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Molecular characterization of rabbit ES and iPS cells

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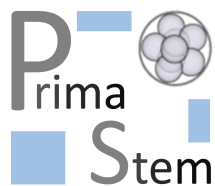
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2nd Plurabbit meeting
Gödöllő Hongria
27/11/2010

***Molecular characterization
of rabbit ES and iPS cells***

**Pierre Osteil, Murielle Godet, Suzy Markossian,
Yann Taponnier, Thierry Joly,
Pierre Savatier and Marielle Afanassieff**

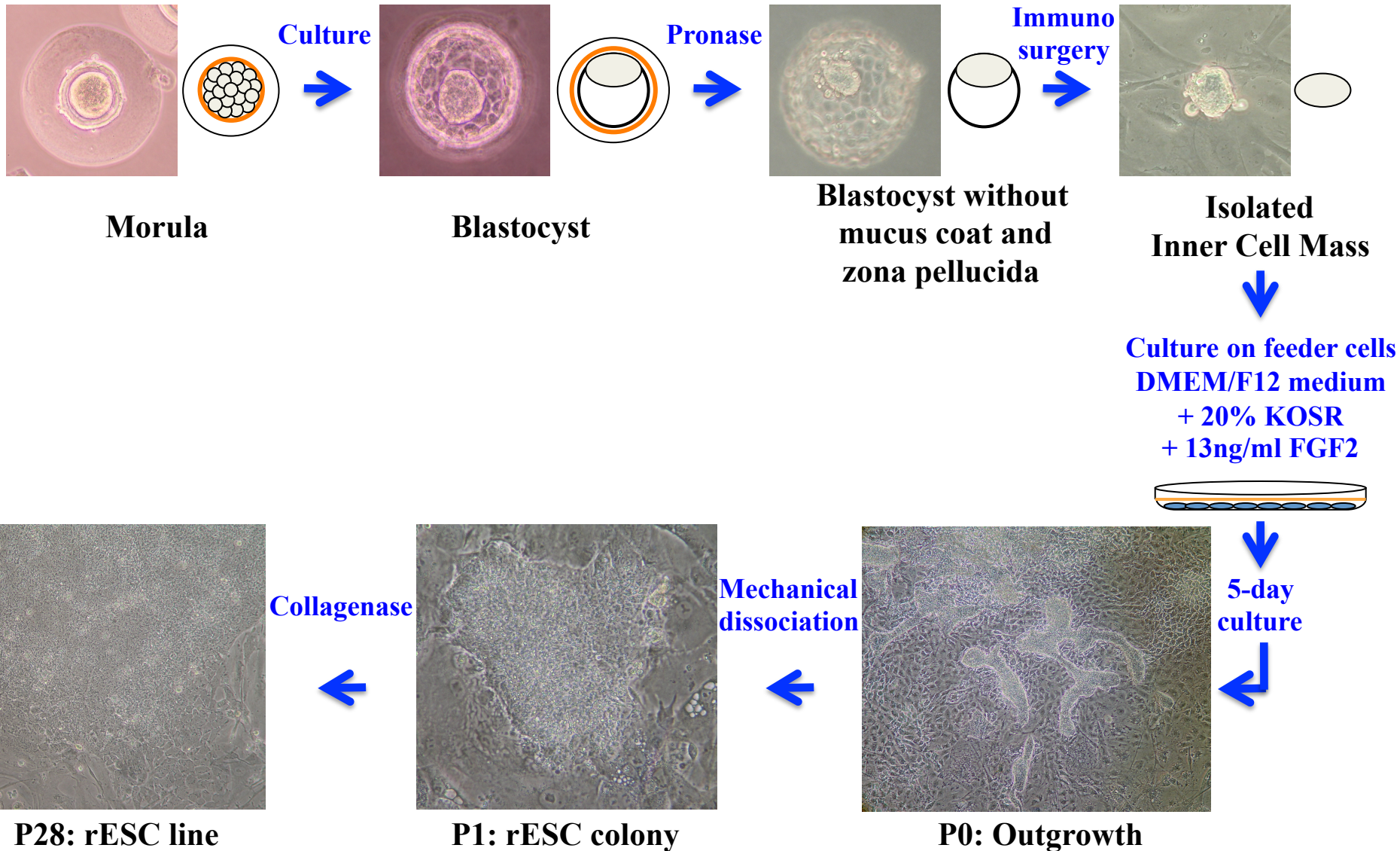
Université Claude Bernard  Lyon 1

Instituts
thématiques 

Inserm

Institut national
de la santé et de la recherche médicale

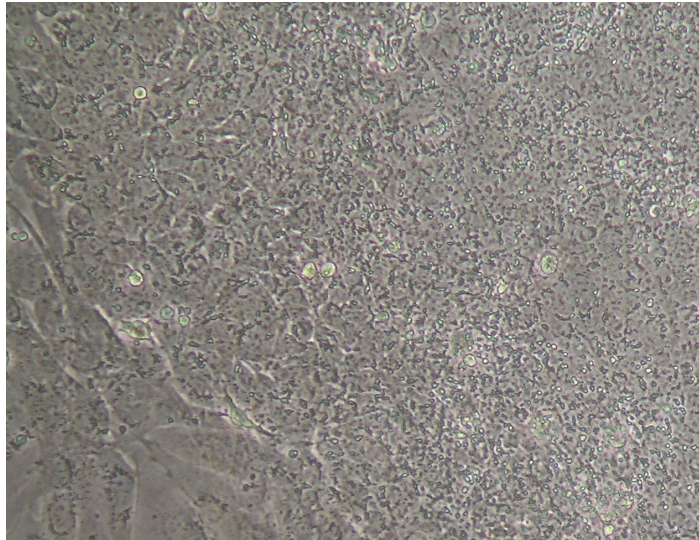
Derivation of rabbit ES cells



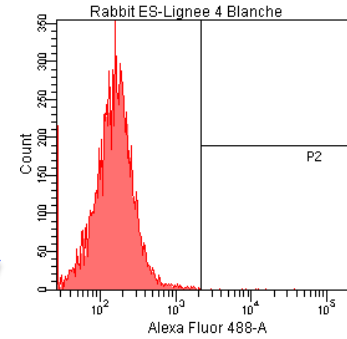
Rabbit ES cell (rESC) lines



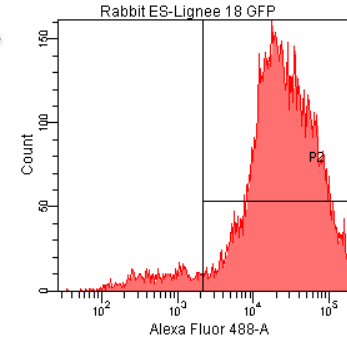
σ GFP^{+/-}



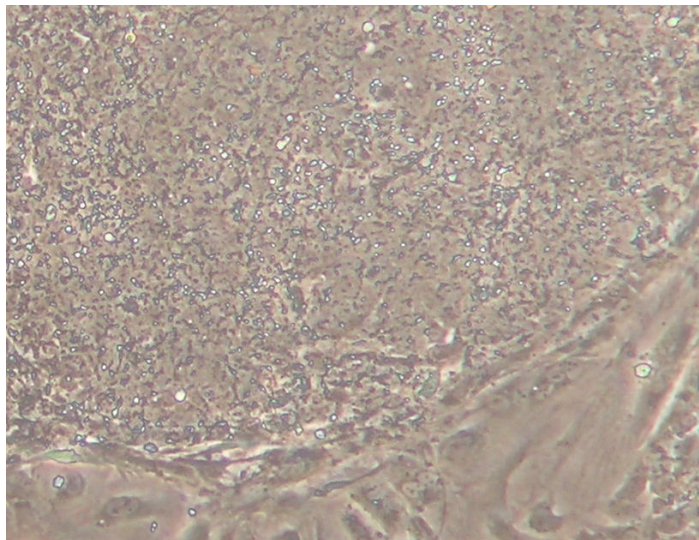
FGF2-dependant rESC



rESC GFP⁻
F4, F8, F19



rESC GFP⁺
F17, F18, F24



LIF-dependant rESC



rESC
L1 and L2

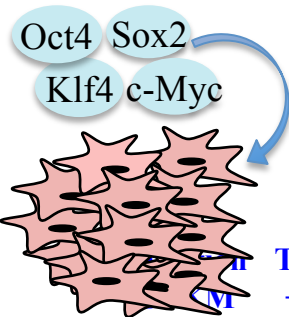
Generation of rabbit iPS cells

Differentiated cells

Loss of morphology

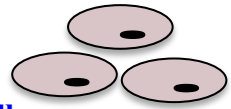
Colony formation

Pluripotent stem cells

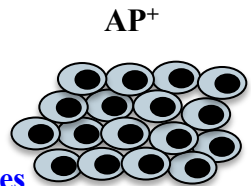


Overexpression

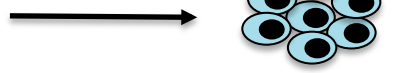
Transfer on feeder cells + iPS culture medium



