UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: BICX04 CURRENT ADVANCES IN BIOTECHNOLOGY
SEMESTER	: Second
EXAM	: November 2018

DATE	: 12 November 2018	**SESSION**	: 08:30-11:30
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]

Targeted genome editing using artificial nucleases has the potential to accelerate plant breeding by providing the means to modify genomes rapidly in a precise and predictable manner. Describe the CRISPR/Cas9 system that can be used as a gene editing method.

QUESTION 2: Molecular Diagnostics [10]

You are a young Researcher interested in investigating virus-plant interactions and you are particularly interested in the gene expression level of a resistance (R) gene, pathogenesis-related protein 5 (PR5), in infected potato leaf tissue after infection with the PVX virus. You design a preliminary study to measure the expression of PR5 relative to the 18S rDNA gene in 3 biological replicates. You generate the following data:

	CT values	
Sample	18S	PR5
Healthy control	17.19	30.57
Healthy control	16.96	30.73
Healthy control	17.07	30.76
PVX-infection	18.04	26.11
PVX-infection	17.99	25.70
PVX-infection	17.90	25.64

2.1. Which nucleic acid-based method did you choose to measure the gene expression levels of PR5? Justify your answer? (2)

2.2. Calculate the gene expression levels of PR5 and indicate whether PR5 expression is up-regulated or down-regulated in PYX-infected leaf tissue compared to the healthy control tissue. Show all calculations. (8)

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

3.1. What is the difference between a small molecule and a biologic? (2)

3.2. What are the main properties of an ideal drug target? (2)

3.3. What is the parameter during lead identification that measures the efficacy of the lead compound? (1)

3.4. Looking at the following 3 technologies, namely RNAi, Microarray and Virtual drug development:

3.4.1. Give a brief description of each. (3)

3.4.2. Which type of "omics" would be a relevant classification for each (there can be more than one)? (3)

3.4.3. Choose two of these technologies and state at which points of the drug discovery process would these be relevant, giving a justification for each (4)

QUESTION 4: Vaccines **[15]**

4.1. Which disease mainly affects children under 5 and remains endemic in 3 countries?

a) Rubella
b) Polio
c) Pneumonia
d) Measles
e) Tetanus

4.2. The biggest challenges to improving global vaccine coverage are:

a) Limited resources
b) Competing health priorities
c) Poor management of health systems
d) All of the above

4.3. Which of the following is NOT a vaccine-preventable disease?

a) Cervical cancer
b) Polio
c) Hepatitis B
d) Asthma
e) Measles

4.4. Successful immunisation can be impaired by

a) Adjuvants
b) Cytokines
c) Cloning the vaccine
d) Maternal antibody

4.5. From this list, the most effective vaccine is against

a) Staphylococci
b) Tuberculosis
c) Tetanus
d) Adenovirus

4.6. The first vaccine for human use produced using recombinant DNA technology was the:

a) Hepatitis B vaccine
b) Hepatitis A vaccine
c) MMR vaccine
d) Polio vaccine

4.7. List any three different types of vaccine antigens, give one advantage and one disadvantage for each, and include an available example of each vaccine. (9)

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

5.1. Comprehensively define 3 (stem)-cell variants and their use in tissue engineering. (9)

5.2. Summarise 3 advantages and 3 disadvantages of generating KO mice by gene targeting in ESC. (6)

QUESTION 6: Biofuels [15]

6.1. Describe (using both schematic and wording) in detail how biodiesel is produced. Is there room for improvement? (5)

6.2. Various feedstocks are used to produce biofuels. Using corn as a feedstock, explain in detail the two processes employed for corn processing. (10 X ½ = 5)

6.3. Biotechnology is the exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms to produce desired products. Using any two genetic manipulation approaches, explain how you will enhance both cellulose break down and sugar fermentation. Use 1 approach for cellulose and 1 approach for sugar fermentation. (5).

