NTNU – Trondheim
Norwegian University of
Science and Technology

Department of Biology

Exam in Bi3013

EXPERIMENTAL CELL AND MOLECULAR BIOLOGY

Contact person during examination:
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Examination time (from-to): 09.00 – 13.00 (4 hours)
Permitted examination support material: None

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Informasjon om trykking av eksamensoppgave
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NOTICE THAT QUESTIONS 1 - 4 ARE WEIGHTED EQUALLY (25 %), BUT SUB QUESTIONS MIGHT BE WEIGHTED DIFFERENTLY (INDICATED IN %). IF NO WEIGHTING IS GIVEN THE SUB-QUESTIONS ARE WEIGTHED EQUALLY. PLEASE START ANSWERING EACH QUESTION (1 - 4) ON A NEW SHEET OF PAPER.

Question 1

You have identified an alga that produce omega 3 fatty acids and you want to find out if you can increase the production of lipids in the algae. Reduction of phosphate in the growth medium has already shown that it can increase lipid production. To learn more about effects of phosphate limitation you have done a phosphate limitation experiment and isolated total RNA for transcriptomic analysis.

a. Describe two sequencing technologies which are often used for transcriptomic analyses and discuss the advantages / disadvantages associated with the methods. (40 %)

b. By using the RNA you have isolated from the algae, describe the various steps in the RNA sequencing (RNAseq) procedure and explain what type information you can get from such an experiment. (30 %)

c. The genome of the alga you are studying are unknown and you decide to run a complete *de novo* genome sequencing. Explain the strategy for de novo genome sequencing and explain how transcriptome data can be used in the assembly process. (30 %)

Question 2.

The transcriptome analysis of the algae identifies a key gene in biosynthesis of carbo-hydrates and you decide to inactivate the gene using the CRISPR/Cas9 technology. You think this can further increase the lipid production in the algae.

a. Explain how the CRISPR/Cas9 system was discovered and how it was developed and adapted for gen editing. (40 %)

b. Explain the experimental setup and the procedure for generating a gene knockout using CRISPR/Cas9 technology. Plasmid delivery and transformation can be done in several ways, describe one of the methods and explain how you will identify the mutations. (40 %)

c. The CRISPR/Cas9 system is efficient to produce indels (insertions or deletions) in the genome, but how can you use the CRISPR/Cas9 system to produce “random” point mutations at defined sites in the genome? (20 %)

Question 3.

Arabidopsis thaliana have been used as a genetic model plant for researchers in several decades and detailed studies of many genes have been performed.

a. Describe how reporter proteins can be used to study gene expression in *Arabidopsis*. (30 %)

b. You have identified an unknown protein in *Arabidopsis* and decide to find out where it is located in the root cells. Explain how you will set up an experiment and examine its cellular location *in-vivo*. (40 %)

c. You suspect that the unknown protein interacts with other proteins. Describe two techniques that can be used to identify interacting proteins. (30 %)

Question 4.

Explain or describe 4 of the 5 following terminologies/techniques, use figures wherever necessary (not more than 200 words for each).

a. Electrophoretic mobility shift assay (EMSA)

b. Bimolecular fluorescence complementation (BIFC)

c. CHIP-Seq

d. MALDI-TOF MS

e. DeadCas9 (dCAS9)

Use figures where appropriate to explain your answers, (questions 1-4).