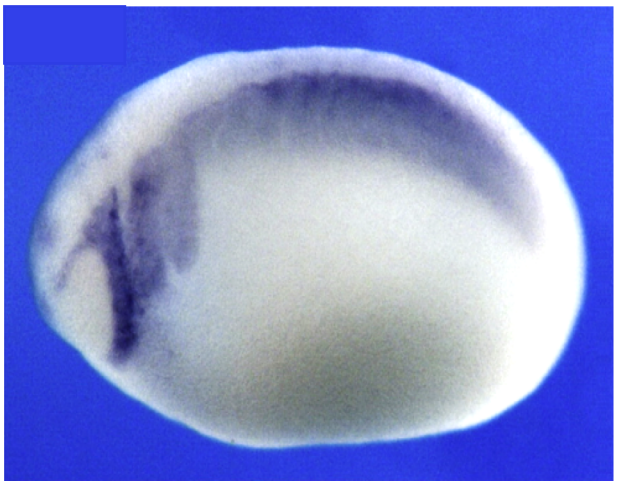
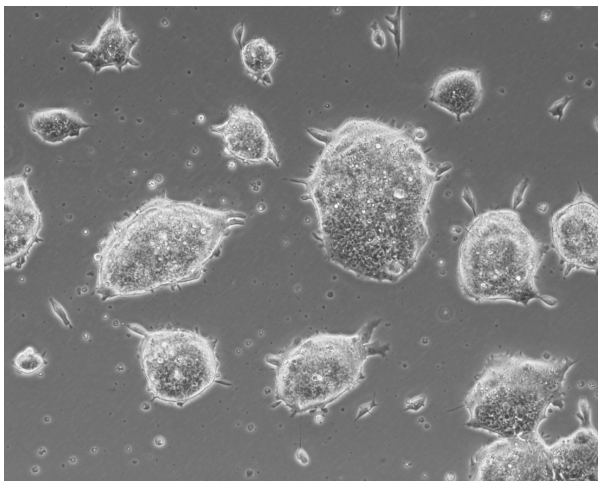
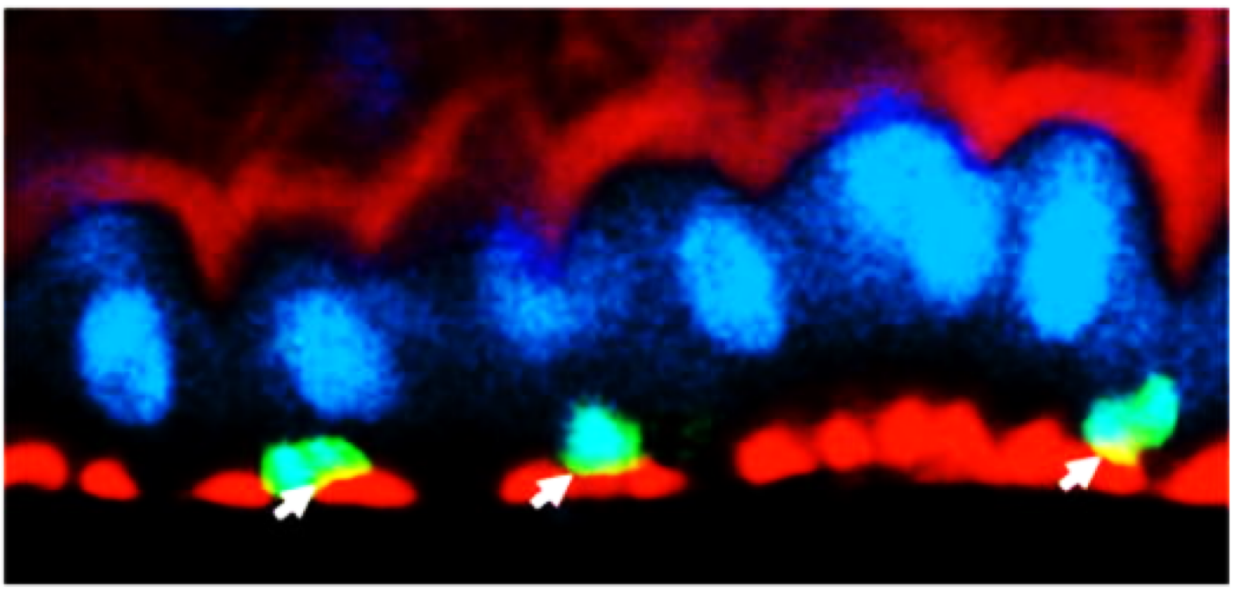
M2 Gene, Cell and Development

Practical course

October 13th – October 27th 2025







CONTENTS

Mini research project 1:
Pluripotent stem cell in culture

Pluripotent stem cells have the ability to self-renew and to differentiate into all cell types of the body including germ cells. Two types of pluripotent stem cells are currently available: embryonic stem cells and induced pluripotent stem cells. Embryonic stem cells (ESC) were first isolated from the epiblast of mouse blastocyst embryos (Evans et al., 1981; Martin, 1981). The team of Shinya Yamanaka further demonstrated in 2006 that somatic cells can be reprogrammed into induced pluripotent stem cells (iPSC) by ectopic expression of a limited number of pluripotent factors (Takahashi et al., 2006). These major breakthroughs and their applications to human have revolutionized the field of medicine.

Your mini-research project is composed of three aims :

- To determine how some extrinsic factors such as the presence of fibroblast feeder cells and Leukemia Inhibiting Factor (LIF) impact the self-renewal activity of mouse ESC. This will be achieved by comparing their clonal growth ability in various cell culture conditions.

- To test whether mouse ESCs can differentiate into multiple cell types. To do this, we will generate embryoid bodies and monitor their differentiation based on morphological criteria and the expression of a fluorescent cell lineage reporter.

- To determine the optimal conditions to reprogram differentiated mouse fibroblasts into iPSCs. Particularly, you will test whether ascorbic acid affect the reprogramming process in genetically modified fibroblasts in which the expression of reprogramming factors can be tightly controlled by doxycyclin.

Mini research project 2:
Homeostasis of adult *Drosophila* midgut

Drosophila midgut is a mono-epithelium composed of only 4 cell types (stem cells (ISC), progenitors (enteroblasts, EB) and two differentiated cell types (enterocytes (EC) and entero-endocrine cells (EE)).

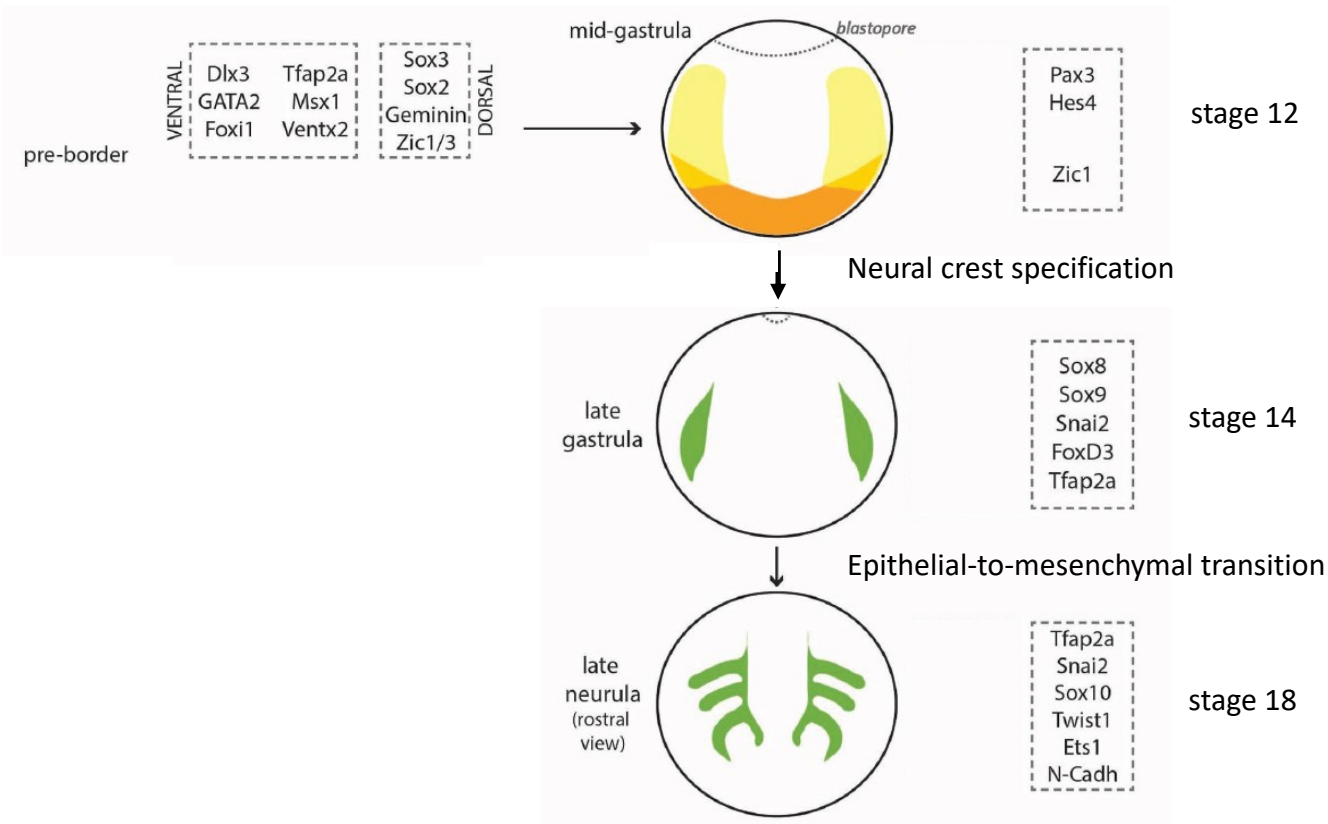
This simplicity coupled to the power of *Drosophila* genetics make this tissue a good model system to study a stem cell-dependent homeostasis.

