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Isolation and characterization of embryonic stem cells in non murin species

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PrimaStem

Research-based biotechnology platform
INSERM, INRA and University Lyon1

Isolation and characterization of embryonic stem cell lines
in non-murine species:
rhesus monkey, Human, goat and rabbit

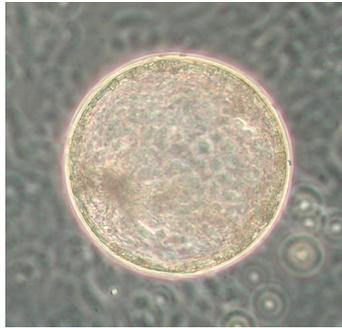
Rhesus monkey: Transversal project on monkey model of Parkinson Disease

Human: ES-based cell replacement therapy

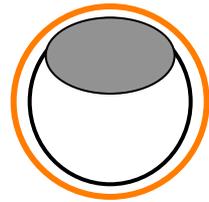
Farm animals: biotechnological tools for transgenesis

1. Fundamental interest: study of physiological functions
(reproduction, lactation, photoperiod, muscle growth).
2. Rabbit: Animal models of human disease
(Atherosclerosis, Tuberculosis, Arrhythmia, Obesity...)
3. Goat: Bioreactor
(Production of pharmacological molecules in milk)

Rabbit and goat ESC derivation

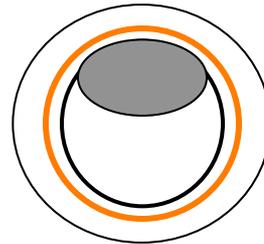


Goat blastocyst



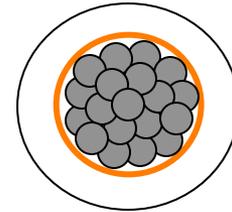
Blastocyst without mucus coat

Pronase



Blastocyst

Culture

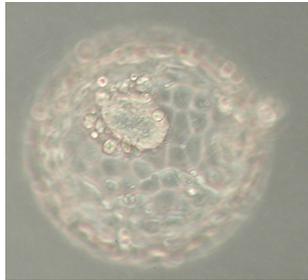


Morula

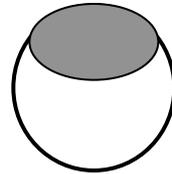


Rabbit morula

Mechanical elimination

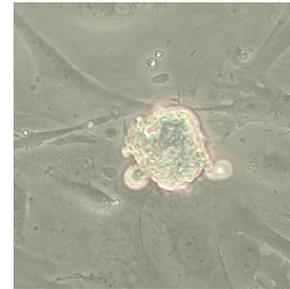


Rabbit blastocyst without zona pellicidae

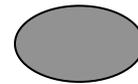


Blastocyst without zona pellucidae

Immunosurgery

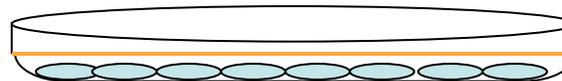


Rabbit ICM

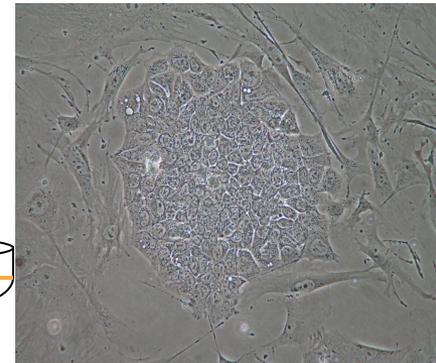


Inner Cell Mass

Culture on Feeder cells



Rabbit ICM after 20h culture on feeder cells

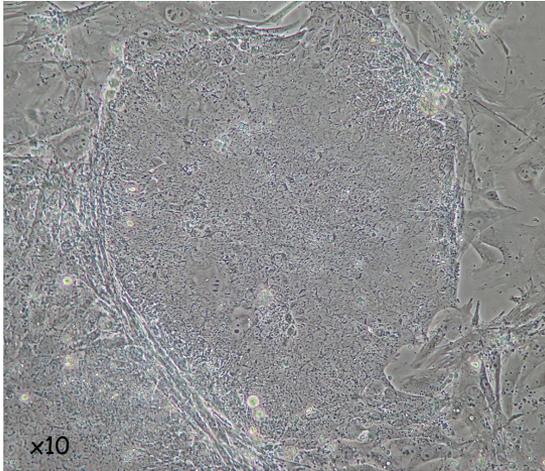


Culture Medium :

