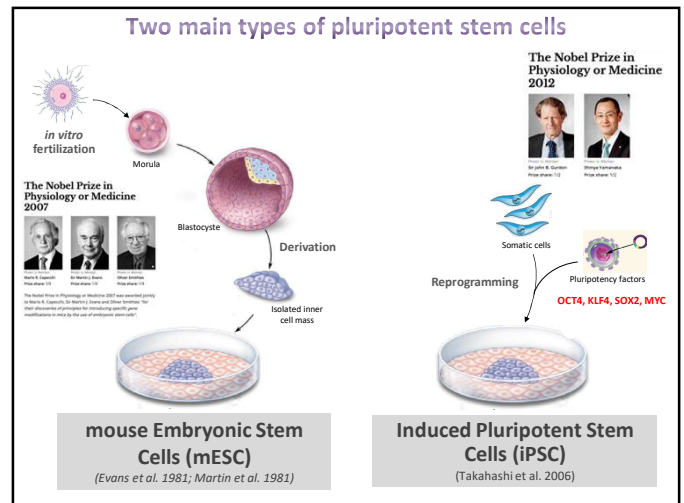


Stem Cell Practical Course - Master GCD  
october 2025

**Mini research project 1:  
*In vitro* Pluripotency from ES to iPS cells**

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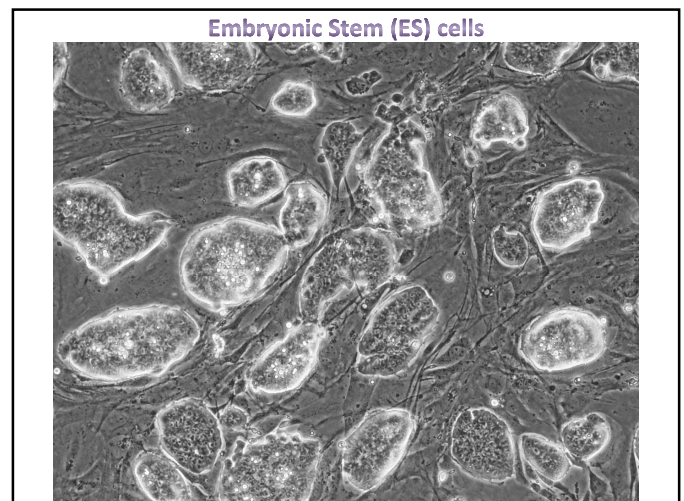
**Mini research project 1: *In vitro* Pluripotency from ES to iPS cells**

**Exp 1. Evaluate the effect of feeder cells and LIF cytokine on the self-renewal capacity of mouse Embryonic Stem (ES) cells**

**Exp 2. Differentiate Sox17<sup>GFP/+</sup> ES cells using embryoid body formation**

**Exp 3. Reprogram MEF into iPS cells and test the effect of Vitamin C**

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### ES cell culture

- DMEM 4,5g/l glucose, glutamax with sodium Pyruvate
- 15% of fetal calf serum
- $10^3$  unit/ml of LIF
- 100  $\mu$ M  $\beta$ -mercaptoethanol
- penicillin/streptomycin

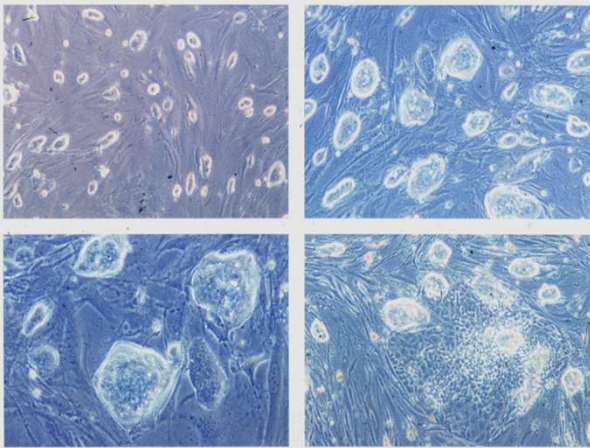
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### ES cell culture