QUESTION 7: Crystallography [15]

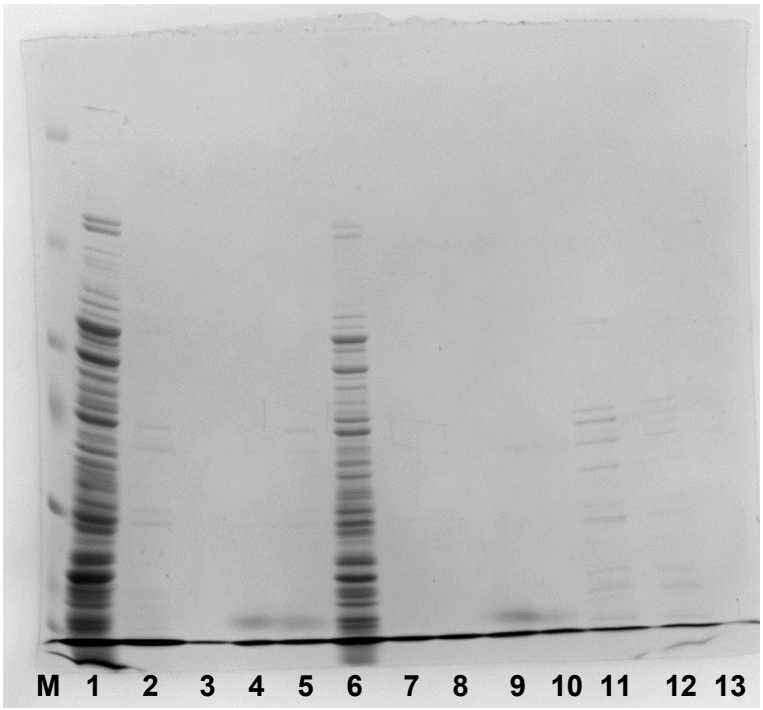

Figure 1: SDS-PAGE of one of three of the protein isolations performed during the practical end of July. M is Molecular weight marker. Lanes 1-5 are group 1: supernatant, lysate flow through, wash buffer, elution 300, elution 500. Lanes 6-10 are group 2, same labels. Lanes 11-13 are group 3: supernatant, 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 in July 2018:

7.1.1. Assess and justify whether you can be confident that any of the targeted proteins were indeed present in cultured bacteria. Point out the evidence clearly. (2)

7.1.2. During the practical, you discovered the NaCl concentration in all buffers was 4x too high (2M instead of 500mM). How did this affect the results discussed in 7.1.1? (2)

7.1.3. Discuss 3 ways in which the above gel fails to yield the information it was meant to provide, and how you would fix this if you repeated the experiment. (3)

7.2. Discuss why crystals are necessary for 3D structure determination using X-rays. (2)

7.3. Discuss how entropy drives both micelle formation and therefore protein folding. (3)

7.4. Describe a general strategy for achieving larger crystals simply by changing concentrations of crystallization components, and explain the principle; sketch a schematic phase diagram if it simplifies your answer. (3)

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]

Targeted genome editing using artificial nucleases has the potential to accelerate plant breeding by providing the means to modify genomes rapidly in a precise and predictable manner. Describe the CRISPR/Cas9 system that can be used as a gene editing method.

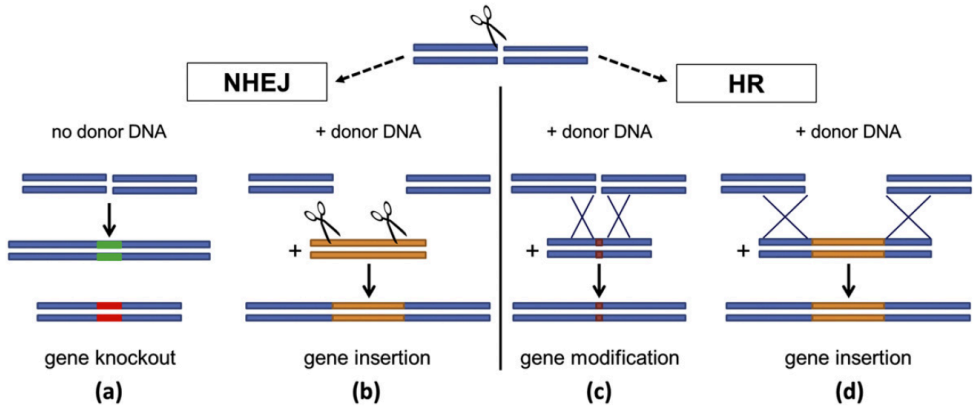

Fig. 1. Genome editing with site-specific nucleases. Double-strand breaks induced by a nuclease at a specific site can be repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR). (a) Repair by NHEJ usually results in the insertion (green) or deletion (red) of random base pairs, causing gene knockout by disruption. (b) If a donor DNA is available, which is simultaneously cut by the same nuclease leaving compatible overhangs, gene insertion by NHEJ can also be achieved. (c) HR with a donor DNA template can be exploited to modify a gene by introducing precise nucleotide substitutions or (d) to achieve gene insertion.