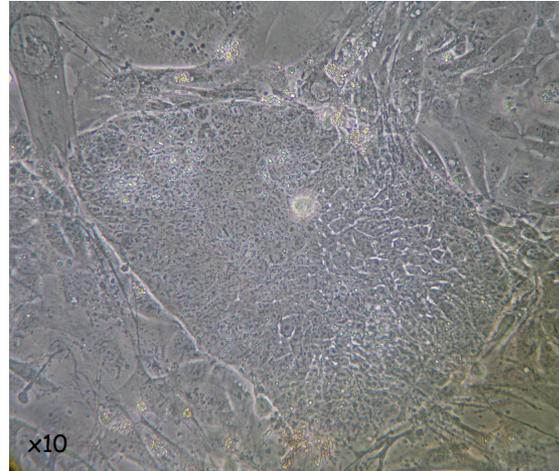
**KO-DMEM + 10% FBS + 10% KO-SR + FGF2
(medium using to derive monkey Lyon-ES line)**

Morphology of secondary ES-like colonies

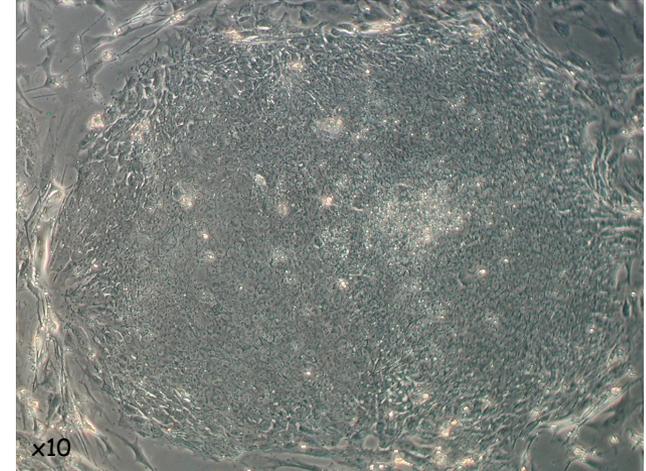
Rabbit ES-like colony



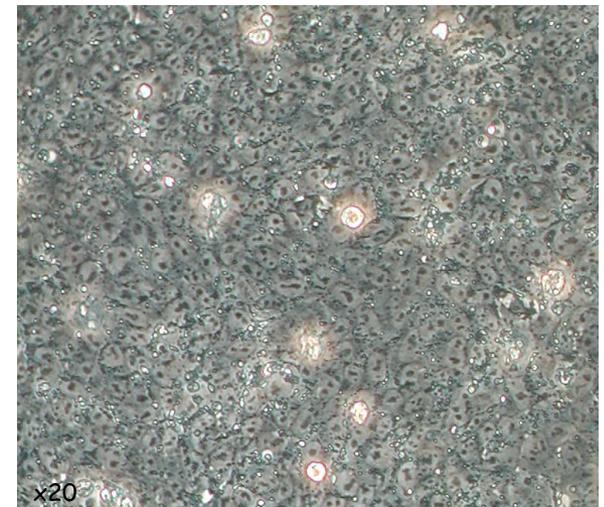
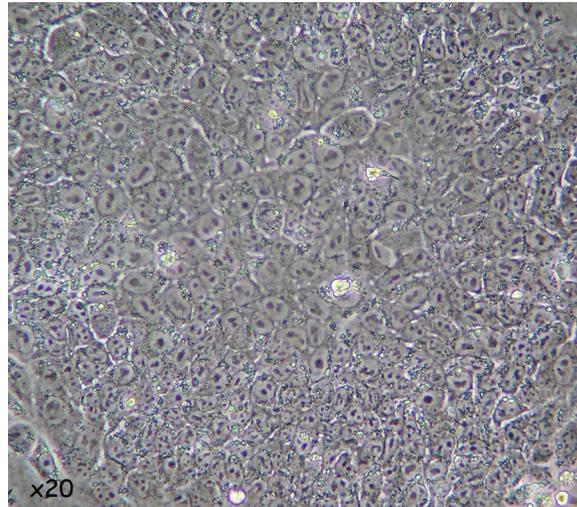
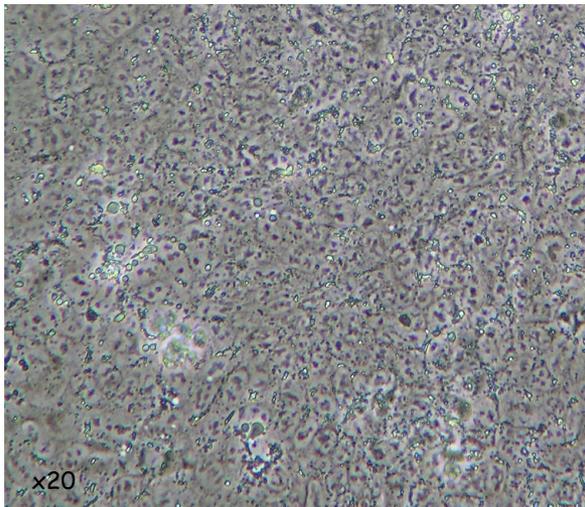
Goat ES-like colony



