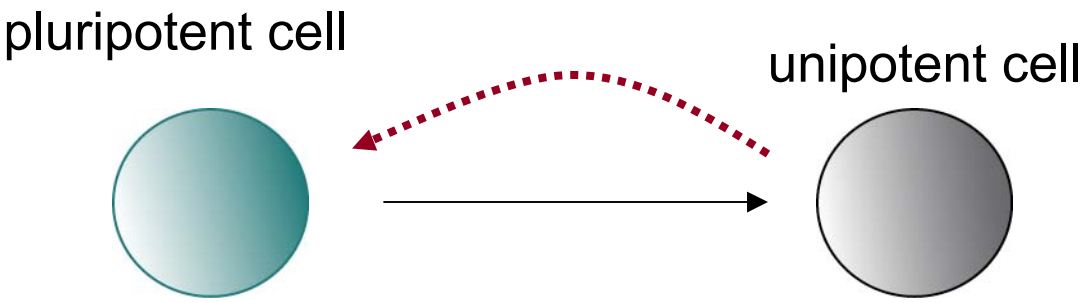
Transcription Factor-Induced Epigenetic Reprogramming

Kathrin Plath

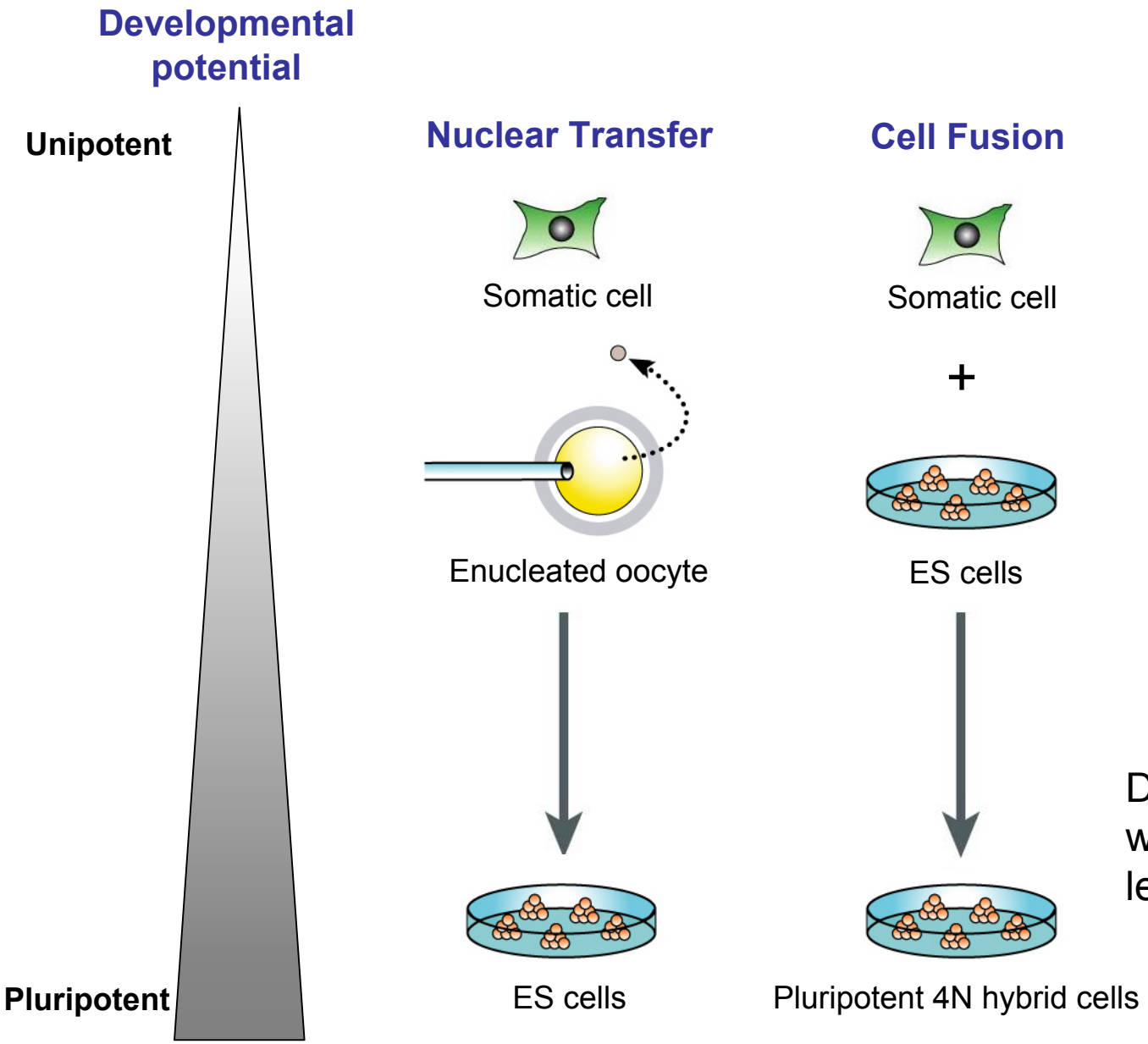
July 16, 2007

nuclear reprogramming



decrease in developmental potential

Nuclear Reprogramming



Goals of reprogramming:

- understand mechanisms
- apply to human system for therapy

Direct reprogramming would potentially have less limitations.

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]

Test for ES cell qualities

Fibroblasts

\+

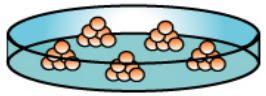
Oct4
Sox2
c-Myc
Klf4

Fbx15-Neo^R

OFF

Retroviral infection

iPS cells
(induced pluripotent stem cells)



Fbx15-Neo^R

ON

embryoid body formation

teratoma formation

contribution to chimera

Limitations…

Takahashi & Yamanaka, *Cell* (2006)

Fbx15 iPS cells are different from ES cells

1. True pluripotent state reached?

   Lack of endogenous pluripotency gene expression

2. Incomplete reprogramming of gene expression

   Transcriptional profile in between that of fibroblasts and ES cells

3. Incomplete epigenetic reprogramming

   Incomplete promoter demethylation of essential pluripotency genes *Oct4* and *Nanog*

4. Limited developmental potency

   Low degree chimeras, no viable pups recovered

Questions

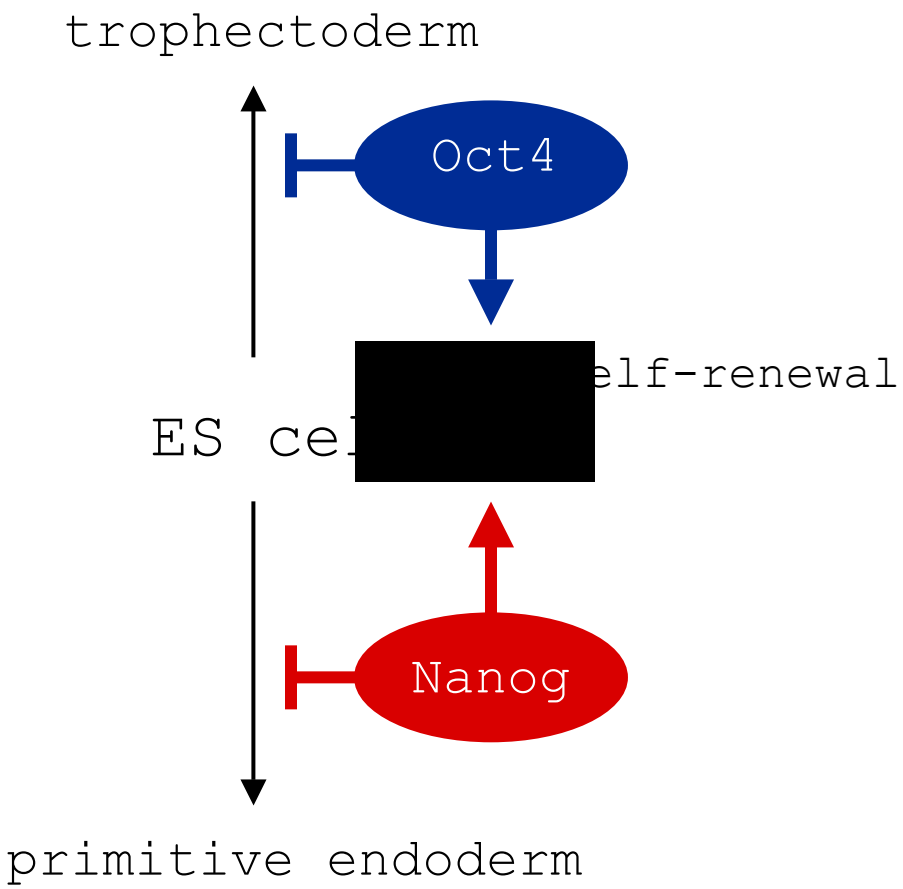
Can iPS cells be generated that are more similar to ES cells?

Why can iPS cells be induced to differentiate despite that four TFs are constitutively expressed?

Does the pluripotent state of iPS cells depend on continuous expression of exogenous factors?

To what extent can four transcription factors reset the epigenetic landscape of a fibroblast into that of a pluripotent cell?

Could selection for expression of an essential gene in ES cells result in better reprogrammed cells?



Oct4 and Nanog are genes essential for the maintenance of the pluripotent state of ES cells and are only expressed in pluripotent cells

Hypothesis

Selection for an *essential* ES cell gene gives rise to “better” iPS cells.

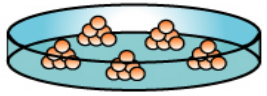
Fibroblasts

+

Oct4
Sox2
c-Myc
Klf4

Retroviral infection

iPS cells
(induced pluripotent stem cells)