RNA guided engineered nucleases: type II clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 (CRISPR-associated) system from Streptococcus pyogenes. CRISPR/Cas systems are part of the adaptive immune system of bacteria and archaea, protecting them against invading nucleic acids such as viruses by cleaving the foreign DNA in a sequence-dependent manner. The immunity is acquired by the integration of short fragments of the invading DNA known as spacers between two adjacent repeats at the proximal end of a CRISPR locus. The CRISPR arrays, including the spacers, are transcribed during subsequent encounters with invasive DNA and are processed into small interfering CRISPR RNAs (crRNAs) approximately 40 nt in length, which combine with the transactivating CRISPR RNA (tracrRNA) to activate and guide the Cas9 nuclease. This cleaves homologous double-stranded DNA sequences known as protospacers in the invading DNA. A prerequisite for cleavage is the presence of a conserved protospacer-adjacent motif (PAM) downstream of the target DNA, which usually has the sequence 5′-NGG-3′ but less frequently NAG. Specificity is provided by the so-called ‘seed sequence’ approximately 12 bases upstream of the PAM, which must match between the RNA and target DNA (Fig. 2). Fig. 1. Genome editing with site-specific nucleases. Double-strand breaks induced by a nuclease at a specific site can be repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR). (a) Repair by NHEJ usually results in the insertion (green) or deletion (red) of random base pairs, causing gene knockout by disruption. (b) If a donor DNA is

available,which is simultaneously cut by the same nuclease leaving compatible overhangs, gene insertion byNHEJ can also be achieved. (c) HRwith a donorDNA template can be exploited to modify a gene by introducing precise nucleotide substitutions or (d) to achieve gene insertion.

