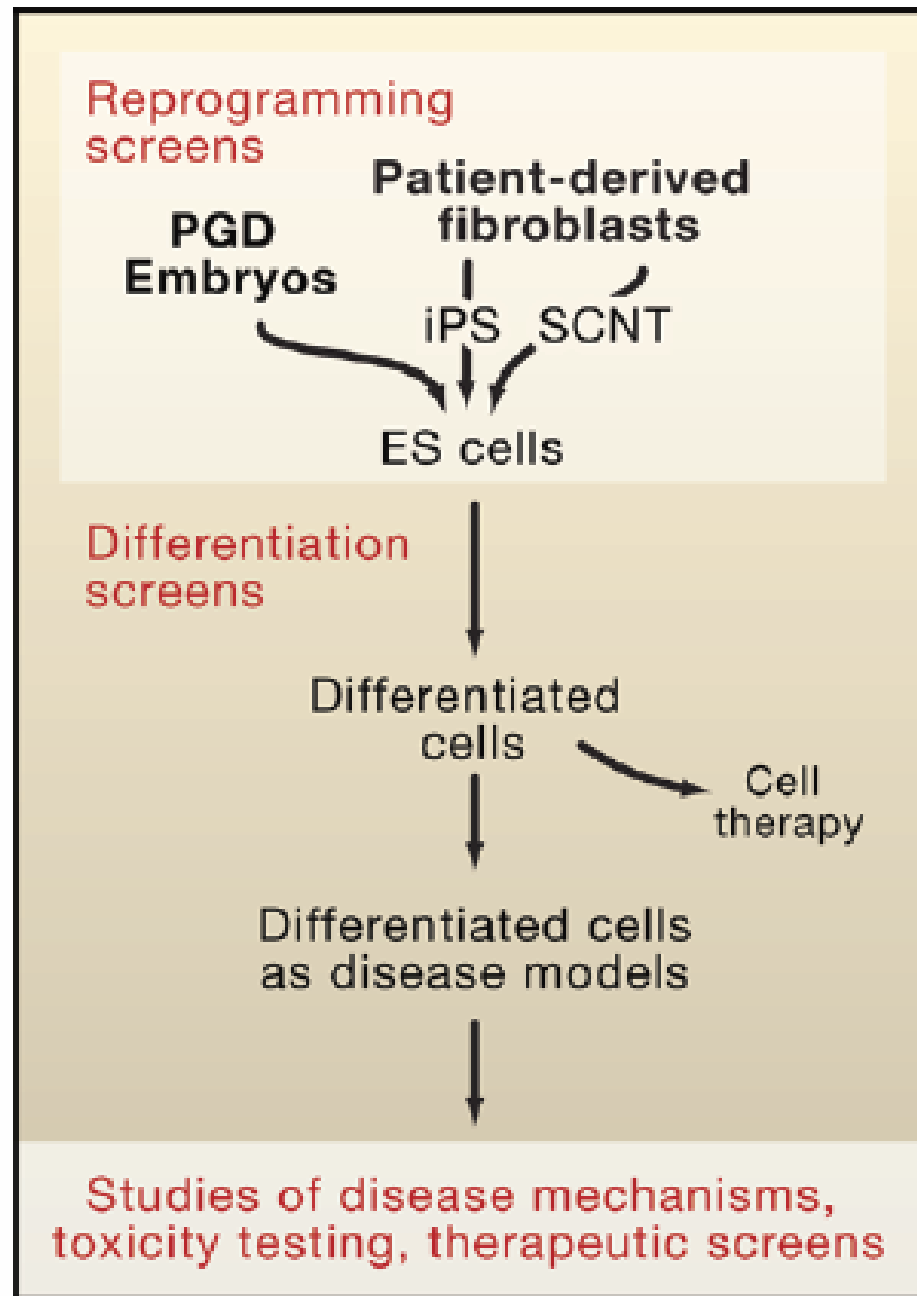


"Le cellule embrionali staminali come sorgenti per la riprogrammazione ed il differenziamento cellulare"

7 marzo 2008

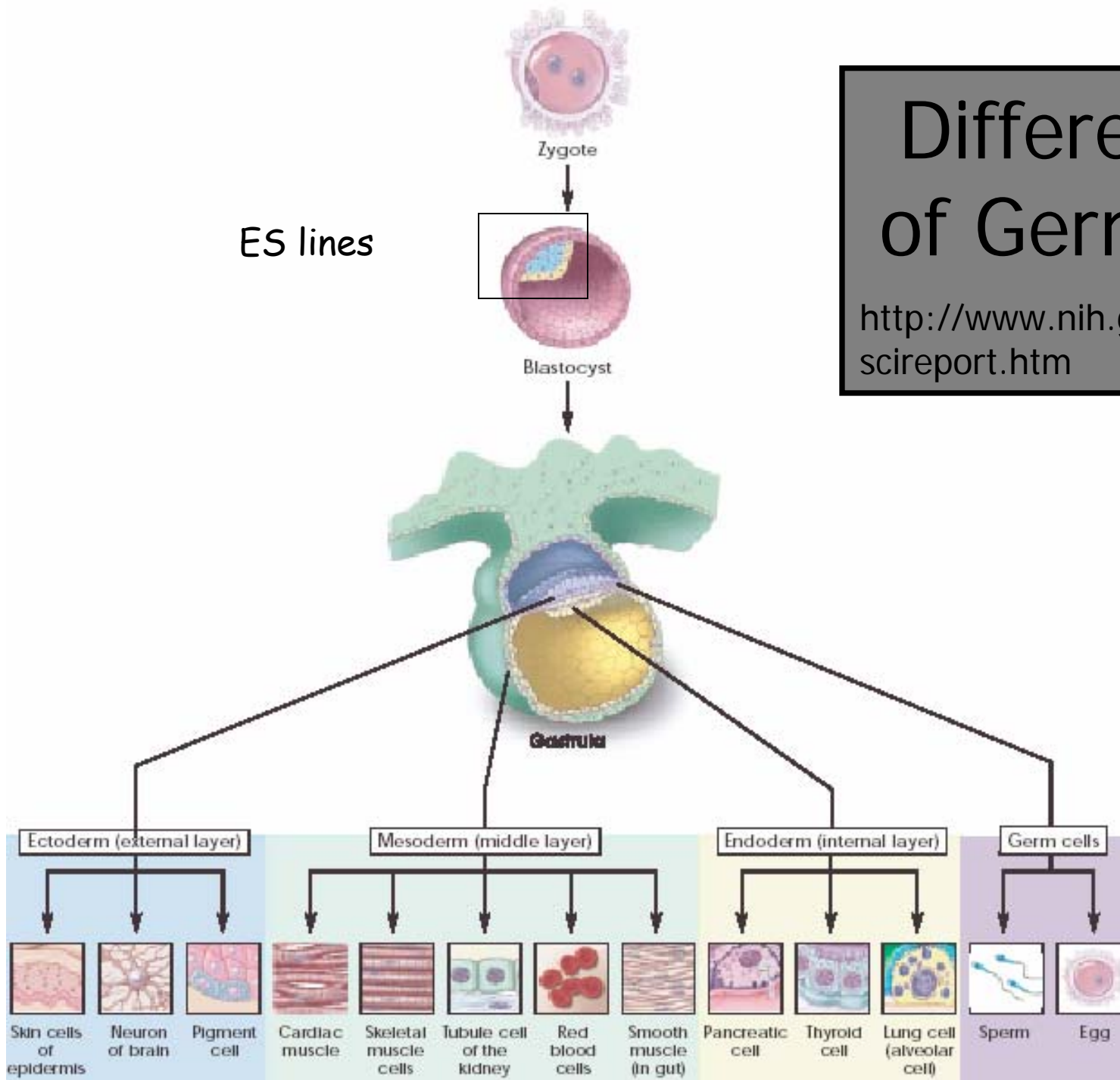


Potency	Sum of developmental options accessible to cell
Totipotent	Ability to form all lineages of organism; in mammals only the zygote and the first cleavage blastomeres are totipotent
Pluripotent	Ability to form all lineages of body. Example: embryonic stem cells
Multipotent	Ability of adult stem cells to form multiple cell types of one lineage. Example: hematopoietic stem cells
Unipotent	Cells form one cell type. Example: spermatogonial stem cells (can only generate sperm)
Reprogramming	Increase in potency, dedifferentiation. Can be induced by nuclear transfer, cell fusion, genetic manipulation
Transdifferentiation, plasticity	Notion that somatic stem cells have broadened potency and can generate cells of other lineages, a concept that is controversial in mammals

