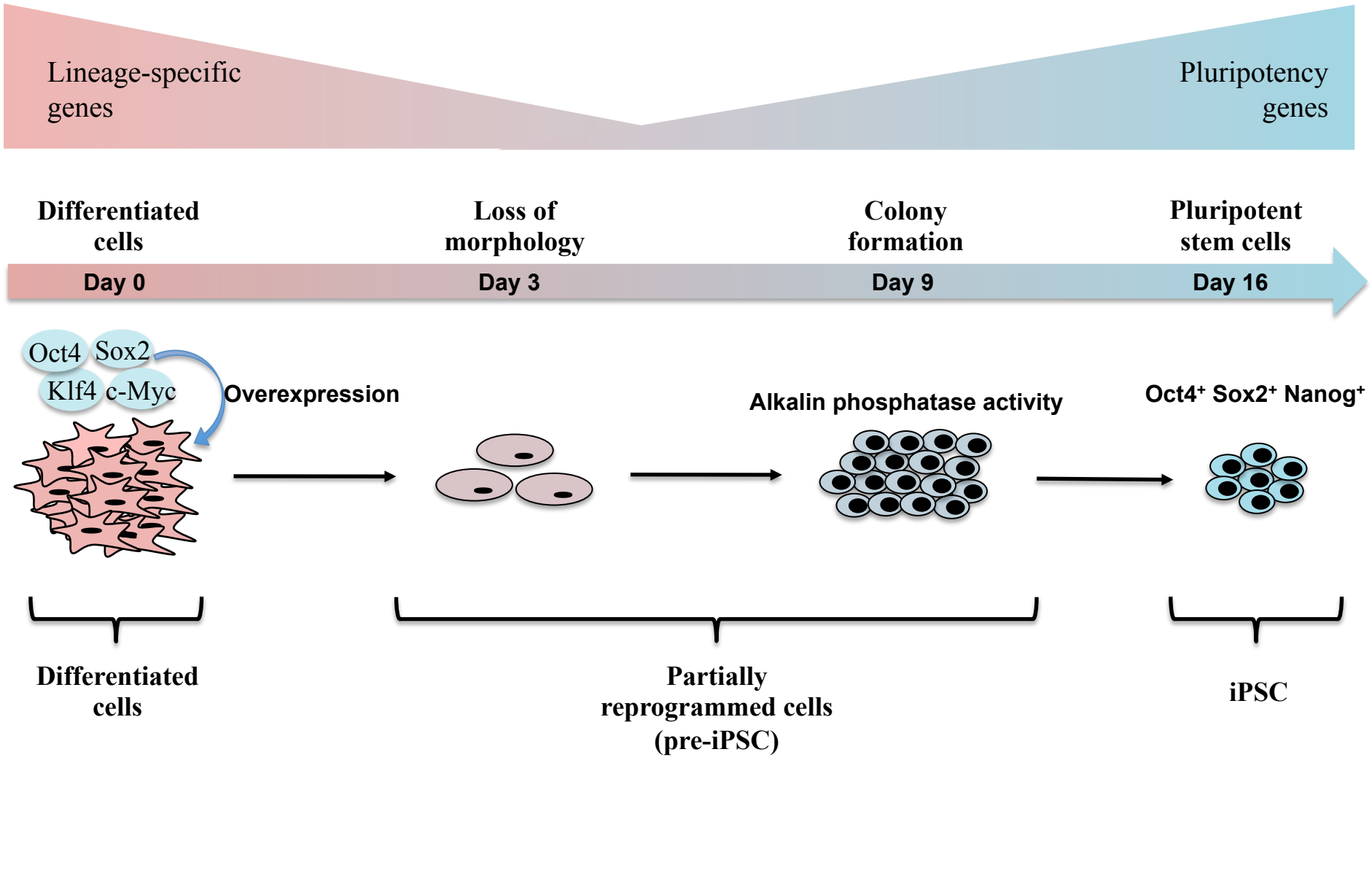
Cell reprogramming in Mouse



Cell reprogramming in Mouse



Cell reprogramming in Mouse



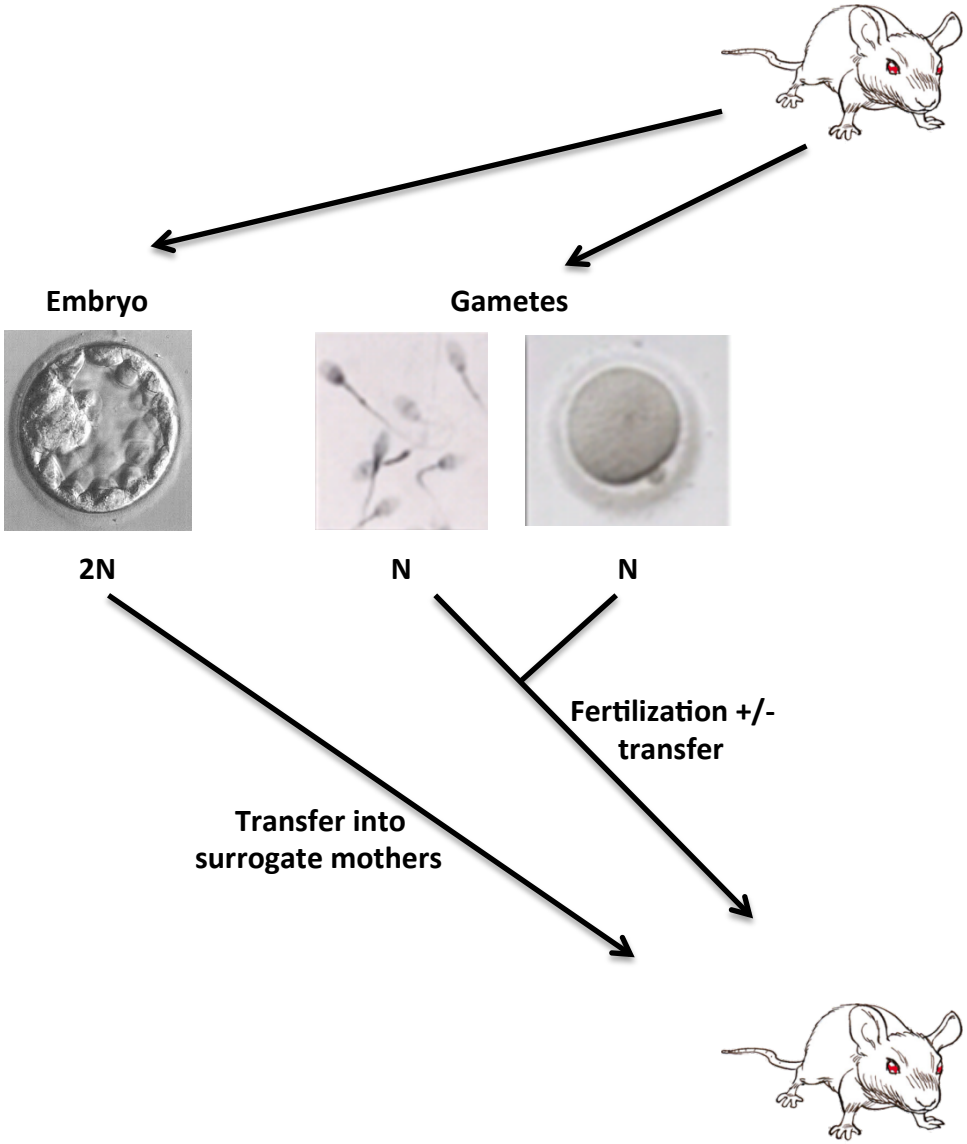
Interest of iPSC for conservation of species

Embryo

2N

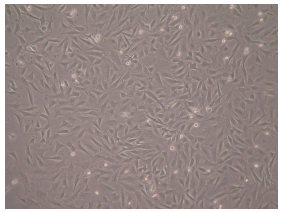
Transfer into
surrogate mothers

Interest of iPSC for conservation of species



Interest of iPSC for conservation of species

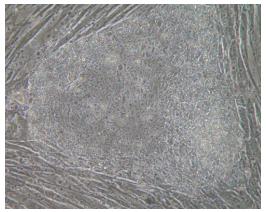
Somatic tissue



2N

Reprogramming

iPSC



2N

Embryo

2N

Gametes

N

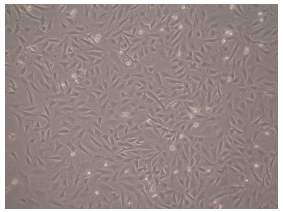
N

Fertilization +/-
transfer

Transfer into
surrogate mothers

Interest of iPSC for conservation of species

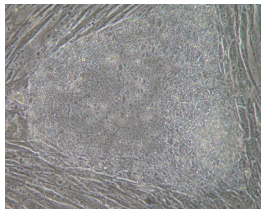
Somatic tissue



2N

Reprogramming

iPSC



2N

Embryo colonization

Embryo

2N

Gametes

N

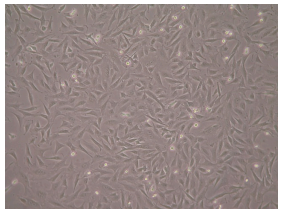
N

Fertilization +/- transfer

Transfer into surrogate mothers

Interest of iPSC for conservation of species

Somatic tissue

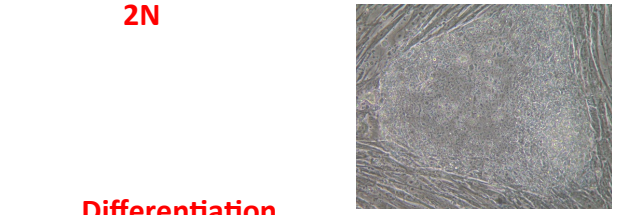


Reprogramming

Embryo

Gametes

2N



iPSC

2N

2N

N

N

Differentiation

Embryo colonization

Fertilization +/- transfer

Transfer into surrogate mothers

Reprogramming somatic cells into rabbit iPSC

- ✓ ***Advantages of the rabbit*** *?*

  small animal, easy and cheap to breed
  prolific animal with a short reproductive interval
  physiologically and genetically closer to other mammals

Reprogramming somatic cells into rabbit iPSC

- ✓ ***Advantages of the rabbit*** *?*

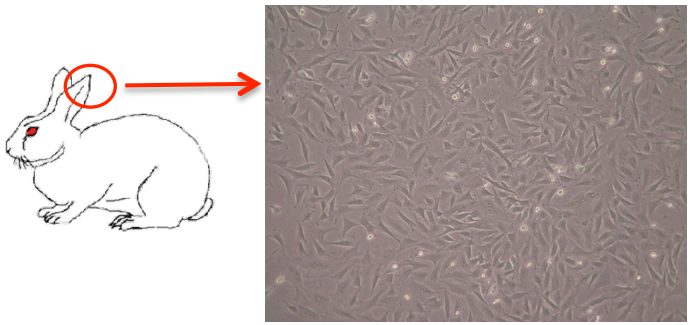
  small animal, easy and cheap to breed
  prolific animal with a short reproductive interval
  physiologically and genetically closer to other mammals

- ✓ ***Development of rabbit cryobanking***

  iPSC technology is available for rabbit
  determination of somatic tissues/cells to be used
  establishment of cryoconservation conditions
  adaptation of reprogramming techniques