Human ES colony

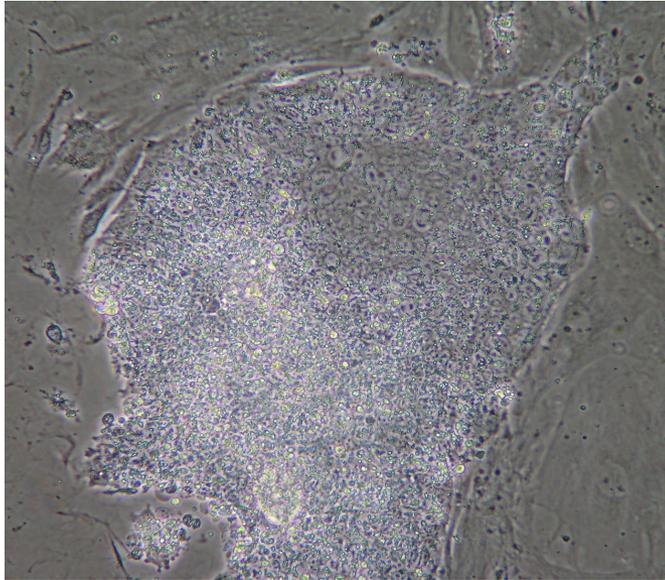


➤ Flat colonies of compact cells

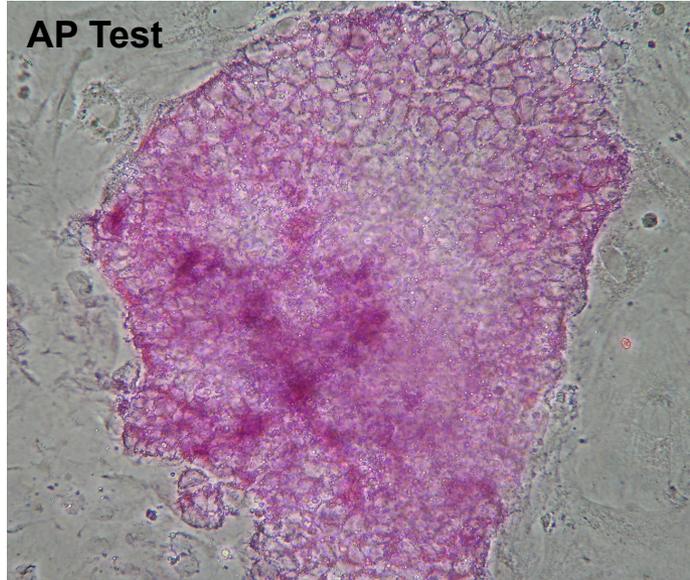


➤ High nucleus/cytoplasm ratio and prominent nucleoli

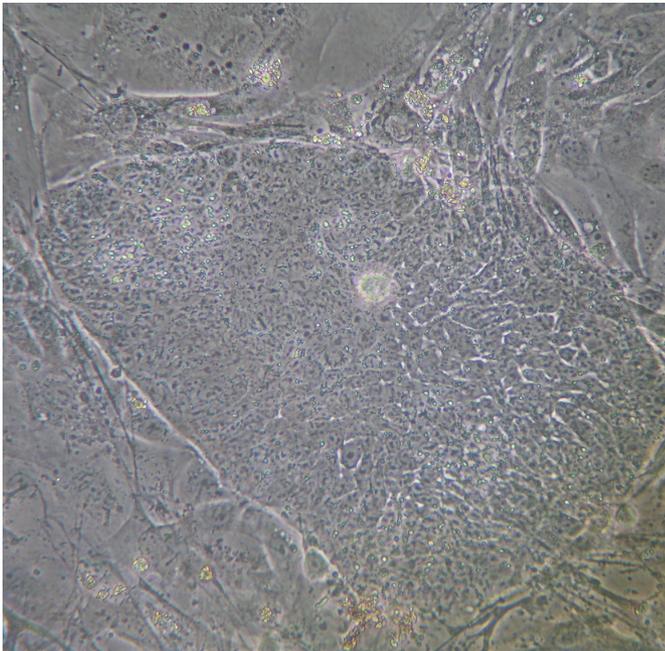
Alkaline phosphatase activity in ES-like cells



