

# EXPLORATION OF RABBIT MICRORNA EXPRESSION PROFILE IN RABBIT EMBRYO AND PLURIPOTENT STEM CELLS

**Elen Gócza**, Kinga Németh, Babett Bontovics, Bence Lázár, Roland Tóth,  
Mahek Anand, Pouneh Maraghechi, Zsuzsanna Bősze

***NARIC, Agricultural Biotechnology Institute, Gödöllő, Hungary***

French - Hungarian bilateral project  
(2010-2014)

ANR PROJECT: PLURABIT  
OMFB-00131/2010

**U846 INSERM, USC1361 INRA (SBRI)**  
Prof. Pierre Savatier

**BioTalentum Ltd., SZIU**  
Prof. Andras Dinnyes

**ABC**  
Prof. Zsuzsanna Bősze



# French - Hungarian collaborations

**Transcriptome analysis and functional characterization of rabbit pluripotent stem cells for the production of novel animal models.**

**INRA UMR 1198, Domaine de Vilvert 78352, Jouy en Josas**

Developmental biology and reproduction  
Véronique Duranthon

**U1208 INSERM, USC1361 INRA, Stem-cell and Brain Research Institute**

Pierre Savatier  
Marielle Afanassieff

**COST RGB-NET / 2012-2014**

BMBS COST Action TD1101

A Collaborative European Network on Rabbit Genome Biology (RGB-Net)

<http://www.biocomp.unibo.it/rabbit/>

**COST SAALAM 2014-2017**

BMBS COST Action BM1308

Sharing Advances on Large Animal Models (SALAAM)

<http://www.salaam.genzentrum.lmu.de>

# Steps toward the improvement of the efficiency of rabbit PS cell establishment

Existing rabbit pluripotent stem cell lines (PSCs: rabESCs or rabiPSCs) aren't capable for germ-line transmission. This problem may be due to in the currently used in vitro culture conditions that cannot support pluripotency maintenance.

## Main goals:

- Examination the expression pattern of factors effecting the rabbit embryonic development and pluripotency in 6-day old rabbit embryos
- Examination the expression pattern of stem cell specific microRNAs in 6-day old rabbit embryos

# Examination the factors influencing the ES cell pluripotency

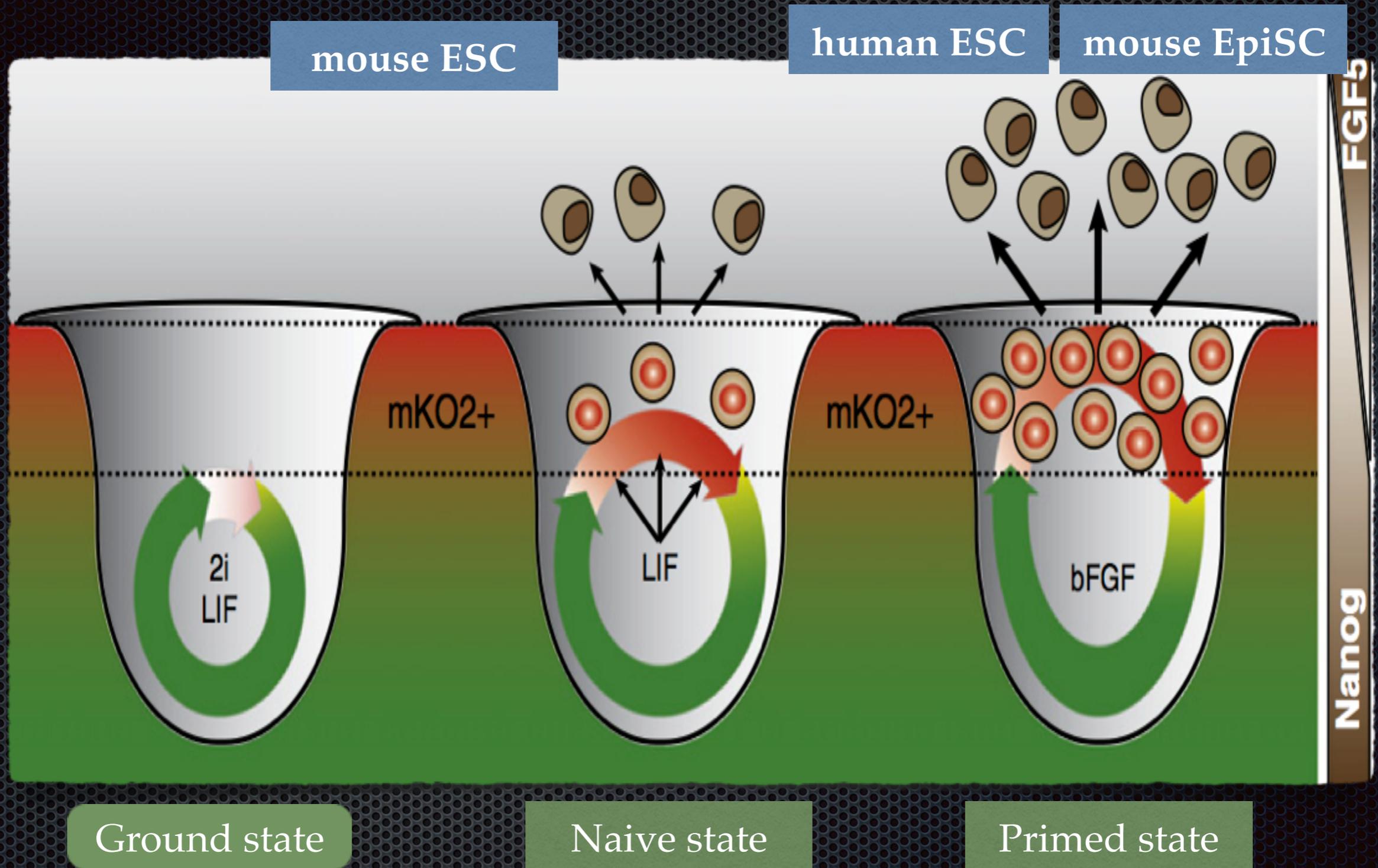


- KO-EM, 15%FBS, mLIF
- CD1 MEF
- trypsin
- ZP, blastocyst
- AP, SSEA-1
- in vitro differentiation
- teratocarcinoma formation
- germ cell chimeras

- LIF ↔ bFGF
- FBS ↔ SRL
- trypsin ↔ ?
- 2i, 3i, 4i ↔ GATA6
- activin
- miRNAs

- KO-EM/F12, 15%SRL, bFGF
- OF1 MEF
- accutase
- no ZP, only ICM
- AP, SSEA-1, SSEA-4
- in vitro differentiation
- teratocarcinoma formation
- chimeras

# Pluripotency in embryos and embryonic stem cells

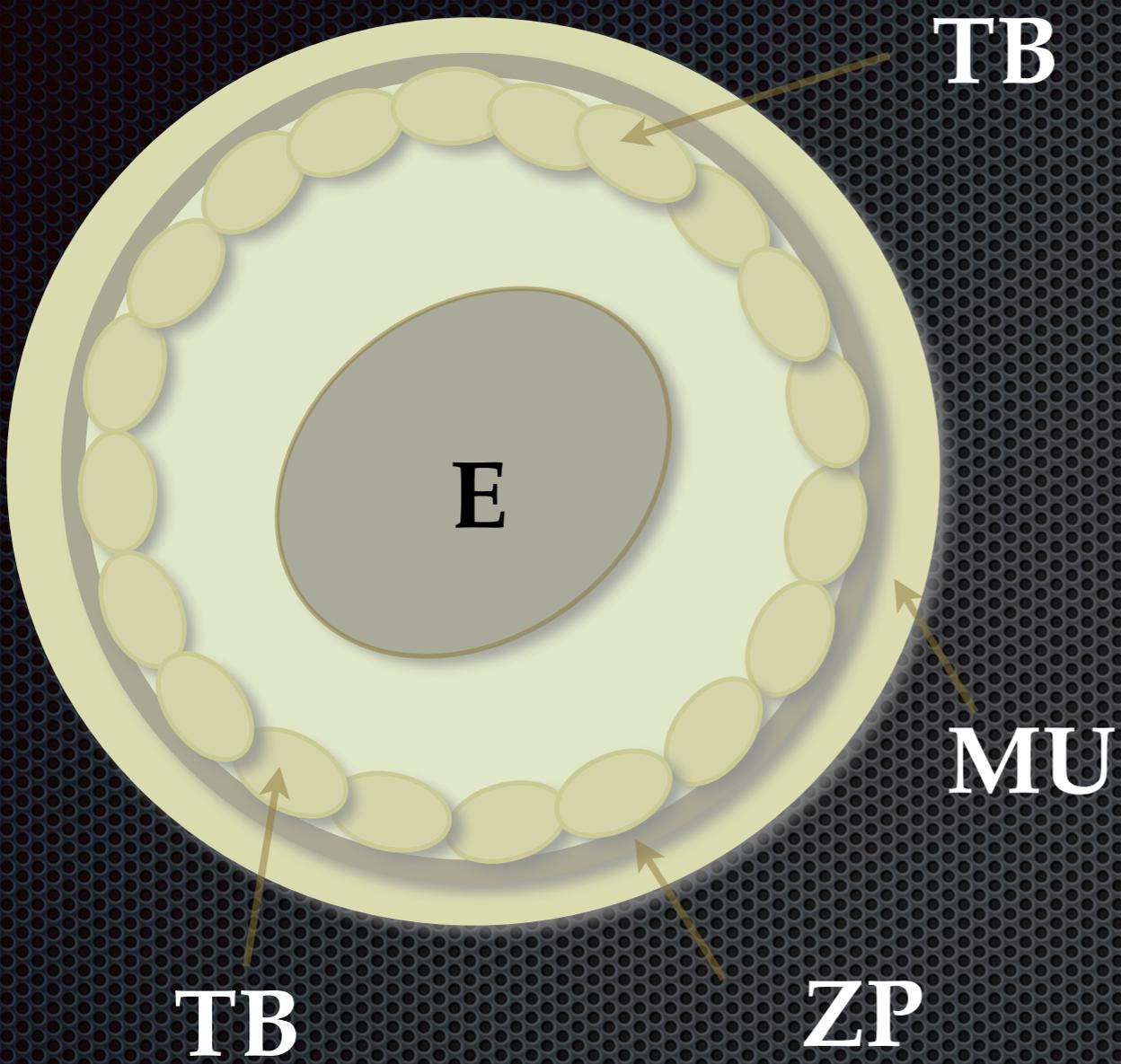


Coronado et al.: A short G1 phase is an intrinsic determinant of naive embryonic stem cell pluripotency, *Stem Cell Research* (2013) 10, 118–131