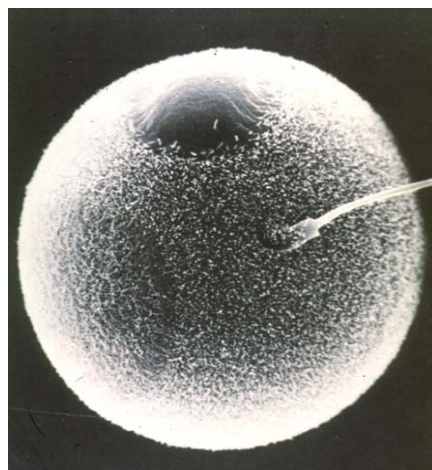
Differentiation of Germ Layers

<http://www.nih.gov/news/stemcell/scireport.htm>

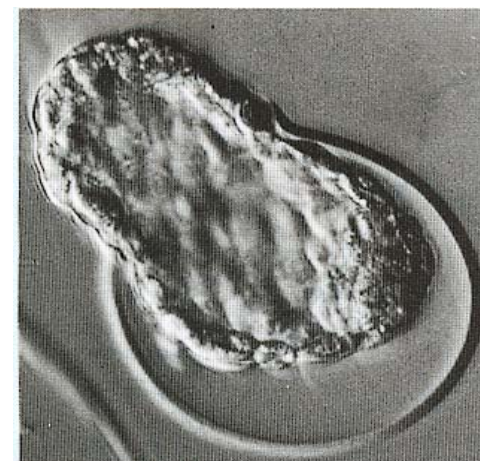
ES lines



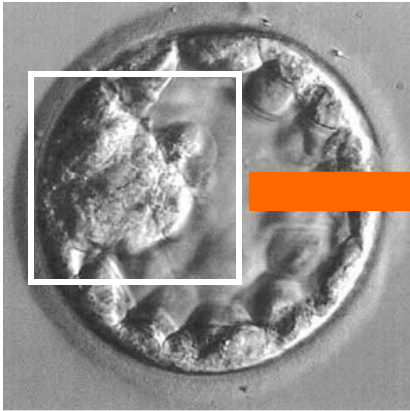
- Fecondazione in vivo - in vitro
- ICSI
- Trasferimento nucleare
- Partenogenesi



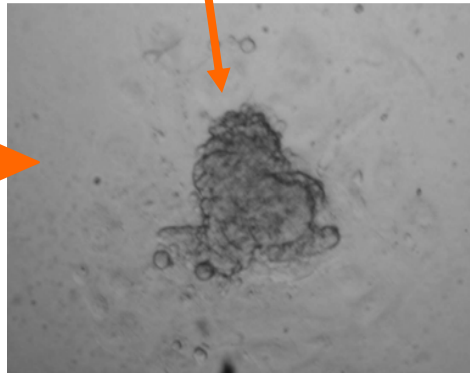
Blastocisti



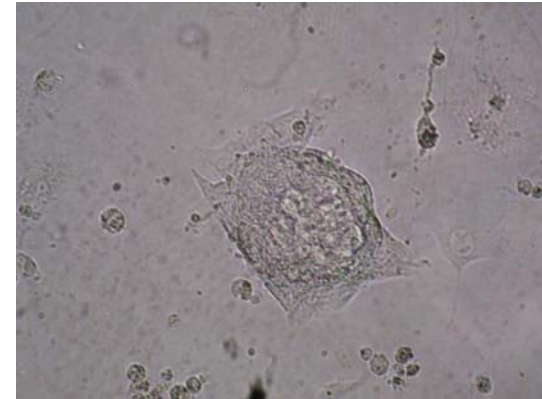
Blastocisti



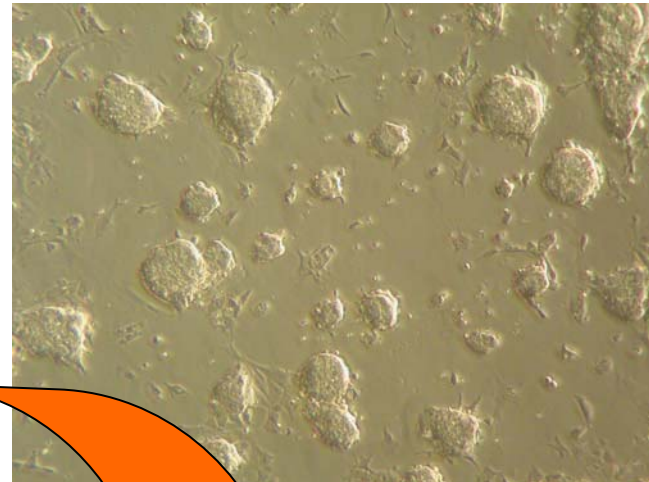
**Distacco della massa
cellulare interna**



