Reprogrammation de cellules somatiques chez les animaux d'élevage

Marielle Afanassieff, Bertrand Pain

Comité Scientifique #3

23 Novembre 2015 (9h15– 17h00) – Mercure Montparnasse Paris

Ordre du Jour

9h15-9h30 : Accueil petit déjeuner

9h30 – 11h30 : Des Go/No Go et des freins (règlementaires, sanitaires, expérimentaux) importants à lever - développements technologiques reproductifs (WP2.2) (2h)

- Introduction, résumé des Go/No Go WP2.2 (10')

Elisabeth Blesbois

- Transferts de tissus gonadiques chez les Mammifères (20' + 7')

Samuel Buff & Loris Commin

- Embryons et larves d'abeilles (20' + 7')

Florence Guignot

- Reprogrammation cellulaire et altérations épigénétiques (20' + 7')

Marielle Afanassieff & Bertrand Pain

- Cryoconservation des gamètes chez l'âne (20' + 7')

Michelle Magistrini

11h30 – 12h30 : Présentation d'EMBRC, CRB biologie marine de Roscoff (40' + 20')
Nathalie Turque & Ian Probert

12h30 – 14h00 Déjeuner

14h00 – 14h15 : Présentation de l'Observatoire des anomalies bovines (15')
Coralie Danchin

14h15 - 15h15 : Projet CRB Animal Tropical – vers une intégration au réseau CRB-Anim ? (40' + 20')
Michel Naves

15h15 – 16h15 : L'appel à idée CRB-Anim – Contenu, forme, modalités d'évaluation (30' + 30')
Michèle Tixier Boichard & Aurélie Delavaud

16h15 - 16h45 : Bilan des développements technologiques en génomique (WP2.1) (30')
Marco Moroldo & Laetitia Lagoutte

16h45 – 17h00 : Reprise Finale *(Présidence du CS)*

Hôtel Mercure Montparnasse
20 Rue de la Gaité, 75014 Paris

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2	Redon	Richard	na	INSERM	Schott	Jean-Jacques	na	INSERM
2	Riquet	Juliette	na	INRA	Pitel	Frédérique	na	INRA
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	Fieni	Francis	na	ONIRIS	Martignat	Lionel	na	ONIRIS
	Reynaud	Karine	p	ENVA	Tiret	Laurent	na	ENVA

Totaux	22	11	Présent	18	4 Présent
Total Gén	40	5	Not applicable		10 Not applicable
		5	Excusé		4 Excusé

1	Tixier-Boichard	Michèle	p	wp1 wp5	INRA GABI
2	Blesbois	Elisabeth	p	wp2	INRA PRC
3	Labbé	Catherine	p	wp2	INRA LPGP
4	André	Catherine	p	wp3	CNRS
6	Amigues	Yves	na		Labogena
7	Thomas	Anne	p	wp7	Antagene
8	Delavaud	Aurélie	na	wp4	FRB
	d'Arbaumont	Maelle	p	wp4	INRA GABI
	Danchin	Coralie	p	wp3 wp7	Idele
	Duittoz	Anne	p	wp6	Univ Tours
	Audiot	Annick	p	pres CPE	
	Probert	Ian	p		EMBRC
	Turque	Nathalie	exc		EMBRC
	Commin	Loris	na	wp2.2	VetAgroSup
	Guignot	Florence	p	wp2.2	INRA
	Danchin	Coralie	p	wp3	IDELE
	Moroldo	Marco	p	wp2.1	INRA
	Lagoutte	Laetitia	p	wp2.1	CNRS
	Naves	Michel	p		CRB Tropical
	Marthey	Sylvain	na	wp4	INRA GABI
	de Renty	Pierre	p		INRA Transfert

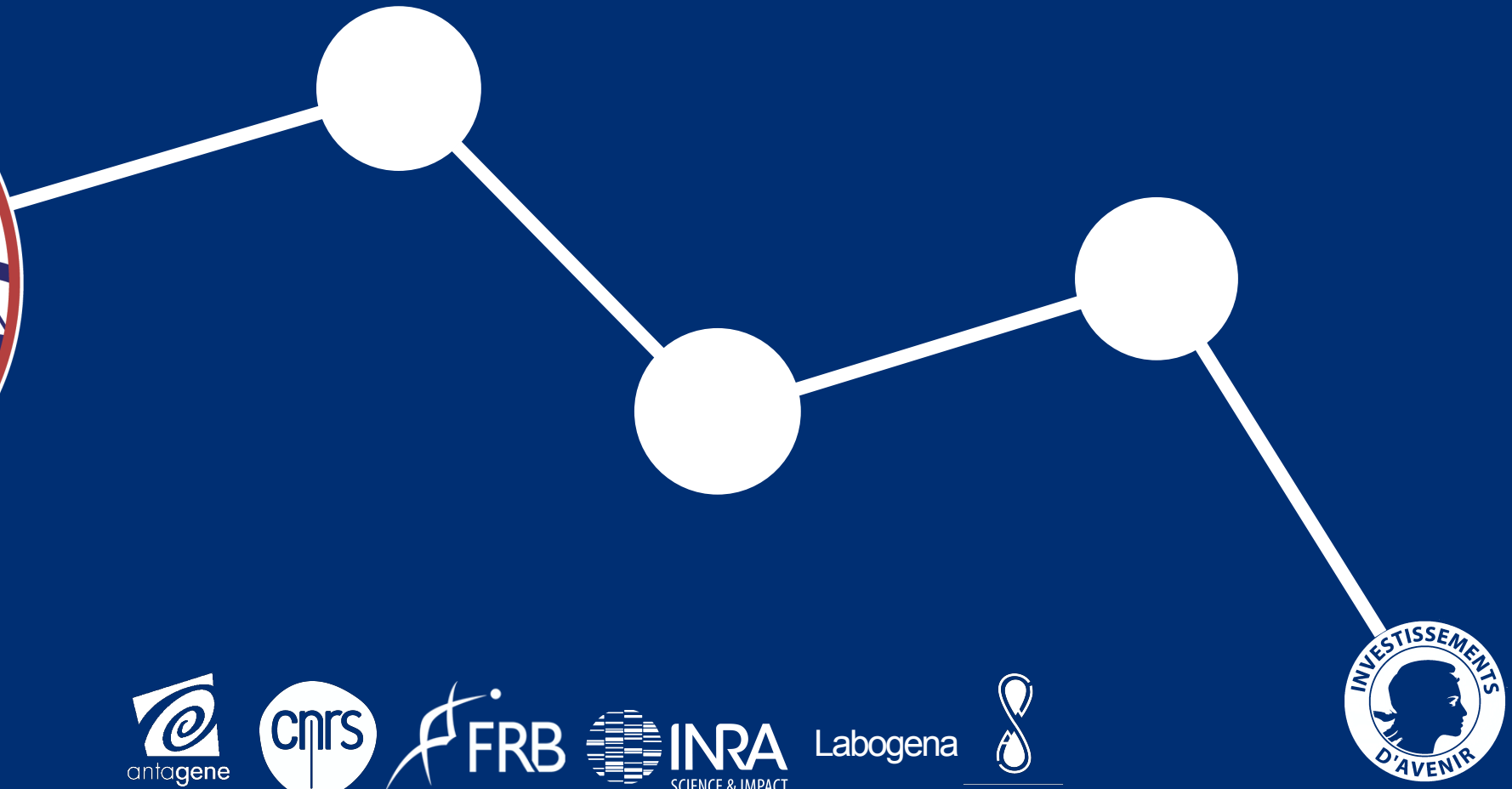
				totaux
	Leader groupe espèce	16	Présent	31
pdt	Présidence CS	4	Not Applicable	19
1	Membre ext International	1	Excusé	10
2	Membre ext Province			

B. Pain - M. Afanassieff - CS #3 23 Novembre 2015

CRB Anim

Centres de Ressources Biologiques

CS# 3 – WP2.2 Go- No-Go



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Labogena

VetAgro Sup

Somatic cell reprogramming in livestock

M. Afanassieff & B. Pain

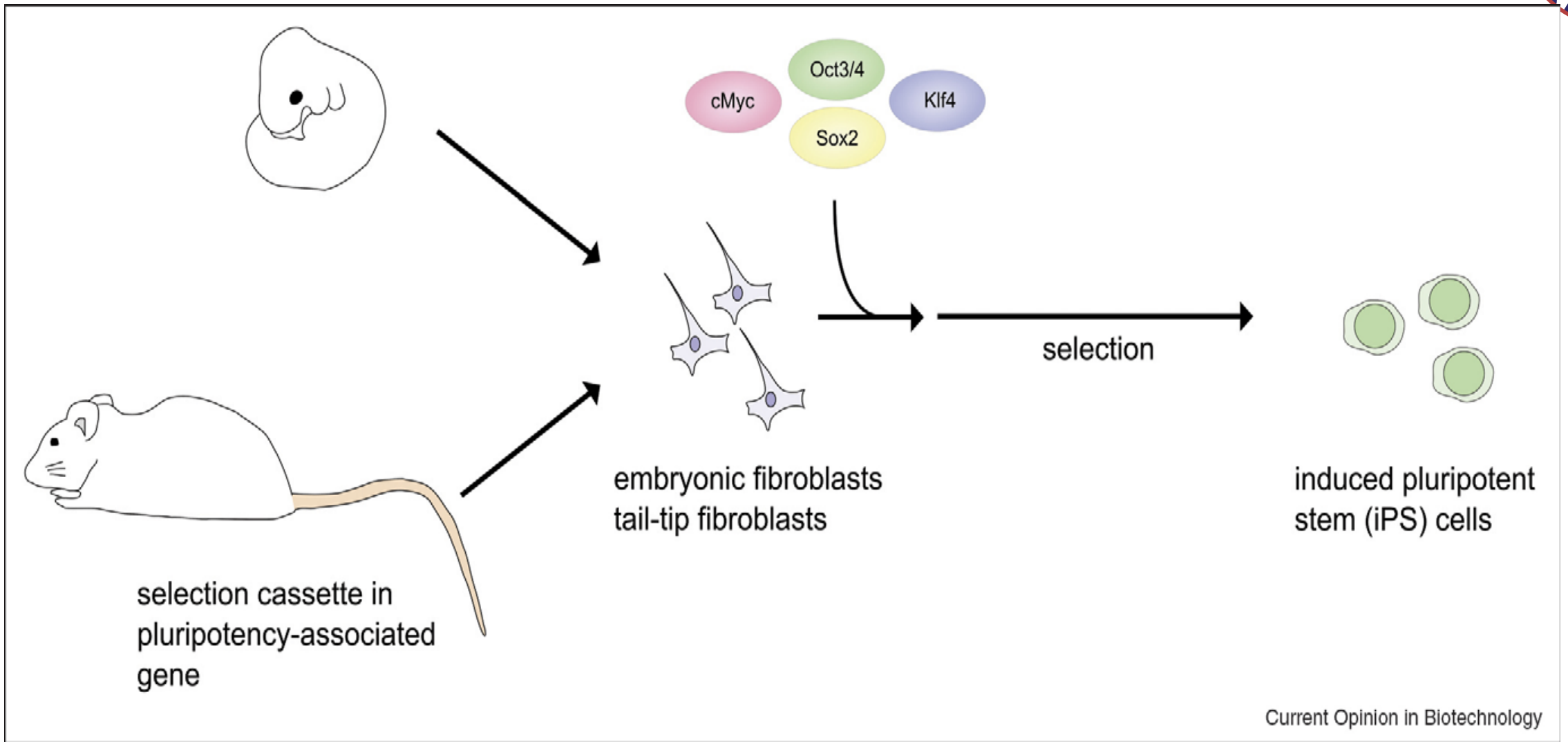
U846, INSERM, USC1361, INRA

Institut Cellule Souche et Cerveau

INRA
SCIENCE & IMPACT

CRB ANIM

Somatic cell reprogramming



Somatic cell reprogramming: Objectives

Using different livestock species – mainly rabbit and avian species-, the project aims to generate and study induced pluripotent stem (iPS) cells for

- Demonstrating that the somatic reprogramming is a usefull, successfull and efficient process in livestock species