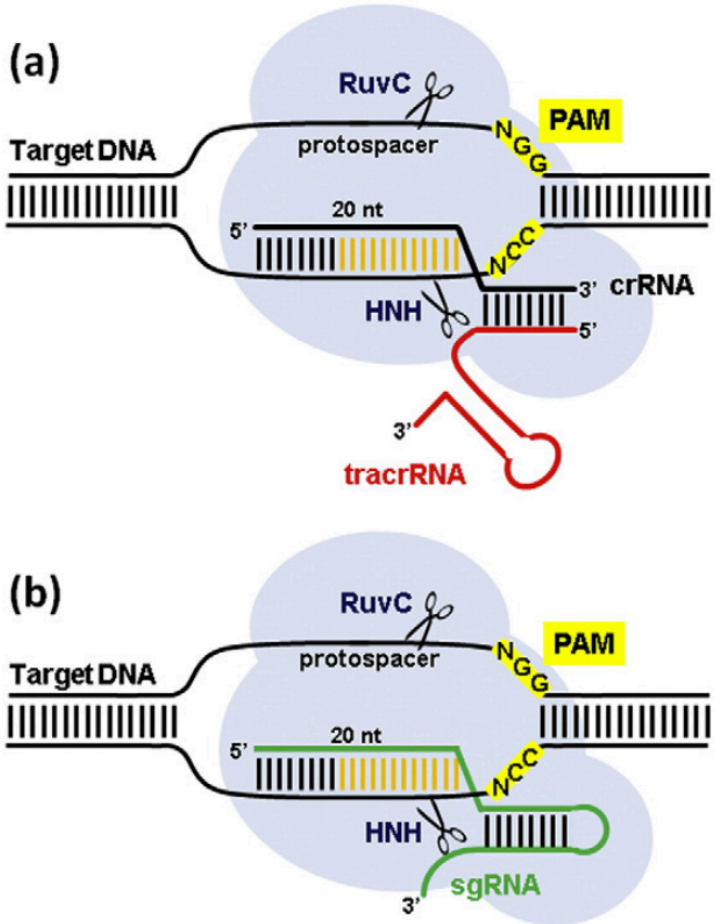

Fig. 2. RNA-guided DNA cleavage by Cas9. (a) In the native system, the Cas9 protein (light blue) is guided by a structure formed by a CRISPR RNA (crRNA, in black), which contains a 20-nt segment determining target specificity, and a trans-activating CRISPR RNA (tracrRNA, in red), which stabilizes the structure and activates Cas9 to cleave the target DNA (protospacer). The presence of a protospacer-adjacent motif (PAM, in yellow), i.e., an NGG (or less frequently NAG) sequence directly downstream from the target DNA, is a prerequisite for DNA cleavage by Cas9. Among the 20 RNA nucleotides determining target specificity, the so-called seed sequence of approximately 12 nt (in orange) upstream of the PAM is thought to be particularly important for the pairing between RNA and target DNA. (b) Cas9 can be reprogrammed to cleave DNA by a single guide RNA molecule (gRNA, in green), a chimera generated by fusing the 3′ end of the crRNA to the 5′ end of the tracrRNA.

QUESTION 2: Molecular Diagnostics [10]

2.1. Which nucleic acid-based method did you choose to measure the gene expression levels of PR5? Justify your answer? (2)

RT-qPCR used to measure gene expression changes in the leaf tissue sample. RT will have to be performed befpre qPCR because the researcher is interested in gene expression; therefore mRNA/RNA needs to be converted to cDNA first.

2.2. Calculate the gene expression levels of PR5 and indicate whether PR5 expression is up-regulated or down-regulated in PYX-infected leaf tissue compared to the healthy control tissue. Show all calculations. (8)

Students need to calculate the fold change using the basic Livak/ΔΔCt method as shown below:

Where:

GOI- gene of interest (PR5)

HK – Housekeeping gene (18S)

PVX-infected | Non-infected sample (Healthy)

ΔΔCT= ΔCT(treated sample) – ΔCT(untreated sample)

$$\Delta Ct_{(sample)} = Ct_{GOI} - Ct_{HK}$$
$$\Delta Ct_{(calibrator)} = Ct_{GOI} - Ct_{HK}$$
$$\Delta\,\Delta Ct = \Delta Ct_{sample} - \Delta ct_{calibrator}$$

Step 1: Find the mean/average of the 3 replicates.

Sample	Average CT Values	
Healthy	17.07	30.69
PVX-infected	17.98	25.82

Step 2: Calculate the ΔCT value for PR5 expression in PVX-infected tissue. Also, calculate the ΔCT value for PR5 expression in healthy tissue.

ΔCT (PVX-infected) → $CT_{PR5} - CT_{18S}$ → 25.82 – 17.98 = 7.84

ΔCT (Healthy) → $CT_{PR5} - CT_{18S}$ → 30.69 – 17.07 = 13.62

Step 3: Then, calculate the difference between ΔCTE and ΔCTC (ΔCTE-ΔCTC) to arrive at the Double Delta Ct Value (ΔΔCT)

Therefore:

ΔΔCT = ΔCT (PVX-infected) - ΔCT (Healthy) → 7.84 – 13.62 = -5.78

Step 4. Since all calculations are in logarithm base 2, every time there is twice as much DNA, your Ct values decrease by 1 and will not halve. You need to calculate the value of 2^-ΔΔCt to get the expression fold change.

$$\text{Normalized } \frac{\text{Unknown}}{\text{Control}} = \frac{2^{-\Delta Ct_{target}}}{2^{-\Delta Ct_{norm}}} = 2^{-\Delta\Delta Ct}$$

$2^{-(\Delta\Delta CT)} \rightarrow 2^{-(-5.78)} \rightarrow$ 54.98 Fold change.

Therefore PR5 highly upregulated. 54.98X higher in PVX-infected leaf tissue compared to healthy control tissue

QUESTION 3: Biotechnology-based Drug Discovery [15]

3.1. What is the difference between a small molecule and a biologic? (2)

Small molecules are usually chemically synthesised compounds that are 500 Daltons or less. Biologics are usually synthesised in living organisms and are large macromolecules such as antibodies or recombinant proteins.

3.2. What are the main properties of an ideal drug target? (2)

Any 2 of the following:

1) Target is disease-modifying and/ or has a proven function in the pathophysiology of a disease
2) A 3-D structure for the protein structure of a close homolog is available
3) Target has a favourable 'assayability'
4) Target expression is not uniformly distributed throughout the body
5) A target/ disease specific biomarker exists to monitor therapeutic efficacy
6) Favourable prediction of potential side effects

3.3. What is the parameter during lead identification that measures the efficacy of the lead compound? (1)

the 50% Inhibitory concentration (IC_{50}) or effective concentration (EC_{50}).