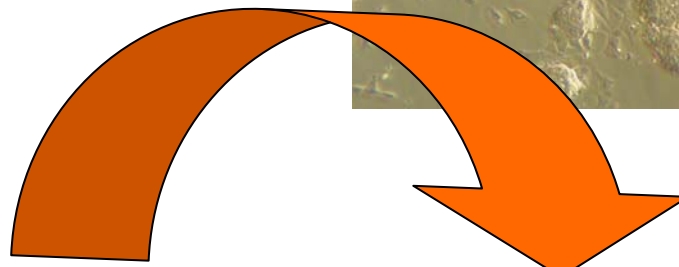
Prima colonia di ES



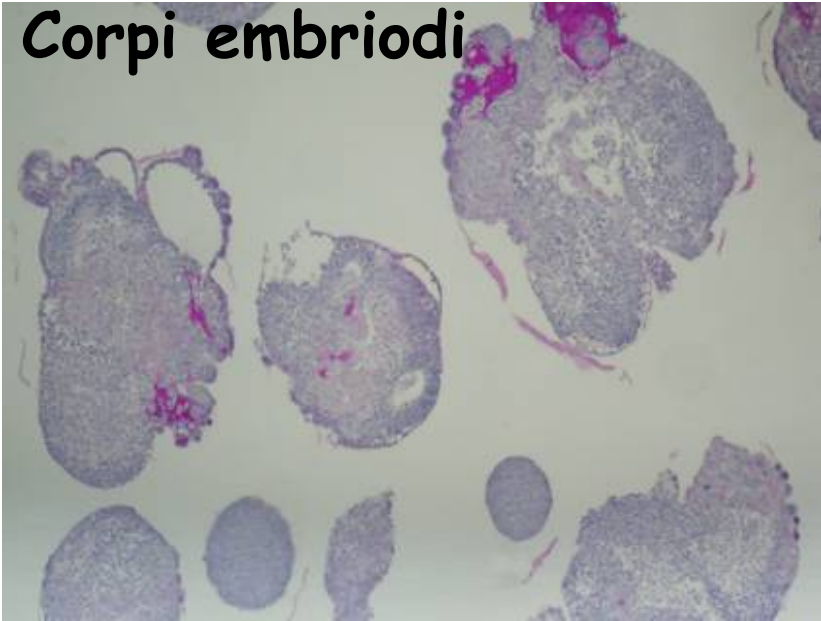
Linea di ES



**Differenziamento
in:**



Corpi embriodi



**Differenziamento e
meccanismi di sviluppo
della malattia**



Megacariociti

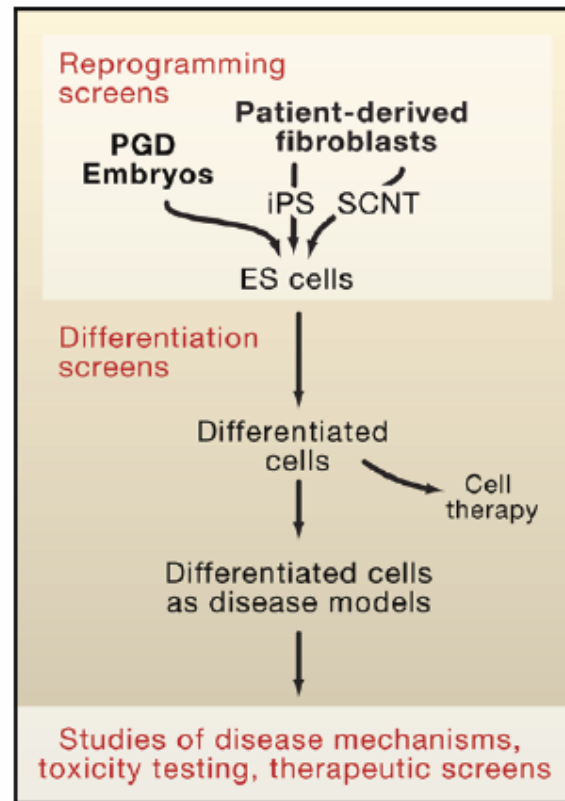


Cardiomiociti

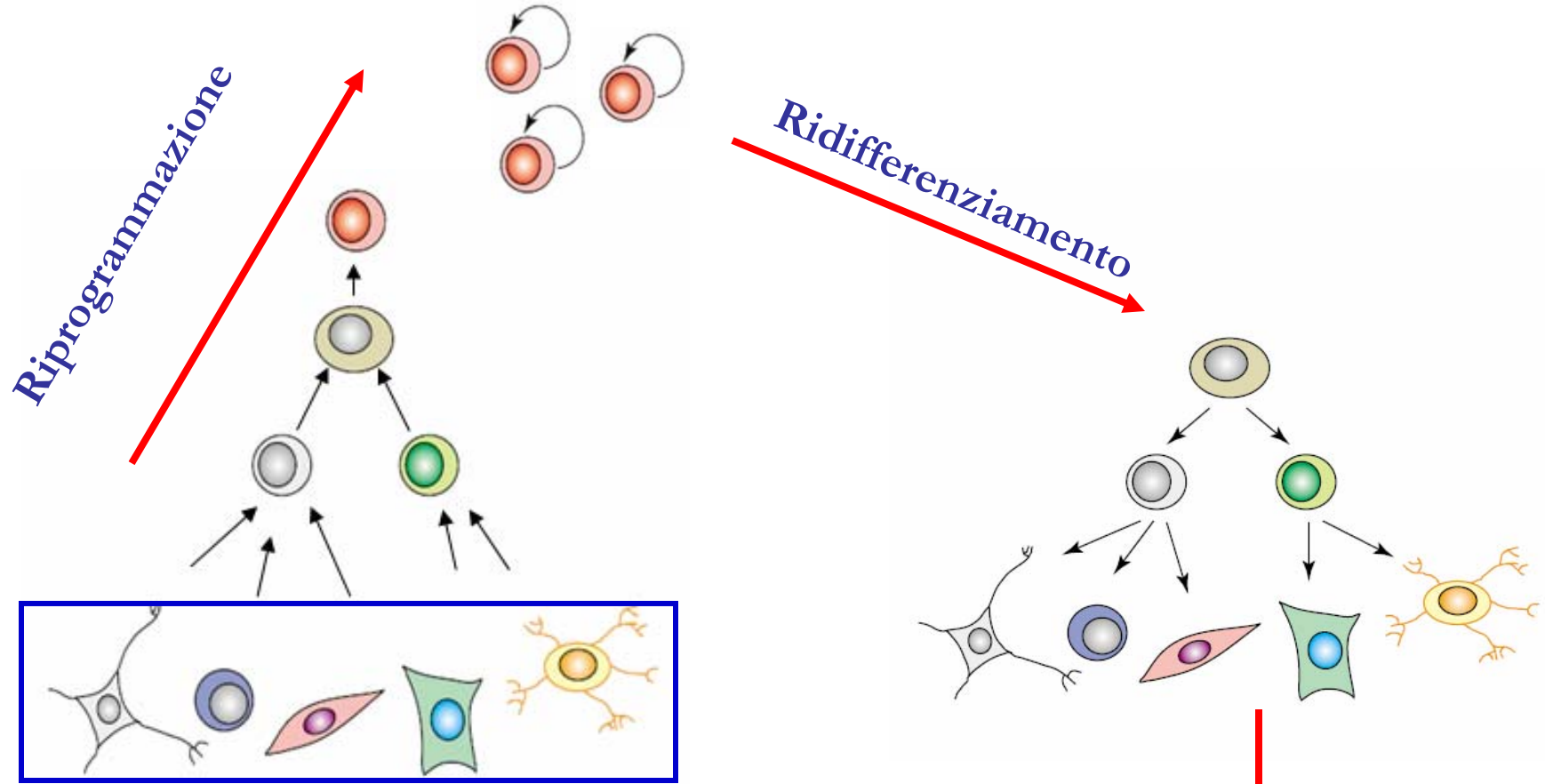
**Effetti della TCDD sul
differenziamento**

•Riprogrammazione di cellule differenziate

riprogrammazione di fibroblasti mediante estratti di cellule ES al fine di far assumere loro una condizione di pluripotenza ES-simile



