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
Originalen er:
1-sidig X **2-sidig** ☐
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Date Signature

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

Phosphate is an essential nutrient for microalgae. How microalgae sense and respond to low phosphate levels are still little known. You have identified a *Phaeodactylum tricornutum* mutant which grows poorly under low phosphate levels and who has a mutation in gene X where a serine codon (TCA) is changed to an alanine (GCA), (both alleles have the same mutation).

a. Explain how you can use the CRISPR/Cas9 technology to change the alanine mutation in gene X back to a wild type serine. The answer should contain a description of the CRISPR/Cas9 technology and how it works, plus an explanation / description of the transformation method used. (60 %)

b. Describe the procedure you will use to confirm that the gene editing has taken place and that the codon has been changed from GCA → TCA. (40 %)

Question 2.

Arabidopsis thaliana is widely used model organism for studies of fundamental physiological, cellular and molecular processes in plants. There are several hundred plasma membrane receptors of the type leucine rich repeat (LRR)-kinases in *Arabidopsis* and based on transcription data from an experiment where plants were exposed to salt stress (200 mM NaCl), you have identified a LRR-kinase which is induced by the treatment.

a. How can you identify the plant tissue and cell types the LRR-kinase gene is induced in due to salt stress? Describe how you want to perform an experiment to study how salt stress affects gene expression patterns of the LRR-kinase in the plant. Use relevant methods and techniques from the lab course.

b. You have identified a *Arabidopsis* mutant with a T-DNA insertion in the LRR-kinase which leads to its inactivation. Describe how a transcriptional analysis using RNAseq can be used to study the effect of gene inactivation. Explain the different steps in the RNAseq procedure, including a description of the DNA sequencing technology.

Question 3.

Sar GTPases are evolutionary conserved proteins and exist in all eukaryotic organisms. You have identified the *SAR1a* gene in salmon (*Salmo salar*) and want to study the cellular location and function of the protein in the salmon cell line SHK-1. cDNA and genomic sequence is known.

a. How can the intracellular localization of the SAR1a protein be determined *in-vivo*? Describe how you will perform this experiment using relevant methods and techniques form the lab course. (40 %)

b. Explain the principle for confocal microscopy and describe the layout of a confocal microscope. (30 %)

c. Describe how various confocal microscopy applications can be used to gain extra information of protein localization and protein-protein interactions. (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. Chromatin immunoprecipitation (ChIP).

b. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS)

c. Electrospray ionization mass spectrometry (ESI MS)

d. Gene drive.

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