In order to pass the exam you need 40% of the total points (12 out of 30 points). The exam contributes 75% to the grade.

Essay questions 1-5: Answer 4 out of the 5 questions, each question gives 5 points, in total 20 points.

1. Why isn't the plasmid that contains the strongest promoter always the best option for expression of a protein? Explain possible consequences of too high expression levels on the host organism(s) and how the organism(s) react. (5p)

2. You are producing a secreted galactosidase enzyme in yeast. The galactosidase contains 3 disulfide bonds, which are essential for its enzymatic activity. Expression of the functional galactosidase can be easily verified with an enzymatic assay. For expression of the enzyme, the gene encoding the galactosidase under control of a strong constitutive promoter is introduced on a high-copy number plasmid into the expression host.
   The analysis of the production indicated that 80% of the total galactosidase activity is detected in the medium, the remaining 20% of the galactosidase activity is detected inside the cell. In addition, it was noticed that a substantial amount of inactive galactosidase enzyme accumulated in the cell.
   Identify potentially rate-limiting steps in the production of the galactosidase and propose solutions to overcome the problem. (5p)

3. The pET expression system is derived from *E. coli* bacteriophage T7, a phage which cannot infect other bacteria. Describe all the elements of the pET expression system and describe how the pET expression system might be used to express proteins in other organisms than *E. coli*. (5p)

4. For each of the products described below, suggest two expression strategies including suitable host organisms. In both cases (a. and b.), you can consider non-engineered and engineered expression systems.
   a. a non-glycosylated, non-disulfide containing mammalian protein, which is via inhibition of a cytoplasmic enzyme hampering growth of the production organism (2.5p)
   b. a glycosylated, non-disulfide containing protein requiring a complex-type N-glycan (2.5p)

5. Figure 1 represents a sketch of the metabolic pathway leading to formation of the Product A in a bacterial organism. All steps take place in the cytoplasm. Product A accumulates within the cell. Point out all possible bottlenecks and describe a strategy for improving productivity. (5p)

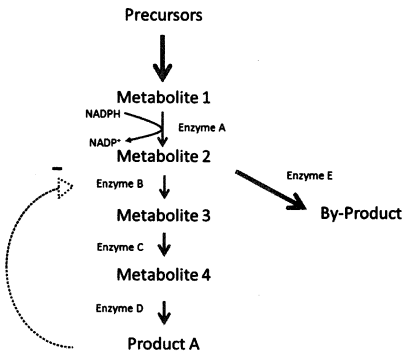

Figure 1. The size of the arrows indicates the capacity of the enzyme to catalyze the reaction. The dotted arrow indicates regulation of Enzyme B by feedback inhibition and repression.

6. Shortly describe/explain with 2 to 5 sentences and/or annotated illustrations (1 point per question, in total 10 points):

a. the function of the signal sequence in protein expression
b. the main difference in the production strategy for a primary and a secondary metabolite
c. the mechanism for formation of a disulfide bond
d. how a gene can be integrated into the genome
e. the difference in the cell structure between a gram$^-$ and gram$^+$ bacteria and its effect on the production of a recombinant protein
f. *Agrobacterium tumefaciens* mediated plant transformation
g. Codon-optimization
h. a prokaryotic expression plasmid
i. the function of the oligosaccharyltransferase (OST)
j. a yeast artificial chromosome (YAC)