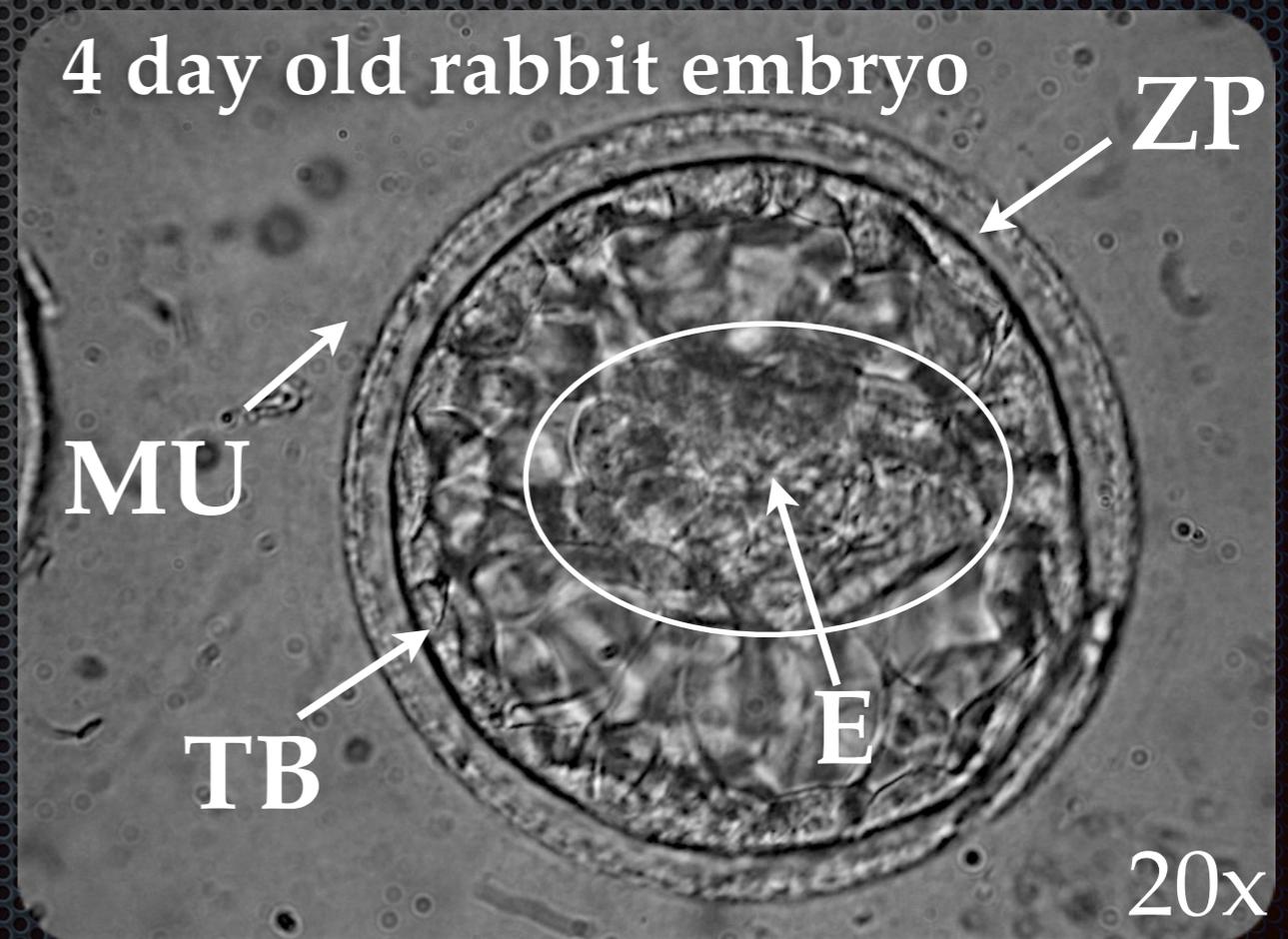
rabbit ESC, iPSC



# Examination the factors influencing the rabbit embryonic development

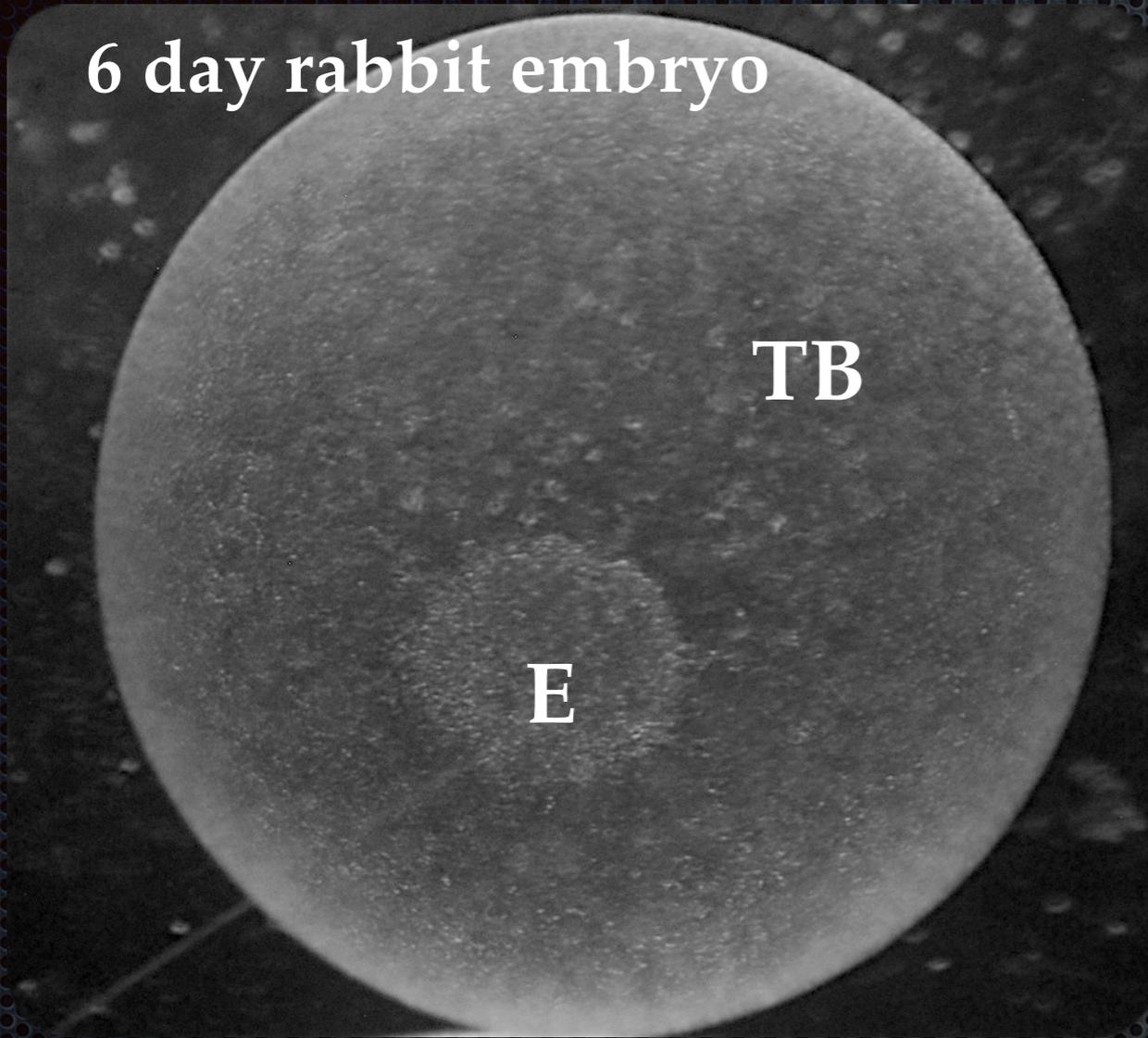


- E: embryo-disk (ICM)
- TB: trophoblast
- ZP: zona pellucida
- MU: mucin coat

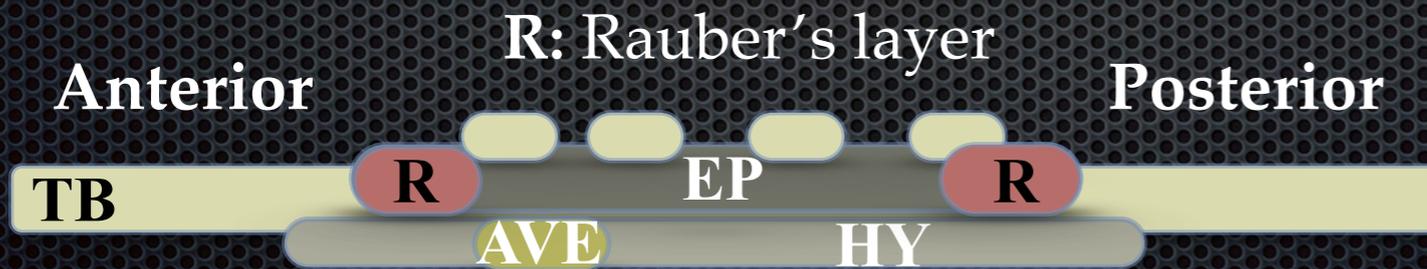
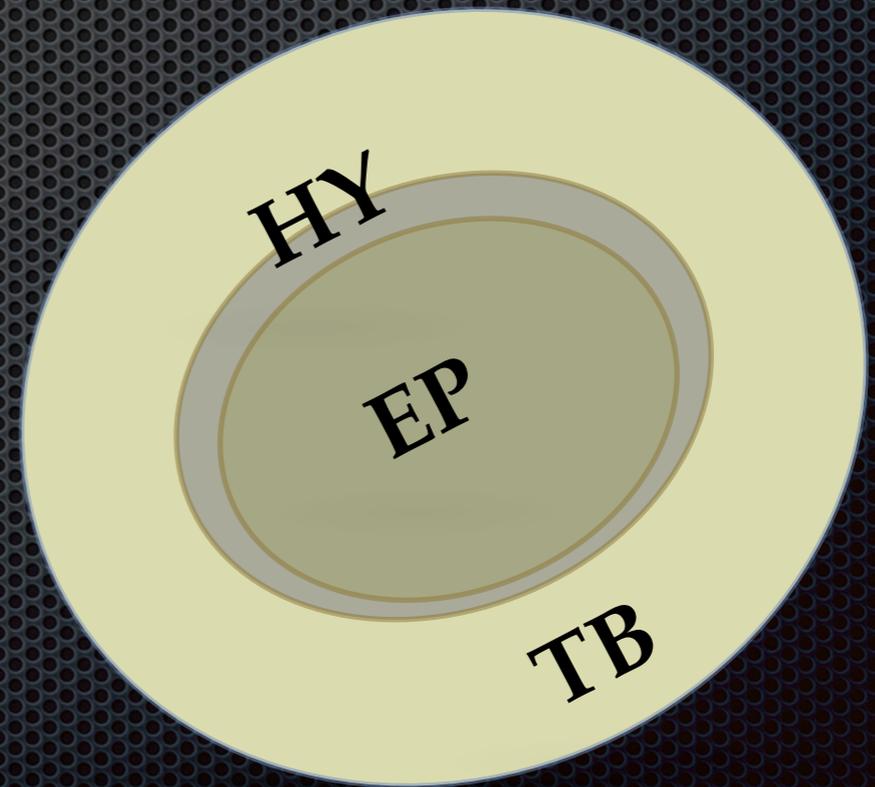