- Establishing the most efficient protocols for cells and tissues cryobanking

- Identifying the most promising somatic cells and tissues for iPS production

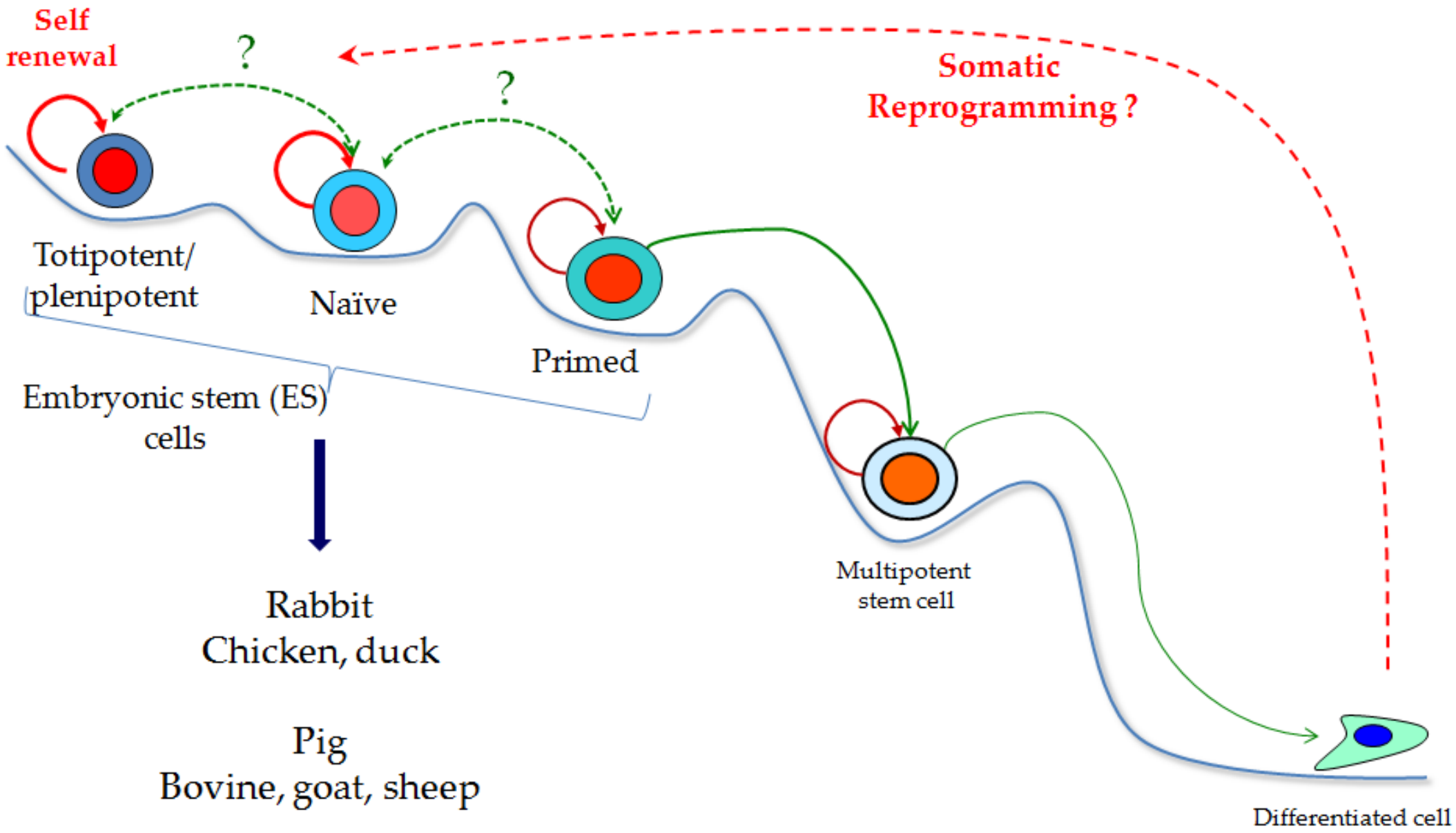
- Generating iPS cells wih robust protocols

- Characterizing molecularly the obtained iPS cells

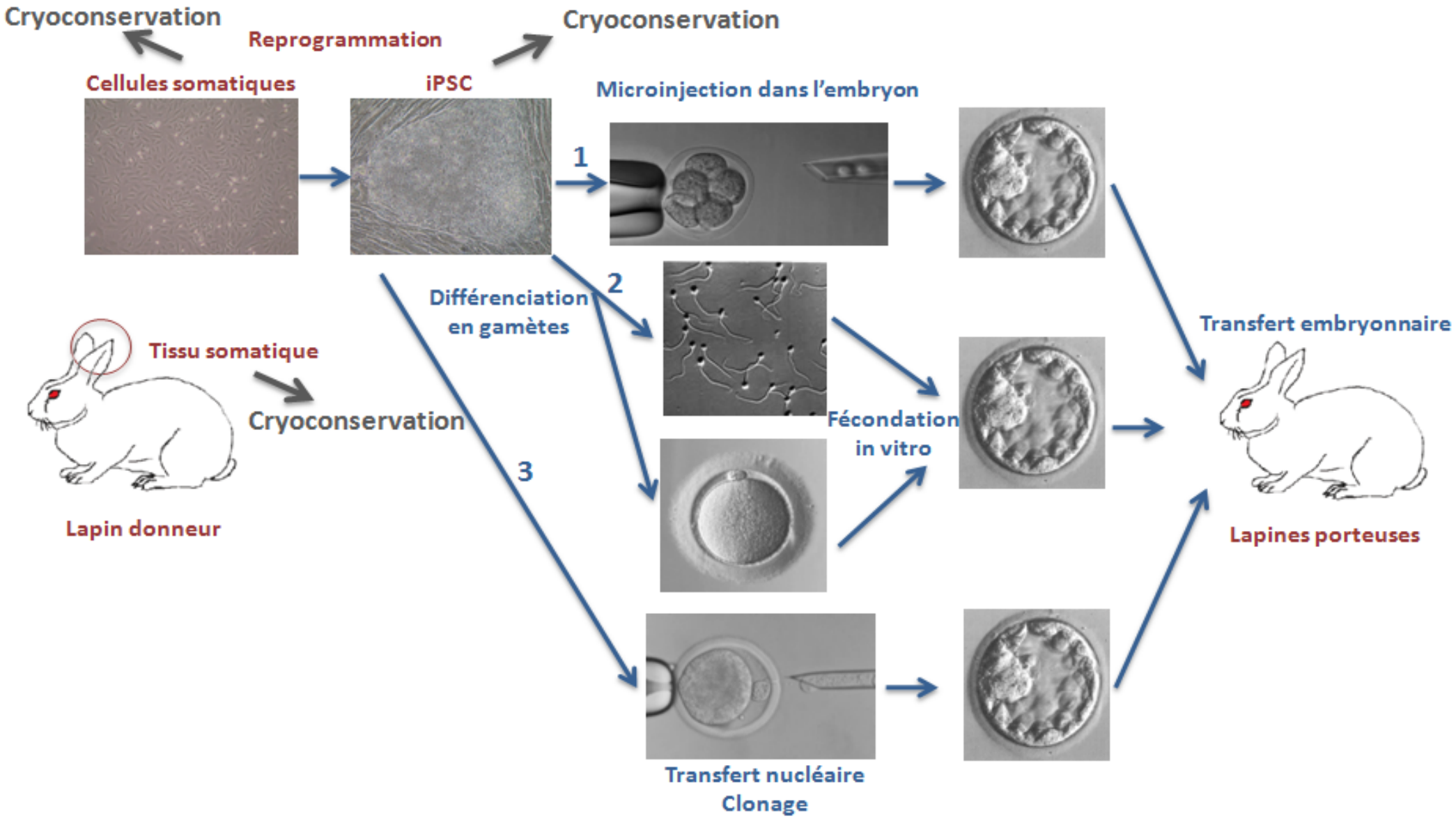
- Evaluating the developmental potential of the produced iPS cells

- ➔ *Establishing germ line competent reprogrammed cells*

Somatic cell reprogramming



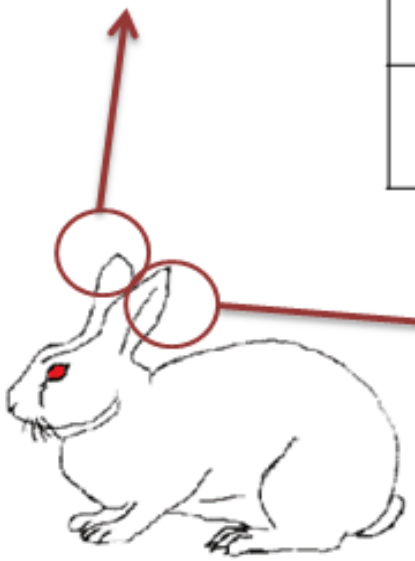
Somatic cell reprogramming: the rabbit model

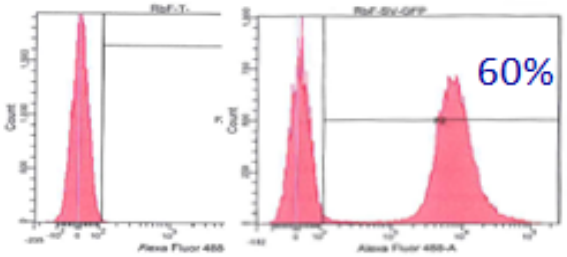


Somatic cell reprogramming: the rabbit model – the goal

Biopsie d'oreille
fibroblastes de peau

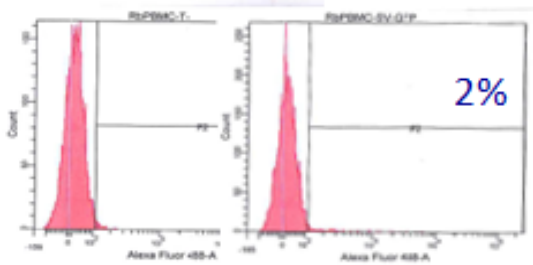
Pour	**Facilité de prélèvement** **Possibilité de congélation sans dérivation** **Reprogrammation fonctionnelle (vecteurs intégratifs)** **Forte sensibilité d'infection aux virus Sendaï (60%)**
Contre	**Instabilité génétique des cellules (?)** **Forte capacité de prolifération des fibroblastes**



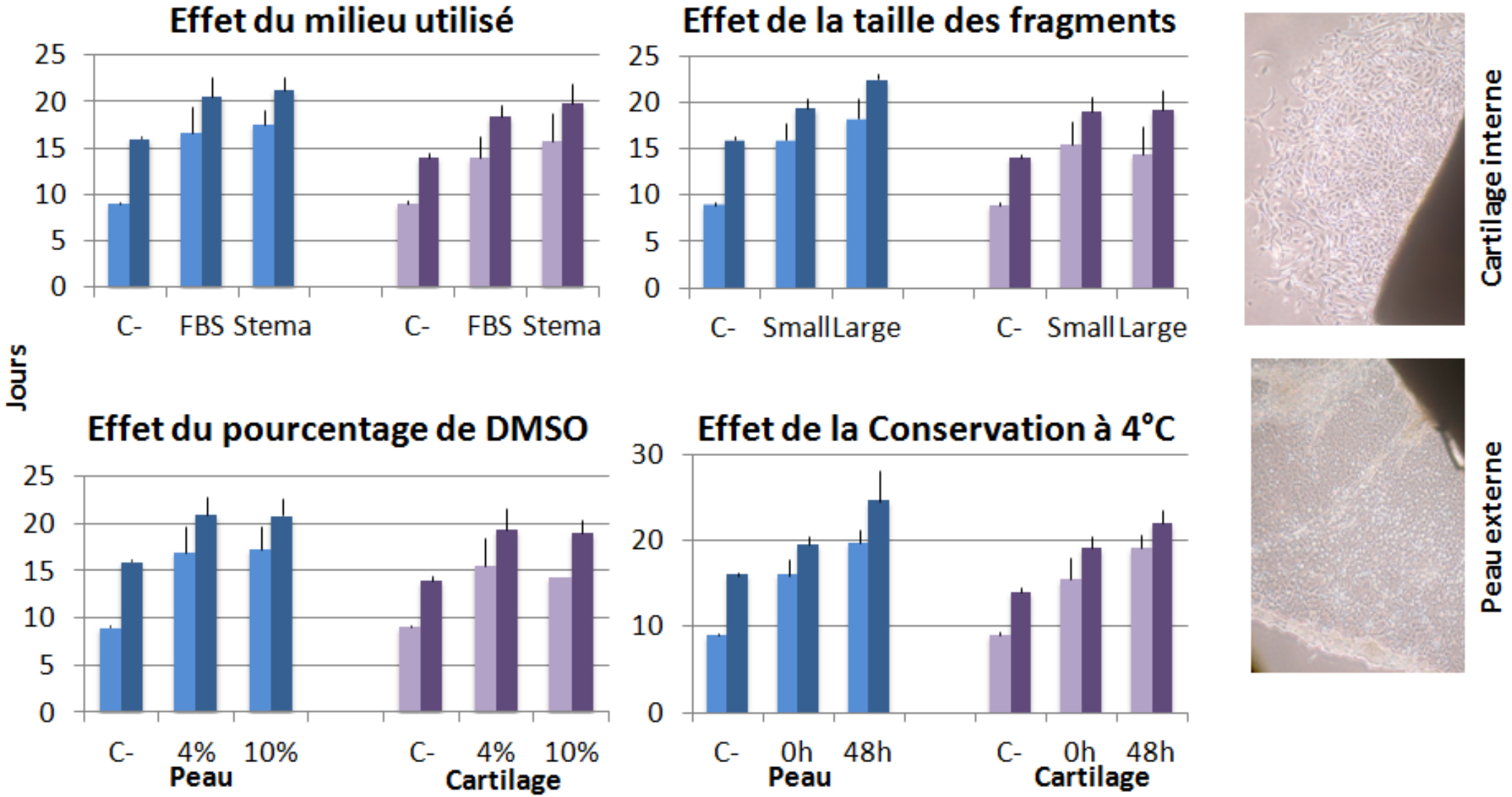


Sang circulant
PBMC

Pour	**Facilité de prélèvement** **Faible prolifération des PBMCs en culture** **Meilleur stabilité génétique des cellules** **Protocole avec les virus Sendaï courant chez l'homme**
Contre	**Impossibilité de congélation sans dérivation** **Faible sensibilité d'infection aux virus sendaï (2%)**