Il dedifferenziamento e la riprogrammazione cellulare



Biopsia da un paziente

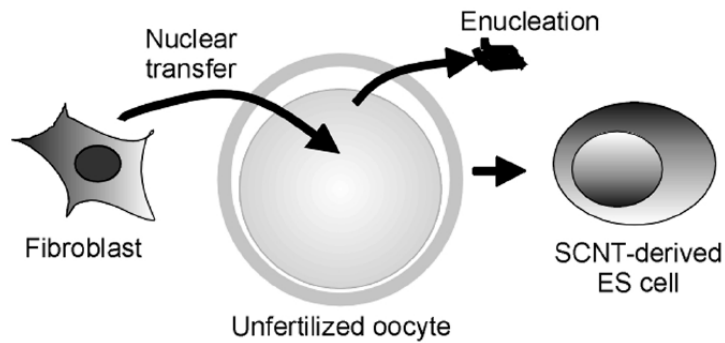


Trapianto

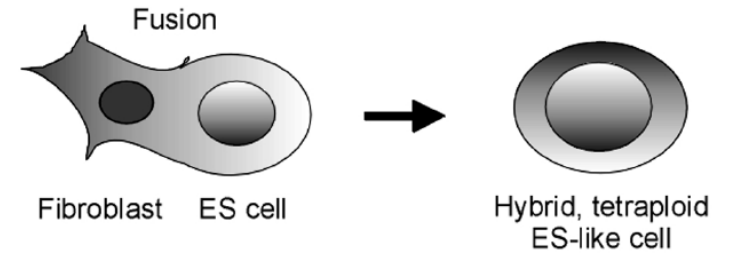
Non c'è rigetto

Diversi metodi per riprogrammare

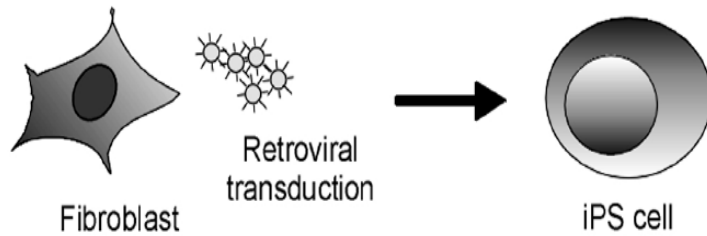
Nuclear transplantation



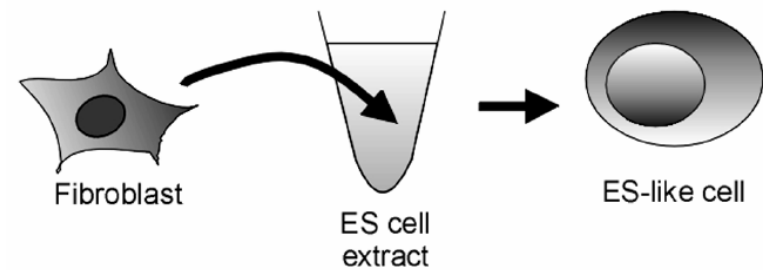
Fusion with ES cell



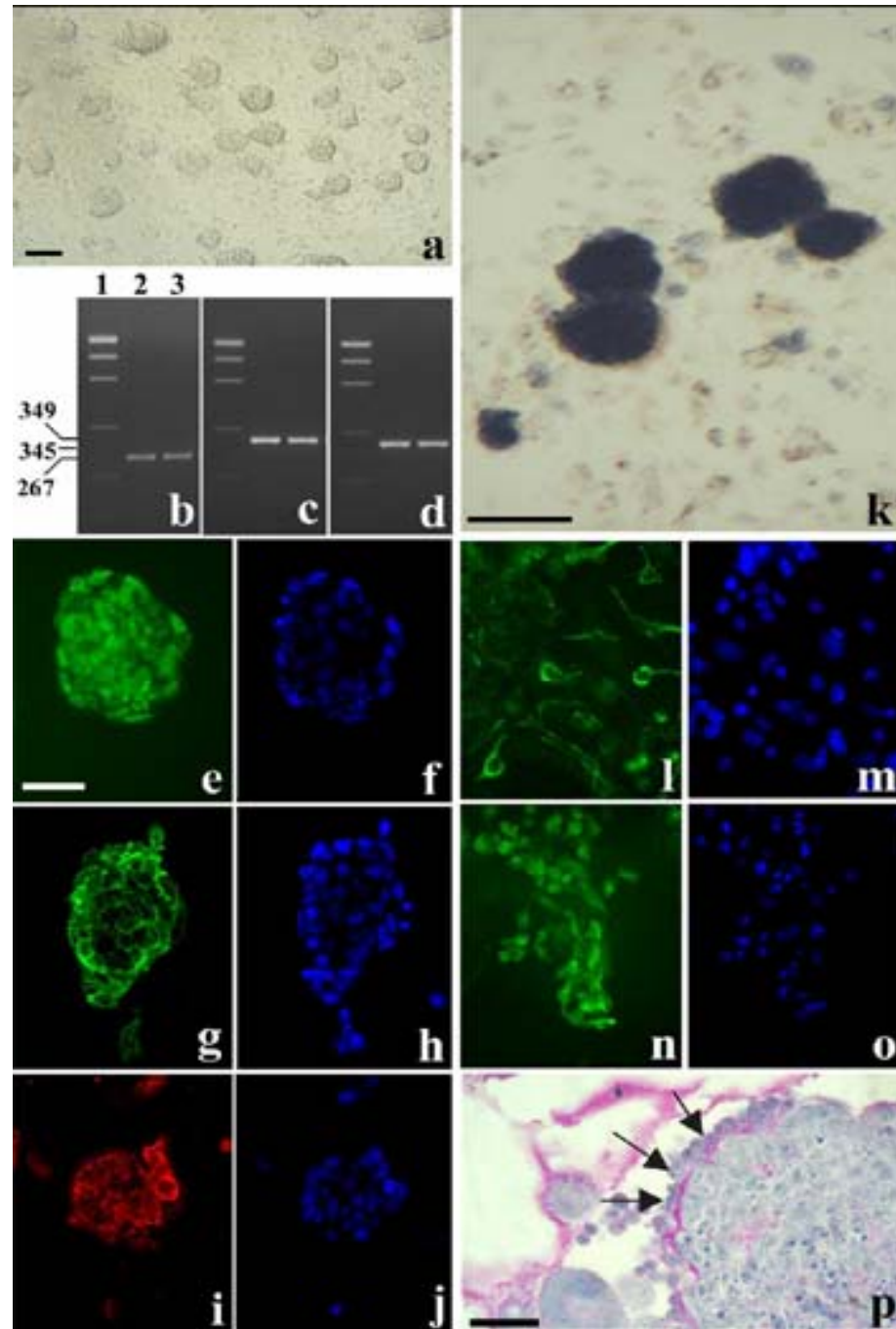
Retroviral transduction of pluripotency genes