Picking colonies + amplification



AP⁺



Oct4⁺ Sox2⁺ Nanog⁺

Analysis at P6

Rabbit skin fibroblasts

D0/D2

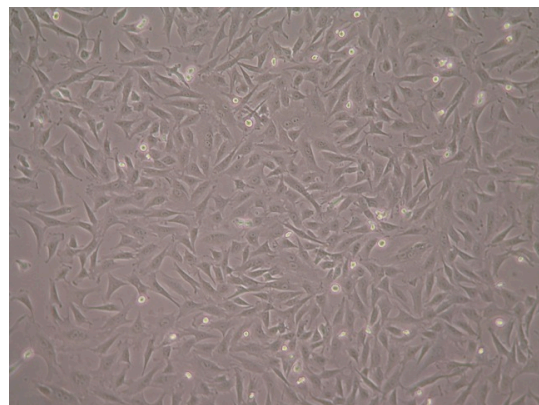
D5/D6

D16

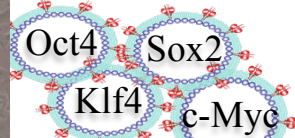
D27

D40/D55

Rabbit iPS cells

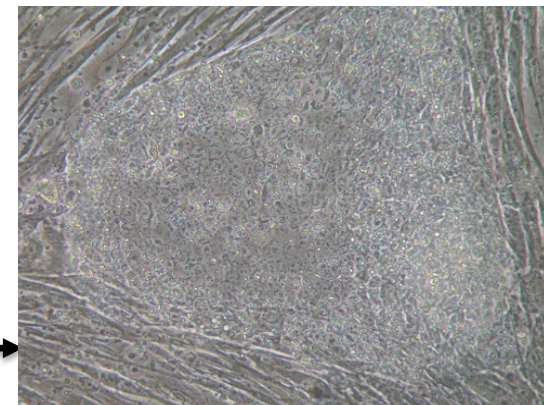


Rabbit skin fibroblasts



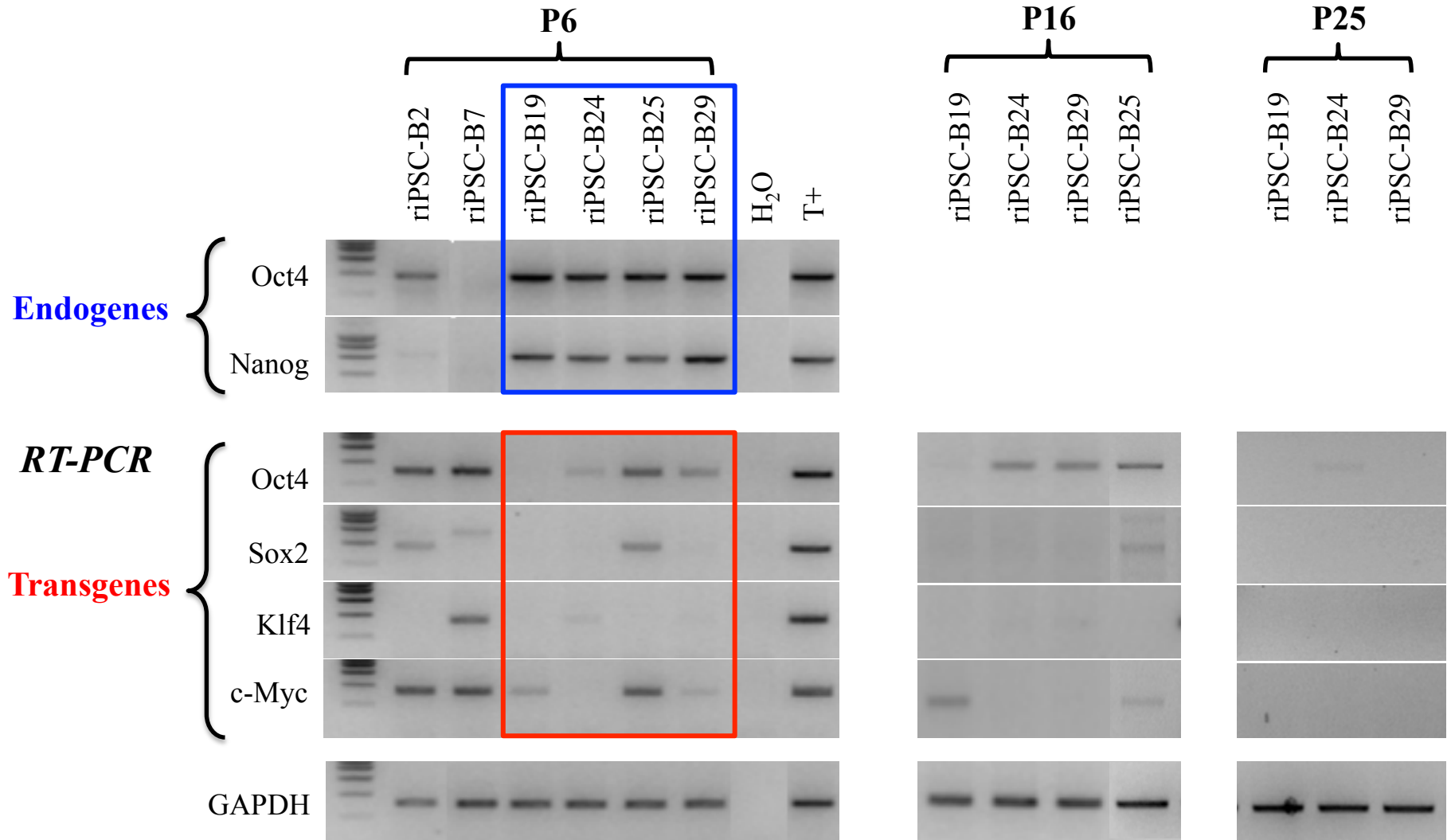
Infection

Reprogramming



Rabbit iPS colony

Rabbit iPSC (riPSC) lines



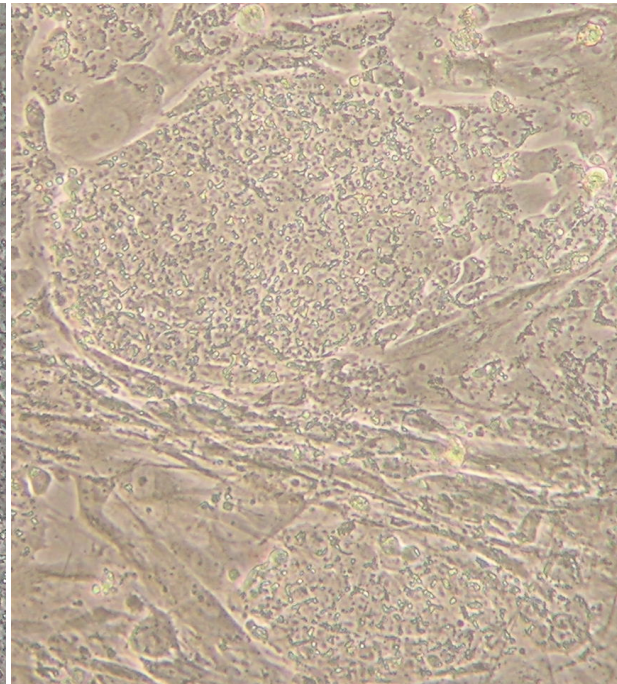
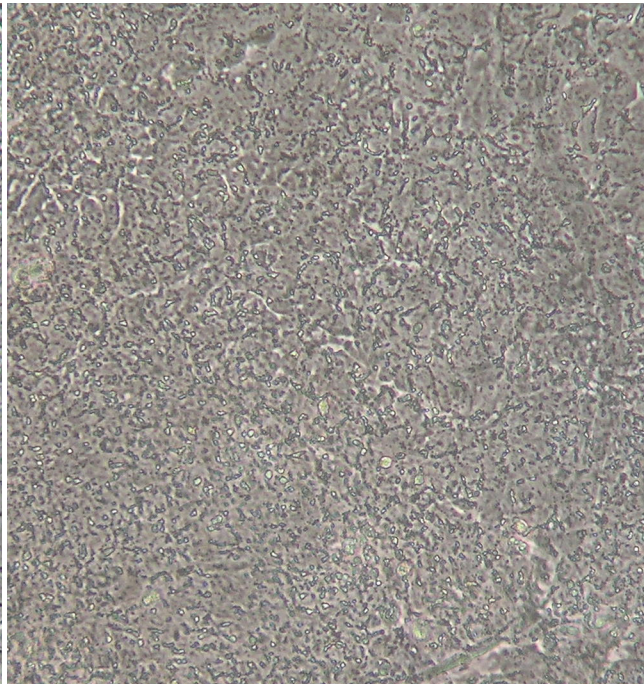
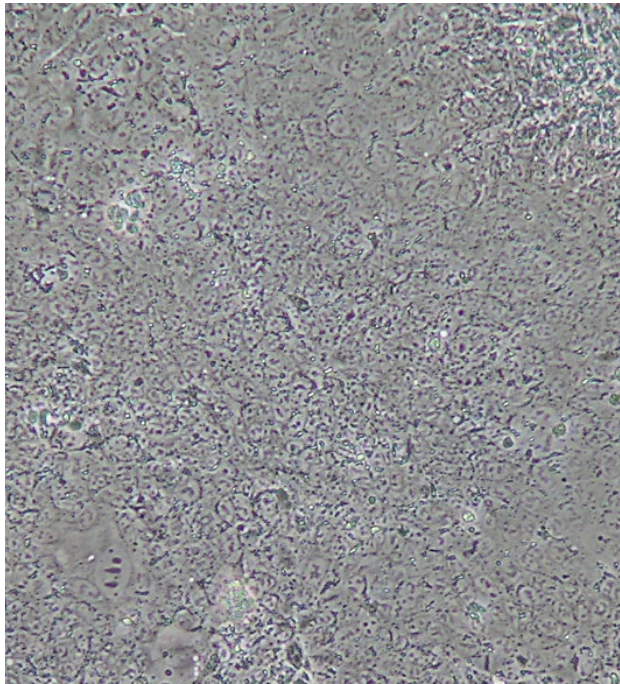
4 lines of FGF2-dependant riPSC: B19, B24, B25 and B29