Nanog-Puro^R

OR

Oct4-Neo^R

OFF

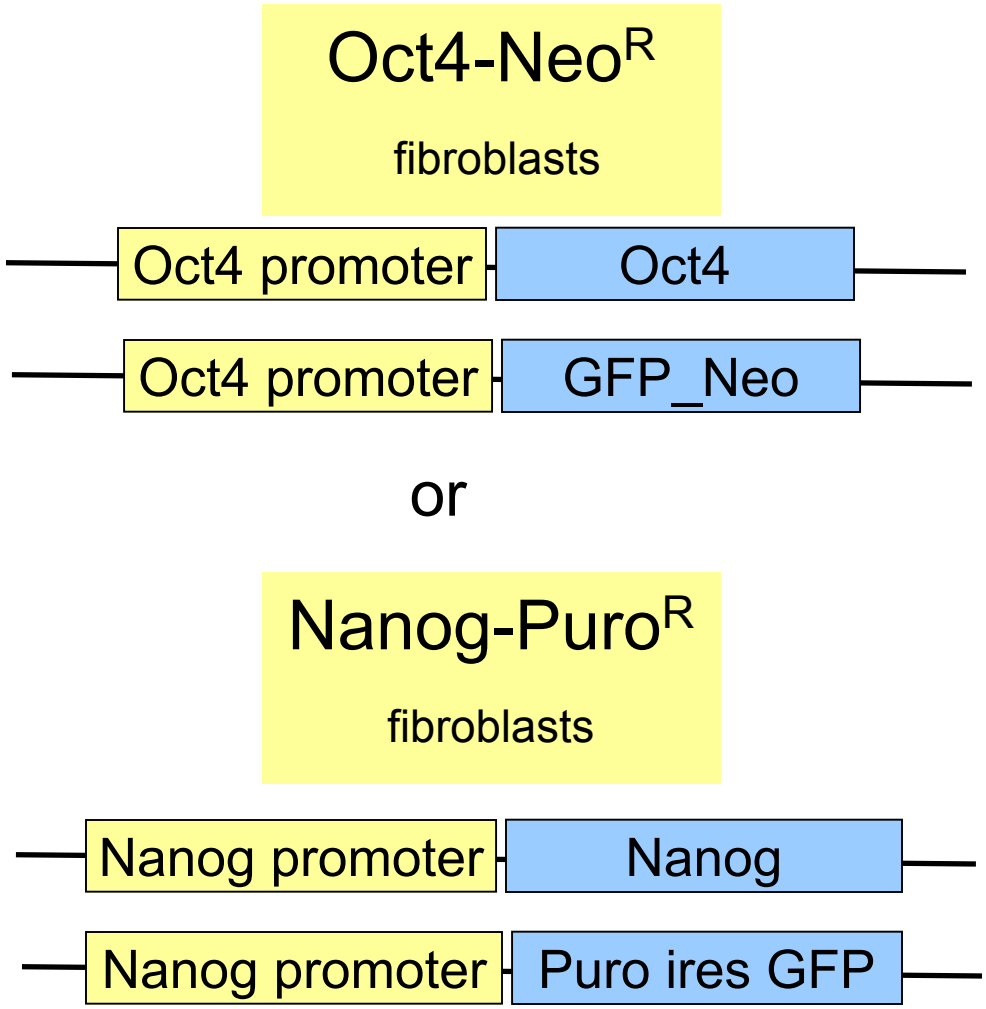
Nanog-Puro^R

OR

Oct4-Neo^R

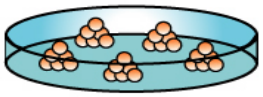
ON

Nanog or Oct4 selection



Assays

iPS cells



Nanog-Puro^{R}

OR

Oct4-Neo^{R}

ON

General characterization

Endogenous vs. viral gene expression

Epigenetic analysis
Gene-specific
Chromosome-wide
Genome-wide

Transcriptional profiling

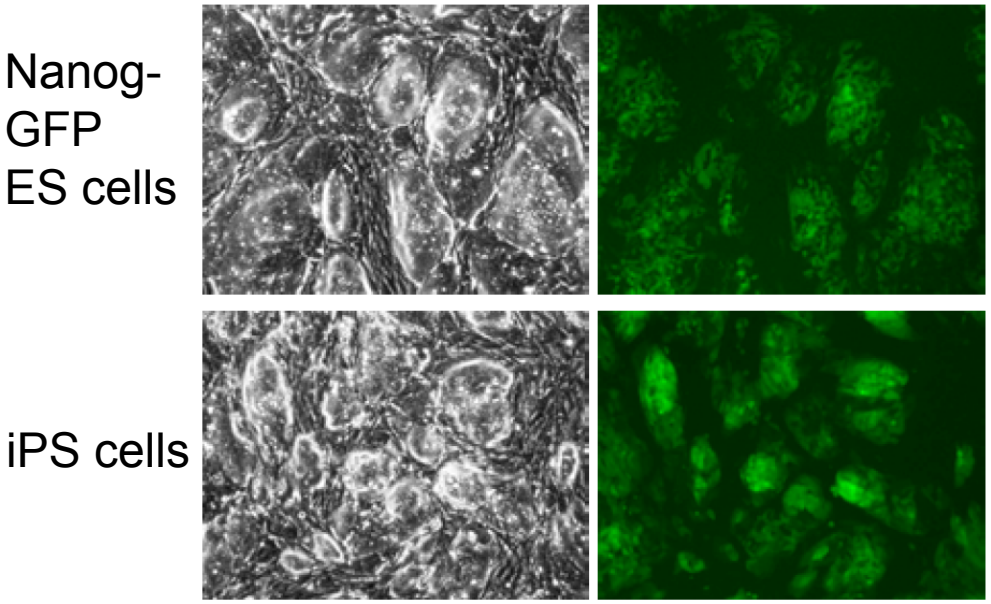
Chimera contribution

Assays

General characterization

Characterization of Nanog-selectable iPS cells

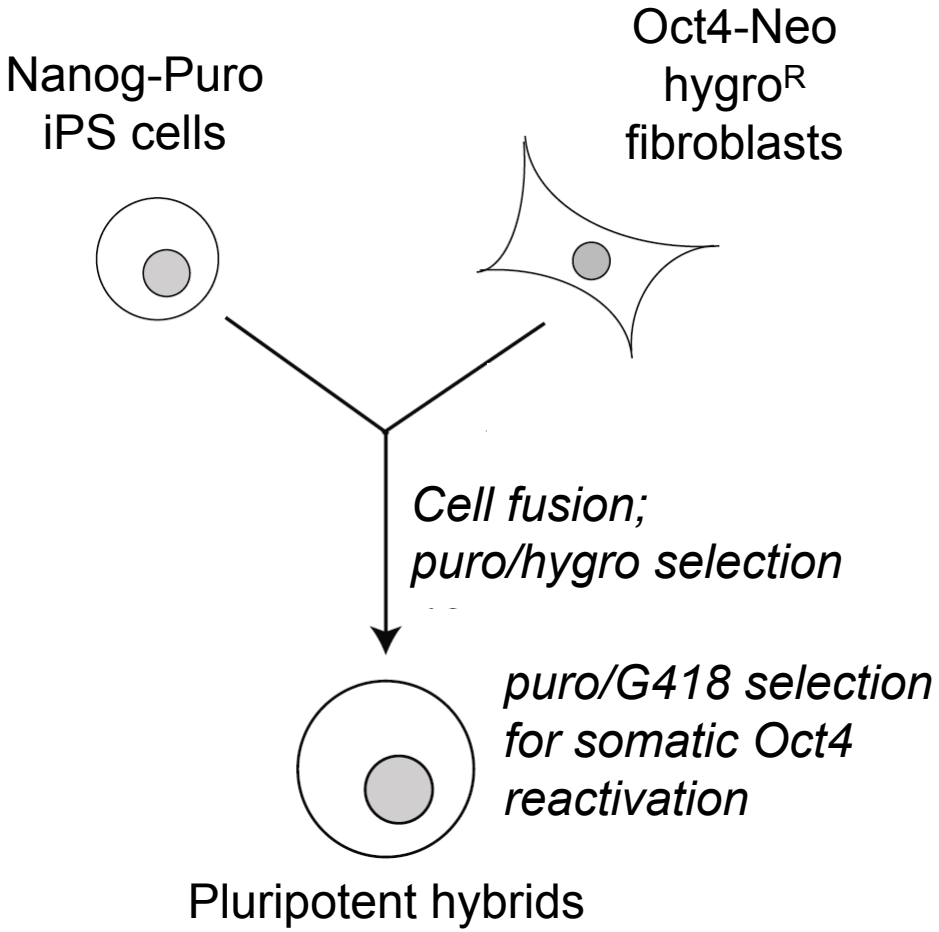
- *ES cell-like morphology*
- *Nanog-GFP expression*

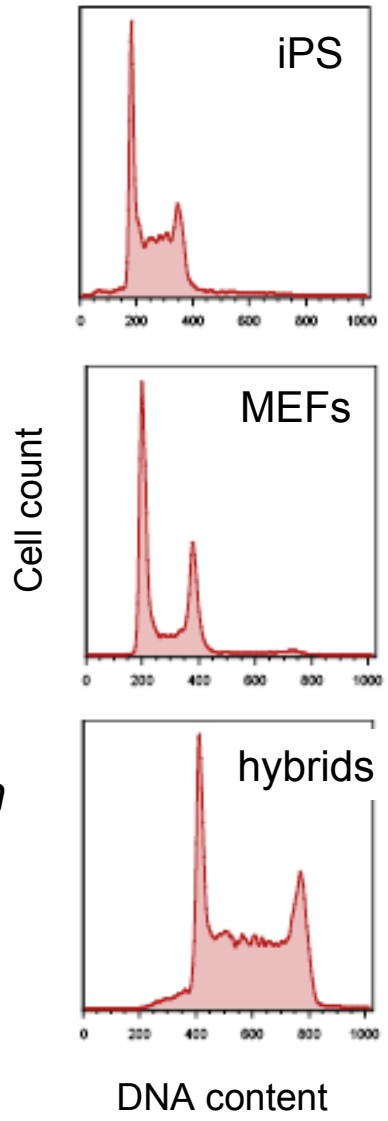


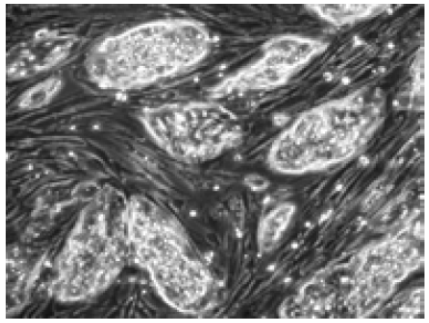
… Do iPS cells possess functional attributes of ES cells?

Dominant reprogramming activity of iPS cells in cell fusion

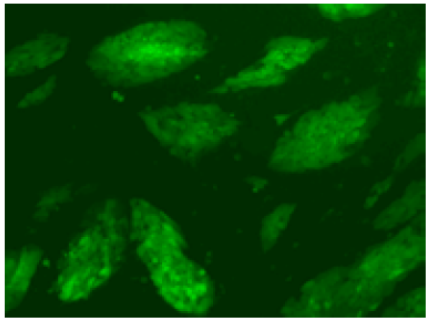
Strategy





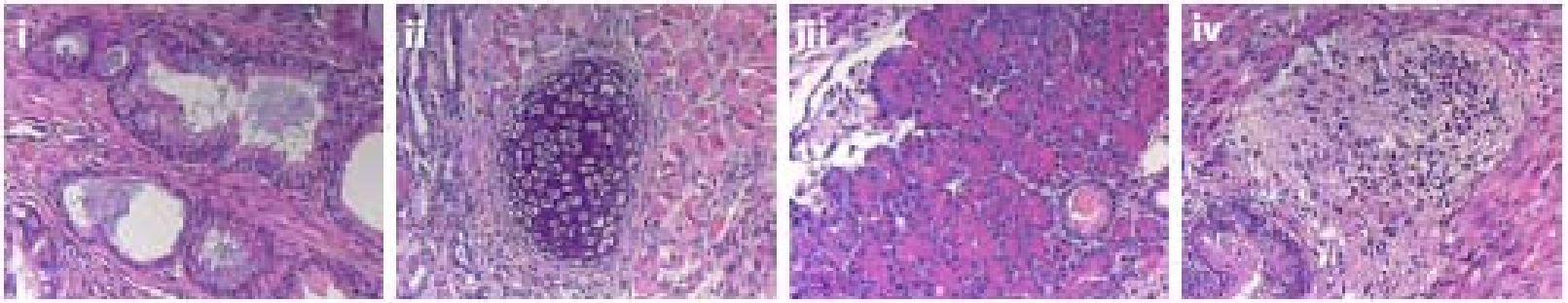


Cell hybrids



Nanog-GFP

Nanog -selected iPS cells are pluripotent (teratoma)