Treatment with ES cell extract

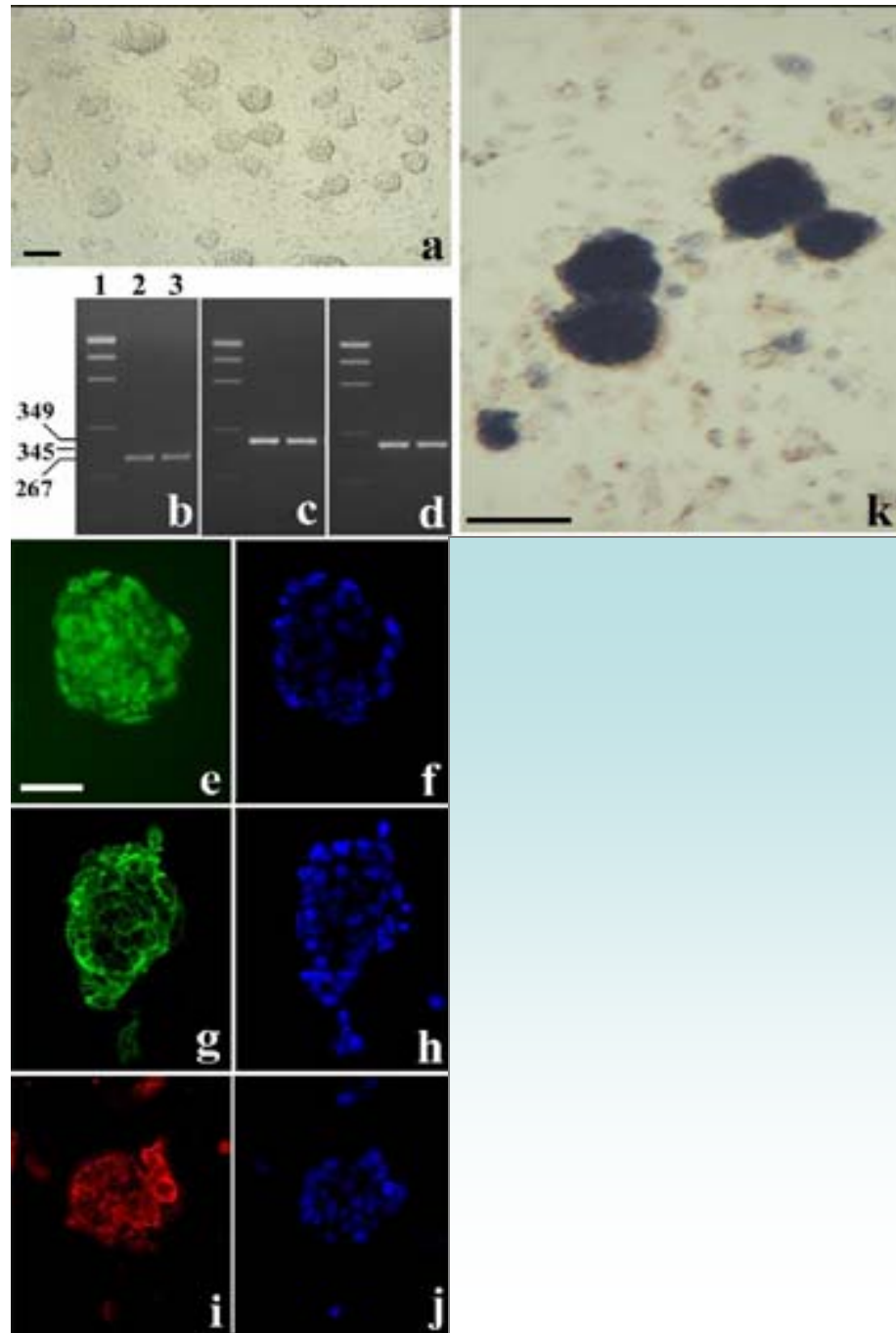


ES cell lines characterization

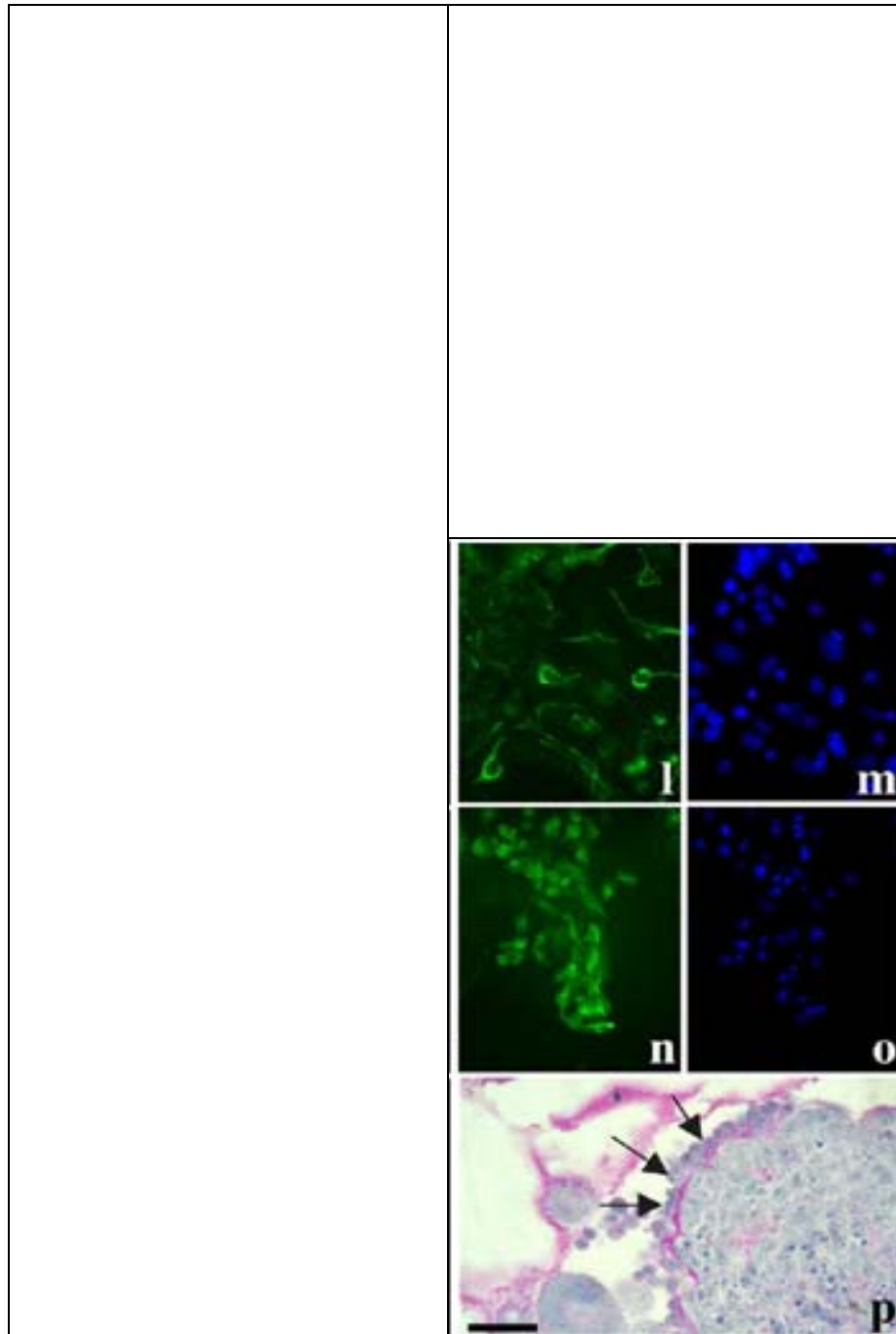


-undifferentiate state

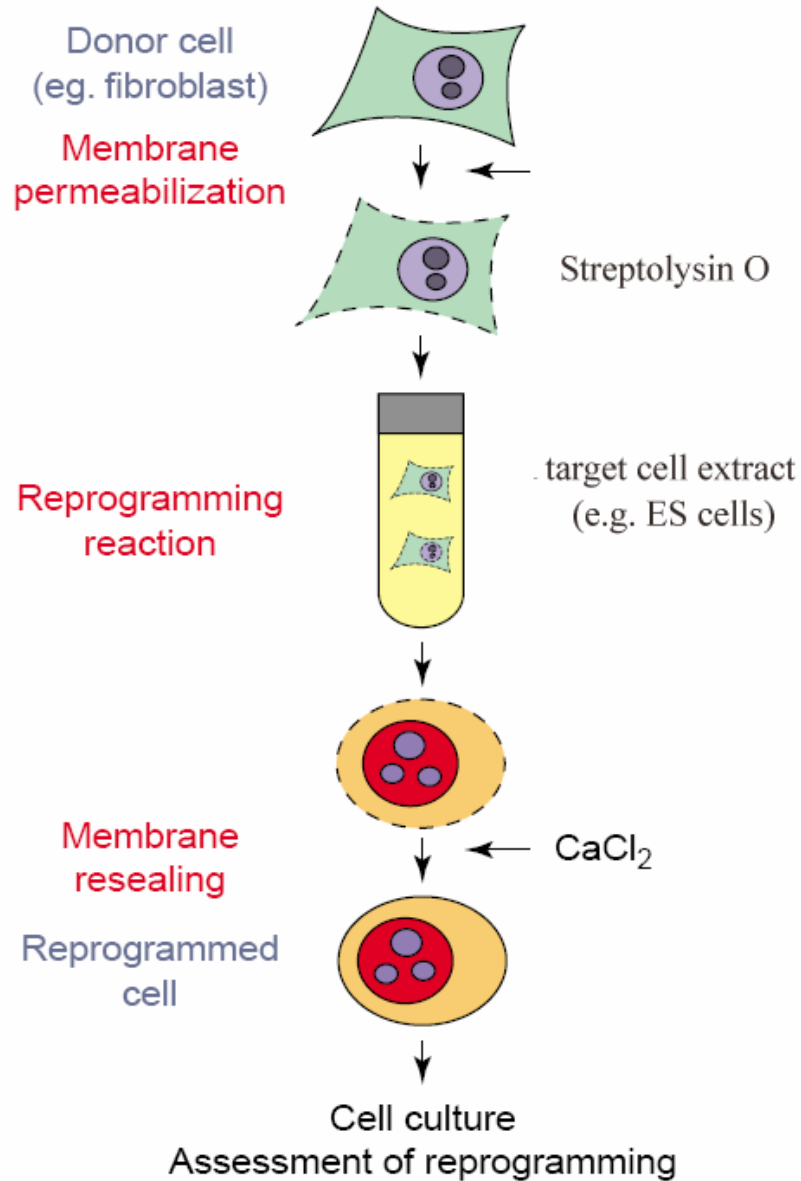
-Differentiation



-Differentiation



Fibroblasts reprogramming di by ES extracts



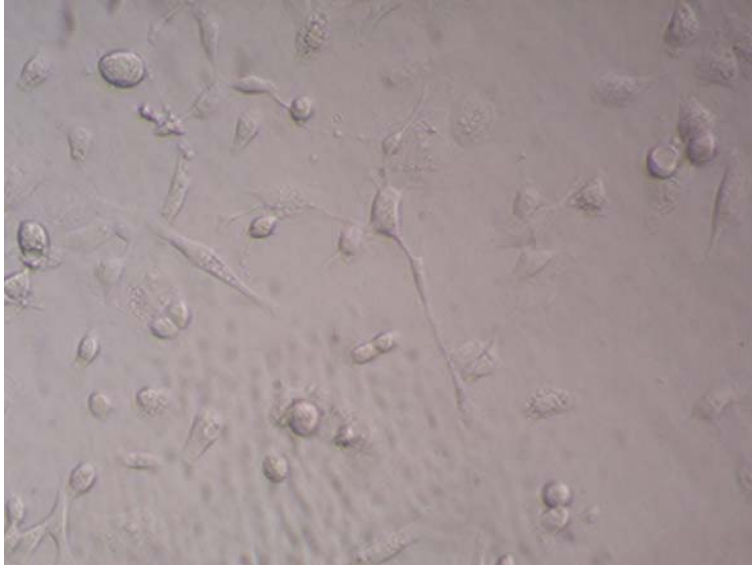
•Due linee cellulari: **STO**

NIH-3T3

•**Fibroblasti fetali**

riprogrammazione di fibroblasti STO

*Contengono un gene per la resistenza alla geneticina (G 418)
producono LIF*



-Trattati con SLO e estratto di ES (**SLO**)

-Coltivati in terreno ES in presenza di geneticina per 10 settimane (3 esperimenti indipendenti)

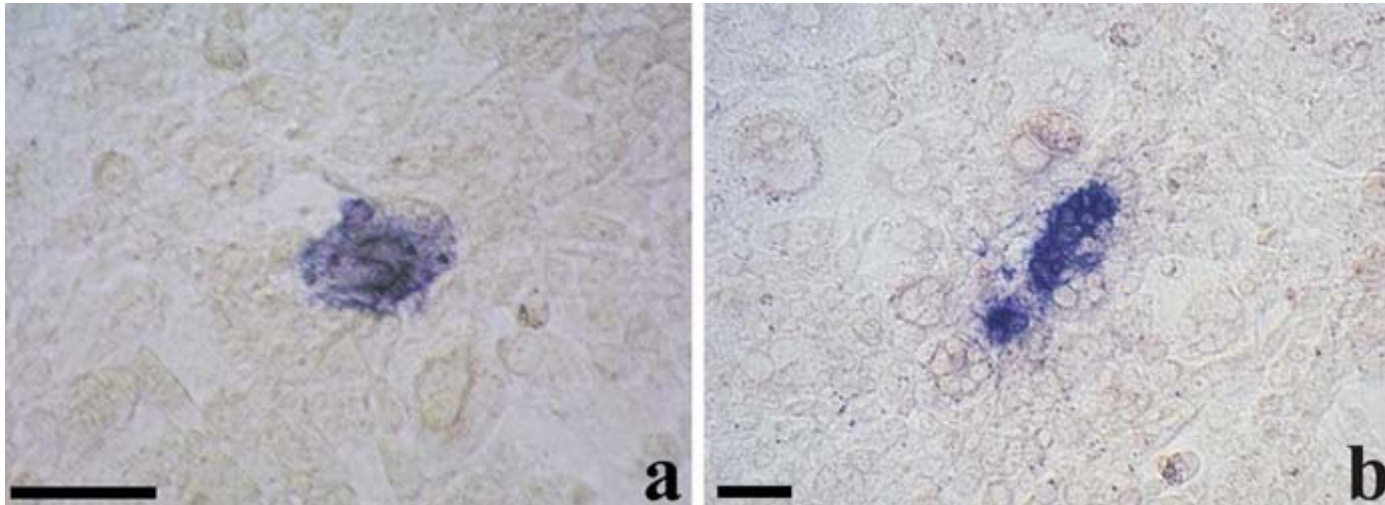
In parallelo sono stati piastrati i controlli

-STO coltivati in terreno ES (**azione terreno**)

