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 date: 26th May, 2016
Examination time (from-to): 09.00 – 13.00 (4 hours)
Permitted examination support material: None

Other information: Language: English
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Number of pages enclosed: 0

Checked by:

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Date Signature

NOTICE THAT QUESTIONS 1, 2, 3, AND 4 ARE WEIGHTED EQUALLY, BUT SINGLE QUESTIONS MIGHT BE WEIGHTED DIFFERENTLY (INDICATED IN %). IF NO WEIGHTING IS GIVEN THE SUB-QUESTIONS ARE WEIGTHED EQUALLY.
PLEASE START ANSWERING EACH QUESTION (1, 2, 3, and 4) ON A NEW SHEET OF PAPER.

Question 1

Phosphate is an essential macronutrient for algae and phosphate limitation is known to affect many cellular processes in algae. You have decided to set up a phosphate limitation experiment in the diatom *Phaeodactylum tricornutum* and you are interested to study changes in gene expression.

a. Describe how you will set up a phosphate limitation experiment for *P. tricornutum*. How would you grow and treat the algae, and how would you harvest the samples? (20 %)

b. To perform transcriptional analyzes you will need to isolate RNA. What type of RNA isolation method will you use? Describe the RNA isolation procedure and what quality analyzes of the RNA you will perform (40 %).

c. You decide to perform a genome wide transcriptional analysis and will to use the Illumina sequencing platform. Describe the principle and technology behind Illumina DNA sequencing and explain the various steps in the process. How will you identify the genes significantly differentially expressed by the phosphate limitation? (40 %)

Question 2.

The transcriptional analysis of *P. tricornutum* identifies several genes showing strong upregulation after phosphate limitation and you decide to study five of those genes in more detail.

a. Gene expression responses to phosphate limitations can be both quick or slow (early and late responses). Describe how you will set up an experiment where you analyze the early gene expression responses of the five selected genes, ex. the first six hours, and explain in detail the method you will use (40%).

b. Two of the five genes you are studying encode phosphate transporters, and you are curious to find out where they are located in the algae. How you can determine their cellular location? Describe how you will perform such an analysis and discuss the various methods used in the experiment (60%).

Question 3.

The CRISPR/Cas9 gene editing technology has in the last few years become a powerful technique with many applications in molecular biology.

a. Explain the principle behind the CRISPR/Cas9 method and why this is a great improvement over previous gene editing methods.

b. Describe how you will use this method to knockout a gene in *P. tricornutum*. You can use methods described and used in the laboratory course.

c. How will you confirm that the gene has been edited?

d. A modified type of the Cas9 protein exists were both nuclease domains are inactivated, so called dead Cas9 (dCas9). Explain what type of applications modified dCas9 proteins are used for in molecular biology?

Question 4.

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

a. Yeast two hybrid system

b. 2D gel electrophoresis and 2D DIGE

c. Nanopore sequencing.

d. Stimulated emission depletion (STED) microscopy

e. Green fluorescent protein (GFP)

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