- Cell density is a critical factor for optimal growth of ES cell : ES cells do not like to be « alone » and suffer in overgrown conditions
- Must be passaged every 2-3 days
- Counting of ES cells number is highly recommended
- For passaging, a single cell suspension is required. Otherwise, ES tend to differentiate
- ES cells are usually grown on MEF feeder layer

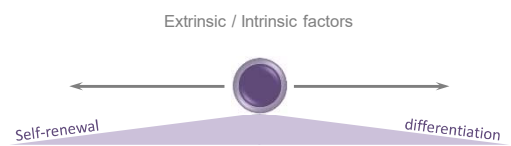
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### Culture of ES cells



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### Embryonic Stem cells



Stem cells are defined according to the **functional demonstration** of their properties (*in vitro*, *in vivo*)

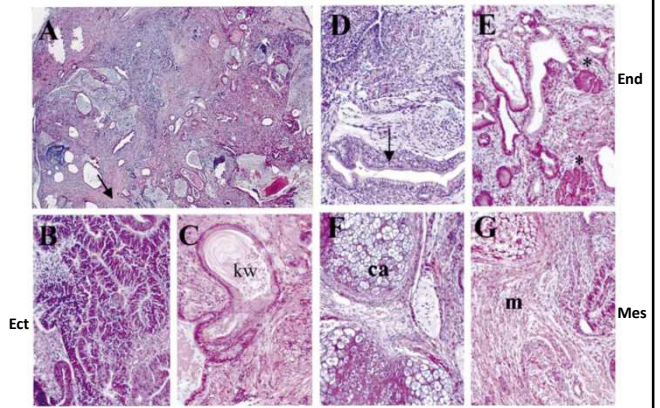
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### Mouse Embryonic Stem cells Assessing pluripotency

- ✧ **Morphology** (*indicative*)
- ✧ **Marker expression**
  - ✧ Cell surface marker SSEA1 (TRA 1-60 in human ES)
  - ✧ Core pluripotency TFs: OCT4/SOX2/NANOG
  - ✧ Additional markers: *Essrb*, *Klf4*, *Stella*, *Prdm14*, ...
  - ✧ Alkaline phosphatase
- ✧ **Differentiation capacities**
  - ✧ *In vitro* differentiation
  - ✧ Teratoma formation
  - ✧ *In vivo* differentiation: production of chimerae, germ line transmission

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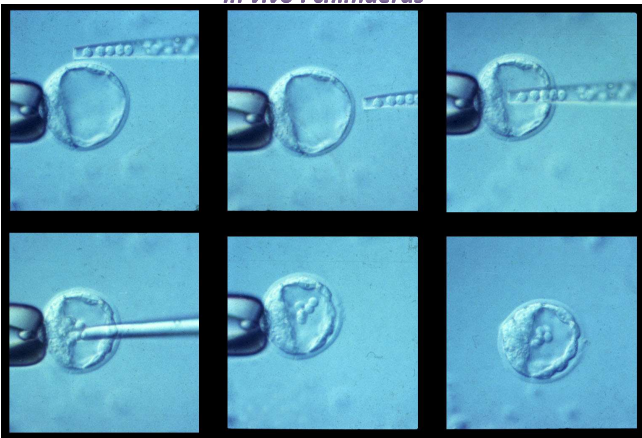
### Mouse Embryonic Stem cells *In vivo* : teratomas



