# Methods in Biochemistry (KB7014)

**start at 9 am on Monday September 2, end on Wednesday October 2.**

**AIM**

'Methods in Biochemistry' will introduce students to the fundamental principles behind several basic and more advanced techniques commonly used in biochemical research. During the course, the strengths and weaknesses of each technique will be covered to explain which techniques are suitable for particular applications. Upon establishing this foundation, the students will be taught how to combine different methods to overcome problems associated with the individual techniques. The theoretical aspect of the course will be supplemented with a lab practical where the students will apply a number of these techniques themselves. In the end, the students should be able to design strategies to address complex biological questions using the techniques they have been exposed to from a theoretical and practical perspective.

**Upon completion of the course you should be able to:**

- Describe the principles behind a number of common biochemical techniques.
- Explain the strengths and weaknesses of a technique for particular applications.
- Combine different biochemical methods to address a complex biological question.
- Troubleshoot biochemical methods based on their scientific principles.
- Analyze generated data and communicate them in writing and orally.
- Read, communicate and critically evaluate course-related scientific literature.

**You will be expected to:**

- Attend and actively participate in the lectures, practicals and tutorials.
- Participate in discussions with other students and the faculty.
- Read the assigned literature and complete the assignments and lab reports on time.
- Present the lab practical results in writing and make the appropriate corrections.

**COURSE CONTENT**

**-Cell homogenization and fractionation** The organization and chemical composition of pro- and eukaryotic cells will be discussed in relationship to different homogenization methods, which are used to enrich for particular fractions from these cells.
-Materials on how to make buffers and solutions (incl. sterilization), pH, the metric system, statistics and making lab. notes are provided at the beginning of the course.

**-'History of molecular biology'** Students will be taught the history behind the main organisms used for molecular biology and how these are handled, genotyped, cultured and stored.

**-Centrifugation** The theoretical basis for multiple centrifugation techniques will be explained as well as their applications. These include: differential centrifugation, density gradient centrifugation and analytical ultracentrifugation.

**-Recombinant DNA techniques** Several modern DNA analysis methods will be covered ranging from DNA isolation and sequencing to PCR and a variety of molecular biology techniques for DNA manipulation. Significant time will be spent on: restriction enzyme digests and other reactions that modify DNA, PCR, primer design, sequencing methods, homology cloning, Gibson cloning, recombineering, CRISPR-based genome editing, forwarded evolution etc.

**-Protein production** Several different eukaryotic and prokaryotic protein production platforms are available. These will be thoroughly explained from both a theoretical and need perspective based

on the current protein production bottlenecks. Specifically, we will address how to choose an organism, the expression system, and the design of the target gene.

**-Protein isolation using physiochemical properties** The biochemical principles for a number of basic protein purification techniques will be covered. This will include the buffer systems and resins that support: affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, and gel-filtration.

**-Antibodies and their applications** We will discuss the factors to consider for generating a polyclonal/monoclonal antibody, alternative binding approaches that can replace antibodies, and several applications such as: immuno-blotting, immuno-precipitations, and ELISAs.

**-Mass spectrometry** Basics of mass spectrometry, MALDI-TOF, MS/MS (incl. sequencing proteins), quantitative proteomics (*e.g.*, iTRAQ, iCAT, spectral counting), characterization of post-translational modifications using MS etc.

**-Gel-electrophoresis and detection methods** Different gel-electrophoresis techniques for DNA and protein will be explained as well as a number of methods for staining and detection.

**-Introduction to Python programming for biochemists** This one-day module comprises lectures and hands-on exercises that provides an introduction to the Python programming language for biochemists. We will cover programming basics, flow of a python program, operators, loops, functions, dictionaries, classes, visualization, plotting and data analysis, modeling biochemical kinetics, numpy, scipy, matplotlib, pandas, jupyter notebooks.

