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

Phosphate is an essential nutrient for algae and reduced availability can affect many cellular processes. You have decided to set up a phosphate limitation experiment using the diatom *Thalassiosira pseudonana* and are interested to study how the gene expression is affected by the treatment. Here the design of the experiment, growth of algae and RNA isolation is of high importance.

a. List some of the most important factors you need to take into consideration when setting up this experiment. How would you grow and treat the algae? (20 %)

b. Describe each step in the isolation of RNA. From harvesting of the samples until you have the purified RNA. Which RNA isolation method would you use and what factors are important to consider for getting high quality RNA? (40 %)

c. The quantity and quality of the isolated RNA have to be determined. Describe what type of analyzes of the RNA you will perform and how this information can be used to evaluate whether the RNA samples are suitable for transcriptional analyses (40 %).

Question 2.

Global gene expression can be analyzed through RNAseq methods or by using DNA microarrays.

a. Explain how you would set up a transcription analysis with DNA microarrays using RNA isolated from the phosphate limitation experiment. Describe the various steps in the procedure and discuss advantages and disadvantages of the method. (40 %)

b. You want to compare the results from the DNA microarray experiment using RNAseq (using the same RNA as in DNA microarray experiment). What sequencing technology would you use? Explain your choice and the describe principle behind this sequencing method. Do you expect results from RNAseq and DNA microarrays analysis to be similar or different? Explain why. (40 %)

c. How will you identify the genes significantly differentially expressed in the phosphate limitation experiment (from RNAseq or DNA microarrays) and what type of cellular responses would you expect to see from the analysis? (20 %)

Question 3.

The transcriptional analysis identifies an unknown gene in *T. pseudonana* that is highly induced by the phosphate starvation.

a. Describe a method that can be used to inactivate the unknown gene and explain the different steps needed to perform this in *T. pseudonana*. (40 %)

b. How will you verify that you have produced a real gene knockout? (20 %)

c. You suspect that the unknown protein is membrane bound. How you can determine its cellular location? Describe how you will perform such an analysis and discuss the various methods / techniques used in the experiment. (40 %)

Question 4.

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

a. Fluorescence resonance energy transfer (FRET)

b. Single molecule real time (SMRT) DNA Sequencing

c. PAM-site

d. Stimulated emission depletion (STED) microscopy

e. MALDI-TOF MS

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