FACULTY OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY

MODULE **BIC0090**
CURRENT ADVANCES IN BIOTECHNOLOGY

CAMPUS **APK**

EXAM **NOVEMBER 2015**

DATE 11/11/2015 **SESSION** 08h30 – 11h30
ASSESSOR(S) **DR LA PIATER**
DR L ESTERHUIZEN

INTERNAL MODERATOR

EXTERNAL MODERATOR **DR DJ OPPERMAN**

DURATION 3 HOURS MARKS 100

NUMBER OF PAGES: 6 PAGES

INSTRUCTIONS: ANSWER ALL THE QUESTIONS
QUESTION PAPERS MUST BE HANDED IN.

QUESTION 1 **[13]**

During the Protein Crystallography course presented by Dr von Delft, you attempted to purify and crystallize recombinant human CHD1 protein. Answer the following questions:

1.1 Consider the adjacent, annotated SDS-PAGE of the aliquots collected during the purification of construct A2 by Group 1.

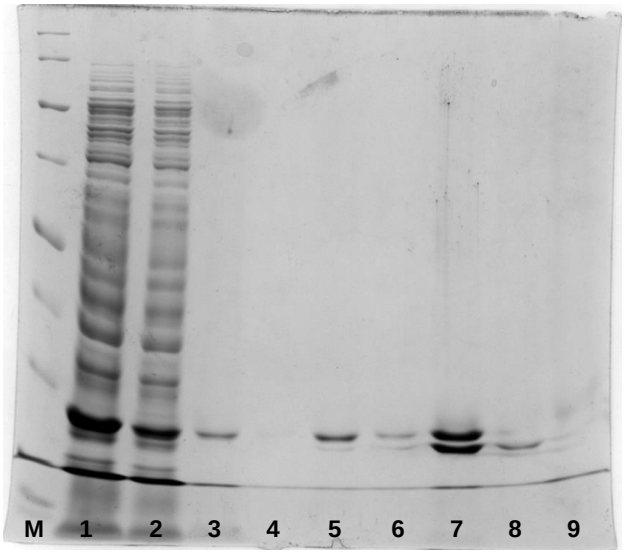

Figure 1: SDS-PAGE of A2 protein isolation M=Molecular weight marker, lane 1:supernatant, lane 2: lysate flow through, lane 3: binding buffer, lane 4: wash buffer, lane 5: elution buffer E300, lane 6: elution buffer E500 , lane 7: After incubation with TEV, lane 8: Protein eluate, lane 9: elution buffer40

1.1.1 Assess and justify whether you can be confident that the desired protein was indeed purified. (1)

1.1.2 A considerable amount of (apparent) product appears in the first flow-through of lysate (lane 2). Discuss what this means for this purification step specifically, and the overall purification procedure. (2)

1.1.3 Comment on the quality of the gel. Describe any conclusions that *can* be drawn, and any questions that *cannot* be answered. (3)

1.2 Group 3 achieved a final protein concentration of 2 mg/ml for their sample (A7). This is considerably lower than the rule-of-thumb protein concentration of 10 mg/ml required in the crystallization drop.

1.2.1 How can you adjust your sitting-drop vapour diffusion experiment to ensure the concentration in the drop approximates the desired concentration? (1)

1.2.2 Describe the physicochemical mechanism you will be exploiting using this adjustment. (2)

1.2.3 Justify your answer in 1.2.1 with a calculation from first principles. (1)

1.3 None of the four protein preparations crystallized, unfortunately. Apart from intrinsic properties of each protein, describe aspects of the experiment that are in your control that you could improve on, and how. (3)

QUESTION 2 **[13]**

2.1 You are asked to sequence the genome of the Moringa plant in order to identify genes that contribute to its medicinal properties. You are given the choice between WGS and hierarchical sequencing. Which one will you select? Motivate your answer and also explain the experimental approach that will be followed in the sequencing strategy. (7)

2.2 Bonitas, a medical aid service provider in SA, has recently proposed genome sequencing for disease genes of persons applying for health insurance. The Minster of Health, Dr Aaron Motsoaledi, contacts you in this regard to enquire your opinion. Scientifically debate three reasons in support of and three reasons opposing such a mandate. (6)

QUESTION 3 **[24]**

3.1 Briefly discuss how a yeast cell may be engineered into (i) producing useful plant secondary metabolites and (ii) "humanizing" (authentic glycosylation) proteins. (7)

3.2 Briefly discuss the application of each of the following techniques:

3.2.1 TAR (1)

3.3.2 Yeast-two hybrid system (2)

3.3 Use an annotated, detailed diagram, or give a written explanation, of how homologous recombination and the selectable/counter selectable nature of the uracil phenotype can be used to introduce a desired mutation into a heterologous gene X carried in a YAC (as in the example figure below). (8)