Somatic cell reprogramming: the rabbit model – the cryobanking conditions



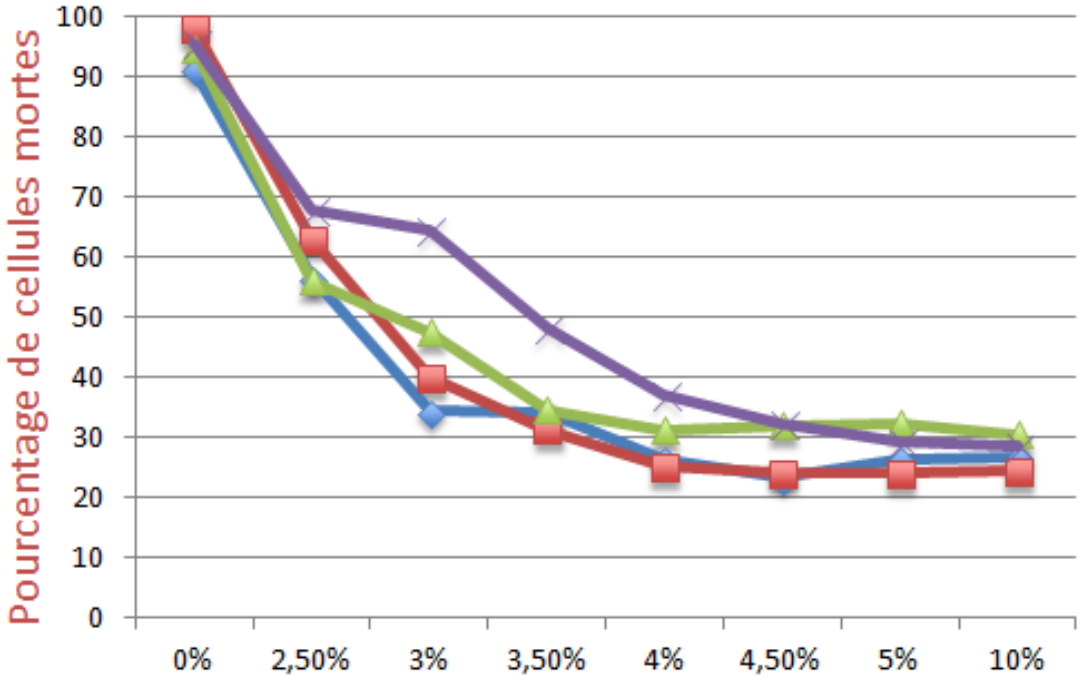
Cartilage interne

Peau externe

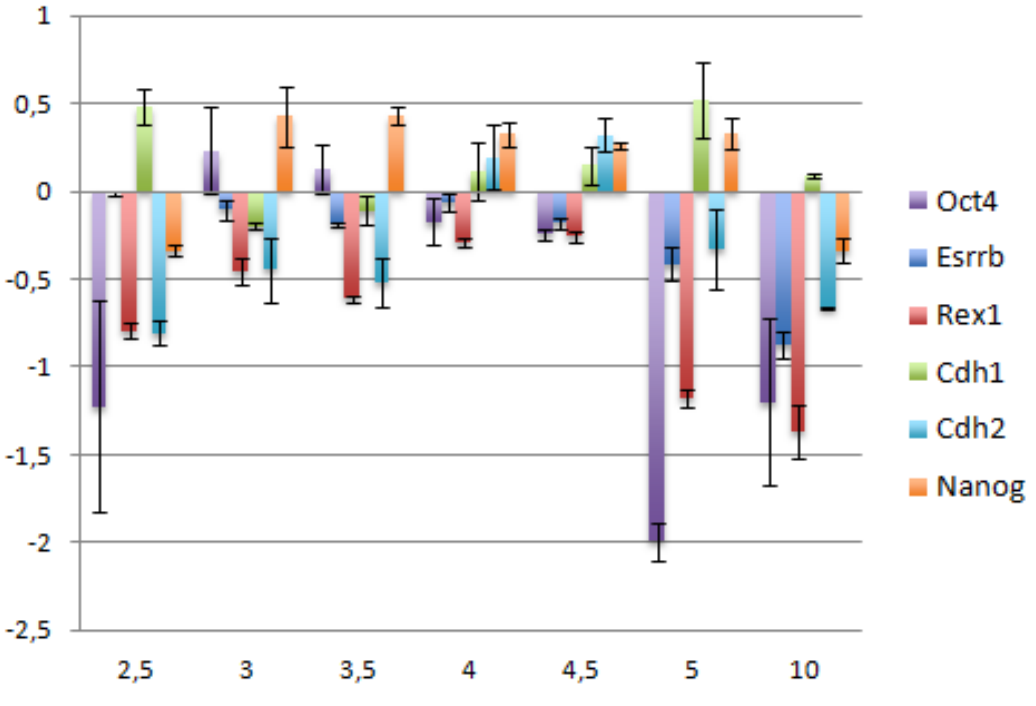
Claire: Premier Passage / Foncé: Second Passage

Somatic cell reprogramming: the rabbit model – the cryobanking conditions

- ✓ **Pourquoi? Cellules fragiles et instables cultivées en KOSR**
- ✓ **Comment? Test d'un milieu synthétique et diminution de la quantité de DMSO**
- ✓ **Etude de la viabilité, de la croissance et de la qualité des RbiPSC**



Courbe de viabilité après décongélation



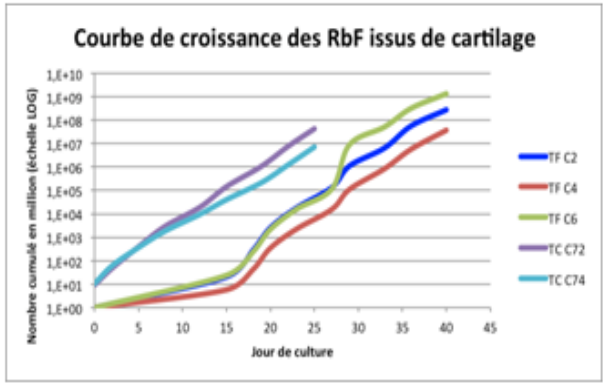
Taux d'expression par rapport à l'ICM

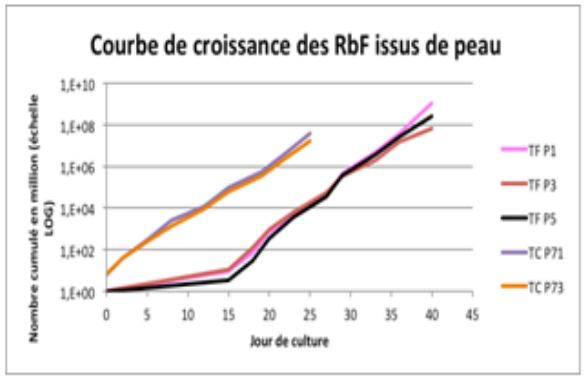
Somatic cell reprogramming: the rabbit model – the reprogramming

Analyses en cours.

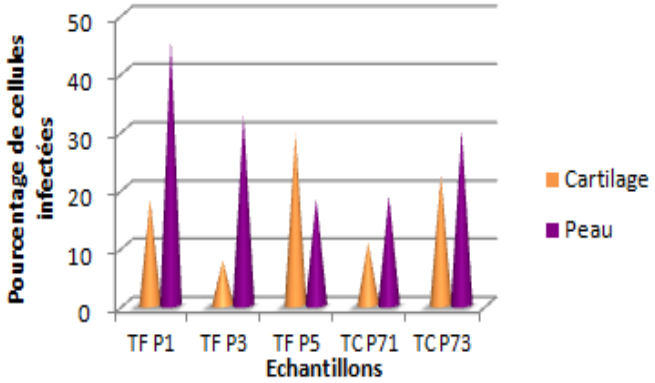
Mise au point des tests sur des fibroblastes issus de tissus non congelés:

✓ Etude de la qualité des fibroblastes après décongélation





✓ Etude du taux d'infection des fibroblastes avec le virus Sendaï



A faire: Choix d'une technique non intégrative

➔ pas de modifications génétiques

✓ **Virus Sendaï** ←

✓ Vecteurs adénoviraux

✓ Episomes

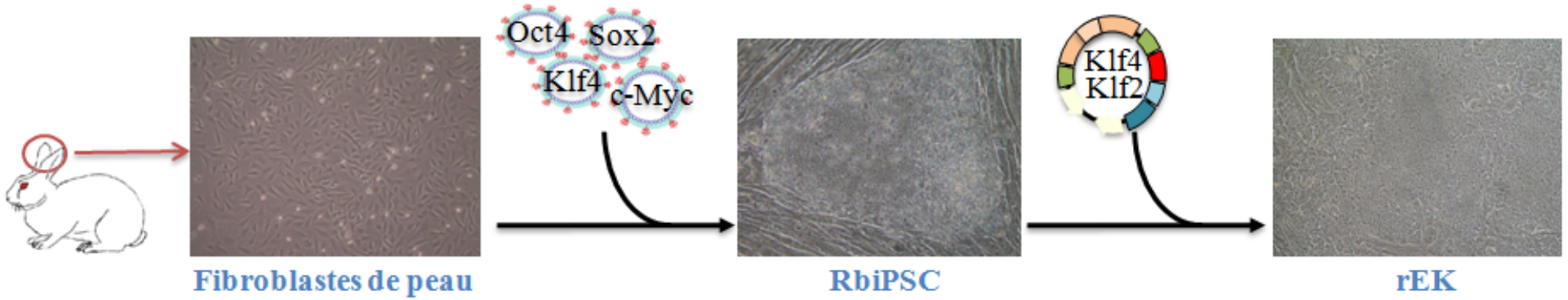
✓ Plasmides polycistroniques loxés

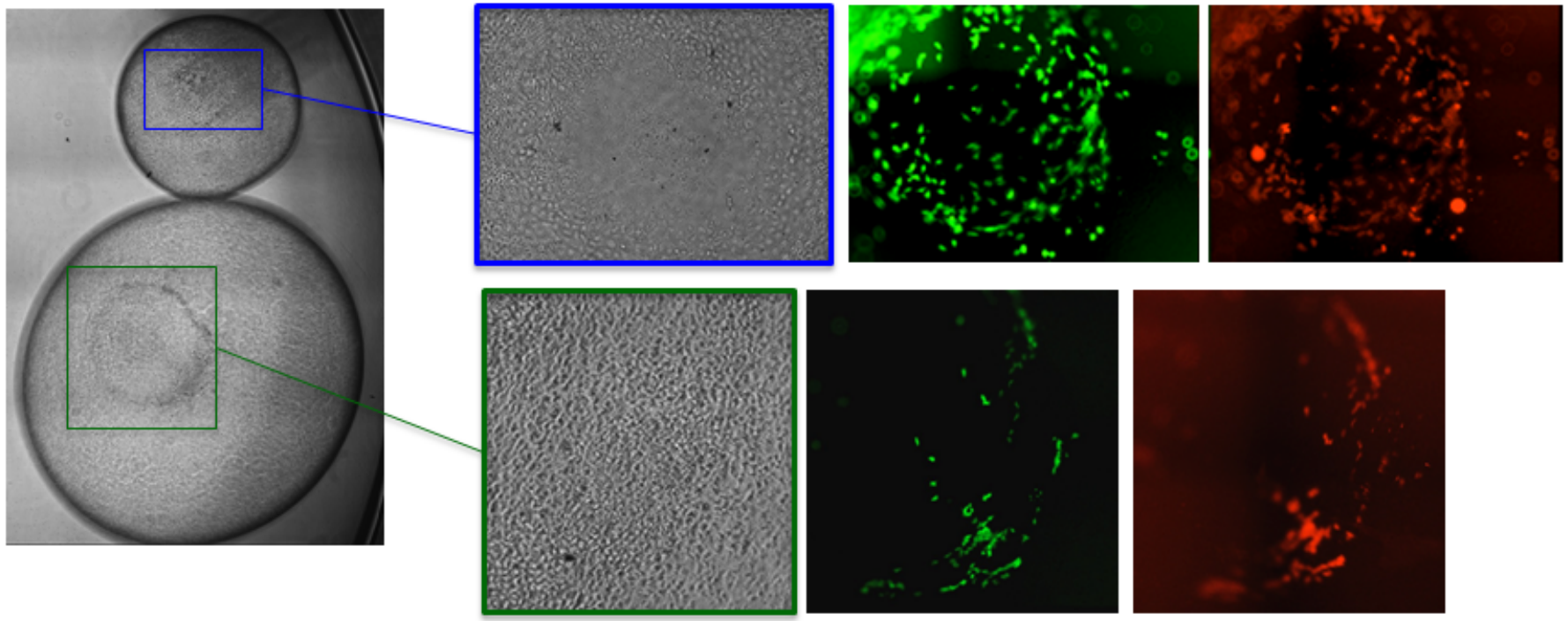
✓ **Transfection d'ARN** ←

✓ Transduction protéique

Somatic cell reprogramming: the rabbit model

➔ **Amélioration de la capacité de colonisation embryonnaire /germinale des iPSC**