3.4. Looking at the following 3 technologies, namely RNAi, Microarray and Virtual drug development:

3.4.1 Give a brief description of each. (3)

3.4.2 Which type of "omics" would be a relevant classification for each (there can be more than one)? (3)

3.4.3 Choose two of these technologies and state at which points of the drug discovery process would these be relevant, giving a justification for each (4)

1) Description of each can be based on information provided in the reading materials. Namely : "Biotechnology-based Drug Discovery" chapter 11 in the Molecular Biology and Biotechnology, 5th Edition.

2 RNAi (genomics, transcriptomics). Microarray (genomics, transcriptomics, proteomics). Virtual drug screening (proteomics, metabolomics)

3) I would expect that the candidates' responses here would be varied depending on their understanding of the various technologies. They can state at least one, and give a general justification that may be acceptable.

So as an example for RNAi, the answer can be any of the following:

- Target selection. siRNA libraries can be used for explorative research by knocking down groups of genes and thereby characterising novel cellular pathways that may be involved in disease progression
- Target validation. Can be used to knockdown specific targets. This may mimic or alleviate disease phenotypes, thereby validating the targets involvement in the disease.
- Assay development. Can serve as a positive control and provide a guideline for hit selection.

QUESTION 4: Vaccines [10]

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a) Rubella
b) Polio
c) Pneumonia
d) Measles
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4.2. The biggest challenges to improving global vaccine coverage are:

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4.3. Which of the following is NOT a vaccine-preventable disease?

a) Cervical cancer
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4.4. Successful immunisation can be impaired by

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4.5. From this list, the most effective vaccine is against

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4.6. The first vaccine for human use produced using recombinant DNA technology was the:

a) Hepatitis B vaccine
b) Hepatitis A vaccine
c) MMR vaccine
d) Polio vaccine

4.7. List any three different types of vaccine antigens, give one advantage and one disadvantage for each, and include an available example of each vaccine. (9)

	Live attenuated	Inactivated (killed)	subunit vaccines	Toxoid vaccines	Polysaccharide	Conjugate	Recombinant	DNA vaccines
Advantage	1. Very low virulence 2. They will reproduce, but very slowly, also means that less virus must be injected to induce protection 3. Can stimulate generation of memory humoral & cellular immune responses 4. Boosters are required less often 5. Risk of reversion to virulence is small, this risk is smaller in vaccines with deletions	1. Cannot replicate 2. Use of the whole virus stimulates immunity to antigens in their natural conformation on the virus surface (essential for neutralizing antibodies) 3. When manufactured correctly the vaccine is not infectious, but improper inactivation can result in intact and infectious particles 4. Booster shots are required periodically to reinforce the immune response	1. Not infectious, so they can safely be given to immunosuppressed people 2. Less likely to induce unfavourable immune reactions that may cause side effects	1. Toxins are inactivated by treating them with formalin Toxoid = "detoxified" toxins, which are safe for use 2. Same as subunit advantages	1. Immune response is typically T-cell independent, which means that these vaccines are able to stimulate B cells without the assistance of T-helper cells 2. Works well in adults, but are poorly immunogenic in children younger than 2 years of age (likely due to immaturity of the immune system) 3. No immune memory 4. Repeat doses usually do not cause a booster response	1. Linkage helps the immature immune system react to polysaccharide coatings 2. T-cell independent to T-cell dependent, leading to increased immunogenicity and antibody booster response to multiple doses of vaccine 3. Effective in infants	1. can't cause disease 2. Genes for desired antigens are inserted into a vector, usually a virus, that has low virulence 3. The vector expressing the antigen may be used as the vaccine, or the antigen may be purified and injected as a subunit vaccine 4. Allows for mass production 5. much safer than using attenuated HBV	1. uses genes, can't cause disease 2. stimulates viral infection and invokes an immune response from the host 3. allows antigen production to occur in vivo, bypassing the need to produce and purify protein antigen in vitro 4. Relatively easy and inexpensive to design and produce
Disadvantage	1. Risk of reversion to virulence 2. Cannot be used by pregnant individuals	1. Cost 2. Adjuvants are CRITICAL for the use of inactivated vaccines	1. Antigens may not retain their native conformation, so that antibodies produced against the subunit may not recognize the infectious organism 2. The isolated protein does not stimulate the immune system as well as a whole organism vaccine	1. Same as subunit disadvantages	1. Antibody induced has less functional activity than that induced by protein antigens. 2. Predominant antibody produced in response to most polysaccharide vaccines is IgM, and little IgG is produced	1. IgG levels are maintained for a long time	Same as subunit disadvantages	Same as subunit disadvantages
Example	tuberculosis (BCG), oral polio vaccine (OPV), measles, mump, rubella vaccine (MMR), yellow fever vaccine, varicella zoster vaccine	inactivated polio vaccine (IPV), whole cell pertussis vaccine (wP), rabies vaccine, hepatitis A vaccine	whooping cough vaccine and acellular pertussis (aP) vaccine	Tetanus, diphtheria and pertussis toxins are inactivated to create toxoids for use in Infanrixhexa, Infanrix-IPV, Boostrix, and ADT Booster Schedule vaccines.	pneumococcal disease (Pneumococcal vaccines), meningococcal disease (Meningococcal vaccine) and Salmonella Typhi	Haemophilus influenza type B (Hib)	HBV vaccine	None commercially available yet