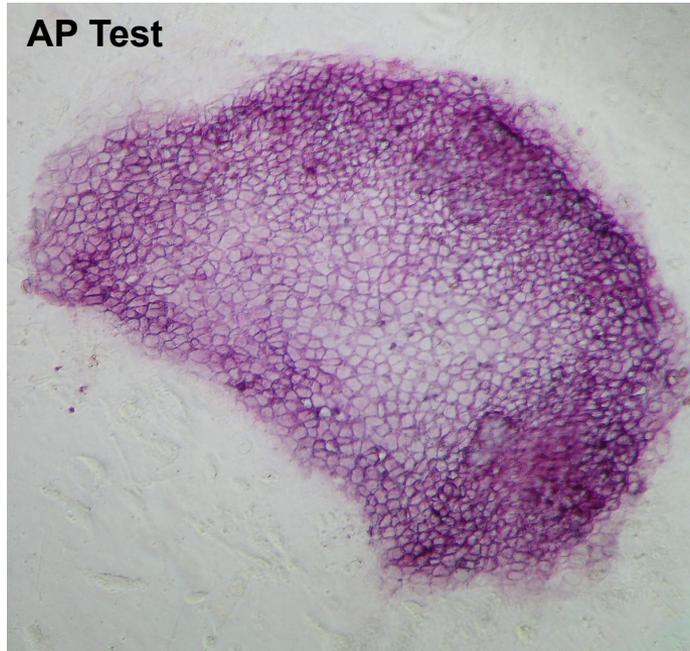
AP Test



Rabbit ES-like colony



AP Test



Goat ES-like colony

Oct4 expression in rabbit ES-like cells

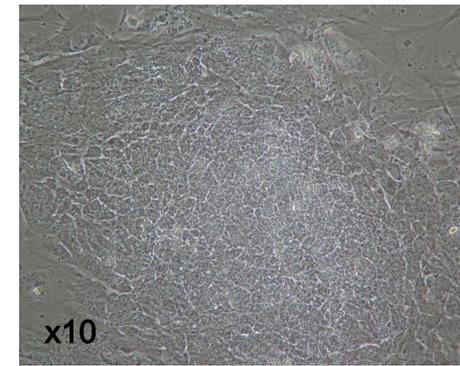
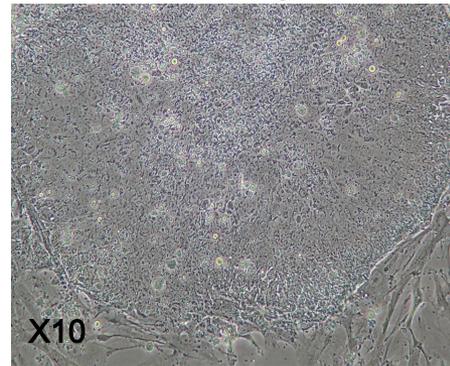