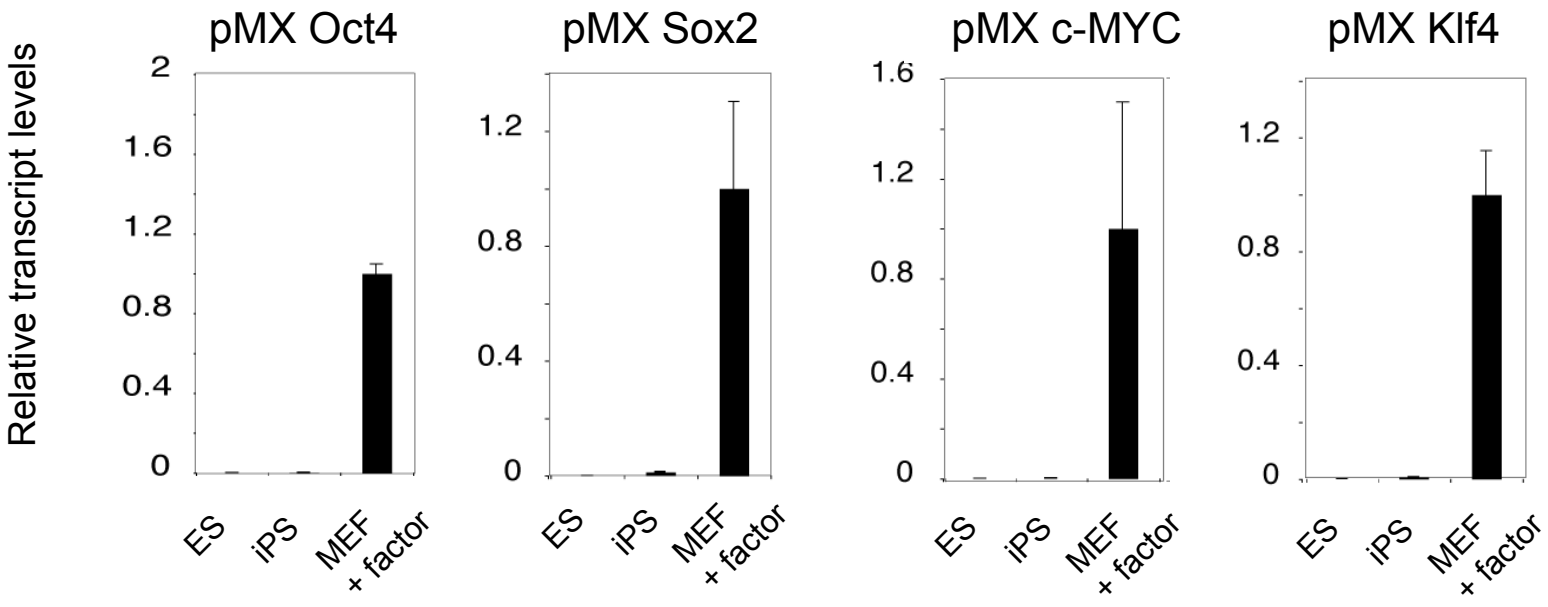


i) epithelial structures
ii) cartilage with surrounding muscle
iii) glandular structures
iv) neural tissues

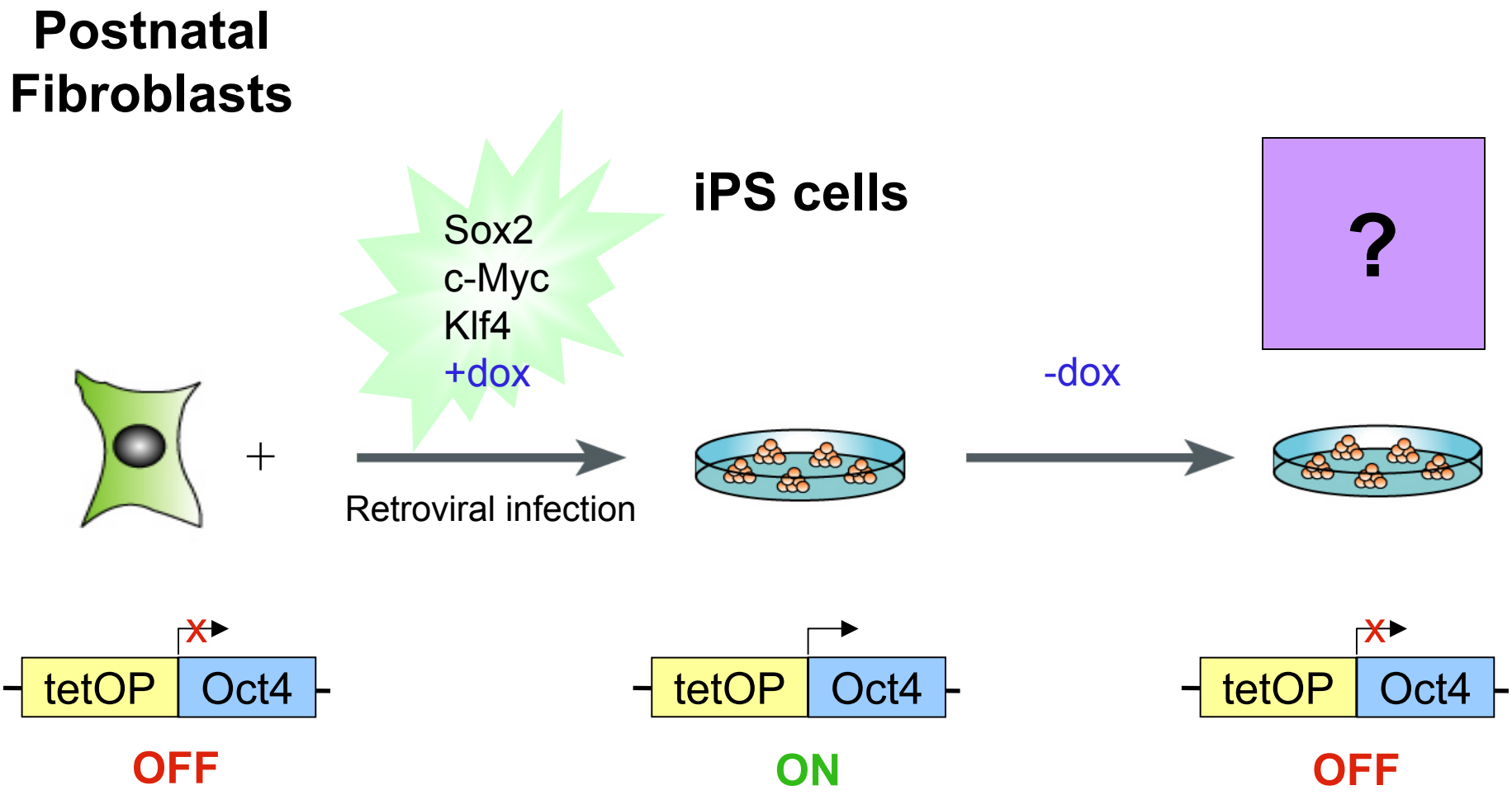
Assays

Endogenous vs. viral gene expression

Retrovirally induced iPS cells don't have persistent viral gene expression (and therefore can differentiate)

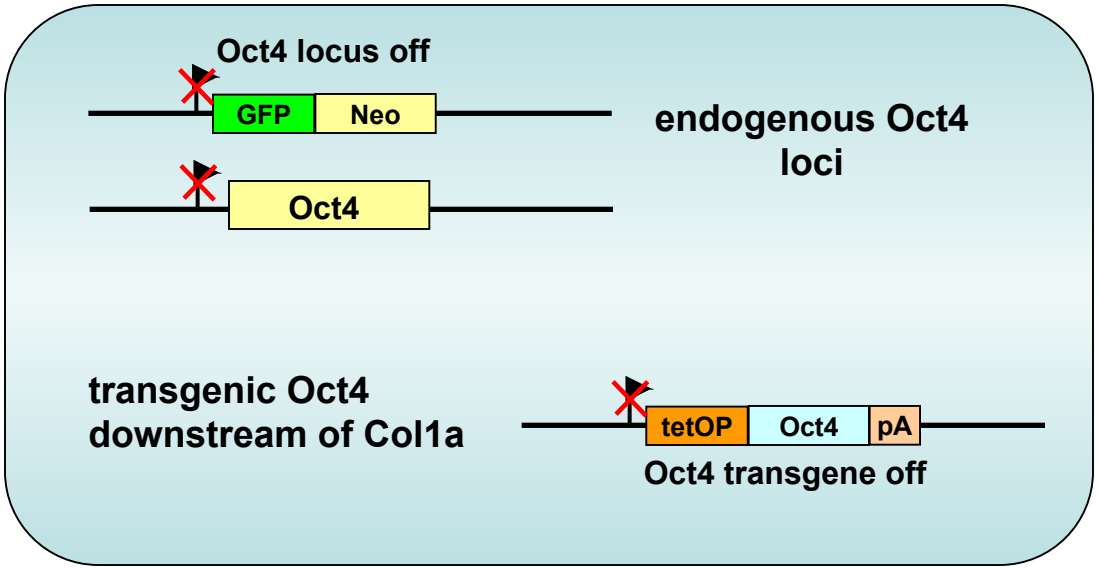


Can iPS cells be generated without retroviral transduction ?



Oct4 transgene is incorporated downstream of the Col1A locus

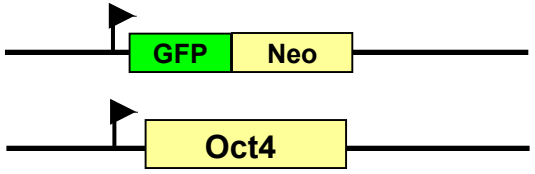
Fibroblast with Oct4 selectable allele and dox-inducible Oct4 transgene



