Laboratory Course in Biosystems and Biomaterials engineering

CHEM-E8110

Period I & II

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General course overview

- The course is tailored for students of Biosystems and Biomaterials engineering major

  The theory for some of the topics that we experimentally address in this course is covered in detail in the Cell Biology course (CHEM-E8120) running in period II

- Timetable is optimized for major students!

- Experimental work is carried out in pairs, except starting days

- The course will be run in two separate groups (A & B)

Important personal

- In lab
  - Part I: Laura Niemelä
  - Part II: Karoliina Elfving and Mengjie Shen
  - Part III: Laura Niemelä and An Nguyen
- Virtual lab
  - Samuel Girmay
- Lectures
  - University lecturer, Heli Viskari (Part I)
  - Postdoc, Salla Koskela (Part III)
- Academy Research Fellow, Rahul Mangayil
  - General course organization, lectures (Part II) and lab backup

Group selection

- Do visit the Mycourse page and select the group
  - For Period I, the page will be opened from 26th – 27th September until 23.59.
  - For Period II, the page will be opened closer to the date. Will keep you updated.

Period I: Learning outcomes

- Practice accurate pipetting (Dilution series)
- Conducting an enzyme activity assay
  - Prepare standard curve
  - How to calculate enzyme catalysis turnover & turnover rate using the prepared standard curve
- Practice working with microbes
  - Working in sterile bench
  - Microbe handling
    - Will be working with
      - » *Saccharomyces cerevisiae*
      - » *Bacillus subtilis*
  - Streak plating

Period I: Schedule

Although grouped up, the introductory experimental works are conducted individually

Timetable - Part I (Introductory experiments)				
	Date	**Time**	**Group A**	**Group B**
Week 39	26.09 (Tuesday)	10:15 - 12:00	**Kick-off lecture: Course introduction**	
	29.09 (Friday)	10:15 - 12:45	Enzyme kinetics: Acid Phosphatase	No lab work
		13:30 - 16:00	No lab work	Enzyme kinetics: Acid Phosphatase
Week 40	03.10 (Tuesday)	10:15 - 12:00	**Lecture: Microbial genetics (Postdoc Salla Koskela)**	
	06.10 (Friday)	10:15 - 17:00	Yeast crossing & Natural transformation in Bacillus	
Week 41	10.10 (Tuesday)	10:15 - 12:00	**Lecture: Introduction to BioBricks (University Lecturer Heli Viskari)**	
	13.10 (Friday)	10:15 - 17:00		Yeast crossing & Natural transformation in Bacillus

Lecture room for 29.09, 03.10 & 10.10: Ke5 - D311

Period II

- Comprises of Parts II & III of the lab course

- Experimental work is carried out in pairs.

- Timetables for the groups take into account the 1st year courses of the different tracks.
  - Group A: Students of the **Biosystems track**
    - Fridays not used, except Friday December 1st, 12:15-14:00 for final discussion.
  - Group B: Students of the **Biomaterials track**
    - Mondays are not used.
  - Students of the **Chemistry of Life track** can select group A or B. There will be an overlap with one course.

Period II- Part II: Learning outcomes

- Introduction to standardized gene assembly technique: BioBricks
- Explore the potential of BioBricks and how the use of standardized building blocks revolutionized genetic engineering
- Introduce to basic stages involved in cloning
  - Plasmid isolation, restriction
  - Agarose gel run & DNA purification
  - Competent cell preparation
  - Ligation & transformation
  - Colony PCR
  - Recombinant protein expression and analytics

Part II: Learning outcomes

- Use these techniques to assemble of a genetic construct comprising a promoter and fluorescent reporter protein.