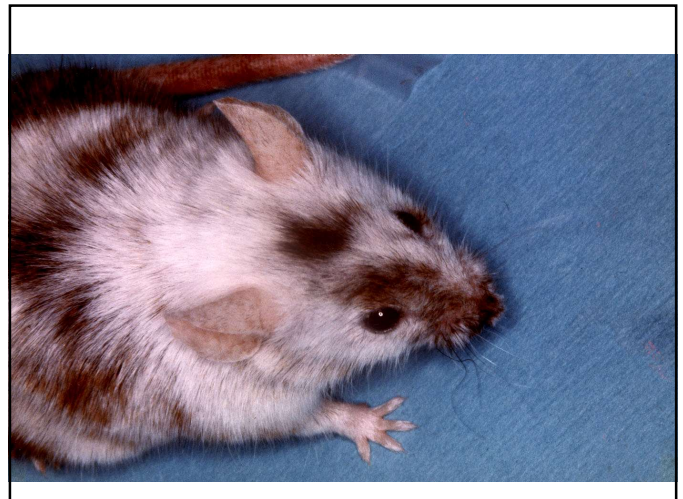
Lanpa et al., J cell Science 2000

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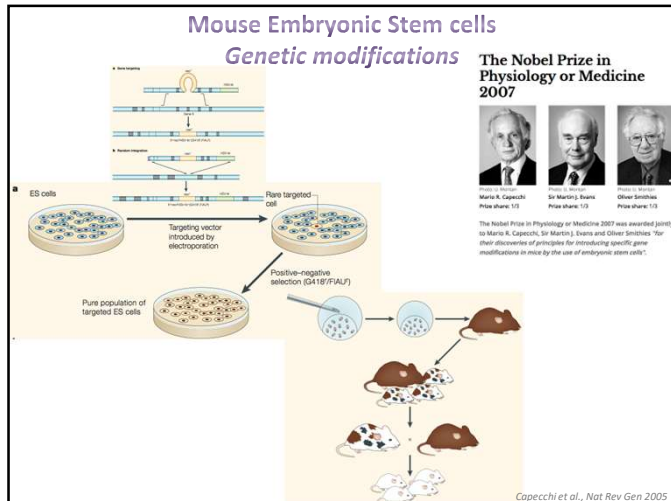
### Mouse Embryonic Stem cells *In vivo* : chimaeras



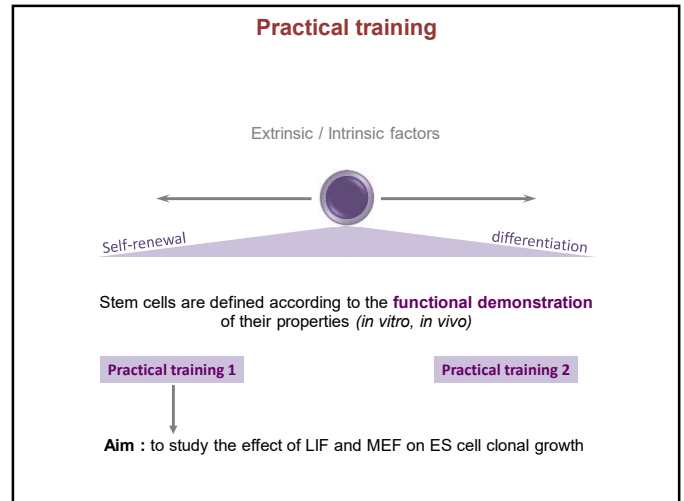
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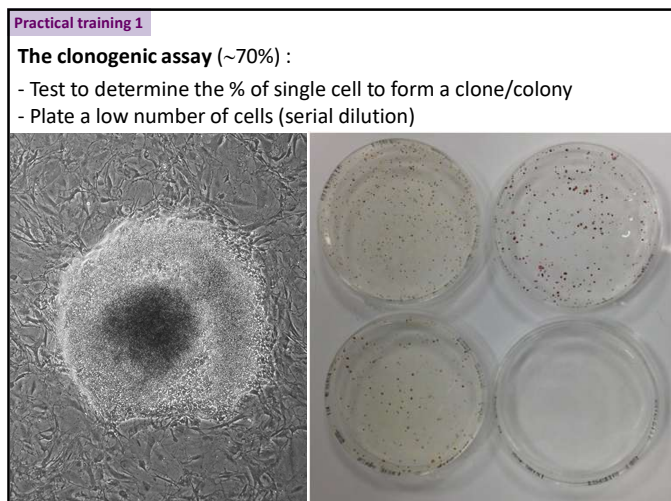
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**Practical training 1**