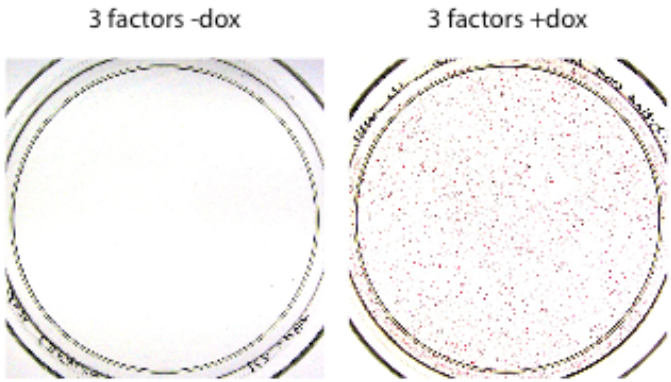
Sox2
Klf4
c-myc*
+ dox

G418 selection

iPS cell

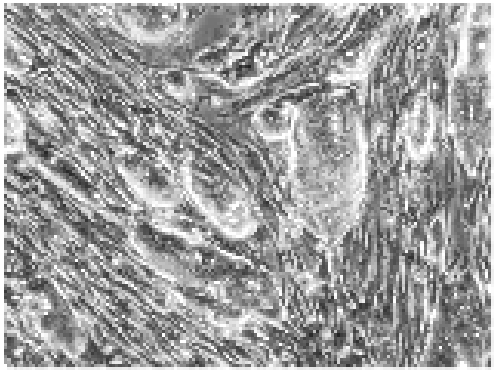


endogenous Oct4 loci

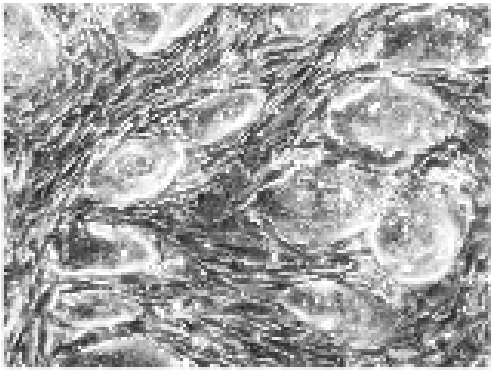


iPS cells are stable and pluripotent in the absence of transgenic Oct4

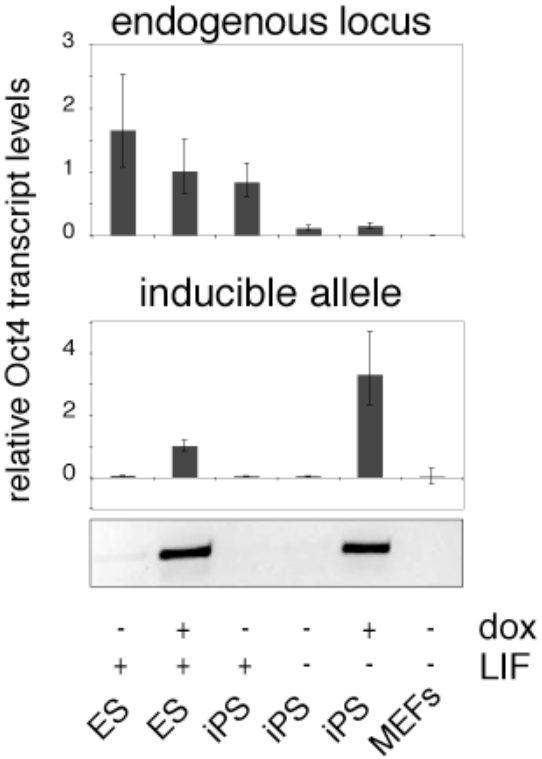
Dox-independent self-renewal



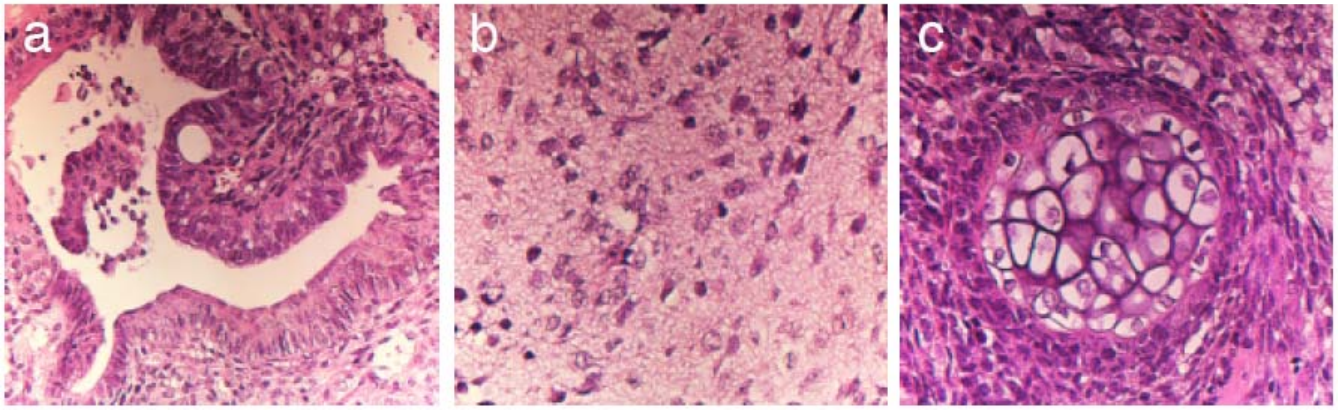
+dox



-dox



Pluripotency (teratoma formation)



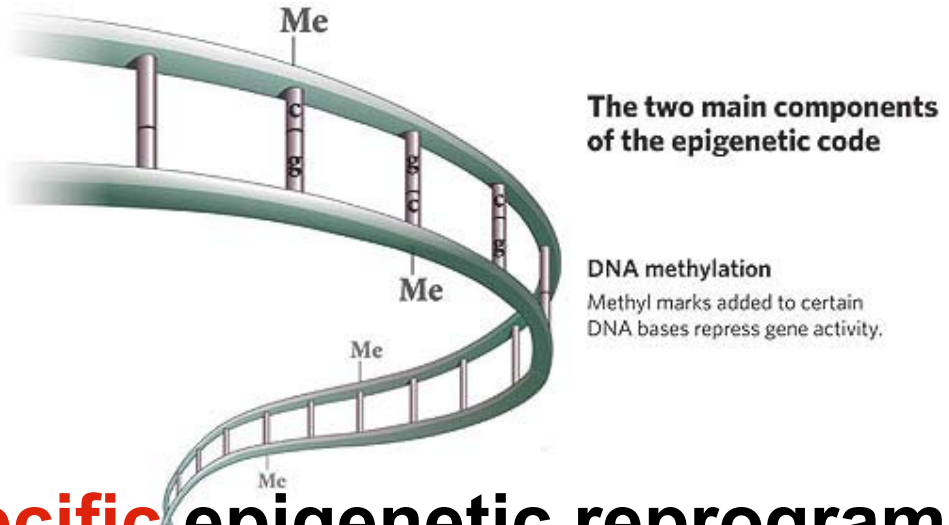
Assays

Epigenetic analysis

Gene-specific

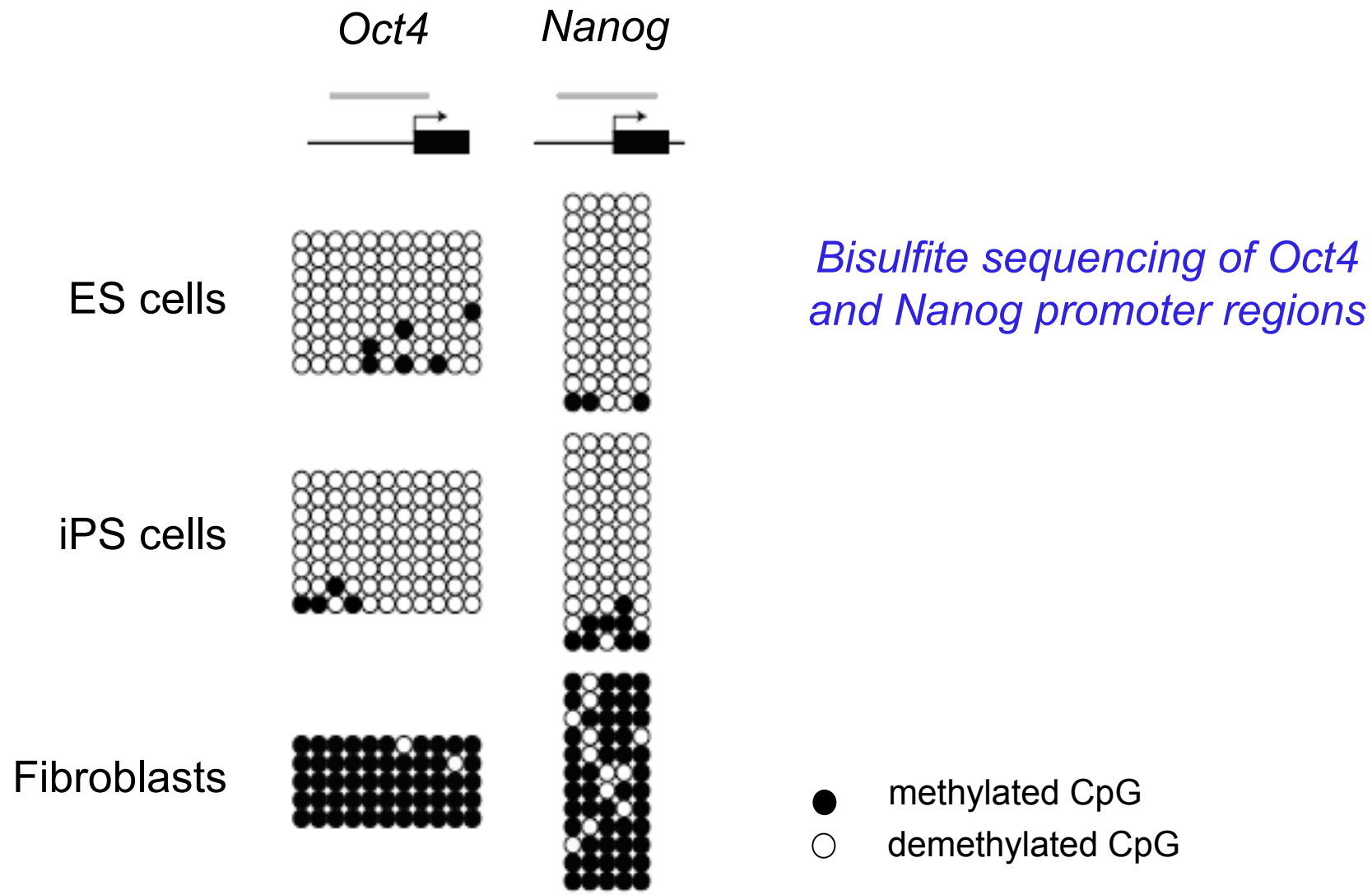
Chromosome-wide

Genome-wide



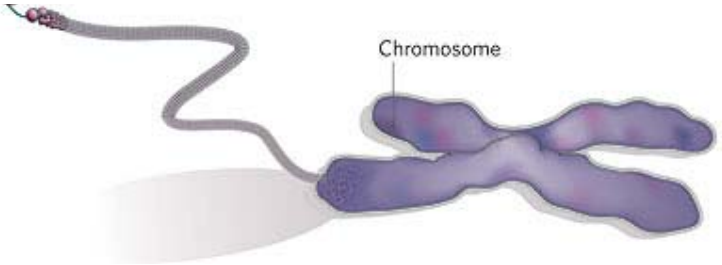
Gene-specific epigenetic reprogramming?