Morphology of rESC and riPSC

FGF2-dependant rESC

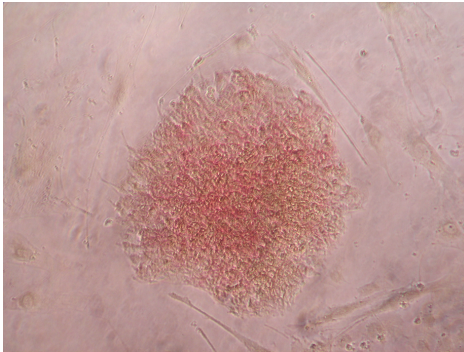
LIF-dependant rESC

FGF2-dependant riPSC

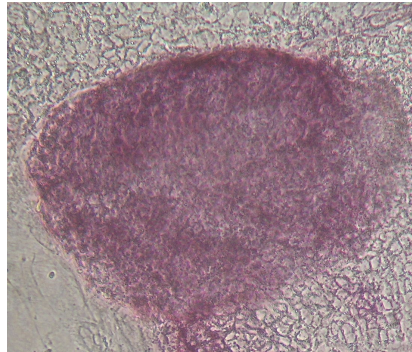


Alkaline phosphatase activity

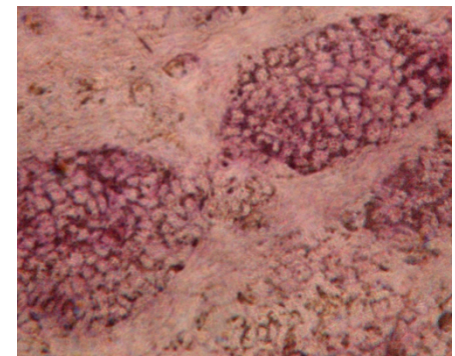
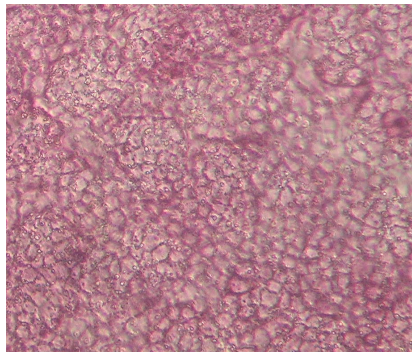
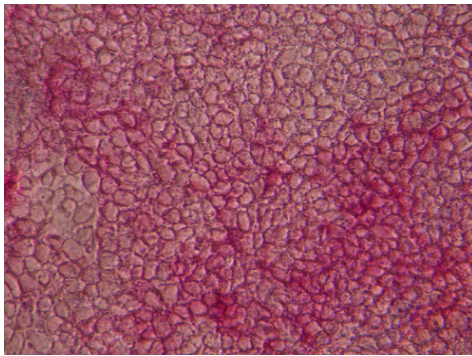
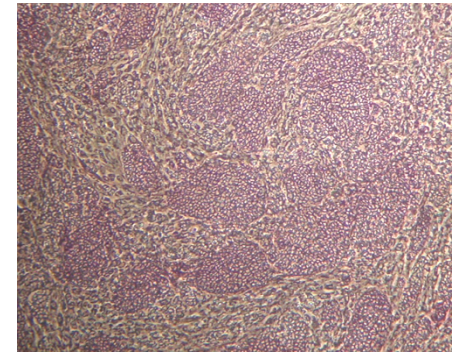
FGF2-dependant rESC



LIF-dependant rESC



FGF2-dependant riPSC



Expression of pluripotency markers

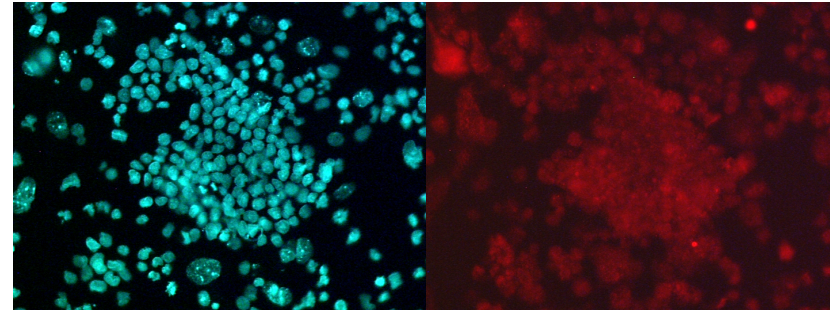
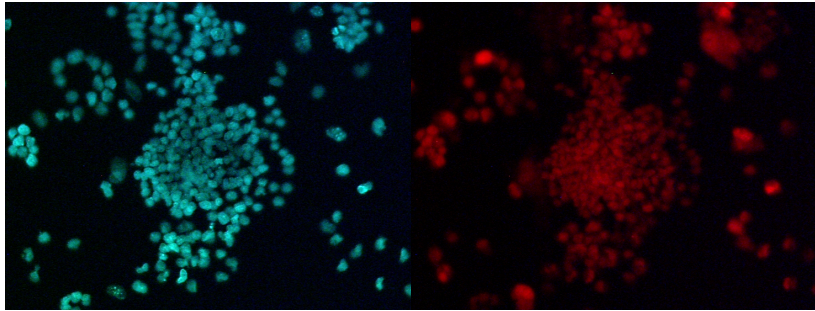
Hoechst

Oct-4

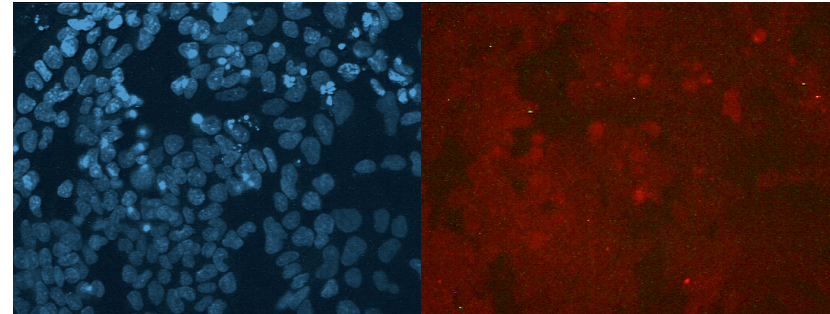
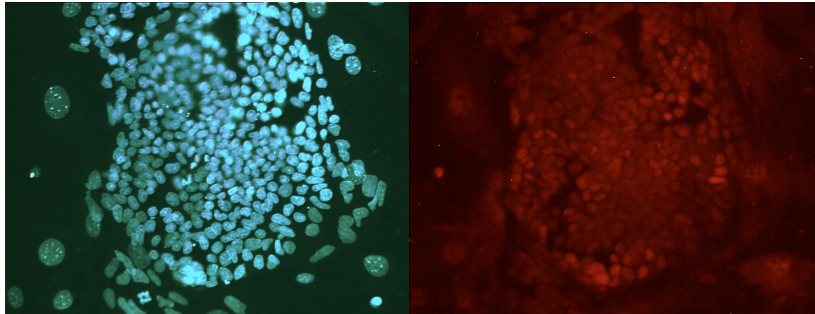
Hoechst