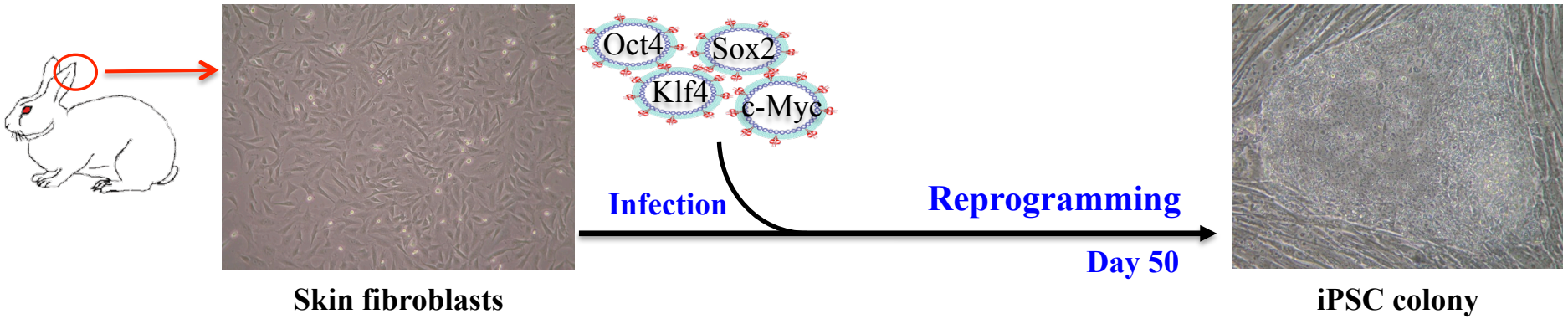
- ✓ ***Good model for farm animals***

Rabbit iPSC



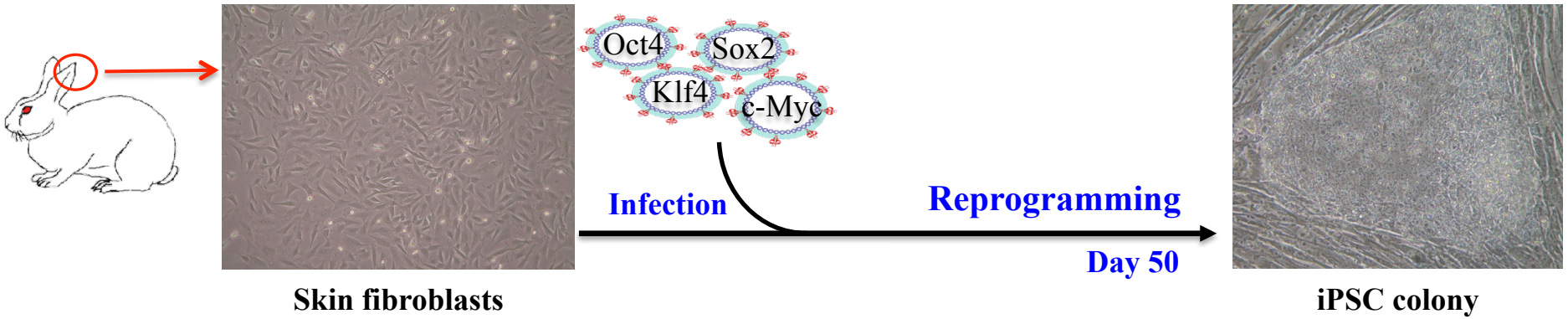
Skin fibroblasts

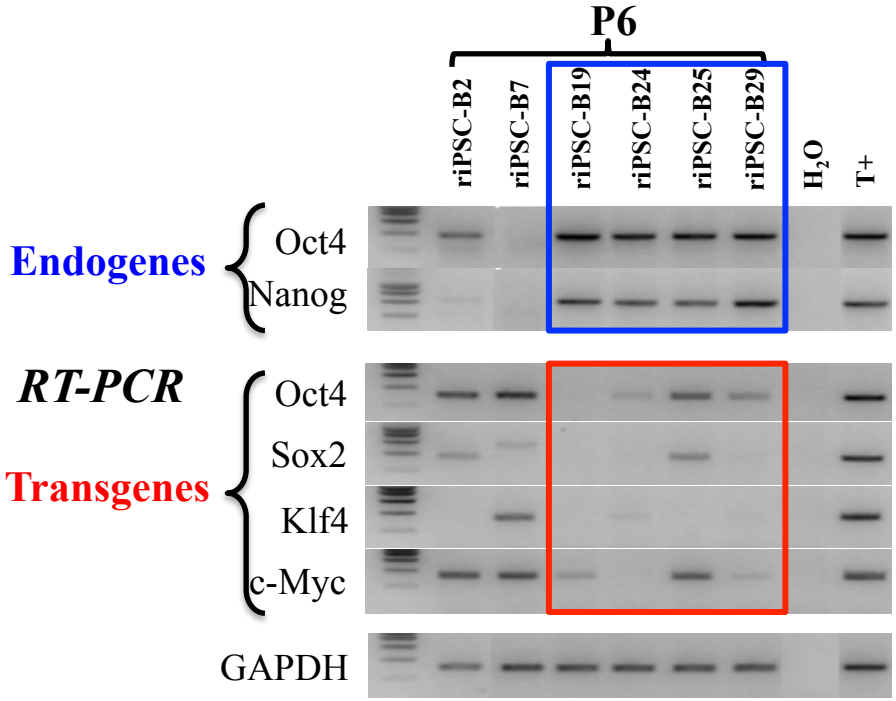
Rabbit iPSC



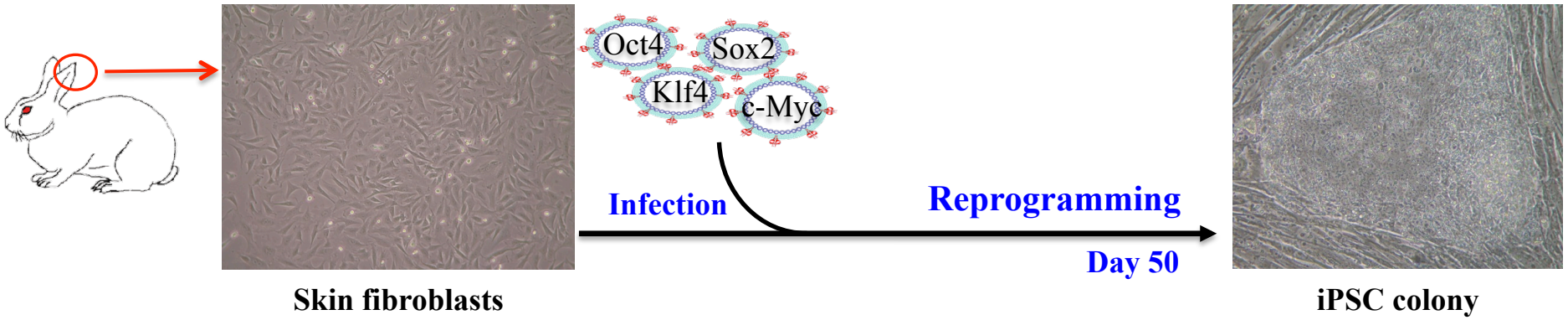
Skin fibroblasts **iPSC colony**

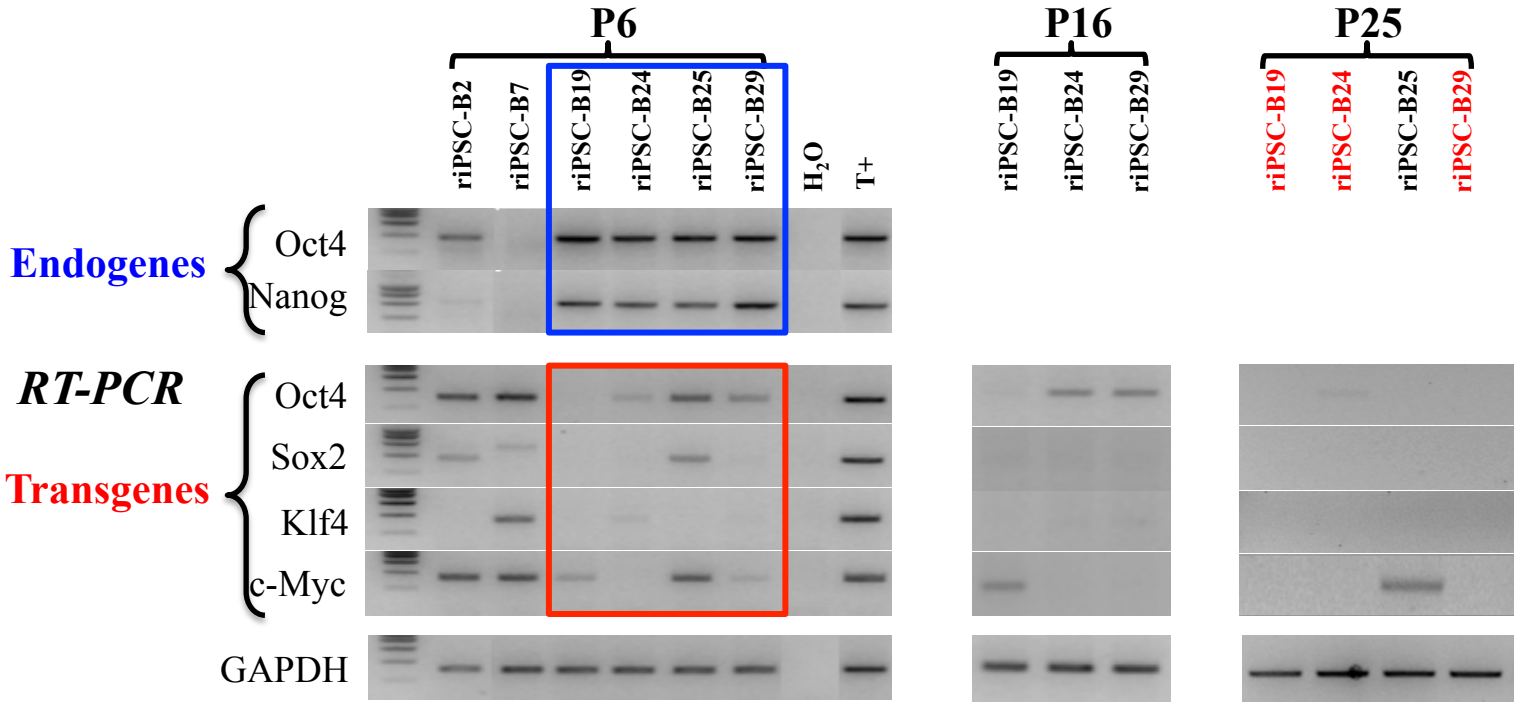
Rabbit iPSC


Oct4
Sox2
Klf4
c-Myc
Infection
Reprogramming
Day 50
Skin fibroblasts
iPSC colony

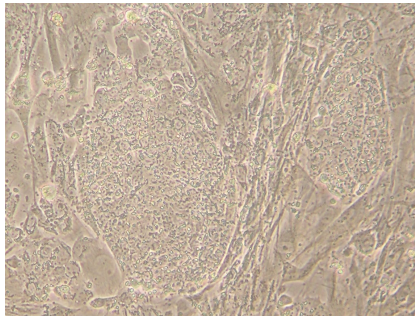

P6
riPSC-B2
riPSC-B7
riPSC-B19
riPSC-B24
riPSC-B25
riPSC-B29
H2O
T+
Endogenes
Oct4
Nanog
RT-PCR
Transgenes
Oct4
Sox2
Klf4
c-Myc
GAPDH

Rabbit iPSC


Oct4
Sox2
Klf4
c-Myc
Infection
Reprogramming
Day 50
Skin fibroblasts
iPSC colony

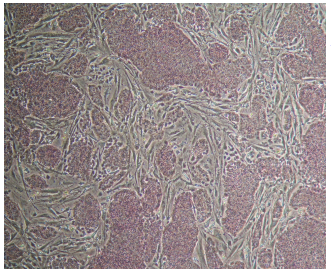

P6
P16
P25
riPSC-B2
riPSC-B7
riPSC-B19
riPSC-B24
riPSC-B25
riPSC-B29
H2O
T+
riPSC-B19
riPSC-B24
riPSC-B29
riPSC-B19
riPSC-B24
riPSC-B25
riPSC-B29
Endogenes
Oct4
Nanog
RT-PCR
Transgenes
Oct4
Sox2
Klf4
c-Myc
GAPDH