- Explore the potential of BioBricks and how the use of standardized building blocks revolutionized genetic engineering

Part II: Schedule

Timetable - Part II (BioBrick cloning, recombinant protein expression and analytics)						
	Date	**Time**	**Day**	**Group A**	**Day**	**Group B**
Week 43	23.10 (Monday)	10:15 - 17:00	1	Plasmid isolation & restriction Agarose gel preparation & run DNA excision and purification		No lab work
	24.10 (Tuesday)	08:15 - 10:00		**Lecture: Molecular cloning techniques**		
	25.10 (Wednesday)	10:15 - 17:00	2	Competant cell preparation Setting ligation reaction & Ligation Transformation & streak plating		
	26.10 (Thursday)	10:15 - 17:00		No lab work	1	Plasmid isolation & restriction Agarose gel preparation & run DNA excision and purification
	27.10 (Friday)	10:15 - 17:00		No lab work	2	Competant cell preparation Setting ligation reaction & Ligation Transformation & streak plating

Lecture room for 24.10: Ke4 - C301

Note I: not all of the reserved times will be used.

Note II: The tentative timetable for period II is available, but small changes might still be needed

Timetable - Part II (BioBrick cloning, recombinant protein expression and analytics)						
	Date	**Time**	**Day**	**Group A**	**Day**	**Group B**
Week 44	30.10 (Monday)	10:15 - 17:00	3	Transformant screening • Colony PCR and agarose gel • Restreaking positive clones		No lab work
	31.10 (Tuesday)	08:15 - 10:00		**Lecture: Mining of genome sequences, primer design and DNA sequencing**		
	01.11 (Wednesday)	10:15 - 17:00	4	Inoculation of positive clones for recombinant protein expression test Note: 1 hour work. Do agree the exact time with the teaching assistant		No lab work
	02.11 (Thursday)	10:15 - 17:00	5	Expression test in microtiter plates • Setup the experiment to follow the growth and fluorescence data using Cytation		No lab work
	03.11 (Friday)	10:15 - 17:00		No lab work		No lab work

Lecture room for 31.10: Ke4 - C301

Timetable - Part II (BioBrick cloning, recombinant protein expression and analytics)						
	Date	**Time**	**Day**	**Group A**	**Day**	**Group B**
Week 45	6.11 (Monday)	10:15 - 17:00		No lab work		No lab work
	07.11 (Tuesday)	08:15 - 10:00		**Instructions on the written report** **Lecture: Yeast as an expression system (Postdoc Salla Koskela)**		
	08.11 (Wednesday)	10:15 - 17:00		No lab work	3	Transformant screening • Colony PCR and agarose gel • Restreaking positive clones
	09.11 (Thursday)	10:15 - 17:00			4	Inoculation of positive clones for recombinant protein expression test Note: 1 hour work. Do agree the exact time with the teaching assistant
	10.11 (Friday)	10:15 - 17:00		No lab work	5	Expression test in microtiter plates • Setup the experiment to follow the growth and fluorescence data using Cytation

Lecture room for 07.11: Ke4 - C301

Part III: Learning outcomes

- Learn about yeast as an expression system
  - Analyze if and how *Saccharomyces cerevisiae* can be genetically modified to enable production of recombinant proteins

- Learn how specific aspects of cellular production systems can be tested and targeted for improvements.

- You will use different analytical methods (SDS-PAGE, ELISA; Immunoblotting) in the characterization

Part III: Schedule

Timetable - Part III (Recombinant antibody production from yeast and analytics)						
	Date	**Time**		**Group A**		**Group B**
Week 46	13.11 (Monday)	10:15 - 17:00	Day	No lab work	Day	No lab work
	14.11 (Tuesday)	08:15 - 10:00		**No Lecture**		
			1	• Preculture inoculation (1 group member; Morning) Note: 1 hour work. Agree the exact time with the teaching assistant		
	15.11 (Wednesday)	10:15 - 17:00	2	• Inoculation of experimental cultures Note: 1 hour work. Do agree the exact time with the teaching assistant		No lab work
	16.11 (Thursday)	10:15 - 17:00	3	Cell culture harvest Sample preparation for analysis ELISA using the supernatent Extraction of total cellular protein Preparing SDS-PAGE gels		No lab work
	17.11 (Friday)	10:15 - 17:00		No lab work		No lab work

