UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: BICX04 CURRENT ADVANCES IN BIOTECHNOLOGY
SEMESTER	: Second
EXAM	: SSA January 2019

DATE	: January 2019	**SESSION**	: 08:00-11:00
ASSESSOR(S)	: PROF LA PIATER		
MODERATOR	: DR L STEENKAMP		
DURATION	: 3 HOURS	**MARKS**	: 100

NUMBER OF PAGES: 6 PAGES (including this page)

INSTRUCTIONS:

1. Answer ALL THE QUESTIONS.
2. Number your answers clearly

QUESTION 1: Plant Biotechnology **[10]**

Briefly explain the genetic engineering strategies that can be used to create **virus resistance** in transgenic crops and illustrate using appropriate examples.

QUESTION 2: Molecular Diagnostics **[10]**

2.1. Provide at least two applications of real time PCR. (2)

2.2. Describe the chemistry of a Taqman probe and the advantages of using them in qPCR over SYBR green. (8)

QUESTION 3: Biotechnology-based Drug Discovery **[15]**

3.1. State the drug discovery process in sequence and indicate how long this process takes on average. (5)

3.2. Looking at the following 3 terms/technologies, namely Antisense, Biomarker, Screening:

3.2.1 What is the main difference between antisense (oligonucleotide technologies) and RNAi? (1)

3.2.2 Explain how antisense technologies aid in target validation. (2)

3.2.3 How can biomarkers be used during drug discovery, and where are they relevant? (2)

3.2.4 Give an example of a specific biomarker and how it can used to assess drug efficacy. (2)

3.2.5 What are the properties of an ideal high throughput screening procedure and mention at least 1 advantage and 1 disadvantage of this technology? (3)

QUESTION 4: Vaccines **[15]**

4.1. Disease caused by which one of the following bacteria can be prevented by a toxoid vaccine?

a) *Neisseria meningitidis*
b) *Corynebacterium diphtheriae*
c) *Salmonella typhi*
d) *Haemophilus influenzae*

4.2. The two most common types of viral vaccines are killed vaccines and live, attenuated vaccines. Regarding these vaccines, which one of the following statements is the MOST accurate?

a) Killed vaccines induce a longer-lasting response than do live, attenuated vaccines.
b) Killed vaccines induce a broader range of immune responses than do live, attenuated vaccines.
c) Killed vaccines are no longer used in this country because they do not induce secretory IgA.
d) Killed vaccines are safer to give to immunocompromised patients than are live, attenuated vaccines.

4.3. The MOST important protective function of the antibody stimulated by tetanus immunization is:

a) To prevent adherence of the pathogen
b) To opsonize the pathogen (Clostridium tetani)
c) To neutralize the toxin of the pathogen
d) To prevent growth of the pathogen

4.4. Passage of antibodies from mother to infant is called

a) Passive immunity
b) Active immunity
c) Innate immunity
d) Defense

4.5. What is vaccine efficacy, and how can it be measured? (2)

4.6. Explain how conjugate vaccines are able to generate a T-cell dependent immune response. (3)

4.7. Give any two mechanisms of antibody action. (2)

4.8. Give any four mechanisms of adjuvant action. (4)

QUESTION 5: Tissue Engineering [15]

Discuss, in detail, the procedure for generating a knockout mouse through gene targeting by homologous recombination.

QUESTION 6: Biofuels [15]

6.1. A dairy farmer situated in a rural area struggles to run his farm due to electricity cut off now. However, the dairy cows produce a lot of cow dung.

6.1.1. Explain how this waste can be used to produce biomethane. (2)

6.1.2. Explain how the biomethane can be converted to electricity to run the farm? (2)

6.2. Elaborate on the differences, advantaged and disadvantages of SHF and SSCF approaches to biofuel production. (6)

6.3. Define the term ‘Metabolic engineering’ and explain how this method can be used to improve enzymatic hydrolysis and conversion of biomass for ethanol production. (5)