DNA within promoters of pluripotency genes are demethylated in iPS cells

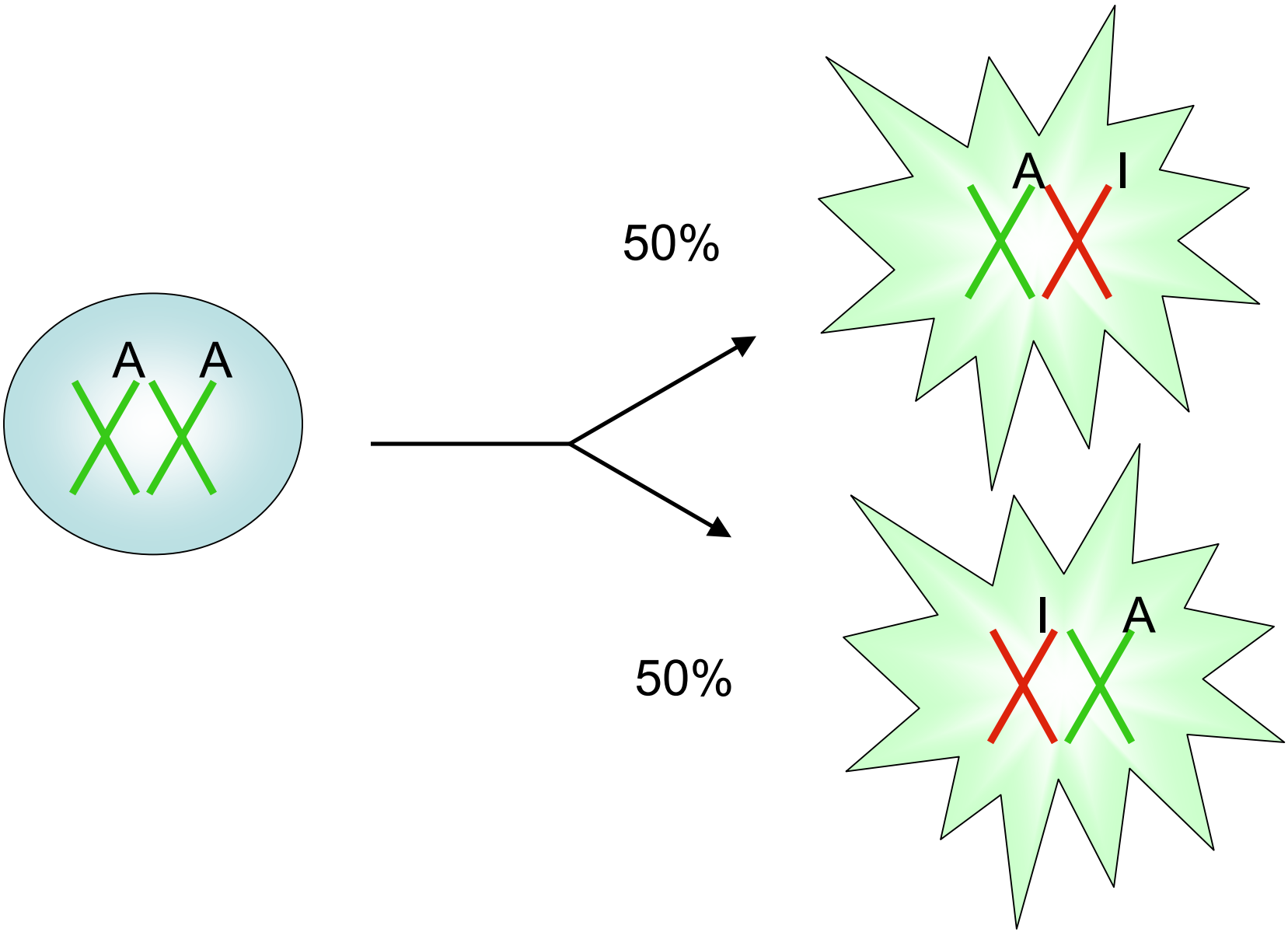


Bisulfite sequencing of Oct4 and Nanog promoter regions

Chromosome wide epigenetic reprogramming?



X inactivation as an example of chromosome -wide silencing



X-inactivation is regulated by a non-coding RNA

***Xist* RNA:**

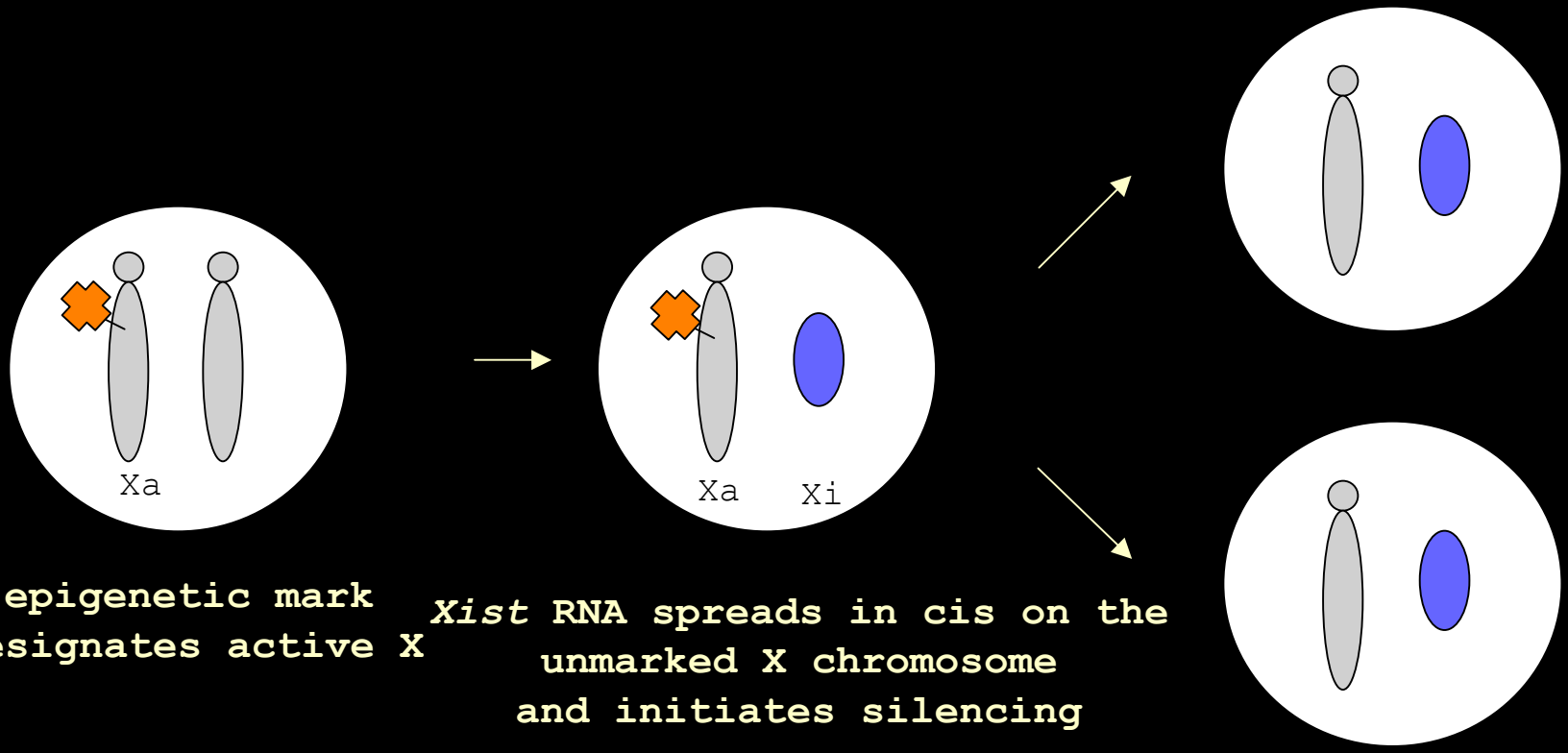
- non-coding, 17.5 kb in length, spliced, and poly
- encoded by an X-linked gene
- stable expression only from the inactive X-chrom
- "coats" the inactive X chromosome in female cel

X chromosome paint
Xist RNA
dapi

Xi = inactive X chromosome

Xa = active X chromosome

Xist RNA is required for initiation of X chromosome silencing



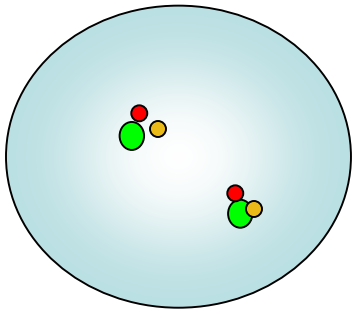
epigenetic mark designates active X

Xist RNA spreads in cis on the unmarked X chromosome and initiates silencing

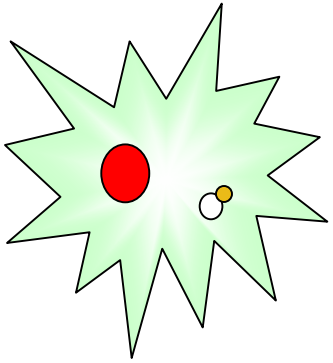
stable propagation of the Xi and *Xist* RNA coating through all subsequent cell divisions

Do female iPS cells reactivate the inactive X chromosome?

Embryonic stem cell



Differentiated cell



Tsix (antisense transcript to Xist)

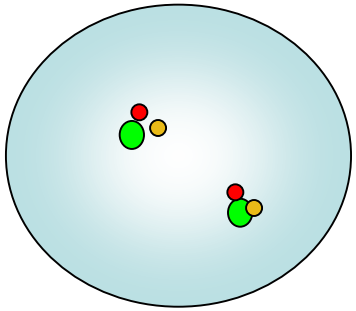
Xist (expressed at very low levels as repressed by Tsix)

Pgk-1 (X-linked gene transcript)

Xist (high level Xist expression and coating of the Xi)

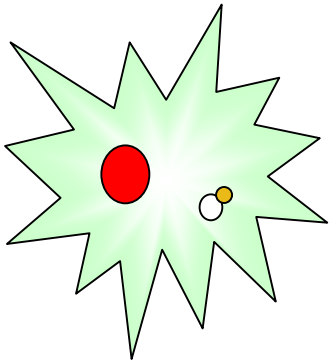
Pgk-1 (X-linked gene transcript)

Embryonic stem cell (two Xa)



iPS reprogramming ?

Differentiated cell (one Xi and one Xa)