Characterization of rabbit iPSC



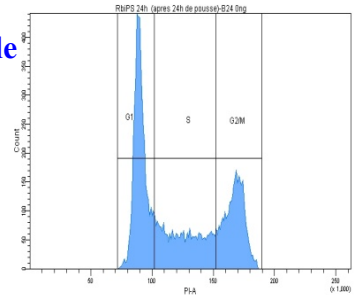
Rabbit iPSC

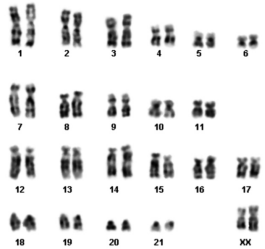
Characterization of rabbit iPSC



AP activity

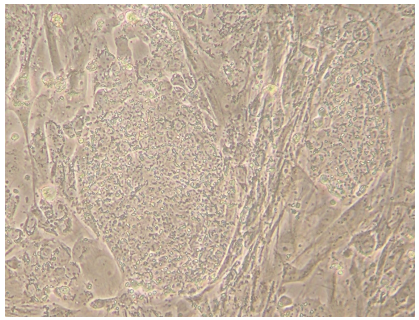
Cell cycle





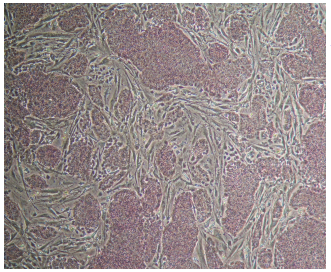
42XX

Self-renewal



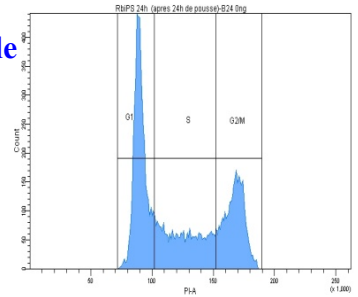
Rabbit iPSC

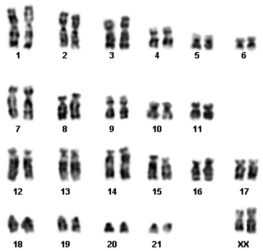
Characterization of rabbit iPSC



AP activity

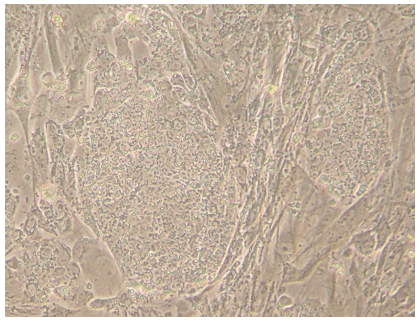
Cell cycle





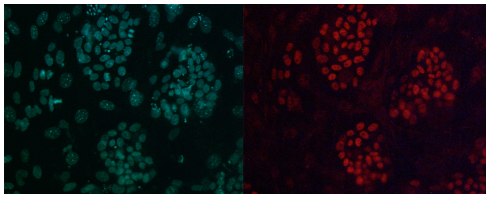
42XX

Self-renewal

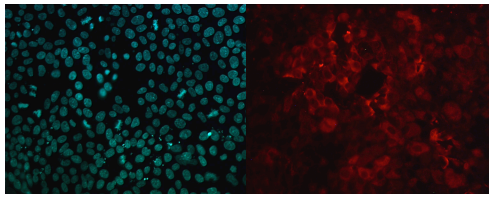


Rabbit iPSC

Pluripotency markers

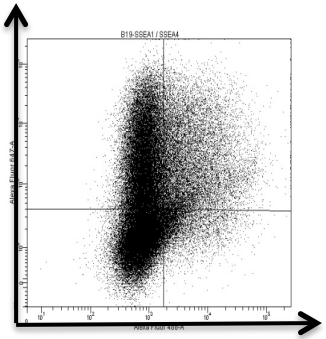


Oct4



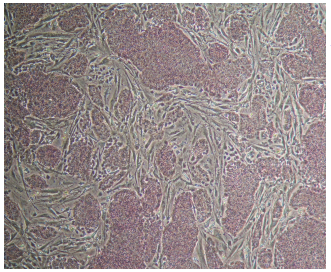
E-Cadherin

SSEA1



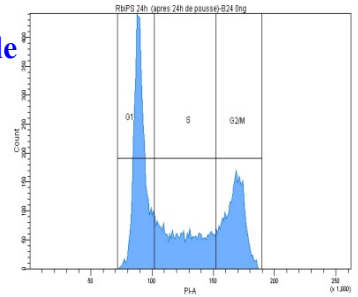
SSEA4

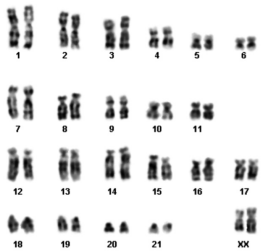
Characterization of rabbit iPSC



AP activity

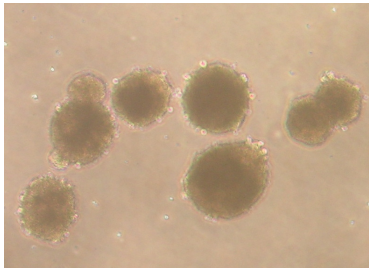
Cell cycle



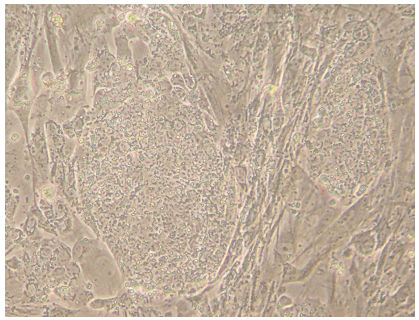


42XX

Self-renewal



Embryoid bodies

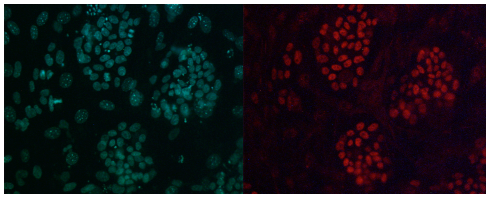


Rabbit iPSC

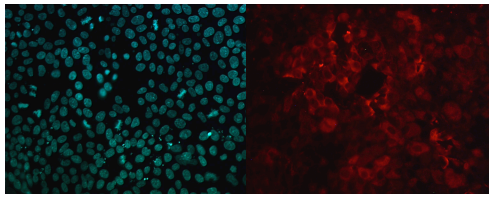
Differentiation and oncogenesis

Teratoma

Pluripotency markers

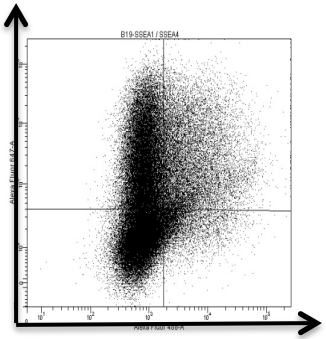


Oct4



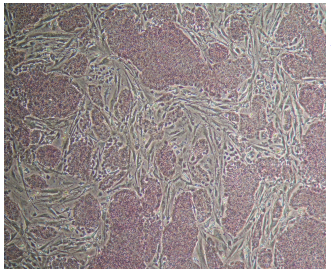
E-Cadherin

SSEA1



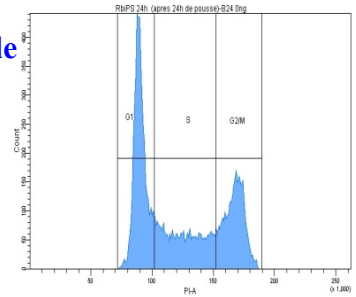
SSEA4

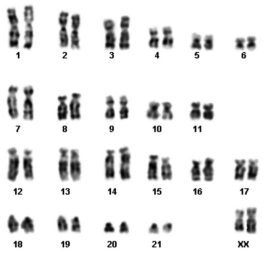
Characterization of rabbit iPSC



AP activity

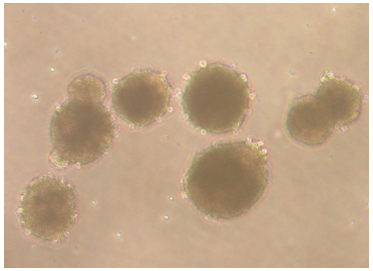
Cell cycle





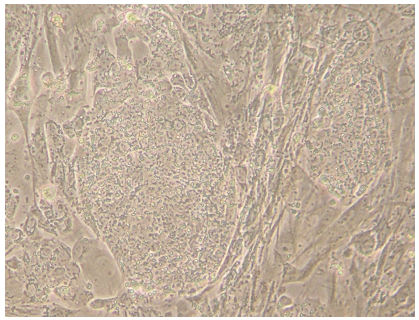
42XX

Self-renewal



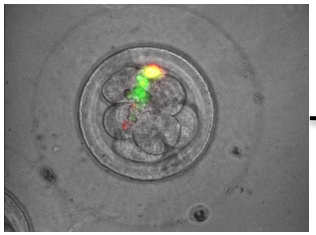
Embryoid bodies

Differentiation and oncogenesis

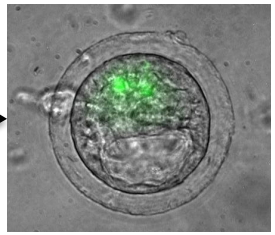


Rabbit iPSC

Embryo colonization ?



