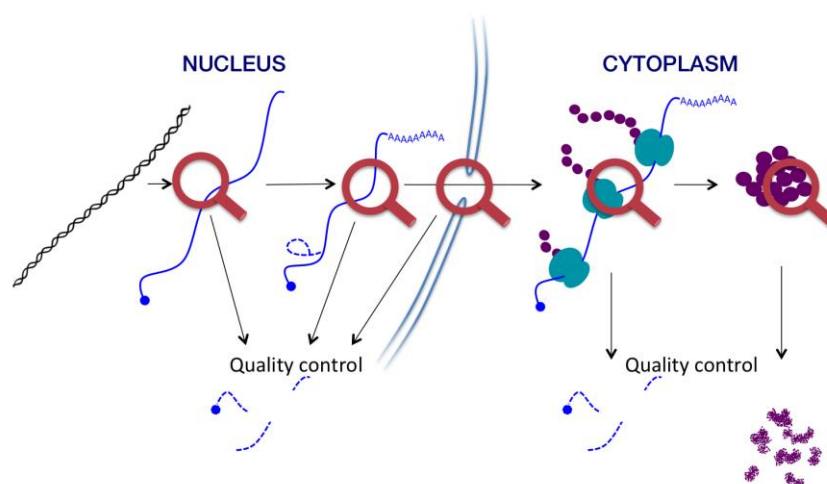


MULTIPLE ROLES OF RNAs

PROGRAM



2024-2025
From February 10th to 21st, 2025

CENTRE D'ENSEIGNEMENT DE L'INSTITUT PASTEUR

28 rue du Docteur Roux
75724 PARIS Cedex 15

DIRECTEUR.RICES DU COURS

Cosmin SAVEANU

Unité de Biologie des ARN des pathogènes fongiques
Institut Pasteur, Paris

Claire TORCHET

Equipe Régulation Fonctionnelle et Surveillance de l'ARN
Institut de Biologie Physico-Chimique, Paris

TECHNICIENNES PEDAGOGIQUES

Isabelle LEQUEUTRE (Technicienne pédagogique)

Angélique GODFIN (Technicienne de laboratoire de préparation)

Centre d'enseignement
Institut Pasteur, Paris

CONFERENCES

Salle 6 - Bâtiment 5 - Sous-sol

TRAVAUX PRATIQUES

Salle TP RdC - Pavillon Louis Martin - Bâtiment 09

« MULTIPLE ROLES OF RNAs » COURSE 2024-2025
(February 10th to 21st 2025)

Scientific programme and supervision: Cosmin Saveanu (IP) and Claire Torchet (SU)

CONFERENCES

- 1 « Nuclear and cytoplasmic RNA degradation ensure robust gene expression in eukaryotes»**

GWENAËL BADIS-BREARD
(ENS, Paris)

- 2 « RNAseq general presentation »**

RACHEL LEGENDRE
(Institut Pasteur, Paris)

- 3 « Differential analysis of RNA-Seq data: design, describe, explore and model »**

STEVENN VOLANT
(Institut Pasteur, Paris)

- 4 « Epitranscriptomic response to antibiotics »**

Zeynep BAHAROGLU
(Institut Pasteur, PARIS)

- 5 « RNAseq : Illumina libraries preparation »**

ODILE SISMEIRO
(Institut Pasteur, Paris)

- 6 « Small RNAs in epigenetic inheritance »**

GERMANO CECERE
(Institut Pasteur, Paris)

- 7 « First step with Unix »**

RACHEL LEGENDRE
(Institut Pasteur, Paris)

- 8 «Analysis of a nucleic acid population using RT-qPCR»**

MATHILDE GARCIA
(IBPS, Sorbonne Université, Paris)

PRACTICAL COURSE

- | | |
|---------------|--|
| EXP. 1 | Extraction of total RNA from WT and mutant yeast strains |
| EXP. 2 | RNA-Seq |
| EXP. 3 | RT-qPCR |
| EXP. 4 | Northern blot |

PLANNING WEEK 1

Supervision during the week: Cosmin Saveanu and Claire Torchet

Practical part: Laurence Decourty, Isabelle Lequeutre, Cosmin Saveanu and Claire Torchet

Monday February 10th 2025

WEEK 1

- 9:00-10:00 Welcome to students **Cosmin SAVEANU & Claire TORCHET** (Co-Directors of the Course)
General presentation of the course
- 10:00-10:30 General presentation of the room and safety rules **Isabelle LEQUEUTRE**
- 10:30-11:30 - **Exp.1** : Culture of yeast strains
- 11:30-12:30 **①** « Nuclear and cytoplasmic RNA degradation ensure robust gene expression in eukaryotes »
Gwenaél BADIS-BREARD
- Lunch -
- 13:30-14:30 **②** « RNAseq, general presentation » **Rachel LEGENDRE**
- 14:30-18:30 - **Exp.1** : OD measurement of yeast strains **Practical Team**
- Extraction of total RNA and measure of the amount of total RNA

Tuesday February 11st 2025

WEEK 1

- 9:00-11:00 **③** PART I « Differential analysis of RNA-Seq data: design, describe, explore and model » **Stevenn VOLANT**
- 11:00-12:00 - **Exp.1** : Preparation of agarose gel and run
- Lunch -
- 13:00-14:30 - **Exp.1** : Picture of agarose gel and analysis
- **Exp.1** : DNase treatment of total RNA and precipitation for the following **Exp.2** and **Exp.3** **Practical Team**
- 14:30-16:00 **④** « RNA modifications and translation fidelity in eukaryotes » **Zeynep BAHAROGLU**
- 16:00-16:30 - **Exp.1** : End of the precipitation for Exp.2 and Exp.3
- 16:30 - **Exp.2** : Bioanalyzer **Odile SISMEIRO**
(AT THE PLATFORM)
- 16:30-17:30 **③** PART II «Differential analysis of RNA-Seq data: design, describe, explore and model » **Stevenn VOLANT**