-STO trattati con SLO e estratto e coltivati in terreno STO (**azione terreno**)

-STO non trattati con SLO e trattati con estratto, coltivati in terreno ES (**NO-SLO**) (**azione SLO**)

Alkaline phosphatase (4 weeks)



a) single cell or doublets

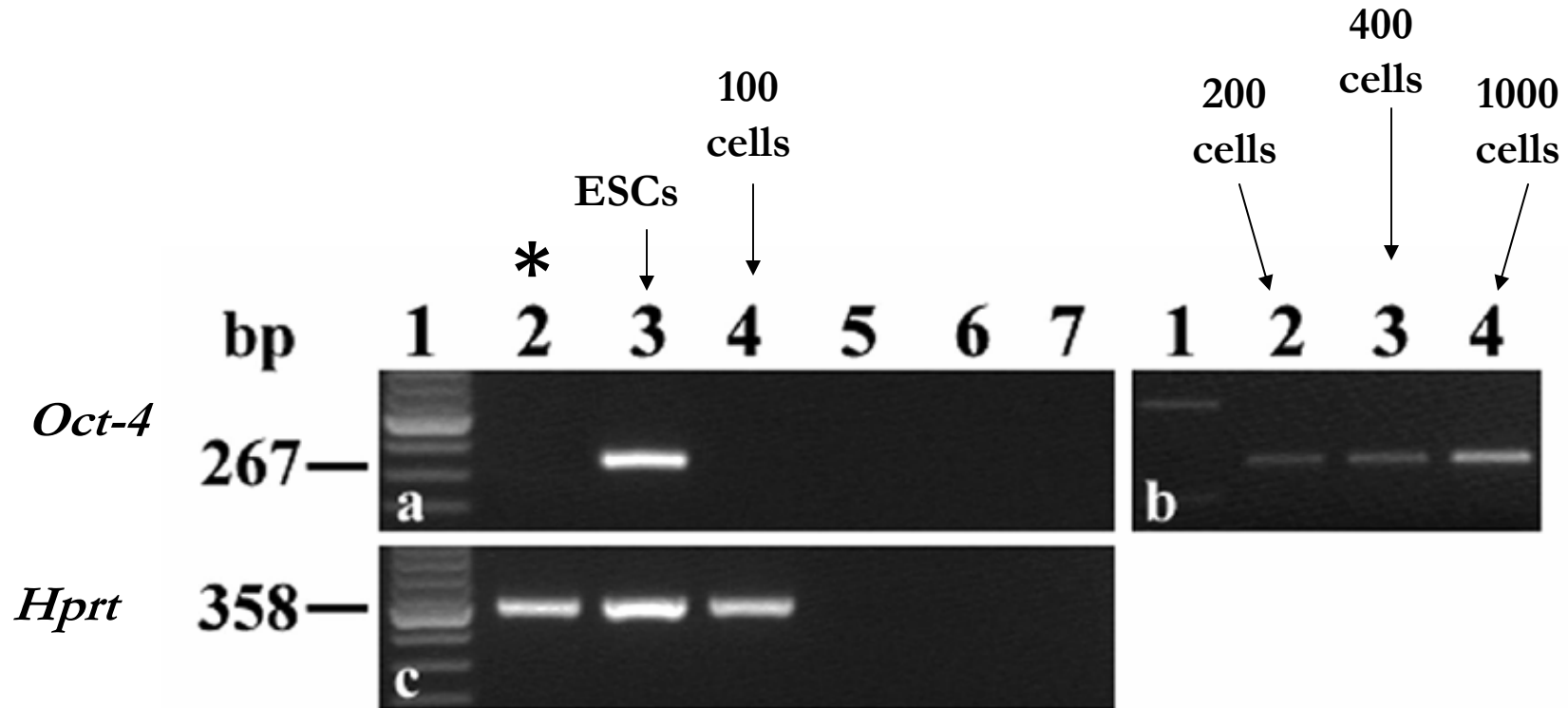
b) small colonies

-SLO: positivity 0.004%-0.04%

-NO-SLO: positivity 0%-0.004%

What's about RT-PCR sensitivity?

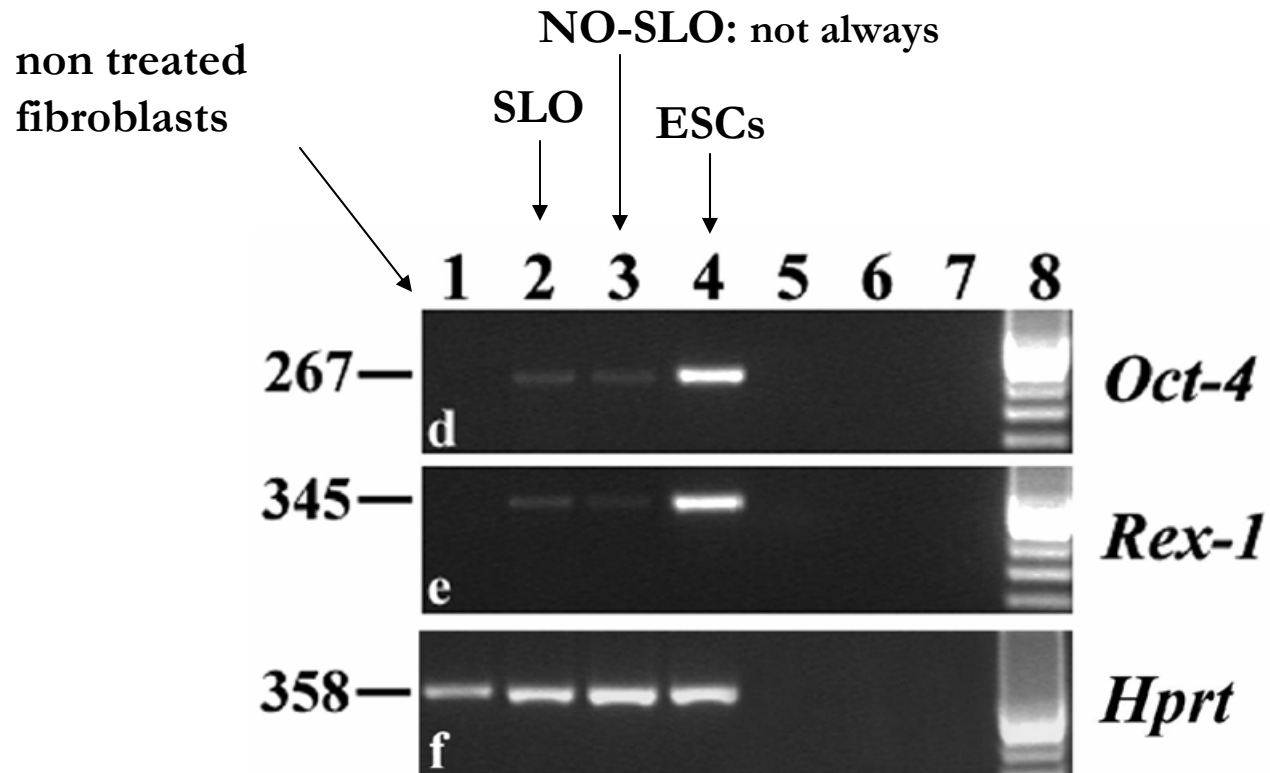
Different number of ES in 700,000 STO :



Oct-4, *Rex-1* e *Nanog* are detectable when ESCs are at least 200 cells in 700,000 fibroblasts (0.003%)

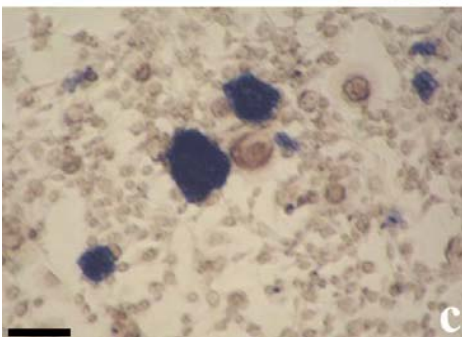
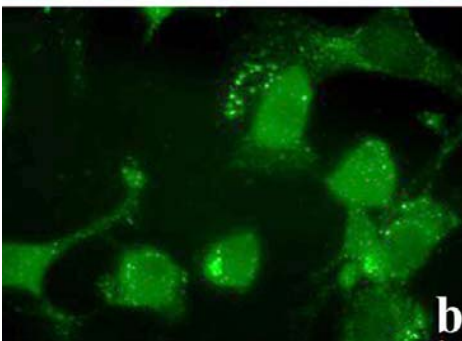
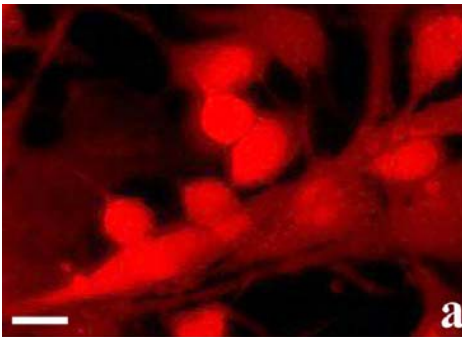
*negative control Fibroblasts cultured in STO medium

RT-PCR stemness genes detection (4 weeks)



Possono esserci contaminazioni da ESCs nell'estratto?

senza geneticina: fino all'80% di cellule positive per OCT-4 (a), SSEA-1 (b) e fosfatasi alcalina (c).