The aim of your mini-research project is to describe, using the Gal4 technique for real-time and clonal expression (G-TRACE) system, confocal microscopy and flow cytometry, the alterations of this homeostasis induced by infections with *Erwinia carotovora spp. carotovora,* a Gram-negative bacteria.

Mini research project 3: Are Hedgehog and Wnt signaling pathways involved in neural crest cell specification and migration?

- The neural crest (NC), a migratory and multipotent cell population in vertebrate embryos, forms many differentiated cell types, including pigment cells, craniofacial skeleton, peripheral neurons and glia (Bronner and Le Douarin, 2012). NC development starts during gastrulation, at the edges of the neural plate, and continues until late organogenesis. In early gastrulas, signals from the adjacent neural plate, non-neural ectoderm, and underlying mesoderm specify the neural border (NB), a transition area located between the neural plate and the non-neural ectoderm (Pla and Monsoro-Burq, 2018). In late gastrulas and early neurulas (neural plate stage), NC specification begins within the NB and during the second half of neurulation, as neural folds elevate, the immature NC is further specified into functional premigratory NC, ready to undergo epithelium to mesenchyme transition (EMT) and migration.
- All steps in the development of the neural crest result from the coordinated action of different signaling pathways leading to, in particular, the activation of the expression of a specific combination of transcription factors.
- Hedgehog and Wnt trigger two master signaling pathways known to be involved in many developmental processes.
- We will here investigate whether these pathways might also act during (1) specification and/or (2) migration of neural crest cells.

Early phases of neural crest development

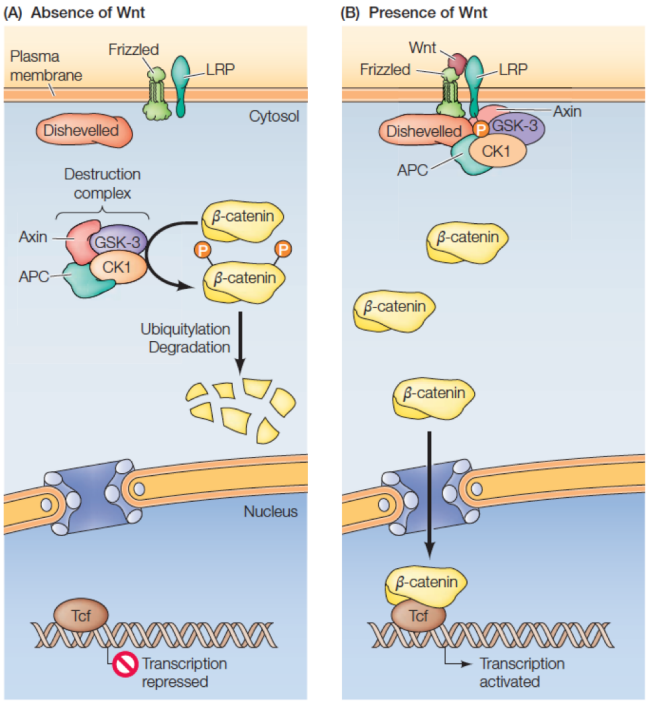


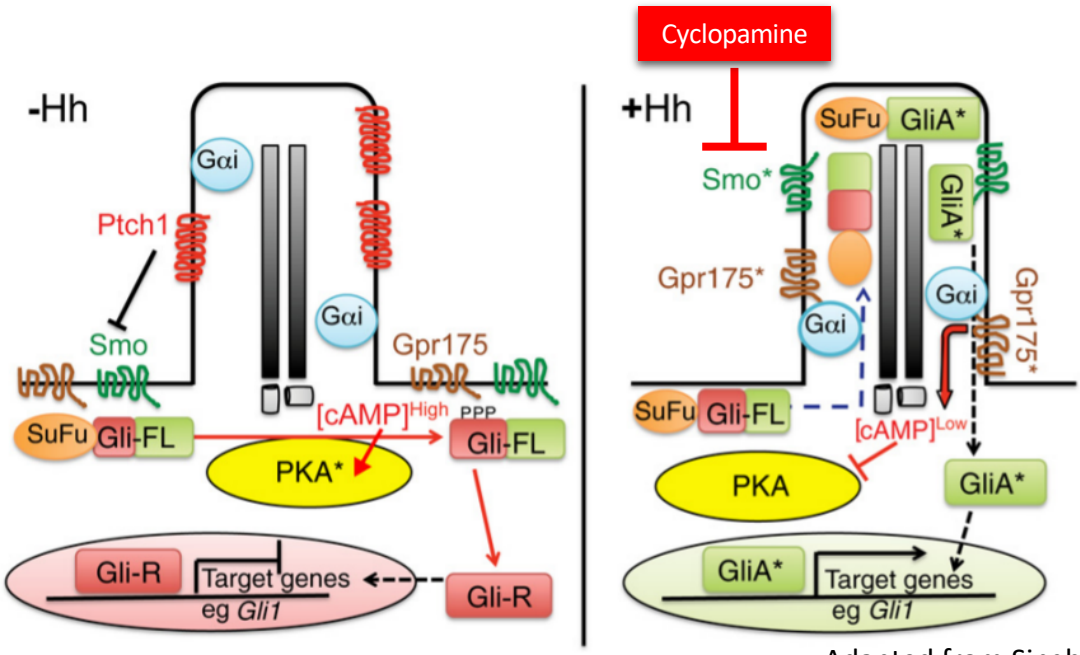
Adapted from Seal and Monsoro-Burq, 2020

Genes controlling neural crest (NC) development. The combined effects of signaling pathways and transcription factors (TFs) lead to the development of different tissues in a temporally and spatially regulated manner. Here, the major genes involved at each stage have been indicated. Genes have been selected according to their conserved functions in various vertebrate animal models and to the availability of detailed studies about their regulation and function in ectoderm patterning. At the mid-gastrula stage (pre-border stage), orange labels the anterior neural border (NB), and yellow depicts the posterior NB. At later stages, green depicts the NC.
im., intermediate; var., variable.

Wnt and Hedgehog signaling pathways

Canonical Wnt signaling pathway.
(A) In the absence of Wnt, β-catenin is phosphorylated by GSK-3 in a complex with casein kinase-1, axin, and APC (the destruction complex), leading to β-catenin ubiquitylation and degradation. (B) Wnt polypeptides bind to Frizzled and LRP receptors, leading to recruitment of Dishevelled, inactivation of the destruction complex, and stabilization of β-catenin. β-catenin then translocates to the nucleus and forms a complex with Tcf transcription factors, converting them from repressors to activators of their target genes.





Adapted from Singh et al., 2015

Hedgehog (Hh) signaling pathway. Left: In the absence of Hh, Patched1 (Ptch1) in the primary cilium suppresses Smoothened (Smo) activity and prevents its localization in the cilium. Gpr175 is not present in the cilium and therefore does not interact with ciliary Gα-i leading to high local concentrations of cAMP which stimulates PKA (asterisks denote active forms of all proteins) which phosphorylates full-length Gli3 (and Gli2; Gli-FL), triggering the cleavage of Gli3 into its repressor form (Gli-R). Gli3-R represses transcriptional activation of Hh pathway target genes**. Right:** In the presence of Hh, Ptch1 is removed from the cilium, allowing Smo to enter the cilium and be activated. This leads to the accumulation of Gpr175 in the cilia, where it interacts with Gα-i which inhibits local production of cAMP, preventing PKA activity and Gli3 cleavage. The SuFu/Gli-FL complex accumulates at the tip of the primary cilium, dissociates and activated Gli-FL (GliA*) exits the cilium and enters the nucleus where it activates the transcription of Hh target genes such as *Gli1*.
Cyclopamine is a plant steroid alkaloid that inhibits Smo (even in the presence of Hh ligands).

