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 01/12/2023

Examination time 08:15 - 12:30

Examination responsible Nada Mahmoud

Teachers concerned Magnus Fagerlind and Nada Mahmoud

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 Total 35 points graded as follows:

A > or = 90 % , B 80 %, C 70 %, D 60 %, E 50 %, and F < 50 %

Examination results should be made public within 18 working days

Good luck!

Total number of pages 2

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Re-Exam: NGS library: preparation and quality control BV704A- HT23

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

1) Figure 1 below shows the steps of sample and library preparation for a specific sequencing method. Which sequencing method is the below library is suitable for? Explain the library preparation for this method in detail. **(4P)**

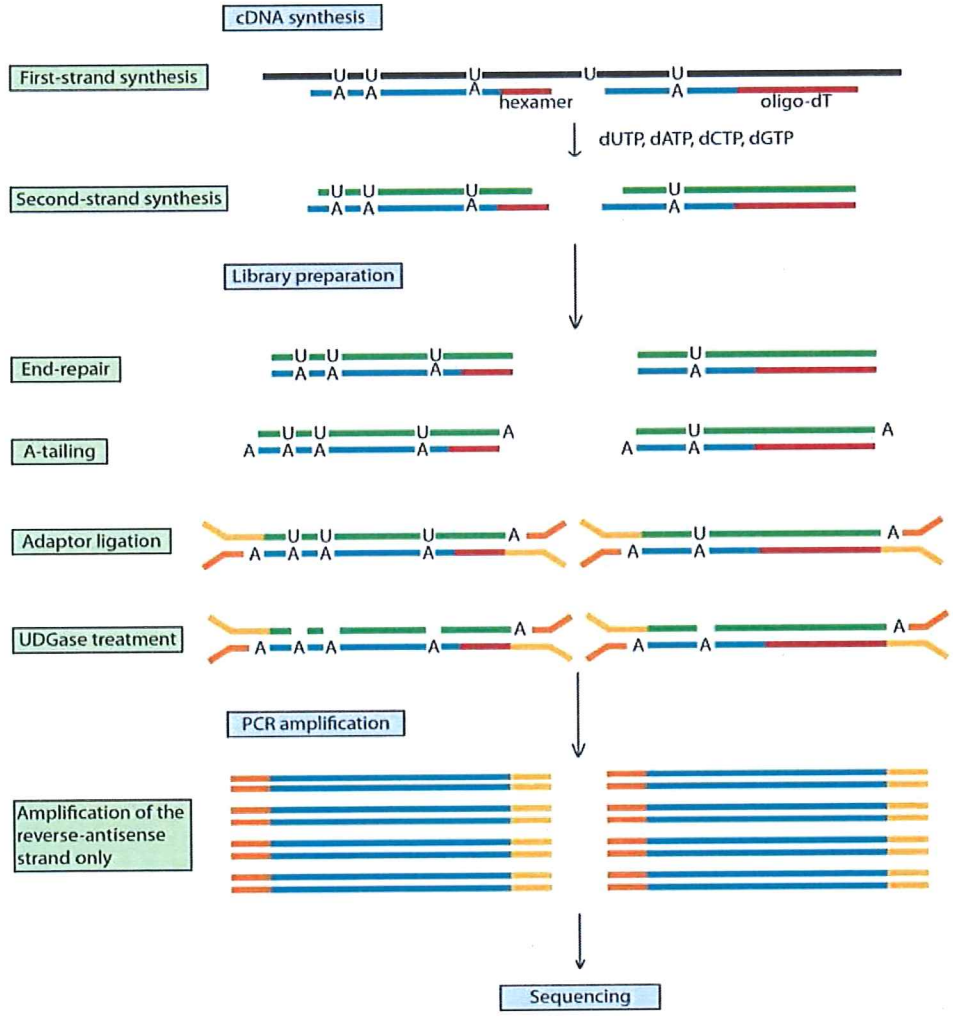

Figure 1. Sample and library preparation for a certain sequencing method. Read the question above.

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2) Describe in a brief detail **two methods** used for DNA fragmentation for Illumina sequencing library preparation. **(4P)**

3) One of the major advantages of Next-generation sequencing (NGS) is the possibility of Multiplexing.

   A. What is multiplexing, and what is the benefit of employing this technology in sequencing? **(1P)**
   B. At which step of library preparation can multiplexing be performed? **(1P)**

4) An alternative library preparation method is the Illumina Nextera DNA Sample Preparation Kit.
   A. Provide a brief description of this method. **(2P)**
   B. Enumerate the advantages and possible limitations associated with this approach. **(2P)**

5) Describe briefly the general principle method of **MinIon** Oxford Nanopore sequencing. **(4P)**

Learning objective: Design and validate experimental methods for NGS-analysis. (17 Points)

6) Bias and reduced complexity are two significant challenges that an NGS library may encounter.
   A. Explain the concept of bias and the possible reasons that might lead to it. **(2P)**
   B. Clarify the concept of library complexity. **(2P)**

7) In the majority of NGS library preparation protocols, AMPure beads are preferred over column or gel-based purification methods for various purification steps. What are the reasons for this preference? Mention at least four reasons. **(4P)**

8) It is recommended to perform qPCR as the final step in NGS library preparation before sequencing. Answer the following:

   A. What is the purpose of this recommendation? **(1P)**
   B. What are the benefits of this choice? **(1P)**

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9) How can incorrect library quantification affect the sequencing output? **(3P)**

10) Single molecule real-time (SMRT) sequencing is becoming a popular method for sequencing. Answer the following:

A. What are the limitations of SMRT sequencing in comparison to illumine sequencing? **(2P)**

B. What are the advantages of SMRT sequencing technology over Illumina sequencing technology? **(2P)**