Nanog

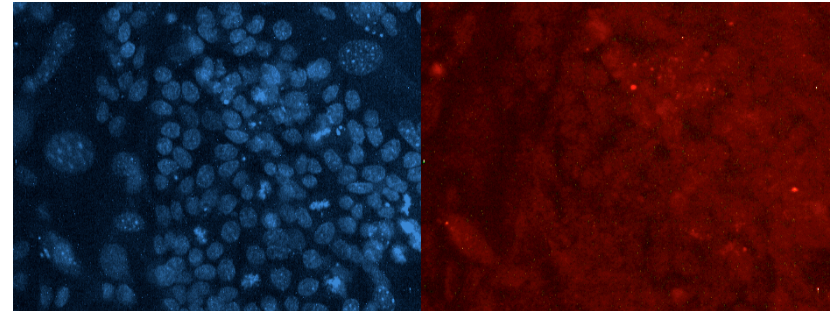
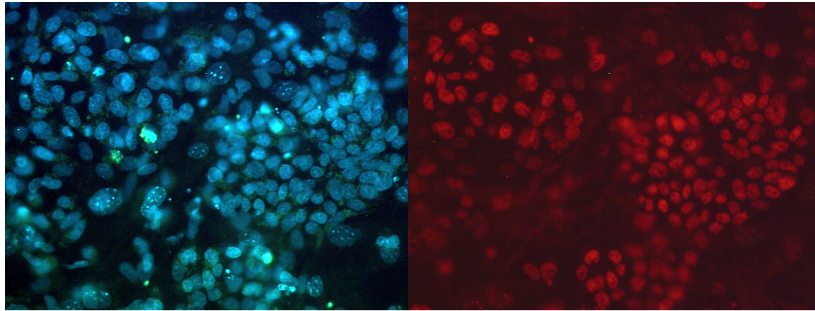
*FGF2-
dependant
rESC*



*LIF-
dependant
rESC*



*FGF2-
dependant
riPSC*



Expression of cell-surface markers

Hoechst

SSEA1

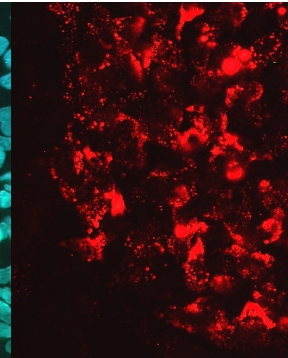
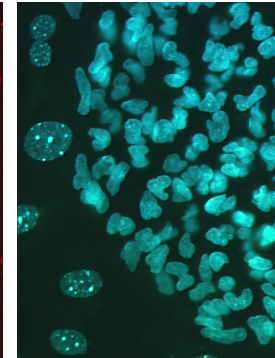
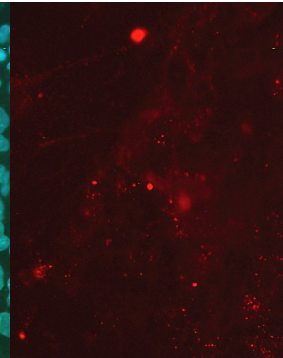
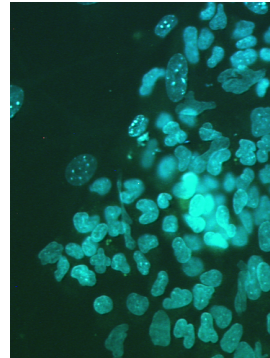
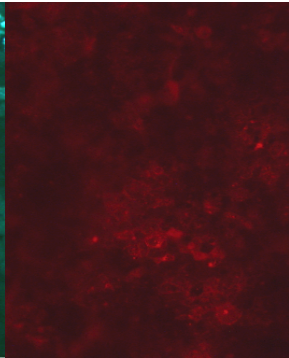
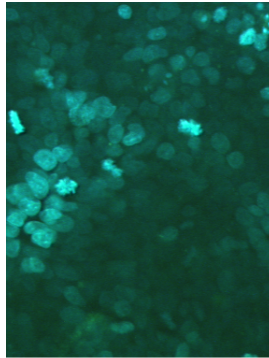
Hoechst

SSEA4

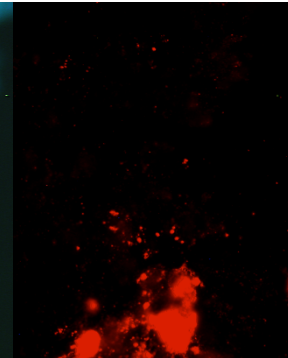
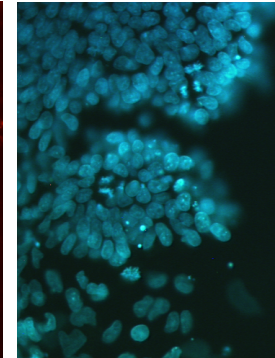
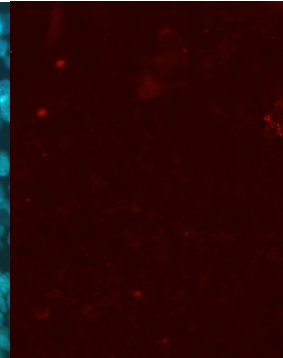
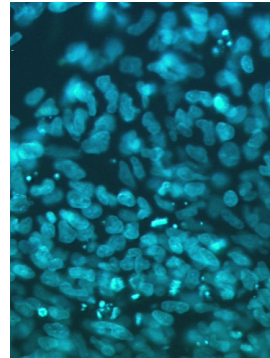
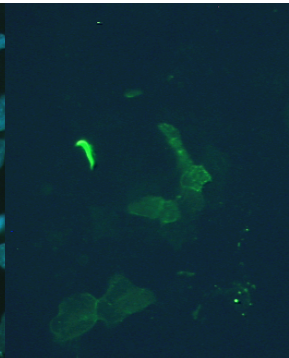
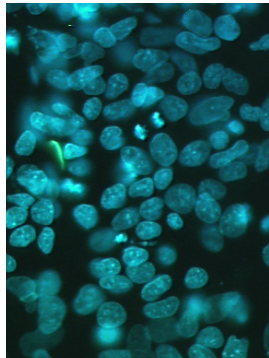
Hoechst

Tra1-60

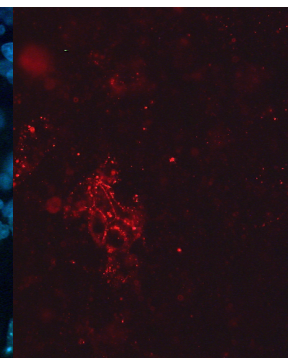
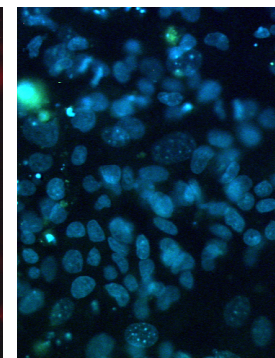
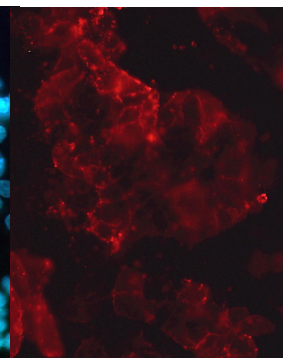
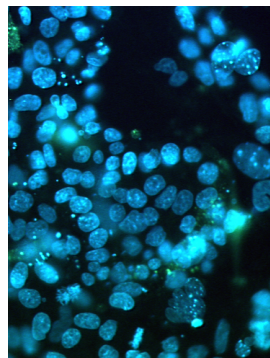
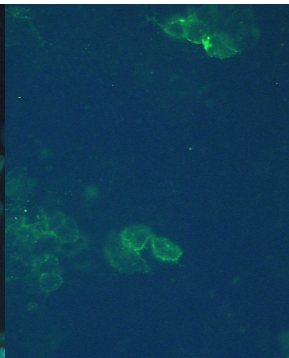
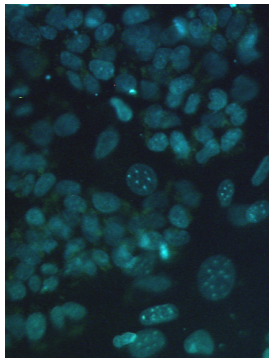
*FGF2-
dependant
rESC*