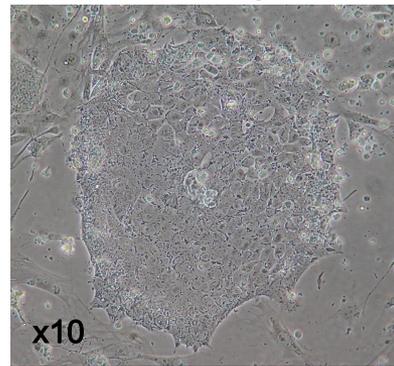
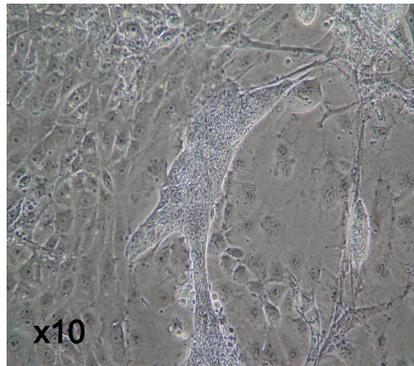
Passage 1

Passage 2

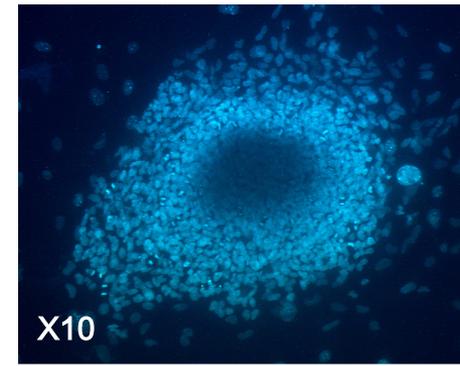
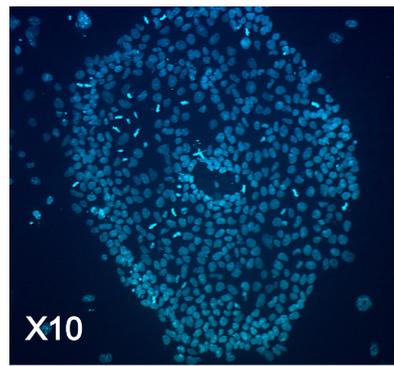
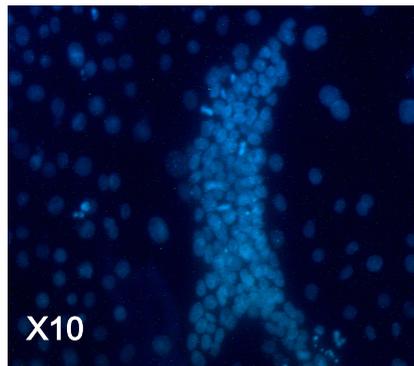
Passage 4