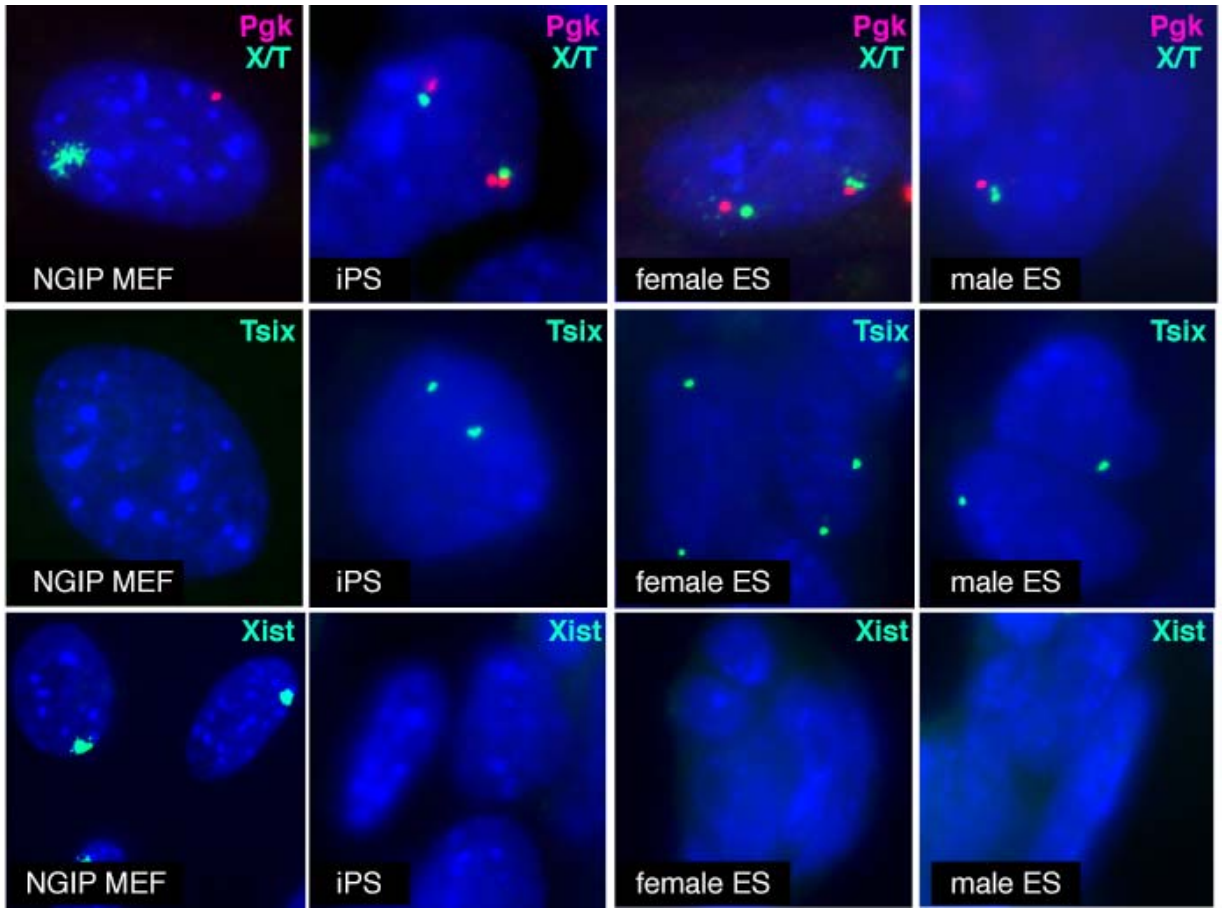


Tsix
Xist
Pgk-1

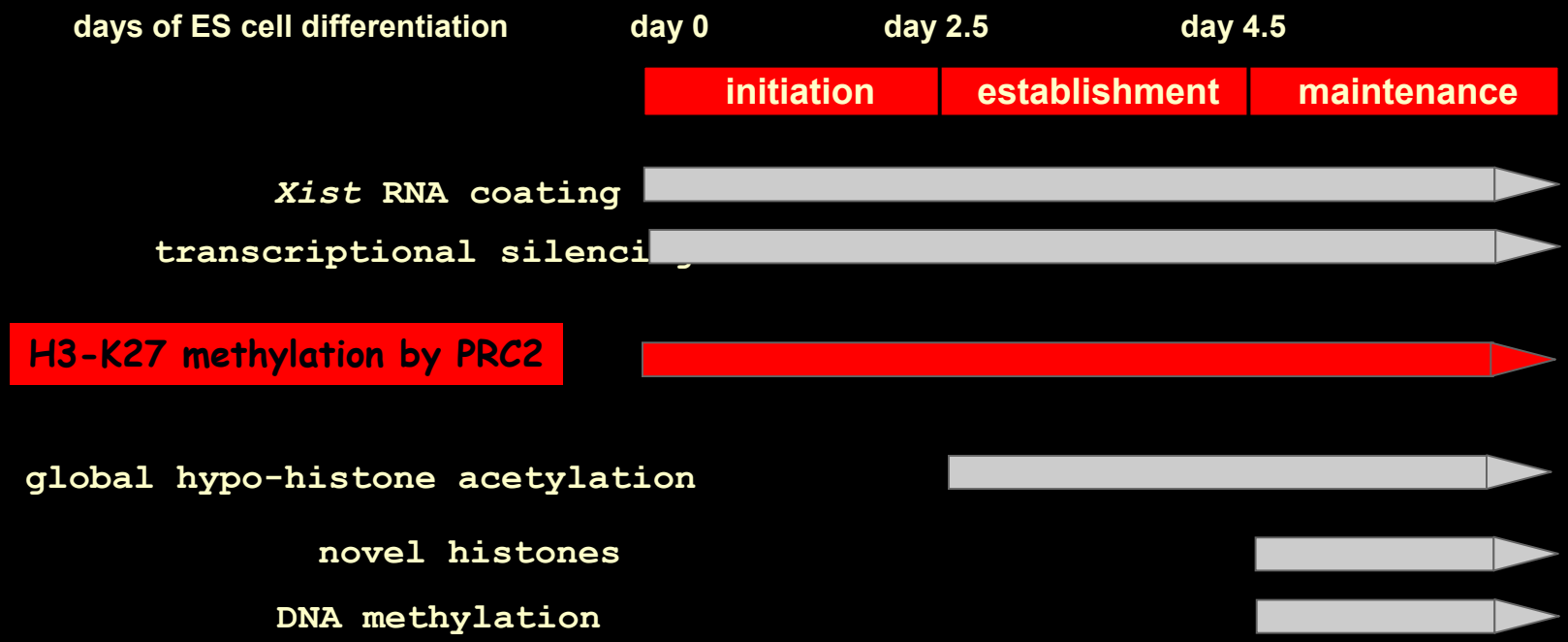
Tsix and Xist
Pgk-1

Tsix

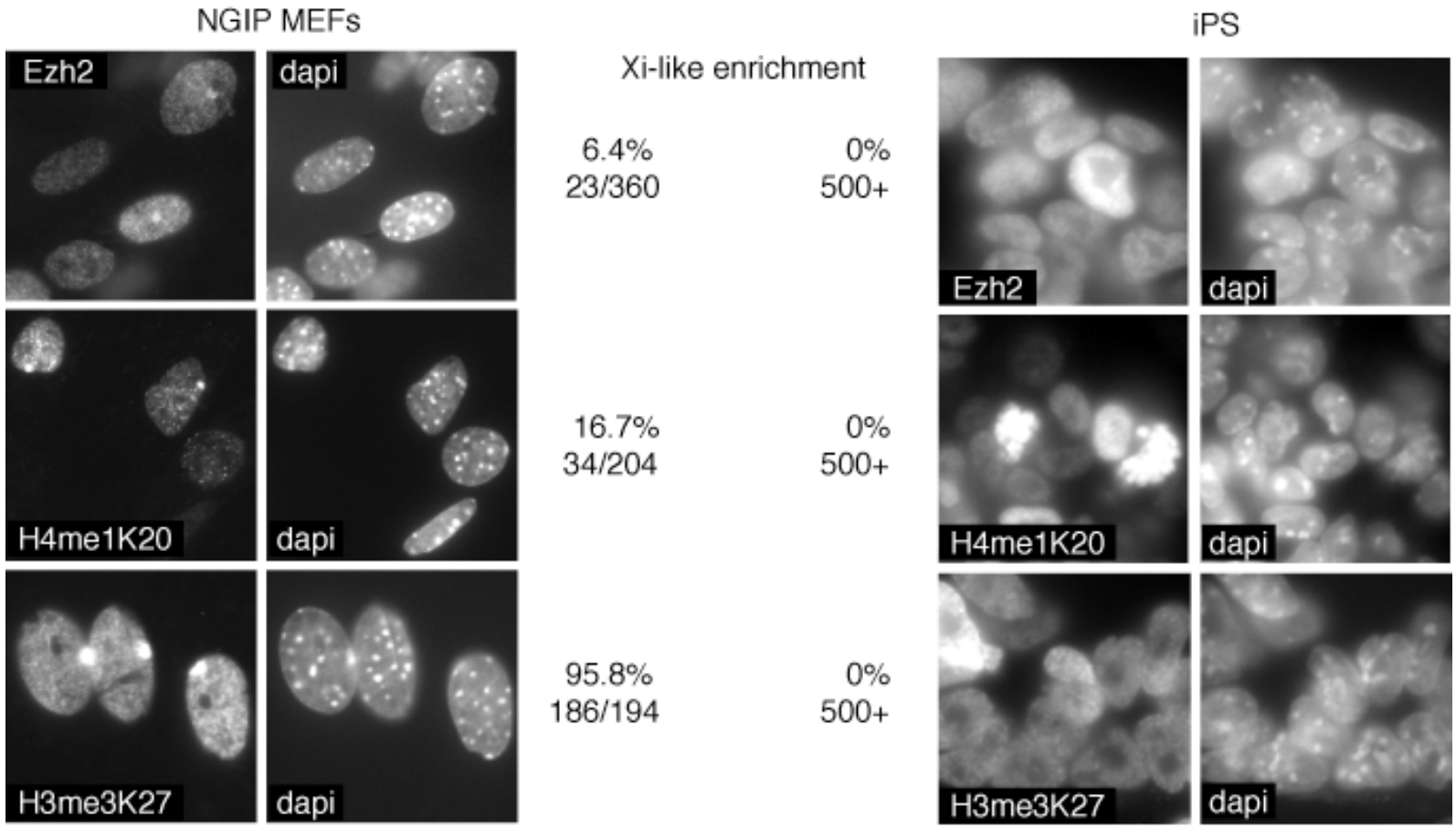
Xist



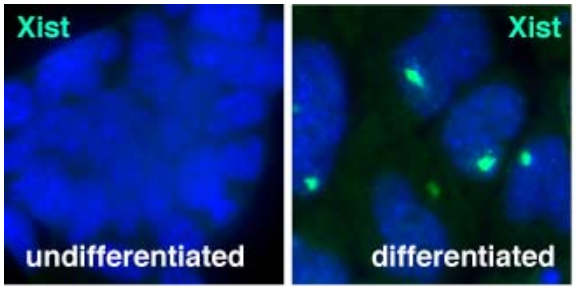
Chromatin modifications accumulate on the Xi

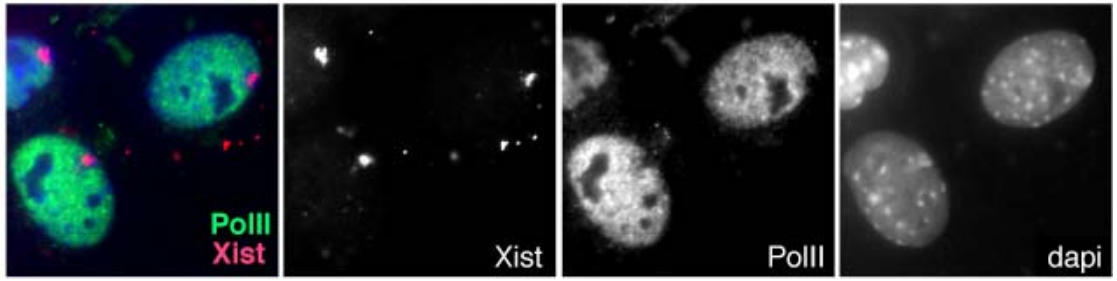


Do female iPS cells change the chromatin state on the X?