Wednesday February 12nd 2025**WEEK 1**

9:00-12:30 **5** «Illumina libraries preparation »

Odile SISMEIRO

Bioanalyzer results

- **Exp.2** : PolyA purification
- **Exp.2** : Fragmentation
- **Exp.2** : First strand cDNA
- **Exp.2** : Second strand cDNA

- Lunch -

13:30-18:00 - **Exp.2** : AMPure beads purification
- **Exp.2** : Adenylate 3' ends
- **Exp.2** : Adapters ligation + AMPure beads purification
- **Exp.2** : PCR amplification

Odile SISMEIRO

Thursday February 13rd 2025**WEEK 1**

9:00-11:00 - **Exp.2** : AMPure beads purification
- **Exp.2** : Bioanalyzer.

- **Exp.1** : Analysis

Odile SISMEIRO (AT THE PLATFORM)

11:00-12:00 **6** « small RNAs in epigenetic inheritance»

Germano CECERE

- Lunch -

13:00-15:30 - **Exp.3** : cDNA for RT-qPCR,
- RT inactivation

15:30-17:30 **7** « First Steps with Unix »

Rachel LEGENDRE

Friday February 14th 2025**WEEK 1**

9:00-10:00 *Data analysis : sequence treatment and bioinformatic*

Rachel LEGENDRE

10:00-10:30 **Breakfast**

10:30-13:00 - **Exp.2** : Quality check, aptamers trimming, mapping on the yeast genome **Practical Team**

- Lunch - **Group photo at 13:45**

14:00-16:00 - **Exp.2** : Statistics, graphics and modeling using the R programming language

Cosmin SAVEANU

16:00-18:00 - **Exp.2** : Looking for targets to test by RT-qPCR and Northern blot

Cosmin SAVEANU

PLANNING WEEK 2

Supervision during the week: Cosmin Saveanu and Claire Torchet

Practical part: Laurence Decourty, Mathilde Garcia, Isabelle Lequeutre, Cosmin Saveanu and Claire Torchet

Monday February 17th 2025

WEEK 2

9:00-13:00 **8** - Analysis of a nucleic acid population using qRT-PCR

Mathilde GARCIA

- **Exp.3** : RT-qPCR design and experiment
- **Exp.4** : Northern blot: agarose gel preparation

- Lunch -

14:00-17:00 - **Exp.4** : Northern blot: agarose gel run
- **Exp.4** : RNA probe preparation : transcription reaction

17:00-18:00 - **Exp.4** : Northern blot: transfer

Tuesday February 18th 2025

WEEK 2

9:00-12:00 - **Exp.3** : RT-qPCR data analysis

Mathilde GARCIA

12:00-12:30 - **Exp.4** : Northern pre-hybridization

- Lunch -

13:30-14:00 - **Exp.4** : Northern hybridization

14:00-18:00 - **Exp.2** : RNA seq data analysis and **Exp.3** : RT-qPCR data analysis

Wednesday February 19th 2025

WEEK 2

9:00-12:00 - **Exp.4** : Northern washing
Visit to the Biomix platform by groups of 8 students (Chloé Baum)

Practical Team

- Lunch -

13:00-17h30 - **Exp.4** : Northern signal detection, Northern analysis with Image J

Thursday February 20th 2025

WEEK 2

9:30-12:30 - Data analysis and preparation of the oral presentation

*Cosmin SAVEANU
Claire TORCHET*

- Lunch -

13:30-17:30 - Data analysis and preparation of the oral presentation

*Cosmin SAVEANU
Claire TORCHET*

9:00-12:30 - Data analysis and preparation of the oral presentation

Cosmin SAVEANU
Claire TORCHET

- Lunch -

13:30-17:00 - **Oral presentation:** Salle 1 RdC – Pavillon Louis Martin – Bâtiment 09

17:00 **Farewell party**

ORGANISATION OF THE EXAMINATIONS

Oral examinations (40/100)

Continuous student assessment is based on the participation during the practical course and the quality of the work at the bench.

- Oral examination

Friday February 21st 2025, presentation by groups of 3 or 4 students, of the results and conclusions of the different experiments obtained during the practical course.

- Slides (Powerpoint, LibreOffice or pdf file)

- The presentation should last 20 minutes, followed by 20 minutes for questions

Each student must present at least an aim of an experiment, a technical aspect, including data analysis and an obtained result, together with a critical opinion on these methods and data. A selection of the results, instead of presenting every one of them, is preferred.

Written examinations (60/100)

A report of the practical course

The report should be structured in the form of a **scientific paper**, with careful illustration of the main results and with the following sections:

- **Introduction**, stating the problem and the questions in the context of previous work and general knowledge.
- **Results**, briefly including the principle of the method and how the results were obtained and analysed with a critical discussion of the problems, difficulties, issues or variability observed.
- **Discussion/Conclusion** in which the merits of the different methods and their limitations are critically discussed. Also, possible further experiments, or even general ideas about RNA degradation pathways can be included.

There is no need for a detailed *Materials and methods* section. The basis of each experiment should be mentioned together with its main aim.

Maximum 12 pages (front side only). Times 12.

The scientific convention is to use present tense for previously published results and general principles and the past tense for any of the results obtained during this course.

NO LATER THAN MARCH 3rd 2025