VANDERBILT UNIVERSITY

Jens Meiler, Ph.D.
Professor & Stevenson Chair
Vanderbilt University

Syllabus – CPBP 8330 – Special Topics in Protein Biochemistry

NEW APPROACHES FOR PRACTICAL AND TIME EFFICIENT CLONING, CONSTRUCT DESIGN AND THE STRATEGIC SELECTION OF PROTEIN-PROTEIN INTERACTION TECHNIQUES.

Course Description:

Research techniques are always changing, they develop and improve and sometimes it is hard to stay fully informed. In this module we will talk about different methods and techniques you want to use to be most efficient in your project.

In the first half of the module we will introduce you to the basics and the latest cloning techniques. First, we will talk about vector design and what to consider for your perfect plasmid for cell culture experiments or protein expression. Second, we will show you where to get your plasmids and how to modify them by e.g. introducing tags or mutations. This will be accompanied with a practical part where we will compare the different techniques and strategies in a lab setting.

In the second half of the module we will talk about different techniques to study protein-protein interactions. These interactions can be very versatile and not every biophysical method can be applied for every system. Therefore, we will compare advantages and disadvantages of different techniques which are available at Vanderbilt like NMR spectroscopy, X-ray crystallography, MST (microscale thermophoresis), SPR (surface plasmon resonance), ITC (isothermal titration calorimetry) and fluorescence spectroscopy. We will address for example: which technique to use for a weak or strong interaction; what to do when you get different affinities with different techniques; what to do when you want to characterize a membrane protein or an unstable protein. In the final part of the module, we will introduce *in-cell* assays which are helpful to verify your *in-vitro* findings for your protein-protein interactions.

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Date	Program
09-04-2019	Introduction to cloning: Theory and techniques (Cut/Ligation, Gibson Assembly, Recombineering), cloning vectors, promoters, protein tags, transfection methods, viral transduction, insert verification
	Practical part: Students design their own cloning approach (group work)
09-05-2019	Practical part: In the morning, set up of PCR reactions for Quickchange or Gibson and in the afternoon, analysis of the PCR products by agarose gel electrophoresis, clean-up and restriction cut.
	Theory in-between: Discussion of cloning strategy, approaches to mutate, Strategies to analyse proper protein expression and location (ELISA, IHC)
09-06-2019	Protein-protein interactions: Comparison of NMR spectroscopy and X-ray crystallography – high affinity vs low affinity systems.
	Practical part: Clean-up of restriction cuts, transformation into bacteria
09-09-2019	Protein-protein interaction techniques: MST (microscale thermophoresis), SPR (surface plasmon resonance), ITC (isothermal titration calorimetry) and fluorescence spectroscopy – when to use what technique and how to troubleshoot.
	Practical part: Clone picking, Colony PCR, mini-culture over night
09-10-2019	*In vitro* and *in cell* read-out techniques: expression, signal transduction (eukaryotes and yeast), FRET, BRET, live cell imaging
	Practical part: Mini prep and sequencing setup
	Multiple choice test

Coordinator:

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Instructors:

Dr. Dr. Ines Liebscher
Leipzig University, Medical Faculty
Institute of Biochemistry

Dr. Sandra Berndt
Vanderbilt University
Department of Pharmacology

Date and Time:

The class will meet on 5 days in the week of September 4th always 9am-12pm and 1-4pm Additionally, relevant, recent literature will be assigned for reading. Additionally, students will develop a cloning strategy for a protein of their interest. This is a one credit course.

Room:

5131 MRBIII

Registration:

Students who wish to take the class for credit should register as soon as possible! Students, postdocs, and faculty who wish to audit the class are welcome. Please send a note to Jens Meiler (jens.meiler@vanderbilt.edu).

Grading:

There will be a multiple choice exam (50%) at the end of the course and the cloning strategy developed as a class assignment will be graded (50%).

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