**Lectures and tutorials** All the lectures are linked to tutorials, which are a significant part of the course. For the tutorials the students get assignments ('homework'), *i.e.*, they have to answer questions based on the lectures and articles they have to read. The assignments are handed in, checked (feedback) and graded before the tutorial (-: fail, +: good, ++: very good). In case the tutorial is graded with a 'fail', the tutorial has to be corrected until it is graded with at least a 'good'. To be able to finish the course all tutorials should have been graded with a 'good' or 'very good'. There is one 'special' tutorial: the cloning tutorial. In the cloning tutorial the students design constructs (primer design, plasmid design, they learn how to use different computer programs to facilitate this).

**Practical**.
**5 people max per group. Bring your own lab coat!**
Group A: 5 people max
Group B: 5 people max
Group C: 5 people max

**Block 1**
Day 1 (0.5 Day): Transformations + Setting up a PCR reaction + Setting up a DNA sequencing reaction (spectra will be mailed to you when they become available). Set up an ON culture for plasmid isolation (colonies will be taken from a prepared plate).
Day 2 (0.5 Day): Pour a DNA gel (we will do this for you, and show you how it is done) and analyze the PCR reaction. Assess the transformations. Plasmid isolation and put the isolated plasmid DNA on gel (load the plasmid DNA and PCR reactions at the same time!).
**Block 2**
Day 1 (0.5 Day*): Determine the concentration of the LacZ sample handed out using the BCA assay. Make a dilution series for a calibration curve on a gel and run a gel with dilutions of a total cell lysate of cell producing LacZ. Stain gel ON in Coomassie. Set up culture using a premade ON culture. Monitor growth, split the culture in two and induce recombinant protein production in one half (the other half is a control).

Day 2 (0.5 Day*): Destain the gel and scan it. Determine the amount of LacZ produced by the cells. Monitor growth of cells producing/not producing the recombinant protein.
*Some students will do this practical during one day.
Day 3 (0.5 Day): Help with processing the data of blocks 1 + 2. For all three groups at the same time if help is needed.

The students write a lab. report covering block 1 and block 2 that is corrected (until the level of the reports is acceptable). Instructions for writing the report will be given on Thursday 1/9 in the afternoon.

**Assessment**
Written open book home exam, and the student have to complete all tutorials and lab. reports on time.

**Final mark:**
-Exam 100% (at least 50% of the points are needed).
-Tutorials should have been handed in and either be '+' or '++' plus the lab report should be done (given that it has been handed in on time)

**Practical matters**

**There will be no more than 15 students.**

**Setup**
There is an Athena link where we will put all course materials: **https://athena.itslearning.com** (if you are registered as a student at SU, you should have access to this link). I will send you an Email when new materials have been uploaded. Importantly, I have pre-recorded parts of some of the lectures. You send your tutorial answers to me by Email (degier@dbb.su.se), label your Email and file with answers with your name and the topic of the tutorial). I will send you information/updates using Email. I have made a recurring Zoom link (see below). The first day I will give a general introduction and we will go through different methods to break cells. The remaining days of the week we will go through methods in molecular biology (mainly DNA oriented).

**Zoom link for the course (in case we need extra lectures/time/focused meeting etc.):**
https://stockholmuniversity.zoom.us/j/61163789321
In case you cannot make it contact through Zoom send an Email to degier@dbb.su.se.

**The teachers:**
-JWdG: Jan-Willem de Gier (degier@dbb.su.se)
-Liselotte Antonsson (liselotte.antonsson@dbb.su.se) (safety intro)
-Ville Kaila (ville.kaila@dbb.su.se) (Python)
-Renuka Kudva (host (+lecturer) of the SciLifeLab visit)

**Tutorials:**
The students have to hand in the answers before the tutorials and they have to attend the tutorials. The teachers have a look at the answers before the tutorial. **A mark will be given; - (not ok),+ (ok),++ (good). The students have to hand in the answers two days (48 hrs) before the tutorial** (send your answers to me by Email as described above)**. Answers that are handed in too late won't be considered.**

**PRACTICAL:**
Room A373 (wet labs) and K343 (Python)

**Assistants:**
Wet labs: Olivia Anden (olivia.anden@dbb.su.se) & Urska Kasnik (urska.kasnik@dbb.su.se)
Python lab: Hyunho Kim (hyunho.kim@dbb.su.se)

**Access Card:**
KÖL will prepare access cards that will give you access to both the lecture rooms at KÖL and the lecture rooms and lab. for the practicals at the department.