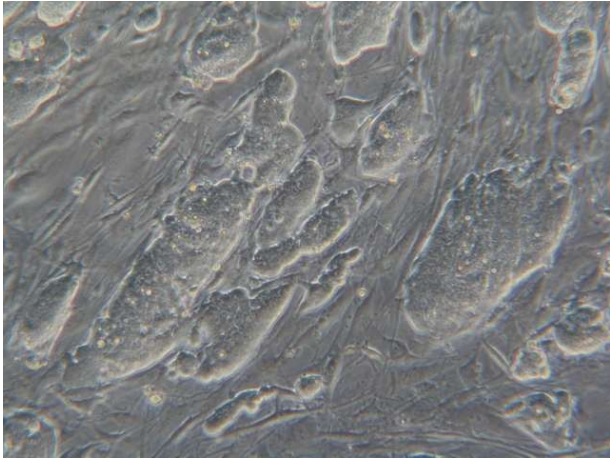
**Timeline**

<b>Monday Oct 13</b>	Plate 200 ES cells in B60 dish containing 5mL of medium
1x B60	with feeder layer in ESC medium
1x B60	without feeder layer in ESC medium
1x B60	with feeder layer in EB differentiation medium
1x B60	without feeder layer in EB differentiation medium
<b>Wednesday Oct 15</b>	Observe ES cells, change media (5mL medium per B60)
<b>Friday Oct 17</b>	Observe ES cells, change media
<b>Monday Oct 20</b>	Change media
<b>Wednesday Oct 22</b>	Observe ES cells, change media
<b>Friday Oct 24</b>	Observe ES cells. Take pictures. Alkaline phosphatase detection. Count, analyze

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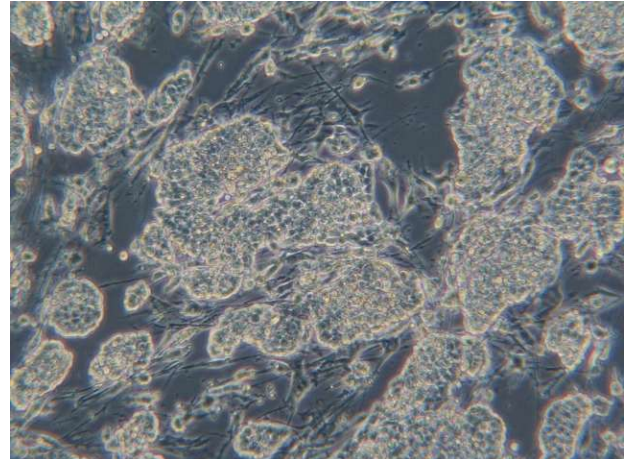


Practical training 1



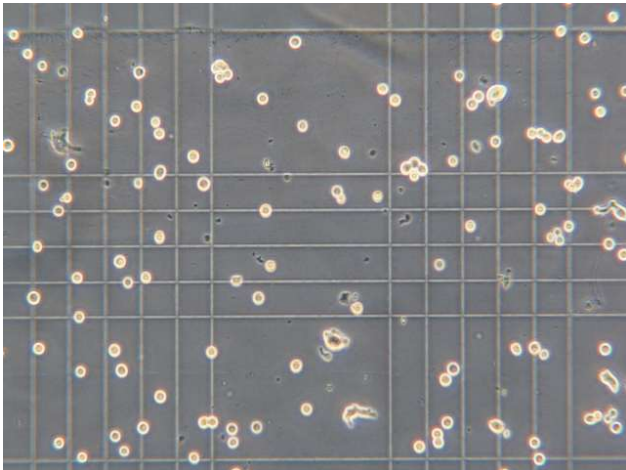
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Practical training 1



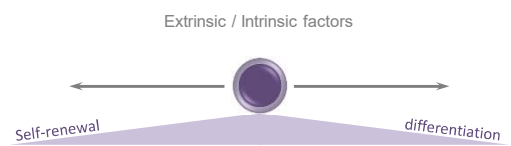
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Practical training 1



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### Practical training



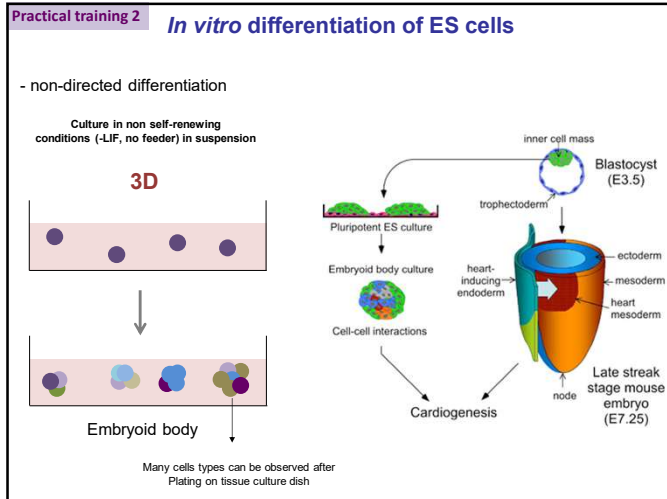
Stem cells are defined according to the **functional demonstration** of their properties (*in vitro*, *in vivo*)