Timetable - Part III (Recombinant antibody production from yeast and analytics)						
	Date	**Time**		**Group A**		**Group B**
Week 47	20.11 (Monday)	10:15 - 17:00		No lab work		No lab work
	21.11 (Tuesday)	08:15 - 10:00		**Lecture: Immunological methods (Postdoc Salla Koskela)**		
	22.11 (Wednesday)	10:15 - 17:00	4	SDS-PAGE run Transfer protein to nitrocellulose membrane Prepare coat plates for ELISA	1	• Preculture (1 group member) Note: 1 hour work. Agree the exact time with the teaching assistant
	23.11 (Thursday)	10:15 - 17:00	5	Immunoblotting ELISA	2	• Inoculation of experimental cultures (afternoon) Note: 1 hour work. Agree the exact time with the teaching assistant
	24.11 (Friday)	10:15 - 17:00		No lab work	3	Cell culture harvest Sample preparation for analysis ELISA using the supernatent Extraction of total cellular protein Preparing SDS-PAGE gels

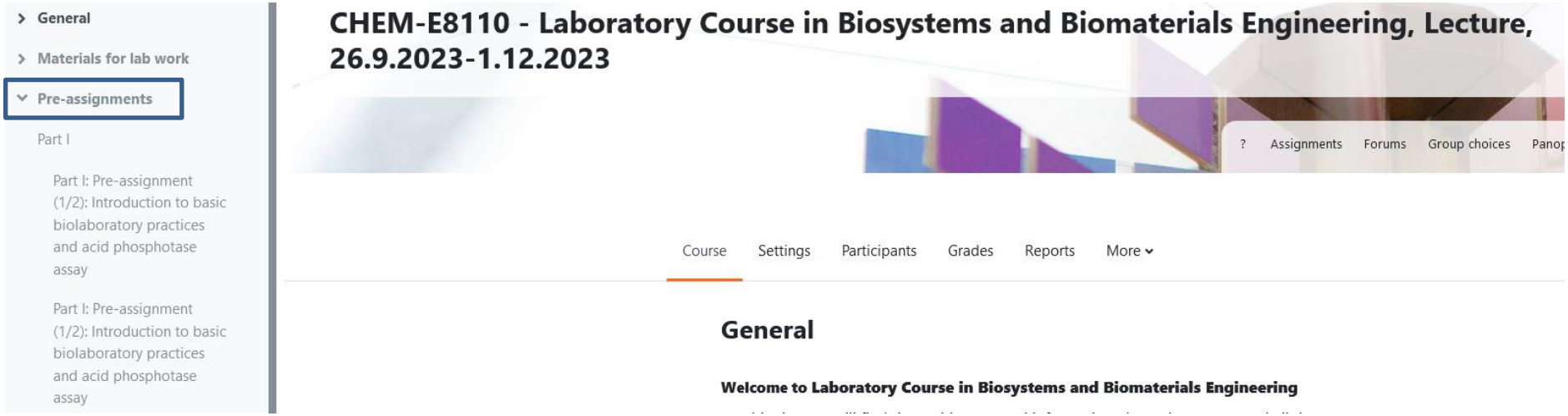
Lecture room for 21.11: Ke4 - C301

Timetable - Part III (Recombinant antibody production from yeast and analytics)						
	Date	**Time**		**Group A**		**Group B**
Week 48	27.11 (Monday)	10:15 - 17:00		No lab work		Not available
	28.11 (Tuesday)	08:15 - 10:00		**No lecture**		
	29.11 (Wednesday)	10:15 - 17:00		No lab work	4	SDS-PAGE run Transfer protein to nitrocellulose membrane Prepare coat plates for ELISA
	30.11 (Thursday)	10:15 - 17:00		No lab work	5	Immunoblotting ELISA
	01.12 (Friday)	10:15 - 17:00		Final discussion		