# Stem cell specific markers in 6-day old rabbit embryos

6 day rabbit embryo

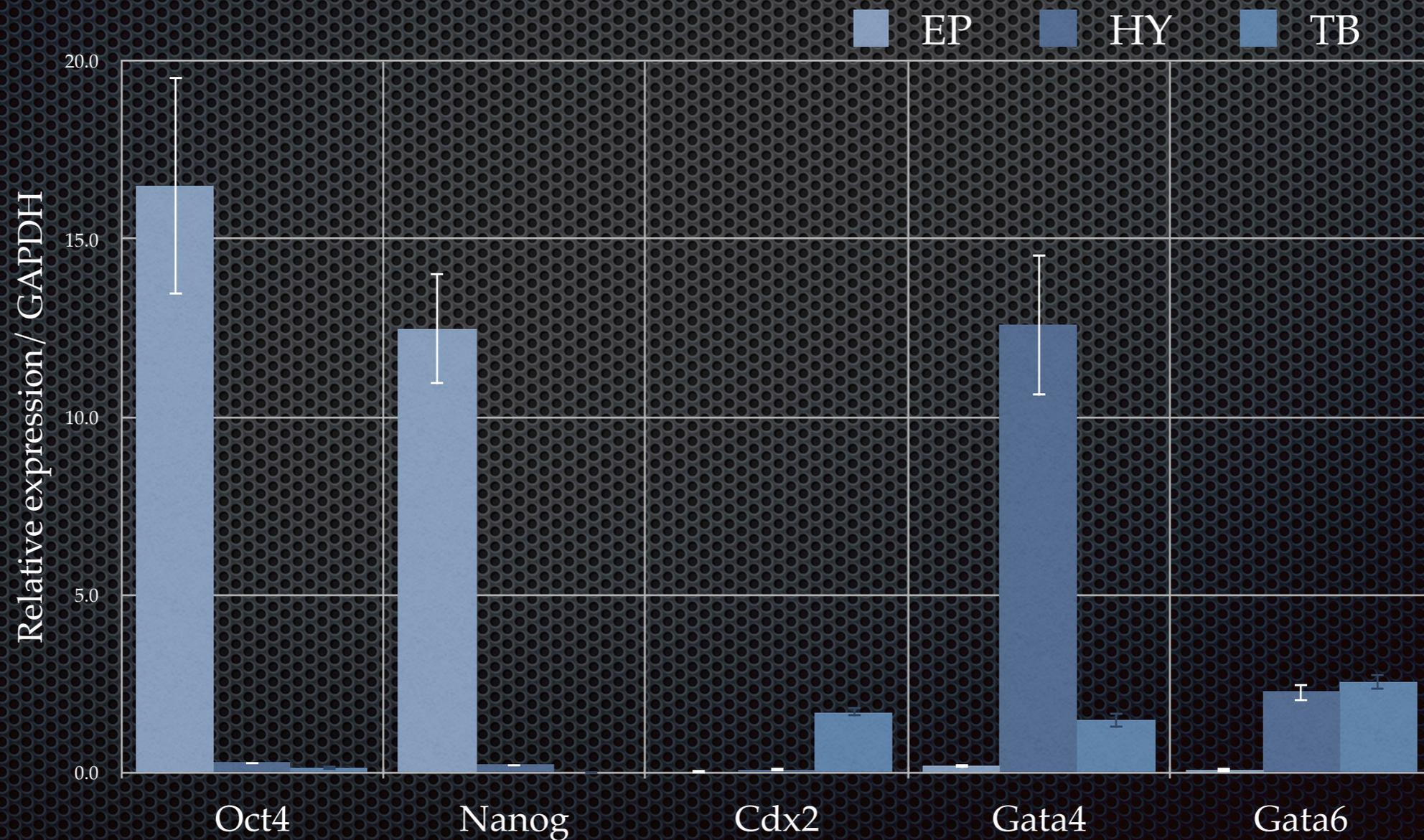


- E: embryo-disk (ICM)
- TB: trophoblast
- HY: hypoblast
- EP: epiblast



# Stem cell specific markers in 6-day old rabbit embryos

- E (4d): *OCT4*
- TB (4d): *OCT4*, *LIFR*, *GATA6*
- TB (6d): *CDX2*, *GATA6*, *GATA4*, *LIFR*
- HY (6d): *GATA6*, *GATA4*
- EP (6d): *OCT4*, *SOX2*, *SSEA-1*, *LIFR*, *SSEA-4*



