School of Life Sciences

WRITTEN EXAMINATION

Course   NGS library: preparation and quality control

Sub-course

Course code   BV704A   Credits for written examination   3

Date   15/11/2024   Examination time   08:15 - 12:30

Examination responsible   John Baxter

Teachers concerned   John Baxter and Magnus Fagerlind

Aid at the exam/appendices

Other

Instructions

☐	Take a new sheet of paper for each teacher.
☐	Take a new sheet of paper when starting a new question.
☒	Write only on one side of the paper.
☒	Write your name and personal ID No. on all pages you hand in.
☒	Use page numbering.
☒	Don´t use a red pen.
☒	Mark answered questions with a cross on the cover sheet.

Grade points

Examination results should be made public within 18 working days

Good luck!

Total number of pages   1

UNIVERSITY OF SKÖVDE

Exam: NGS library: preparation and quality control BV704A

Learning objective: Describe methods for NGS library preparation and sample quality control. (18p)

1. What are the four major steps in preparing Illumina sequencing libraries for RNA or DNA? **(2p)**

2. Which major approaches are available to fragment nucleic acid chains? Describe them in short. **(3p)**

3. When performing multiplexing, barcodes/indexes are used. What is multiplexing? How and when are they added to sequences within Illumina sequencing? **(3p)**

4. When using the Illumina Nextera DNA sample prep kit, undertagmentation and overtagmentation are two possible problems. What is meant by that, what problems do they create and how can it be detected and prevented? **(6p)**

5. What is the RNA Integrity Number (RIN), how is it calculated and why is it important to consider during RNA-Seq library construction? **(4p)**

Learning objective: Design and validate experimental methods for NGS-analysis. (18p)

6. How can incorrect library quantification affect the sequencing output? **(2p)**

7. What are the advantages of SMRT sequencing technology over Illumina sequencing technology? **(3p)**

8. In most NGS library preparation protocols, AMPure beads are used during the different purification steps, instead of using column or gel-based purification methods. Why? **(2p)**

9. In general, describe the applications of and sample preparation of targeted and amplicon sequencing? Are there pros and cons of this method? **(5p)**

10. The goal when preparing NGS libraries is to maximize complexity. How can maximum complexity be achieved and/or maintained? **(3p)**

11. If comparing only NGS and Sanger sequencing in bacterial community analysis, what are the benefits and disadvantages/limitations with each method? When is each method is preferred? **(3p)**