QUESTION 7: Crystallography **[15]**

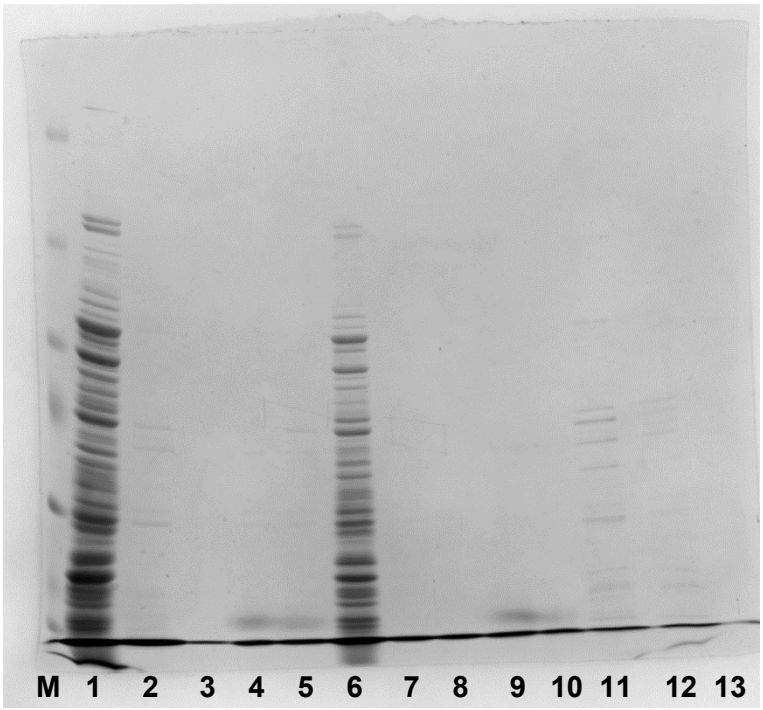


Figure 1: SDS-PAGE of one of three of the protein isolations performed during the practical end of July. M is Molecular weight marker. Lanes 1-5 are group 1: supernatant, lysate flow through, wash buffer, elution 300, elution 500. Lanes 6-10 are group 2, same labels. Lanes 11-13 are group 3: supernatant, lysate flow through, wash buffer.

7.1. Consider the annotated SDS-PAGE above, of the aliquots collected during the purification of three different constructs by three groups in the practical.

7.1.1. The bands in lanes 4 and 5 are weak, presumably because the buffers contained 2M NaCl (instead of 500mM), causing the protein to be eluted with the washes. Explain why this protein is then not visible in lane 3 (wash step). (2)

7.1.2. During the practical, you discovered the aliquots with SDS had not been boiled, because this was overlooked in the protocol. Discuss why this would cause the gel to show misleading results, and point to examples of this in the gel. (2)

7.1.3. During the practical, you concluded the gel needed to be prepared at higher % acrylamide. Discuss what aspect of the gel prompted this conclusion, describe why the higher % acrylamide would mitigate this, and suggest one alternative for achieving the same mitigation. (3)

7.2. Discuss why it is necessary to use X-rays in order to observe atomic detail. (2)

7.3. Discuss why the presence of bubbles in your protein solution indicates presence of low-quality protein. (3)

7.4. Some of the groups achieved final protein concentrations of only 5 mg/ml for their sample, which is considerably lower than the rule-of-thumb protein concentration of 10 mg/ml required in the crystallization drop.

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

7.4.2. Describe the physical chemical mechanism you will be exploiting using this adjustment. (2)

QUESTION 8: Application of knowledge in project [5]

During your individual BICX00 research project, you made use of “omics” (genomics, proteomic, metabolomics, *etc.*) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.

MEMO

QUESTION 1: Plant Biotechnology [10]

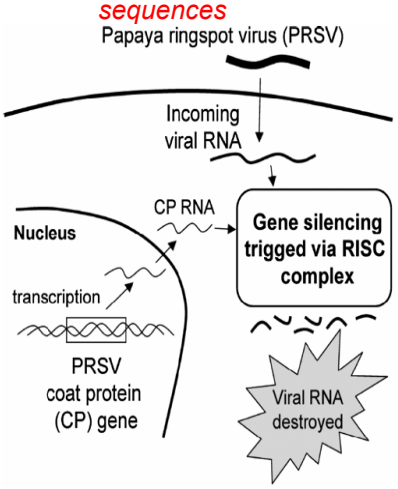
Briefly explain the genetic engineering strategies that can be used to create **virus resistance** in transgenic crops and illustrate using appropriate examples.