# Biogenesis of miRNAs

*RNA polymerase II*  
miRNA gene → Pri-miRNA

*Drosha, DGCR8*  
Pri-miRNS → Pre-miRNA

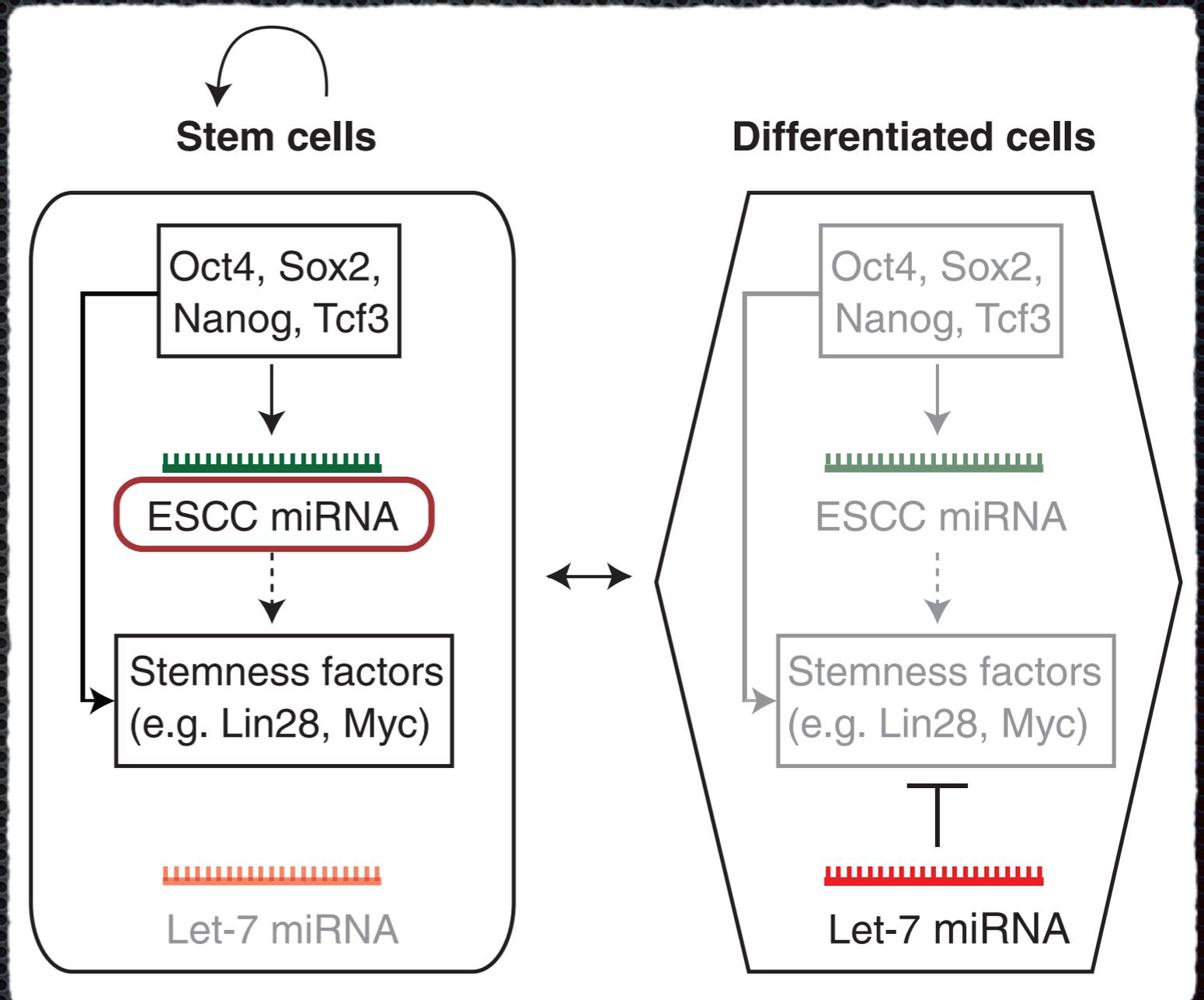
*Dicer*  
miRNA duplex → miRNA

*RNA induced silencing complex*  
*RISC*

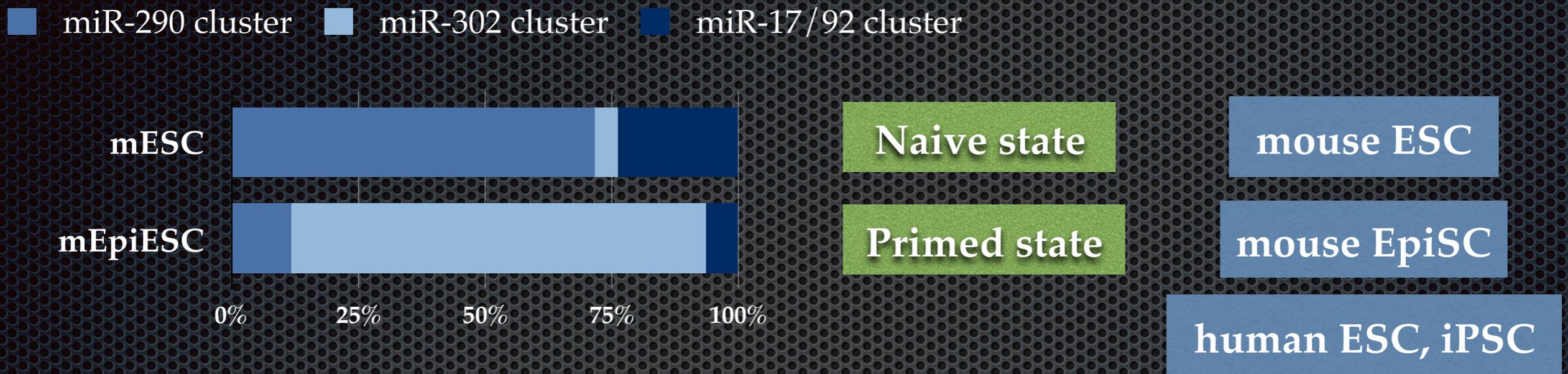
mRNA translation repression at 3'UTR

RNA cleavage

# ES cell-specific cell-cycle (ESCC) miRNAs



# Stem cell specific miRNA clusters in mouse



# Stem cell specific miRNA clusters in rabbit





# Stem cell specific miRNA clusters in rabbit

Naive state



rabbit ESC, IPS



Primed state

GENOMIC CHR-UN0226 (REVERSE STRAND): 228819 – 215892

294-3P

292-1-3P

290-5P

520e

498

512-I-5P

512-3P

512-II-5-P

512-3P

miR-290 cluster

C19MC homologue cluster

CHR8 (+): 93067688-93068482

17-5P

92-1-3P

miR-17/92 cluster

miR-302b

mir-302c

mir-302a

mir-302d

mir-367

ocu-miR-302/367 cluster

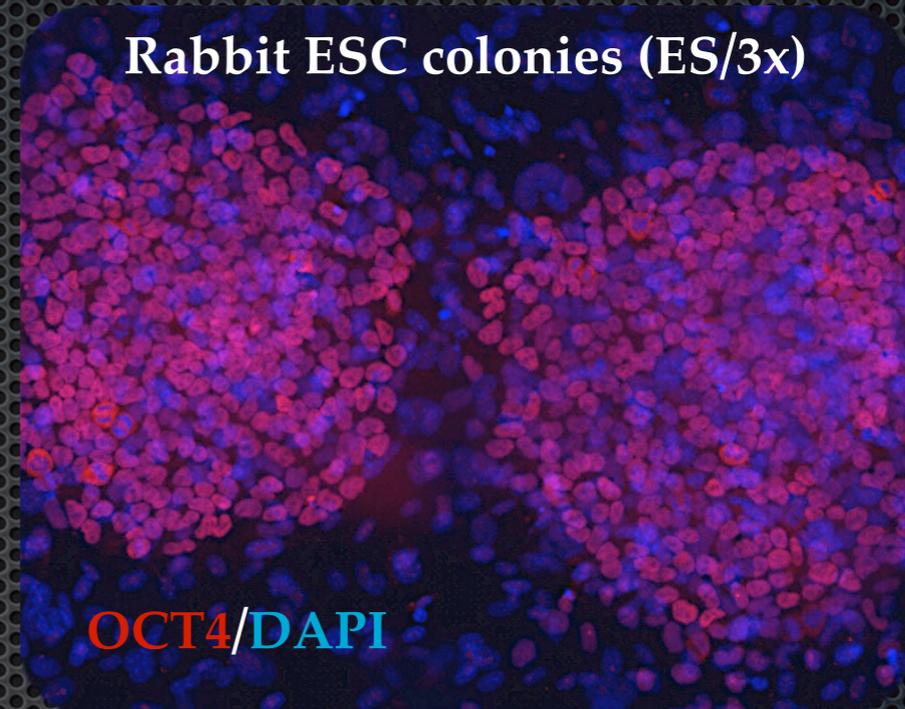
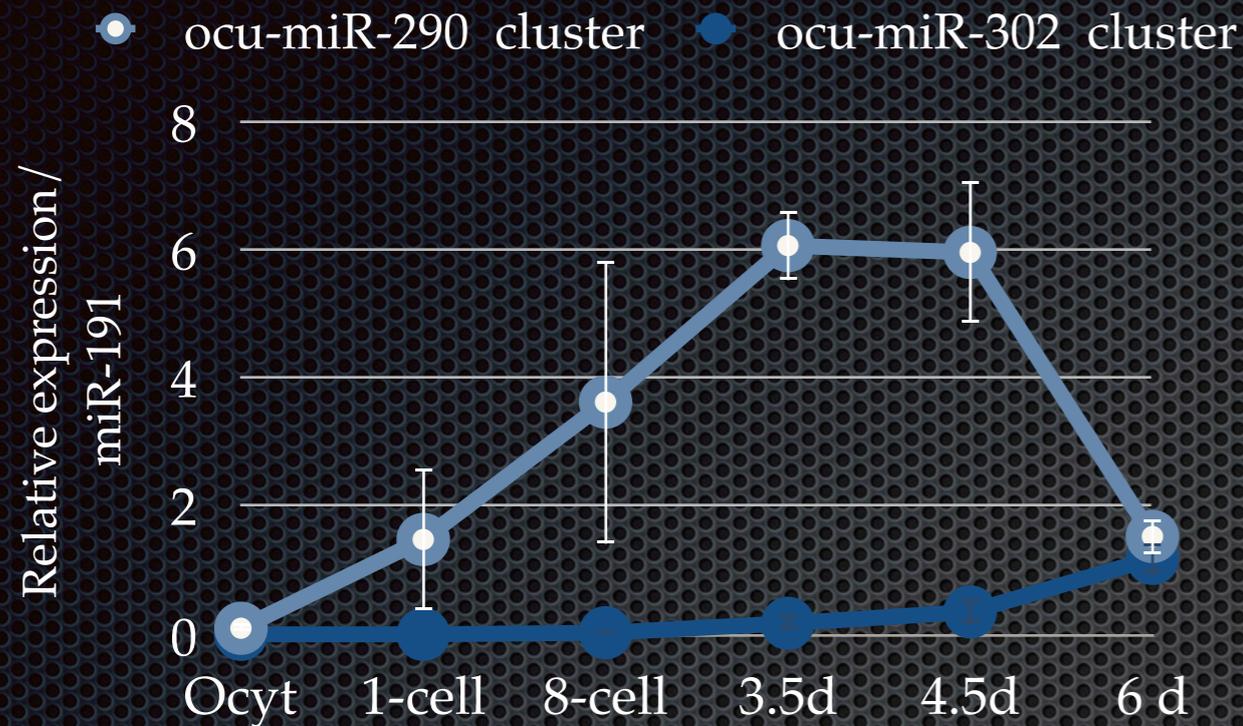
# Rabbit stem cell specific miRNAs array

	1	2	3	4	5	6	7	8	9	10	11	12
A	miR-302a	miR-302a	miR-302a	miR-294	miR-294	miR-294	miR-99a	miR-99a	miR-99a	miR-191	miR-191	miR-191
B	miR-320b	miR-302b	miR-302b	miR-512	miR-512	miR-512	miR-125b	miR-125b	miR-125b	miR-423	miR-423	miR-423
C	miR-302c	miR-302c	miR-302c	miR-498	miR-498	miR-498	miR-145	miR-145	miR-145	miR-302a	miR-302a	miR-302a
D	miR-302d	miR-302d	miR-302d	miR-520e	miR-520e	miR-520e	miR-1	miR-1	miR-1	miR-292	miR-292	miR-292
E	miR-367	miR-367	miR-367	miR-let-7b	miR-let-7b	miR-let-7b	miR-191	miR-191	miR-191	miR-294	miR-294	miR-294
F	m-miR-290	m-miR-290	m-miR-290	miR-let-7c	miR-let-7c	miR-let-7c	miR-423	miR-423	miR-423	miR-512	miR-512	miR-512
G	miR-290	miR-290	miR-290	miR-17	miR-17	miR-17	snoRNA135	snoRNA135	snoRNA135	miR-92	miR-92	miR-92
H	miR-292	miR-292	miR-292	miR-92	miR-92	miR-92	miR-191 NTC	miR-191 NTC	miR-191 NTC	miR-145	miR-145	miR-145

