



MOLECULAR AND CELLULAR ANALYSIS

essencial toolkit for in vitro cell cultures, gene expression and microscopy

15th May-2nd June 2017

T – Lecture; P – Laboratory class

Week 1: Cell and Tissue Culture: From Basic Principles to Advanced Techniques

Coordinators: António Salgado, Sandra Costa and Luisa Pinto

Goals: Provide a practical and theoretical framework on basic and advanced techniques on cells and tissue culture

Day 1:

09:20-10:30 Presentation, Introduction and Objectives

[Room G2.02] **T1:** Cell and Tissue Culture I: Purpose, Advantages and Applications.

António Salgado, ICVS

T2: Cell and Tissue Culture II: Lab Design, Equipment and Aseptic Technique.

António Salgado, ICVS

11:00-11:45 **T3:** Defined Media and Supplements.

[Room G2.02] António Salgado, ICVS

13:30-17:30 **P1:** Working with Cell Lines – Starting Cultures.

[Lab I2.04] Sofia Serra, Rita Silva - ICVS

18:00-19:30 *STUDY PERIOD*

Day 2:

08:30-09:15 **T4:** An Introduction to Primary Cell Cultures.

[Room G2.02] Sandra Costa, ICVS

09:15-10:00 **T5:** Organotypic Cultures.

[Room G2.02] António Salgado, ICVS

10:00-13:00 **P2:** Isolation and culture of epithelial and endothelial cells from fetal mouse lungs:

[Lab I2.04] Lung cells dissociation, separation and culture

Sandra Costa, ICVS

14:00-17:30 **P3:** Organ Cultures - Dorsal root ganglia explants

[Lab I2.04] António Salgado, ICVS / Rita Silva, ICVS

17:30-19:30 *STUDY PERIOD*



Day 3:

- 10:00-11:00 **T6:** Neuronal cell fate in the adult brain
 [Room G2.02] - Identification of neural stem cells and their anatomical locations
 - Differentiation potential *in vitro* and *in vivo*
 Luisa Pinto, ICVS
- 11:00-12:00 **T7:** The Stem Cell Niche in the adult brain
 [Room G2.02] - The dominant nature of the niche and the control of stem cells homeostasis
 - The role of intrinsic and extrinsic factors
 Luisa Pinto, ICVS
- 13:00- 19:00 Neural Stem Cells in the lab – Luisa Pinto, ICVS / Patricia Patricio, ICVS
 [Lab I2.04] **P4:** Isolation of adult neural stem cells
P5: Preparation and observation of neurospheres cultures
P6: Differentiation protocols and immunocytochemistry for the identification of neurons, astrocytes and oligodendrocytes

Day 4:

- 09:30-12:00 **T8:** Microfluidics - Ramiro Almeida
 [Room G2.02] **P7:** Animal Cell and Tissue Culture: New Trends | hands on - Andreia Carvalho, ICVS
- 14.30-17:30 **P8:** Isolation and culture of epithelial and endothelial cells from fetal mouse lungs:
 [Lab I2.04] Identification of isolated epithelial and endothelial cells by immunofluorescence microscopy
 Sandra Costa, ICVS

Day 5:

- 09:30-12:30 Animal Cell and Tissue Culture: New Trends
 [Room A2.09] **T9:** Bioreactors - Fábio Teixeira, ICVS
T10: Optogenetics - Ana João Rodrigues, ICVS
T11: An introduction to transfection techniques in cell and tissue culture - Andreia Carvalho, ICVS
- 14:00-17:00 *SELF-STUDY*



Week 2: Gene expression with a focus on qPCR

Coordinators: Fernanda Marques, João Sousa

Goal: Provide a practical and theoretical framework on basic aspects of molecular biology, with a focus on gene expression

	22 nd May	23 rd May	24 th May	25 th May	26 th May
Morning	Gene expression analysis; RNA extraction methods; cDNA synthesis strategies	RNA extraction; RNA analysis	qPCR	Self-study (exercises)	Self-study
Afternoon	Primer Design; qPCR normalization and data analysis	cDNA synthesis	Data Analysis	Self-study (exercises)	Self-study

Day 1 – qPCR gene expression analysis: theoretical framework [Room G2.02]

RNA extraction

- Guidelines for the manipulation of RNA
- RNA extraction protocols (alternatives)
- Trizol vs column kits vs others

RNA analysis

- Quantification
- Quality assessment (agarose, bioanalyser) and output analysis

cDNA synthesis

- Different strategies
- Amount of sample needed

Primers

- How to design primers for qPCR

qPCR

- Different Techniques
- Comparison of advantages/disadvantages
- Normalization (housekeeping genes)
- How to report data from qPCR

Day 2 – Hands-on wet lab [Lab I2.04]

- Sample RNA extraction and quality analysis
- cDNA synthesis

Day 3 – Hands-on wet lab [Lab I2.04]

- qPCR preparation;
- primer design exercises;
- data analysis.

Day 4 – Self-study

- exploring other gene expression methods;
- data analysis;
- RNA quality assessment and troubleshooting exercises

Day 5 – Self-study



Week 3: Microscopy in Cellular and Molecular Analysis

Coordinators: Andreia Castro

Goals: Provide a practical and theoretical framework on basic and advanced microscopy techniques in Cellular and Molecular Analysis.

Day 1:

09:30-10:00 **T1:** Light Microscopy

[Room G2.02] Andreia Castro, ICVS

10:00-11:00 **T2:** Principles of Optical and fluorescence microscopy (part 1)

[Room G2.02] Andreia Castro, ICVS

11:30-12:30 **T3:** Principles of Optical and fluorescence microscopy (part 2)

[Room G2.02] Andreia Castro, ICVS

14:00-18:00 **P1:** Exploration of light microscope anatomy, setup Koller illumination, Brighthfield,

[Lab I1β.04] Phase contrast, DIC and Fluorescence.

Goreti Pinto, ICVS / Andreia Castro, ICVS

Day 2:

09:30-11:00 **T4:** Optical section and Confocal microscopy.

[Room G2.02] Andreia Castro, ICVS

11:30-12:30 **T5:** Applications of confocal microscopy.

[Room G2.02] Andreia Castro, ICVS

14:00-18:00 **P2:** Laser Scanning Confocal Microscope setup, image acquisition settings.

[Lab I1β.04] Nuno Vilas Boas, Olympus / Andreia Castro, ICVS / Goreti Pinto, ICVS

Day 3:

09:30-10:30 **TP1:** How to build a microscope.

[Room G2.02] Christian Maibohm, INL.

11:00-12:30 **TP2:** How to build a microscope.

[Room G2.02] Christian Maibohm, INL.

14:30-15:30 **T6:** Super-resolution microscopy.

[Room G2.02] TBA

16:00-18:00 **P3:** Laser Scanning Confocal Microscope- hands-on on applications.

[Lab I1β.04] Nuno Vilas Boas, Olympus / Andreia Castro, ICVS / Goreti Pinto, ICVS

Day 4:

SELF-STUDY

Day 5:

[Room A2.09] *EXAM*