Somatic cell reprogramming: the rabbit model – the achievements

✓ Conclusion:

Les résultats obtenus sont très prometteurs à la fois:

- **pour la cryopréservation de tissus somatiques**
- **pour la capacité de colonisation embryonnaire des iPSC**

➔ GO

✓ Publications:

En révision après une soumission à Devlopment (DEVELOP-2015-137035v1-Savatier)

Transcriptional reconfiguration of rabbit iPS cells with Krüppel-like factors confers chimeric competency

Yann Tapponnier[1,2,3], Marielle Afanassieff[1,2,3,4], Thierry Joly[5,6], Maxime Aubry[1,2,3], Anaïs Moulin[1,2,3], Luc Jouneau[7], Catherine Archilla[7], Barbara Schmaltz-Panneau[7], Harmonie Barasc[8,9], Pierre Osteil[1,2,3,4], Jérome Lecardonnel[10], Alain Pinton[8,9], Elen Gocza[11], Véronique Duranthon[7], and Pierre Savatier[1,2,3,£]

En préparation:

Afanassieff, Pain & Joly (2016) Cryopreservation of pluripotent stem cells in synthetic medium

Somatic cell reprogramming: the avian model

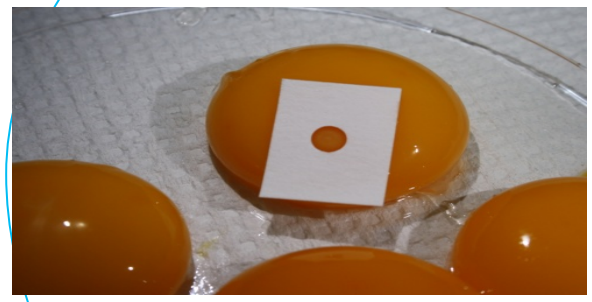
Blastodermal Cell

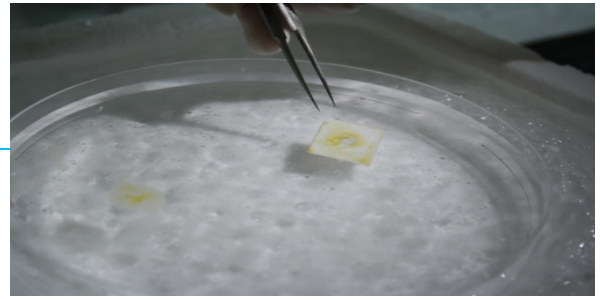
BC

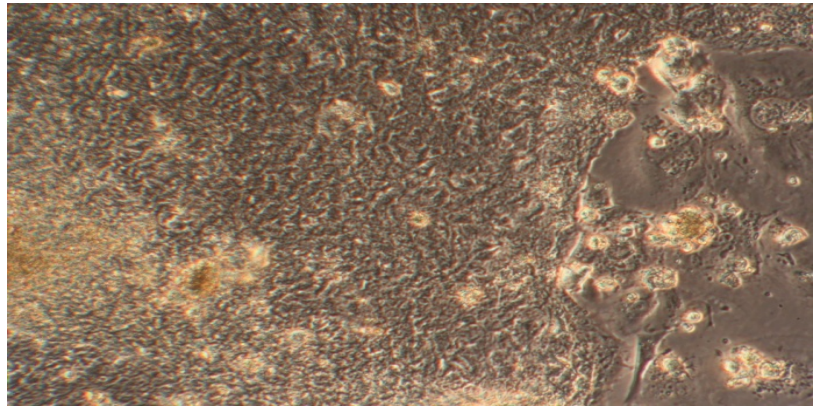
LIF / IL6

in vitro
establishment

chicken
Embryonic stem cell

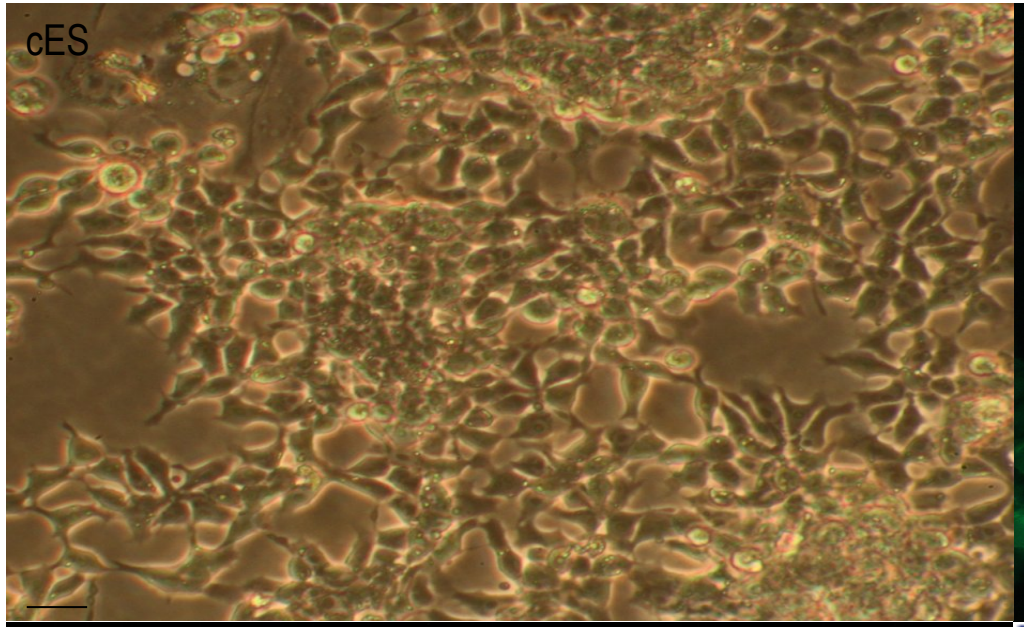


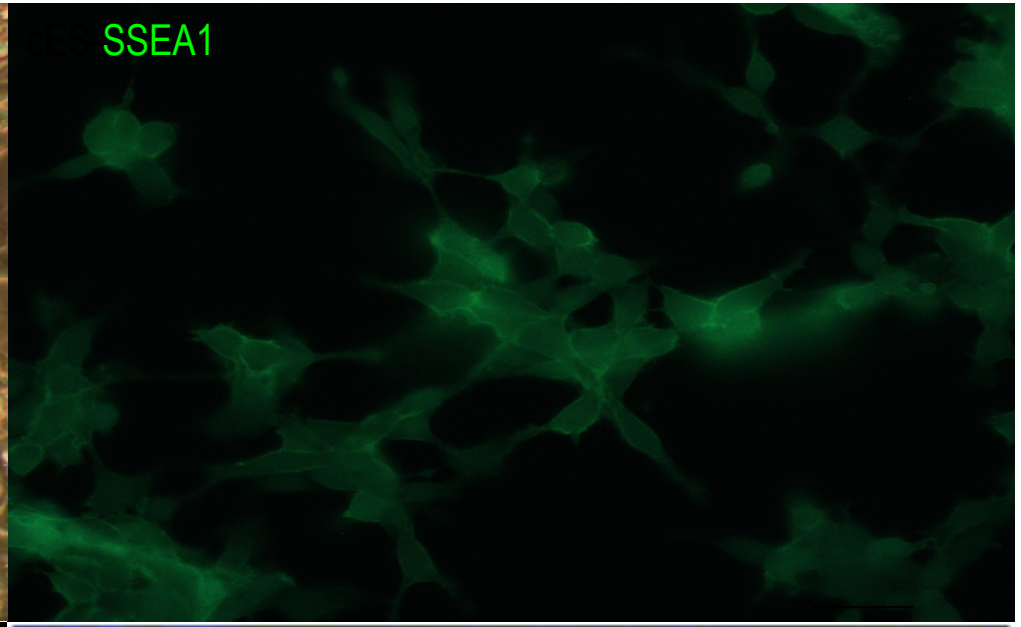


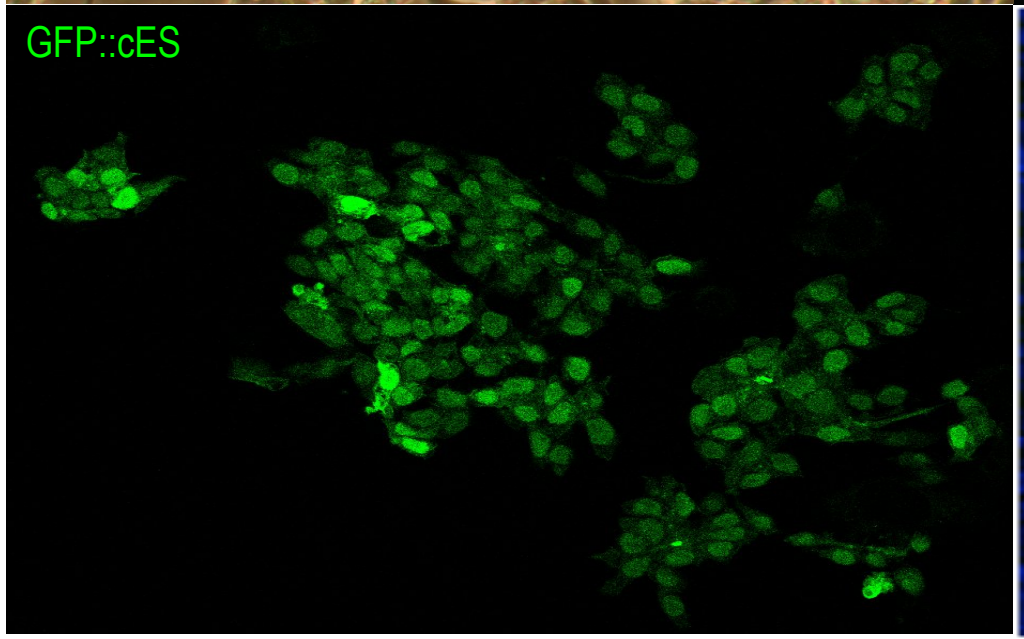


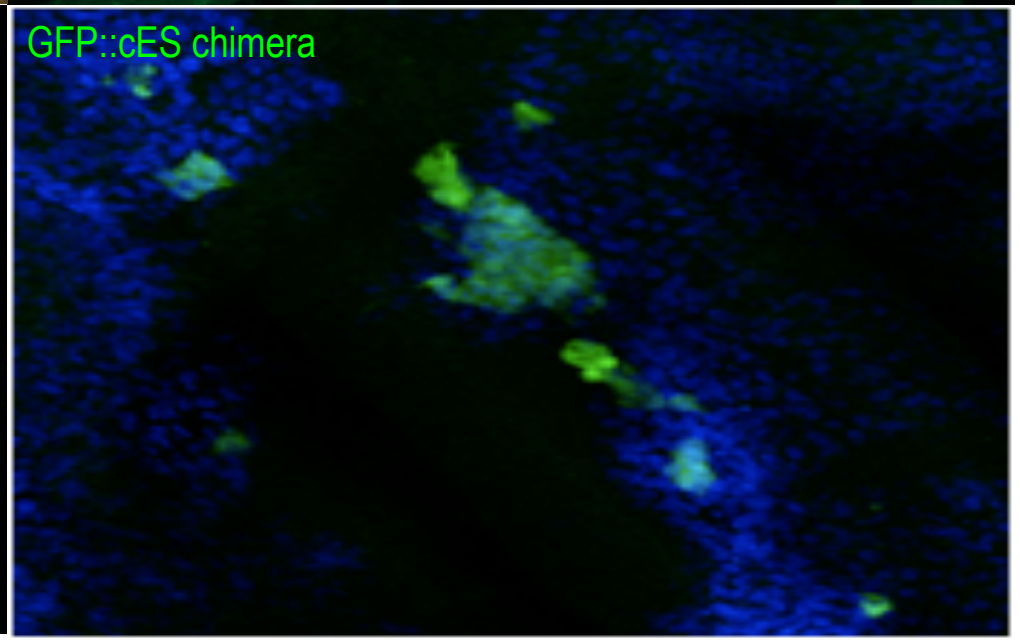
Adapted from Aubel & Pain, 2013

Somatic cell reprogramming: the avian model

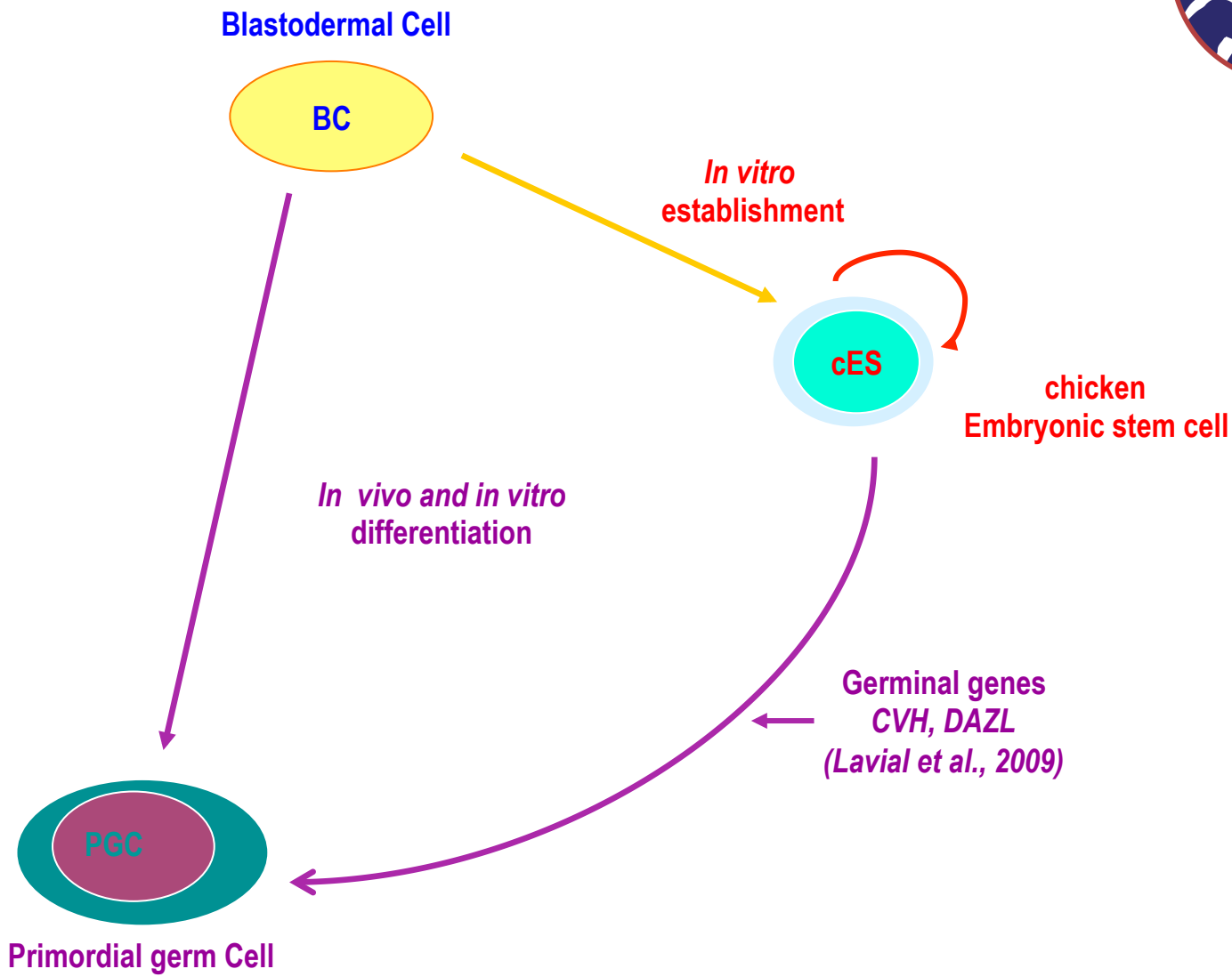




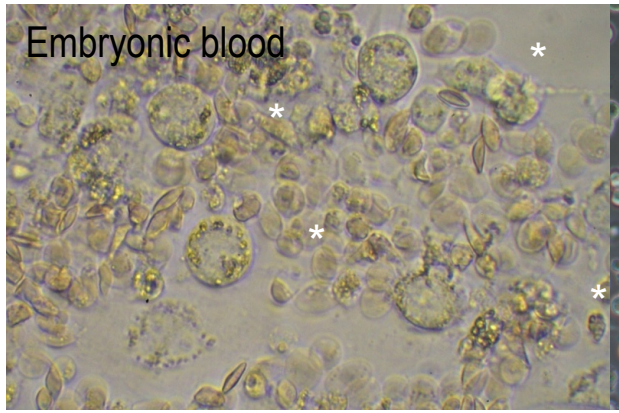


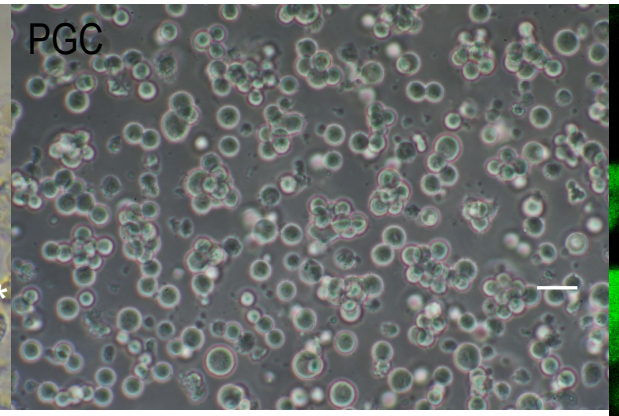


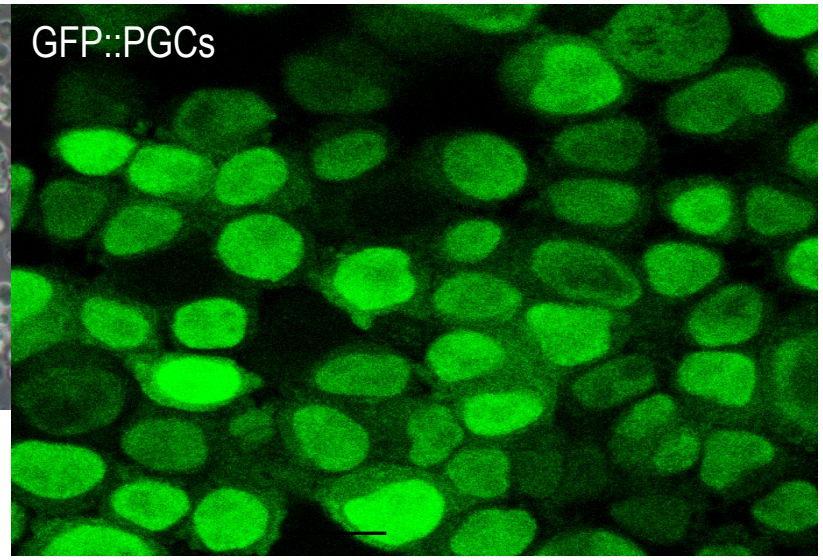
Somatic cell reprogramming: the avian model

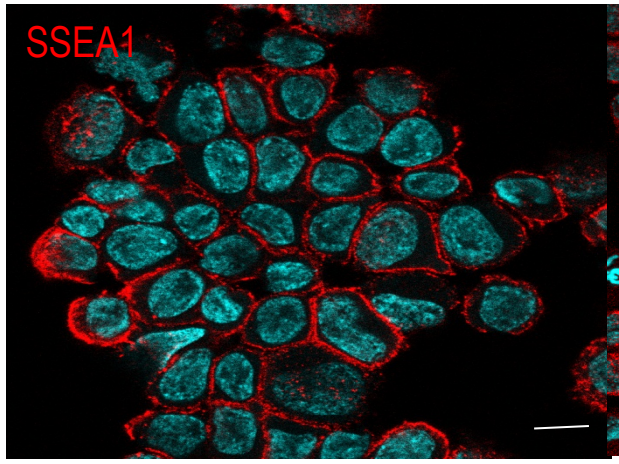


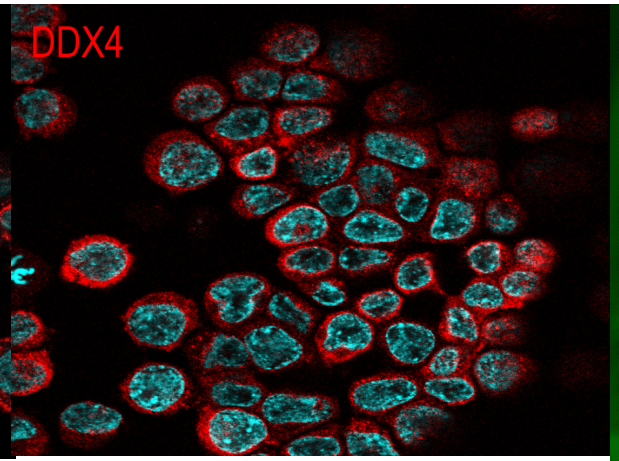
Somatic cell reprogramming: the avian model