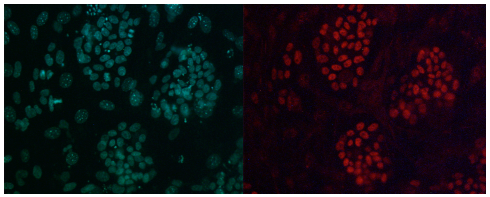
8-cell stage embryo



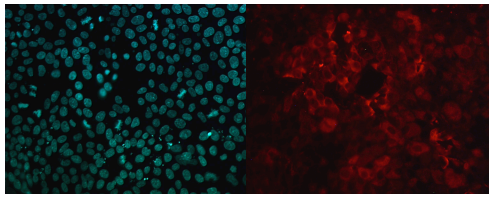
Blastocyst

Teratoma

Pluripotency markers

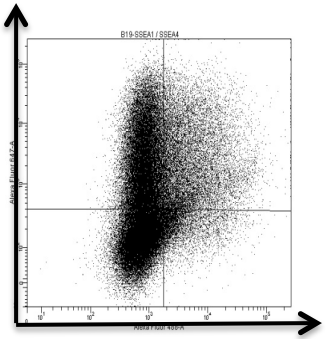


Oct4



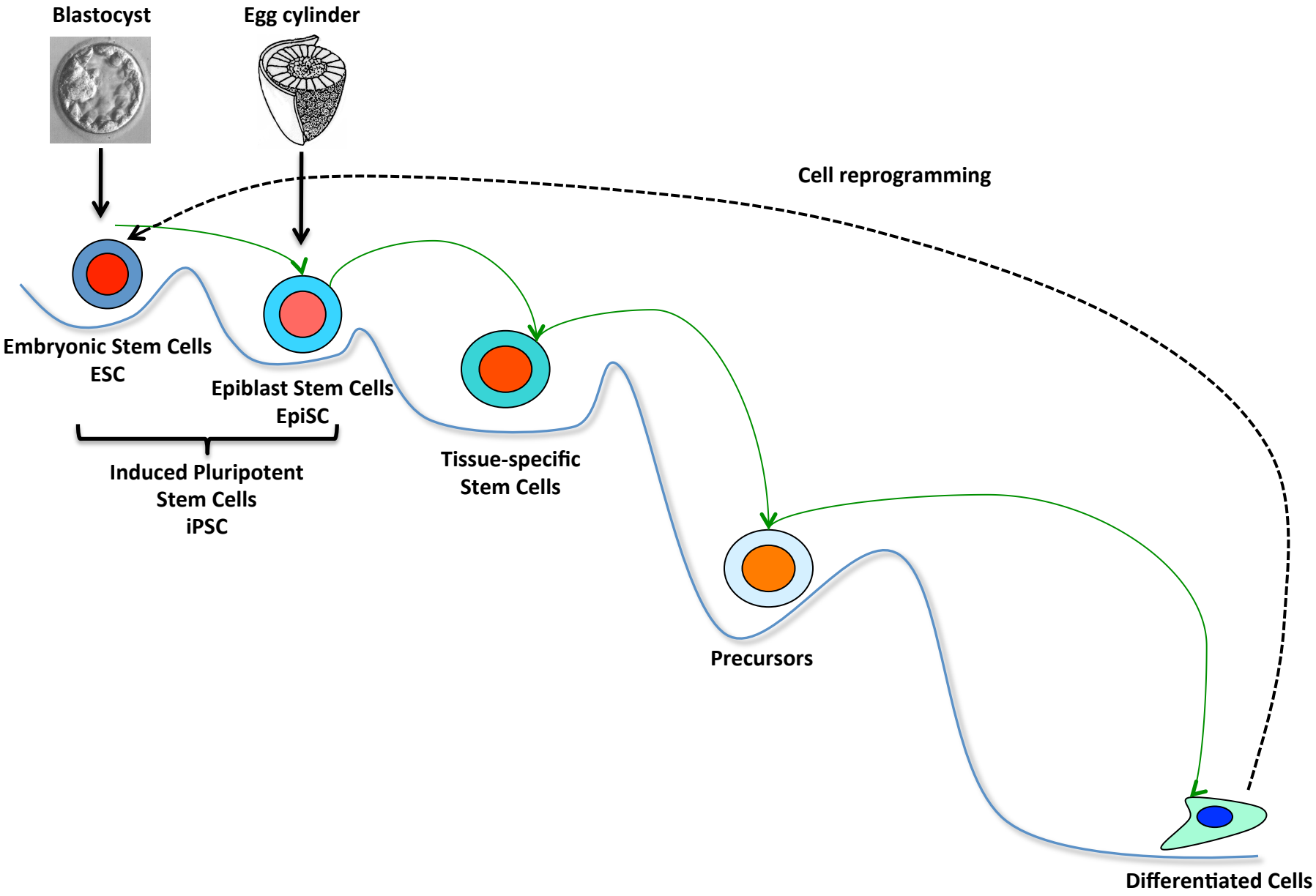
E-Cadherin

SSEA1

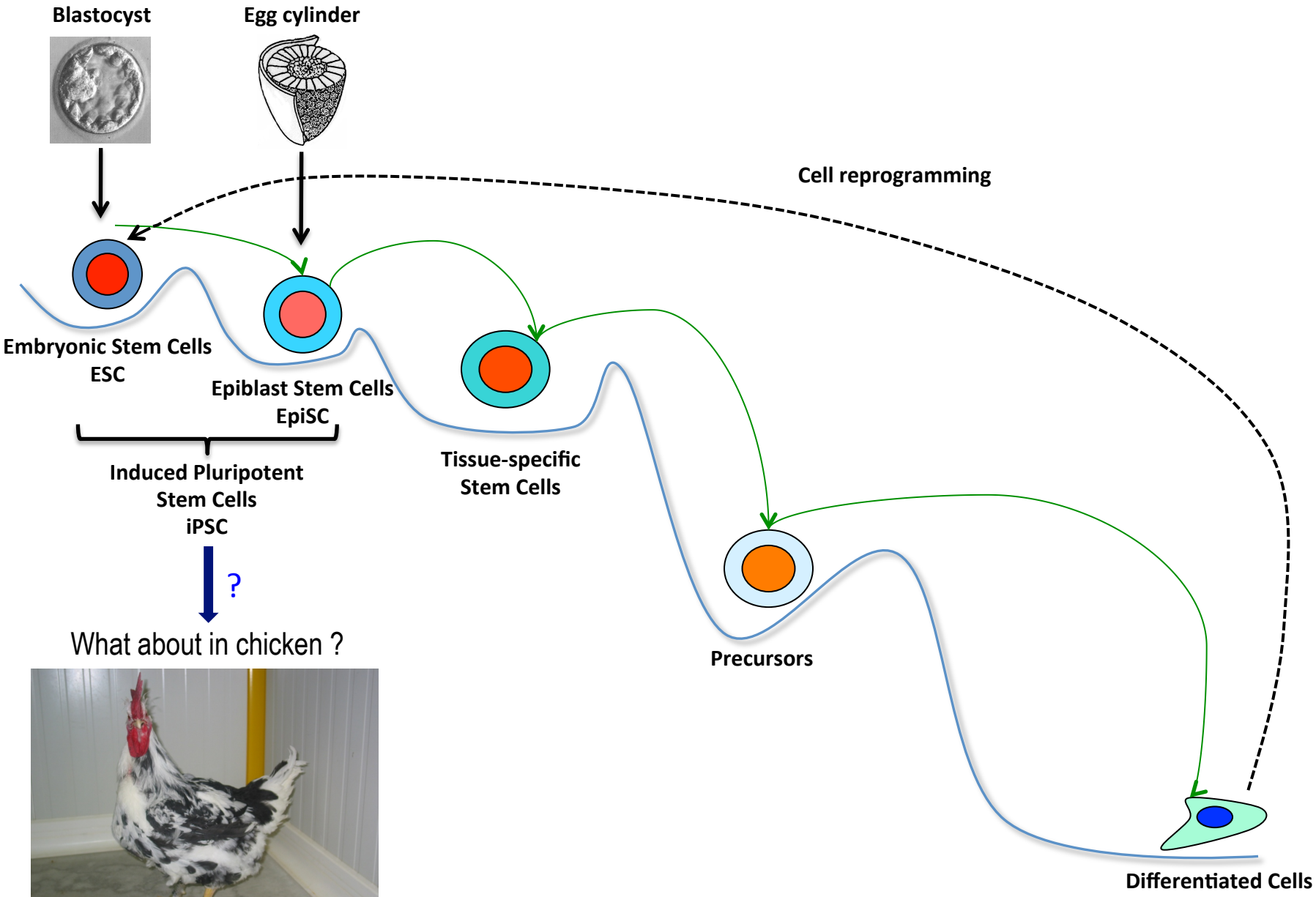


SSEA4

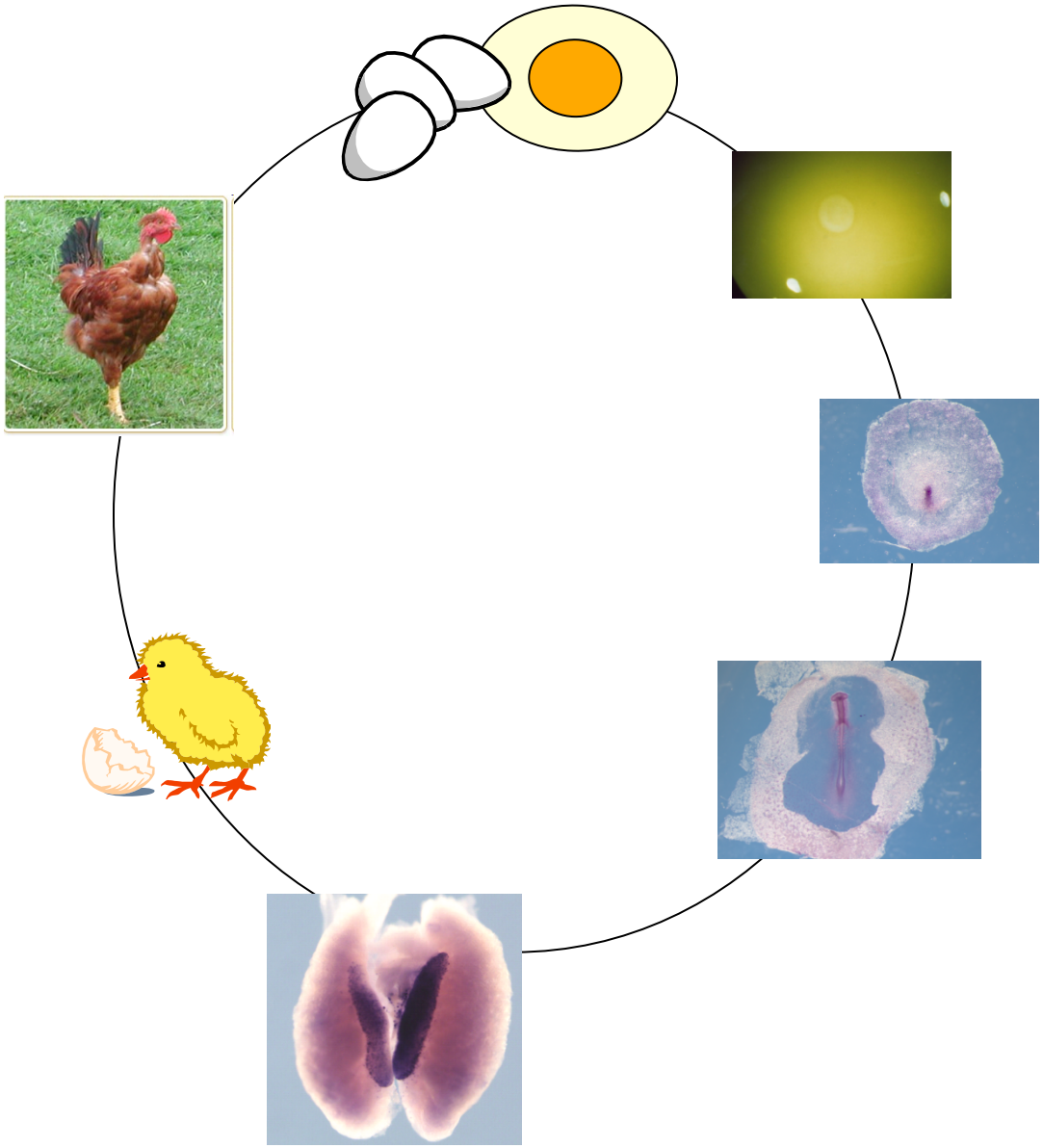
Reprogramming somatic cells into avian iPSC