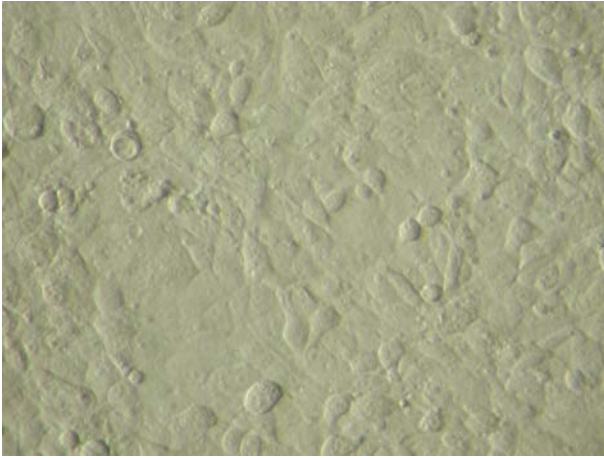


Geneticina (0.2 mg/ml)

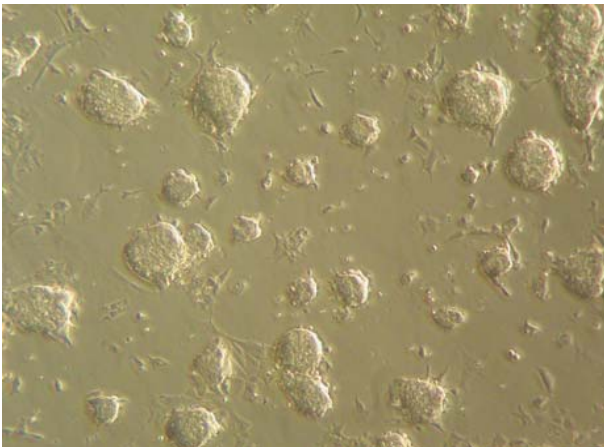


a 2 settimane dal trattamento: nessuna positività

reprogramming fibroblasts NIH-3T3

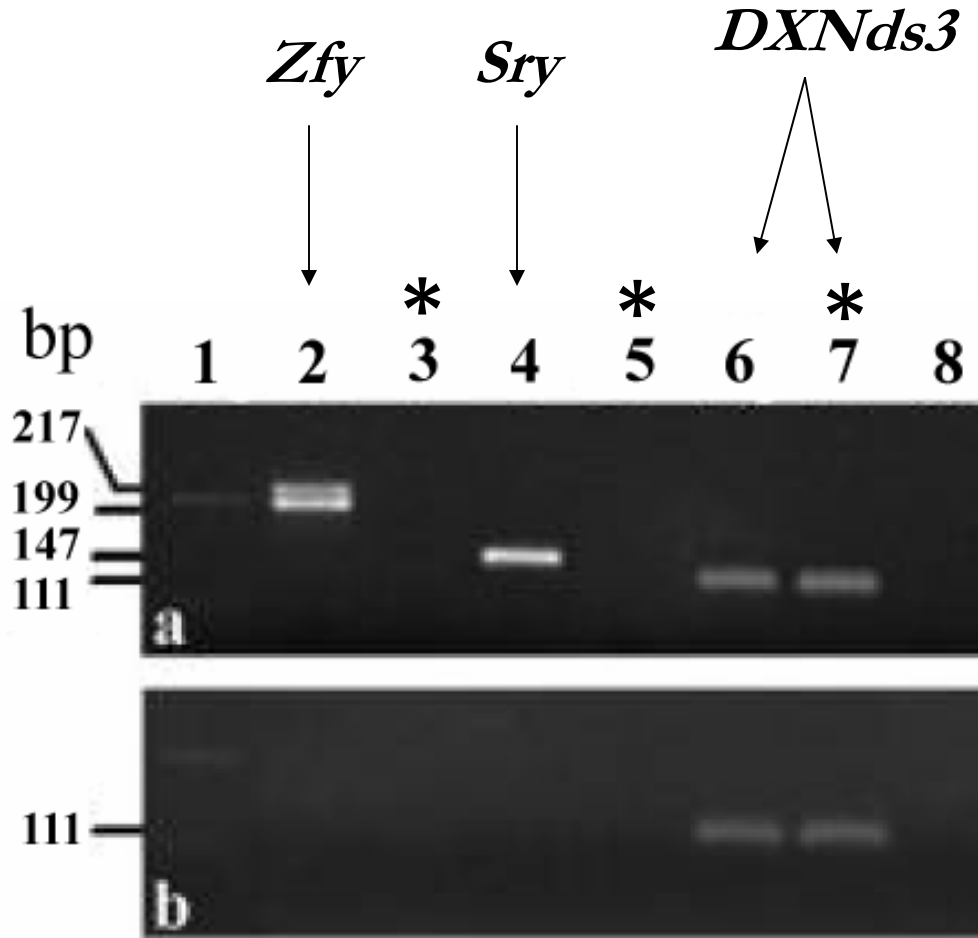


NIH-3T3: from a mixed population we derived clones **XX**



ES: derived from blastocysts **XY**

Estracts contamination by ESCs ?

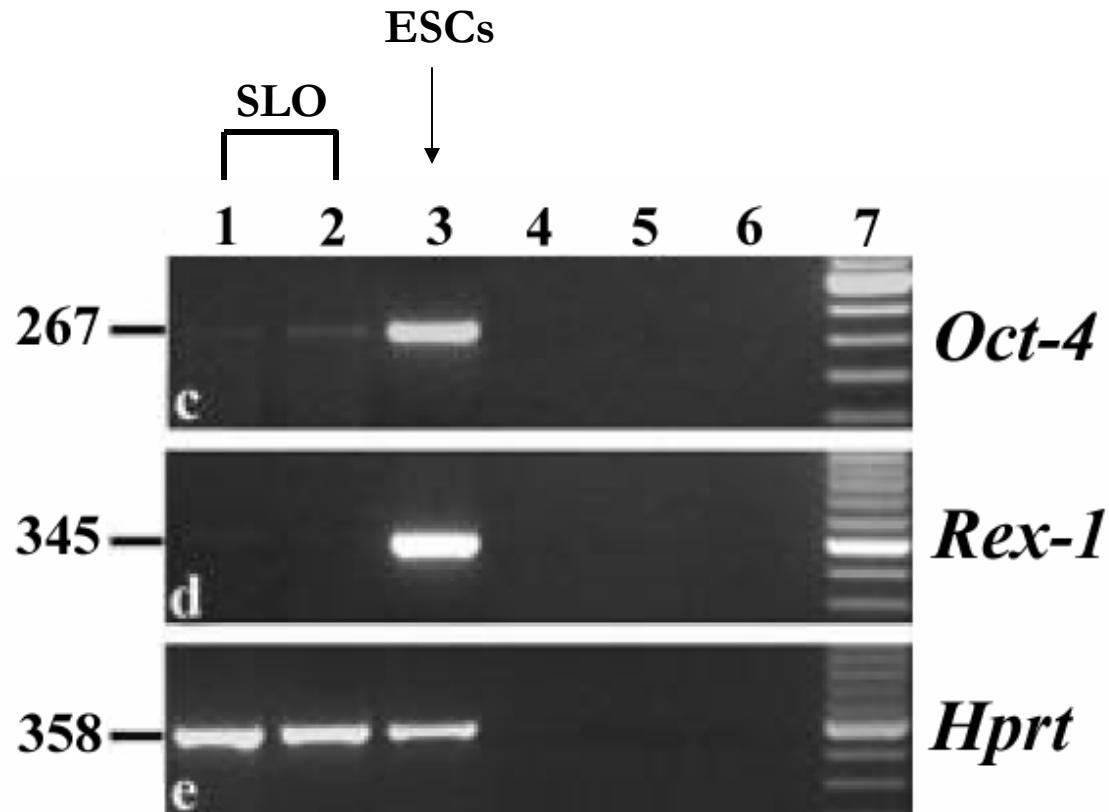


Y-linked genes before stemness genes analysis

OK

* non treated fibroblasts, negative controll (XX)