3.4 Use a detailed diagram, or give a written explanation, to describe how a PCR-based deletion strategy, relying on homologous recombination, may be used to knock-out the yeast ORF in the figure below. (6)

QUESTION 4 **[10]**

Read the abstract from the following article and answer the questions below:

Transgenic Res
DOI 10.1007/s11248-011-9510-1

ORIGINAL PAPER

Functional stacking of three resistance genes against *Phytophthora infestans* in potato

Suxian Zhu · Ying Li · Jack H. Vossen · Richard G. F. Visser · Evert Jacobsen

Abstract: Functional stacking of broad spectrum resistance (R) genes could potentially be an effective strategy for more durable disease resistance, for example, to potato late blight caused by the fungus, *Phytophthora infestans* (Pi). For this reason, three broad spectrum potato R genes (Rpi), Rpi-sto1 (*Solanum stoloniferum*), Rpi-vnt1.1 (*S. venturii*) and Rpi-blb3 (*S. bulbocastanum*) that belong to the coiled-coil NBS leucine-rich repeat (LRR) class of plant *R* genes were selected, combined into a single binary vector pBINPLUS and transformed into the susceptible potato cultivar Desiree, using *Agrobacterium* mediated transformation. The presence of the Rpi genes was determined by PCR. Biological activity of the Rpi genes was determined by Pi inoculation in detached leaf assays or by agro-infiltration using Avr effectors matching the introduced Rpi genes. Among the 550 kanamycin resistant regenerants, 28 were further investigated by gene specific PCRs. All regenerants were positive for the nptII gene and 23 of them contained the three Rpi genes, referred to as triple Rpi gene transformants. Detached leaf assay and agroinfiltration of avirulence (Avr) genes showed that the 23 triple Rpi gene transformants were resistant to the selected isolates and showed hypersensitive response (HR) with the three Avr effectors indicating functional stacking of all the three Rpi genes. It is concluded that Avr genes, corresponding to the R genes to be stacked, must be available in order to assay for functionality of each stack component. The resistance spectrum of these 23 triple Rpi gene transformants was, as expected, a sum of the spectra from the three individual Rpi genes. This is the first example of a one-step approach for the simultaneous domestication of three natural R genes against a single disease by genetic transformation.

4.1 The above article is an example of which generation of transgenic crops. Motivate your answer. (2)

4.2 Which method was used to transform the R gene into the potato cultivar? Why can this method be considered a '**natural plant genetic engineering'** method? (3)

4.3 Which **selectable marker gene** was used? What are 'selectable markers' and why are they used in Plant Biotechnology? (2)

4.4 Which method was used as a 'molecular marker' to confirm the presence of the three resistance gene in the transformed lines? (1)

4.5 Explain a method that can be used by companies to control the spread of this genetic material from transgenic crops. (2)

QUESTION 5 **[10]**

5.1 For pathogen detection via PCR, DNA or RNA needs to be isolated form the clinical samples, such as human secretions, fluids, tissue, etc. Problems may be encountered due to inhibitors of the *Taq* DNA Polymerase within the samples. Explain one method that can be used to verify/confirm the presence of such chemicals in a sample. (2)

5.2 The **BD Affirm™ VPIII** is a microbial identification system based on an automated DNA probe sandwich assay and allows the quick identification of the three major causes of vaginitis/bacterial vaginosis, i.e. *Candida, Gardnerella* and *Trichomonas.* Explain the principle of the **sandwich hybridization** detection technique used by this system in detail. (8)

QUESTION 6 **[10]**

Drug Discovery is the fundamental first phase of the Drug Discovery and Development pipeline and involves target identification, assay development, LEAD identification and LEAD optimisation.

6.1 Describe why the well-known paradigm of "fail early and fail cheap" specifically applies to the drug discovery phase. (3)

6.2 During Target Identification, two major decisions need to be made. Name these two decisions and describe the considerations that need to be made in each. (5)

6.3 What is the end goal of the drug discovery phase? (2)

QUESTION 7 **[10]**

Name three types of vaccines currently in commercial use. Briefly explain the basis upon which each type of vaccine elicits immunity and list the major advantage and disadvantage of each of the three types of vaccines. (10)

QUESTION 8 **[10]**

8.1 Discuss the differences, advantages and disadvantages of SHF and SSCT approaches to biofuel production. (6)

8.2 Define the term ‘systems biology approaches’ and explain how these methods can be used to improve biofuel production. (4)