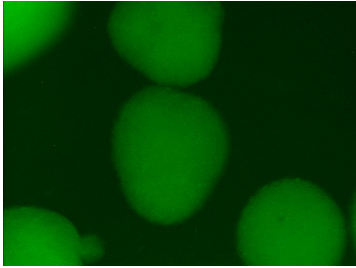
*LIF-
dependant
rESC*



*FGF2-
dependant
riPSC*

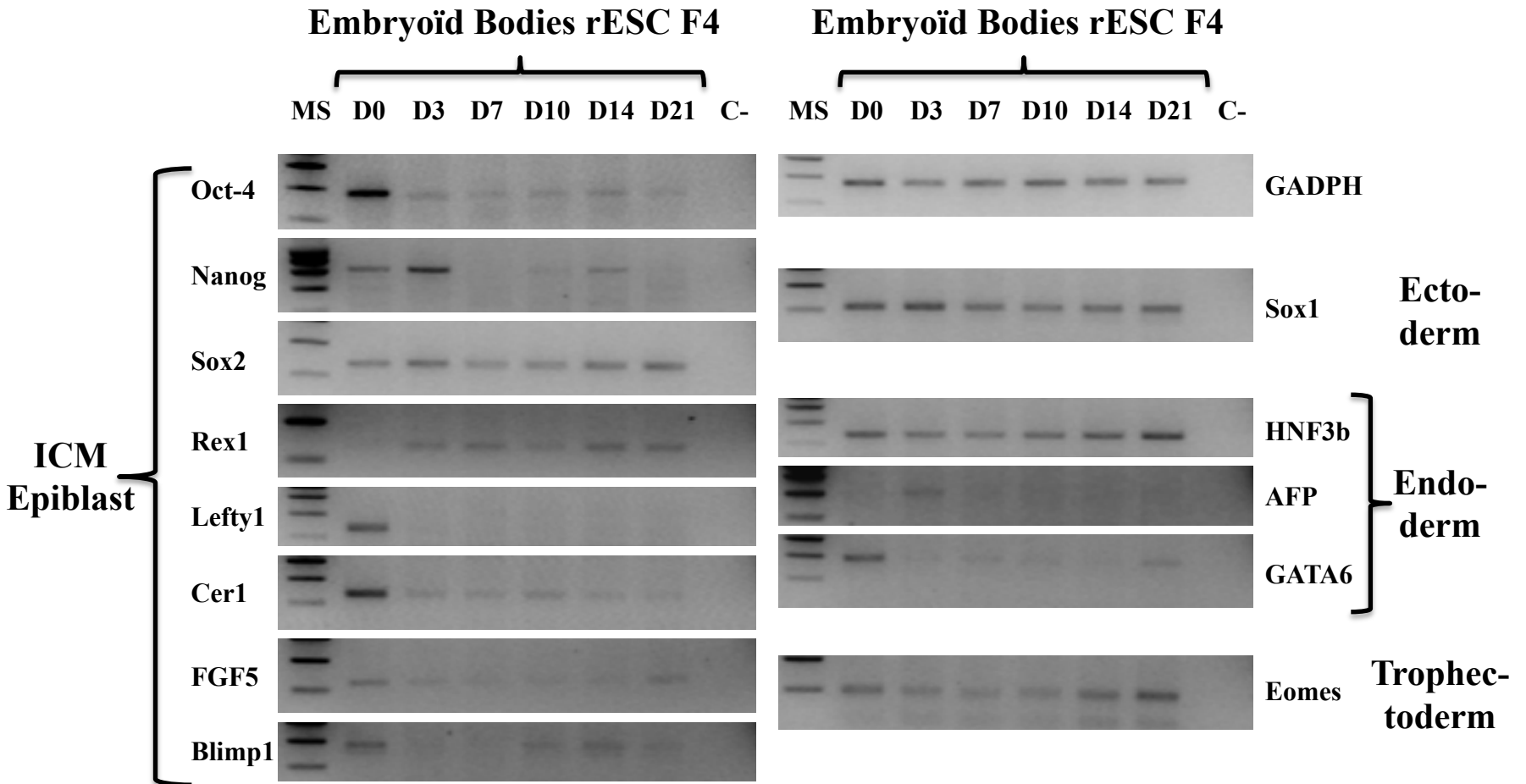
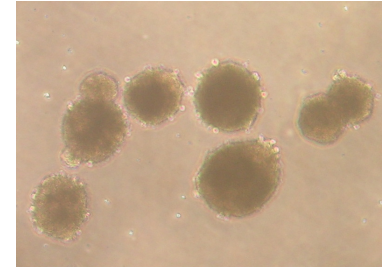