RT-PCR analysis of stemness genes

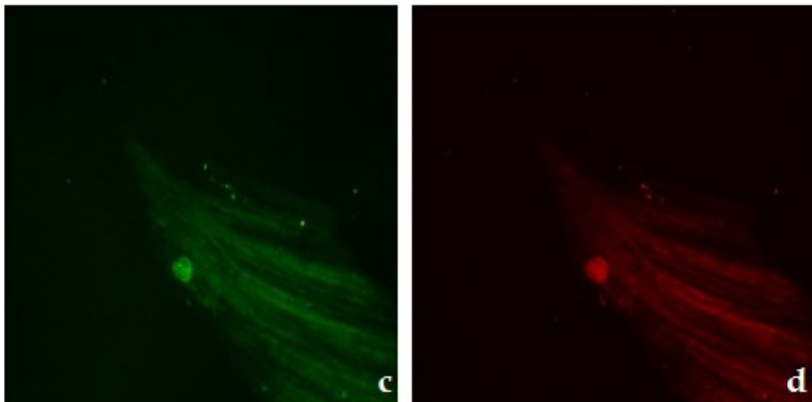
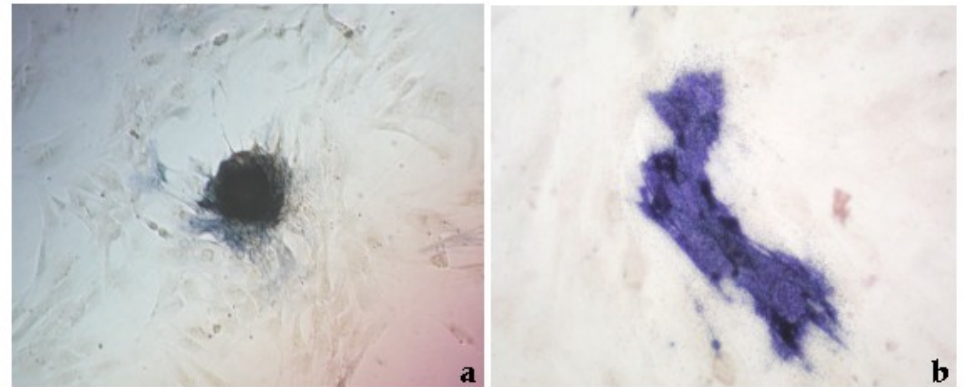
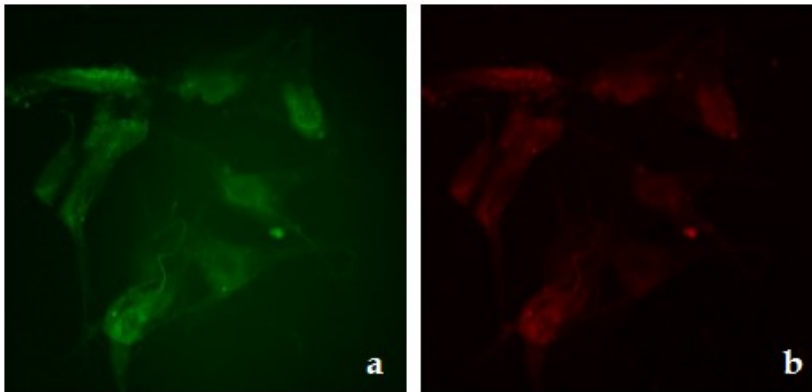


- *Oct-4* and *Rex-1* are slightly expressed after 7 weeks
- 0.003% of cells are alkaline phosphatase positive

riprogrammazione di fibroblasti fetali

Sono direttamente derivati dall'embrione: come dal paziente

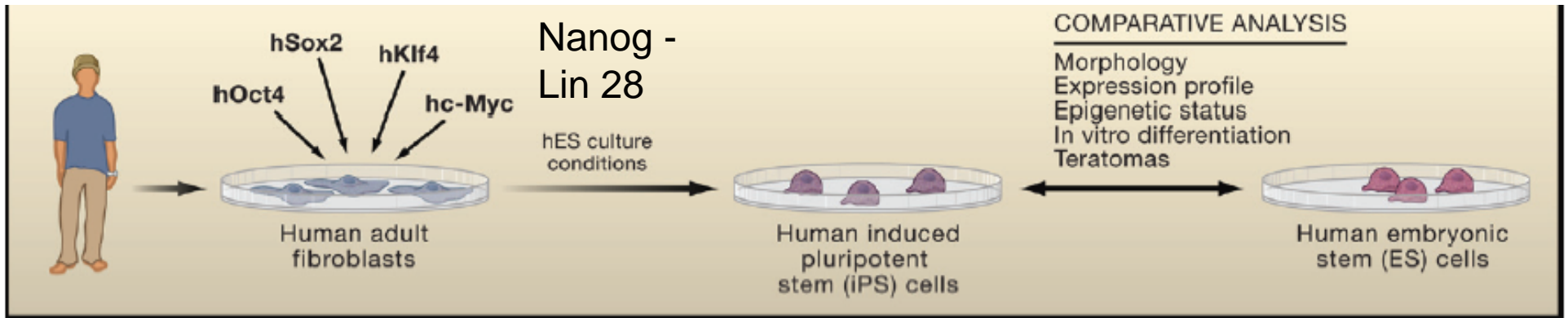
SLO



NO-SLO

Risultati

attività riprogrammante duratura dell'estratto di ES su un basso numero di cellule (0.003%-0.04%) e limitata all'espressione di *Oct-4*, *Rex-1*, *SSEA-1*, *Forsmann* e fosfatasi alcalina

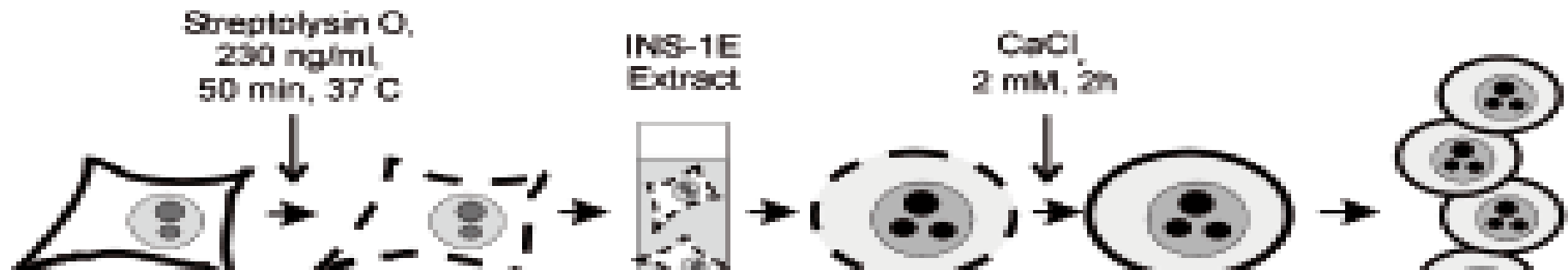


retrotransfections (stemness & oncogenes)

Yamanaka et al. (2006/07/08)	≈	0.02%
Thomson et al. (2007)	≈	0.02%
Jaenisch et al. (2007)	≈	0.05-0.1%

cytoplasts (insulinoma & stem cell extracts)

Collas et al. (2004)	≈	50%
Neri et al. (2007)	≈	0.003- 0.04%



Perché un numero basso di cellule riprogrammate?

- Esiste una piccola popolazione di fibroblasti che risponde meglio al trattamento (Yamanaka?)
- Il grande numero di riarrangiamenti del cariotipo può rendere più difficile la riprogrammazione
- Può dipendere dalla differente capacità riprogrammante della linea di ES utilizzata per preparare l'estratto

Treatment of Sickle Cell Anemia Mouse Model with iPS Cells Generated from Autologous Skin

Jacob Hanna,¹ Marius Wernig,¹ Styliani Markoulaki,¹ Chiao-Wang Sun,²
Alexander Meissner,¹ John P. Cassady,^{1,3} Caroline Beard,¹ Tobias Brambrink,¹
Li-Chen Wu,² Tim M. Townes,^{2*} Rudolf Jaenisch^{1,3*}

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therapeutic potential of such induced pluripotent stem (iPS) cells remained undefined. By using a humanized sickle cell anemia mouse model, we show that mice can be rescued after transplantation with hematopoietic progenitors obtained in vitro from autologous iPS cells. This was achieved after correction of the human sickle hemoglobin allele by gene-specific targeting. Our results provide proof of principle for using transcription factor–induced reprogramming combined with gene and cell therapy for disease treatment in mice. The problems associated with using retroviruses and oncogenes for reprogramming need to be resolved before iPS cells can be considered for human therapy.