Reprogramming somatic cells into avian iPSC

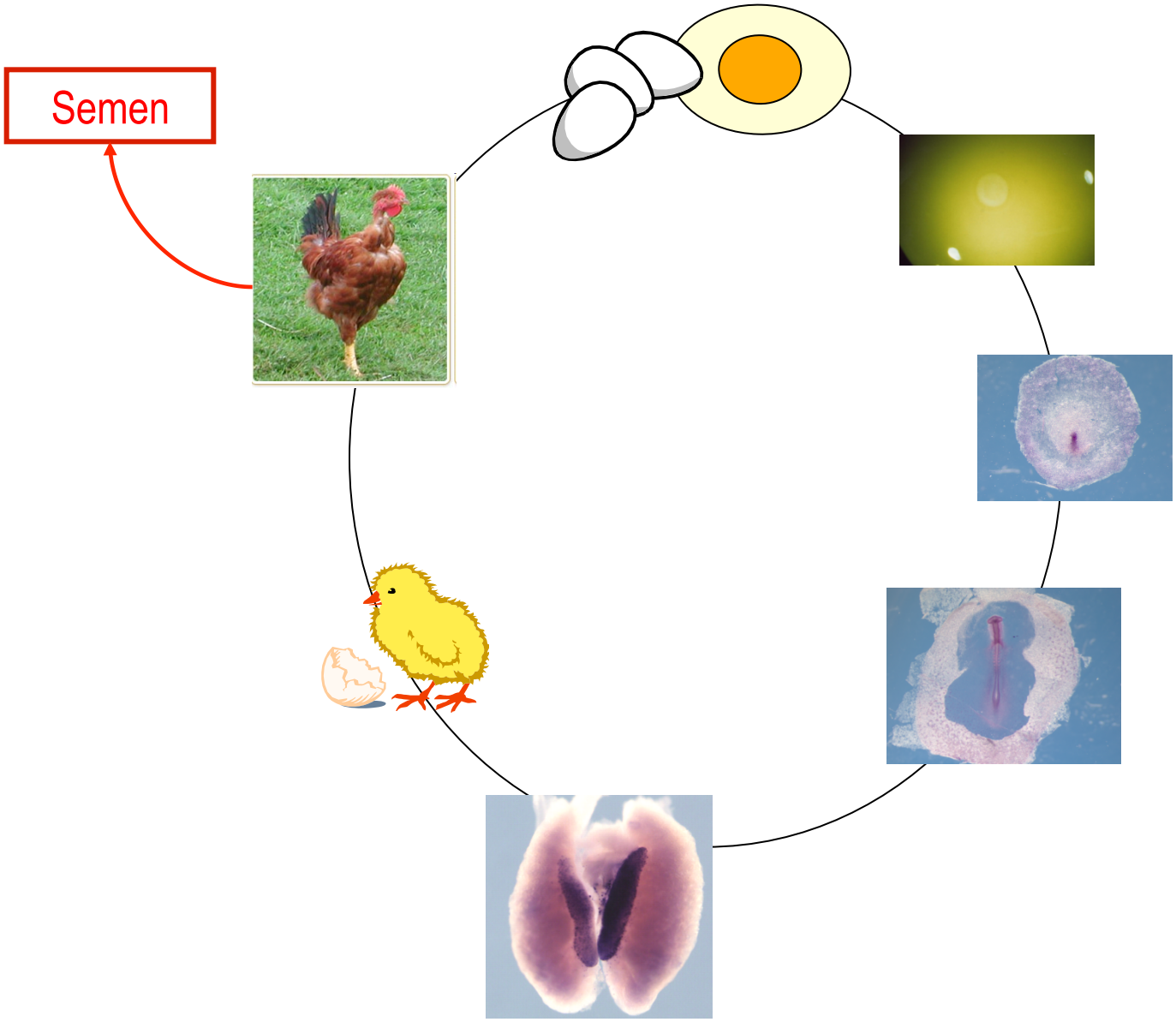


Stem cells in chicken



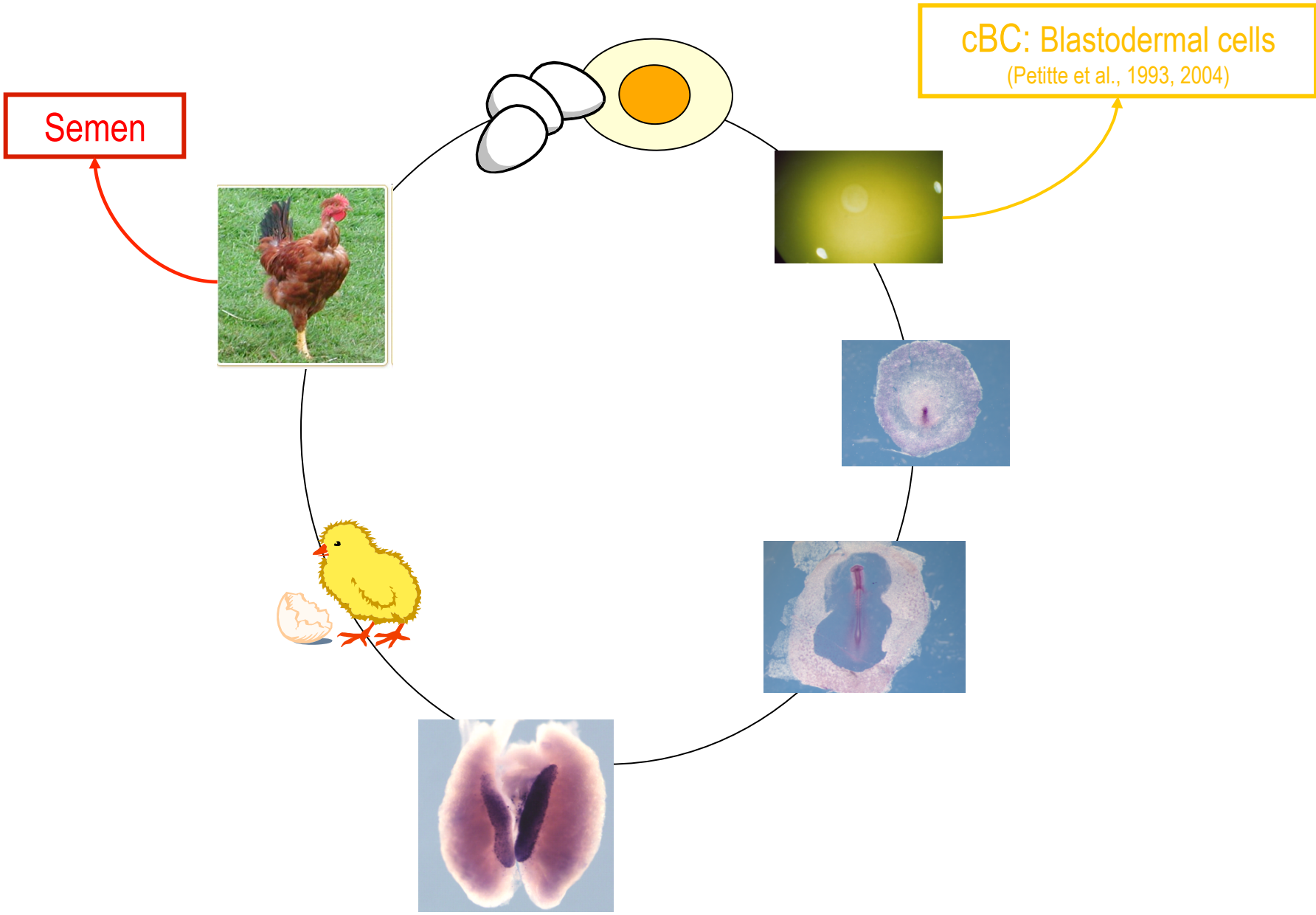
Adapted from Lavial & Pain, 2010

Stem cells in chicken



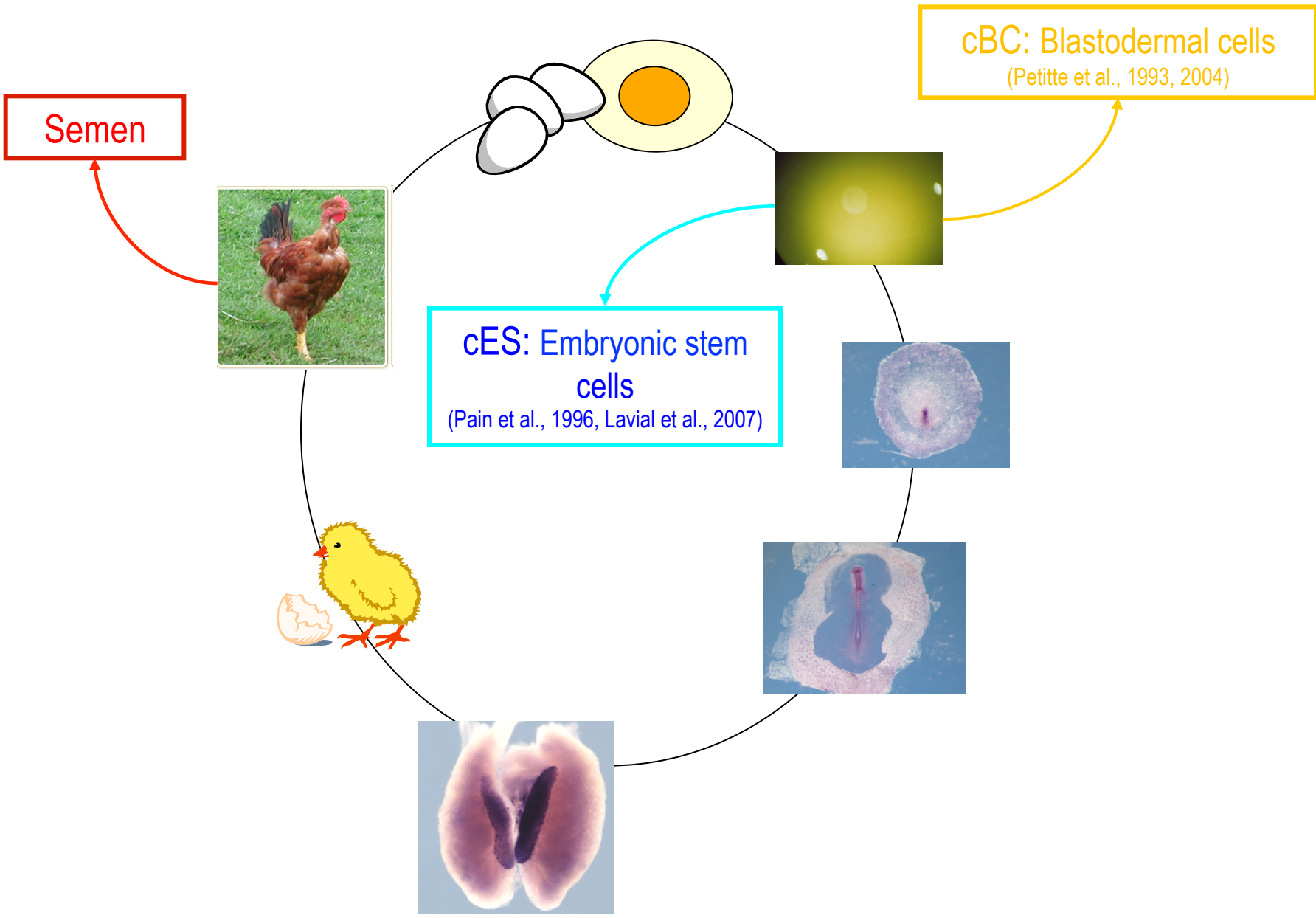
Adapted from Lavial & Pain, 2010

Stem cells in chicken



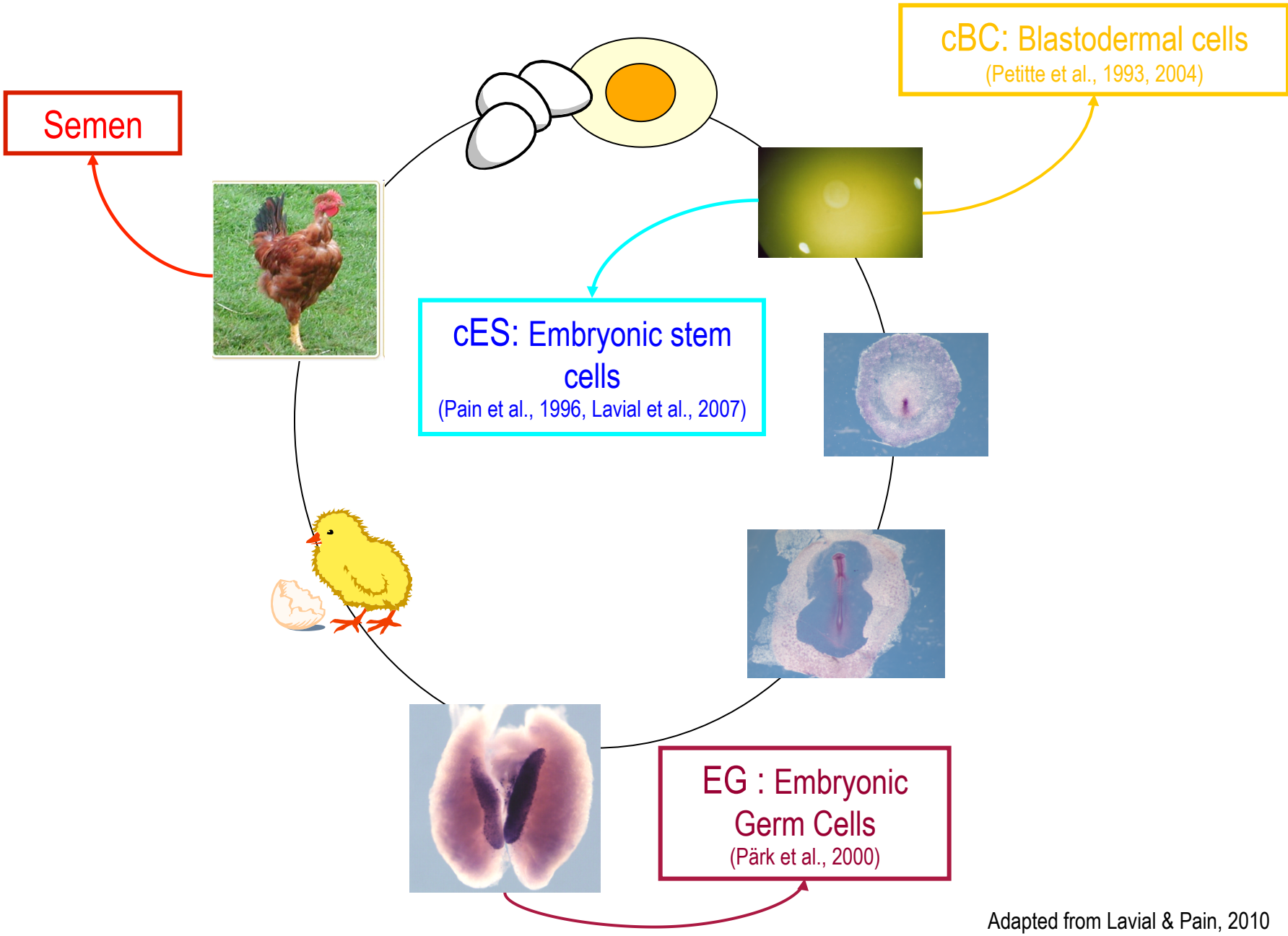
Adapted from Lavial & Pain, 2010

Stem cells in chicken



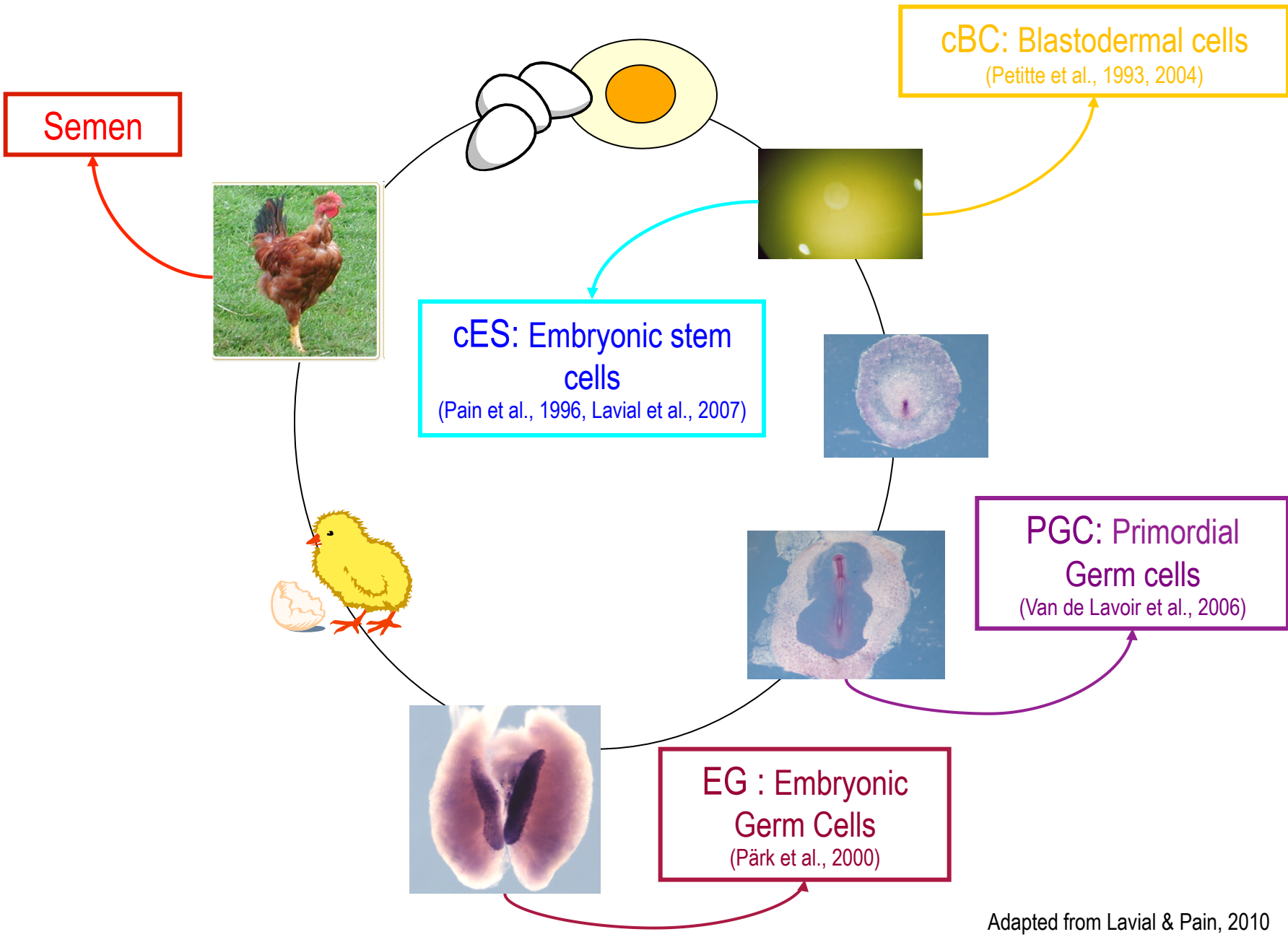
Adapted from Lavial & Pain, 2010

Stem cells in chicken



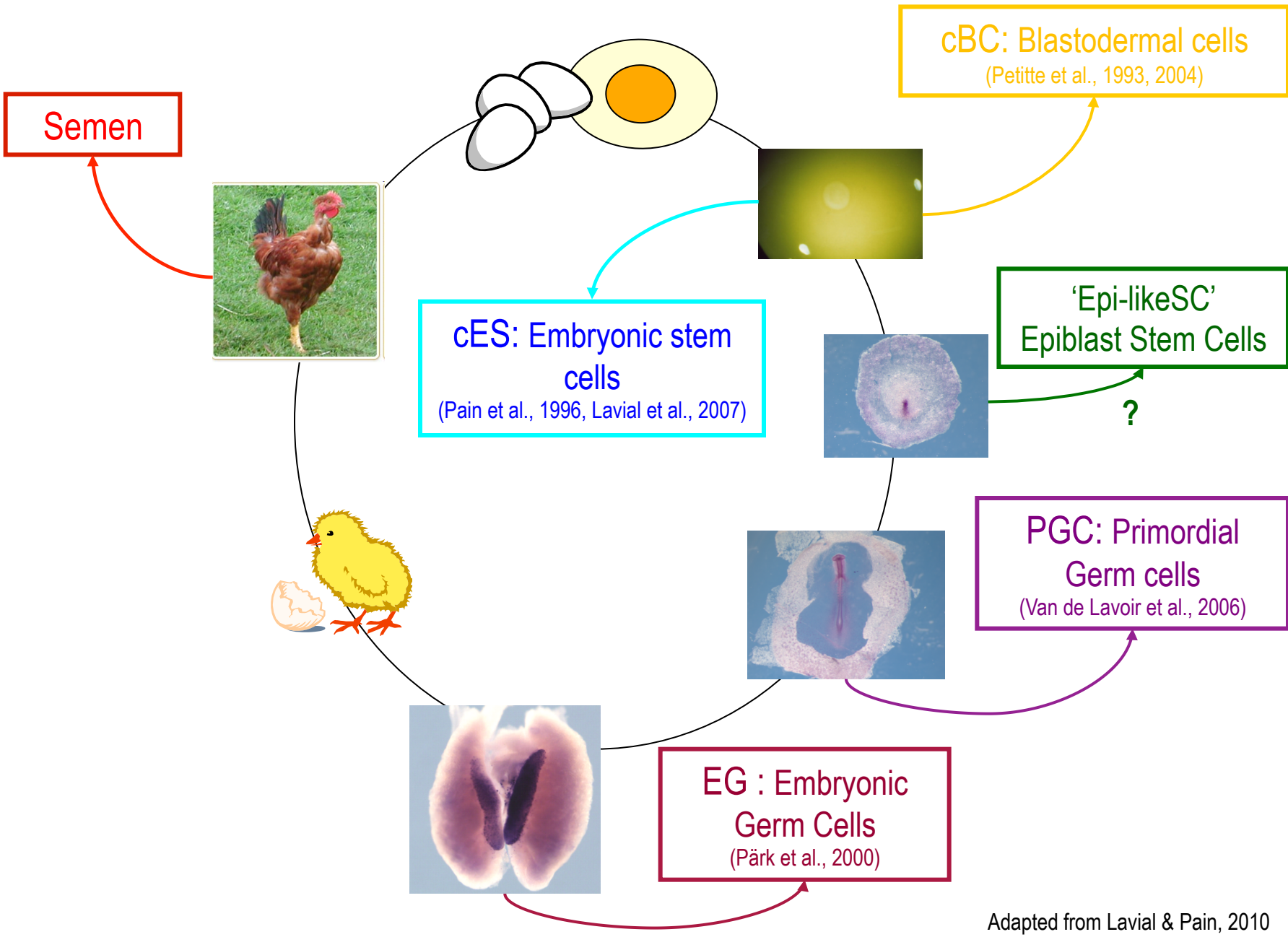
Adapted from Lavial & Pain, 2010

Stem cells in chicken



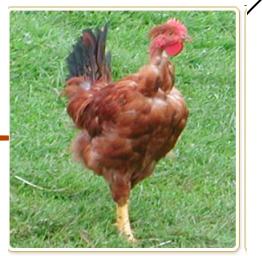
Adapted from Lavial & Pain, 2010

Stem cells in chicken



Adapted from Lavial & Pain, 2010

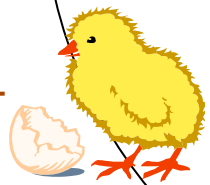
Stem cells in chicken



Semen

???