rESC F18 GFP+



Formation of embryoid bodies

riPSC B29 GFP-



Production of teratoma



rESC F18

31 days



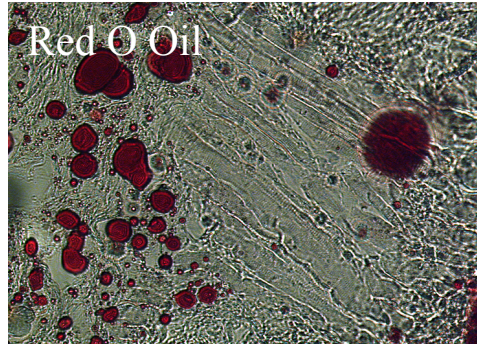
32 days



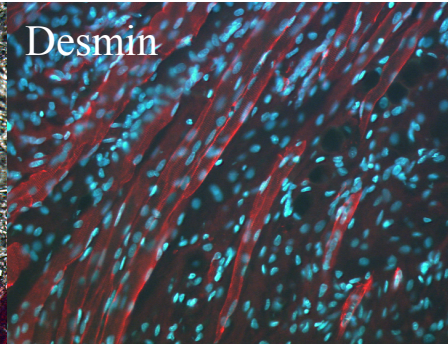
riPSC B29

Mesoderm

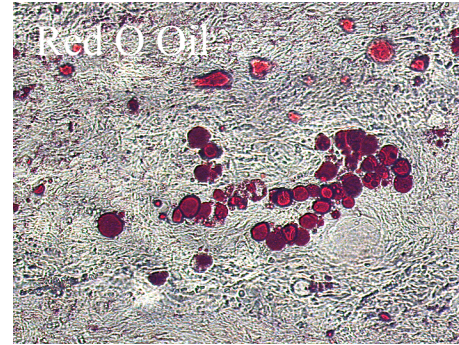
Mesoderm



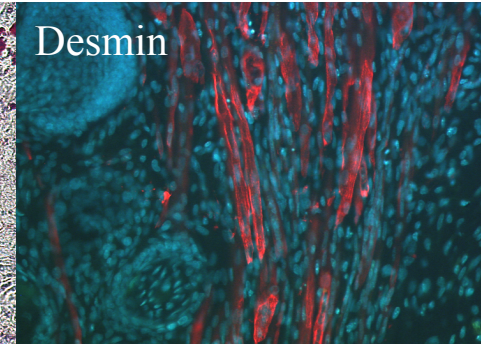
Red O Oil



Desmin



Red O Oil

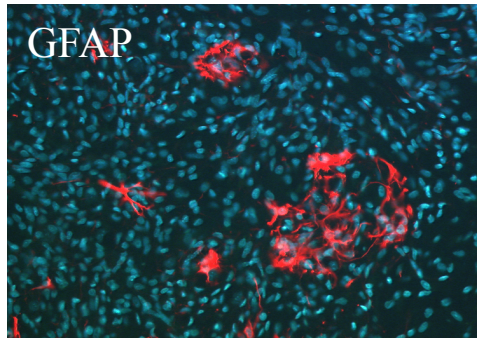


Desmin

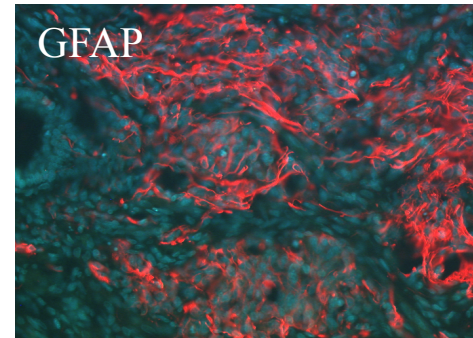
Ectoderm

Ectoderm

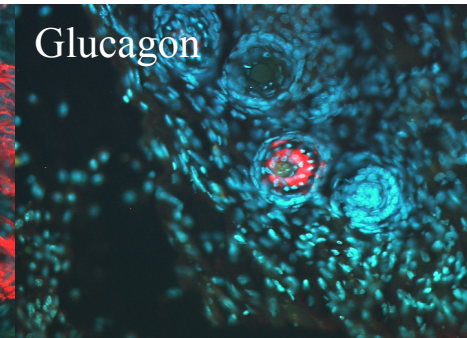
Endoderm



GFAP



GFAP



Glucagon

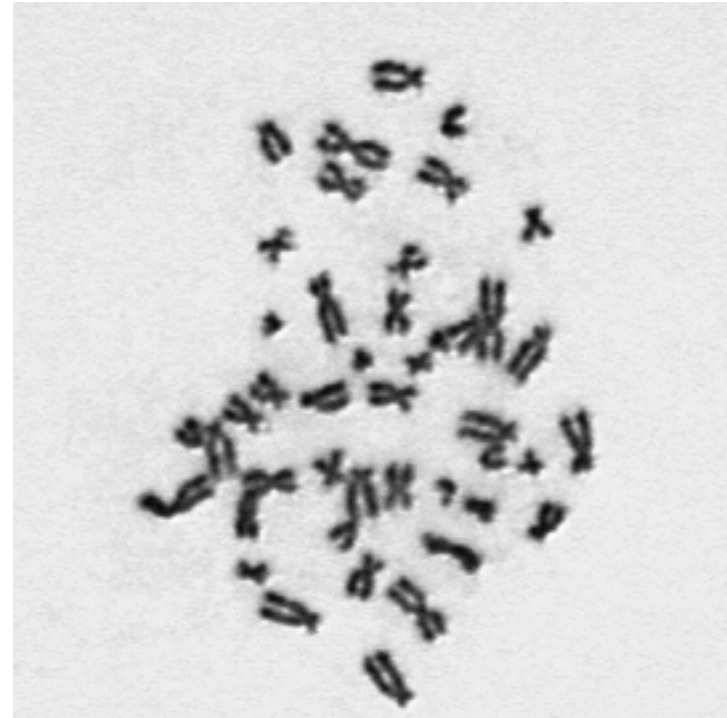
Analysis of karyotype

rESC F18



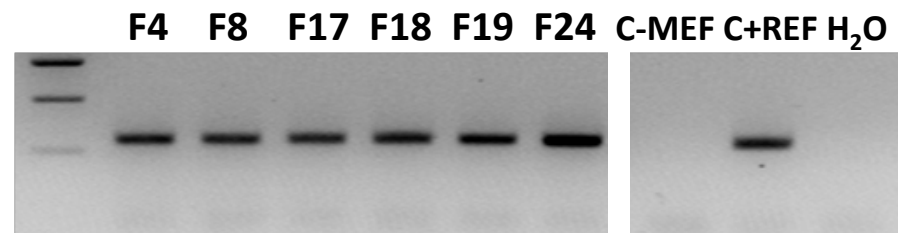
44 XY

riPSC B19



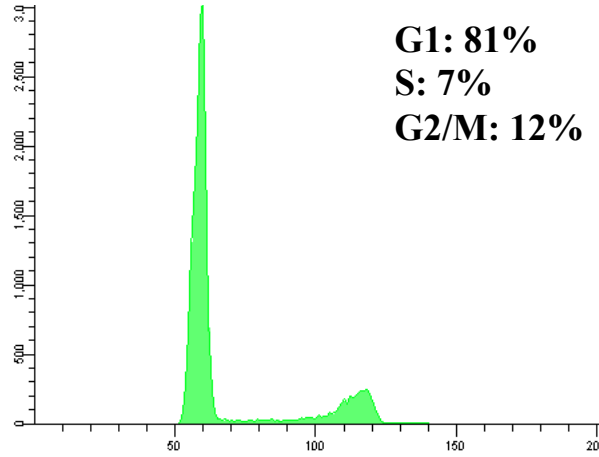
44 XX

*SRY gene detection by PCR
on FGF2-dependant rESC lines*

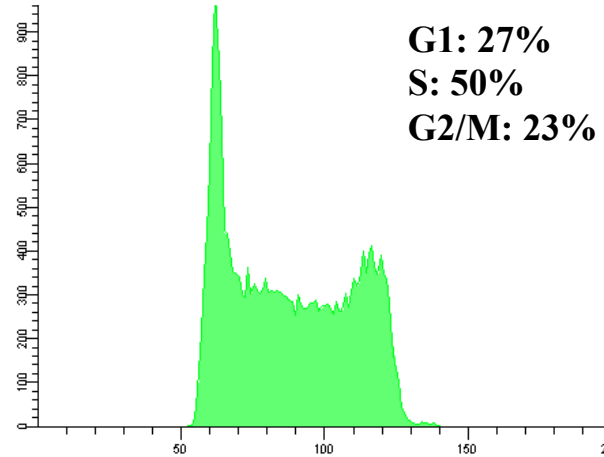


Cell cycle profiles

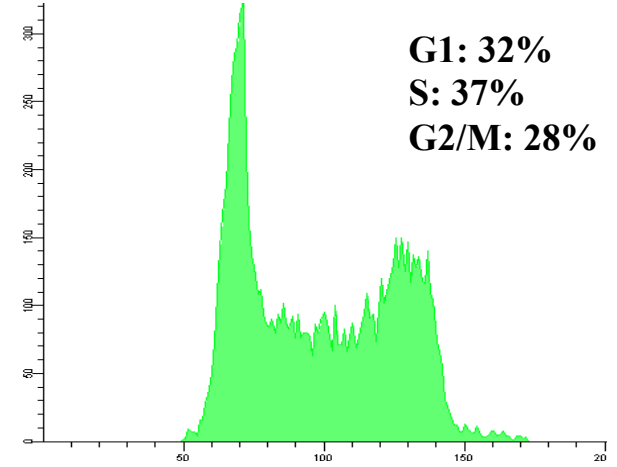
Rabbit Fibroblasts



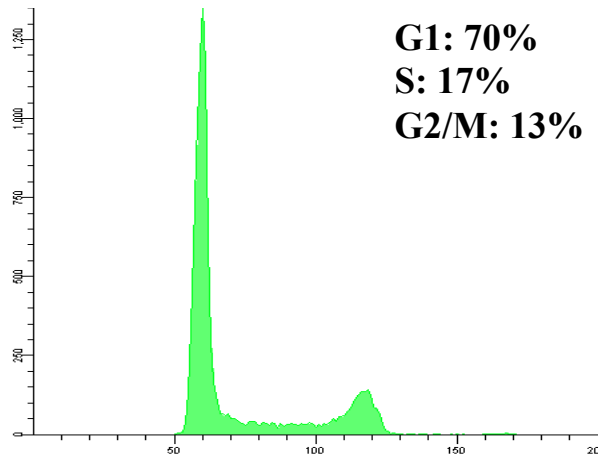
Mouse ESC



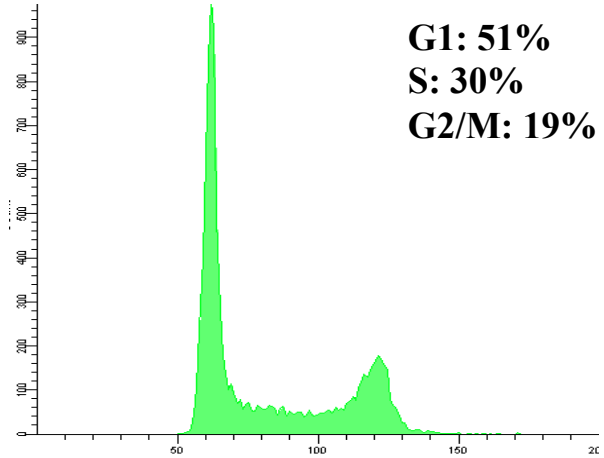
Rhesus ESC



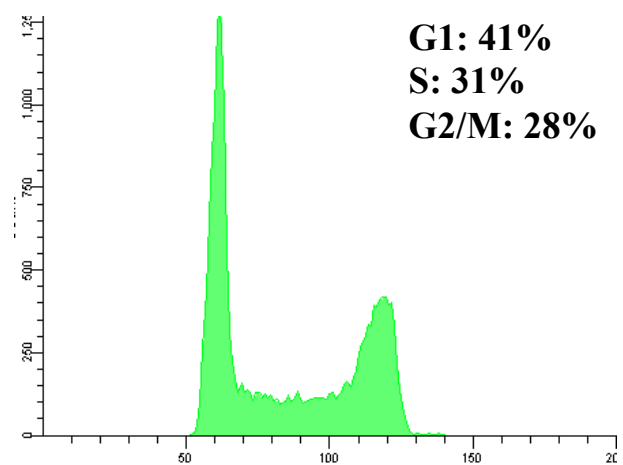
rESC F18



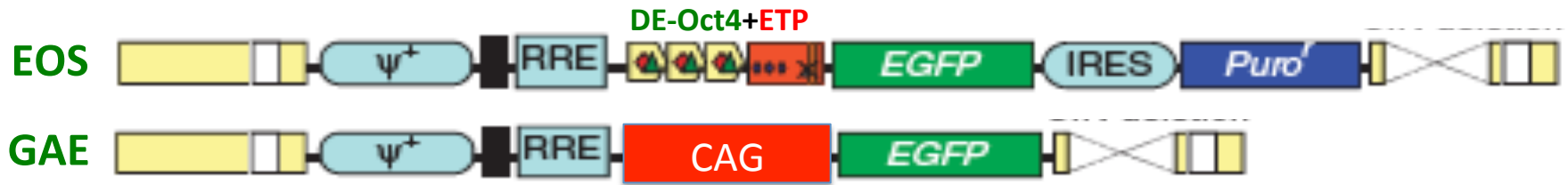