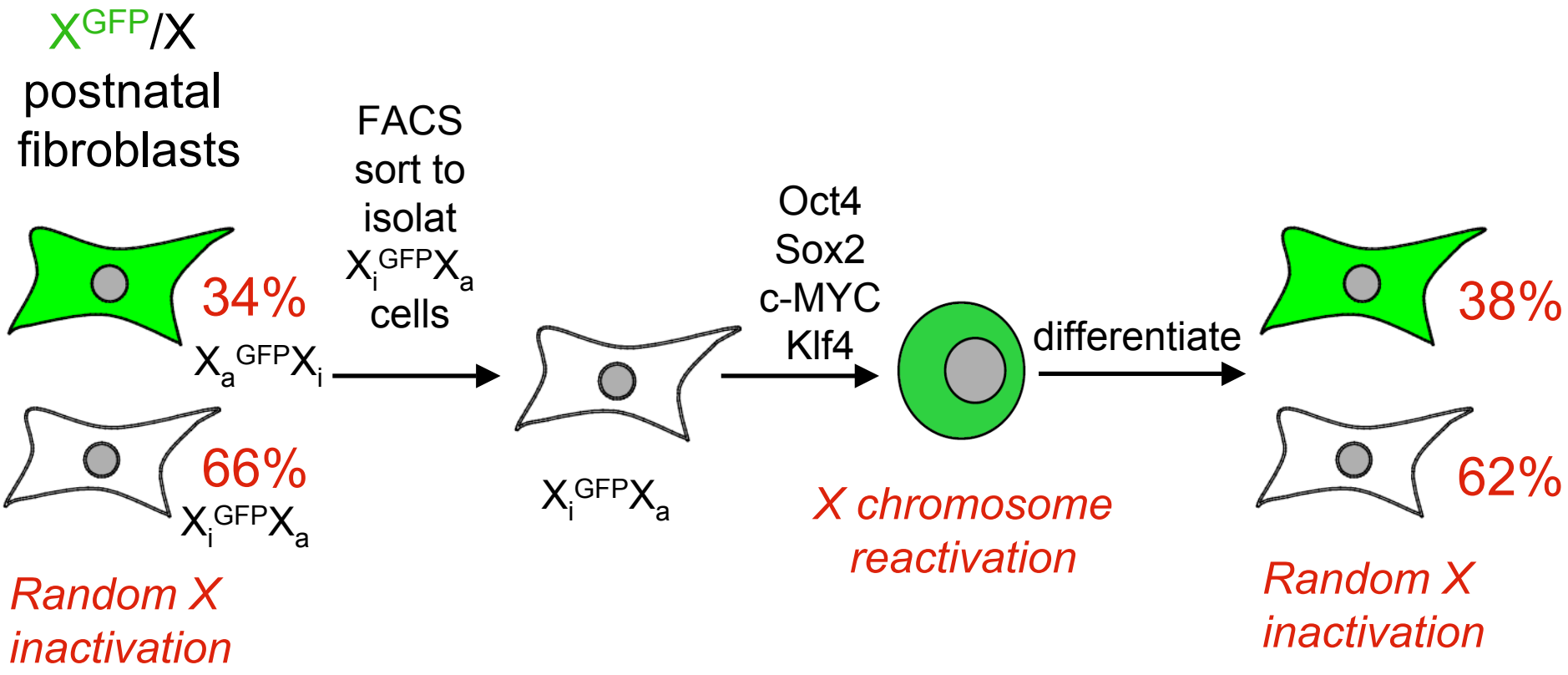
iPS cells undergo X-inactivation





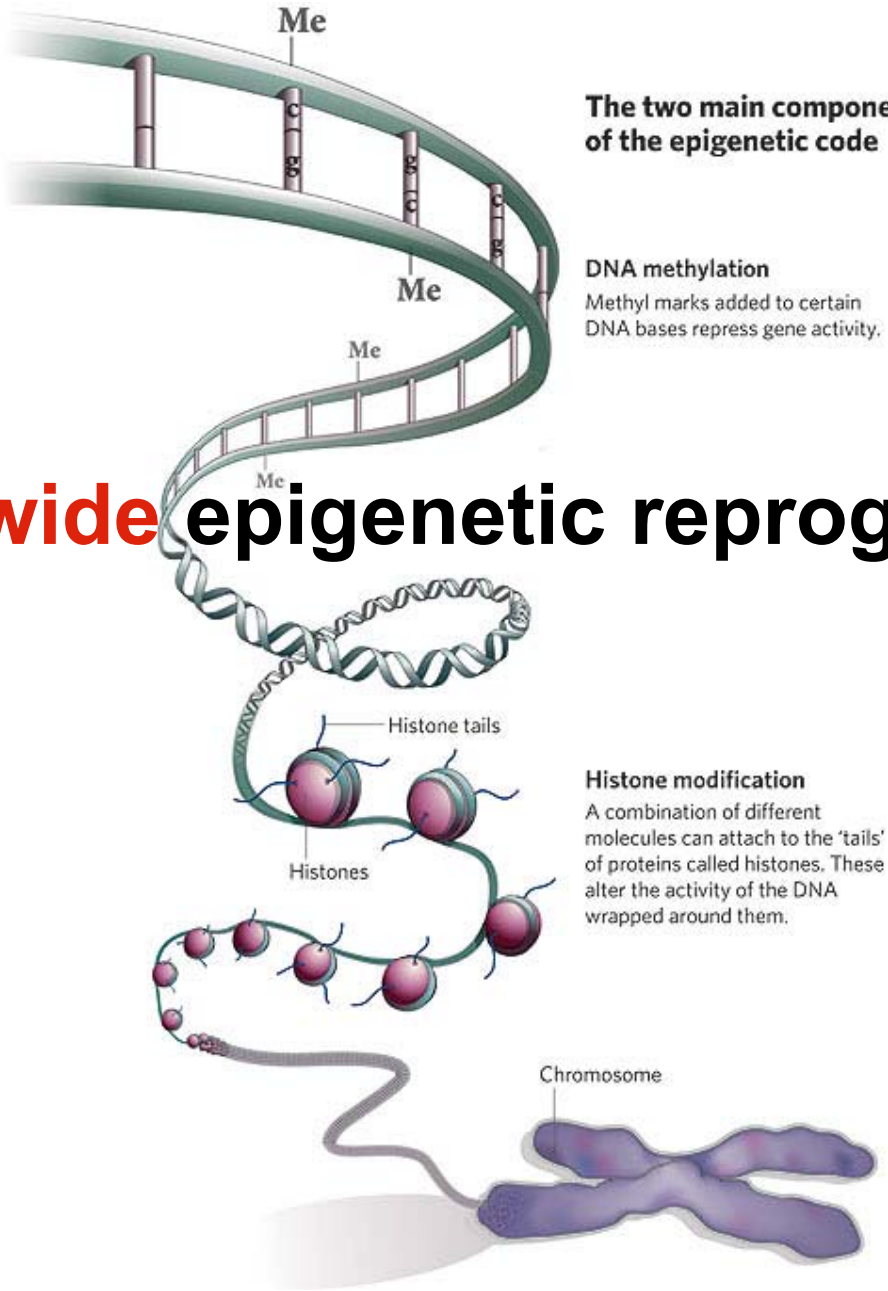
Is X-inactivation, like in ES cells, random?

Proof of random X inactivation in female iPS cells

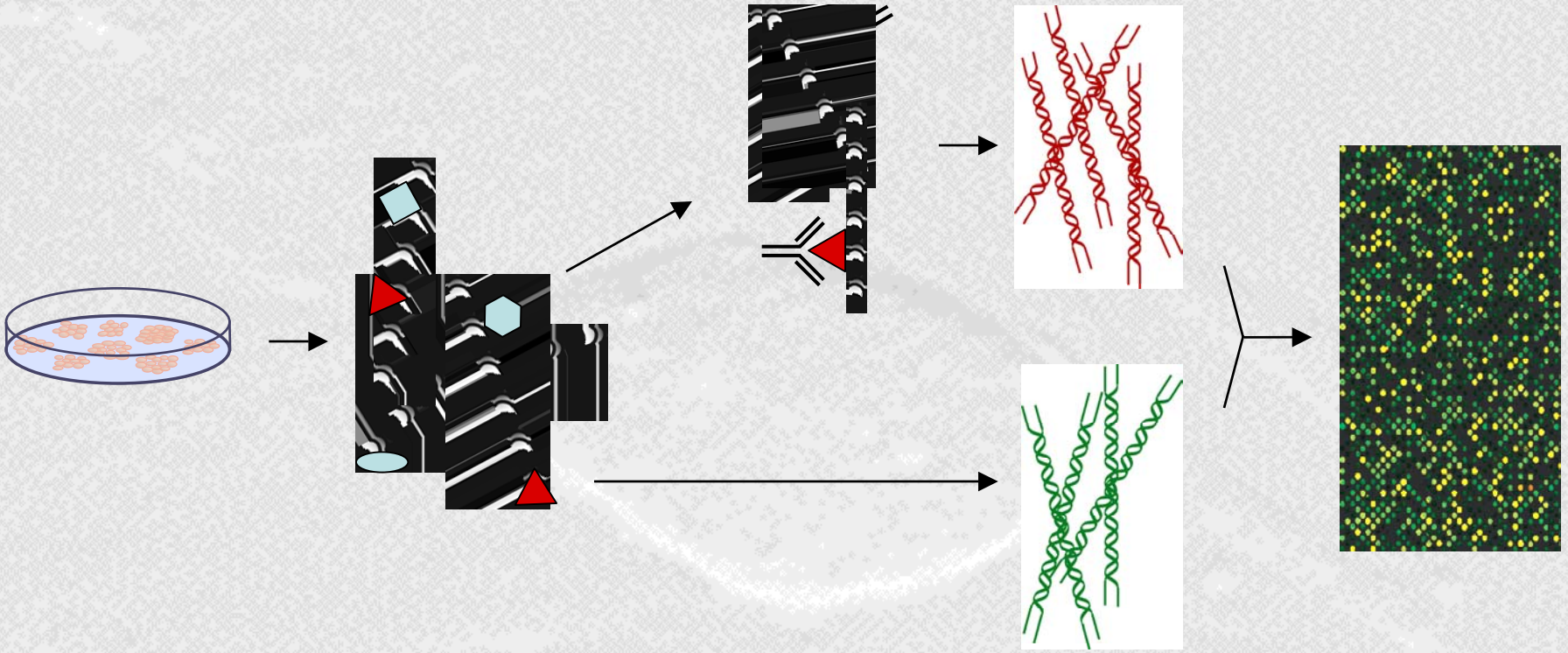


Erasure of epigenetic memory for previously inactive X chromosome

Genome-wide epigenetic reprogramming?



ome-scale location analysis of histone modificati



crosslinking of proteins to DNA-binding sites in ES cells

harvesting of cells and fragmentation DNA

enrichment of DNA fragments x-linked to modified histones with antibodies

differentia l labeling of total and Chip-enriched DNA

hybridizatio n to microarrays and comparison of intensity ratios

Binding data at high resolution

mouse arrays (Agilent)

60mer oligonucleotide probes: ~ 3 probes/kb

covering the region from -8kb to +2kb relative to the transcript start sites for 15,742 annotated mouse genes

Global epigenetic reprogramming in iPS cells

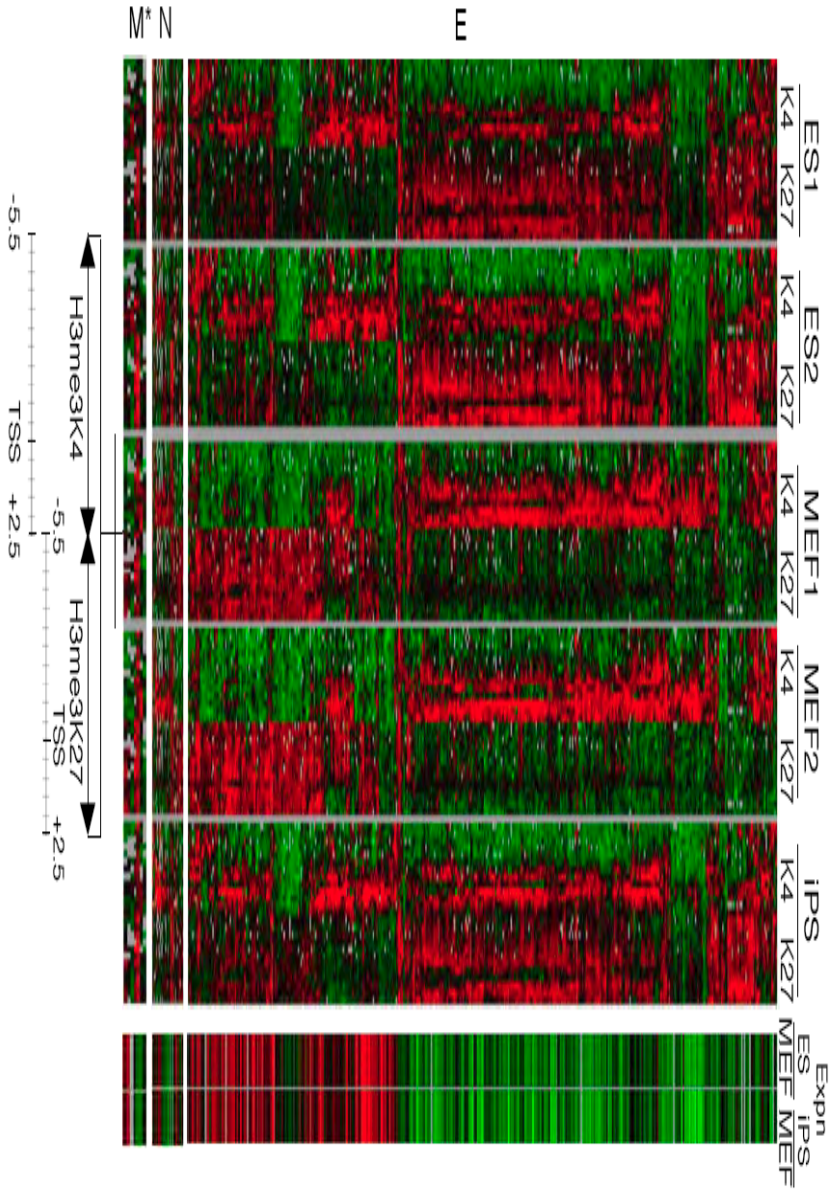
Approach

Genome-wide ChIP-chip analysis of K4/K27 trimethylation (16,500 promoters)