Workshop UE Practical course

Stem cells

October 13th – October 27th 2025

PLANNING AND PROTOCOLS

GENERAL PLANNING

	Monday oct 13rd **UVSQ**	*Tuesday oct 14th* **NeuroPSI**	*Wednesday oct 15th* **UVSQ**	*Thursday oct 16th* **NeuroPSI**	*Friday oct 17th* **UVSQ**
9h-10h	General introduction	Xenopus FIV and injection training	personal work	Fixation of the first batch of embryos at stage 18 / induction by DEX treatment of the second batch of embryos at stage 18 / cyclopamine treatment at stage 18	Cell culture & observation
10-11h	Experimental design of the Xenopus project		Cell culture & observation / dissection		
11h-12h	Experimental design of the Drosophila project				
12h-13h	*lunch*	*lunch*	dissociation		
13h-14h	Design of the cell culture projects	Xenopus FIV and mRNA injection	*lunch*	*lunch*	*lunch*
14h-15h	Cell Culture / *Drosophila* dissection training		cytometry acquisition	WISH probe synthesis	cytometry analysis
15h-16h					
16h-17h					
17h-18h					personal work
made by teachers	Inducing Ovulation / *Drosophila* infection	*Drosophila* infection	checking of the embryos / induction by DEX treatment at stage 12,5 / cyclopamine treatment at stage 12,5		fixation of the second batch at stage 24

	Monday oct 20th **NeuroPSI**	*Tuesday oct 21st* **NeuroPSI**	*Wednesday oct 22nd* **UVSQ**	*Thursday oct 23rd* **NeuroPSI**	*Friday oct 24th* **UVSQ**	*Monday oct 27th* **NeuroPSI**
9h-10h	WISH day 1: pre-treatments	WISH day 2: probe washes and incubation with anti-DIG antibody	Cell culture & observation / Dissection fixation mounting	WISH day 4: Post-fixation, pictures of whole mount embryos	Cell Culture & observation	Presentation of the results
10-11h						
11h-12h					Cell Culture analysis / Drosophila analysis	
12h-13h					*lunch*	
13h-14h	*lunch*	*lunch*	*lunch*	*lunch*	Perconal work, Cell Culture analysis / Perconal work, Drosophila analysis	social event
14h-15h	WISH day 1: pre-treatments, personal work, **QUIZZ**	WISH day 2: probe washes and incubation with anti-DIG antibody	Cell culture & observation / confocal acquisition/ analysis	personal work, analysis of the results		
15h-16h		Conference on organoids				
16h-17h						
17h-18h	WISH day 1 : o/n hybridization	WISH day 2 : o/n washes				
made by teachers	medium changes / *Drosophila* infection	*Drosophila* infection	WISH day 3: start NBT/BCIP coloration			

Part I – Pluripotent stem cell in culture

Location: UVSQ

Exp 1. Maintenance of mouse Embryonic Stem (ES) cells

MONDAY 13TH OF OCTOBER 2025

- Treat 2 B60 dishes with 3mL 0.1% gelatin at least 1h at room temperature
- Observe ES cells
- Remove medium
- Wash with 1X PBS
- Add 1 ml 1X Trypsin-EDTA
- Incubate 5 min at 37°C
- Dissociate cells by pipetting and transfer into 9 ml of EB differentiation medium
- Count cells (using a haemocytometer)
- Plate $2x10^2$ cells in 5ml medium per B60 in the following conditions:

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

- Place into the incubator

WEDNESDAY 15TH OF OCTOBER 2025

- Observe ES cells
- Change media (5mL medium per B60)

FRIDAY 17TH OF OCTOBER 2025

- Observe ES cells
- Change media (5mL medium per B60)

Monday Oct 20 **(made by teachers)**

- *Change medium (same protocol as described previously)*

WEDNESDAY 22ND OF OCTOBER 2025

- Observe ES cells
- Change media (5mL medium per B60)

FRIDAY 24TH OF OCTOBER 2025

- Remove media
- Replace with 1X PBS
- Transfer dishes into room 77-79 2nd floor
- Remove PBS and replace with 3mL 4% **PFA (Toxic***)
- Incubate 20 min at room temperature
- Take back the PFA solution into a 50 ml tube (elimination in chemical trash)
- Rinse twice with 1X PBS
- Incubate in 25mM Tris-maleate
- Prepare the following **staining solution** (take care of the order of reagents)

Reagents	Final concentration	Volume
285 mM Tris-maleate pH=9	25 mM	
100 mg/ml α-naphthyl phosphate	0.4 mg/ml	
1M $MgCl_2$	8 mM	
H_20	Quantity to 9 mL final	
100 mg/ml Fast-Red TR	1 mg/ml	

- Remove 25 mM Tris-maleate and replace by staining solution (2 mL per B60)
- Incubate 15 to 30 min at room temperature
- Rinse twice with 1X PBS and once with water
- Air dry
- Observe the aspect of the clones and count colonies

Exp 2. Embryoid body differentiation of mouse Embryonic Stem cell

MONDAY 13TH OF OCTOBER 2025

• Transfer 1x10^6 ES cells (same ES cells used for Exp 1) into 15 ml tube

• Spin at 300 g for 5 min

• Resuspend cells in 10 ml of EB differentiation medium and transfer into a 100 mm Petri dish (not treated for cell culture)

• Place into the incubator

WEDNESDAY 15TH OF OCTOBER 2025

• Observe fluorescence of EBs

• Transfer EBs in a 15 ml conical tube and let them decant for 5 min

• Carefully remove the supernatant

• Resuspend (EBs that will have settled on the bottom of the tube) gently with 10 ml EB differentiation medium

• Replate onto a new Petri dish

• Place into the incubator

FRIDAY 17TH OF OCTOBER 2025

• Observe fluorescence of EBs

• Transfer EBs into a 15 ml conical tube and let them decant for 5 min

• Carefully remove the supernatant

• Resuspend (EBs that will have settled on the bottom of the tube) gently with 10 ml EB differentiation medium

• Replate onto a new Petri dish

• Place into the incubator

Monday Oct 20 **(made by teachers)**

• *Change medium (same protocol as described previously)*

WEDNESDAY 22ND OF OCTOBER 2025

• Observe fluorescence of EBs

• Transfer EBs in a 15 ml conical tube and let them decant for 5 min

• Carefully remove the supernatant

• Resuspend (EBs that will have settled on the bottom of the tube) gently with 10 ml EB differentiation medium

• Replate onto a new Petri dish

• Place into the incubator

FRIDAY 24TH OF OCTOBER 2025

• Observe fluorescence of EBs

Exp 3. Reprogramming MEF into iPS cells

MONDAY 13TH OF OCTOBER 2025

Observe reproMEFs.

Remove medium

Wash with 1X PBS

Add 1.5 ml 1X Trypsin-EDTA

Incubate 5 min at 37°C

Dissociate cells by pipetting and transfer into 3.5 ml of ES cell medium

Count cells (using a haemocytometer)

Seed 6x10^3 cells per B60 with feeder cells in ES cell medium with the following conditions:

1x B60	*no Dox*
1x B60	*Dox (1 µg/ml)*
1x B60	*Dox (1 µg/ml) and ascorbic acid 50 µg/ml*
1x B60	*ascorbic acid 50 µg/ml*

WEDNESDAY 15TH OF OCTOBER 2025

- Observe cells
- Change media (5mL medium per B60)

FRIDAY 17TH OF OCTOBER 2025

- Observe cells
- Change media (5mL medium per B60)

Monday Oct 20 **(made by teachers)**

- *Change medium (same protocol as described previously)*

WEDNESDAY 22ND OF OCTOBER 2025

- Observe cells
- Change media (5mL medium per B60)

THURSAY 24TH OF OCTOBER 2025

- Observe cells

Alkaline phosphatase staining can be performed on extra iPS colonies to assess for pluripotency-associated marker expression.

Material and Reagents

1. Media

ES cell medium: 15% Fetal Calf Serum; 100 µM ß-mercaptoethanol (Sigma, M7522); 10^3 units/ml LIF (PAA, F001-008); 0.1 mg/ml Penicillin/Streptomycin (Invitrogen, 15070-008) in DMEM high-Glucose (Invitrogen, 31966021)

EB differentiation medium: 15% Fetal Calf Serum; 100 µM ß-mercaptoethanol (Sigma, M7522); 0.1 mg/ml Penicillin/Streptomycin (Invitrogen, 15070-008) in DMEM high-Glucose (Invitrogen, 31966021)

2. Cell lines

- ES *Sox17-GFP*
- ReproMEFs
- Feeder cells. *Mouse embryonic fibroblast treated with mitomycin C to arrest their growth.*

3. Reagents

- Gelatin from porcine skin, Type A (Sigma, #G1890) 0.1%
- Trypsin-EDTA 0.25%
- Doxycycline (Sigma, #D9891) 1 mg/ml
- Ascorbic acid (Sigma, #A4403) 50 mg/ml

References

Experiment 1

- Evans, M. J. And Kaufman, M. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292,** 154-156.

- Martin, G. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *PNAS* **78**-12, 7634-7638

Experiment 2

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- Kim, I., Saunders, T.L., and Morrison, S.J. (2007). Sox17 Dependence Distinguishes the Transcriptional Regulation of Fetal from Adult Hematopoietic Stem Cells. Cell *130*, 470–483.

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- Martin GR, Evans MJ. Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. Proc Natl Acad Sci USA. 1975. 72(4):1441-5.

- Martin GR, Wiley LM, Damjanov I. The development of cystic embryoid bodies in vitro from clonal teratocarcinoma stem cells. Devopmental Biology. 1977. 61(2):230-44.