rESC L2



riPSC B19



Activity of mouse Oct-4 distal enhancer

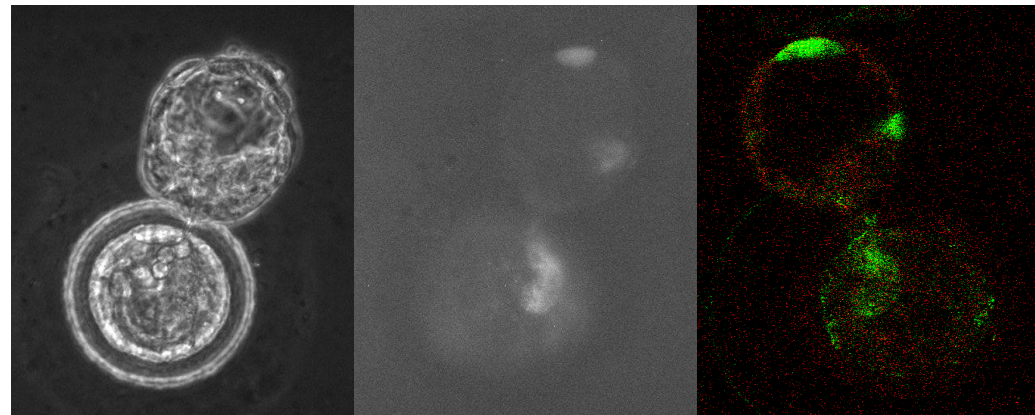


Bright light

GFP
fluorescence

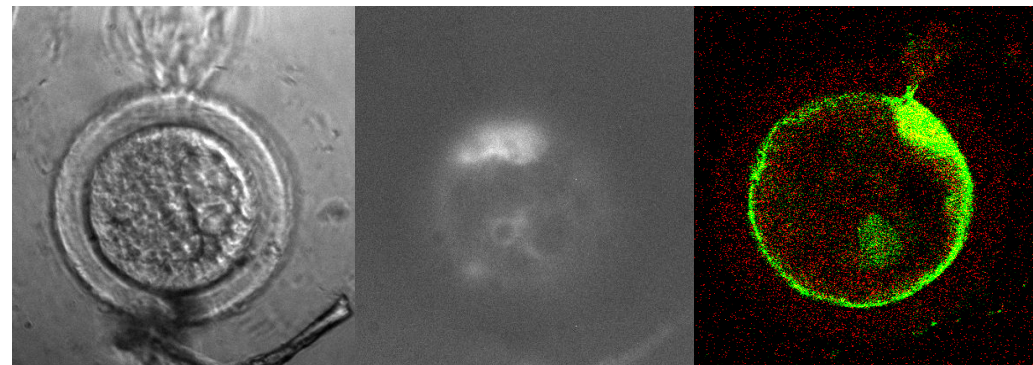
GFP
immuno-labelling

GAE



*Infection of 16-cell
embryos and analysis
at blastocyst stage*

EOS



Expression of EOS vector in rESC and riPSC

C⁺ mESC

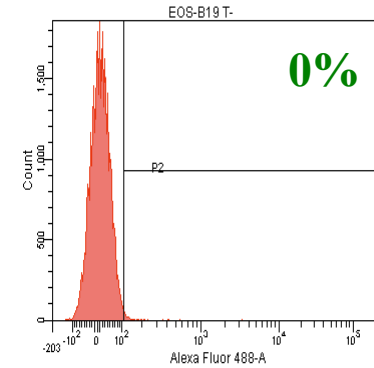
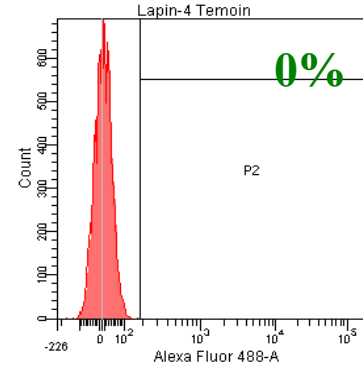
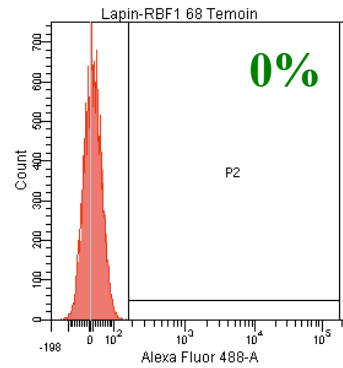
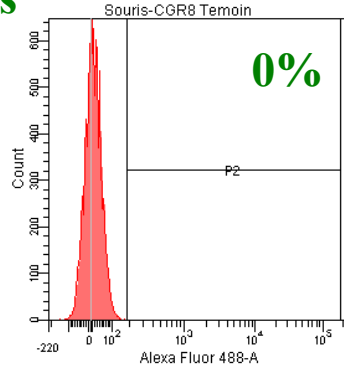
C⁻ rbF

rESC F4

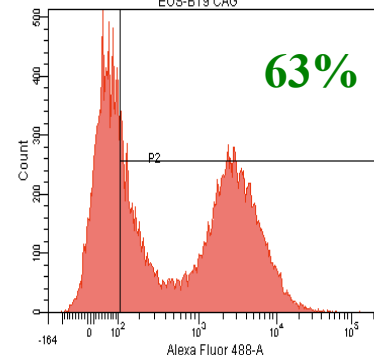
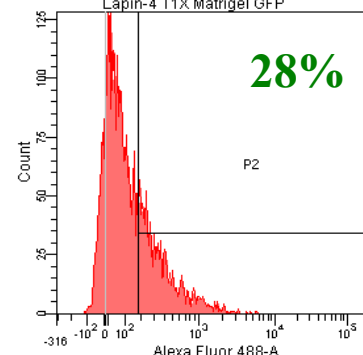
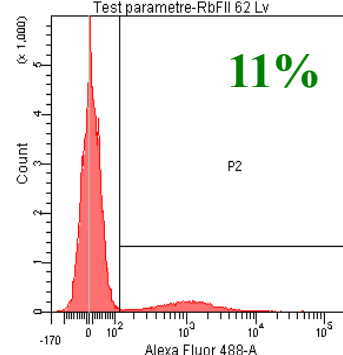
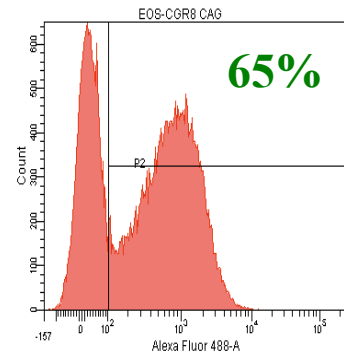
riPSC B19

% of GFP⁺ Cells

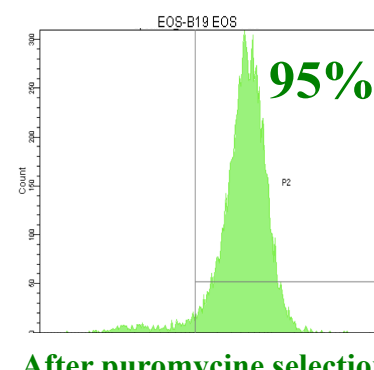
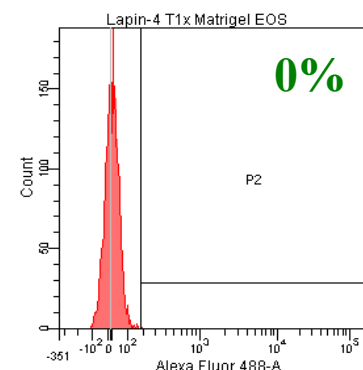
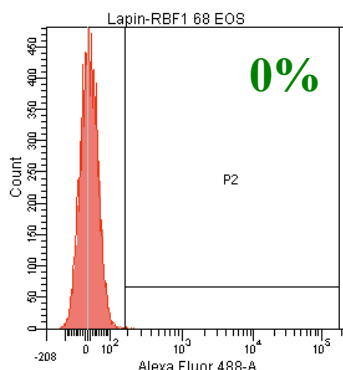
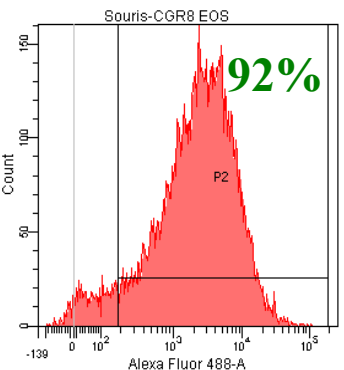
Non infection



GAE infection



EOS infection



After puromycin selection

Loss of mouse Oct-4 distal enhancer during rESC derivation

*Infection of 16-cell embryos with GAE or EOS lentiviruses
and analysis of GFP⁺ cells in ICM-derived outgrowths*