Lecture room for final discussion: Will be updated later

Grade requirements for credit points – active participation and completion of the experimental work (40%)

- Regular and active attendance in the course work is a must for receiving credit points
  - Completed pre-assignments
    - Virtual lab assignments (+feedback)
    - Written pre-assignments (for Part II)



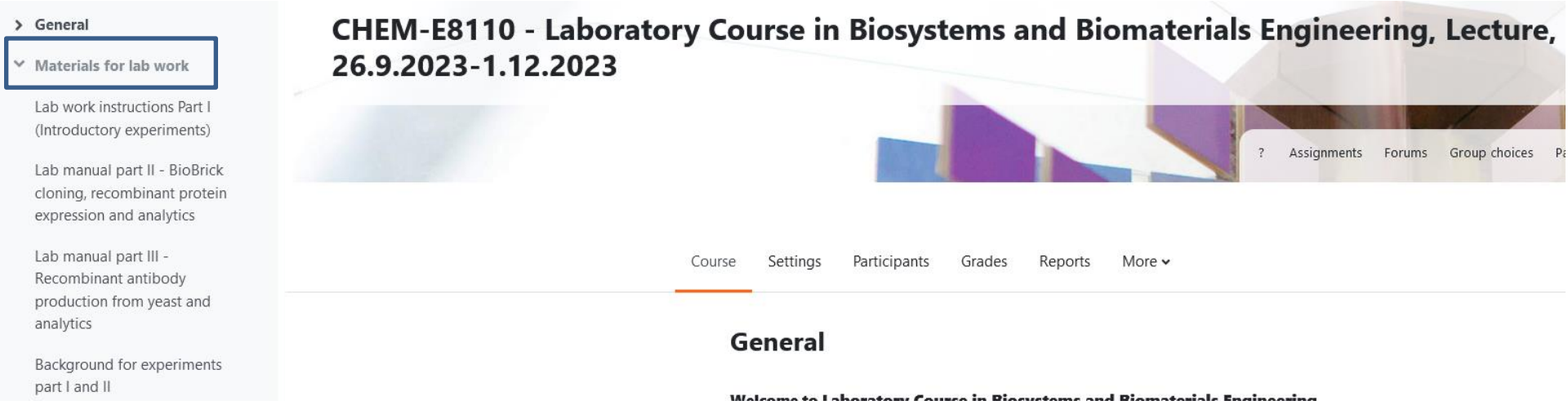
Pre-assignments

- **The submission deadlines for each pre-assignment are indicated within their respective sections**.

- Detailed information and instructions by Samuel Girmay.

Grade requirements for credit points – active participation and completion of the experimental work (40%)

- Prepare yourself for the experiments by reading the manual before each course day
  - Be prepared to introduce & discuss the work before the lab starts
  - if something is unclear have specific questions ready



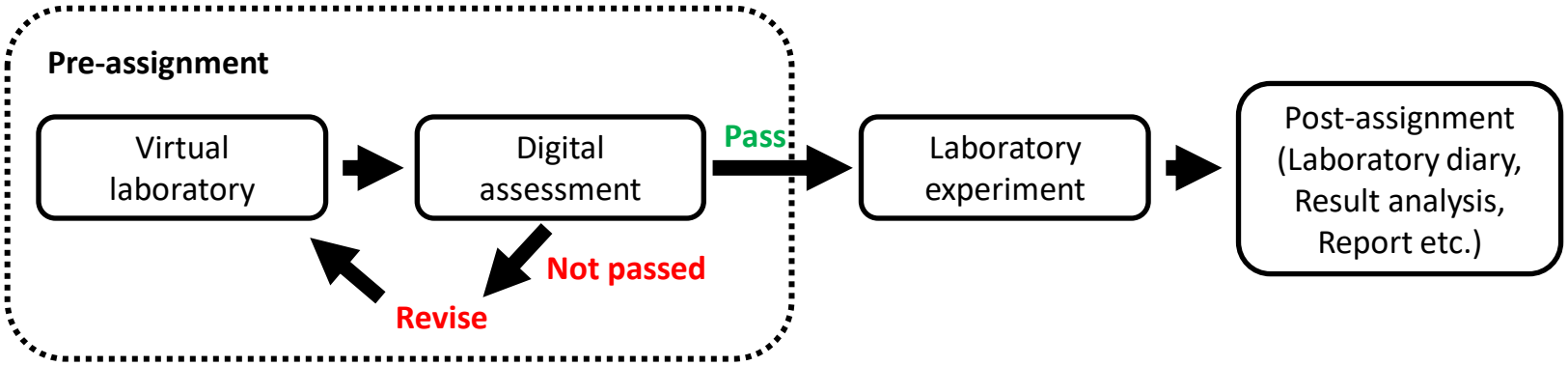
- Missing lab days must be compensated with extra assignments!
  - **1 day of absence = 2 pages of written essay, topic related to the work**

Pre-assignments (Part I and III)

Learning outcomes

- Able to familiarize with basic terms, methods and concepts related to biolaboratory.
- Able to apply pre-assignment materials to week's experiments.
- Able to analyze experimental data from week's experiments in basic level.

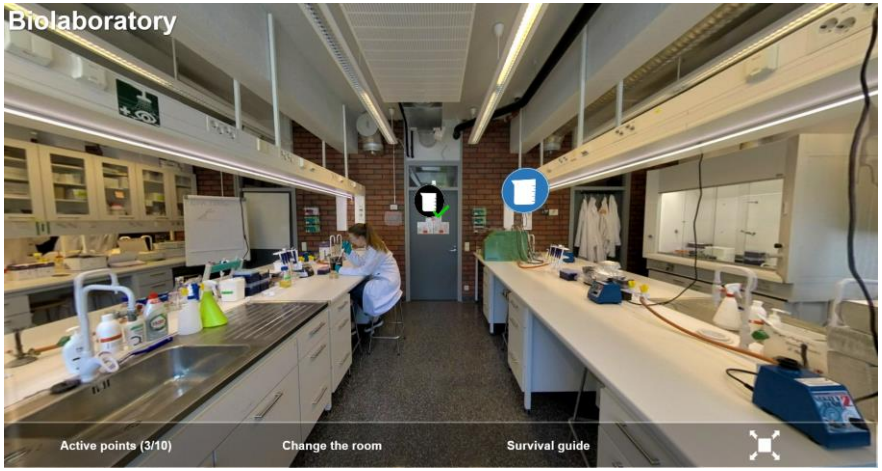
Overview of the week (In part I and part III)

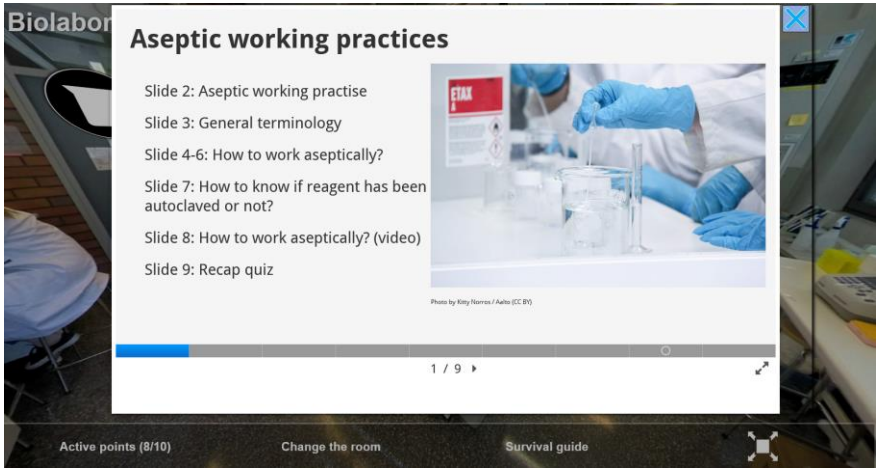


Pre-assignments (Part I and III)

Virtual laboratories

- 360 degree learning environment which contains activities (text, pictures, interactive videos and recap quizzes).





Pre-assignments

Digital assessments

- For you and the teachers to see that you have understood the topic according to the learning outcomes of pre-assignment.
- Pass/Fail
- Can be done multiple times

Part	Group A	Group B
Part I (week 1)	Multiple choice quiz	Short text
Part I (week 2)	Short text assessment	Multiple choice quiz
PART II (written pre-assignment (Heli))		
Part III (week 1)	Answer with voice recording	''Peer-review'' assessment
Part III (week 2)	''Peer-review'' assessment	Answer with voice recording

Pre-assignments

Research study

- To investigate the use of virtual laboratories and assessment methods in biochemical engineering education.

- Contains multiple choice and open feedback questions about virtual laboratory and assessments
    - Learning outcomes
    - Learning experience
    - Workload
    - Feedback from assignments and virtual laboratory
    - What was good? What needs to improve? What is feasible? What is not?

- Feedback is anonymous and **strongly recommended to answer honestly**.

- Feedback quiz is available in MyCourses at the end of course (end-Nov).

- Any questions related to virtual laboratories, digital assessment or study, contact: **samuel.girmay@aalto.fi**

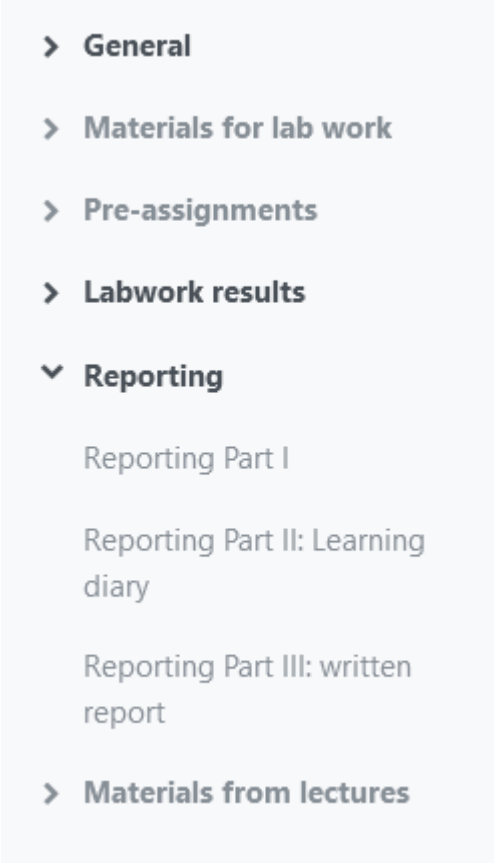
Grade requirements for credit points – reporting (60%)

- Reporting is done individually.

- Part I
  - Summary of the results
    - Day 1 (Enzyme kinetics)
    - Day 2 (Microbial genetics)

- Part II
  - Learning diary

- Part III
  - Written report that includes
    - Abstract
    - 1 composite figure (3 or 4 panels) with figure legend



Grade requirements for credit points – Summary of results (10%)