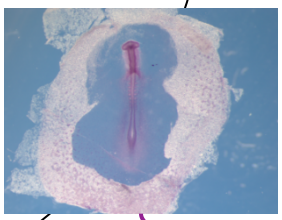
SSC: Spermatogonial Stem cells



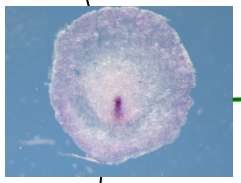
???

iPS: Induced Pluripotent Stem

EG : Embryonic Germ Cells
(Pärk et al., 2000)

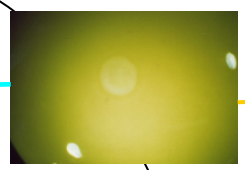


PGC: Primordial Germ cells
(Van de Lavoir et al., 2006)

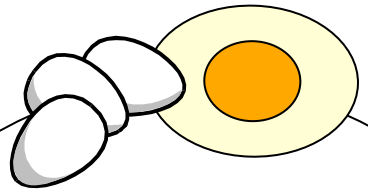


?

‘Epi-likeSC’ Epiblast Stem Cells



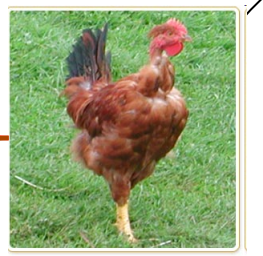
cES: Embryonic stem cells
(Pain et al., 1996, Lavial et al., 2007)



cBC: Blastodermal cells
(Petitte et al., 1993, 2004)

Adapted from Lavial & Pain, 2010

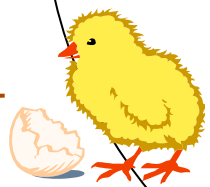
Stem cells in chicken



Semen

???

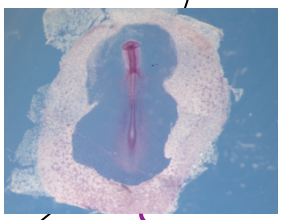
SSC: Spermatogonial Stem cells



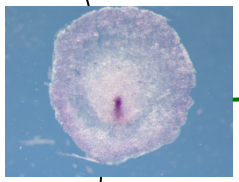
???

iPS: Induced Pluripotent Stem

EG : Embryonic Germ Cells
(Pärk et al., 2000)

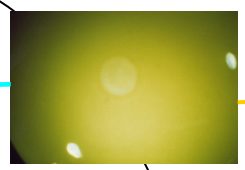


PGC: Primordial Germ cells
(Van de Lavoir et al., 2006)



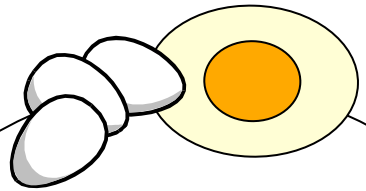
'Epi-likeSC' Epiblast Stem Cells

?



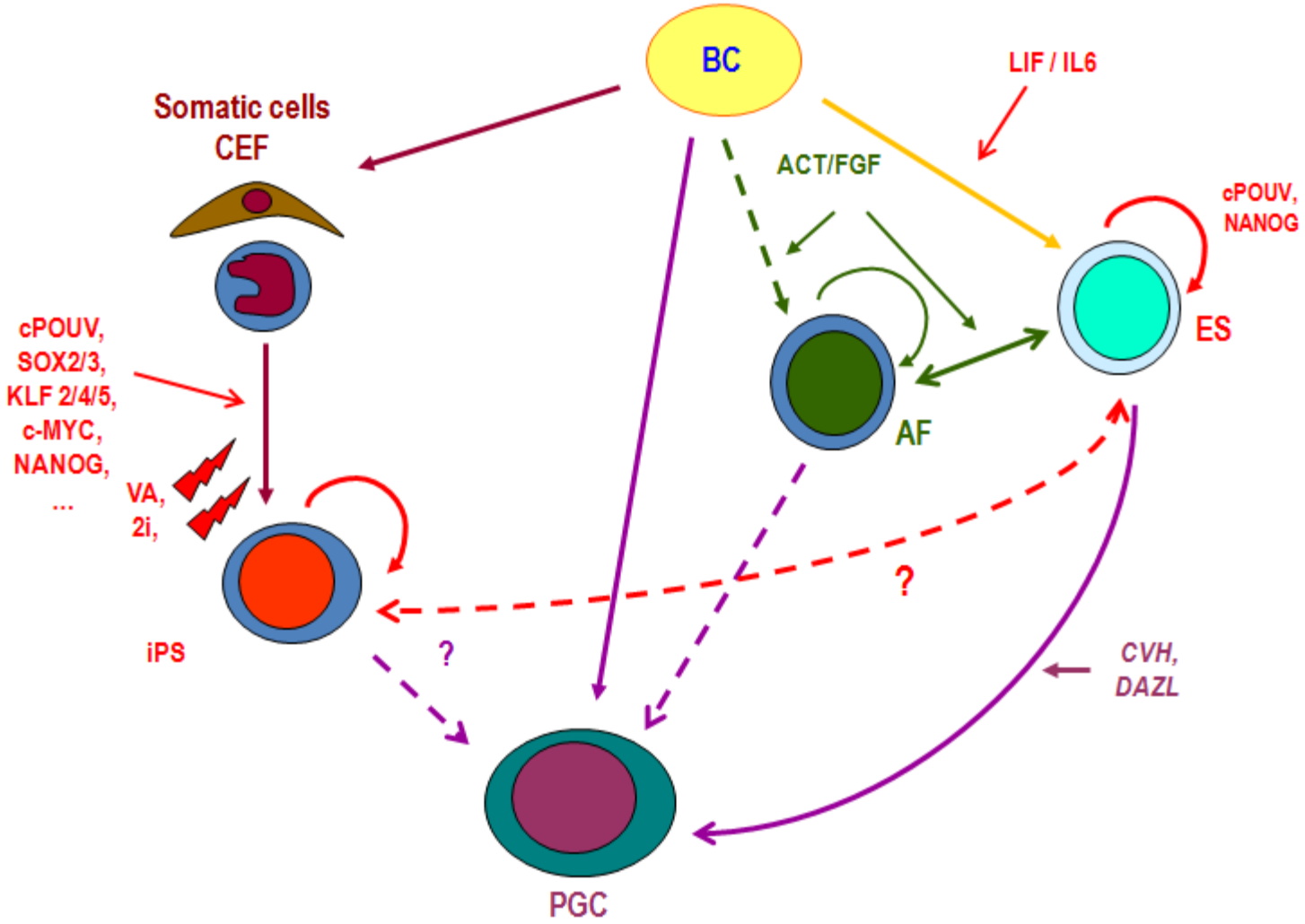
cES: Embryonic stem cells
(Pain et al., 1996, Lavial et al., 2007)

cBC: Blastodermal cells
(Petitte et al., 1993, 2004)