Practical training 1

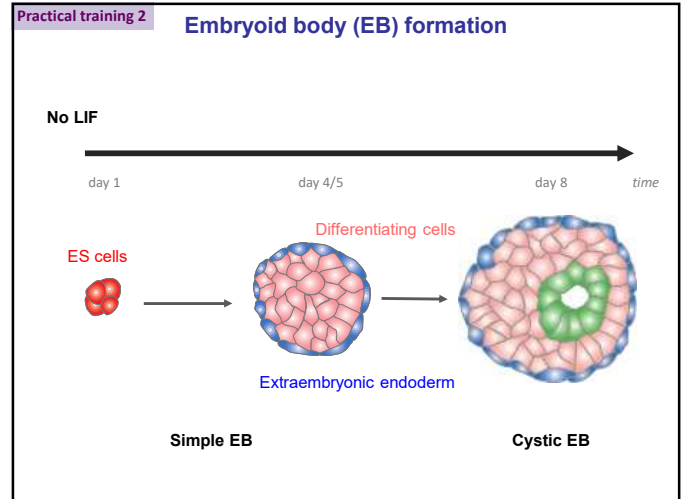
Practical training 2

**Aim :** to study the differentiation potential of ES cell

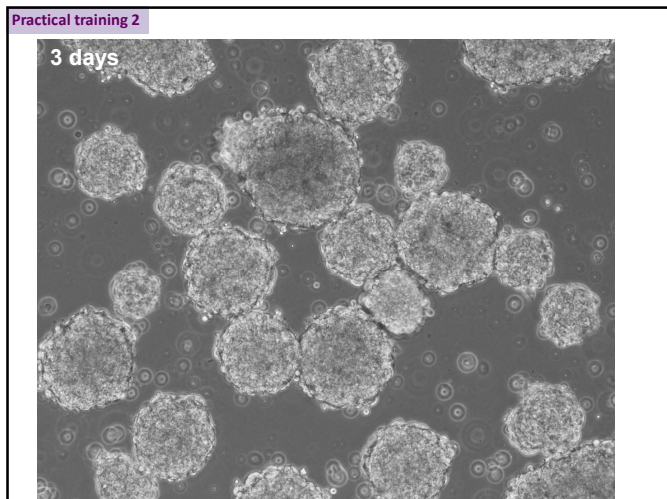
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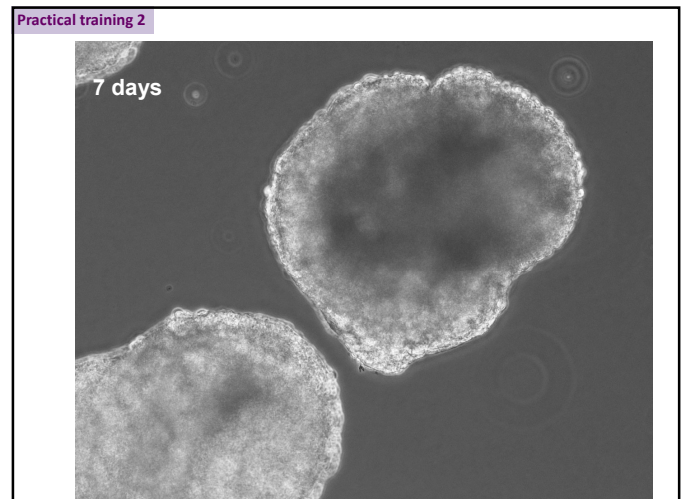
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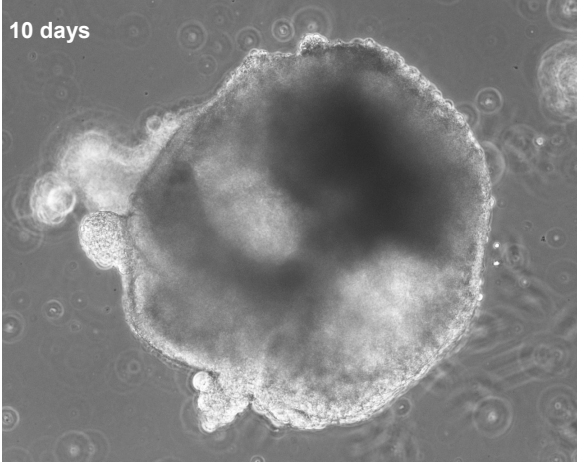
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## Practical training 2