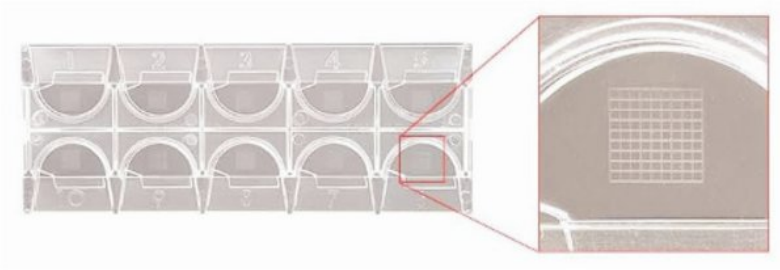
Experiment 3

- Beard, C., Hochedlinger, K., Plath, K., Wutz, A., & Jaenisch, R. (2006). Efficient method to generate single-copy transgenic mice by site-specific integration in embryonic stem cells. Genesis, 44(1), 23–28.

- Stadtfeld, M., Maherali, N., Borkent, M., and Hochedlinger, K. (2010). A reprogrammable mouse strain from gene-targeted embryonic stem cells. Nature Methods *7*, 53–55.

- Takahashi, K., & Yamanaka, S. (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell, 126(4), 663–676.

Counting cells : haemocytometer type chamber



KOVA Glasstic Slide 10 with Grid Chamber

The volume within the grid (9x9 small grids) is 0.9 µL

Cells / mL = Average number of cells per small grid x 90 (multiplication factor) x 1000

- Deposit 12 µL to fill the chamber

- You must perform two independent counting

- Count at least 100 cells each time

Example :

I counted *104 cells in 9 small grids*

113 cells in 9 small grids

*[(104/9*90*1000) + (113/9*90*1000)] /2 = 1.1x10^6 cells/mL*

Part II – Homeostasis of adult *Drosophila* midgut

Location: UVSQ

Sunday Oct 12th **(made by teachers)**

Bacterial culture

Put 2 µL of frozen Pectobacterium carotovorum / Erwinia carotovora ssp. carotovora (Ecc15) to grow in 150 mL of LB (Luria-Bertani/Lysogeny broth) at 28°C for 24 hr.

This volume allows the infection of 30 fly tubes.

MONDAY 13TH OF OCTOBER 2025

Dissection training

Flies collection, in the fly room

• Put to sleep 6-8 female flies on CO2 pad.

The large size of female flies facilitates the dissection and allows us to obtain more cells of each cell population

• Cut the head of a fly with a scalpel or a pair of forceps.

• Transfer the headless fly to a plastic dish of 1x PBS.

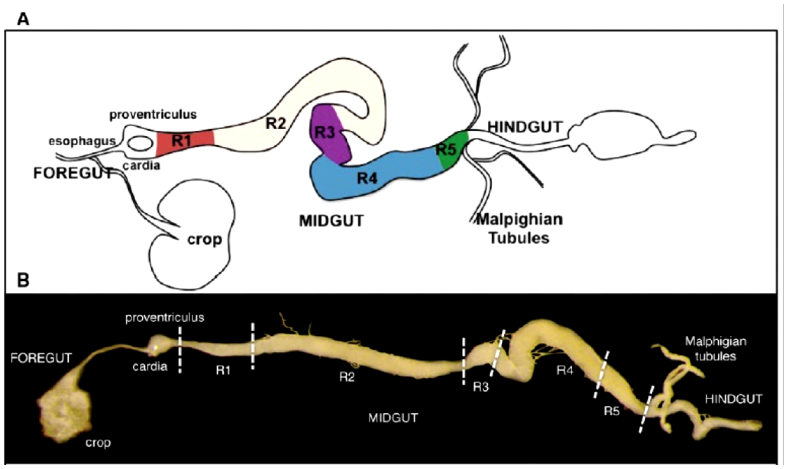
Dissection (less than 30 min per genotype),

• Drown the fly with one forceps.

• Carefully tear abdomen with the other forceps.

• Remove the intestine. Try to gently straighten it out so that it is no longer coiled.

• Cut the piece of interest and reduce the size of the Malpighian tubules (see below)



The *Drosophila* midgut consists of five morphologically and functionally distinct regions or compartments

(**A**) Schematic drawing showing the *Drosophila* midgut and the five regions (**B**) Picture showing the midgut regions.

The white dotted lines show the start and end of each region.

R1 starts from heproventricular boundary to the first hinge, where the crop physically interacts with the midgut.

R2 starts from the end of R1 and ends just before the retrograde folding of the gut from where the copper cell region starts. R3 covers the copper cell region.

R4 starts at the end of R3 and endsin the posterior midgut where midgut starts to narrow.

R5 starts at the end of R4 and ends at the midgut hindgut junction, where the Malphigian tubules physically interact with the gut.

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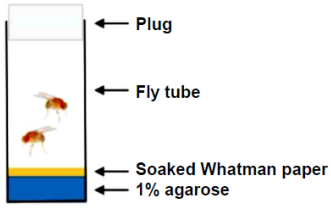
Oral infection **(made by teachers)**

- *Pour 2.5 mL of 1% agarose in each fly tube.*
- *After agarose solidification, put a pre-cut Whatman paper on it.*
- *Centrifuge the bacterial culture for 15 min at 3220 g, 20°C.*
- *Replace the supernatant with 500 µL of 5% sucrose. (do not resuspend).*
- *Invert the tube to eliminate the PBS which is toxic for the flies.*
- *Resuspend the pellet with an equivalent volume of 5% sucrose.*
- *Put 80 µL of resuspended bacterial solution (infected condition) or 80 µL of 2.5 % sucrose (uninfected) on the Whatman paper.*
- *Add 20 females per tube.*



Tuesday Oct 14 **(made by teachers)**

WEDNESDAY 15TH OF OCTOBER 2025

Flow cytometry

• Put to sleep 20 female flies on CO2 pad protected by a tissue paper.

• Cut the head of a fly with a scalpel or a pair of scissors.

• Transfer the headless fly to plastic dish of 1x PBS.

• Dissect on ice the whole gut and remove the Malphigian tubules and trachea

Keep total dissection time <2 hr

• Transfer the 20 guts into a 1.5 mL microcentrifuge tube containing 400 µl of a cooled solution of collagenase type XI (50mg/mL) at 1/4 in PBS 1X

The dissected midguts should be maintained at 4°C until the dissociation step.

• Incubate the tubes for 1 hr 30 min at 29°C with shaking at 800 rpm in the dark (heating block ThermoMixer Eppendorf).

• Add 400 µl working solution into the microcentrifuge tube containing the collagenase. Agitate by pipetting up and down (using a cut blue tips coated with SVF for this step) at least 50 times to fully dissociate the guts.

Working solution = Trypsin (10X) at 1/2 and DRAQ5 at 1/2000 in PBS 1X

⚠ Don't make any bubble ⚠

• Incubate the tubes for 30 min at 29°C with shaking at 800 rpm in the dark (heating block ThermoMixer Eppendorf).

• Add 700µl of PBS1X

• Centrifuge the tubes for 5 min at 600g at 20°C.

• Re-suspend the pellet in 400 µL fresh 1x PBS

• Place a 40 µm filter on the top of a flow cytometry tube and add 20µL of 1x PBS to humidify the filter.

• Add the dissociated cells on the 40 µm filter to remove clumps, which might otherwise block the nozzle of the flow cytometer, and pipet up and down

• Rinse the Eppendorf tube, in which cells were dissociated, with 400 µL of 1x and put it on the filter.

• Keep the flow cytometry tube containing the cells in solution for 20 min. at room temperature then on ice (4°C).

• Go to the flow cytometer (Becton Dickinson Fortessa).

Sunday Oct 19 **(made by teachers)**

Bacterial culture

Monday Oct 20 **(made by teachers)**

Oral infection

Tuesday Oct 21 **(made by teachers)**

Oral infection

WEDNESDAY 22ND OF OCTOBER 2025

Preparation of *Drosophila* midguts for microscopic observation

Flies collection, in the fly room

• Put to sleep 6-8 female flies on CO2 pad protected by a tissue paper.

• Cut the head of a fly with a scalpel or a pair of forceps.

• Transfer the headless fly to a plastic dish containing some 1x PBS.

Dissection (less than 30 min per genotype)

• Drown the fly with one forceps.

• Carefully tear abdomen with the other forceps.

• Remove the intestine. Try to gently straighten it out so that it is no longer coiled.

• Cut the piece of interest (R4&5) and reduce the size of the Malpighian tubules.

• Transfer the intestines (6 to 8) to a glass dish containing 300 µL of 1x PBS.

• Add 100 µL of 16%Formaldehyde MeOH free *(UNDER THE FUMEHOOD).*

• Incubate for 1 hour to 1.5 hours in the dark.

• Remove the formaldehyde 4% and rinse briefly with 500 µL PBT.

• Rinse two times 5 min with 500 µL PBT.

• Incubate for 1h in 300 µL PBT + Phalloïdin (1/1000) + Hoechst (1/1000), in the dark.

• Rinse briefly with 500 µL PBT.

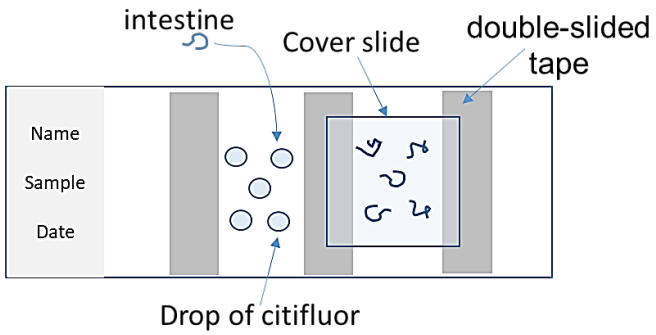
• Rinse two times 5 min with 500 µL PBT.