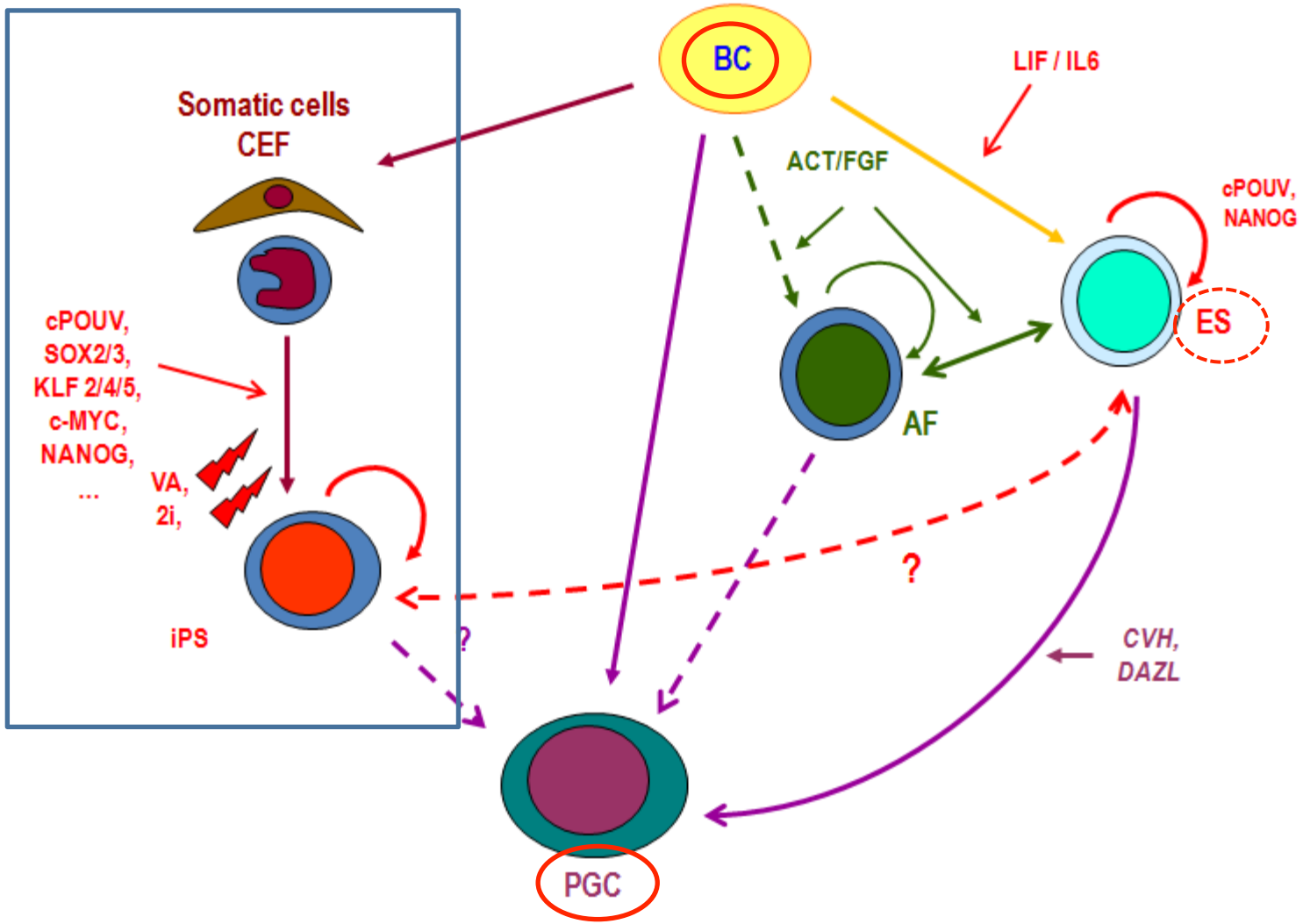


Adapted from Lavial & Pain, 2010

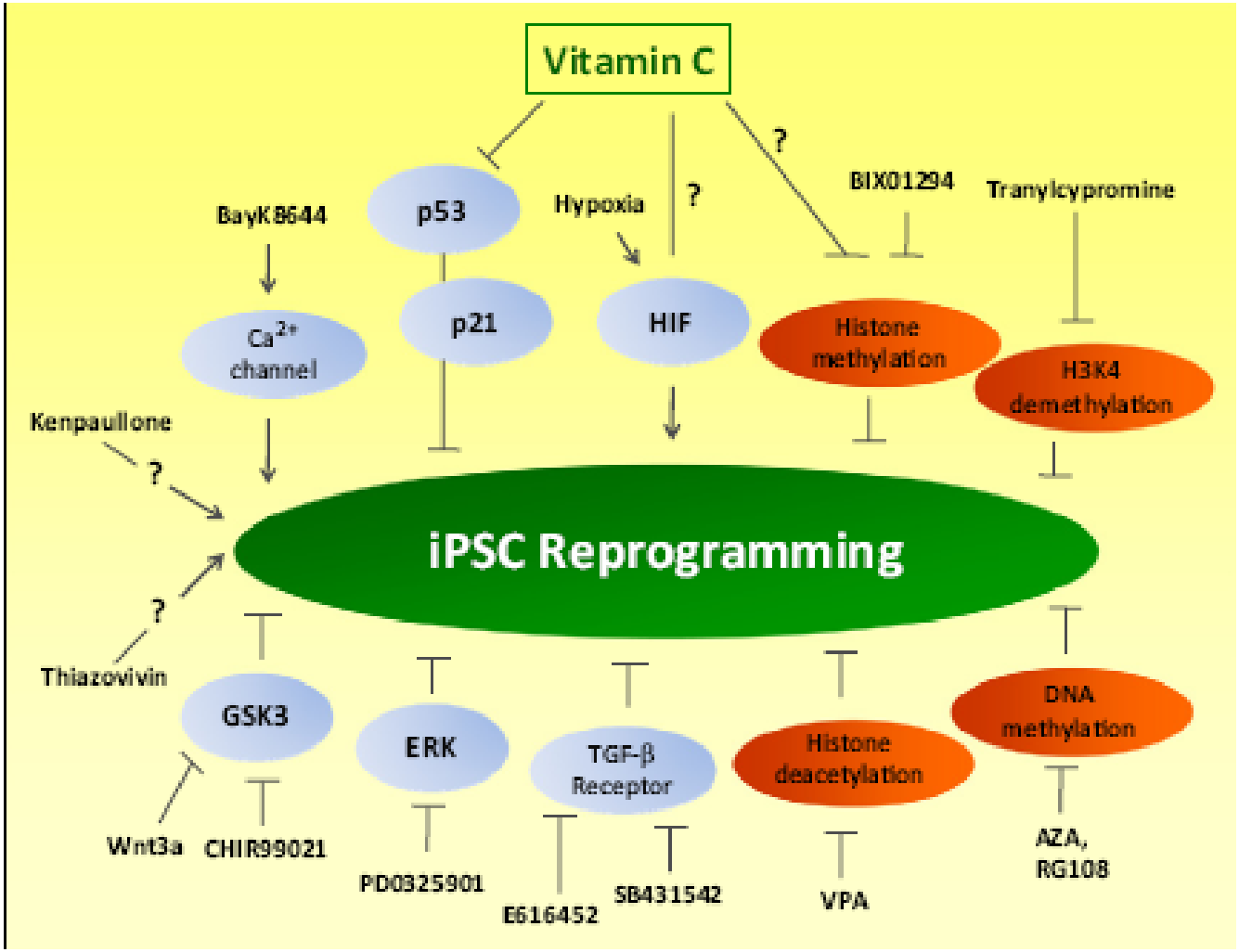
Stem cells in chicken



Stem cells in chicken

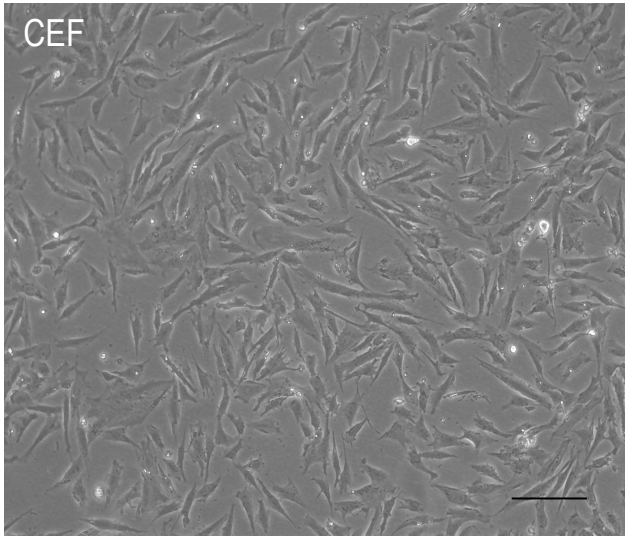


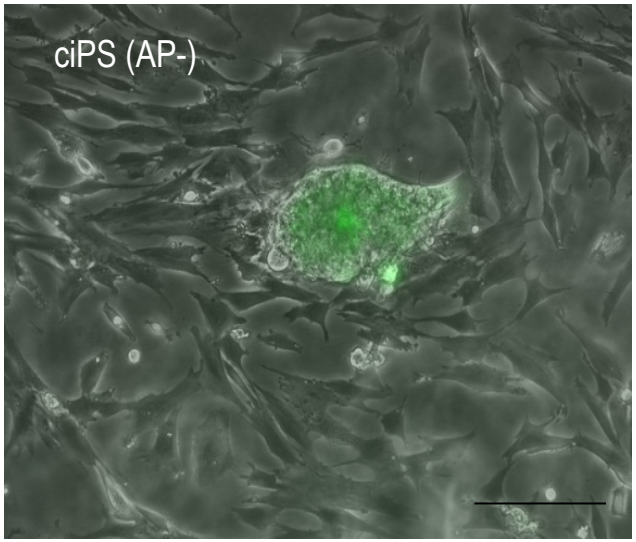
Reprogramming: a stochastic and complex process

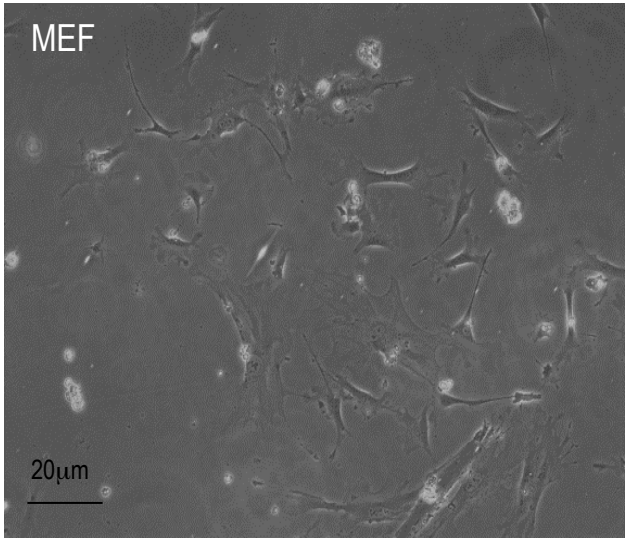


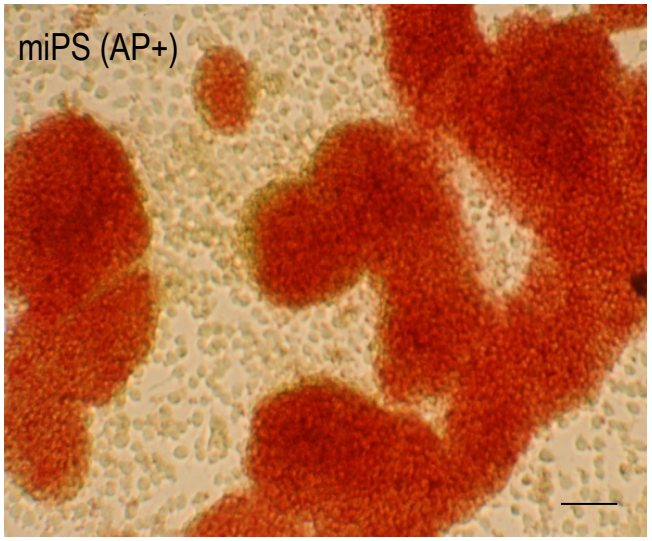
First tests

The direct infection with the polycistronic vector (pLent-KOSM) leads to small colonies unable to proliferate









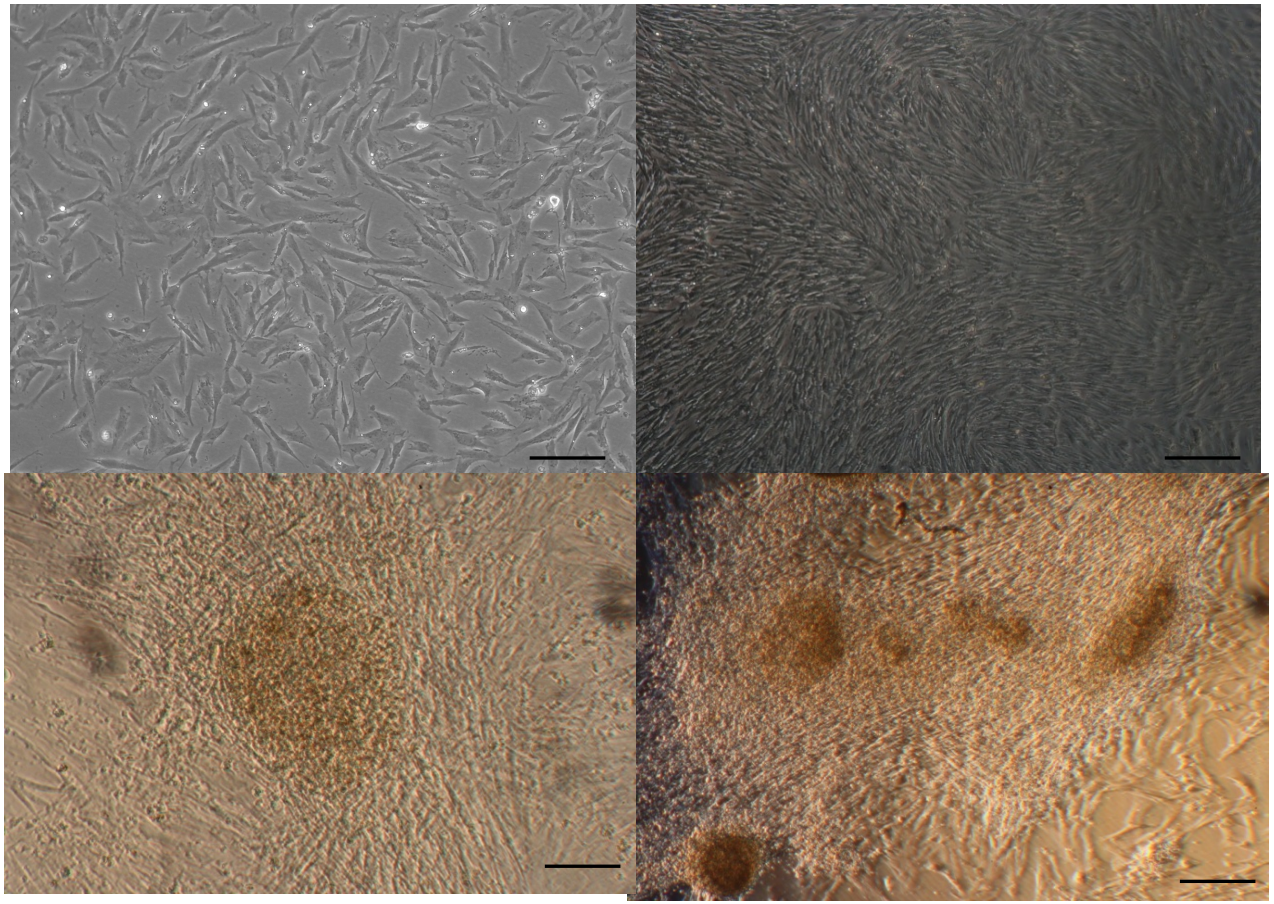
New strategy

➔ Development of a two step procedure using pPB and lentiviral vectors

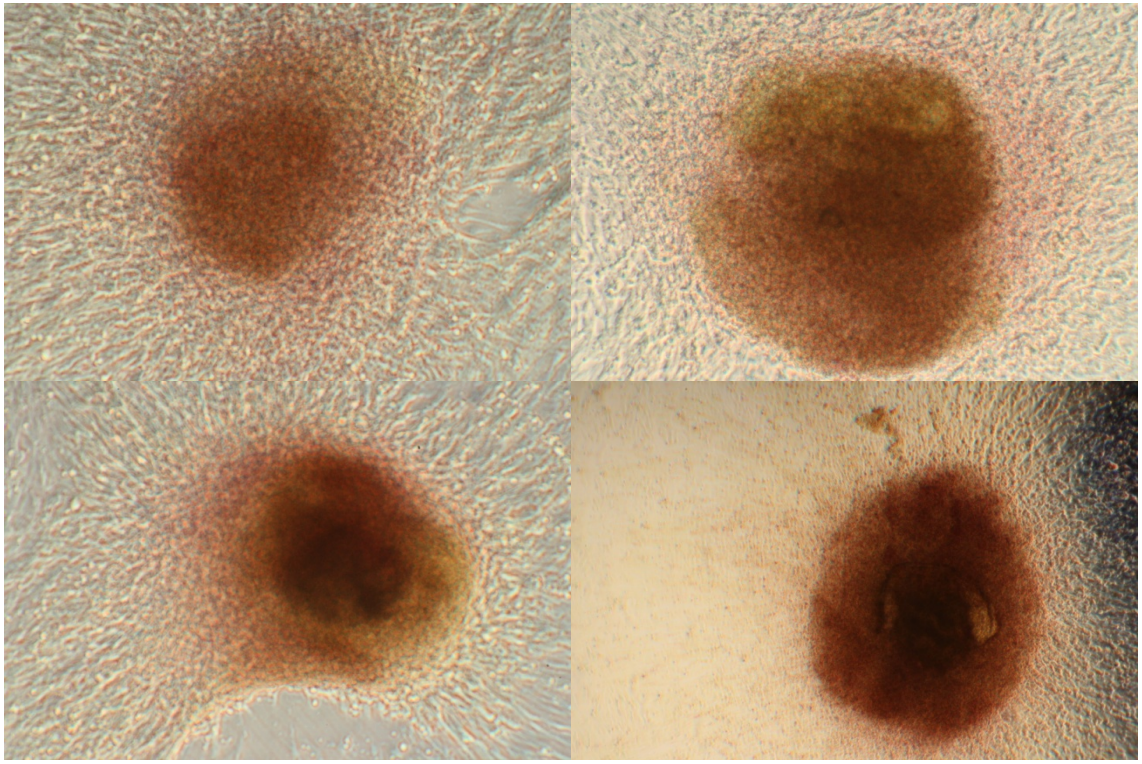
J0 CEF I platting
J4 passage
J5 transfection
J6 infection
J7 VA treatment
J9 selection
J17 passage
J22 passage
J26 passage
J30