Resistance against viral pathogens generated using:

(A) PDR (pathogen derived resistance) based approaches, where protection is achieved by (i) expression of native/modified viral proteins or (ii) Protection at transcriptional level (gene silencing).
(B) Non-pathogen derived antiviral agents

Examples :

1) PDR (pathogen derived resistance) based approaches by **gene silencing** - protection from **Papaya Ringspot Virus**
PTGS =RNA silencing or RNA interference
Seq-specific breakdown mechanism in plants & eukaryotes which represents a natural antiviral defense mechanism.
Involves cleavage of dsRNA to 21-25 nt siRNAs by Dicers
siRNAs interact with host proteins to form RNA-induced silencing complexes (RISC) - ds siRNAs are unwound and used as guides in the specific binding and destruction of targeted mRNA molecules.
PTGS activated in transgenic plants by the introduction of dsRNAs homologous to viral sequences



2) PDR (pathogen derived resistance) based approaches where protection is achieved by (1) expression of native/modified viral proteins

Coat protein mediated resistance (CPMR) - tomato plants expressing the CP of TYLCV exhibited delayed symptom development that was dependent on the expression levels of the transgenic CP (Kunik *et al.,* 1994)

Rep protein (viral replication and transcription) tolerance or strain specific immunity to ACMV, TYLCV-SV, ToLCNDV, BGMV, MSV -broader resistance

3) Non-pathogen derived antiviral agents

Mimicked the hypersensitive reaction (HR) used by plants at the initial site of infection.
Using the cell death-inducing **ribonuclease barnase** and **barstar** genes from *Bacillus amyloliquefaciens* (Taylor *et al.,* 2004; Pakniat *et al.,* 2010).

Barnase is a ribonuclease (RNase) and **barstar** its inhibitor. In the absence of geminivirus infection the 2 transgenes expressed at similar levels, *-no RNase production*. By placing barnase under the control of a viral virion-sense promoter that is activated during geminivirus infection, and barstar under the control of a viral complementary-sense promoter that is repressed during geminivirus infection, an infected cell should over-express barnase relative to barstar and die before the infecting virus can replicate and move.

Successfully applied for ACMV and Tomato leaf curl virus-Australia (TLCV-AU)
Expression of DNA binding proteins (Rep-based artificial zinc finger proteins, Sera, 2005),

Peptide aptamers that binds strongly to the Rep protein and interferes with its intracellular function

Use of **GroEL** homologues that bind to the viral CP as a tool to trap or capture viral particles and disrupt geminivirus infections or lessen their harmful effects (Akad *et al.,* 2007).

QUESTION 2: Molecular Microbial Diagnostics [10]

2.1. Provide at least two applications of real time PCR. (2)

- Gene Expression Studies
- Methylation Specific PCR (HRM)
- Microarray Validation
- Transgenic Analysis
- GMO Testing
- Viral/Bacterial Load Studies
- Chromatin Immunoprecipitation (ChIP)
- Allelic Discrimination/SNP (HRM)

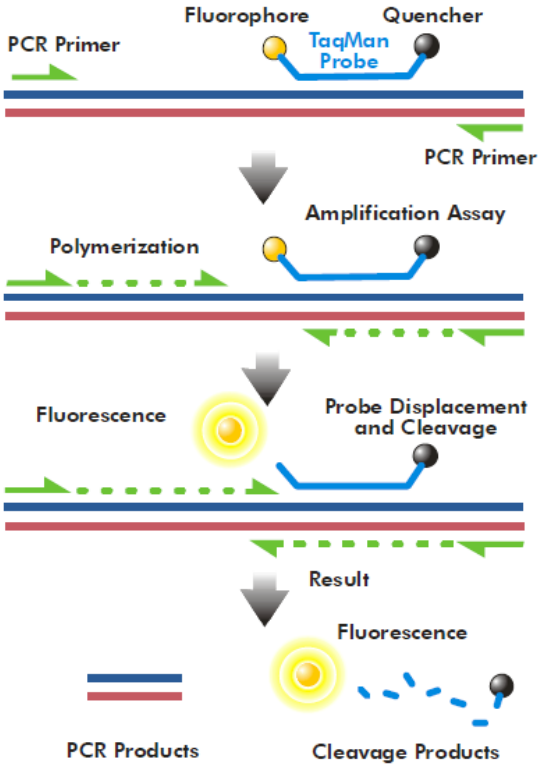
2.2. Describe the chemistry of a Taqman probe and the advantages of using them in qPCR over SYBR green.

