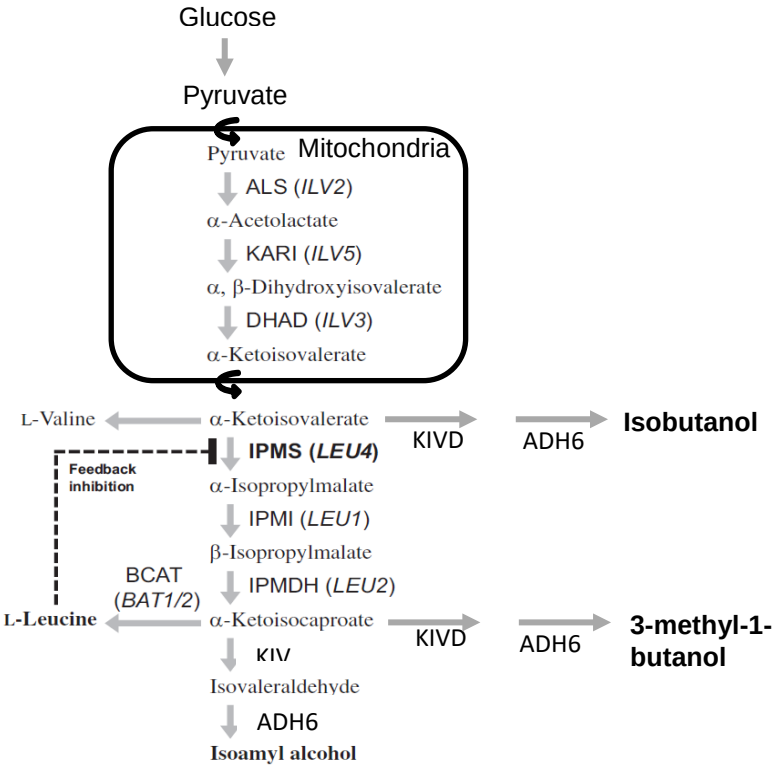
Exam Cell Factory, 07.04.2020.

- **Your answers should be based on the subjects discussed in the course!**
- In order to pass the exam you need 40% of the total points (10 out of 25 points). The exam contributes 70% to the final grade.
- Return your answers as a single PDF file via MyCourses.

Essay questions.

Question 1 (**max. 6 points**)

Besides Ethanol yeast is producing naturally a number of higher alcohols, such as isobutanol, isoamylalcohol or 3-methyl-1-butanol. Typically, these alcohols are produced at very low concentrations, but using genetic engineering the amounts produced have increased. Two key enzymes in these engineered strains are *Lactococcus lactis* 2-keto-acid decarboxylase (KIVD) and the NADPH-dependent medium chain alcohol dehydrogenase ADH6 from *S. cerevisae*. Both enzymes have a broad substrate specificity.



Discuss and explain the following three statements:

a) "If ILV2, ILV5 and ILV3 activities were retargeted to the cytoplasm, the yields of isobutanol would increase." Is this a good idea? How could it be achieved? **(max. 2 points)**

b) “Replacing ADH6 with a NADH-dependent alcohol dehydrogenase would increase productivity” Is it a good idea? Why / why not? How could it be achieved? **(max. 2 points)**
c) “Feeding of α-ketoisovalerate to the growing cultures increases the yield of isobutanol, but not of the two other products.” Why? How could this problem be solved? **(max. 2 points)**

Question 2 (max. 5 points)

The pL expression system is derived from *E. coli* bacteriophage λ, a phage which cannot infect other bacteria.

a. Describe all the elements of the pL expression system and how it is used for protein expression **(1 point)**.
b. Describe a strategy and the necessary modifications to the pL expression system that allow its use in another prokaryote and a eukaryote **(4 points)**.

Question 3 (max. 8 points)

Your company is investigating the possible use of genetically-engineered cells to produce a vaccine against SARS-CoV 2 virus. As vaccines, two different fragments of the spike protein (S protein) which is located on the surface of the virus are considered. Fragment 1 contains the N-terminal part of the S protein including the signal peptide. Fragment 2 is the C-terminal part of the S protein. Fragment 1 is highly N-glycosylated and contains disulfide bonds. Fragment 2 is non-glycosylated but contains disulfide bonds. To enable rapid production the proteins should be secreted.

a. List and discuss the pros and cons of using *prokaryotes (gram- and gram+), Saccharomyces*, and mammalian cell lines to produce the two protein fragments that could be used as vaccines **(max. 5 points)**.
b. In case you consider one or more of these expression systems unsuitable for production of these proteins, explain how cell engineering could add the missing functionalities. **(max. 3 points)**

Question 4. Shortly describe/explain (1 point per question, in total 6 points):

a) explain the similarities and difference(s) between a replicative and integrative plasmid
b) explain the difference between feedback inhibition and repression in biological systems
c) What are the advantages of using inducible promoters compared to constitutive promoters?
d) Which genetic elements can be utilized in order to keep the chromatin in an open conformation? How do they function?
e) explain the rationale behind “laboratory evolution approaches” for strain engineering.
f) explain the rationale behind activating the unfolded protein response for enhancing recombinant protein production.