Non infected

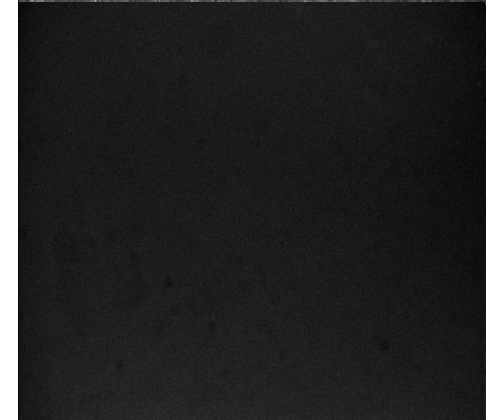
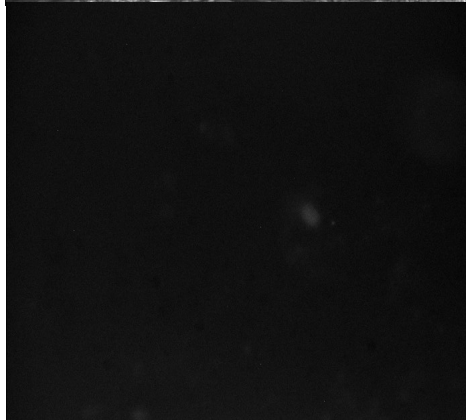
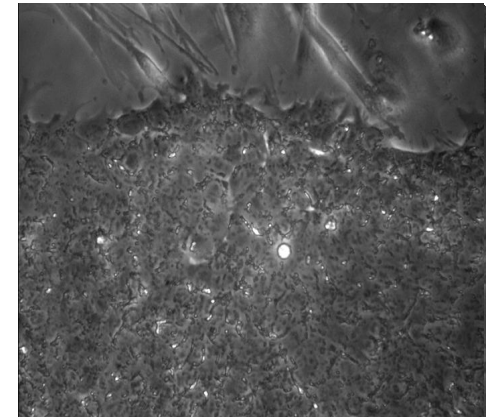
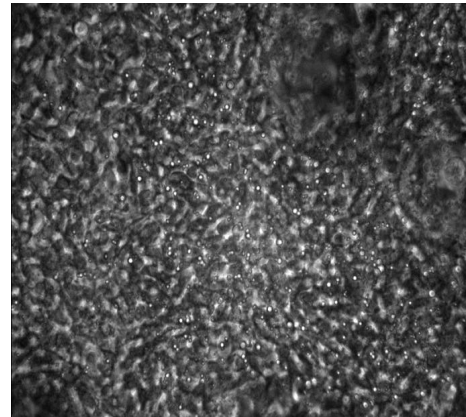
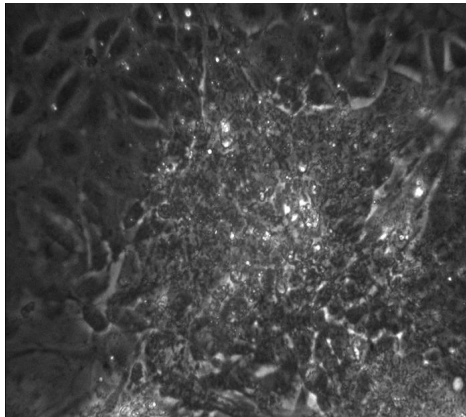
GAE

EOS

Bright Light

*66 days
post-
infection*

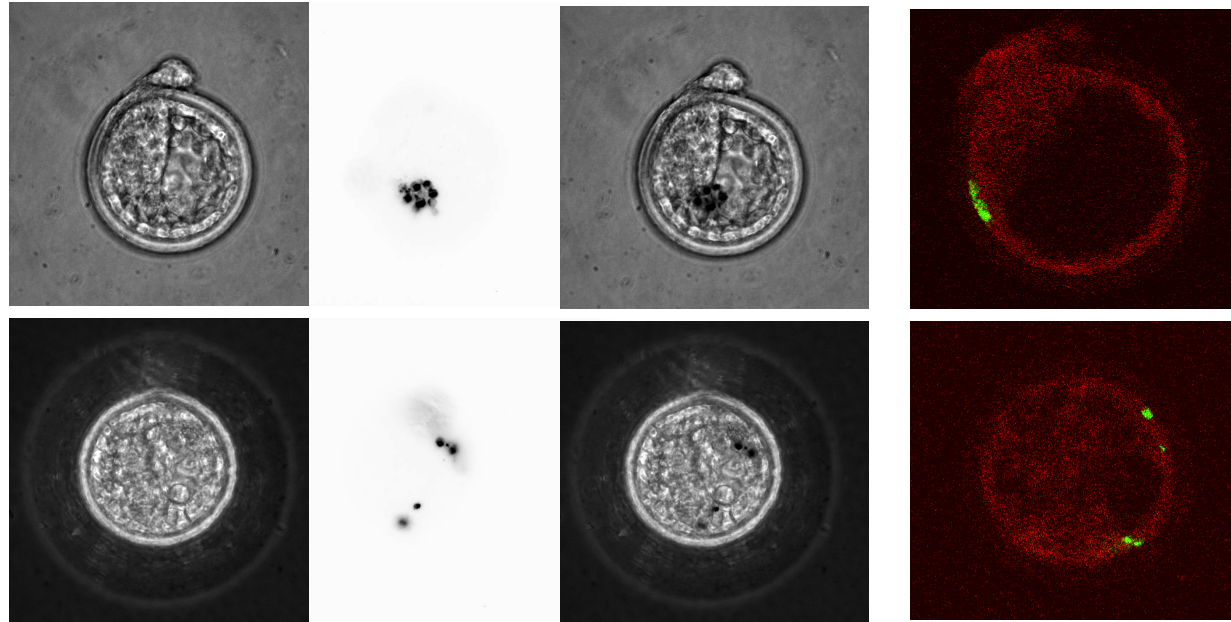
GFP
Fluorescence



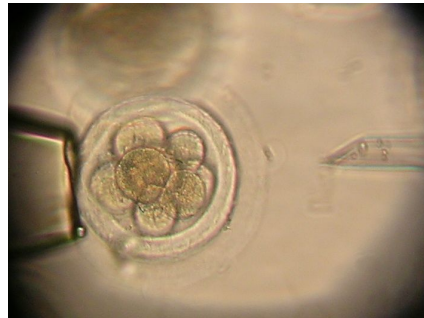
Production of chimera

Bright Light *GFP*
Fluorescence *Merged* *Confocal*
analysis

analysis of fluorescence
at blastocyst stage (E72h)

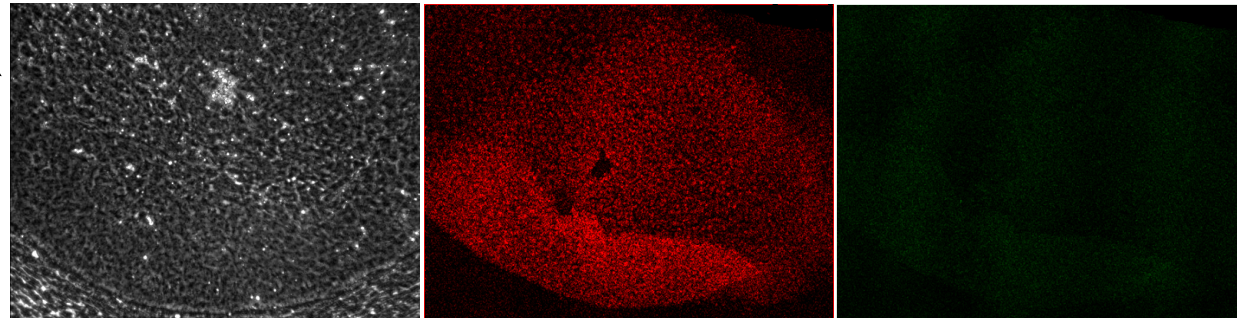


Injection of
GFP⁺ rESC in
8-cell embryos



Bright Light *TOPRO-3* *GFP*
Fluorescence

analysis of fluorescence
at eggcylinder stage (E6.5d)



Conclusion

- ***Rabbit pluripotent cell lines:***

- Six FGF2-dependant ESC lines

- Two LIF-dependant ESC lines

- Four FGF2-dependant iPSC lines

- ***All these lines show:***

- morphology of primate ESC

- high alkaline phosphatase activity

- expression of pluripotency genes Oct4 and Nanog

- ability to differentiate into the three embryonic lineages

- normal 2N karyotype

- ***These lines differ by:***

- signaling pathways which sustain self-renewal

- expression of hESC-specific cell surface markers

- activity of mouse Oct4 distal enhancer

- cell cycle profile

- ➔ *pluripotency state?*

- ➔ *ability to colonize host blastocysts and to produce chimera?*

Naïve and primed pluripotent states