Maintenance
AP staining
RNA

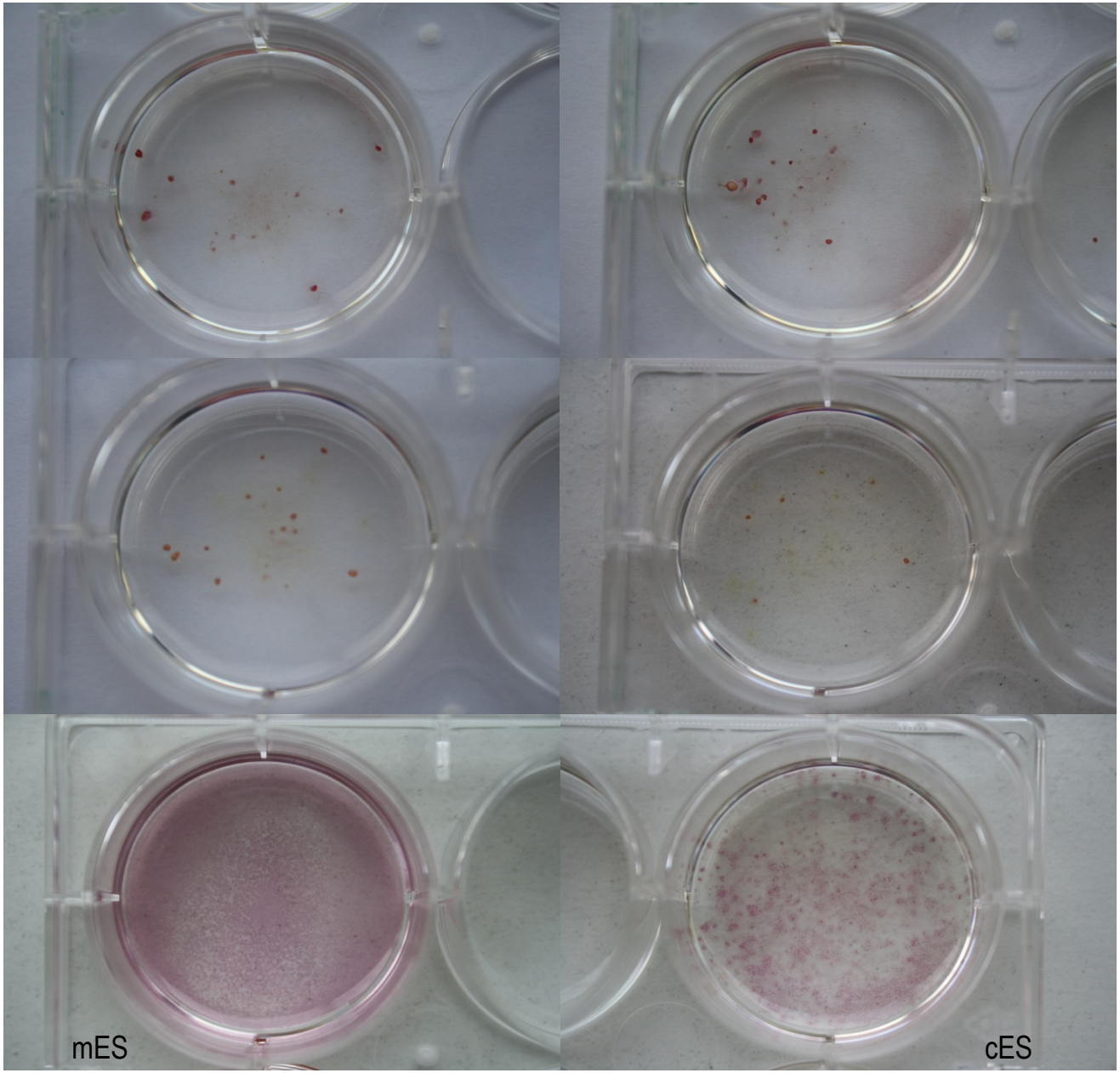
Amplification
&
characterization



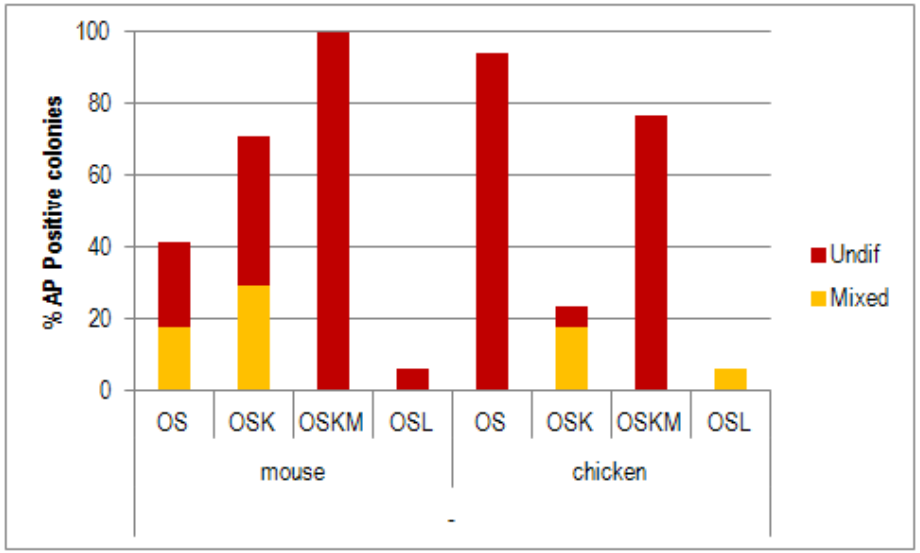
New strategy

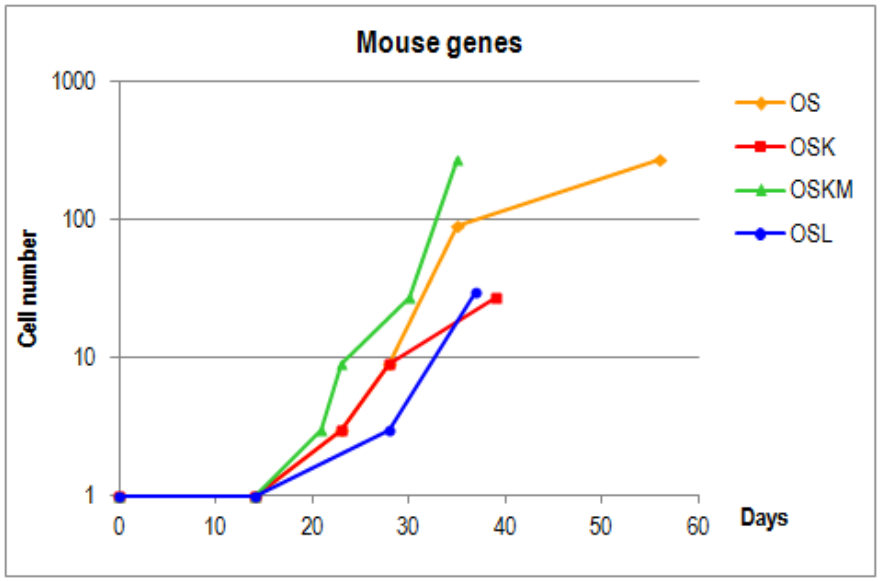


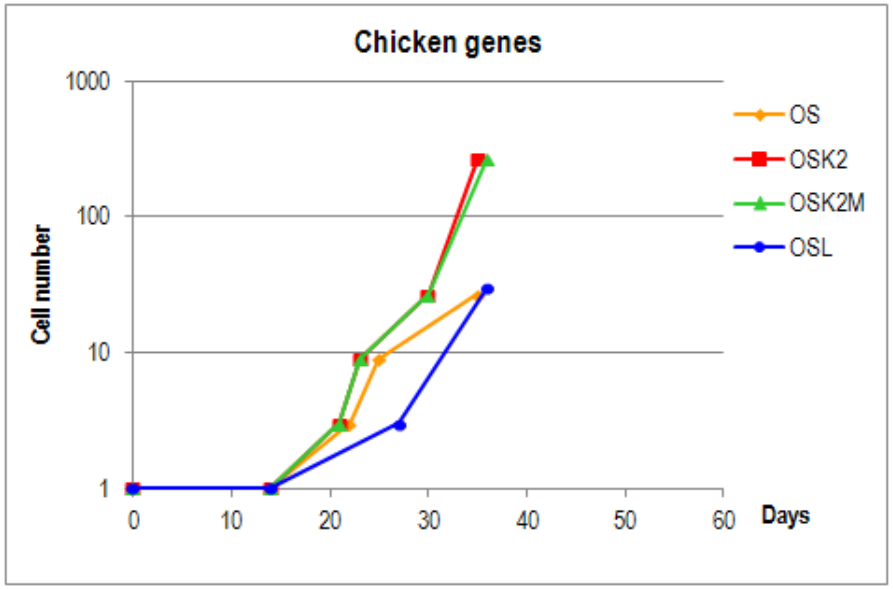
New strategy



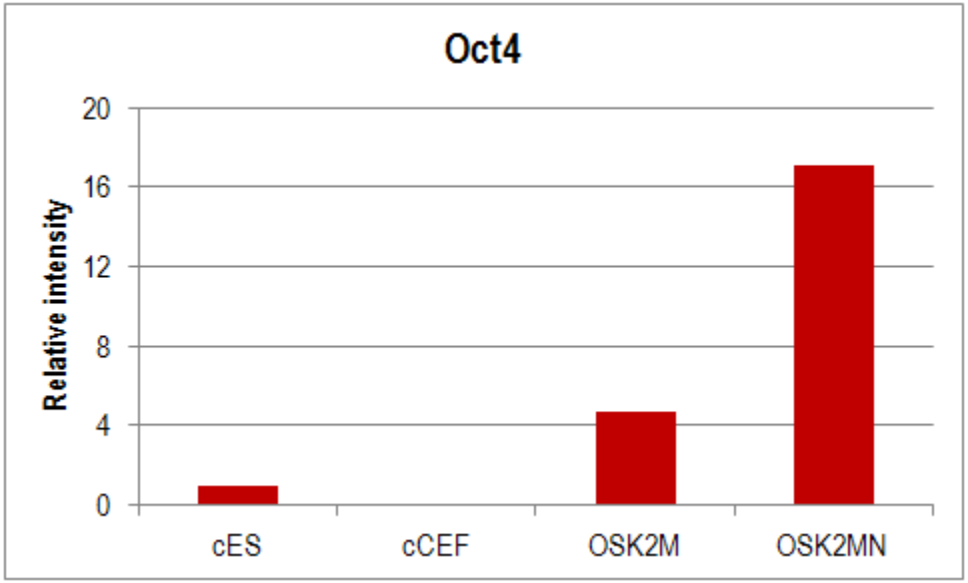
New strategy

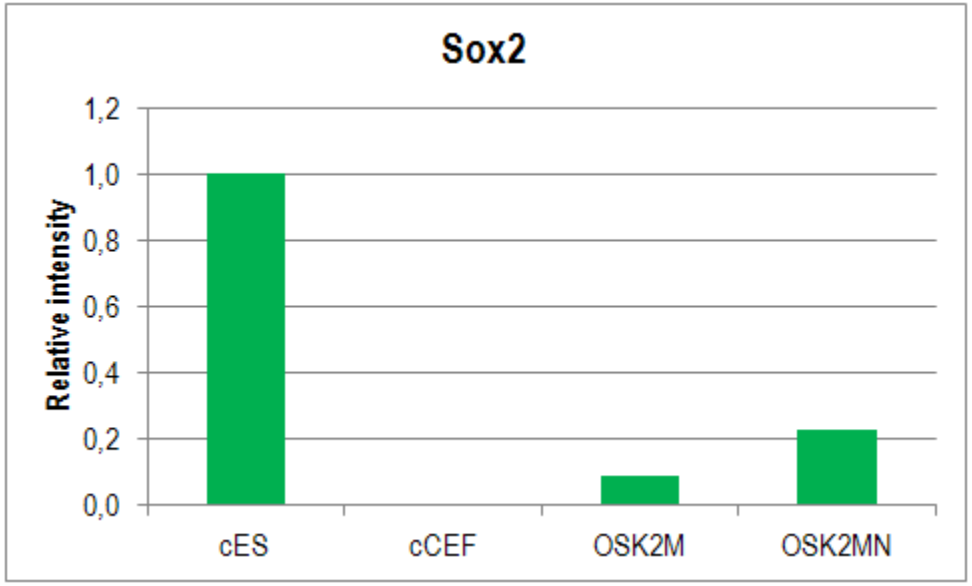






New strategy





Still to be done…

- To standardize the reprogramming protocol ➔ the optimal combination
- To fully characterize the reprogrammed cells
- To inject the reprogrammed cells into recipient embryos
- To evaluate their developmental potential
- To determine the somatic tissues/cells to be used
- To establish the optimum cryobanking conditions

Thanks to

SBRI - Stem Cell & Brain Research Institute,

U846, INSERM, USC1361, INRA, UCB Lyon 1

AgroBioStem

http:/http/www.sbri.fr

Rabbit Group

P. Savatier
T. Joly
P. Osteil
Y. Tapponnier
S. Markossian
M. Al-Khtib

Chicken Group

P. Aubel
A. Fuet
C. Jean
F. Raucci
S. Voisin
C. Kress

INRA

UNIVERSITÉ DE LYON

Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale