

## **Loi de bioéthique (révisée en 2004):**

1. Interdit le clonage (thérap. et repro.)
2. Interdit la production et l'utilisation d'embryons surnuméraires pour la recherche médicale
3. Autorise sous dérogation l'utilisation de lignées ES humaines à visée thérapeutique
4. Autorise sous dérogation la dérivation de lignées ES humaines à visée thérapeutique à partir d'embryons surnuméraires.

# Rejet immunitaire des ES

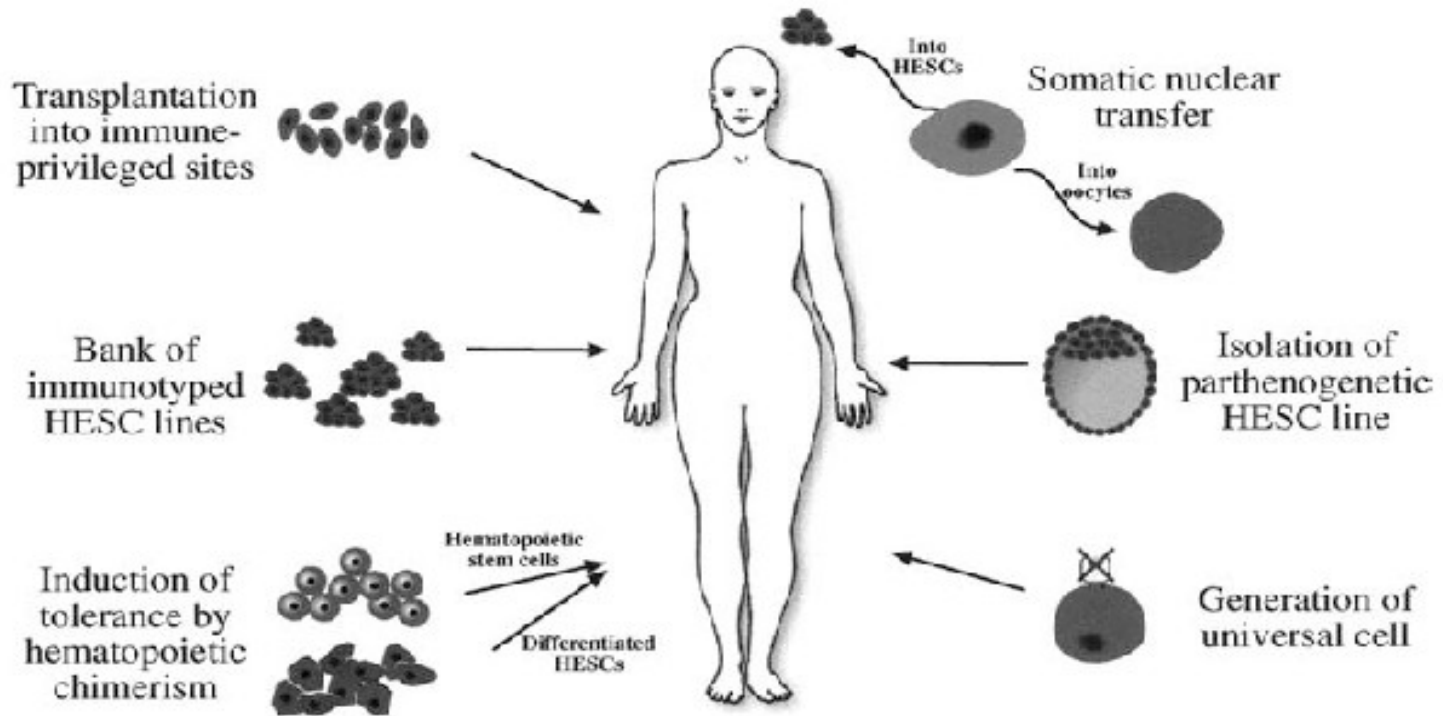
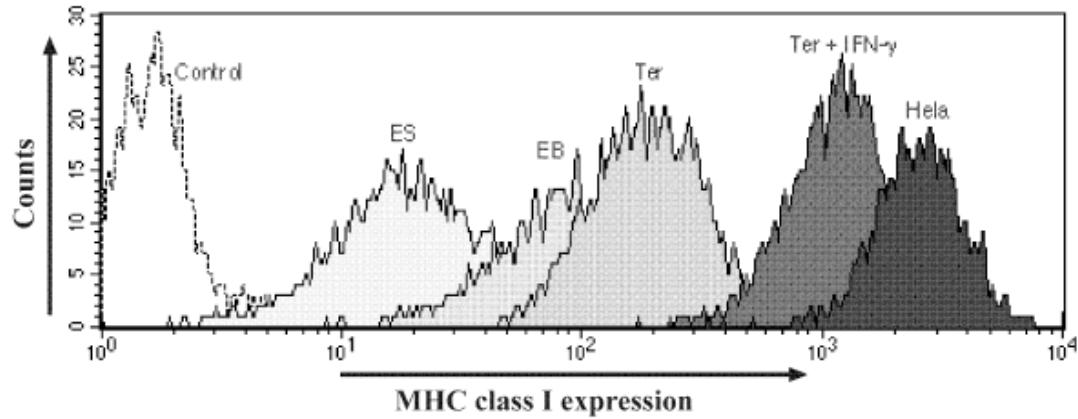
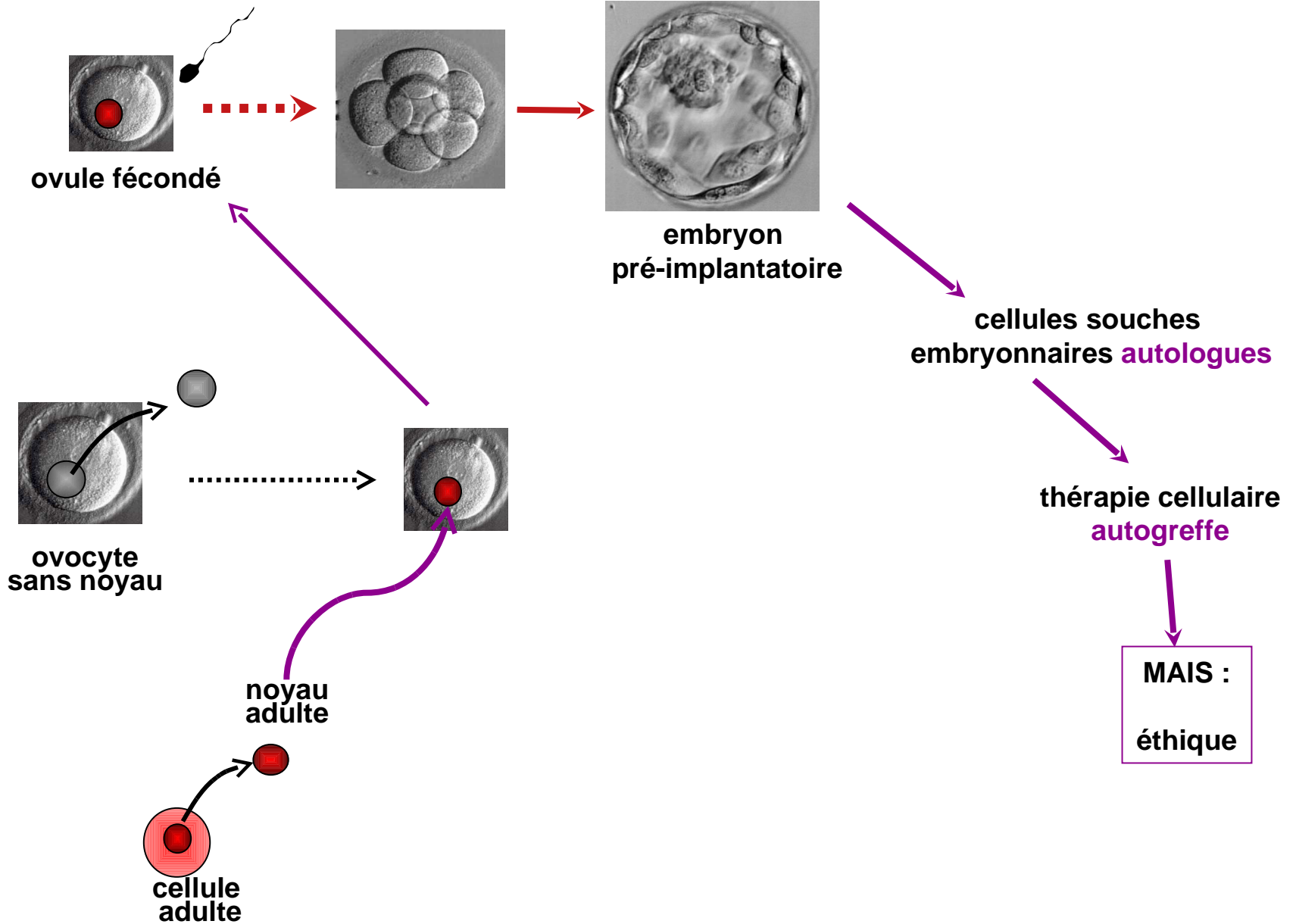
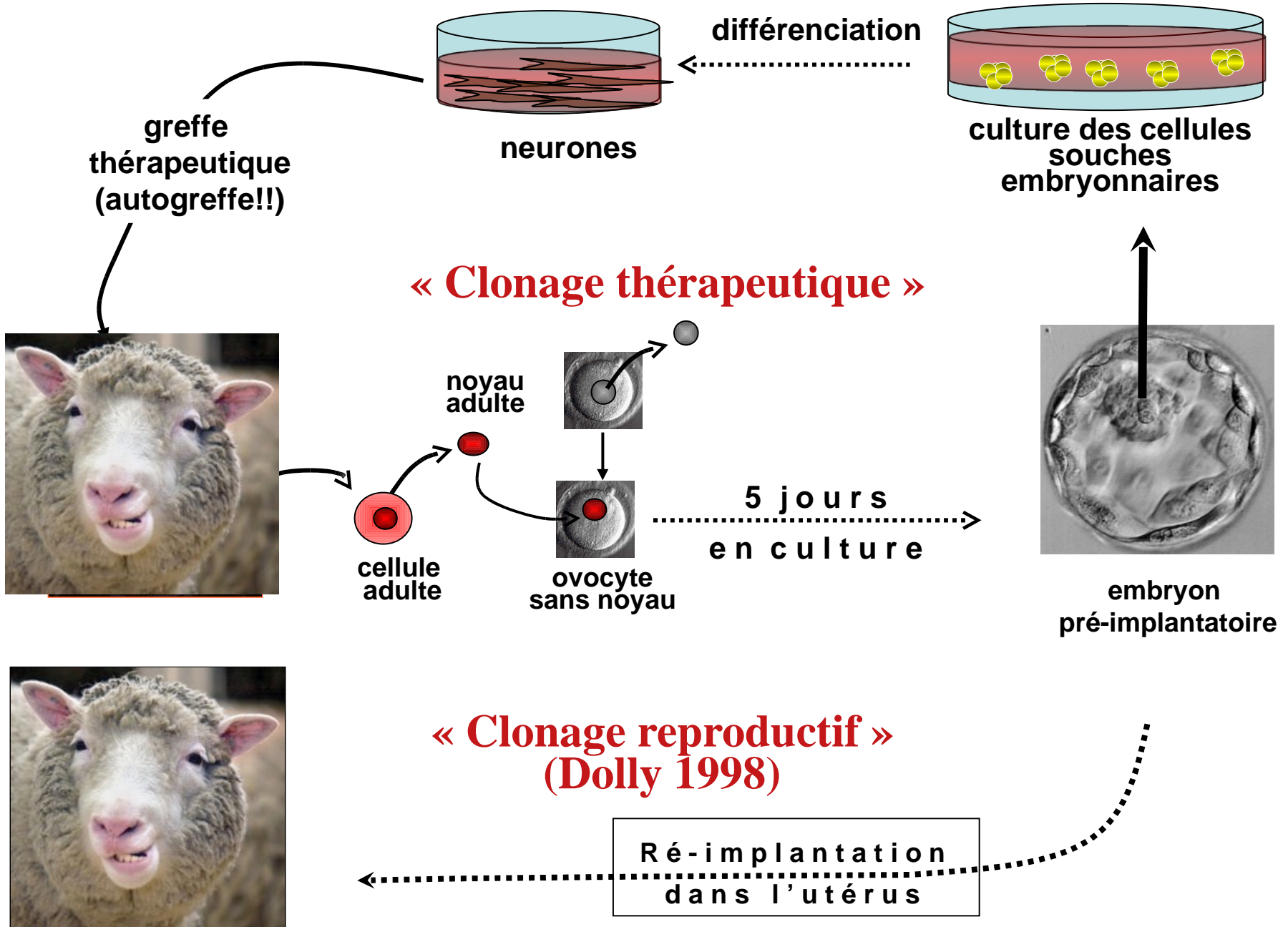
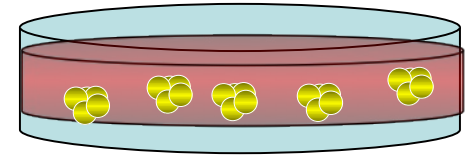


Fig. 2 Suggested routes for tolerance induction

# clonage nucléaire à visée thérapeutique

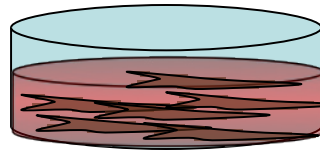






culture des cellules souches embryonnaires

différenciation



neurones

greffe thérapeutique (autogreffe!!)

# « Clonage thérapeutique »

- mar...
- (Hwar...
- statu...
- digni...
- espèc...

Une ba  
à une c



(NT)  
(es)

réponse



embryon pré-implantatoire

# « Clonage reproductif »

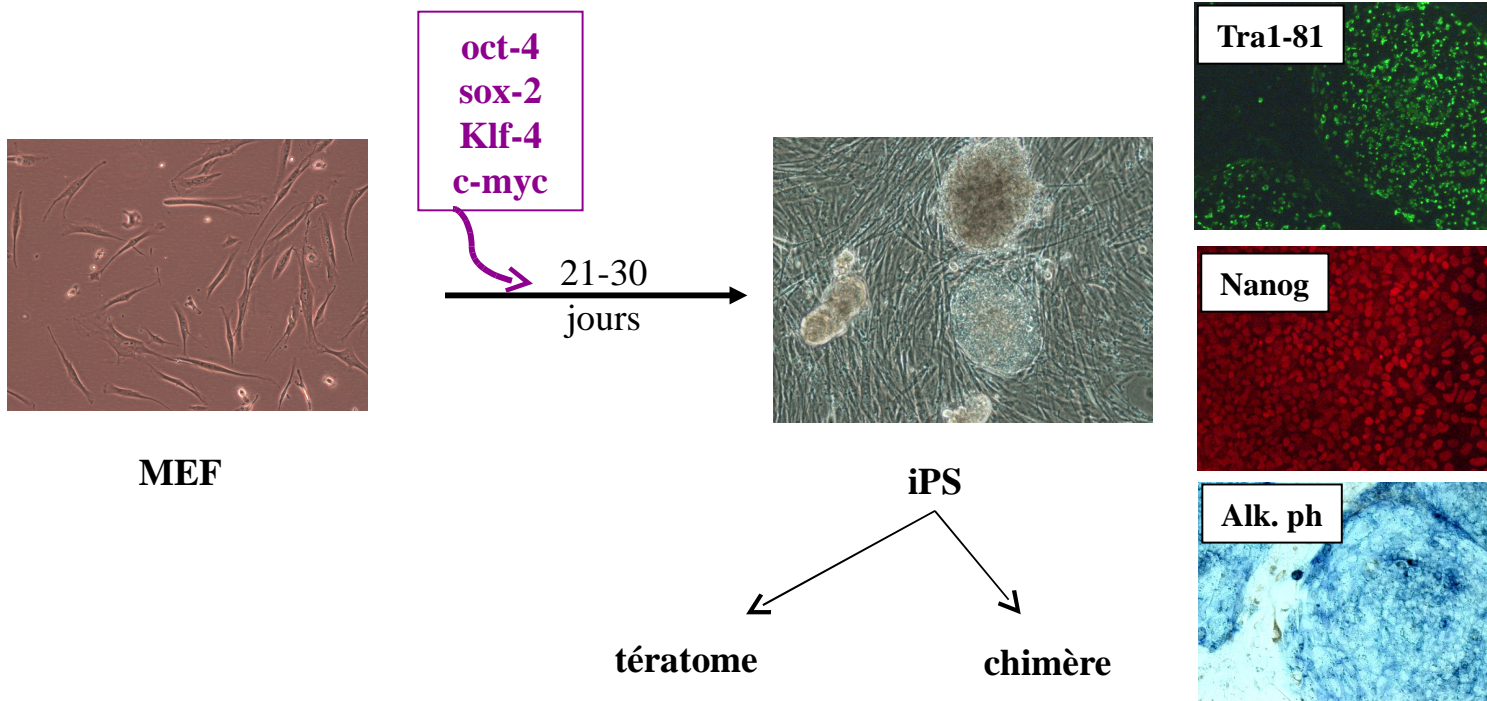
Ré-implantation dans l'utérus



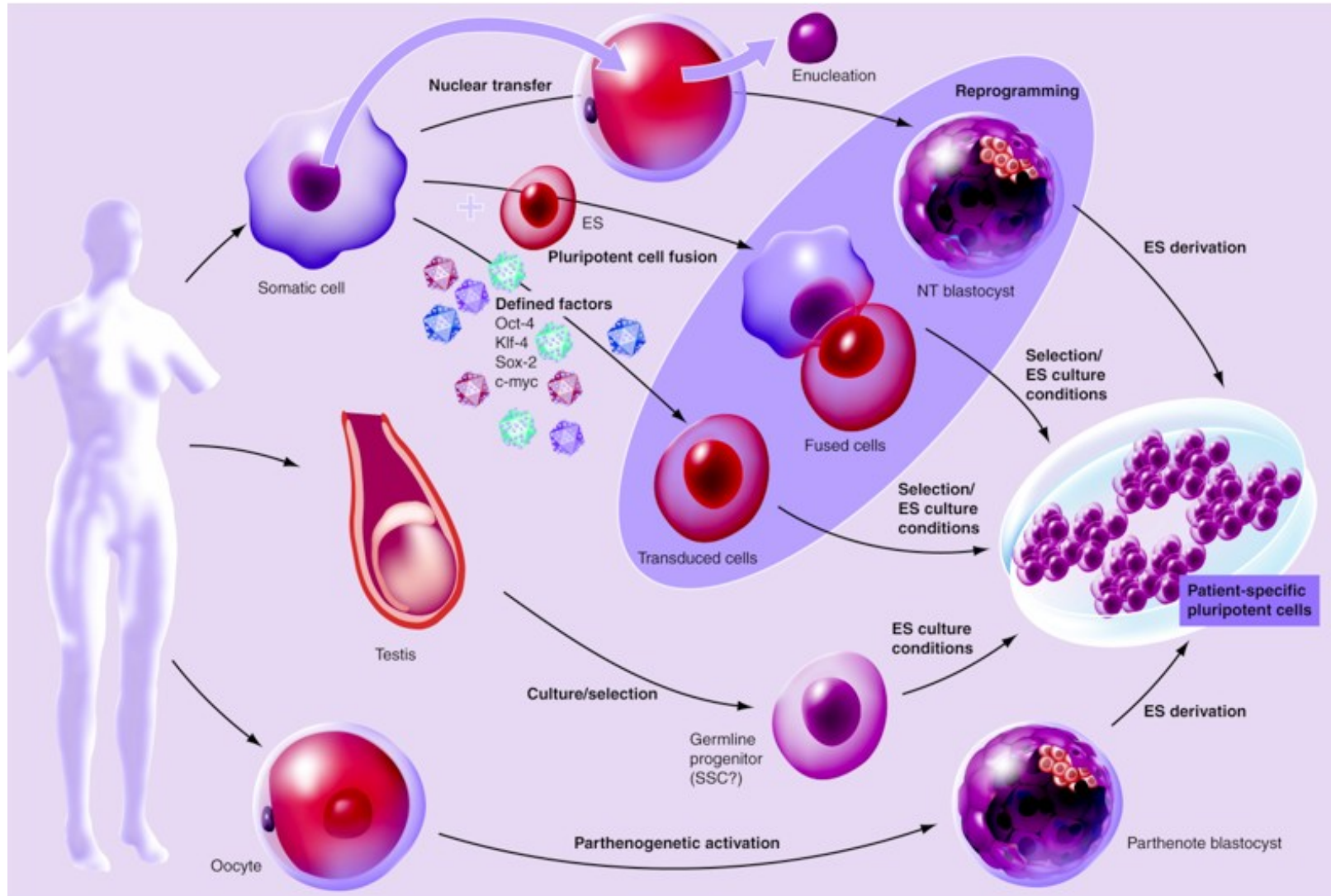
# Comment reprogrammer une cellule somatique en cellule pluripotente?

- mélange cellulaire: fusion cellulaire
- transfert de noyau: noyau ES dans cellule somatique (allogénique)
- Reprogrammation génique: induced pluripotent stem cells:  
Yamanaka (Kyoto) 2006 in mice

Criblage de gènes de pluripotence sur des fibroblastes (combinaison de cocktails)



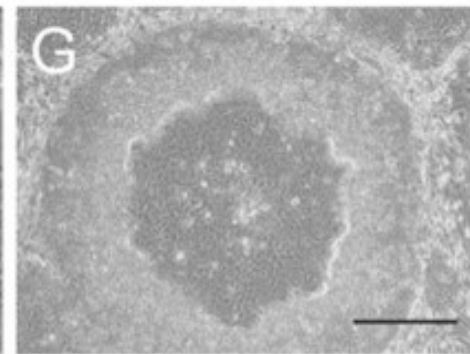
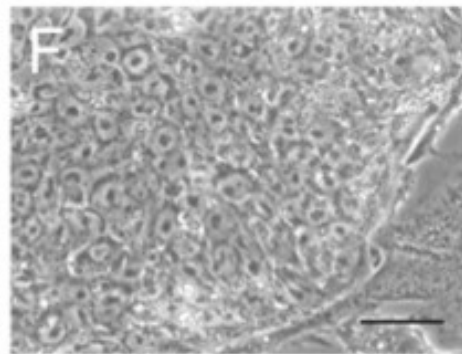
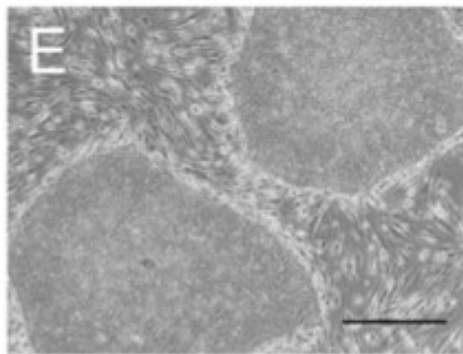
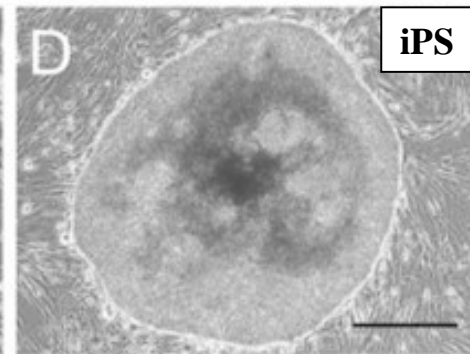
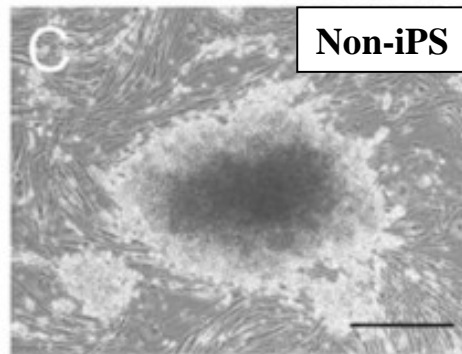
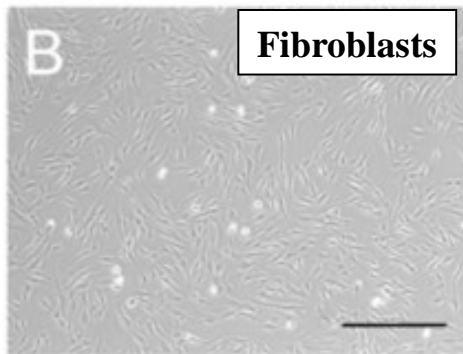
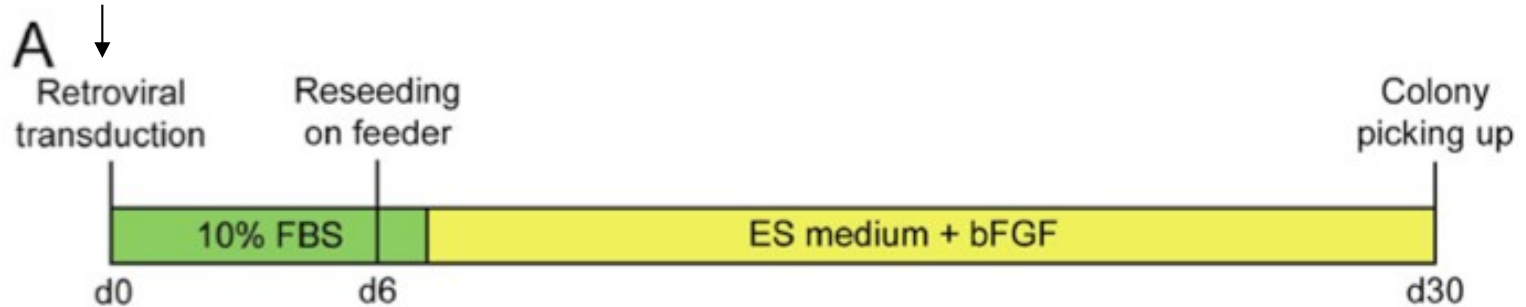
# Obtention of autologous pluripotent cells: The iPS technical breakthrough



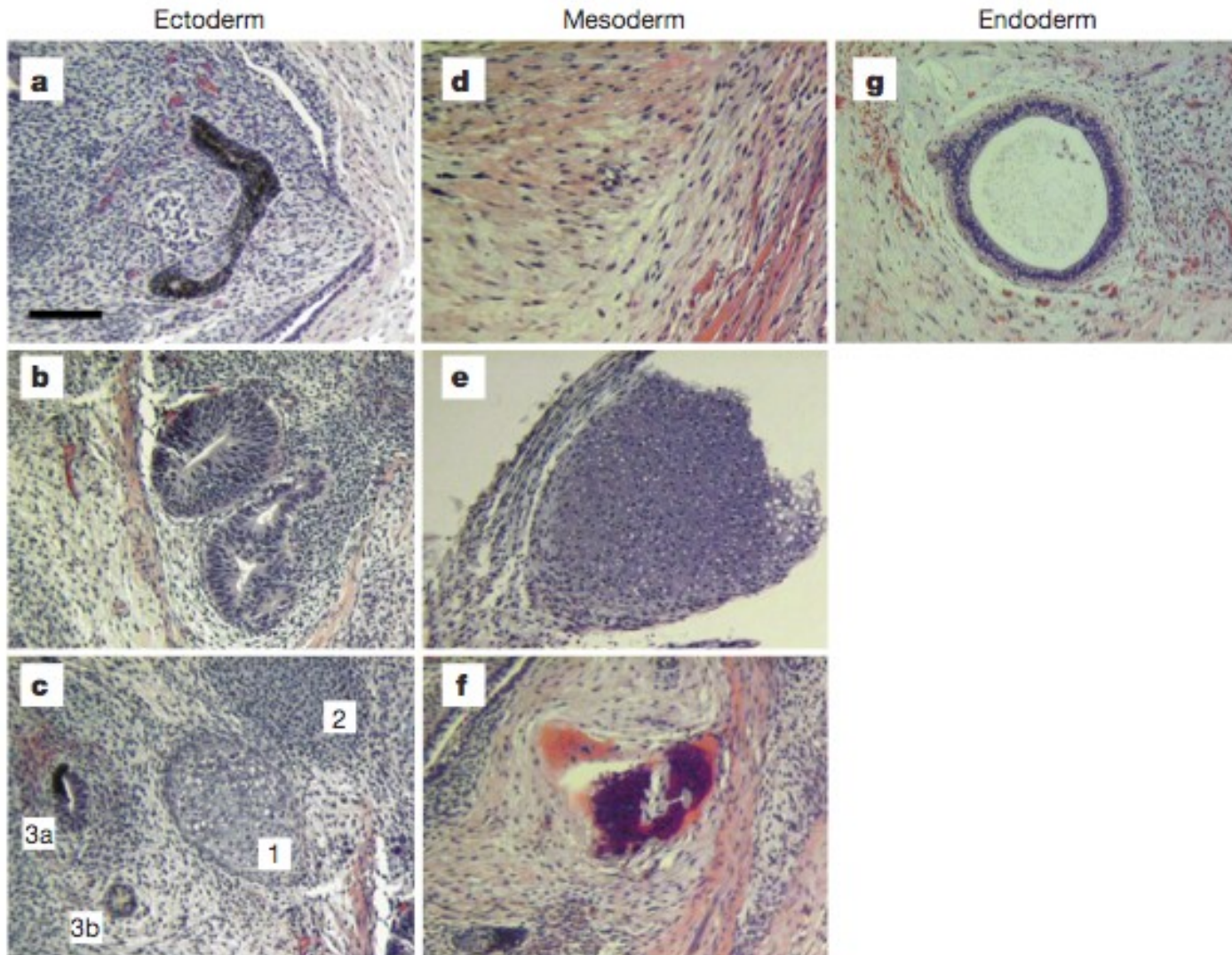
# Derivation of iPS cells from human fibroblasts

Oct4/Sox2/Klf4/c-myc

Oct4/Sox2/Nanog/Lin28



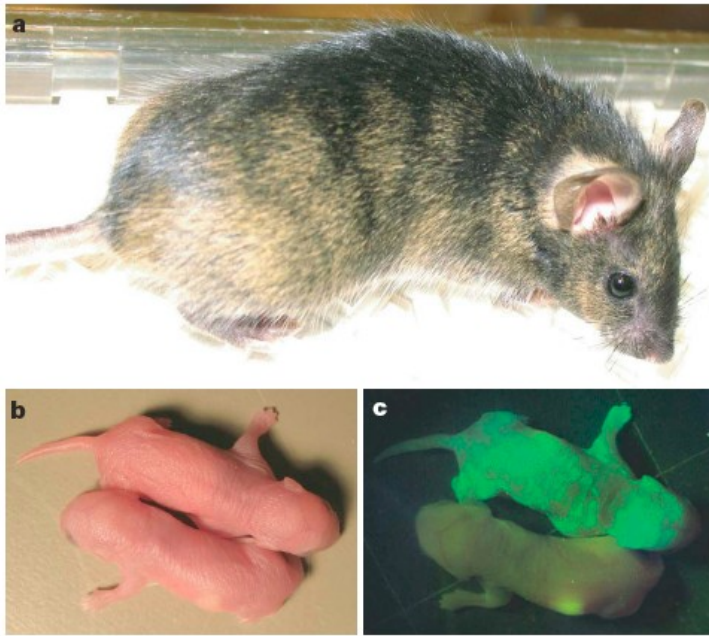
# Pluripotency of iPS cells: 2- Teratoma



*(Daley et al, Nature 2008)*

# Pluripotency of (murine) iPS cells:

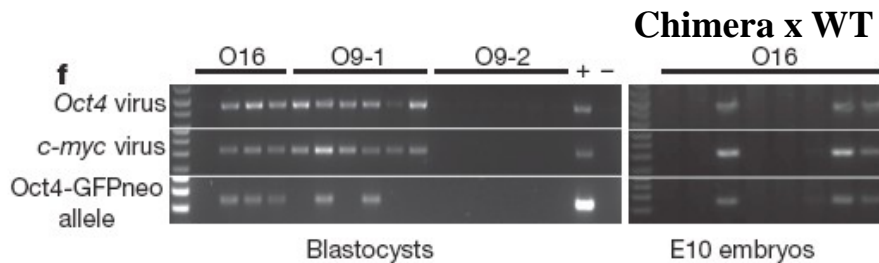
## 3- Chimeric mice/germline transmission



Nanog-GFP iPS Chimera



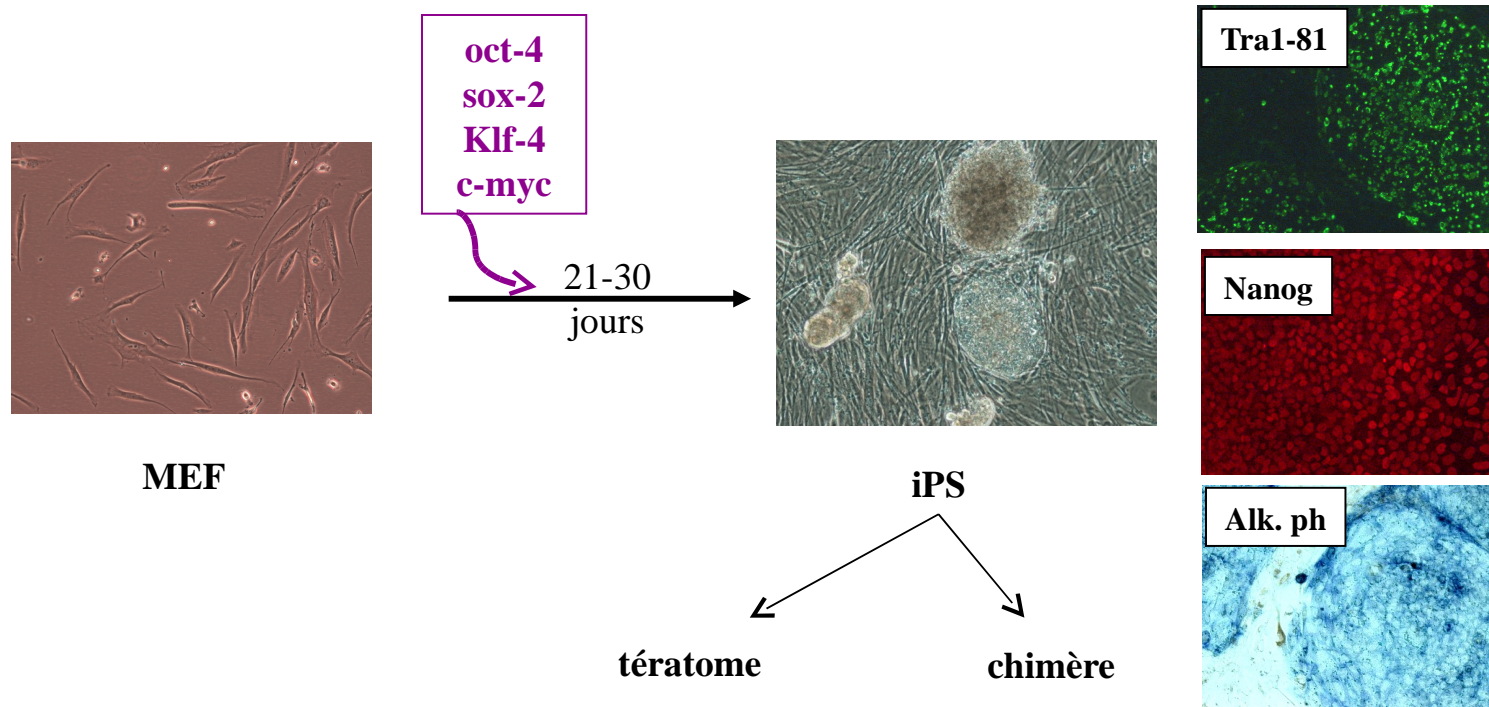
Oct4-GFP iPS Chimeras



(Jaenisch, Cell Stem Cell 2007)

(Jaenisch, Nature 2007)

# Comment reprogrammer une cellule somatique en cellule pluripotente?



**iPS de souris en 2006**

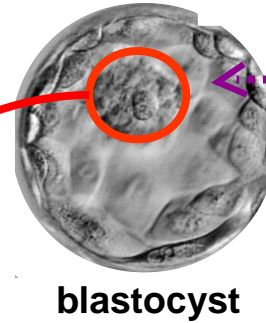
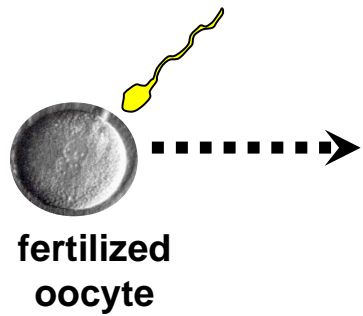
**iPS humaines en 2007. Conf rmé par 2 labo US**

**utilisation de rétrovirus et c-myc oncogène**

**eff cacité très faible (0.05%)**

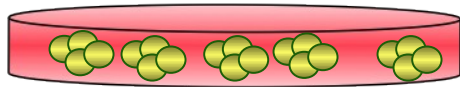
**Entre 2007 et 2010:**

- **iPS sans insertion rétrovirale (adénovirus, protéines recombinantes, molécules chimiques,...)**
- **amélioration de l'eff cacité (de 0.05 à 1%)**
- **reprogrammation de différents types cellulaires et de cellules de patients**



iPS

(induced Pluripotent Stem cells)



embryonic stem cells

Self renewal  
Pluripotent



Cell therapy

Cellular model:

- Embryonic development
- Toxicological tests
- Drug design

ectoderm

mesoderm

endoderm

neurons  
epidermal  
cornea  
mammary  
glands

dermis (fibroblasts)  
muscle  
adipocytes  
endothelial  
hematopoietic

pancreas  
liver  
intestine  
thymus

c-myc

klf-4

oct-4

sox-2

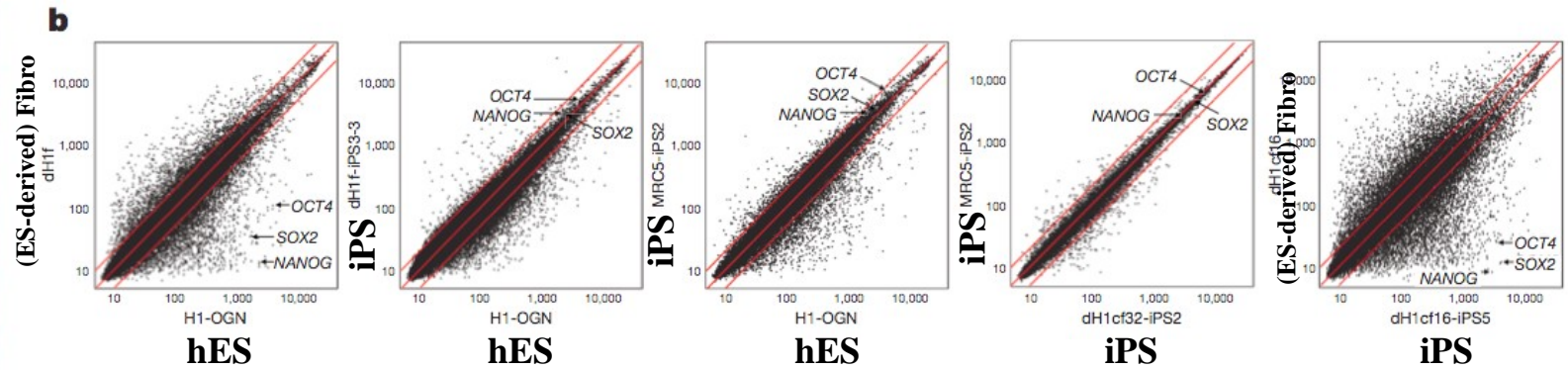
(nanog)

(LIN 28)

- Cellular proliferation (c-myc, Klf4)
- Chromatin remodeling (Klf4)
- Reactivation of ES cell genes (oc-4/sox-2)
- Establishment of ES transcriptomal circuitry

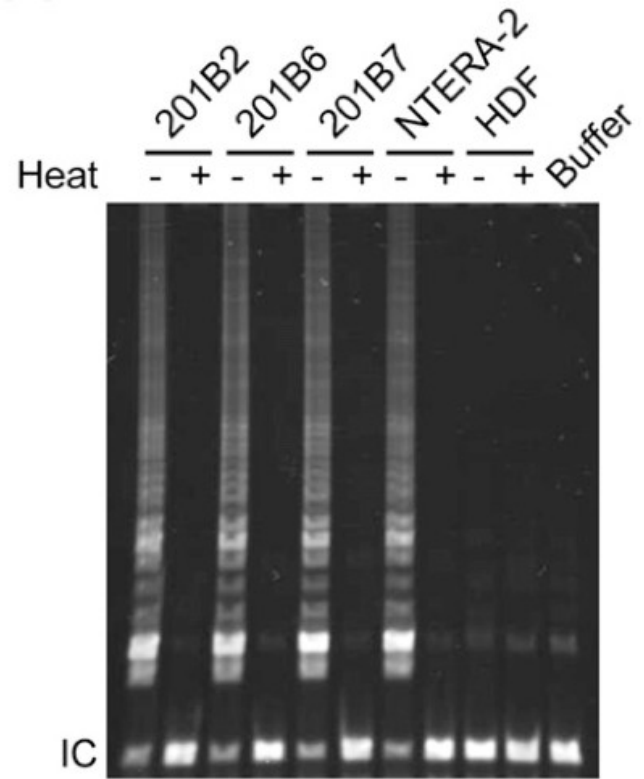
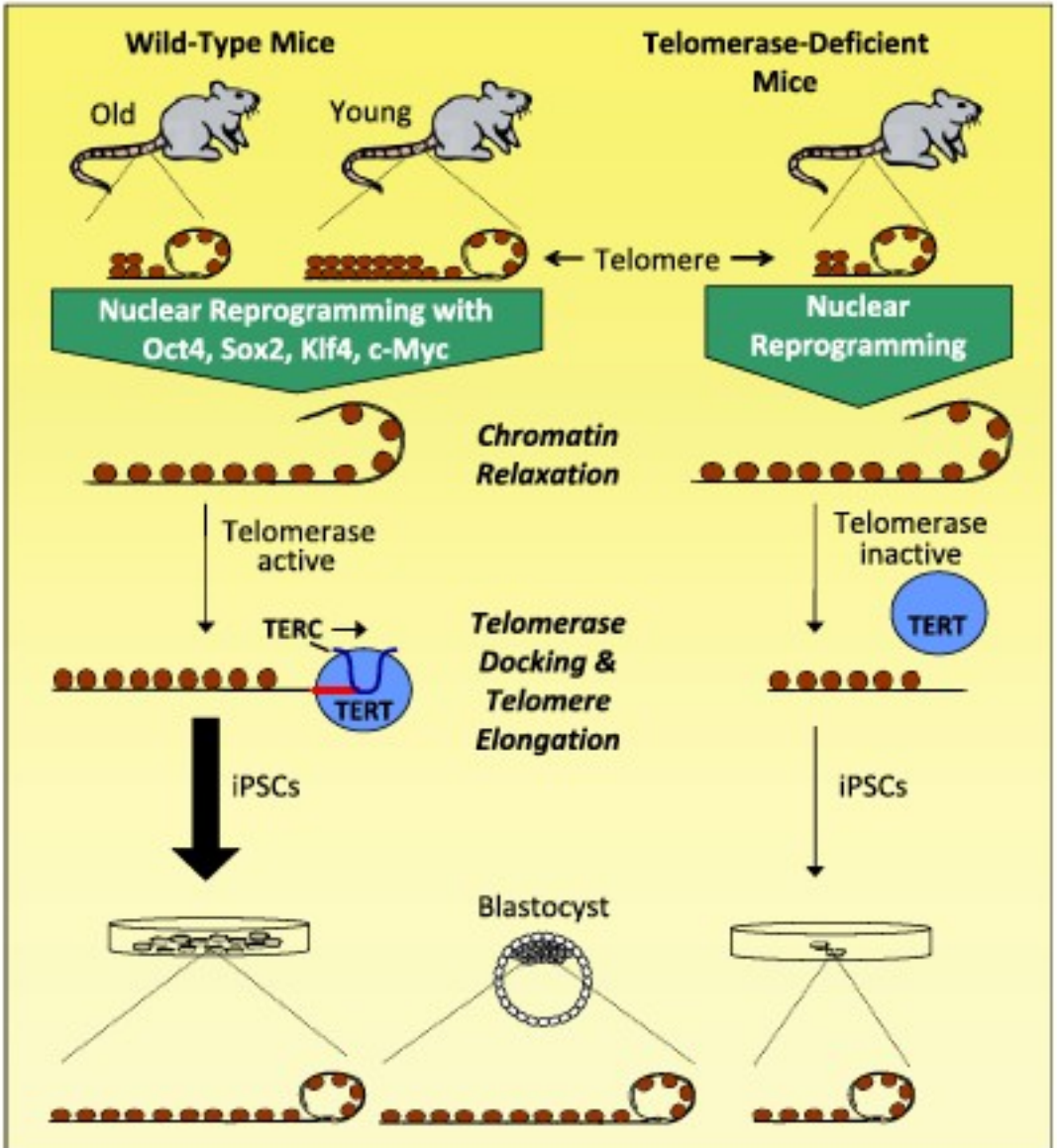
# Pluripotency of iPS cells: 6- Gene expression

ES MEF iPS

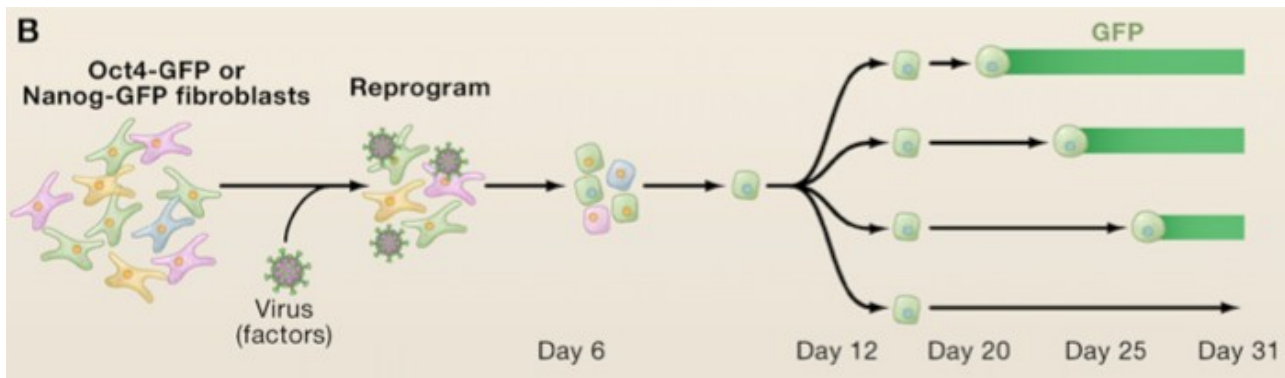
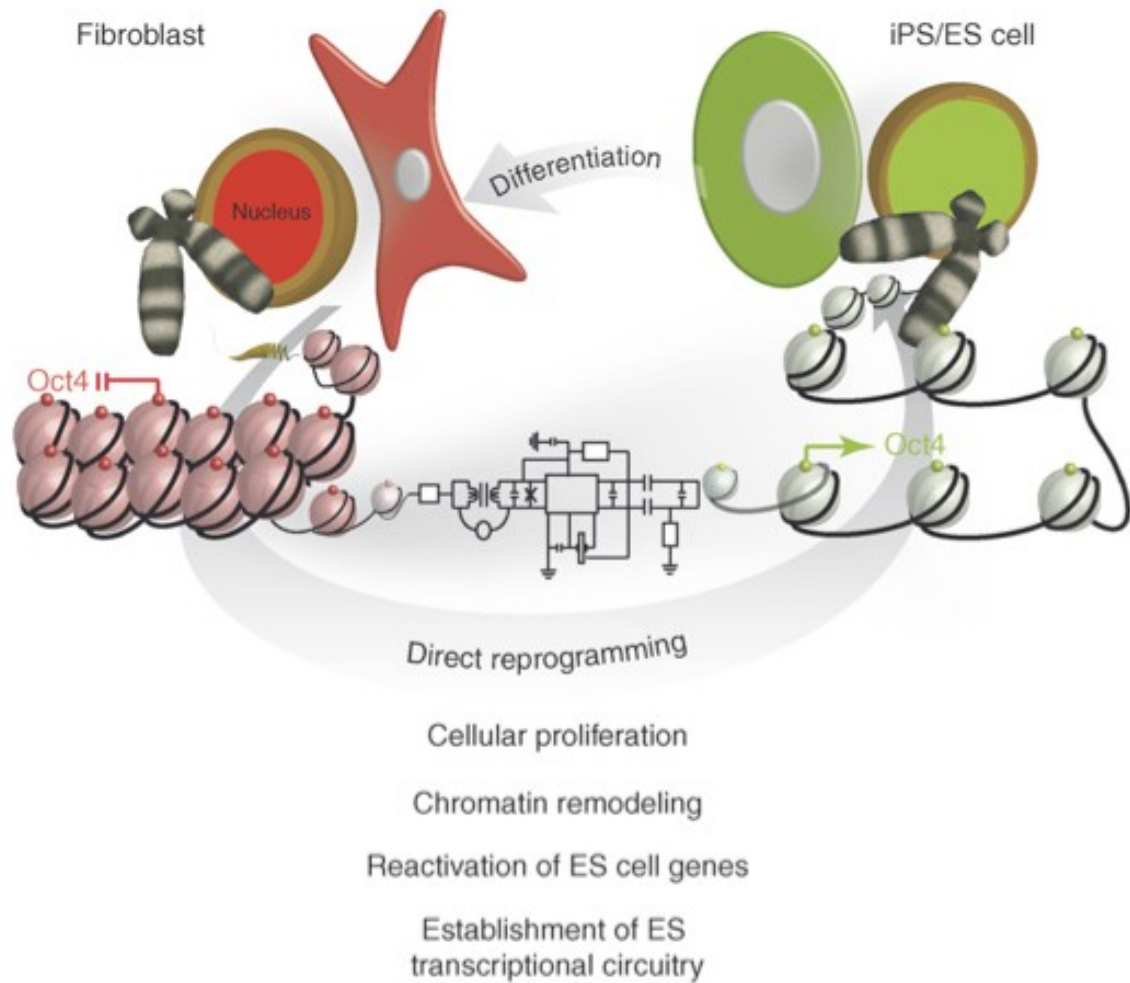


(Hochedlinger, *Cell Stem Cell* 2007)

(Daley et al, *Nature* 2008)



(Yamanaka, Cell 2007)



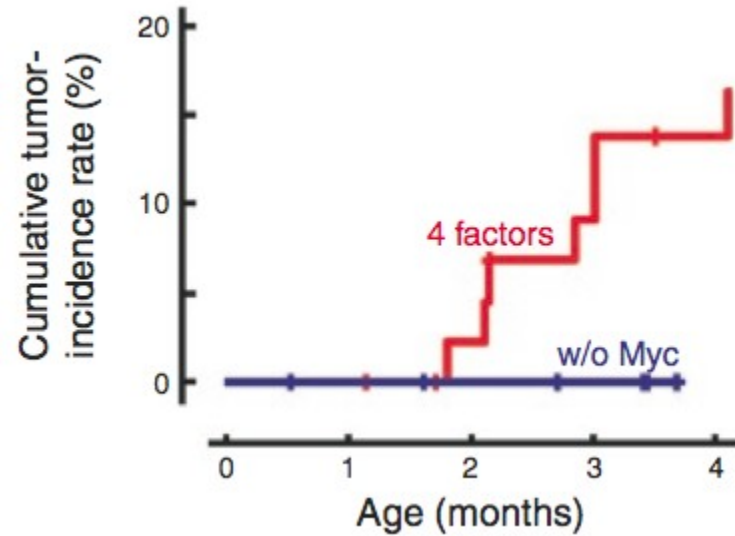
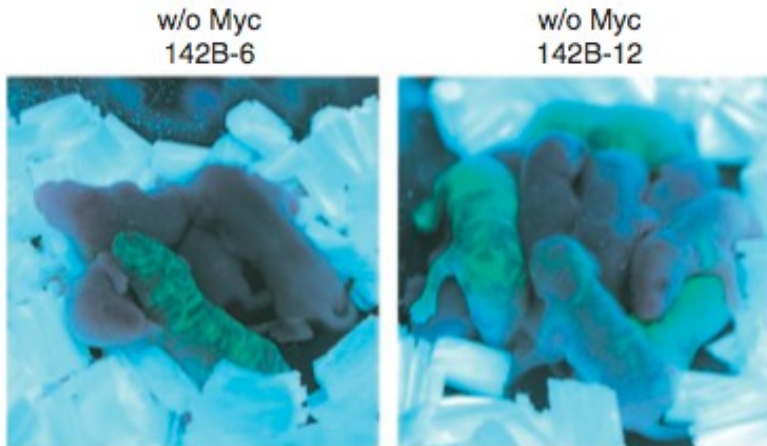
**Table 2****Reprogramming factors**

Factor	Description	Expression	Function	Dispensible/Replaceable for iPS generation?
Oct4	POU-domain containing transcription factor	Oocytes, fertilized embryo, ICM, epiblast, ES cells, EC cells, and germ cells	Crucial for the maintenance of pluripotency; homodimerizes and heterodimerizes with other co-factors such as Sox2 to regulate the ES cell state; deletion in ES cells results in loss of pluripotency and embryonic lethality (blastocyst stage)	No
Sox2	SRY, HMG-box DNA-binding protein	Oocytes, ICM, epiblast, germ cells, multipotent cells of extra-embryonic ectoderm, cells of neural lineage, brachial arches, and gut endoderm	Regulates the pluripotent state; deletion in ES cells results in loss of pluripotency; deletion in mouse results in embryonic lethality (E6.5) because of failure to maintain epiblast	Yes; Sox1, Sox3, Sox15, and Sox18 can replace Sox2 with reduced efficiency
Klf4	Member of the Kruppel-like factor family of transcription	Enriched in the gut, skin, and ES cells; also expressed in cells of the blood such as B cells and monocytes	Tumor suppressor or oncogene that functions in regulating cell differentiation, cell growth, and cell cycle; deletion in the mouse leads to death postnatally because of skin barrier deficiencies; mice also have an intestinal and hematopoietic phenotype	Yes; Klf2 reprograms with a similar efficiency
c-Myc	Basic helix-loop-helix transcription factor	Begins at the morula stage and to varying degrees in proliferating cells throughout development; multiple tissues including the heart, liver, intestine, spleen, kidney, lung, and mammary gland	Involved in cell cycle progression, apoptosis, and cellular transformation; loss of function in mouse leads to embryonic lethality (E10.5) because of defects in growth/cardiac and neural development; c-Myc null ES cells have impaired tumor progression	Yes; not necessary but affects kinetics and efficiency; replaceable with N-Myc, L-Myc to similar efficiencies
Nanog	Divergent homeodomain protein	Morula, ICM, epiblast, ES cells, and germ cells	Nanog is an important regulator of ES cell pluripotency and germ cell development; deletion in the mouse results in embryonic lethality (E5.5) and Nanog null ES cells lose pluripotency	Yes, replaced by c-Myc and Klf4 in human study and not required for reprogramming in either human or mouse
LIN28	Putative RNA-binding protein	Oocyte, zygote, blastocyst and to varying degrees during organogenesis; in adult, expressed in the bone, liver, and gonads	May regulate translation or stability of mRNA during differentiation; regulator of developmental timing; localizes to P-bodies (sites of mRNA and miRNA regulation)	Yes; improves efficiency of reprogramming in combination with Nanog, Oct4, and Sox2

*pluripotency**Cell cycle**pluripotency*

?

*RNA stability,  
Let-7g miR  
processing*



*Yamanaka, Nat. Biot, 2007*

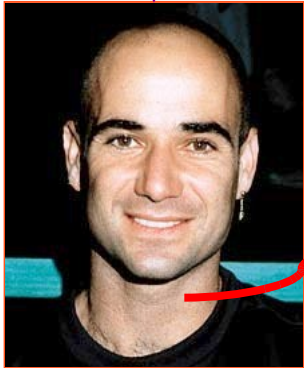
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- *Reduced efficiency*
- *Delayed reprogramming*

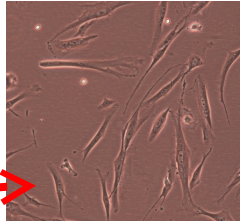
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- *Increased specificity*
- *No tumor*

**Pas de problème d'éthique**  
**Autogreffe (?)**



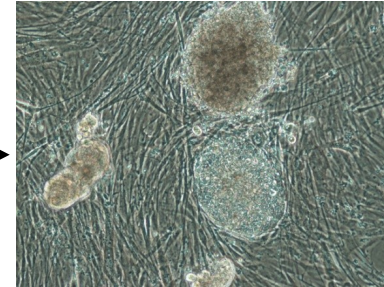
**neurogenerative  
disease**



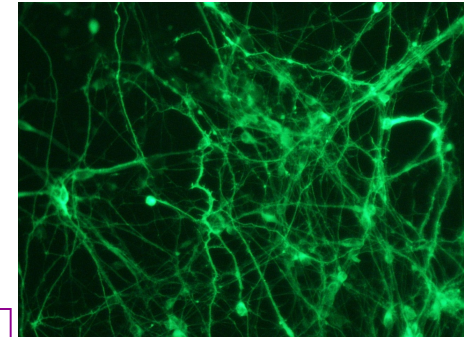
**cellules de  
patients**

**oct-4  
sox-2  
Klf-4  
c-myc**

**21-30  
jours**



**iPS**



**neurones**

**A résoudre:**

- 1. extinction des gènes**
- 2. c-myc**
- 3. épigénétique??**
- 4. coût**
- 5. allogénique (comme sang)**

# Low efficiency of reprogramming

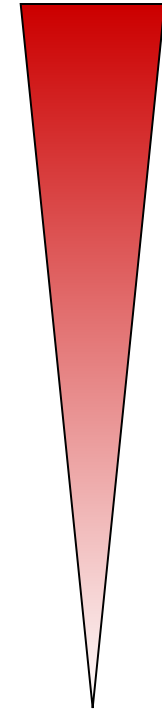
- **Differentiated hES** cells  
(CD45+ cells, fibroblasts-like) (0.1%)

- **Fetal** cells  
(MR90, Detroit551...)

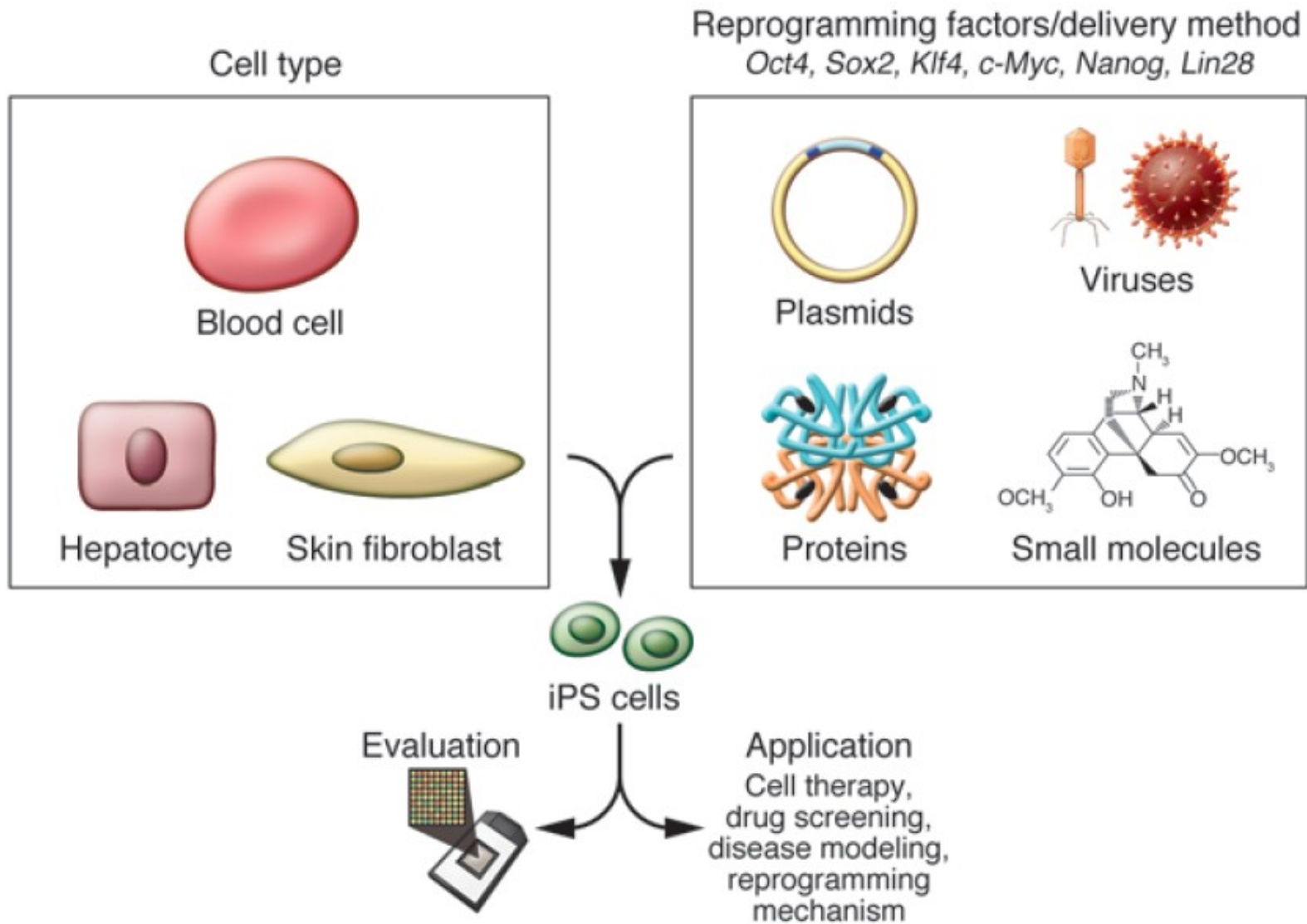
- **Neonatal** foreskin cells

- **Adult** dermal fibroblasts (0-0.02%)

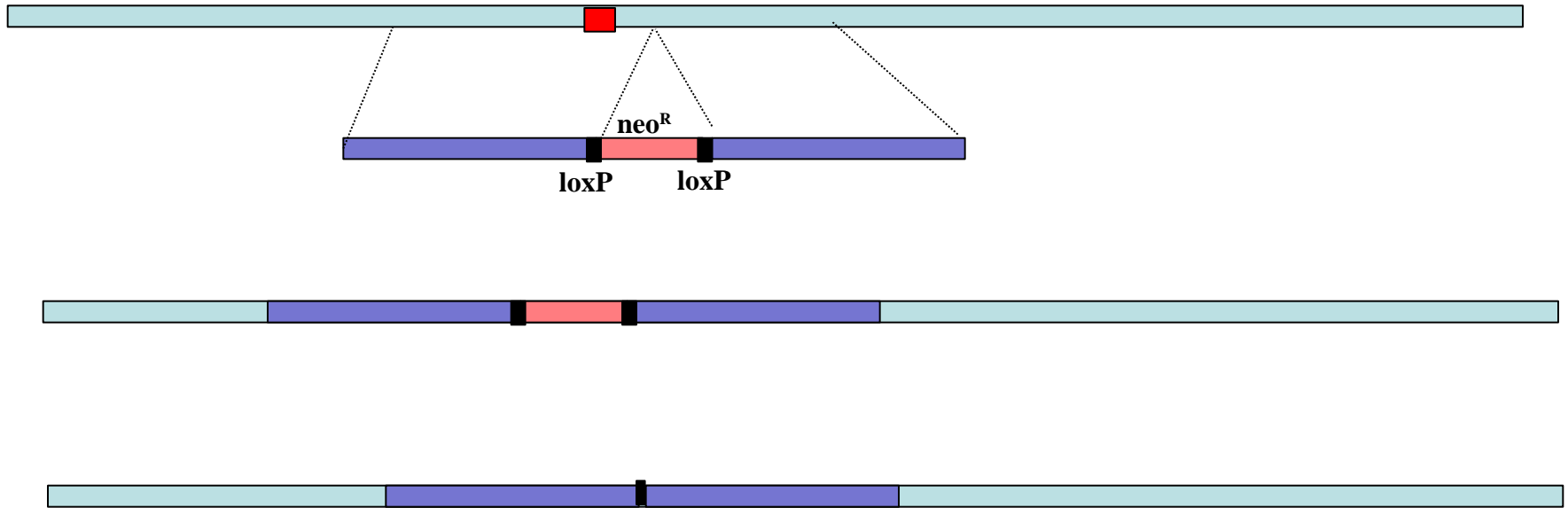
    Mouse B cells (3.3%): quite efficient?  
(MEF: 0.5%)