• Prepare slide compartmentalized with 3 double-slided tape (see figure below).

• Put 5 separated drops of 10 µL of Citifluor in each of the two compartments.

• Place one intestine in each drop of Citifluor.

• Mount on slide by adding a coverslip. Keep in the dark at 4°C.

Solutions:	
1X PBS PBT (1X PBS + 0.1% Triton) Hoechst 33342 (1mg/mL) Phalloïdin 655 (28,2 µg/mL) 16 % Formaldehyde MeOH free Citifluor	

Confocal acquisitions @ Cymages facility

Material and Reagents

Miller's LB medium

Peptone 10 g/L

Yeast extracts 5 g/L

NaCl 10 g/L

1X PBS solution

NaCl	137 mM
KCl	2.7 mM
Na2HPO4	10 mM
KH2PO4	1,8 mM

Collagenase XI

Collagenase XI (Sigma C7657 500MG) at a concentration of 50 mg/ml in PBS1X stored at -20°c

Avoid multiple freeze-thaws.

Used at 1/4 (final concentration).

Trypsin

Trypsin (T0303) stored at 10X in PBS1X at -20°c

Avoid multiple freeze-thaws.

Used at 1/2 (final concentration).

DRAQ5 stock solution (5mM)

Used at 1/2000

References

Evans CJ, Olson JM, Ngo KT, Kim E, Lee NE, Kuoy E, Patananan AN, Sitz D, Tran P, Do MT, Yackle K, Cespedes A, Hartenstein V, Call GB, Banerjee U. G-TRACE: rapid Gal4-based cell lineage analysis in *Drosophila*. Nat Methods. 2009 Aug;6(8):603-5. doi: 10.1038/nmeth.1356.

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Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B. *Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host Microbe. 2009 Feb 19;5(2):200-11. doi: 10.1016/j.chom.2009.01.003.

Part III – Neural crest in *Xenopus*

Location: NeuroPSI

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Monday Oct 13th **(made by teachers)**

- *Hormonal stimulation of Xenopus laevis females (meiosis completion and egg laying).*

TUESDAY 14TH OF OCTOBER 2025

9 AM - 12 AM: Xenopus FIV and mRNA injection training

- *In vitro* fertilization and dejelling of the embryos
- Initiation: injection needle calibration
- mRNA microinjection training

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1 PM- 5 PM: Xenopus FIV, mRNA injection, control of injection

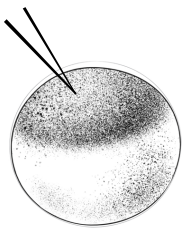
2 batches of embryos:

- Batch 1 for pharmacological treatments to manipulate Hh signaling

- Batch 2 for micro-injections to manipulate Wnt signaling

Plasmids used to synthetize RNAs to inject: pCS2-lacZ; pCS2-dnTCF3-GR

3 groups

Groups	RNAs to inject	at 1-cell stage
Groups 1-3	▪ *nlacZ* (100pg/nL) + DFL (1µL) (dextran fluorescein lysin) ▪ *dnTCF3GR* (100pg/nL) + DFL (1µL) ▪ inject into 1-cell stage embryos ▪ control: non-injected embryos	

- *In vitro* fertilization and dejelling of the embryos
- mRNA microinjection
- Selection of *Xenopus* embryos: remove undivided embryos, check fluorescence
- Preparation of the day2 (that will be made by teachers)

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IN VITRO FERTILIZATION OF *XENOPUS* EGGS

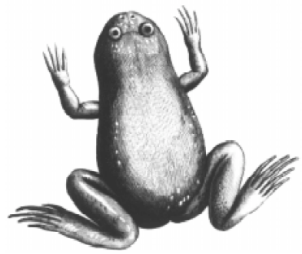

Fig. 1. The first published picture of *Bufo laevis*, from Daudin (1802/03).

INDUCING OVULATION: **MADE BY TEACHERS**

- Inject 700 units of Human Chorionic Gonadotropin (HCG) into the dorsal lymph sac of a female frog, approximately 12 hours before eggs are needed.
- Frogs begin laying oocytes about 12 hours after induction of ovulation.
- After ovulation, frogs need to rest 3-4 months before being induced again.

TESTES DISSECTION

- Anesthetize the male in 0,1% Benzocaine for 20–30 min at cold temperature (12-15°C).
- Dissect out the testes and remove any remaining blood vessel contamination with forceps.
- Transfer testes in a 1ml microcentrifuge tube filled with **1xMBS**, and store at 4°C.

IN VITRO FERTILIZATION

- Label Petri dishes on the bottom **and** on the lid according to which frog the oocytes come from.
- Squeeze the female by gentle massage. After about an hour of rest, the females can be re-squeezed for a second fertilization if needed.
- Crush a testis in the microcentrifuge tube and transfer it in a 15mL tube and add 7mL of **1xMBS**
- Add a few drops of the spermatozoid-containing solution on the oocytes.
- Incubate at room temperature for 5 minutes with gentle mixing.
- Carefully add **0,1xMBS** to submerge the eggs.
- Cover the dish and note the **time of fertilization** on the dish lid.
- Note that the 1-cell embryo should rotate within the jelly coat and the animal pole (pigmented side) should face up after about 20 minutes. Otherwise, eggs have not been fertilized.

DEJELLING

- Decant excess 0,1xMBS with a plastic pipette and add fresh 2% cysteine solution (prepared in 0,1xMBS).
- Incubate for about 5 minutes at room temperature. This step allows the complete removal of the jelly coat surrounding the embryos.
- Decant cysteine solution and wash twice with water and 3 times with 0,1xMBS.
- Incubate the embryos in 0,1xMBS at the desired temperature, preferably at 14-18°C. The embryos cultured at room temperature (18°C) should reach the 2-cell stage 1.5 hours after fertilization.
- Remove dead embryos (white).

SOLUTIONS

- **MBS (Modified Barth's Saline)**

Two solutions: 0.1M $CaCl_2$ **AND** 10x MBS salts.

10x MBS salts
880 mM NaCl
10 mM KCl
10 mM $MgSO_4$
50 mM HEPES (pH 7.8)
25 mM $NaHCO_3$
Adjust final pH to 7.8 with NaOH, autoclave

1x MBS
100 mL 10X MBS salts
7 mL 0.1M $CaCl_2$
Adjust the volume to 1 L with H_2O

0.1x MBS
Dilute 1x MBS 10 times with H_2O

Check pH 7.8-8.0 the same day of fertilization

- **2% Cysteine Solution**

Weight 8 g of L-Cysteine Hydrochloride Monohydrate
Add 400 mL of 0,1xMBS
Adjust pH to 7.8 with NaOH

MICRO-INJECTIONS

⚠ ***RNAse free conditions - Wear gloves*** ⚠

PREPARATION OF CAPILLARIES

We use Sutter Instrument Company's model p87 micropipette puller to make our needles/capillaries. They are pulled from borosilicate glass capillaries with inner filament

using **program 5** under the following settings: *heat 293, pull 100, velocity 60, time 40, and pressure 500*.

Capillaries making

- Turn on the micropipette puller on the left bottom.
- Enter the number of the program: 1.
- Push on ENTR.
- Install the capillary and push on PULL.
- Remove the two needles.
- Switch off with the left ON/OFF bottom.
- Put back the protection plastic lid.

Loading RNA in the capillaries

- Fill a microtip with 2.5 µL of your RNA or Morpholino solution.
- Load the capillary.
- Install the capillary in the capillary holder of the micromanipulator.

PICOSPRITZER USE

- For microinjection, we use the Picospritzer II and III from Parker Hannifin Corporation.

- Our **settings** are:
  - Duration 800 milliseconds
  - Pressure 60-80 psi

- Calibrate your needle in paraffin oil using a 800ms time of injection: To know the volume of the drop, measure its diameter (in mineral oil) with the reticule at the highest magnification (x50 on Stemi 2000 Zeiss). You can then calculate the volume of the sphere: $V= 4\pi r^3/3$. For V=10nl, diameter should be: -- . Cut the tip of the needle until the drop diameters reaches -- graduations.

- Place the embryos in an injection Petri dish (about 20 embryos) in MBS0,1X containing 0,25% Methylcellulose.
- Inject the RNA: 10 nl in an embryo at 1-cell-stage.

1 mm = 5.3 grad (highest magnification)				
Volume of the drop in mineral oil (nL)	**10**	7.5	5	2.5
Diameter (graduations)	**1.42**	1.29	1.13	0.89

- Place the Petri dish with the injected embryos in MBS0.1X in the incubator at 16 °C.