10 days



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## Practical training 2

&gt;2 weeks



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## Practical training 2 Monitoring ES cell differentiation

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## Practical training 2 Monitoring ES cell differentiation

- Morphology
- Enzymatic activity (ex : alkaline phosphatase)

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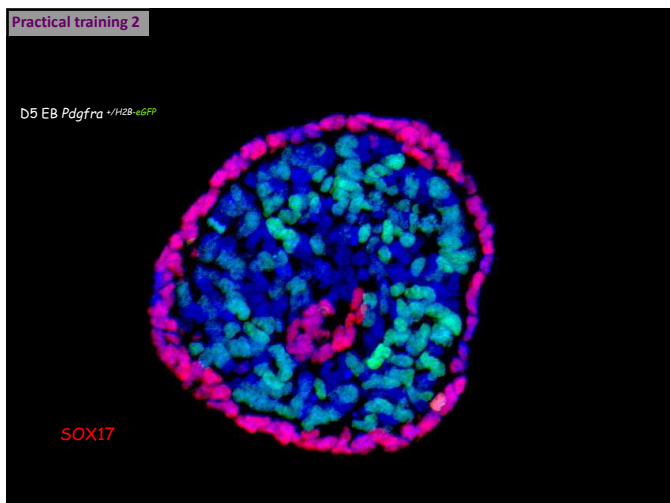


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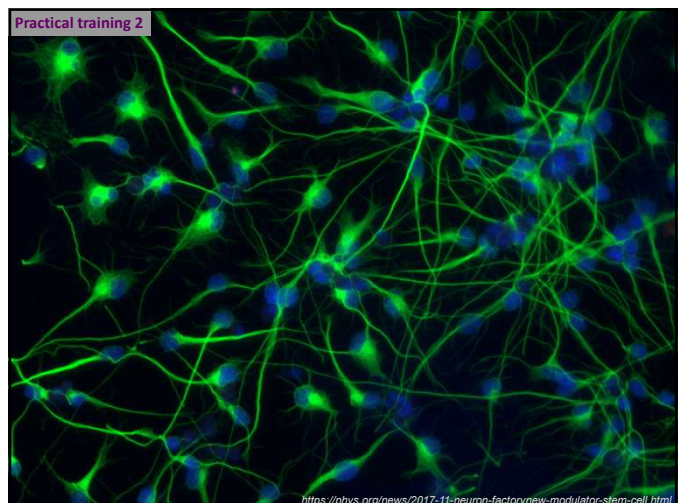
#### Practical training 2 Monitoring ES cell differentiation

- Morphology
- Enzymatic activity (ex : alkaline phosphatase)
- Marker expression
  - (F)ISH, RT(q)PCR, microrray, RNAseq
  - Immunostaining

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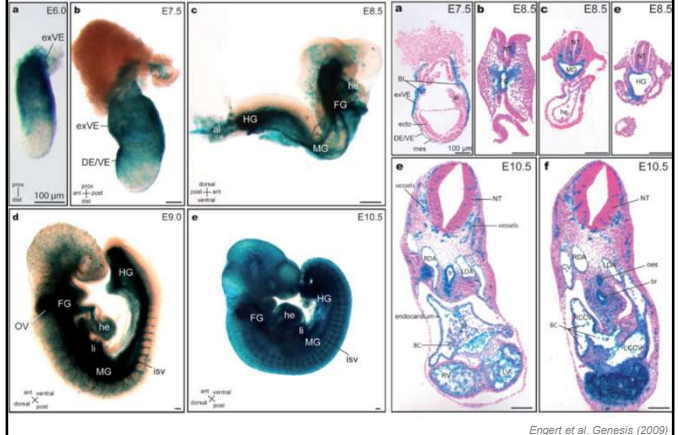
### Practical training 2 Monitoring ES cell differentiation