Passage 6

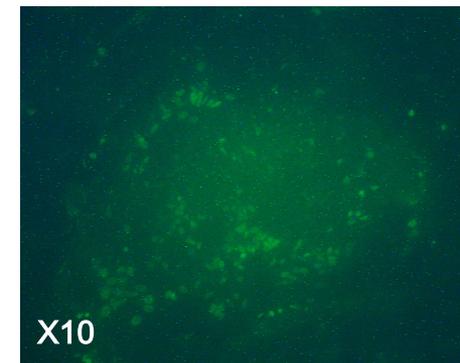
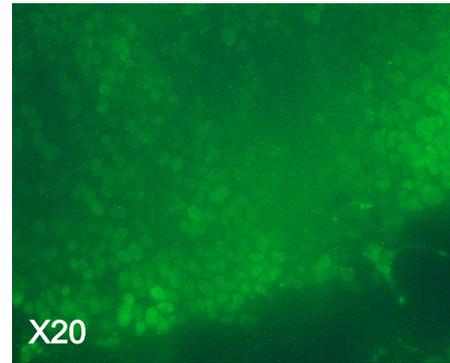
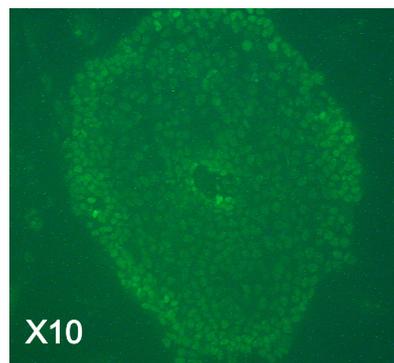
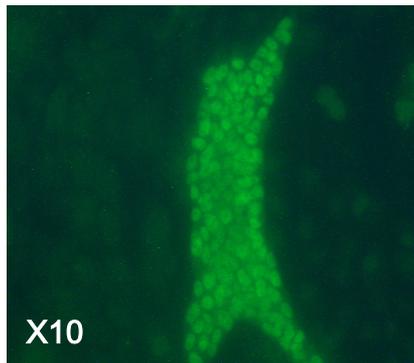
Contrast of Phase



Hoechst

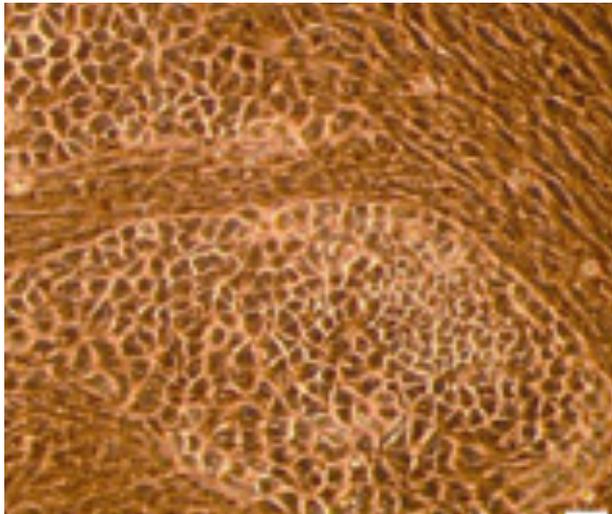


Anti-Oct4



- Loss of Oct4 expression with ES-like cell differentiation
- Self-renewal of ES-like cells is not sustained in applied culture conditions

1. Improvement of derivation and culture conditions
2. Surexpression of self-renewal genes using
 - lentiviral vectors
 - TAT-mediated protein transduction



Generation and Characterization of Rabbit Embryonic Stem Cells
Shufen Wang, Xianghui Tang, Yuyu Niu, Hongwei Chen, Bin Li, Tianqing Li, Xiuzhen
Zhang, Zhixin Hu and Weizhi Ji
Stem Cells published online Oct 12, 2006;
DOI: 10.1634/stemcells.2006-0226

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Jouy-en-Josas
Biology of Development and Reproduction
INRA
Jean-Paul Renard
Cloning in rabbit

China
Beijing
Labiocem
INRA-Science Academy of China
Zhou Chi
Cloning in mouse



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PrimaStem
INSERM INRA
Pierre Savatier et Colette Dehay
ESC in monkey and rabbit

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Kunming
Animal Reproduction Biology
Institute of Zoology
Weizhi Ji
ESC and Cloning in monkey





Yunnan Key Laboratory of Animal Reproduction Biology
Institute of Zoology, Kunming, China
Professor Weizhi Ji