Wednesday Oct 15th **(made by teachers)**

9 AM - 1 PM

- *Checking of Xenopus embryos: remove dead or malformed embryos, check fluorescence, analyze embryo morphology*
- *Batch 1 (non-injected embryos): **for one half of the embryos**: **stage 12.5**: start of cyclopamine treatment; Controls with EtOH in the same quantity as for cyclopamine*
- *Batch 2 (injected embryos): **for one half of the embryos**: **stage 12.5**: dnTCF activity induction by DEX treatment; Controls with EtOH in the same quantity as for DEX.*
- *Place embryos at 14°C*

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CYCLOPAMINE TREATMENT

- In 0.1xMBS: Dilute Cyclopamine to 100 µM final (stock at 20mM, 200x). Prepare 5 ml of medium per well.
- Dilute EtOH in 0.1xMBS for sibling control embryos. Prepare 5 ml of medium per well.
- Put the mediums into 6 wells plates to incubate your embryos.
- Place your embryos in the wells at the desired stage.
- When the treatment is over, rinse the embryos several times in 0.1x MBS before fixing them.

INDUCTION BY DEXAMETHASONE

- In 0.1xMBS: Dilute Dexamethasone to 4µg/mL final (stock at 10mg/mL, 2500x). Prepare 5 ml of medium per well.
- Dilute EtOH in 0.1xMBS for sibling control embryos. Prepare 5 ml of medium per well.
- Put the mediums into 6 wells plates to incubate your embryos.
- Place your embryos in the wells at the desired stage.
- When the treatment is over, rinse the embryos several times in 0.1x MBS before fixing them.

THURSDAY 16TH OF OCTOBER 2025

9 AM - 1 PM

- Checking of Xenopus embryos

- Batch 1 *(non-injected embryos)*: **for the second half of the embryos**: **stage 18**: start of cyclopamine treatment; Controls with EtOH in the same quantity as for cyclopamine

- Batch 2 *(injected embryos)*: **for the second half of the embryos**: **stage 18**: dnTCF activity induction by DEX treatment; Controls with EtOH in the same quantity as for DEX.

- Place embryos at 14°C

- Fixation at stage 18 of embryos treated from stage 12.5 and dehydration of embryos in MetOH

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2 PM - 7 PM

- Plasmid linearization for probe synthesis

Group number	Plasmids to linearize	Restriction Enzyme
Group 1	▪ *Twist* (pBS)	▪ EcoRI
Group 2	▪ *Ptc1* (pCS2)	▪ BamHI
Group 3	▪ *CyclinD1* (pBS)	▪ BamHI

- Linearized DNA purification

- 1% agarose gel preparation and electrophoresis of linearized DNA

- *In vitro* transcription of antisense RNA

Group number	Linearized plasmids	RNA polymerase
Groups 1 (2 tubes)	▪ *Twist*	▪ T7
Group 2	▪ *Ptc1*	▪ T3
Group 3	▪ *CyclinD1*	▪ T3

- Antisense RNA probe purification, 1% agarose gel preparation and electrophoresis

ANTISENSE PROBE SYNTHESIS

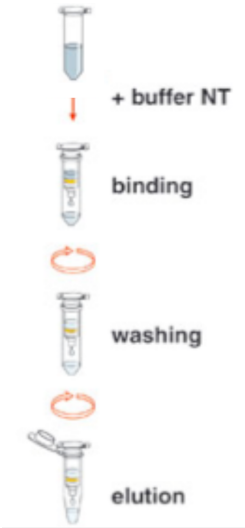
1. PLASMID LINEARIZATION

- Digest your plasmids with the appropriate restriction enzyme and its corresponding buffer: here is the protocol for Fast Digest enzyme

H_2O milliQ	Final volume 50 µL
Buffer 10x	5 µL
Plasmid	**5 µg**
Enzyme	5 µL

- Incubate 30min at 37°C in the water bath.

2. DNA PURIFICATION – PCR CLEAN-UP GEL EXTRACTION KIT (MACHEREY-NAGEL)



PRINCIPLE: The PCR/plasmid clean-up gel extraction procedure allows a fast and easy removal of enzymes, nucleotides, salts, and other impurities.

PROCEDURE:

Before starting the preparation

- Check if Wash Buffer NT3 is already prepared (ethanol added).

Adjust DNA binding condition

- Mix 1 volume of sample with 2 volumes of Buffer NTI (e.g., mix 50 µL DNA solution and 100 µL Buffer NTI).

Bind DNA

- Place a PCR clean-up gel extraction Column into a Collection Tube (2 ml) and load the sample on the column.
- Centrifuge for 30 sec at 11,000 x *g*.
- Discard flow-through and place the column back into the collection tube.

Wash silica membrane

- Add 700 µl Buffer NT3 to the PCR clean-up gel extraction Column.
- Centrifuge for 30 sec at 11,000 x *g*.
- Discard flow-through and place the column back into the collection tube.

Dry silica membrane

- Centrifuge for 1 min at 11,000 x *g* to remove remaining Buffer NT3.

Elute DNA

- Place the Column into a new 1.5 ml microcentrifuge tube.

- Add 30 µL Buffer NE and incubate at room temperature for 1 min.
- Centrifuge for 1 min at 11,000 x g.
- Keep the flow-through, your DNA is in there!

Control quality and quantity by loading 2 µL on an agarose gel (see below)

3. AGAROSE GEL ELECTROPHORESIS

- Weigh out 1 g of agarose into a 250 mL flask (1% agarose gel)
- Add 100 mL of 0,5x TBE, swirl to mix.
- Microwave for about 1 minute to dissolve the agarose. ⚠ *It can become superheated and NOT boil until you take it out, whereupon it may boil out all over your hands!!!*
- Leave it to cool on the bench for 5 min.
- While the agarose is cooling, prepare the gel tank with the comb.
- Add 5 µL of GelRed and swirl to mix.
- Pour the gel slowly into the tank. Rinse out the flask immediately.
- Leave to set for at least 30 min, until solidified.
- Put 0,5x TBE buffer into the gel tank to submerge the gel (same buffer as the one you used for the gel).
- Transfer 2 µL of the purified DNA into a fresh microfuge tube + 3 µL H_2O + 1 µL loading buffer (which also contains dyes: bromophenol blue and xylene cyanol).
- Load your samples and a molecular weight marker. *Write in your lab-book (or take a photo of) the physical order of the tubes so that you can identify the lanes on the gel photograph.*
- Close the gel tank, switch on the power-source and run the gel at 5V/cm. For example, if the electrodes are 20 cm apart then run the gel at 100 V.
- Stop the gel when the bromophenol blue has run 3/4 the length of the gel.
- Switch off, unplug the gel tank and carry the gel in its holder to the UV light-box. ⚠ *UV are **carcinogenic** and must not be allowed to shine on naked skin or eyes.*
- Take a picture for the lab-book.
- Store plasmids at -20 °C

4. PROBE SYNTHESIS

⚠ ***RNAse free conditions - Wear gloves*** ⚠

- Transcribe *in vitro* your linearized DNA with the appropriate RNA polymerase:

Linearized DNA	1 µg
Transcription buffer 10x (Roche)	2 µL

DIG RNA Labelling MIX (Roche)	2 µL
T3, T7 or SP6 RNA polymerase (Roche)	2 µL
H_2O milliQ	Final volume 20 µL

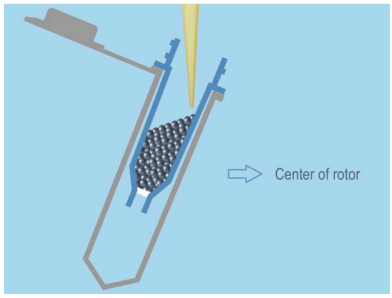
- Incubate 2 hrs at 37°C in the water bath.
- Stop the reaction with 1µL EDTA 0.2M

5. RNA PROBE PURIFICATION – MOBISPIN G-50 (MOBITEC)

PRINCIPLE: Chromatographic separation of unincorporated nucleotides from labelled nucleic acids. The MobiSpin G-Columns are prepacked with Sephadex® G-50 resin, equilibrated and ready to use. Beads have a pore size of 700 Da that allow, e.g., hydrated salt ions or unincorporated nucleotides to enter into the pores while DNA/RNA > 20 bases and most other biomolecules stay outside.

PROCEDURE

- Resuspend the resin in the column by vortexing.
- Bend off the tip of the column and loose the cap one fourth turn.
- Place the column in a 1.5 ml microcentrifuge tube and use this as collecting vessel.
- Pre-spin the column 1 minute at 735 x g in a microcentrifuge with a fixed-angel rotor. Do not pulse as this will override the variable speed setting. Please consider centrifugation note below!
- Use the column immediately after removing the equilibration buffer from the resin to avoid drying up!
- Place the column in a new 1.5 ml tube and slowly apply the sample (50 µl or less) to the upper side of the slanted matrix surface. Take care not to disturb the resin bed!



- Spin the column 2 minutes at 735 x g. The purified sample is collected in the bottom of the support tube.
- Store the tubes at -20°C.

EMBRYO FIXATION

⚠ *RNAse free conditions - Wear gloves* ⚠

- At the desired stage, transfer embryos into a glass vial with a plastic pipette after removal of dead ones.
- Remove the solution without letting the embryos dry (i.e. immediately put fixative).
- Add about 3 mL of PFA 3.7%; carry out fixation for **2 hours at RT** with gentle

shaking.

- Wash 3 times in 1X PBS
- Dehydrate in 50% methanol/PBS and twice in 100% methanol (can be stored at -20°C for about 2 years). **Methanol should be manipulated under the hood**.