**Efficiency  
of  
reprogramming**

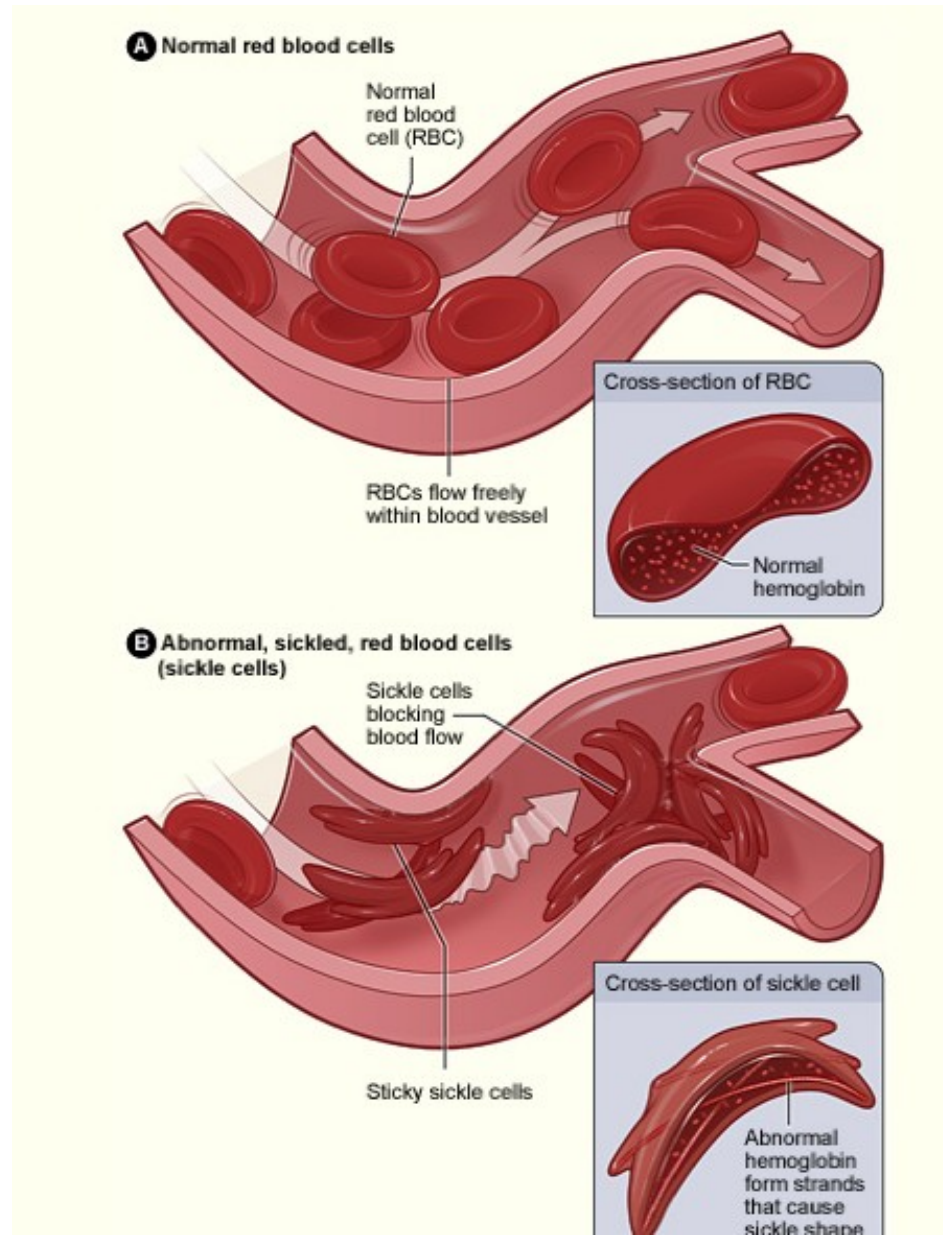


## Recombinaison homologue (KO)



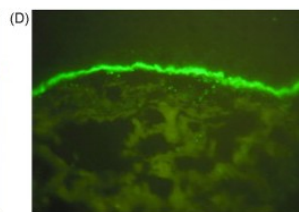
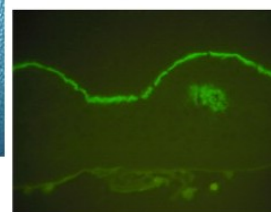
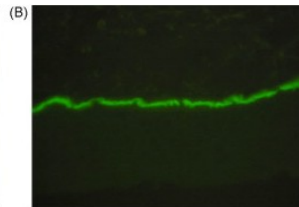
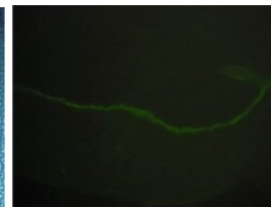
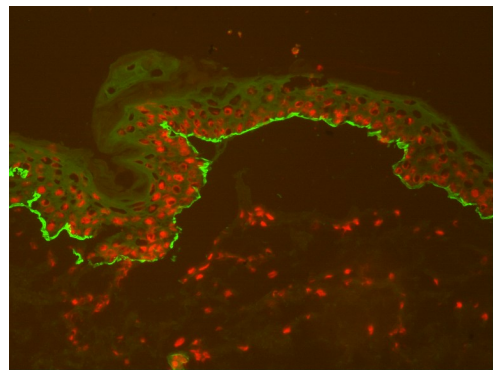
thérapie génique non insertionnelle ?

# anémie à hématies falciformes, drépanocytose





# Dystrophic Epidermolysis Bullosa



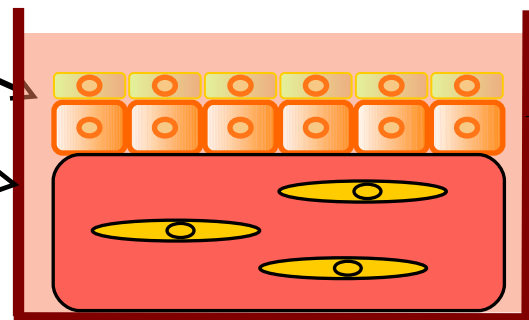
Coll. VII

DEB keratinocytes



R- DEB keratinocytes

DEB-f broblasts



H. R

DEB-f broblasts

DEB-iPS



R-iPS

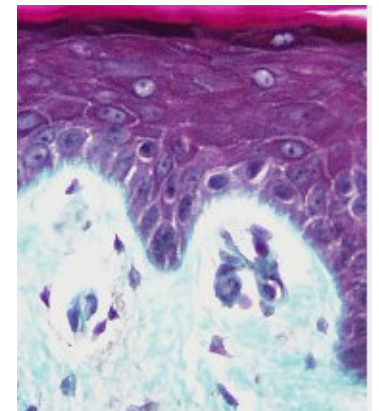
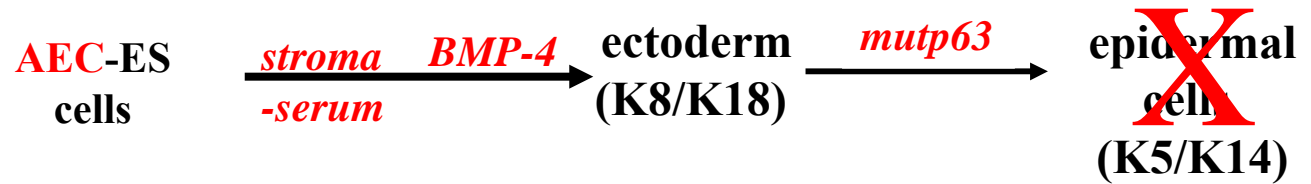
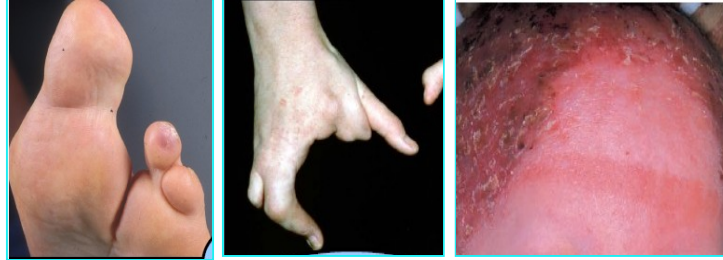
R-iPS-derived f broblast  
(coll. VII)

R-iPS-derived keratinocytes  
(coll. VII)

DEB-keratinocytes

- A résoudre:**
1. c-myc, rétro,...
  2. HR effect?
  3. épigénétique (ageing) ??
  4. coût

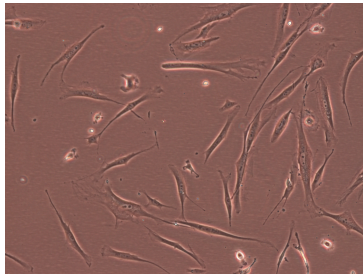
# Cellular models: Human AEC-iPS (R304W)



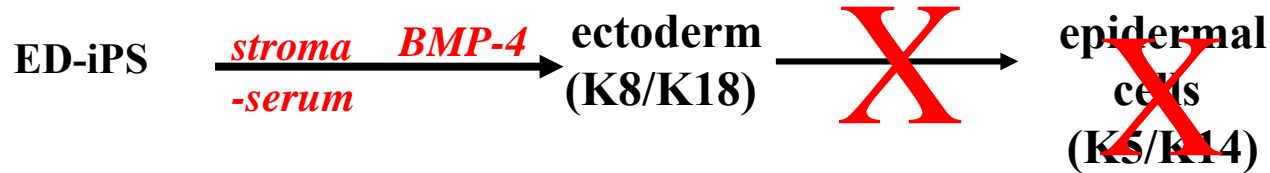
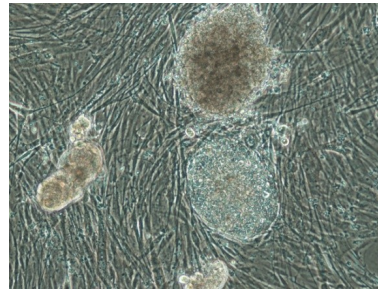
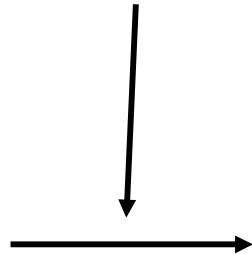
# Cellular models: Human AEC-iPS (R304W)



ED (R304W)  
f broblasts



H. van Bokhoven

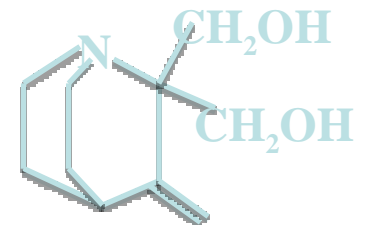


*Nature Medicine* **8**, 282 - 288 (2002)  
doi:10.1038/nm0302-282

**Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound**

Klas Wiman (Sweden)

PRIMA-1

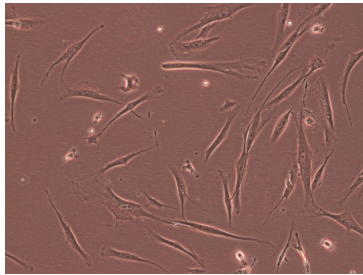


Isabelle Petit

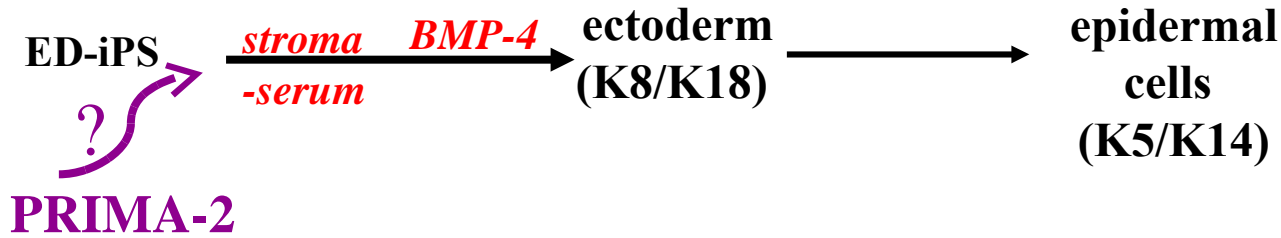
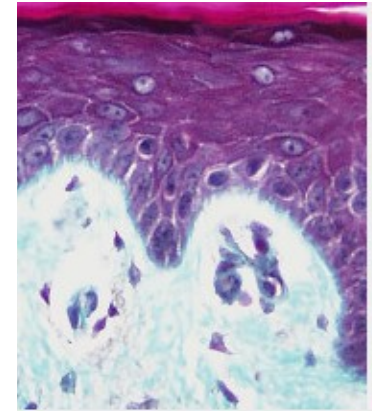
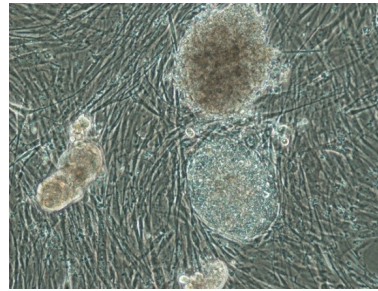
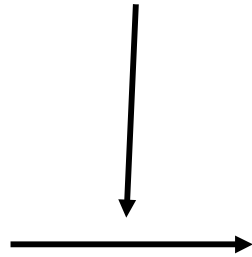
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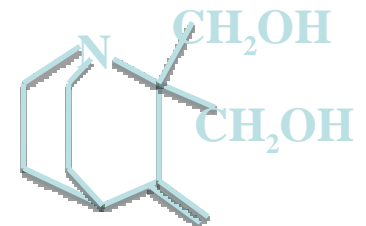


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