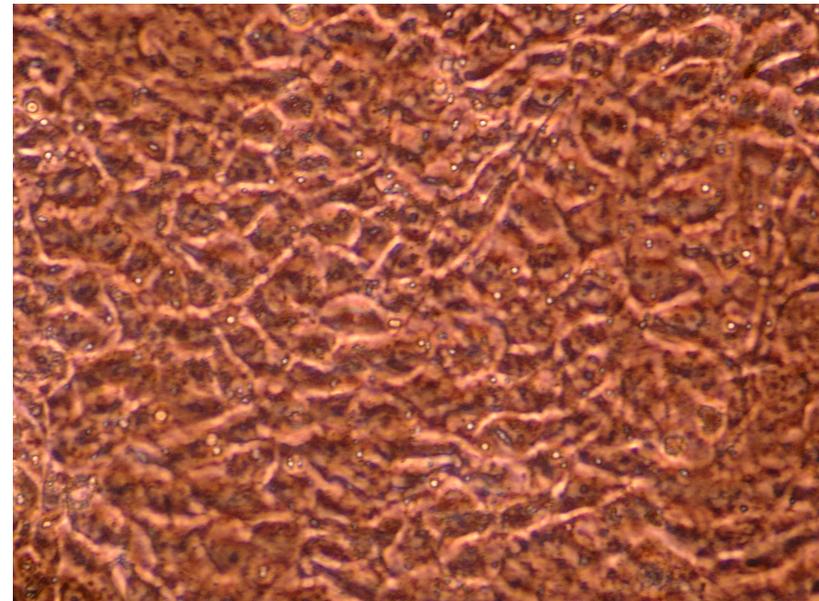
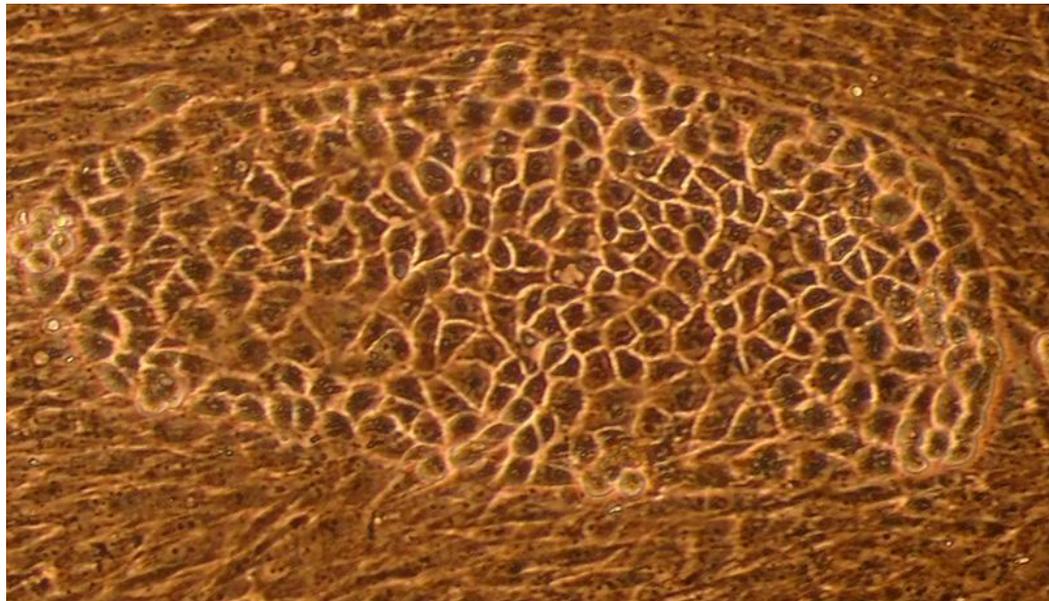






Rabbit ES line derived by Shufen Wang

- Monkey ES morphology (mix of differentiated and undifferentiated cells)
- Very unstable (rapid differentiation)
- Very dependant of quality and density of feeder cells
- Used for cloning but not to obtain chimera