*Friday Oct 17 (***made by teachers)**

- *Fixation at stage 24 of embryos treated from stage 18 and dehydration of embryos in MetOH*

Summary of conditions

conditions		Induction by DEX or start of cyclopamine/EtOH treatment	Fixation	Probes
nlacZ	-Dex	St 12.5	St 18	Twist / CyclinD1
	+Dex			
dnTCF	-Dex			
	+Dex			
EtOH		St 12.5	St 18	Twist / Ptc1
cyclopamine				
nlacZ	-Dex	St 18	St 24	Twist / CyclinD1
	+Dex			
dnTCF	-Dex			
	+Dex			
EtOH		St 18	St 24	Twist / Ptc1
cyclopamine				

MONDAY 20TH OF OCTOBER 2025
TO
WEDNESDAY 22ND OF OCTOBER 2025

20TH OF OCTOBER

9 AM – 7 PM: Whole mount *in situ* hybridization (WISH), day1

- Rehydration and prehybridization of embryos
- Hybridization (at least 8 embryos per condition)

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21ST OF OCTOBER

9 AM – 7 PM: Whole mount *in situ* hybridization, day2

- WISH probe washes
- incubation with anti-DIG antibody
- WISH washes

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22ND OF OCTOBER (made by teachers)

Start WISH coloration (check during the day and stop when needed!)

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WHOLE MOUNT *IN SITU* HYBRIDIZATION

DAY 1: HYBRIDIZATION

⚠ ***RNAse free conditions - Wear gloves*** ⚠

- Rehydrate the embryos in decreasing methanol concentration (100%, 75%, 50%, 25% in PBT): 3 minutes for each bath.
- Wash the embryos 3 times for 3 min in PBT.
- Bleach pigment in Bleaching Solution for 5-10 min on a light box.
- Wash 3 times for 5 min in PBT.
- Treat embryos with Proteinase K 10 µg/ml (3 min at stage 18; 5 min at stage 24) with gentle shaking.
- Wash twice for 5 min in Glycine 2 mg/ml and then twice for 5 min in PBT.
- Post-fix in 4% PFA + 0,2% glutaraldehyde for 20 min.
- Wash 3 times for 5 min in PBT, with gentle shaking.
- Prehybridize in Hybridization buffer (25ml per container or 2mL per vial, minimize hybridization buffer volumes!) for at least 1.5 hrs at 65°C, with gentle shaking.

- Transfer embryos in a 24 wells plate with 500 µL of RNA probe 1X (diluted in Hybridization buffer)
- Incubate overnight at 63 °C, with gentle shaking.

DAY 2: HYBRIDIZATION WASHES AND ANTIBODY INCUBATION

- Take the probes, store the probes back at -20 °C, and incubate embryos for 15 min in Hybridization buffer at RT.
- Wash the embryos in Hybridization buffer/2x SSC (50:50) for 15 min.
- Wash the embryos 2 times in 2x SSC for 30 min at 65°C.
- Wash the embryos 2 times in 0.2x SSC for 30 min at 65°C.
- Wash the embryos in TNX for 15 min at RT.
- Incubate in 2 mL blocking buffer for 1 hr at RT.
- Incubate in 2 mL blocking solution containing anti-DIG antibody (1/4000 dilution) for 2 hrs at RT.
- Wash the embryos 3 times in TNX for 5 min at RT.
- Continue washing the embryos in TNX overnight at 4 °C.

DAY 3: WASHES AND ALKALINE PHOSPHATASE STAINING

- Wash the embryos in TNX for 10 min at RT.
- Wash the embryos 2 times for 10 min in freshly made NTMT buffer.
- Stain with NTMT containing NBT-BCIP staining solution (3,5 µL/mL NTMT).
- Monitor staining along the day and adjust duration and temperature accordingly.
- Stop the staining reaction by washing 3 times in PBT+ EDTA 1mM. Check with teacher.
- Wash with 1X PBS at least 3 times.
- Image the embryos under the stereomicroscope.

SOLUTIONS

- **PBS 10x**
  - Weight:
    80 g of NaCl
    2.0 g of KCl
    14.4 g of Na_2HPO_4
    2.4 g of KH_2PO_4
  - Dissolve in 800ml distilled H_2O
  - Adjust pH to 7.4
  - Adjust volume to 1 L with additional distilled H_2O
  - Sterilize by autoclaving

- **PBT**
  For 1 L:
  - 100 mL 10x PBS (1x final)
  - 1 mL Tween-20 (0.1% final)
  - Adjust volume to 1 L with H_2O

- **Bleaching Solution**

  For 10 ml add in the **EXACT ORDER** *(⚠ Otherwise it can explode!)*:

- 500 µL Formamide (5%) *Careful: toxic solution, needs to be diluted before being discarded in waste bottle*
- 6,45 mL H_2O
- Mix
- 250 µL 20xSSC (0,5%)
- Mix
- 2,8 mL H_2O_2 30% (10%)
- Mix

- **Proteinase K**

  For 50 mL Proteinase K 10 µg/ml:

  - 25 µL of proteinase K stock 2000X (20mg/mL)
  - 50 mL PBT

- **Glycine**

  For 10 mL 2 mg/mL:

  - 20 mg de glycine
  - 10 mL PBT

- **Hybridization buffer (Hyb Mix)**

  For 100 mL:
  - 25 mL SSC 20x (5X)
  - 50 mL Formamide (50%)
  - 1 g Blocking Reagent (1%)
  - 100 mg RNAt (1mg/mL)
  - 200 µL Heparin 50mg/mL (0.1 mg/mL)
  - 1 mL Tween-20 10%(0.1%)
  - 1 ml EDTA 0.5M (5mM)
  - 100 mg Chaps 10% (0.1%)
  - Adjust with H_2O, Store at –20°C

- **20X SSC**

  - Weight: 175.3 g of NaCl and 88.2 g of sodium citrate
  - Dissolve in 800 ml distilled H_2O
  - Adjust pH to 7.0 with a few drops of 1M HCl
  - Adjust volume to 1L with additional distilled H_2O
  - Sterilize by autoclaving

- **TN 10x**

  For 2L:
  - 242.2 g Tris (2M)
  - 175.32 g NaCl (3M)
  - H_2O
  - Adjust to pH 7.5
  - Filter and sterilize

- **TNX**

  For 100mL:
  - 10mL TN10X (1X)

- 1mL TritonX100 10% (0,1%)
- Adjust with H_2OmqA

- **Blocking Buffer 2%**

  For 100 mL:
  - 100 mL TNX
  - 2 g Blocking Reagent
  - Heat at 60°C to dissolve
  - Aliquot and store at –20 °C

- **NTMT**

  For 100 mL:
  - 10 mL Tris 1M pH 9,5
  - 2 mL NaCl 5M
  - 5 mL $MgCl_2$ 1M
  - 1 mL Tween-20
  - 82 mL H2O
  - 200 µL levamisole 1M