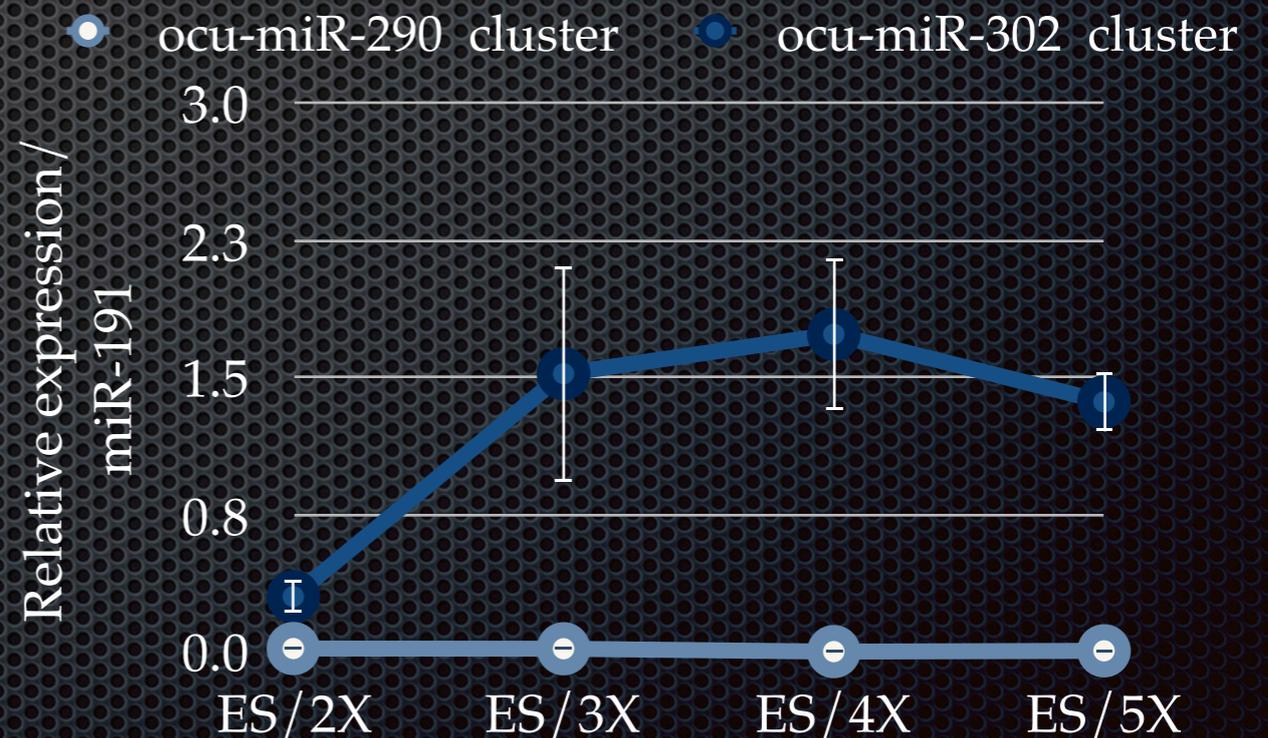
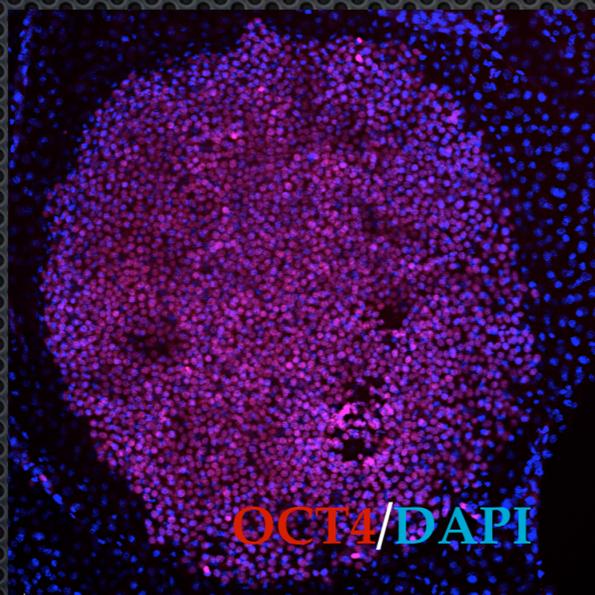
rabbit embryo, ESC, iPSC

6 day old rabbit embryo

# Embryonic stem cell specific miRNA expression in rabbit embryo and ES cells



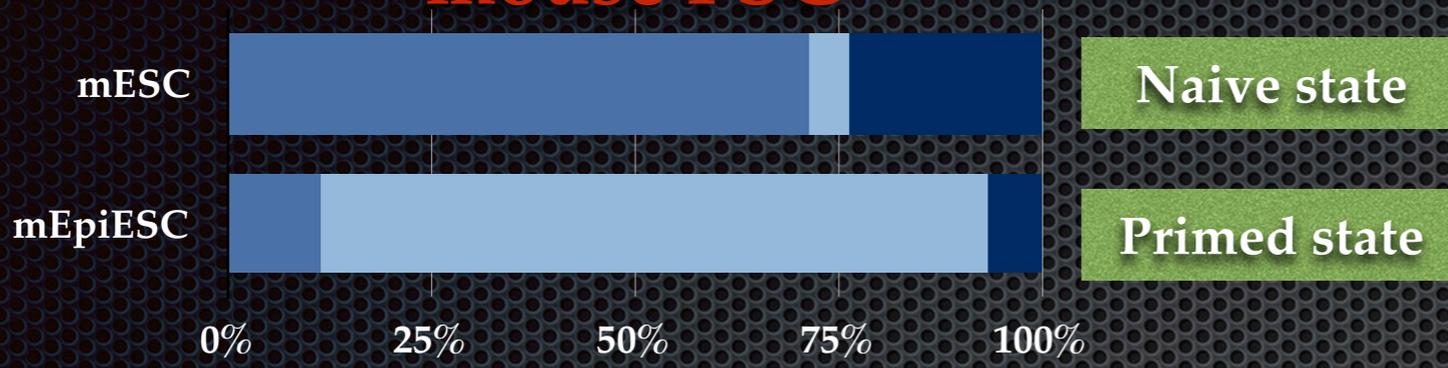
6day old rabbit embryo



# Conclusions

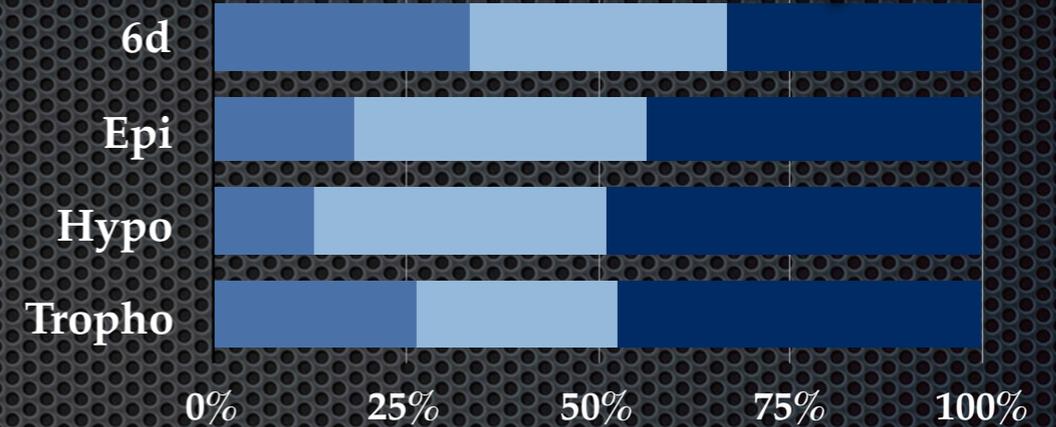
■ miR-290 cluster   
 ■ miR-302 cluster   
 ■ miR-17/92 cluster