**Book + course materials:**
Principles and Techniques of Biochemistry and Molecular Biology (8th edtion). Edited by Andreas Hofmannn and Samuel Clokie (Cambridge University Press). Hand-outs, articles etc. will be provided.

**Exam:**
Open book/home exam during the last 3 days of the course.

**Jan-Willem de Gier**
**Department of Biochemistry and Biophysics**
**Stockholm University**
**S-106 91, Stockholm**
**Sweden**
**tel: +46-8-162420 (office)**
**Email: degier@dbb.su.se**

**Some golden rules:**
**-Be on time (9 o'clock we start).**
**-Lectures, tutorials and the practicals are compulsory: i.e., you must attend them. Not attending seminars (lectures) and tutorials, and not handing in the assignments before the deadline will result in being excluded from doing the exam. If there is a problem you contact Jan-Willem de Gier (degier@dbb.su.se). Attendance of seminars (lectures), tutorial and the practical will be monitored using lists that have to be signed.**
**-If the score of a tutorial was '-' a corrected version of the assignement has to be handed in within 2 days after the tutorial. All tutorials should be '+' or '++' to be able to do the exam.**
**-Lab reports handed in after the deadlines won't be considered. No lab reports means no final mark.**
**-All the students should have signed the SU papers stating what SU considers plagiarism and what happens if plagiarism is detected before they start with KB7014. If plagiarism is detected in first versions of lab reports and assignments this will also be reported to the university.**
**-Students make their own assignments and lab. report (this of course should not prevent students from having discussions etc.).**

# Schedule

| **WEEK 36** | |
|---|---|
| **Morning (9:00-12:00)** | **Afternoon (13:00 ->)** |
| **2/9 Start/cell disruption K433-K439** | **Molecular biology I K441-K447 + Mingle/Fika 2:30 clock** |
| **3/9 Molecular biology II K433** | **study time** |
| **4/9 Molecular biology III K433-K439** | **study time** |
| **5/9 Molecular biology IV K317** | **Introductions cloning tutorial/Practical/Protein production I K438** |
| **6/9 study time** | **study time** |

| **WEEK 37** | |
|---|---|
| **9/9 Safety intro (9:30)/Protein production II K433** | **Group A block 1/hand in mol. biology tutorial (+ calculations)/study time** |

**Please, sign the 'safety form' and send it: Liselotte.Antonsson@dbb.su.se (mention your name and KB7014 in the subject)**

| | |
|---|---|
| **10/9 Protein production III K433-K439** | **Group A block 1/study time** |
| **11/9 Centrifugation K433-K439** | **Group B block 1/study time** |
| **12/9 Protein isolation I K317** | **Group B block 1/hand in cloning tutorial/study time** |
| **13/9 Mol Biology tutorial A515** | **study time** |

| **WEEK 38** | |
|---|---|
| **16/9 Cloning tutorial K433-K439** | **Group C block 1/hand in protein production tutorial/ study time** |
| **17/9 Protein isolation II K441-K447** | **Group C block 1/study time** |
| **18/9 Mass spectrometry K441** | **Group A block 2/study time** |
| **19/9 Antibody based techniques K438** | **Group A block 2/study time** |
| **20/9 Group B block 2/study time** | **Group B block 2/study time** |

| **WEEK 39** | |
|---|---|
| **23/9 Group C block 2/study time** | **Group C block 2/study time** |
| **24/9 Groups A, B, C block 2 + other questions K433-K439** | **Recap Prot sol/MS/Ab lectures K438** |
| **25/9 10:00 SciLifeLab NGS*** | **Groups A, B, C block 2 + other questions K433-K439** |
| **26/9 Python K438** | **Python (Practical) K343** |
| **27/9 study time hand in lab reports** | |

| **Week 40** | |
|---|---|
| **30/9 Test** | **Test** |
| **1/10 Test** | **Test** |
| **2/10 Test** | **Test** |

***SciLifeLab: Tomtebodavägen 23A.**