This chemistry uses a fluorogenic probe to enable the detection of a specific PCR product as it accumulates during PCR.

How it works:
- The probe is designed to anneal to one strand of the target sequence just slightly downstream of one of the primers.
- Light emission from the reporter fluorophore (R) is quenched because of its proximity to the quencher (Q).Cleavage by Taq polymerase separates the reporter and quencher allowing fluorescence
- When the probe is intact, the proximity of the quencher reduces the fluorescence emitted by the reporter dye. During PCR, the forward primer is extended by the Taq DNA polymerase until it reaches the probe.

• At this point, the exonuclease activity of the Taq DNA polymerase displaces and cuts up the probe, releasing the reporter dye and the quencher. Once they are no longer in close proximity, the reporter dye emits fluorescence of a particular wavelength that can be detected.

Advantages:
•Increased specificity due to presence of primers and probe.
•Use when the most accurate quantitation of PCR product accumulation is desired.
•Option of detecting multiple genes in the same well (multiplexing).



QUESTION 3: Biotechnology-based Drug Discovery **[10]**

3.1. State the drug discovery process in sequence and indicate how long this process takes on average. (5)

Target Identification (or Selection); Target Validation; Assay (or Screen) development; Lead identification (or Hit to Lead) **(half mark)**; Lead optimization. **(half mark)**This process takes 5-6 years on average.

3.2. Looking at the following 3 terms/technologies, namely Antisense, Biomarker, Screening:

3.2.1 What is the main difference between antisense (oligonucleotide technologies) and RNAi? (1)

Antisense technology deals with single strand antisense oligonucleotides, while RNAi utilises a double strand oligonucleotide

3.2.2 Explain how antisense technologies aid in target validation. (2)

Can be used to knockdown specific targets. This may mimic or alleviate disease phenotypes, thereby validating the targets involvement in the disease.

3.2.3 How can biomarkers be used during drug discovery, and where are they relevant? (2)

Biomarkers can be used to identify a target, predict and confirm target binding of the drug, determine mechanism of action of a drug, pharmacokinetics and toxicity of a drug. This makes them relevant throughout the drug discovery process.

3.2.4 Give an example of a specific biomarker and how it can used to assess drug efficacy. (2)

I will specifically mention to the candidates the HIV RNA biomarker, and how it is used to monitor viral load in response to therapy

3.2.5 What are the properties of an ideal high throughput screening procedure and mention at least 1 advantage and 1 disadvantage of this technology? (3)

Advantages	Disadvantages
Enhances speed of assay	Requires expensive instrumentation
Significantly increases the quantity of compounds screened	Necessitates a highly skilled operator
Improves reproducibility of assays	Necessitates the acquisition of compound libraries
Increases researcher 'walk-away' time	Necessitates large quantities of reagents

Increases the likelihood of identifying a LEAD compound	

QUESTION 4: Vaccines **[11]**

4.1. Disease caused by which one of the following bacteria can be prevented by a toxoid vaccine?

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4.4. Passage of antibodies from mother to infant is called

a) Passive immunity
b) Active immunity
c) Innate immunity
d) Defense

4.5. What is vaccine efficacy, and how can it be measured? (2)

It is the percentage reduction of disease in a vaccinated group of people compared to an unvaccinated group, using the most favourable conditions. It can be best measured using double-blind, randomized, clinical controlled trials.

4.6. Explain how conjugate vaccines are able to generate a T-cell dependent immune response. (3)

Polysaccharide molecules are chemically conjugated to a carrier protein. Linkage helps the immature immune system react to polysaccharide coatings, leading to increased immunogenicity through T-cell help which allows for a higher quantity of memory B and T cells and antibodies which are sustained for longer.