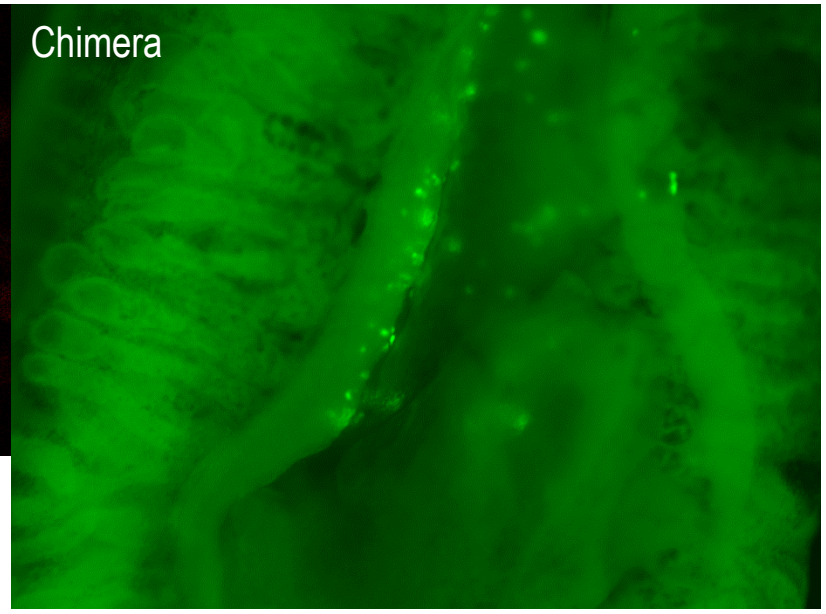






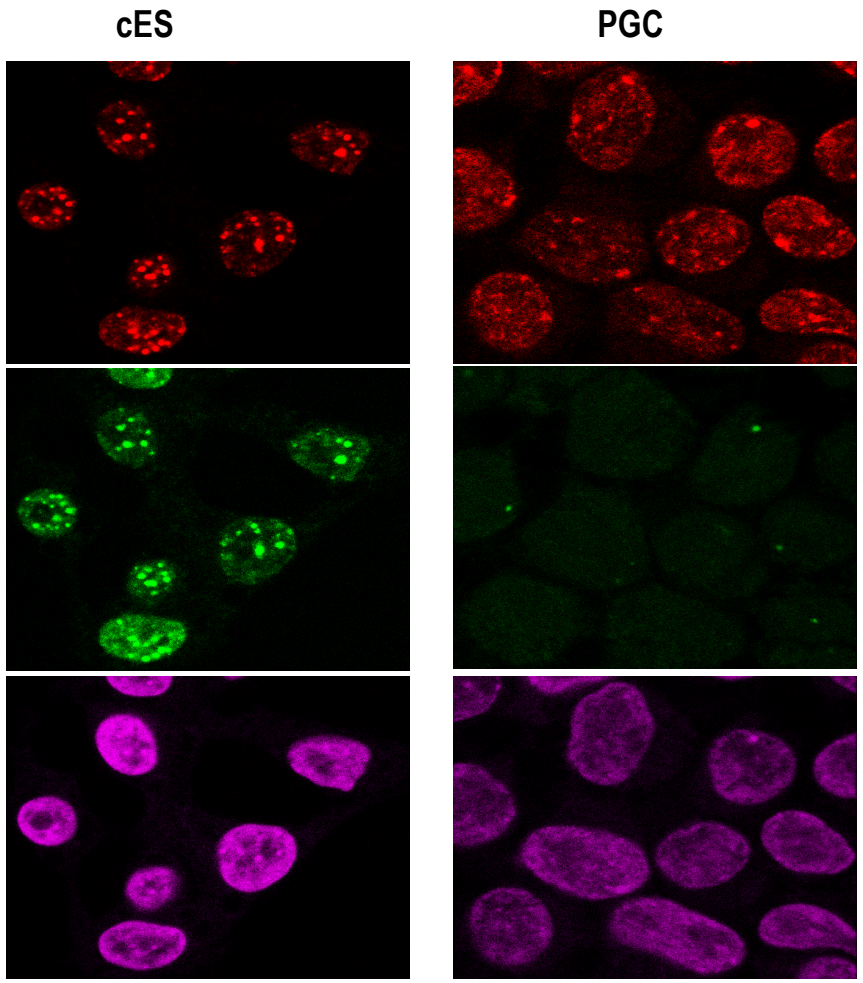


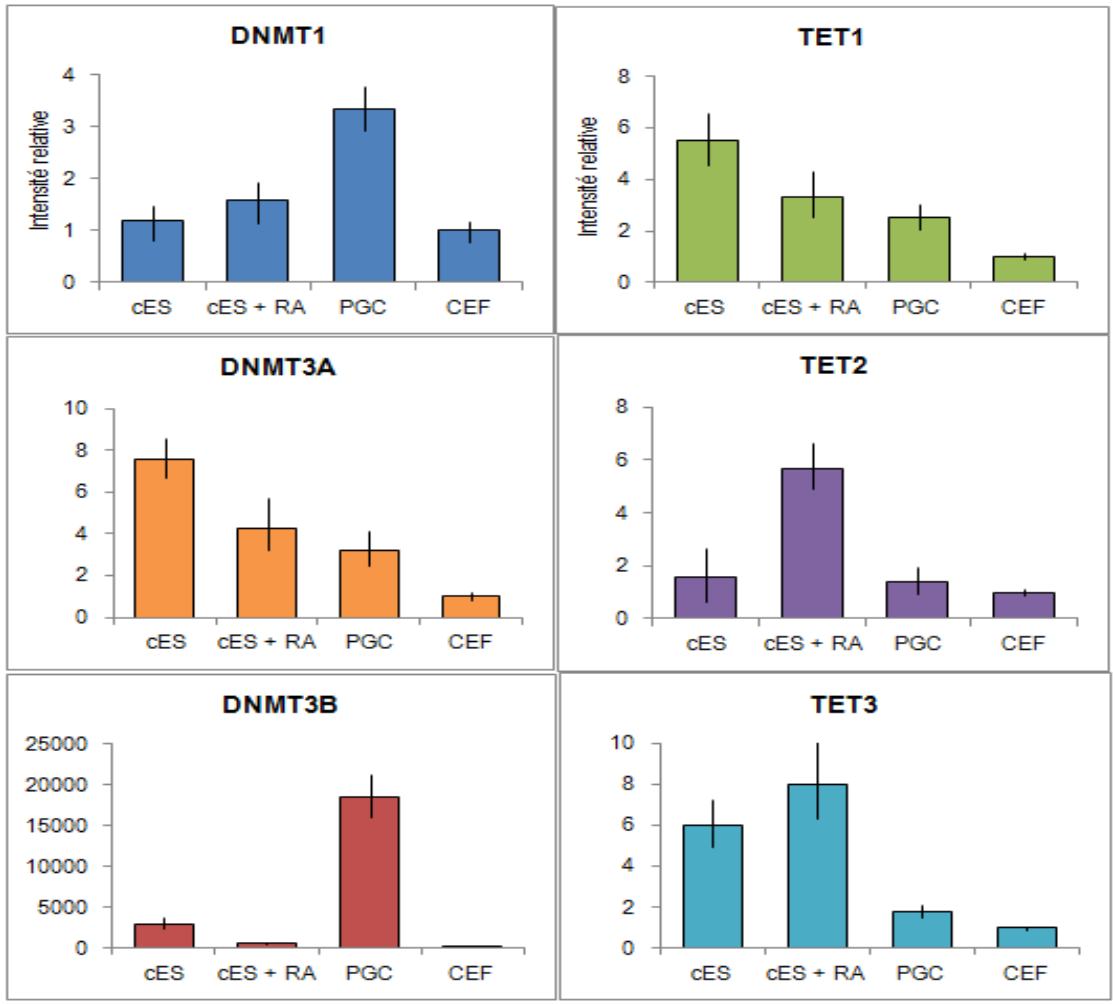




C. Kress

Somatic cell reprogramming: the avian model

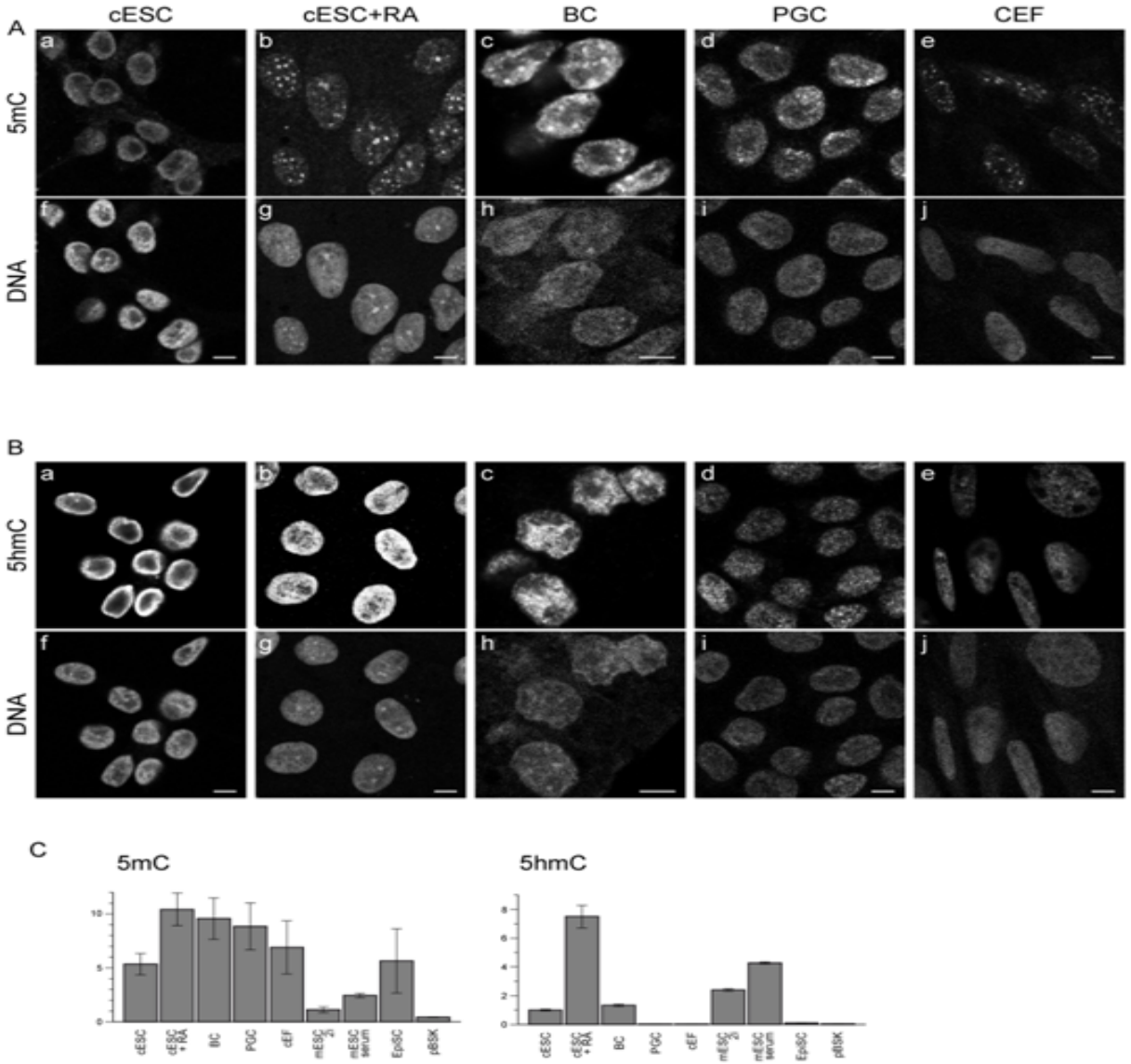




Kress et al., submitted

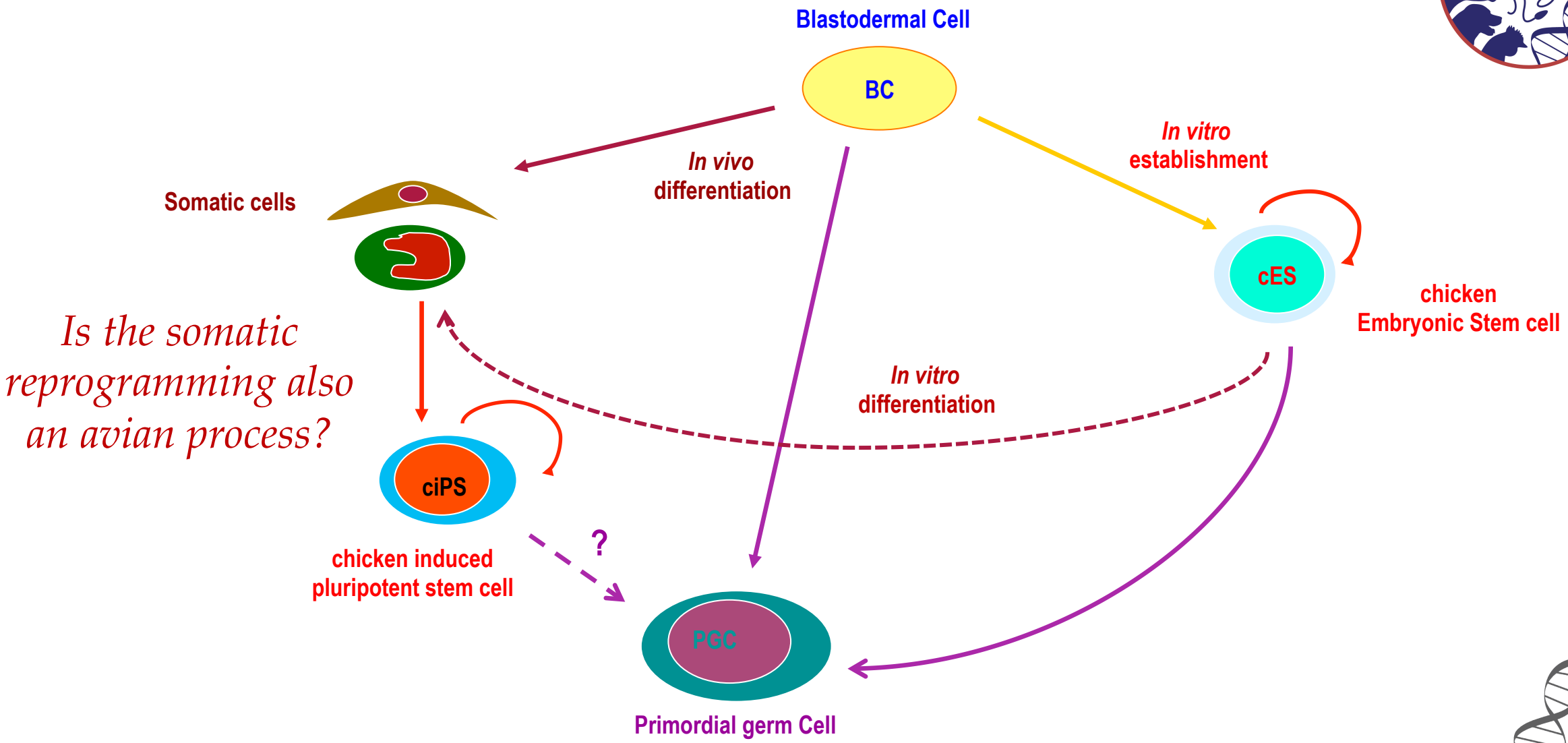
Somatic cell reprogramming: the avian model