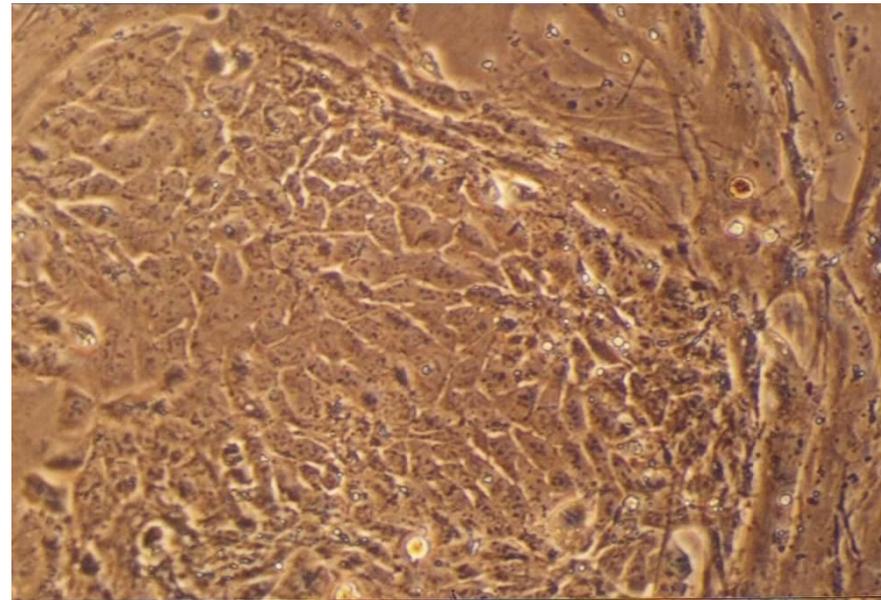
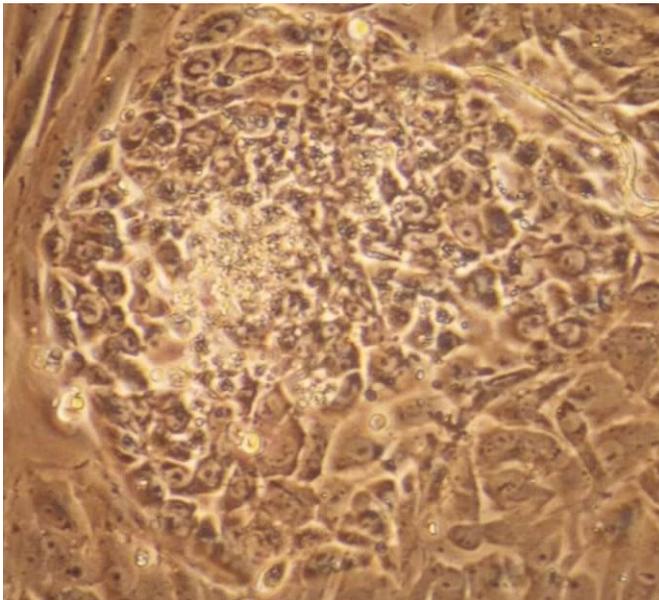
Rabbit ES cell derivation

Six differences between our derivation method and the technique of Shufen:

1. The rabbit line: White Japanese Line (New Zealand)



2. The feeder cells: type (129 and CF1), density and quality
3. The culture medium: could still be improved
4. The stage of embryos before culture: 2-cell embryos
5. The method of ICM isolation: dispase
6. The time between cell passages: shortening the first passages



PrimaStem

Pierre Savatier and Colette Dehay

Derivation of new ES cell lines

Marielle Afanassieff (CR1 INRA)

Suzy Markossian (AI INRA)

Emeline Fontaine (AI INSERM)

Pascal Salvetti (PhD Student, INRA Grand)

Pierre Osteil (EPHE Student)

Murielle Godet (CR1 INRA)

Derivation of new ES cell lines

INRA

Rabbit

T Joly ISARA Lyon
Marielle
Suzy
Pierre

Goat

P Mermillot INRA Tours
Suzy
Characterization of P2 colonies

INSERM

Human

JF Guérin HEH Lyon
Emeline
Pierre
Culture and derivation

Culture of Chinese line

Improvement of derivation method

Comparison of ES cell lines

Pascal

Embryo production

Surexpression of self-renewal genes

Lentiviral vectors

FL Cosset INSERM U758
Marielle
Suzy

TAT-mediated

protein transduction
F Edenhofer Bonn University
Murielle