4.7. Give any two mechanisms of antibody action. (2)

1. Precipitation of soluble antigens
2. Agglutination of foreign cells
3. Neutralization
4. Enhanced phagocytosis
5. Complement activation leading to cell lysis
6. Stimulates inflammation

4.8. Give any four mechanisms of adjuvant action. (4)

1. Sustained release of Ag at the site of injection (depot effect)
2. Up-regulation of cytokines & chemokines
3. Cellular recruitment to the site of injection
4. Increased Ag uptake & presentation to APCs
5. Activation & maturation of APCs
6. Increased Ag processing & presentation by APCs
7. Migration of APCs to lymph nodes to interact with Ag-specific T/ B cells
8. Activation of Ab secreting B cells and/or effector CD8+ T cell responses

QUESTION 5: Stem Cells **[15]**

Discuss, in detail, the procedure for generating a knockout mouse through gene targeting by homologous recombination.

- Generation of a targeting vector
  Two NB aspects are:
  - the vector must contain at least 5-10 kb of isogenic DNA homologous with the sequence to be targeted, and comprises a short (for eary PCR screening) and long arm
  - vector must be a positive and negative cassette. Here a positive selection marker (Neo) is used which allows transgenic ES cells to be selected on neomycin sulphate. With regard to the negative marker, such a gene (HSVtk) is cloned outside of the homologous sequence in the targeting vector and will thus not insert into the genome when homologous recombination occurs. If however, random integration takes place, the expressed gene product will kill the ES cells, thus serving as an additional marker

- ES cell transfection
  - Linearized vector is electroporated into a large number of ES cells which are collected from 3.5 day old blastocysts/embryos having an agouti colour
  - The transfected cells are then plated onto feeder cells (fibroblasts because these secrete LIF which prevents differentiation)
  - 24 h later, the selection process begins

- Identification of ES cells targeted by homologous recombination
  - Discrete colonies are picked and dissociated into single cells by treatment with trypsin
  - Cells are then separated into duplicated in 2 microtitre plates where one plate serves for screeing purposes (isolation of gDNA, PCR, Southern and sequencing), while the other can then be used for the injection into blastocysts

- Injection of ES cells into blastocysts
  - Blastocysts are collected from the uterus of a donor female with black coat and ES cells injected via the blastocoel
  - The embryos are then culture for a few hours afterwhich they are transferred to the uterus of the “foster” mother and pups are born ~ 17 days later

- Identification of chimeric mice and breeding to generate KO mice
  - Chimeric mice initially identified by coat colour (agouti colour). These are then mated with wild-type mice to test for germline transmission
  - Following, the heterozygous mice are mated to produce mice homozygous for the targeted mutation

QUESTION 6: Biofuels **[15]**

6.1. A dairy farmer situated in a rural area struggles to run his farm due to electricity cut off now. However, the dairy cows produce a lot of cow dung.

6.1.1. Explain how this waste can be used to produce biomethane. (2)

Waste comes from the dairy farm in to the biogas plant
Was is pumped into a tank where it is heated at 158℃ for 1 hr.
The sludge created flows in the oxygen free tanks where it remains foe 20-40 days at 100℃. Microbes int tanks convert organic material into biogas and fertilizer (sometimes)
To make biomethane, biogas is upgraded to a higher methane concentration of 97% the quality of natural gas.
Biomethane is used as fuel for transportation.

6.1.2. Explain how the biomethane can be converted to electricity to run the farm? (2)

Biogas technology, the generation of a combustible gas from anaerobic biomass digestion, is a well-known technology. There are already millions of biogas plants in operation throughout the world. Whereas, using the gas for direct combustion in household stoves or gas lamps is common, producing electricity from biogas is still relatively rare in most developing countries.
Theoretically, biogas can be converted directly into electricity by using a fuel cell. However, this process requires very clean gas and expensive fuel cells. The conversion of biogas to electric power by a generator set is much more practical.
In most cases, biogas is used as fuel for combustion engines, which convert it to mechanical energy, powering an electric generator to produce electricity.

6.2. Elaborate on the differences, advantaged and disadvantages of SHF and SSCF approaches to biofuel production. (6)

Separate hydrolysis & fermentation (SHF)
Biomass cellulose hydrolyzed with cellulases
Glc released is then fermented to ethanol

The process concept SHF involves a separation of the hydrolysis and fermentation by running the reactions in separate units. Different factors influence the efficiency of the hydrolysis of lignocellulosic material, including both pre-treatment conditions and process conditions. The factors can be separated in two groups; substrate-related and enzyme-related. The process is cheap to rum, however produces less ethanol due to the above mention factors.