## mouse PSC

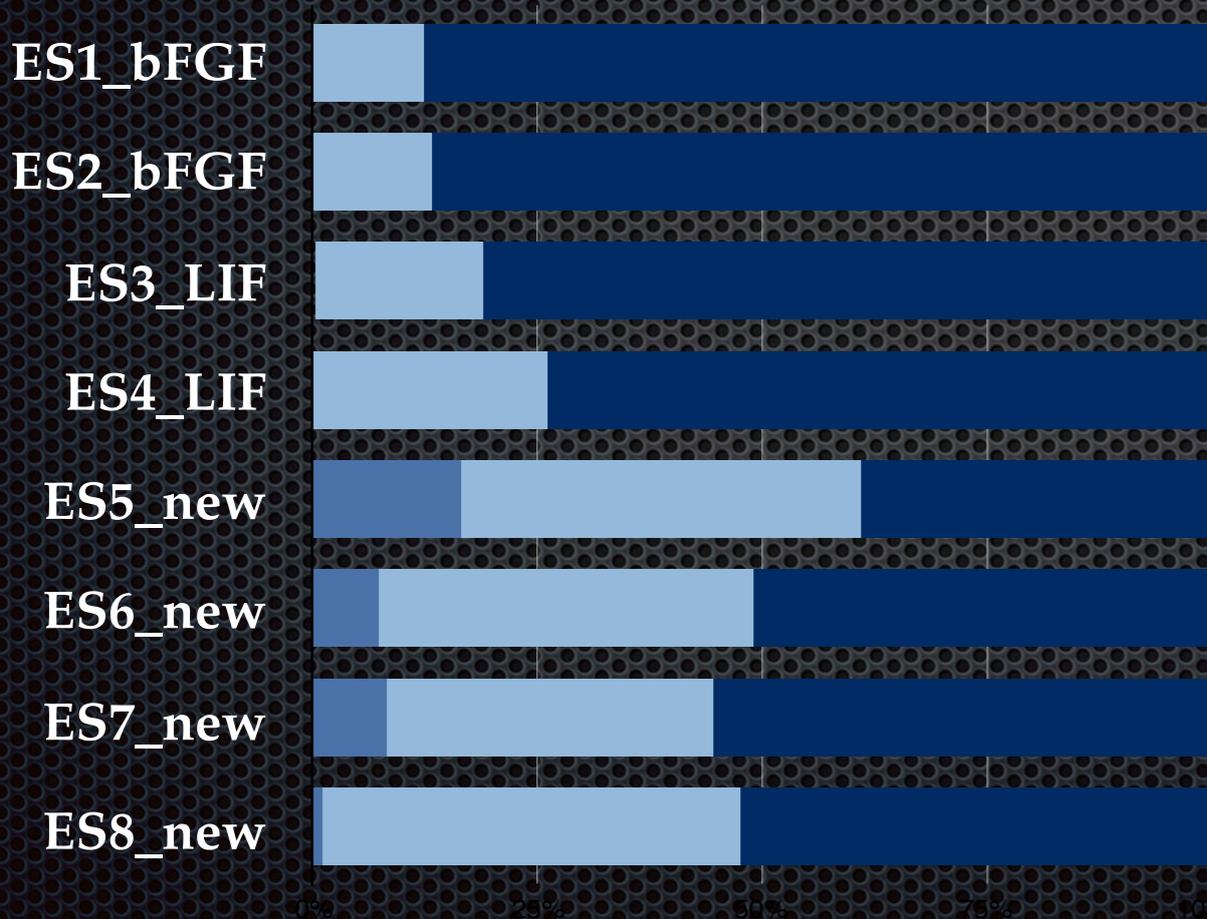


*Jouneau, RNA, 2012.*

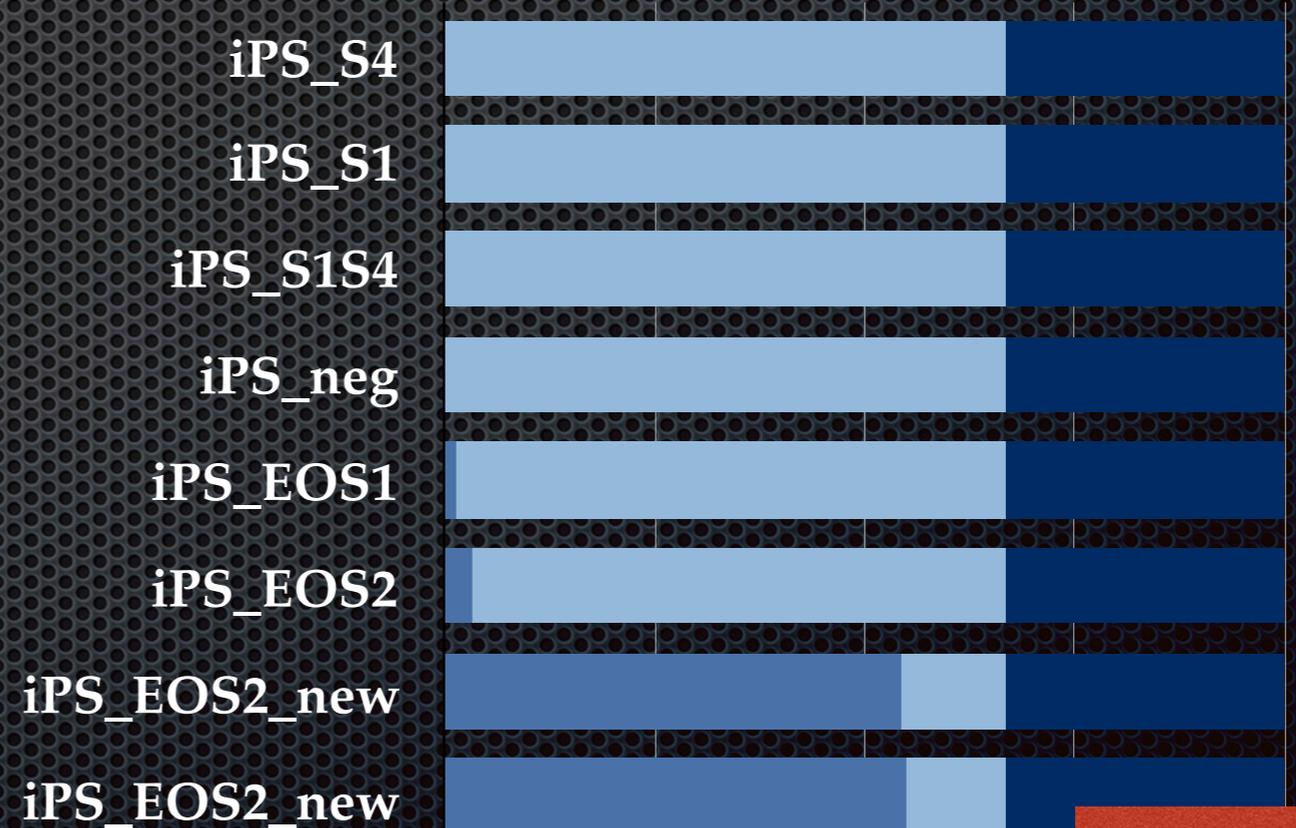
## 6d rabbit embryo



## rabESC



## rabiPS



**3% chimera gastrula**

# French - Hungarian collaboration - publications

1: Osteil P, Moulin A, Santamaria C, Joly T, Jouneau L, Aubry M, Taponnier Y, Archilla C, Schmaltz-Panneau B, Lecardonnel J, Barasc H, Mouney-Bonnet N, Genthon C, Roulet A, Donnadiou C, Acloque H, **Gocza E**, Duranthon V, Afanassieff M, Savatier P.: A Panel of Embryonic Stem Cell Lines Reveals the Variety and Dynamic of Pluripotent States in Rabbits. *Stem Cell Reports*. 2016 Sep 13;7(3):383-398.

2: Taponnier Y, Afanassieff M, Aksoy I, Aubry M, Moulin A, Medjani L, Bouchereau W, Mayère C, Osteil P, Nurse-Francis J, Oikonomakos I, Joly T, Jouneau L, Archilla C, Schmaltz-Panneau B, Peynot N, Barasc H, Pinton A, Lecardonnel J, **Gocza E**, Beaujean N, Duranthon V, Savatier P.: Reprogramming of rabbit induced pluripotent stem cells toward epiblast and chimeric competency using Krüppel-like factors. *Stem Cell Res*. 2017 Oct;24:106-117.

3: Osteil P, Taponnier Y, Markossian S, Godet M, Schmaltz-Panneau B, Jouneau L, Cabau C, Joly T, Blachère T, **Gócza E**, Bernat A, Yerle M, Acloque H, Hidot S, Bosze Z, Duranthon V, Savatier P, Afanassieff M.: Induced pluripotent stem cells derived from rabbits exhibit some characteristics of naïve pluripotency. *Biol Open*. 2013 May 10;2(6):613-28.

4: Maraghechi P, Hiripi L, Tóth G, Bontovics B, Bősze Z, **Gócza E**.: Discovery of pluripotency-associated microRNAs in rabbit preimplantation embryos and embryonic stem-like cells. *Reproduction*. 2013 Apr 15;145(4):421-37.

5: Tancos Z, Nemes C, Polgar Z, **Gocza E**, Daniel N, Stout TA, Maraghechi P, Purity MK, Osteil P, Taponnier Y, Markossian S, Godet M, Afanassieff M, Bosze Z, Duranthon V, Savatier P, Dinnyes A.: Generation of rabbit pluripotent stem cell lines. *Theriogenology*. 2012 Nov;78(8):1774-86.

# Future plan

## French - Hungarian collaborations

### Chicken PGC line establishment

- miRNA expression profile in cPGCs
- epigenetic modification in cPGCs

**U1208 INSERM, USC1361 INRA, Stem-cell and Brain  
Research Institute**

Prof. Pierre Savatier

Prof. Bertrand Pain

*Bilateral Tét/Balaton - 2016, 2017*

*NN OTKA - 2018*

# miRNA expression in cPGCs - PGC line establishment

Partridge colour chicken



(Photo: Somfai S.)

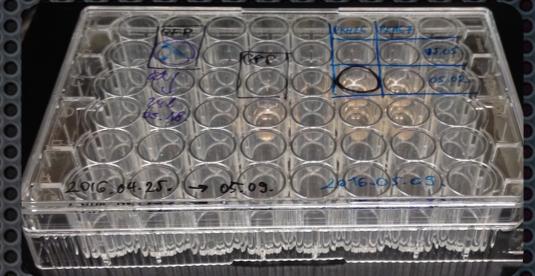
DONOR CHICKEN



80%

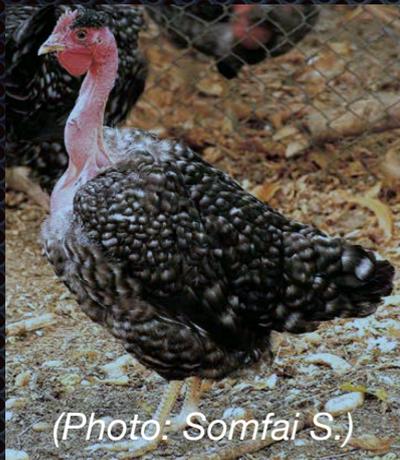


20%



PGC cultivation

PGC freezing



(Photo: Somfai S.)

RECIPIENT ♀



PGCs INJECTION



DONOR PGCs



Transylvanian Black Naked Neck Chicken

X



(Photo: Somfai S.)

INDIGENOUS ♂



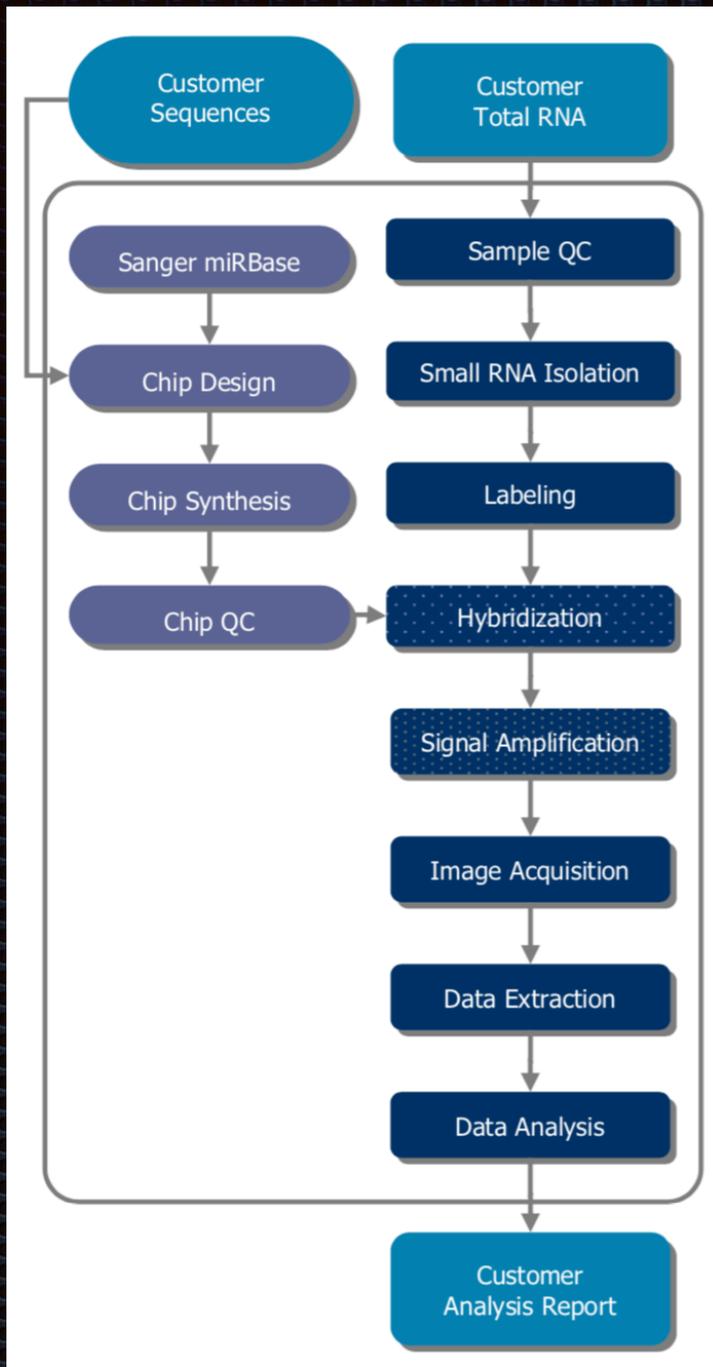
(Photo: Lehoczky István)