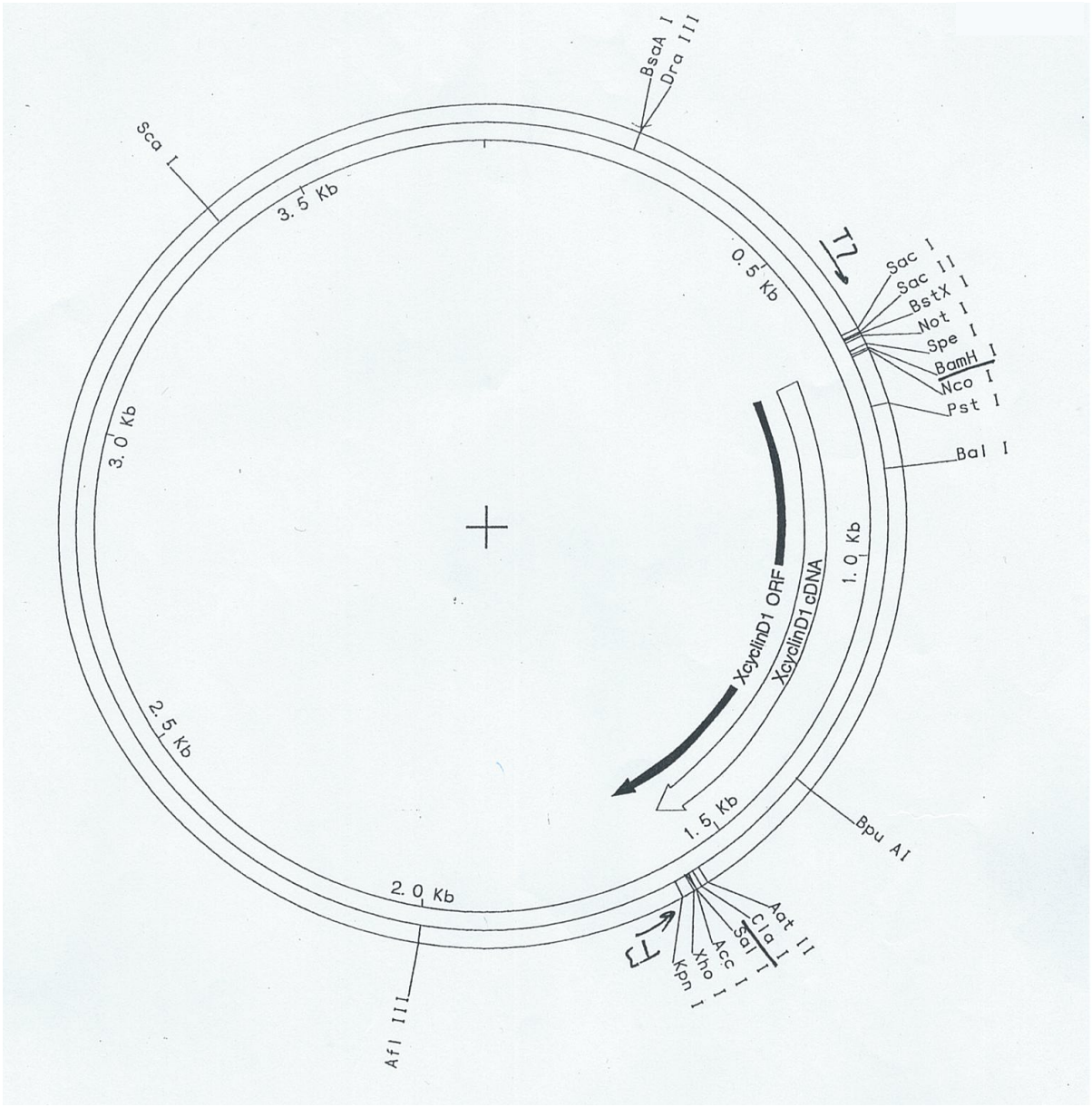
THURSDAY 23RD OF OCTOBER 2025

9 AM (- WISH post-fixation)

- Image capture of whole mount embryos

- Data analysis

PBS-CYCLIN D1 MAP



NIEUWKOOP AND FABER NORMAL TABLE OF XENOPUS LAEVIS DEVELOPMENT

Stage 1 (egg), animal view

Stage 2 (2-cell) ventral view

1 hr 30 min pf @ 23°C

Stage 4 (8-cell) dorso-lateral view

2 hr 15 min pf @ 23°C

Stage 6.5 blastula, animal view

3 hr 30 min pf @ 23°C

Stage 7 blastula, dorsal view

4 hr pf @ 23°C

Stage 8 blastula, ventral view

5 hr pf @ 23°C

Stage 1 (egg), dorsal view

Stage 3 (4-cell) animal view

2 hr pf @ 23°C

Stage 5 (16-cell) animal view

2 hr 45 min pf @ 23°C

Stage 6.5 blastula, dorsal view

3 hr 30 min pf @ 23°C

Stage 7 blastula, ventral view

4 hr pf @ 23°C

Stage 9 blastula, vegetal view

7 hr pf @ 23°C

Stage 1 (egg), ventral view

Stage 3 (4-cell) dorso-lateral view

2 hr pf @ 23°C

Stage 5 (16-cell) dorsal view

2 hr 45 min pf @ 23°C

Stage 6.5 blastula, ventral view

3 hr 30 min pf @ 23°C

Stage 8 blastula, animal view

5 hr pf @ 23°C

Stage 10 early gastrula, veg view

9 hr pf @ 23°C

Stage 2 (2-cell)

1 hr 30 min post-fert (pf) @ 23°C

Stage 4 (8-cell) animal view

2 hr 15 min pf @ 23°C

Stage 6 (32-cell) animal view

3 hr @ 23°C

Stage 7 blastula, animal view

4 hr pf @ 23°C

Stage 8 blastula, dorsal view

5 hr pf @ 23°C

Stage 10.5, vegetal view

11 hr pf @ 23°C

Stage 12, vegetal view

13 hr 15 min pf @ 23°C

Stage 14, lateral view

16 hr 15 min pf @ 23°C

Stage 16, posterior-dorsal view

18 hr 15 min pf @ 23°C

Stage 19, dorsal view

20 hr 45 min pf @ 23°C

Stage 21, dorsal view

22 hr 30 min pf @ 23°C

Stage 10.5, vegetal view

11 hr pf @ 23°C

Stage 12.5, posterior-dorsal view

14 hr 15 min pf @ 23°C

Stage 15, posterior-dorsal view

17 hr 30 min pf @ 23°C

Stage 17, anterior view

18 hr 45 min pf @ 23°C

Stage 19, anterior view

20 hr 45 min pf @ 23°C

Stage 21, anterior view

22 hr 30 min pf @ 23°C

Stage 11, vegetal view

11 hr 45 min pf @ 23°C

Stage 13, posterior-dorsal view

14 hr 45 min pf @ 23°C

Stage 15, anterior view

17 hr 30 min pf @ 23°C

Stage 17, posterior-dorsal view

18 hr 45 min pf @ 23°C

Stage 20, dorsal view

21 hr 45 min pf @ 23°C

Stage 22, dorsal view

24 hr pf @ 23°C

Stage 11.5, vegetal view

12 hr 30 min pf @ 23°C

Stage 14, posterior-dorsal view

16 hr 15 min pf @ 23°C

Stage 16, anterior view

18 hr 15 min pf @ 23°C

Stage 18, anterior view

19 hr 45 min pf @ 23°C

Stage 20, anterior view

21 hr 45 min pf @ 23°C

Stage 22, lateral view

24 hr pf @ 23°C

Stage 23, dorsal view

1 day, 45 minutes pf @ 23°C

Stage 23, lateral view

1 day, 45 minutes pf @ 23°C

Stage 24, dorsal view

1 day, 2 hr 15 min pf @ 23°C

Stage 24, lateral view

1 day, 2 hr 15 min pf @ 23°C

Stage 25, dorsal view

1 day, 3 hr 30 min pf @ 23°C

Stage 25, lateral view

1 day, 3 hr 30 min pf @ 23°C

Stage 26, dorsal view

1 day, 5 hr 30 min pf @ 23°C

Stage 26, lateral view

1 day, 5 hr 30 min pf @ 23°C

Stage 27, dorsal view

1 day, 7 hr 15 min pf @ 23°C

Stage 27, lateral view

1 day, 7 hr 15 min pf @ 23°C

Stage 28, dorsal view

1 day, 8 hr 30 min pf @ 23°C

Stage 28, lateral view

1 day, 8 hr 30 min pf @ 23°C

Scale of images shifts at this point

Stage 29-30, lateral view

1 day, 11 hr pf @ 23°C

Stage 31, lateral view

1 day, 13 hr 30 min pf @ 23°C

Stage 32, lateral view

1 day, 16 hr pf @ 23°C

Stage 33-34, lateral view

1 day, 20 hr 30 min pf @ 23°C

Stage 35-36, lateral view

2 days, 2 hr pf @ 23°C

Stage 37-38, lateral view

2 days, 5 hr 30 min pf @ 23°C

Stage 39, lateral view

2 days, 8 hr 30 min pf @ 23°C

Stage 40, lateral view

2 days, 18 hr pf @ 23°C

Stage 41, lateral view

3 days, 4 hr pf @ 23°C

Stage 41, ventral view

3 days, 4 hr pf @ 23°C

Stage 42, lateral view

3 days, 8 hr pf @ 23°C

Stage 42, ventral view

3 days, 8 hr pf @ 23°C

LABORATORY NOTEBOOK

Why use a laboratory notebook?

- To guarantee research results traceability: identification of the date and authorship of research results
- To benefit from the laboratory's expertise and facilitate in-house knowledge transfer

What should be documented in this notebook?

- The title and date of experiments
- The specific question you assess with each experiment
- Specific description of each stage of experiments as they are carried out (preparation of solutions with calculation of volumes, incubation times, temperatures…)
- Measurements taken and conditions in which they are obtained
- Any new (clearly formulated) working hypotheses
- Assessments, interpretations and comments on the obtained results
- Ideas for improving and completing the results
- Reference to any relevant documents which cannot be included in the laboratory notebook (electronic data, data of colleagues…)

WRITE A PAPER

Article Title

It contains the main message of the article.

Summary

limited length (max 2000 characters)

It gives concise objectives and major results. Specify some keywords.

Introduction

It clearly defines the subject in context, without wanting to do an exhaustive review, identifies the questions and objectives of the work.

Material and Methods

Orderly, this section describes the techniques used and their key indices (concentration of antibodies, pharmacological agents). The description should not be too detailed (washing ...) but experiments should be reproducible by others. Do not forget to specify imaging techniques, statistical methods ...

Results cf UE « Neural stem cells and nervous system development »

This section describes the results of experiments. It is organized into parts with explicit titles, linked through transitions asking the questions sequentially. There are two modes of expression results, tables and figures.

Tables: The title of the painting, always placed at the head must be sufficiently detailed to enable an understanding of the table regardless of the text.

Figures: The legend of the figure, usually at the bottom of figure, must be both accurate and sufficiently explicative. It specifies the abbreviation used in the figure.

- For graphics: Prefer horizontal legends, which must be clearly readable on both axes. Specify the significance of the results (* and correspondence in the legend).
- For photographs: orientate, indicate important structures (eg optic vesicle), do not forget the scales, point the important elements (arrowheads) without invading the figure.

Discussion

Reminder of results and interpretations related to the bibliography.

References