Lecture schedule for the course

"MOLECULAR TECHNIQUES OF RNA ANALYSIS".

winter semester 2023-24r.

(Friday,4:00 p.m., Friday, 4:00 p.m., Room 7, IBB PAN building)

1. (06.10) *"The RNA world"* part 1 - Joanna Kufel, IGiB UW.
The concept of "The RNA world", Nobel prizes in the field of RNA. Catalytic RNA molecules - classes, mechanism, occurrence. SELEX. Remnants of the RNA world - ribosome, spliceosome, RNA viruses. Diversity of RNA classes in Eukaryota and their metabolism. Basic mechanisms of regulation of gene expression.

2. (13.10) "*The world of RNA*" part 2 - Joanna Kufel, IGiB UW.

3. (20.10) *"Overview of reverse transcription-based techniques. Transcription techniques: TRO, ChIP, RIP and DIP. Analysis of polyA RNA ends"* - Michał Koper, IGiB UW.
Reverse transcription (RT) – theory. Overview of RT-based techniques: RT-PCR including semi-quantitative, pulsed RT-PCR, RACE, cRT-PCR, primer extension technique ("primer extension"). Techniques for studying nascent transcripts: nuclear "Transcription Run-On", chromatin immunoprecipitation (ChIP), RNA immunoprecipitation (RiP). RNA Polymerase I transcription termination studies will be used to illustrate the above techniques. Specificity of DNA binding by transcription factors: "ChIP on chip", DNA immunoprecipitation (DIP). Study of polyA RNA ends (PASE technique, isolation of polyA$^+$ RNA fractions).

4. (27.10) *"Real-time PCR (quantitative PCR - qPCR)"* - Michał Koper, IGiB UW.
Theory of qPCR: methods of DNA detection, basics of primer design, hybridization probes, introduction to computation. Applications: determining the level of transcripts under investigation in a cell, detection of nucleic acids of pathogens, detection of single mutations (SNPs). Good laboratory practice for qPCR experiments and the most common "pitfalls" waiting for experimenters.

5. (10.11) *"The RNA world"* part 3 - Joanna Kufel, IGiB UW.

6. (17.11) *"non-coding RNAs"* - Monika Zakrzewska-Płaczek, IGiB UW.
Non-coding RNAs (ncRNAs). Biogenesis and functions of small and long non-coding RNAs. Mechanisms of transcriptional silencing dependent on non-coding RNAs. RNP complexes involved in transcriptional silencing. The role of chromatin in the regulation of gene expression.

7. (24.11) *"Global analysis of ribonucleoprotein complexes"* part 1 - Joanna Kufel, IGiB UW.
Biochemical methods for cleaning ribonucleoprotein complexes (RNPs). Yeast three-hybrid system. RNA chromatography combined with mass spectroscopy. CRAC ("crosslinking and analysis of cRNA") and CLIP ("crosslinking and immunoprecipitation").

8. (01.12) *"RNA metabolism in physiological processes*" - Anna Golisz, IGiB UW.
The role of RNA processes and ncRNAs in cell metabolism in plants and animals: hormone signaling, embryonic and generative development, circadian clock, resistance to stress and pathogens.

9. (08.12) *"RNA in neurons".* - Magdalena Dziembowska, WB UW.
mRNA transport in dendrites and axons, local translation in response to synaptic stimulation. Methods of mRNA visualization in living neuron in real time, *in situ* hybridization, biochemical methods for direct detection of transcripts undergoing local translation in neurons

10. (15.12) *"Methods for RNA structural studies".* - Marcin Nowotny, IICMB.
Methods of studying the structures of RNA-protein complexes, methods of crystallization of RNP complexes (using ribosome, snRNP, RNase H as an example). Comparison of crystallography and NMR studies for RNA. SAXS technique. Chemical (SHAPE) and bioinformatics prediction of RNA structures. Cryo EM.

