Exam questions Cell Factory CHEM-E8115, April 4th, 2017

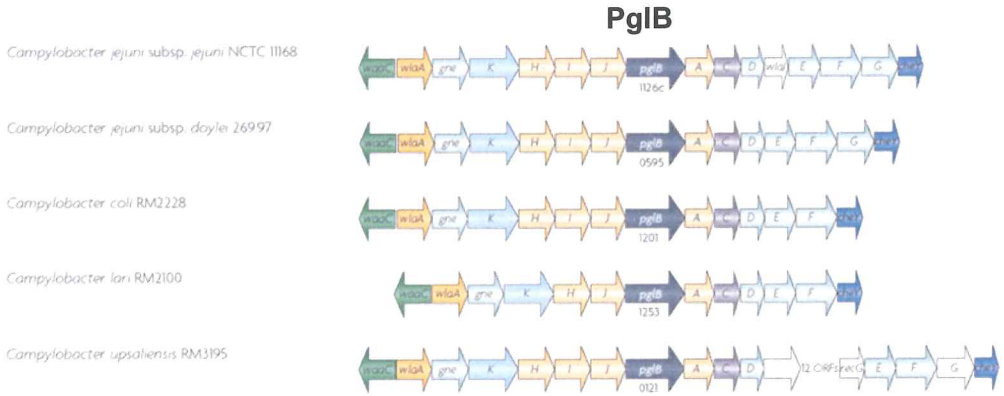
In order to pass the exam you need 40% of the total points (12 out of 30 points). The exam contributes 75% to the final grade.

Essay questions 1-6: Answer 4 of the 6 questions, each question gives 5 points, in total 20 points.

1. You are responsible for the establishment of a new production facility for N-glycosylated proteins in a biotechnology company. The company produces proteins for a wide range of applications ranging from industrial bulk enzymes to therapeutic proteins. You have to inform the company management about different cellular systems available and convince them, in which of these they should invest.
   Describe the pros and cons of two different cellular systems, which are suitable for production of N-glycosylated proteins and give the reasons why you would choose a particular system. In case you believe that the company should invest in more than one cellular system, give your reasoning. (5p).

2. The pL expression system is derived from *E. coli* bacteriophage λ, a phage which cannot infect other bacteria. Describe all the elements of the pL expression system and how it is used for protein expression. Describe a strategy how the pL expression system can be used to express proteins in other organisms than *E. coli*. (5p)

3. The genes encoding the enzymes involved in bacterial N-glycosylation are arranged in clusters (see figure). The clusters comprise the genes encoding the enzymes for the biosynthesis of the individual sugars, for the assembly of the oligosaccharide (glycosyltransferases), for the flipping of the oligosaccharide from the cytoplasm into the periplasm (K) and for the transfer of the glycan onto the protein (here pglB). Each cluster encodes the enzyme for the synthesis of a specific N-glycan structure differing in composition, length and type of linkages. The glycosyltransferases present in the cluster possess different specificities for the acceptor sugar and the linkage type.
   Describe strategies for the generation of novel glycan structures based on these gene clusters (5p).



Schematic representation of the conserved *N*-linked protein glycosylation gene clusters. The gene encoding the essential oligosaccharyltransferase PglB is shown in bold. The other arrows indicate the arrangement of the genes of the biosynthetic enzymes and glycosyltransferases in the cluster. Abbreviations used: Genes encoding the biosynthetic enzymes (gne, D, E, F, and G); glycosyltransferases (A, C, H, I, and J); flippase (K).

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

5. Describe different strategies for improving productivity of an amino acid producing strain. Consider the following points: precursor availability, cofactor availability, compartmentalization, feedback inhibition and repression. Explain how these factors can affect productivity and how to deal with these challenges. (5p)

6. During the integration of DNA into the chromosome of an eukaryotic host organism, often also a selection marker is inserted into the chromosomal DNA. The target gene to be integrated into the chromosome can be present (1) on a linear DNA fragment such as a PCR product or (2) on an entire plasmid. In both cases (1 and 2), also a marker gene is present on the DNA fragment.

   a. Sketch both types of DNA marking all features needed for the integration and selection.

   b. How do you ensure that the plasmid is not maintained in the cell as an episomal (non-integrated) element? However, the plasmid amplification is still needed to produce the plasmid.

   c. What strategy could be used to excise the marker gene after integration?

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

a. A baculovirus
b. The difference between feedback inhibition and repression
c. the main difference in the production strategy for a primary and a secondary metabolite
d. the mechanism of disulfide formation
e. nisin-controlled gene expression in *Lactobacilli*
f. rare codons
g. Why NADH formation and NAD^+ regeneration must be balanced
h. the role of a signal sequence in protein expression
i. The structure of a disarmed Ti-plasmid as used in plant transformations
j. How a scaffold can increase efficiency of a metabolic pathway