Findings

iPS and ES cells are indistinguishable

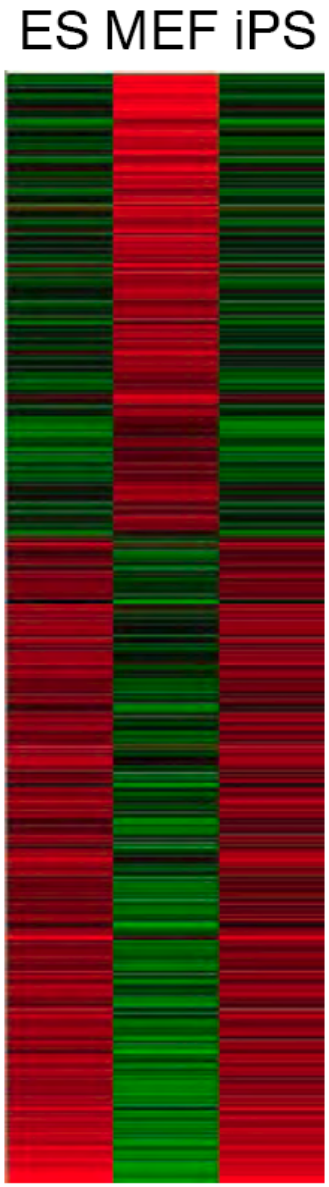
Reprogramming mainly associated with changes in repressive methylation (K27)



Assays

Transcriptional profiling

Reprogramming of transcriptome in iPS cells



Analysis of differentially expressed genes between ES cells and MEFs

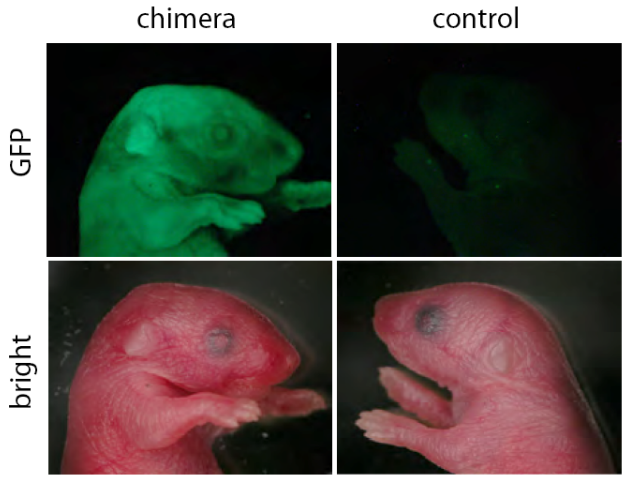
Transcriptional profiles are indistinguishable

Assays

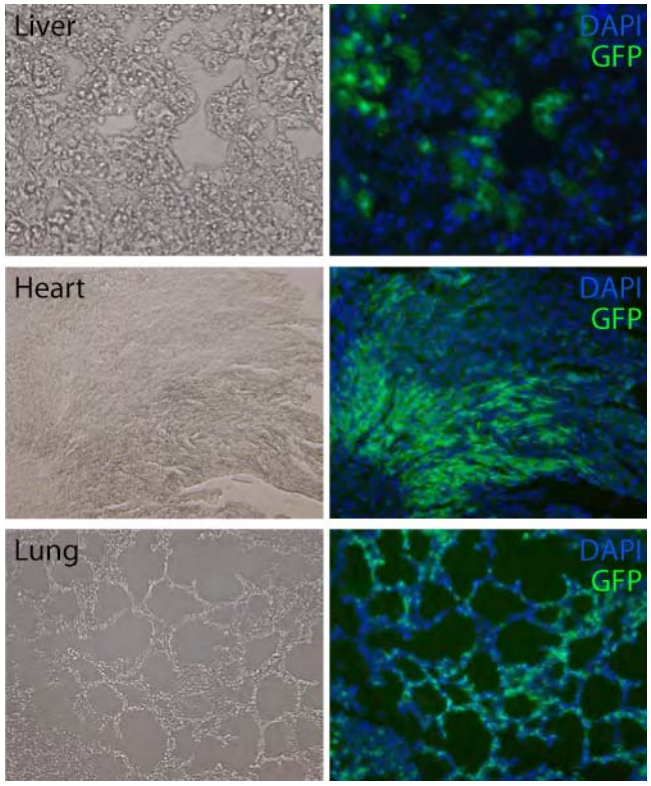
Chimera contribution

In vivo differentiation potential of iPS cells

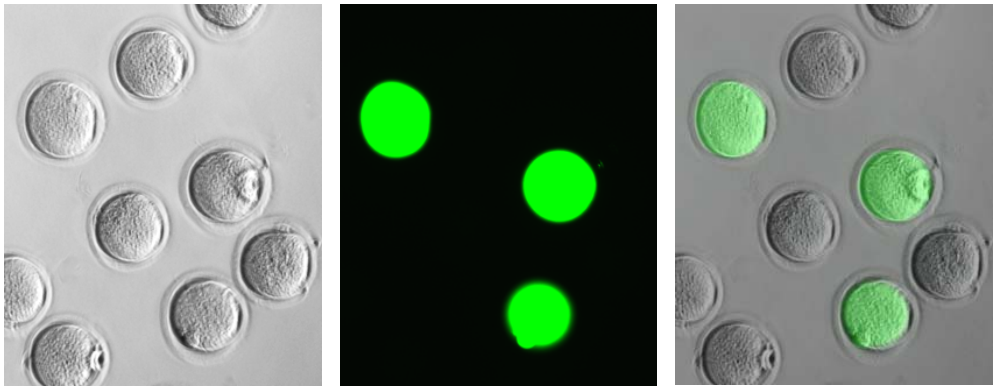
Live-born chimeras (MEF-derived)



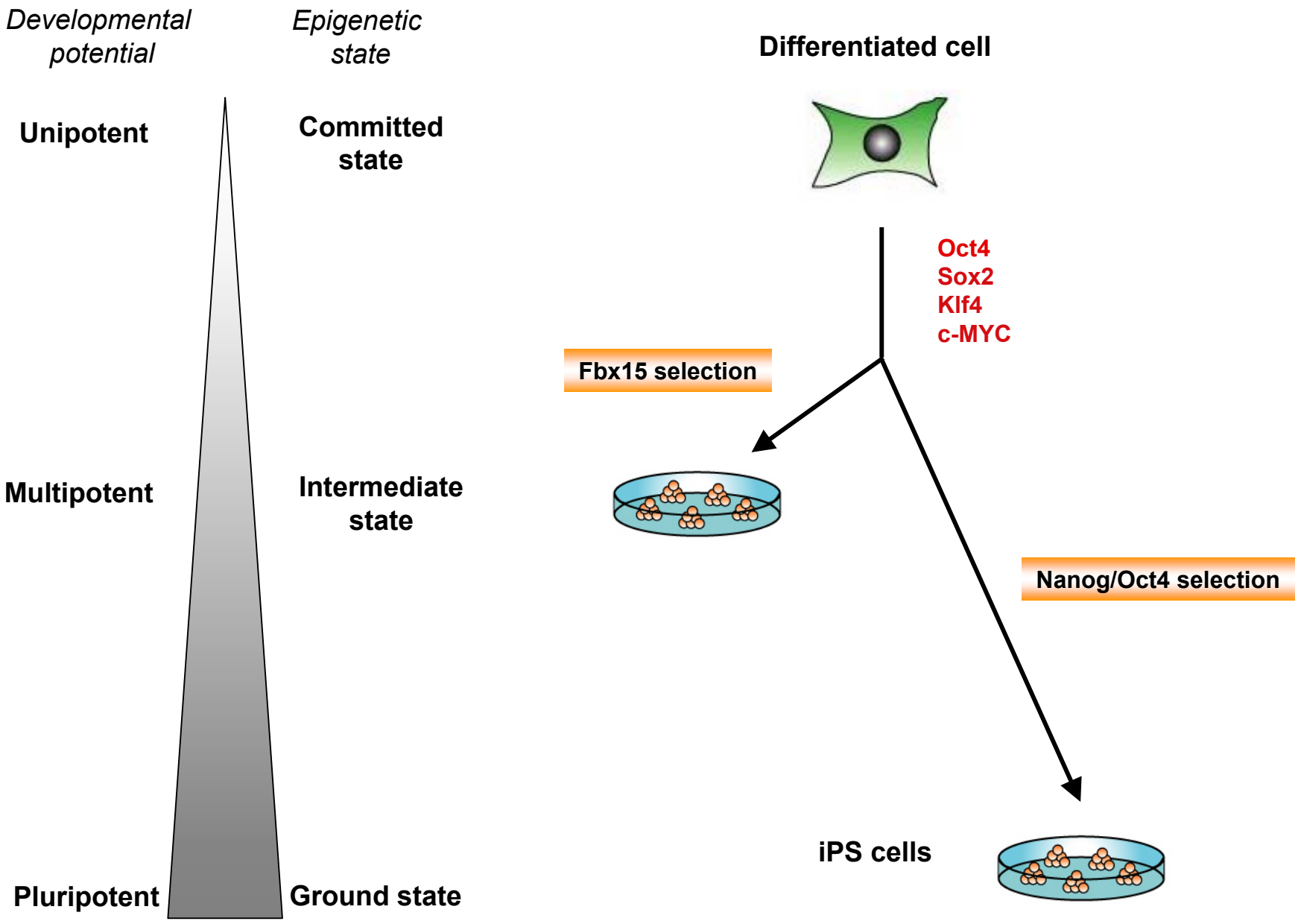
High degree of somatic contribution



Germline contribution



Model


Developmental potential
Epigenetic state
Unipotent
Committed state
Multipotent
Intermediate state
Pluripotent
Ground state
Differentiated cell
Oct4
Sox2
Klf4
c-MYC
Fbx15 selection
Nanog/Oct4 selection
iPS cells

Acknowledgements

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Jason Tchieu
Celine Marban

Grunstein lab

Wei Xie

Harvard/MGH

Hochedlinger lab

Nimet Maherali
Jochen Utikal
Sarah Eminli
Katrin Arnold
Matthias Stadtfeld

Konrad Hochedlinger

MIT

Rudolf Jaenisch