11. (12.01) *"Study of RNA metabolism enzymes on the example of the exosome*" - Rafal Tomecki, IGiB UW/IBB PAN.
RNA degradation pathways in eukaryotic organisms. Exo- and endo- nucleases. The exosome: a multifunctional and multicomponent complex. Structural and functional studies of the exosome in yeast and in human cells. Mechanism of action, cooperation of exo- and endo-nucleolytic activity.

12.(19.01) *"RNA structure vs. function. Mapping RNA structure in vitro. Methods for studying transcriptomes*" - Michał Koper, IGiB UW.
Mapping RNA structure *in vitro*: molecular probes that digest specifically against RNA structure and sequence. RNA switches and aptamers - natural and selected for drugs or biosensors. High-throughput methods for studying transcriptomes based on next-generation sequencing techniques (RNA-seq, ChIP-seq).

13.(26.01) *"Global analysis of ribonucleoprotein complexes"* part 2 - *Joanna Kufel, IGiB UW.*

Schedule of the practical classes for the course

"MOLECULAR TECHNIQUES FOR RNA ANALYSIS".

winter semester 2023-24r.

(group 1 Mondays, 10:15 a.m. to 1:30 p.m.; group 2 Thursdays, 9:30 a.m. to 12:45 p.m. - room 129 IGiB UW)

1. 05 and 09.10. Basics of working with RNA. Isolation of RNA from yeast and from plants.

2. 12 and 16.10. RNA quality assessment. RNA electrophoresis. Radioisotope and fluorescent methods of RNA detection. miRNA detection in plants - introduction. miRNA detection in plants, Northern-blot technique (1) with RNA separation in polyacrylamide gel.

3. 19 and 23.10. Northern-blot technique (1): hybridization with a biotin-labeled oligonucleotide probe. Washing, scanning and analysis of results. Detection of CUT transcripts in yeast: Northern-blot technique (2) with RNA separation in agarose gel.

4. 26 and 30.10. Detection of CUT in yeast: radioactive probe labeling by "asymmetric PCR" and hybridization. Analysis of scientific images.

5. 06 and 09.11. Detection of CUT in yeast: discussion of Northern-blot hybridization results (2). Analysis of the 3' ends of small nucleolar RNAs (snoRNAs) by cRT-PCR. Digestion of RNA-oligo nucleotide duplexes with RNase H and ligation (circularization) of RNA.

6. 13 and 16.11. Analysis of the 3' ends of the snoRNA: RT + PCR.

7. 20 and 23.11. cRT-PCR: separation of products in the gel and analysis of results. mRNA detection by RT-qPCR. Designing primers for qPCR - theory and practice. “Competition” for the most efficient pair of starters, i.e. each student will design at least 1 pair of starters, which will be ordered and tested during the next class.

8. 27 and 30.11. Performing the qPCR reaction: testing the designed primers for chosen genes.

9. 04 and 07.12. Analysis of qPCR reaction results.

10. 11 and 14.12. Exploratory analysis of transcriptomic and translatomic data from RNA-seq experiments – bioinformatics. Introduction.

11. 18 and 21.12. Exploratory analysis of transcriptomic and translatomic data from RNA-seq experiments - bioinformatics. Practical part.

12. 08 and 11.01 EMSA. Interactions of proteins and nucleic acids. Binding of yeast Nsi1 protein to rDNA. Fluorescent "gel-shift" (incubation of samples, separation in native polyacrylamide gel, scan).

13. 15 and 18.01. Biochemical determinations of the activity of RNA degrading enzymes. Analysis of the ribonucleolytic activities of different versions of the Dis3 protein - the main catalytic subunit of the exosome complex; testing the sensitivity of the 5'-3' exoribonucleolytic activity of the Xrn1 protein to the phosphorylation status of the 5' end of the substrate; degradation reactions of fluorescently labeled synthetic RNA oligonucleotides, separation of reaction products in a denaturing polyacrylamide gel.

14. 22 and 25.01. Students’ presentations to pass the practical part of the course.

Rules for passing the course

Practical classes: the grade consists of points obtained for student presentations on scientific publications in which RNA techniques were used (weight in the final grade - 2/3) and points obtained from 4 home works (weight in the final grade - 1/3), Entire subject: written exam (test questions).

Course coordinator:

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