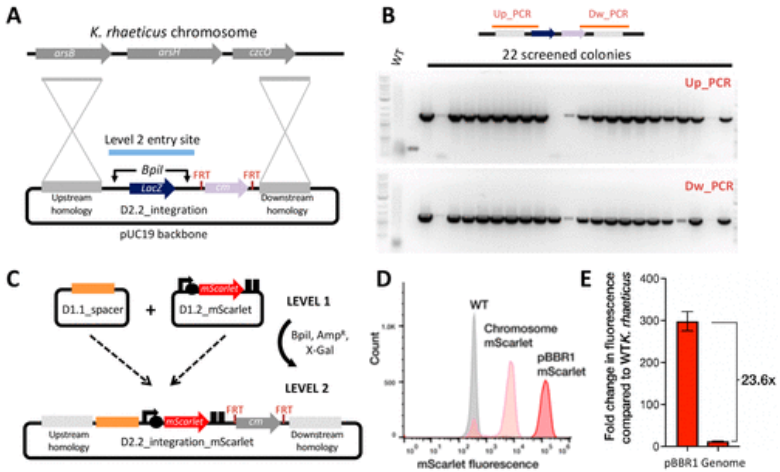
- Summary of results of part I
  - Summary is limited to reporting the obtained results
- Enzyme kinetics
  - Prepare a standard curve & calculate enzyme activity
- Microbial genetics
  - Report the results.
  - Explain whether the obtained results are according to the expectations or not. (number of transformants & genetic complementation)
  - If not, you may hypothesize the reason.

Grade requirements for credit points – learning diary (20%)

- For Part II
  - Detailed instructions for learning diary are provided in the lecture on 10.10.
  - Briefly, the diary should contain
    - Overview & workflow
    - Daily reporting of the results (What does the observations say?)
    - Finally discuss the results (Did everything go as planned?)

Grade requirements for credit points – Written report Part III (20%)

- Written report should include
  - Abstract (150 to 200 words)
  - 1 composite figure (3 or 4 panels) (objective & results)
  - Appropriate figure legend
  - Style according to a scientific publication summarizing the objectives of the work and showing selected key findings.



https://pubs.acs.org/doi/10.1021/acssynbio.1c00358

Figure 3. A KTK system for genome integration. (A) The D2.2_integration plasmid is based on the ampicillin resistant pUC19 backbone with homologous recombination into the *K. rhaeticus* chromosome guided by two regions of homology (upstream: 1000 bp, downstream: 921 bp) to the arsenic resistance operon. Between these regions of homology is a KTK Level 2 entry site, with a LacZ dropout cassette, and a FRT-site-flanked chloramphenicol resistance gene for selection in *K. rhaeticus*. (B) Confirmatory PCR analysis of 22 screened colonies. (C) Construction of a KTK Level 2 plasmid for integration into *K. rhaeticus*. D1.1_spacer and D1.2_mScarlet (using J23104 promoter) were built into the D2.2_intergration backbone by standard KTK Level 2 assembly, with the single alteration of antibiotic selection on ampicillin instead of spectinomycin. (D) Representative flow cytometry distributions of *K. rhaeticus* strains expressing the same mScarlet expression construct from the chromosome (pink) and pBBR1 plasmid (red), compared to no expression in wildtype cells (gray). (E) RFP signal from mScarlet expression when on pBBR1 plasmid vs genome-integrated, as determined by flow cytometry. Red fluorescence is measured as fold increase over wildtype reading and is an the average of biological triplicates. The per cell copy number of pBBR1 plasmid (×23.6) is estimated from the difference between fluorescence of the two average values.

Grade requirements for credit points – Final discussion (10%)

- Final discussion: 1st December in KE4 (12 – 14)

- Each group (A & B) need to prepare a 30 minutes presentation, presenting the overall lab work (Goals, results, hypothesis etc)

- Group work
  - Organize the work within the group
  - Prepare a joint presentation

- Mandatory discussion

Overview of deadlines

- Deadline for pre-assignments
  - Part I: Friday 29th September and 6th October at 10:15.
  - Part II: Monday 23rd October at 10:15.
  - Part III: Monday 13th November at 10:15.
- Deadline for submission of results (Part I): Friday 27th October at 23:59
- Deadline for submission of learning diary (Part II): Friday 17th November at 23:59
- Deadline for submission of written report (Part III): Friday 8th December at 23:59