The enzymatic hydrolysis can be performed simultaneously with the co-fermentation of glucose and xylose in a process referred to as simultaneous saccharification and co-fermentation (SSCF). Besides reduced capital cost, SSCF process offers several advantages which include continuous removal of end-products of enzymatic hydrolysis that inhibit cellulases or β-glucosidases and higher ethanol productivity and yield than separate hydrolysis and fermentation.

6.3. Define the term 'Metabolic engineering' and explain how this method can be used to improve enzymatic hydrolysis and conversion of biomass for ethanol production. (5)

Metabolic engineering: this term refers to the use of recombinant DNA technologies to create new metabolic or biosynthetic pathways in host organisms, or to enhance existing pathways, through the engineered, coordinated expression of several heterologous or enhanced enzymes in the desired pathway.
- Improved cellulase and hemicellulase production economics via microbe-based production systems,
- Improved fermentation strains that efficiently utilize both hemicellulose (C5) and cellulosic (C6) sugars. *Either by engineering microbes or single micro-organism that can ferment bot C5 and C6 sugars*

In the fermentation platform, plant biomass substrate cost is reduced by using all the plant-derived sugars available, C5 and C6, for conversion to ethanol. There is no single naturally occurring microbial biocatalyst that effectively and efficiently converts all C5 and C6 plant-derived sugars to one product with high yields, be it lactic acid or ethanol.

QUESTION 7: Crystallography **[15]**

7.1. Consider the annotated SDS-PAGE above, of the aliquots collected during the purification of three different constructs by three groups in the practical.

7.1.1. The bands in lanes 4 and 5 are weak, presumably because the buffers contained 2M NaCl (instead of 500mM), causing the protein to be eluted with the washes. Explain why this protein is then not visible in lane 3 (wash step). (2)

Wash volume is very large, so protein would have been very dilute; the aliquot loaded onto gel was too small to reveal the protein.

7.1.2. During the practical, you discovered the aliquots with SDS had not been boiled, because this was overlooked in the protocol. Discuss why this would cause the gel to show misleading results, and point to examples of this in the gel. (2)

Most of protein didn't get denatured, so only small fraction will have migrated as expected. Examples: weak protein bands (lanes 4,5,9,10), no big overexpression band in lysate (lanes 1 or 6), very weak bands in lanes 11, 12.

7.1.3. During the practical, you concluded the gel needed to be prepared at higher % acrylamide. Discuss what aspect of the gel prompted this conclusion, describe why the higher % acrylamide would mitigate this, and suggest one alternative for achieving the same mitigation. (3)

Target protein runs too close to gel front; denser polymer makes proteins run more slowly, so large proteins separate better; run the gel for less time.

7.2. Discuss why it is necessary to use X-rays in order to observe atomic detail. (2)

Resolving atoms requires wavelengths of a similar scale to inter-atom distances. X-rays are ~1A, and bond-lengths are also ~1A

7.3. Discuss why the presence of bubbles in your protein solution indicates presence of low-quality protein. (3)

Folded protein has hydrophobic groups buried; air at the air-water interface is hydrophobic, so protein tends to unfold against the surface and stabilise the surface; bubbles are large air-water interfaces that are stabilised by a surfactant: their presence indicates the protein now acts as a surfactant, meaning some of it has unfolded.

7.4. Some of the groups achieved final protein concentrations of only 5 mg/ml for their sample, which is considerably lower than the rule-of-thumb protein concentration of 10 mg/ml required in the crystallization drop.

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

Change the ratio of the protein and precipitant you add to the drop

7.4.2. Describe the physical chemical mechanism you will be exploiting using this adjustment. (2)

The solution in the drop is at lower concentration than in the reservoir (or water is MORE concentrated in drop), so vapour pressure causes water to leave drop and be absorbed by reservoir, until the two solutions are in equilibrium.

QUESTION 8: Application of knowledge in project **[5]**

During your individual BICX00 research project, you made use of “omics” (genomics, proteomic, metabolomics, *etc.*) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.

Pending of student choice. Aspects to address would be:

Sample type and isolation

Method of analysis

Control *versus* treated

Interpretation of results