- Morphology
- Enzymatic activity (ex : alkaline phosphatase)
- Marker expression
  - (F)ISH, RT(q)PCR, microarray, RNAseq
  - Immunostaining
  - Reporter alleles

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### Practical training 2

*Sox17<sup>2A-iCre/+</sup>;R26<sup>R/+</sup>*



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### Practical training 2

#### Timeline

##### Monday Oct 13

Plate  $1 \times 10^6$  ES cells in 100 mm Petri dish (not treated for cell culture) containing 10mL EB differentiation medium

##### Wednesday Oct 15

Observe fluorescence of EBs, change EB media

##### Friday Oct 17

Observe fluorescence of EBs, change EB media

Monday Oct 20

Change media

##### Wednesday Oct 22

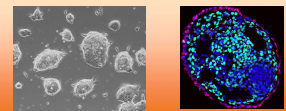
Observe fluorescence of EBs, change EB media

##### Friday Oct 24

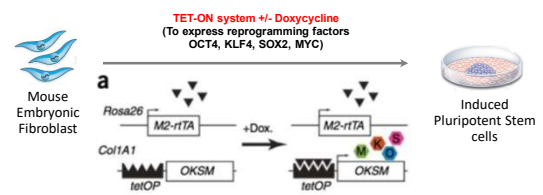
Observe fluorescence of EBs

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### Mini research project 1: *In vitro* Pluripotency from ES to iPS cells

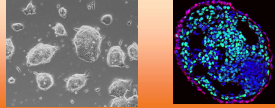


#### Exp 3. Reprogram MEF into iPS cells and test the effect of Vitamin C

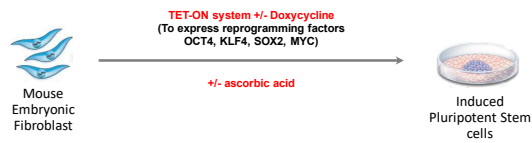


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### Mini research project 1: *In vitro* Pluripotency from ES to iPS cells



#### Exp 3. Reprogram MEF into iPS cells and test the effect of Vitamin C



... carefully monitor the kinetics of Reprogramming

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### Practical training 3

#### Timeline

##### Mon Oct 13

Plate  $6 \times 10^3$  cells per B60 with feeder cells in ES cell medium

1x B60 no Dox

1x B60 Dox (1  $\mu\text{g}/\text{ml}$ )

1x B60 Dox (1  $\mu\text{g}/\text{ml}$ ) and ascorbic acid 50  $\mu\text{g}/\text{ml}$

1x B60 ascorbic acid 50  $\mu\text{g}/\text{ml}$

##### Wednesday Oct 15

Observe cells, change media

##### Friday Oct 17

Observe cells, change media

##### Monday Oct 20

Change media

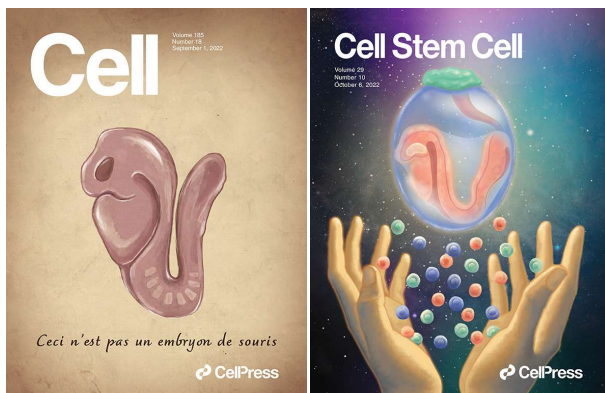
##### Wednesday Oct 22

Observe cells, change media

##### Friday Oct 24

Observe cells

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