UNIVERSITY
OF SKÖVDE

School of Life Sciences

WRITTEN EXAMINATION

Course NGS library: preparation and quality control

Sub-course

Course code BV704A

Credits for written examination 3

Date 17/02/2025

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

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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. How is 'On-Bead tagmentation' an improved method for illumina NGS DNA library preparation? **(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 some advantages of RNA sequencing with third generation technologies? **(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. Short read massive-parallel sequencing is a well-used diagnostic tool in medical research. What are some limitations with this tool? What can be done to prevent these? **(5p)**

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

11. What is the RNA Integrity Number (RIN), how is it calculated and why is it important to consider during RNA-Seq library construction? **(3p)**

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