PGC DERIVED PROGENIES AND HYBRIDS

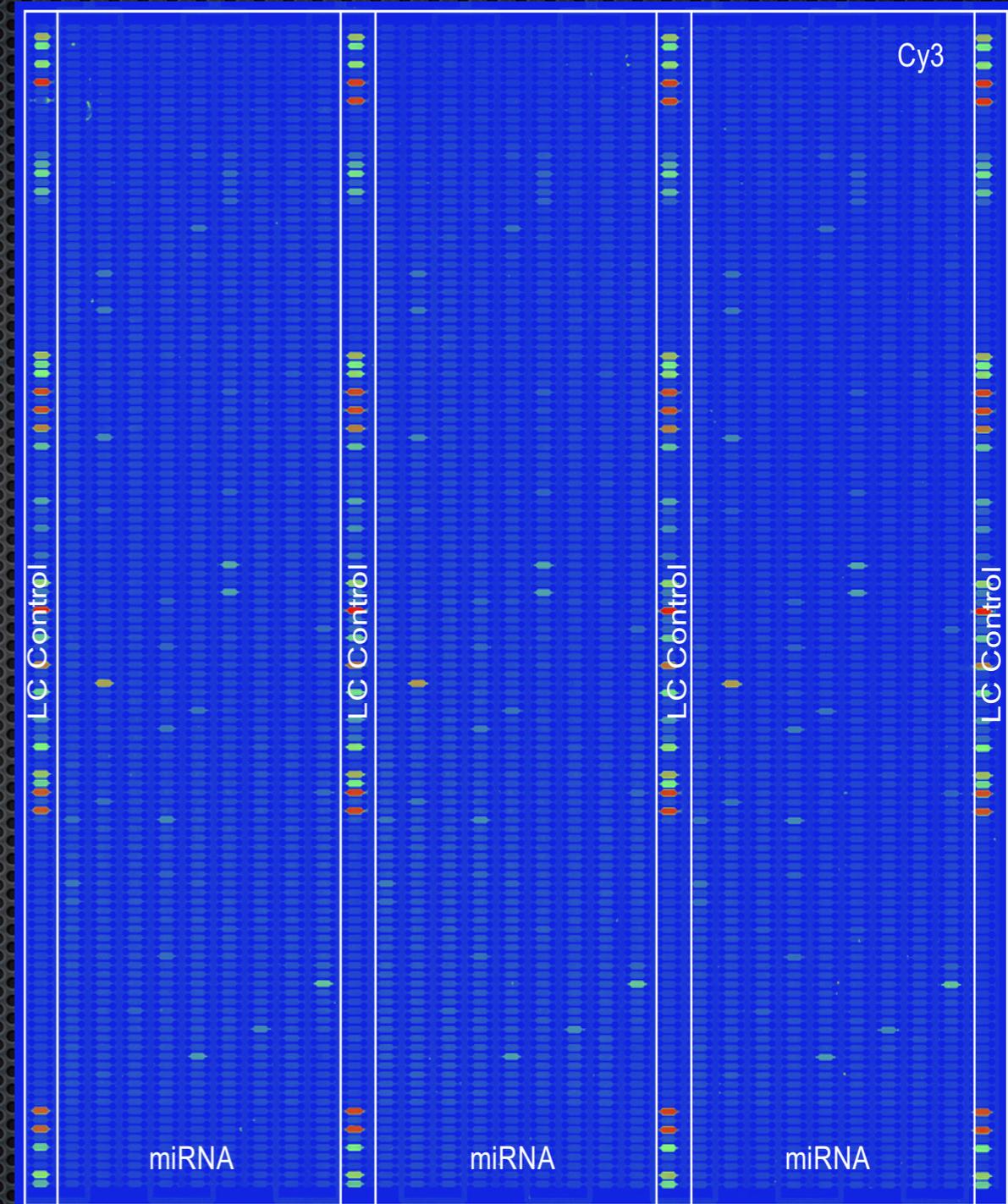
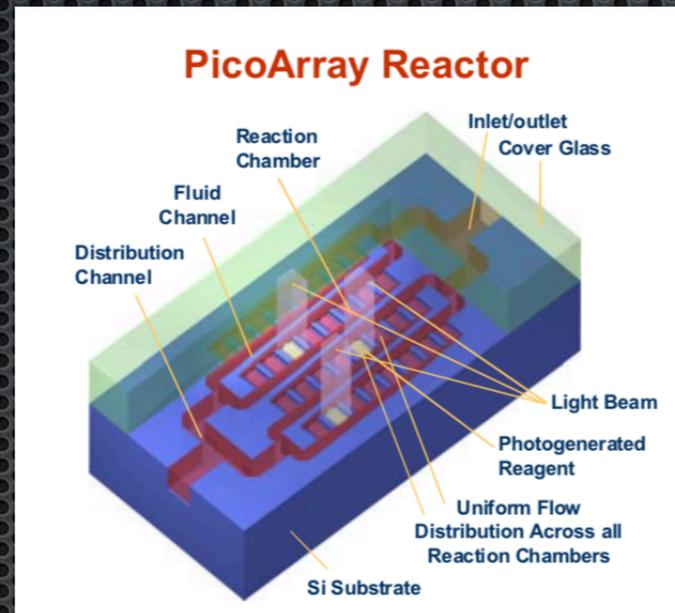
2018.06.20

CHIMERAS ♀

# Introduction the LC Science gga-miRNA array chip



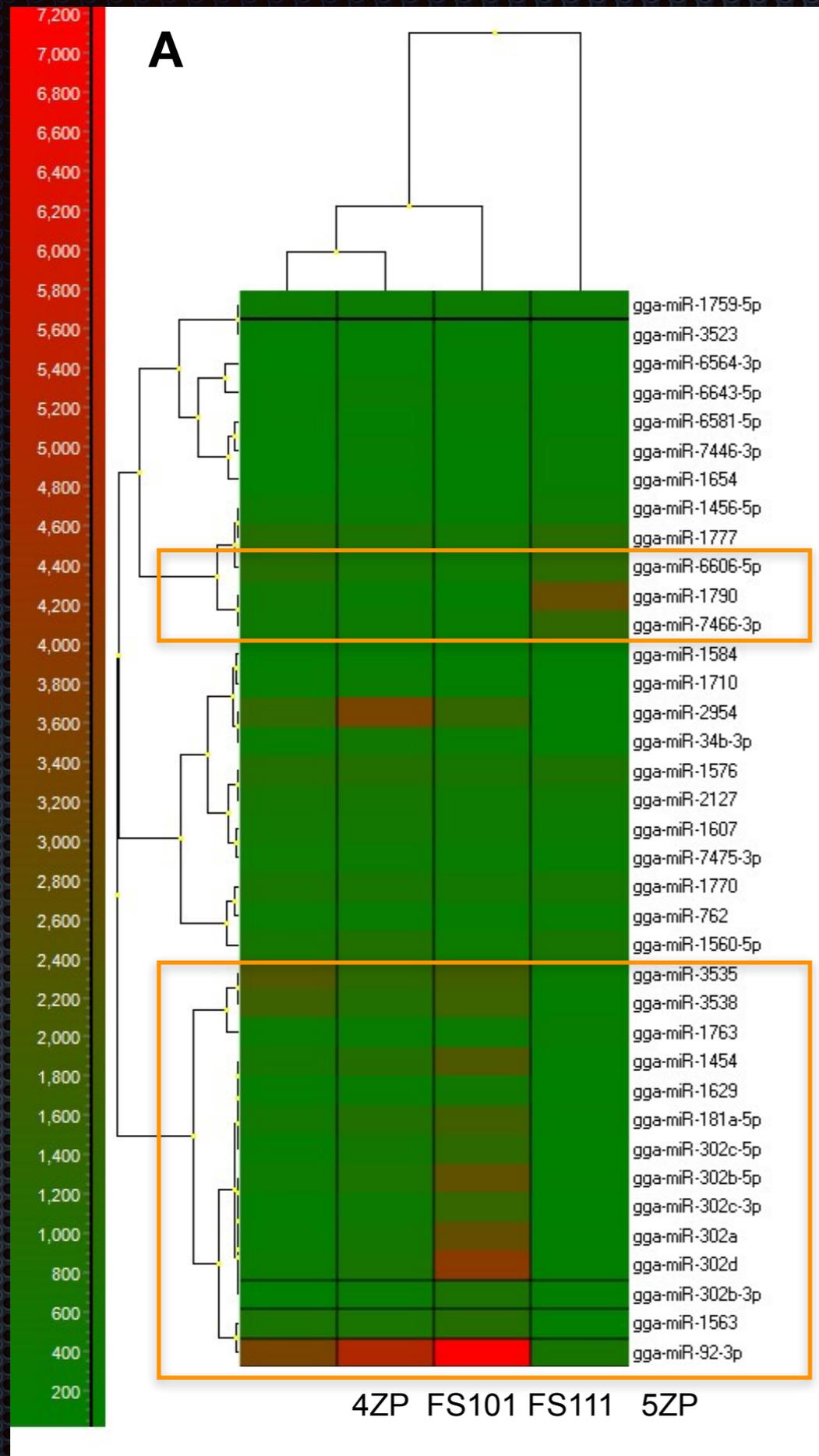
Chip:  
02\_CH21\_170406-171012-  
Gocza-B-375 Cy3: B-(4ZPm)