Figure 7

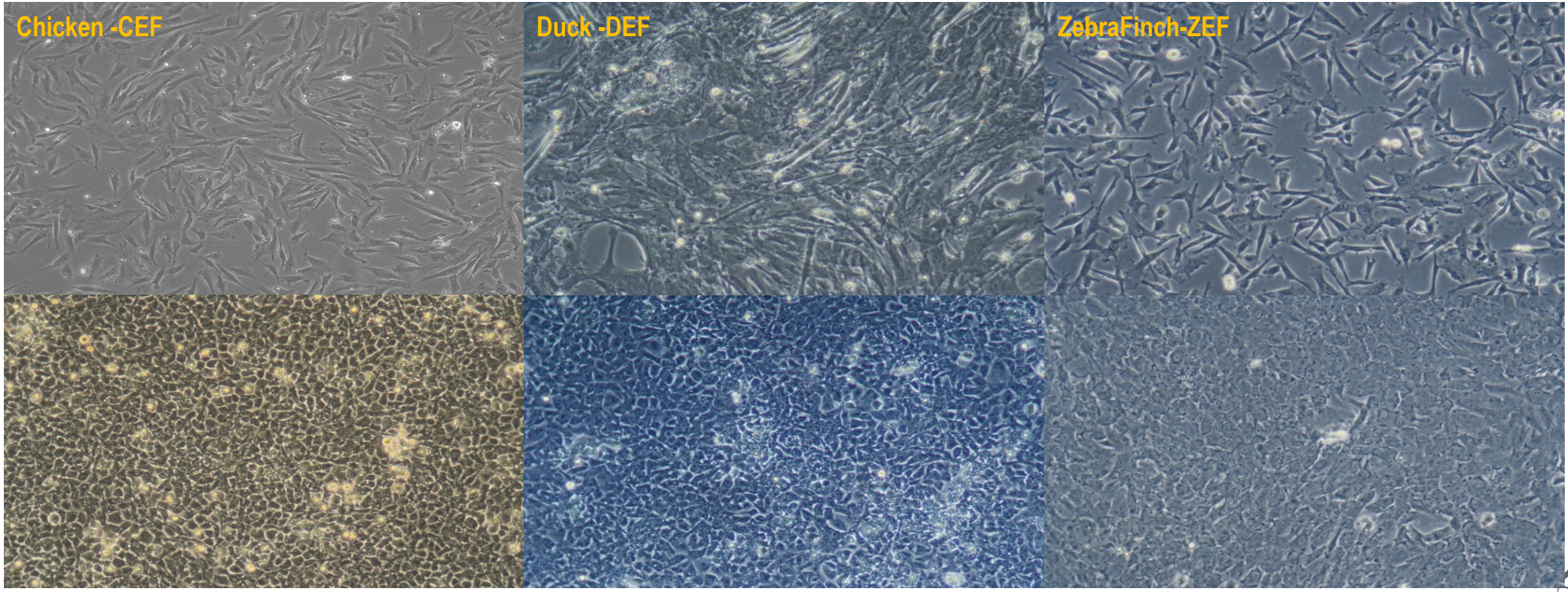


Kress et al., submitted

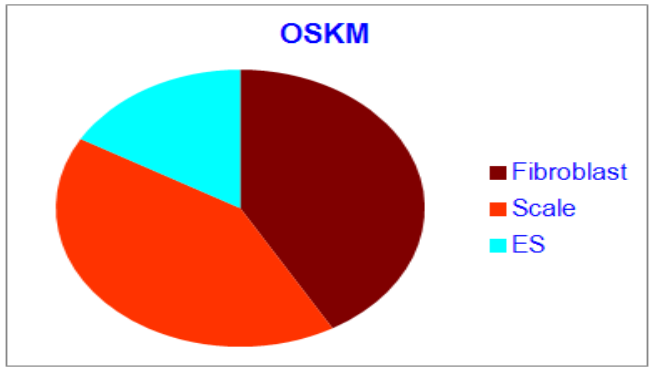
Somatic cell reprogramming: the avian model

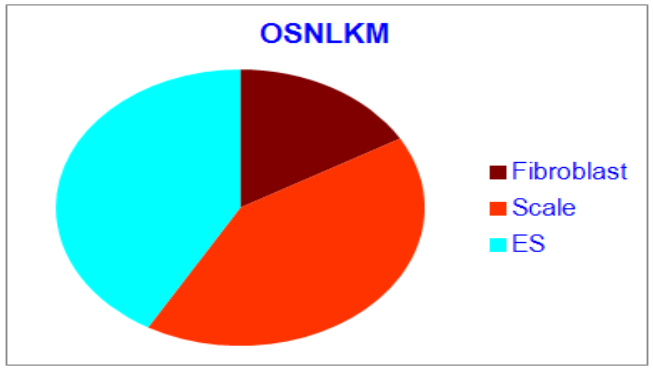


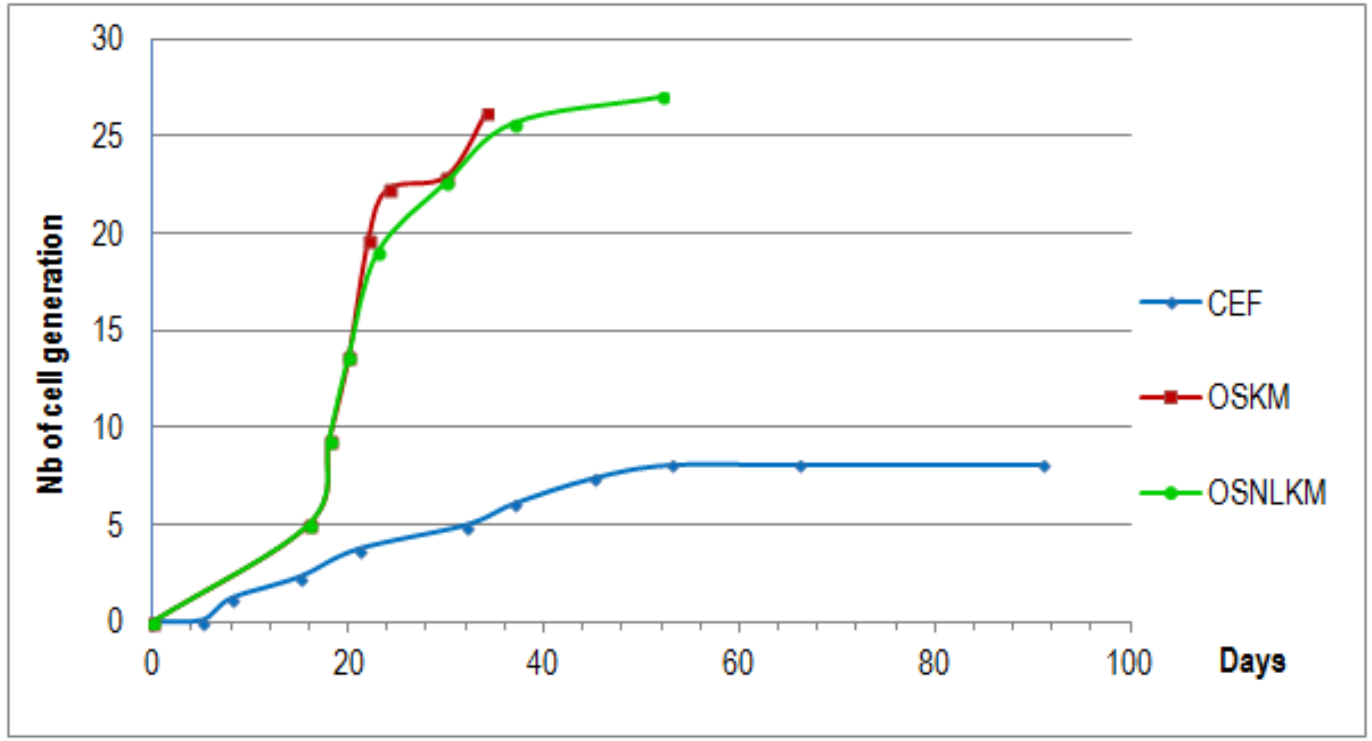
Somatic cell reprogramming: the avian model



Somatic cell reprogramming: the avian model



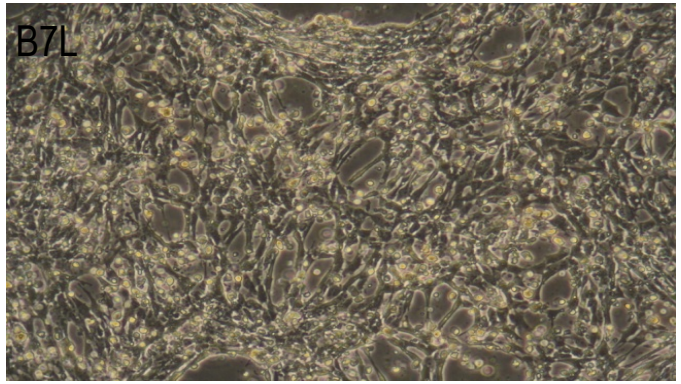


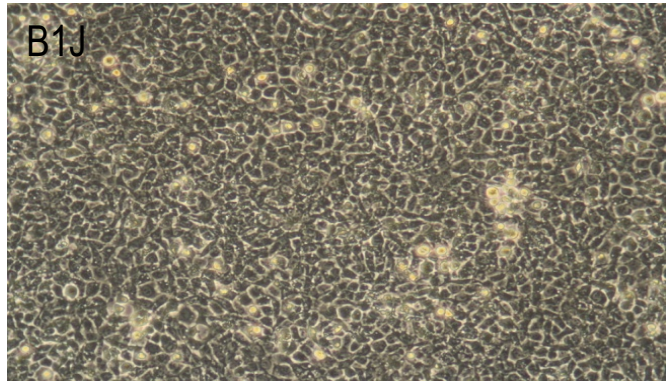


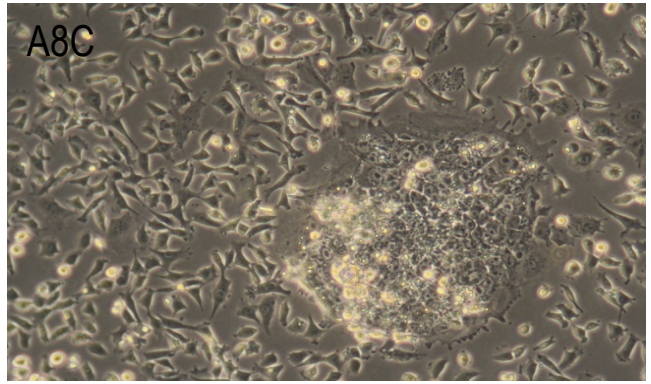
Fibroblast like

Scale

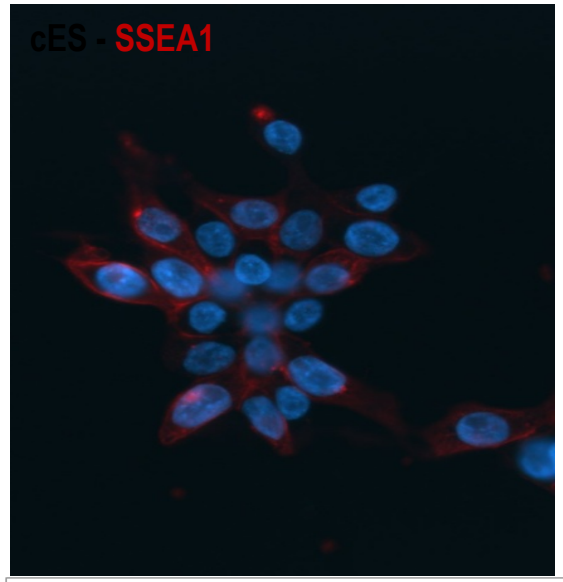
'ES'