**LC Science miRNA array**

RNAqueous<sup>®</sup>-Micro Kit Micro Scale RNA Isolation Kit  $\mu$ Paraflo<sup>®</sup> Biochip Technology

# miRNA expression in cPGCs



**Cluster 1**

**Cluster 2**

**B**

**Cluster 1**  
**Upregulated miRNAs**  
**in 5ZP PGC line**

gga-miR-1790

gga-miR-6606-5p

gga-miR-7466-3p

**C**

**Cluster 2**  
**Upregulated miRNAs in high**  
**proliferation rate PGC lines**  
**(FS111,FS101,4ZP)**

gga-miR-92-3p

gga-miR-3538

gga-miR-3535

gga-miR-302d

gga-miR-302c-5p

gga-miR-302c-3p

gga-miR-302b-5p

gga-miR-302a

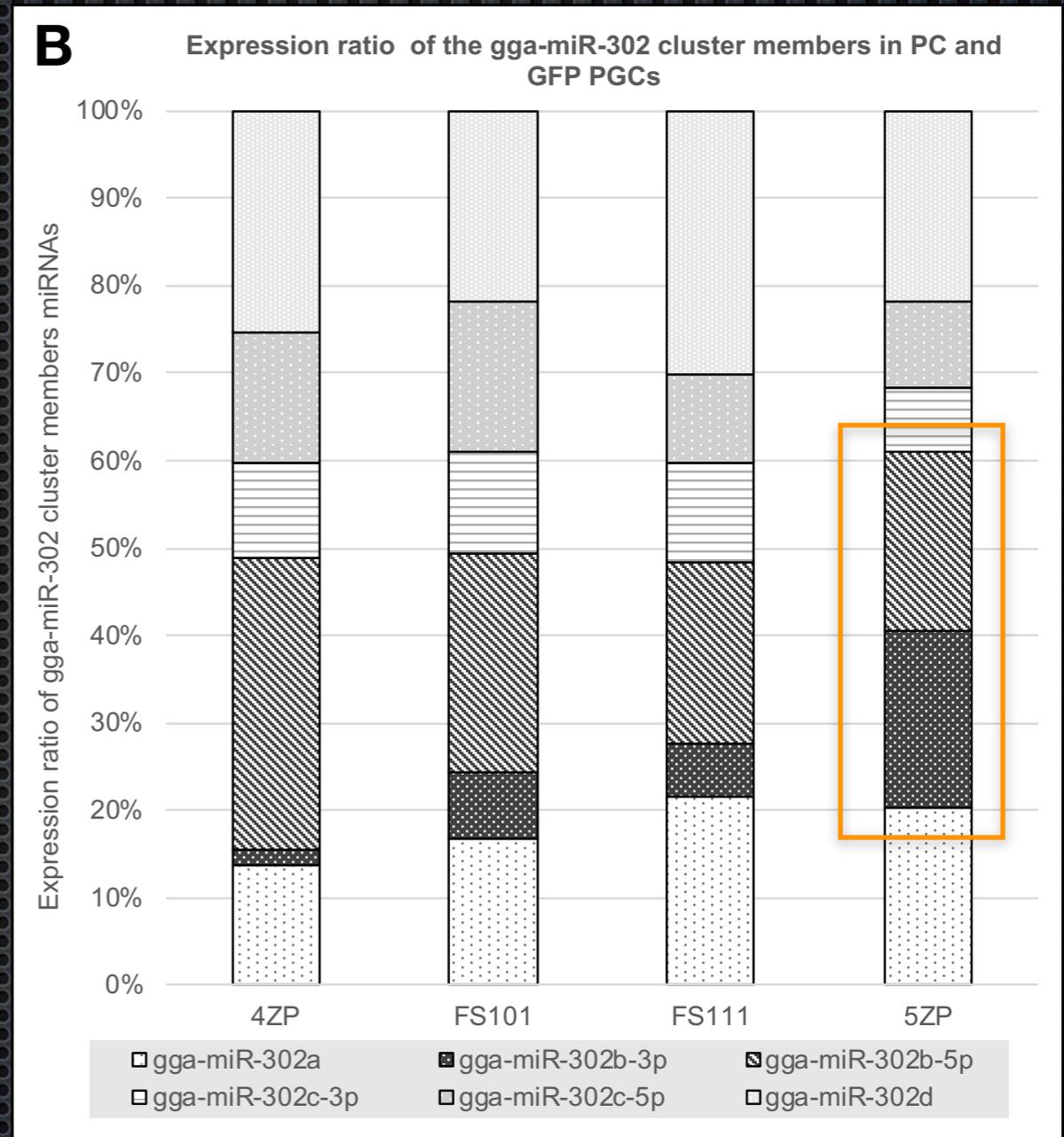
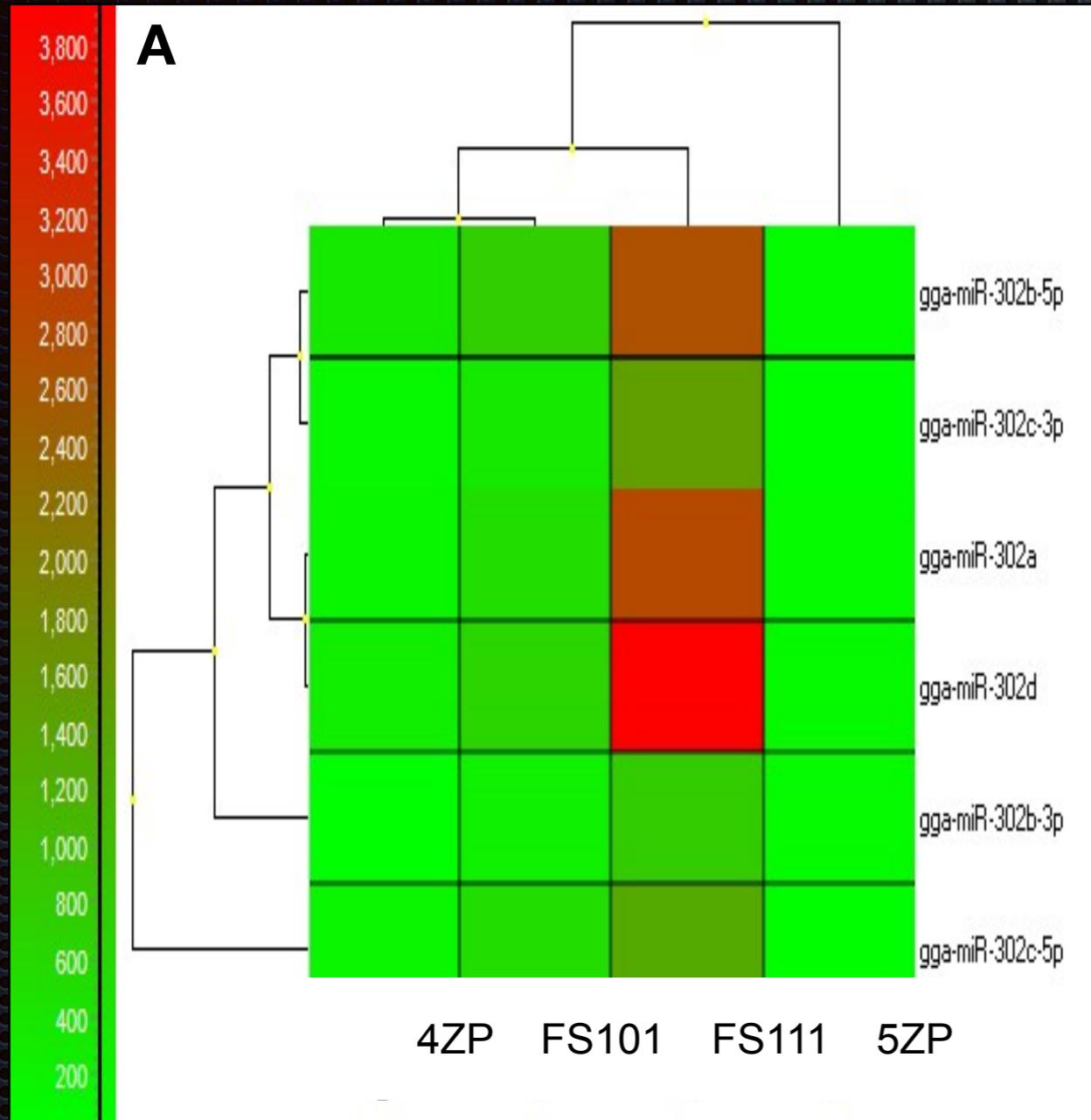
gga-miR-2954

gga-miR-181a-5p

gga-miR-1563

gga-miR-1454

# miRNA expression in cPGCs





NARIC ABC - Agricultural  
Biotechnology Institute  
Gödöllő, Hungary

- ▶ Dr Zsuzsanna Bösze
- ▶ Dr László Hiripi
- ▶ Dr Elen Gócza
- ▶ Bence Lazar
- ▶ Roland Tóth
- ▶ Mahek Anand
- ▶ Németh Kinga
- ▶ Bontovics Babett
- ▶ Renáta Fábián
- ▶ Gergely Iski
- ▶ Dr Pounch Maraghechi
- ▶ Sándor Egedi
- ▶ Zsuzsanna, Galliné

Molecular Cell Biology  
Research Group Momentum  
Program of HAS

- ▶ Dr László Homolya
- ▶ Zoltán Hegyi

BioTalentum Ltd

- ▶ Dr Andras Dinnyes
- ▶ Dr Julianna Kobolak
- ▶ Dr. Táncos Zsuzsanna

2010-2018

- ▶ *ANR - PLURABIT OMFB-00131/2010*
- ▶ *CGIAR/CCAFS project (Agricultural Ministry)*
- ▶ *GÉNNET\_21 (VEKOP-2.3.2-16-2016-00012)*

HáGK - Research Centre for  
Farm Animal Gene  
Conservation  
Gödöllő, Hungary

- ▶ Dr Eszter Patakiné Várkonyi
- ▶ Dr Krisztina Liptói



Thank you for your attention