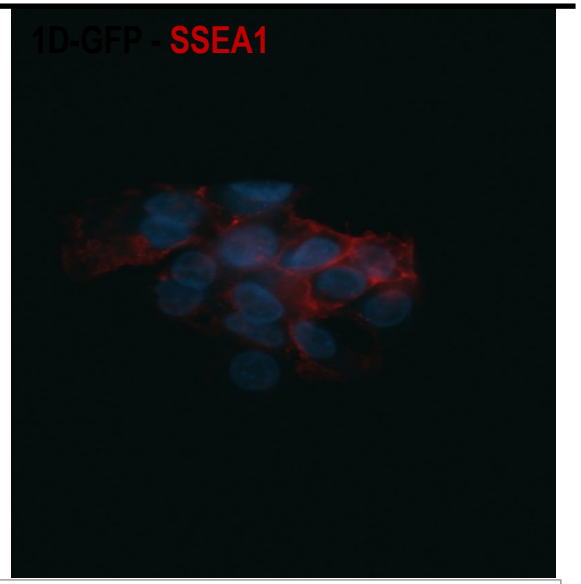


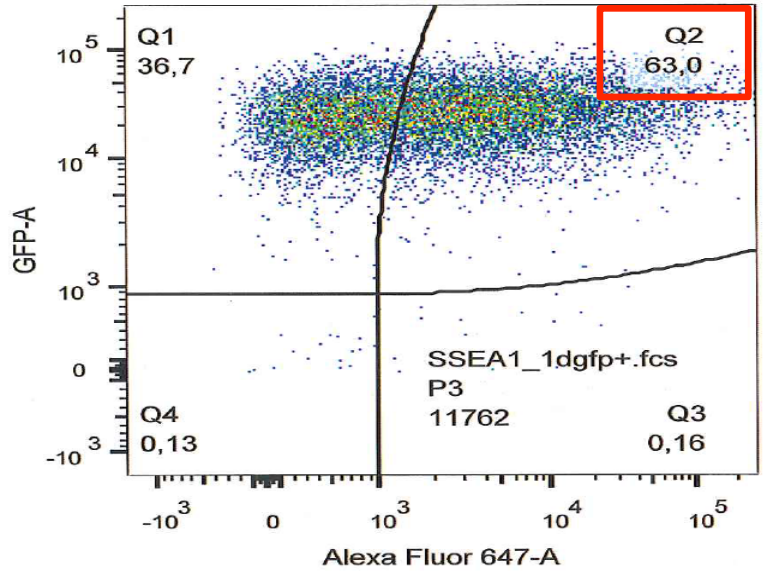


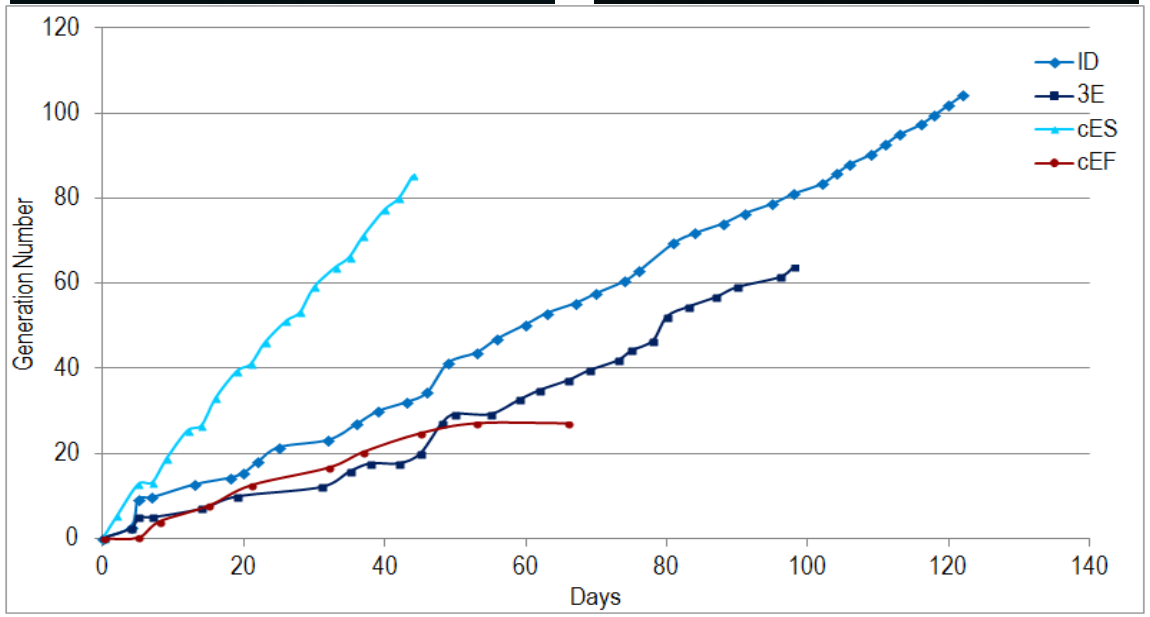


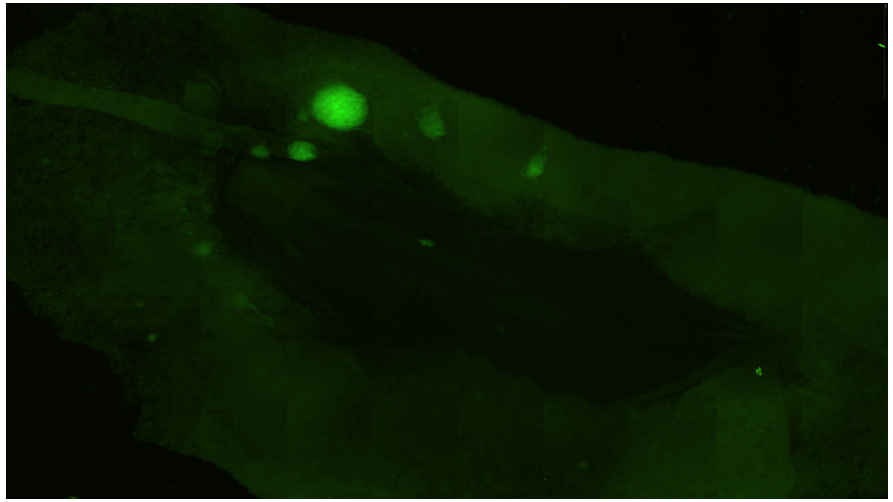
Somatic cell reprogramming: the avian model



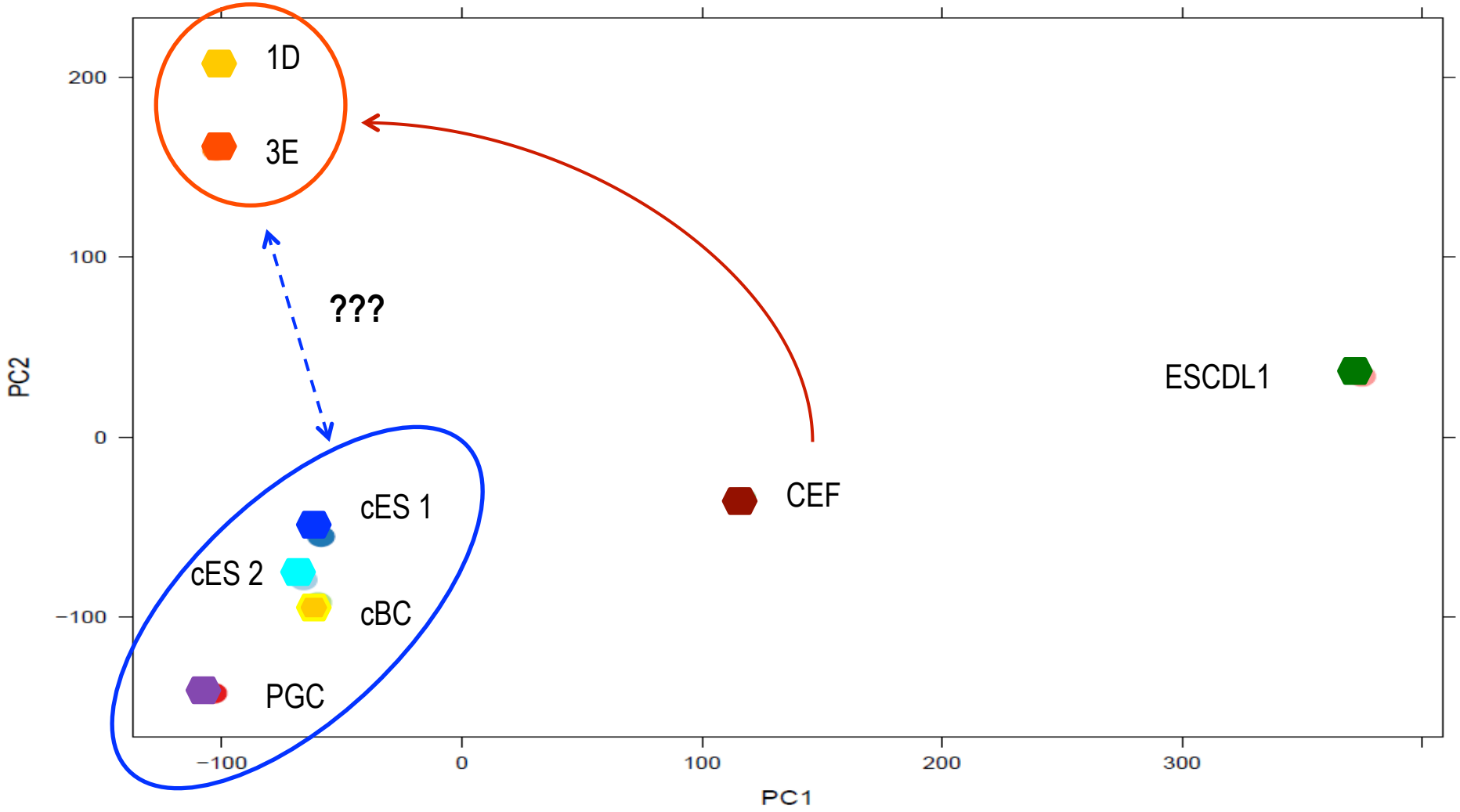






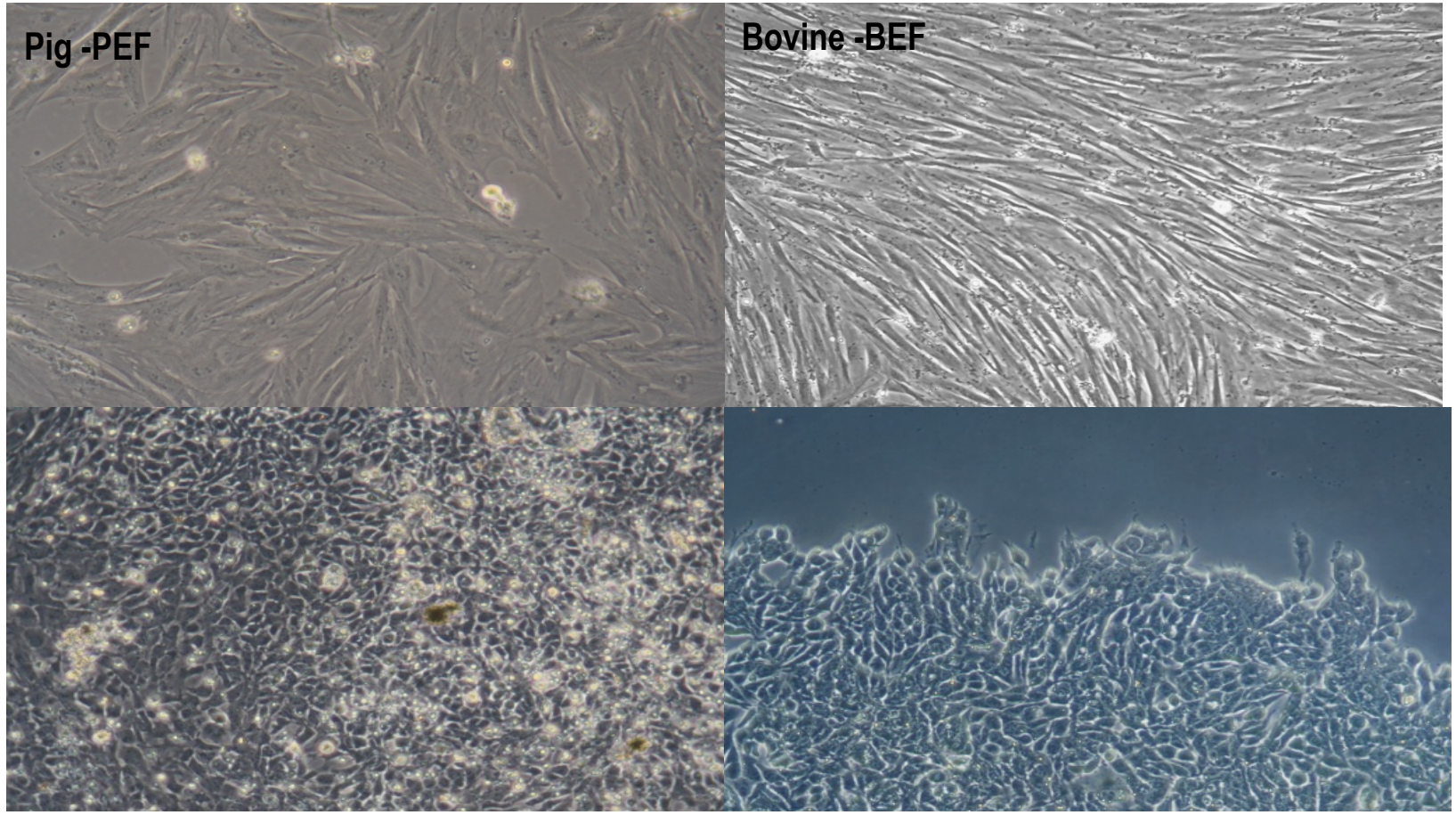


Somatic cell reprogramming: the avian model



Fuet et al., in preparation

Somatic cell reprogramming: livestock species



Somatic cell reprogramming: the avian model – the achievements

We have first established:

- ***A transcriptomic profile of avian stem cells (ES, PGCs, BC) ➔ Jean et al., 2015***
- ***➔ allowed us to identify key germ cell specific genes***

- ***An epigenetic landscape of chicken stem cells : 'Chicken embryonic stem cells and primordial germ cells exibit original epigenetic marks' (Kress et al., submitted)***
- ***➔ allowed us to identify specific epigenetic markers for avian embryos, ES and germ cells***

- ***Avian iPS cells with original reprogramming gene combinations, Fuet et al., (in preparation)***

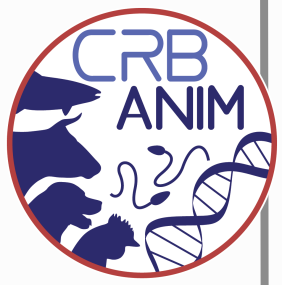
- ***New aseric freezing conditions with Stem Alpha medium for somatic and stem cells in avian, pig and ruminants (bovine, goat and sheep)***

Somatic cell reprogramming: the livestock models – the achievements

With the acquired expertise in reprogramming, we have established:

- *New Pig iPS cells using a classical reprogramming gene cocktail*
- *➔ still to be molecularly and developmentally fully characterized*
- *➔ to be used for new defined phenotypic cell types*

- *New Ruminant 'iPS / iPS-like cells with an original reprogramming cocktail*
- *➔ still to be characterized at the molecular and developmental levels*



Labogena

Color

Labogena

White