QUESTION 5: Stem Cells [15]

5.1. Comprehensively define 3 (stem)-cell variants and their use in tissue engineering. (9)

Embryonic stem cells (ESC) divided
pluripotency, & ability to replicate indefinitely
Divide & generate new stem cell or differentiate into tissue lineage

All hPSCs share two useful theoretical properties:
1 - First, they can be maintained in culture for a large number of passages without loss of genomic integrity, which distinguishes them from standard cultured cell lines that are transformed or immortalized and have severely abnormal karyotypes.
2- Second, hPSCs can be differentiated into any of the myriad of somatic cell types in the human body. [In practice, the ability to differentiate into a desired cell type depends on the availability of an efficient protocol to achieve the differentiation, which at present is only true of a small number of cell types

Possible to derive, expand and differentiate somatic cells that are genetically matched to the patient.

Adult stem cells
Somatic cells

Ethically acceptable alternative (no harvesting of embryos)
Small population of undifferentiated cells
Renew themselves & differentiate into major specialized cells
Maintain proper functioning & repair of tissue
Brain, skin, bone marrow, blood, skeletal muscle & liver
NB adult stem cells:
- Brain (neural)
- Bone marrow (hematopoietic & mesenchymal)
- Digestive tract (epithelical)
- Skin (epidermal)

Not all organs contain adult stem cells

Non-Stem cells (mature cells)
~ 300 different types of differentiated cells, each with own function
Lose ability to divide
Differentiated cells manipulated to multiply *in vitro*
Primary cells from healthy tissue is NB
Reinitiating cell division of differentiated cells:
- Genetically modify cells & immortalize them (proliferation inducing transgenes)
- Reprogramming under controlled conditions (metaplasia e.g. implanting nucleas)
- Transdifferentiation

iPSCs (induced pluripotent stem cells)
The team of Shinya Yamanaka showed that induced PSCs (iPSCs) could be derived from somatic cells through the combined expression of pluripotency-associated transcription factors such as SOX2, OCT4 (also known as POU5F1), Krüppel-like factor 4 (KLF4) and MYC9,10.
induced pluripotent stem cells (iPSCs), which are generated by the introduction of 'reprogramming factors' into fibroblasts or other differentiated somatic cell types

5.2. Summarise 3 advantages and 3 disadvantages of generating KO mice by gene targeting in ESC. (6)

Table 14.2 Advantages and disadvantages of generating a knockout mouse by gene targeting in embryonic stem cells.

Advantages
The integration site and therefore the gene modification are highly specific
A variety of mutations can be achieved including null mutations (gene knockout), deletion/rearrangement of large regions of chromosomes, site-specific mutations, gene knock-in
Recessive alleles can be studied
Disadvantages
Microinjection requires specialist, expensive equipment and highly trained personnel
Process is very time consuming, taking 1.5–2 years to generate a targeting vector, target ES cells, identify homologous recombination events, microinject ES cells and test chimeric pups for germline transmission of mutation
Process is expensive as it is labour intensive, requires expensive equipment and the mouse husbandry costs will be high
Embryonic lethality – if the target gene is essential for development of the embryo, then it will not be possible to study the role of the gene in the adult mouse
Sometimes difficult to determine if the phenotype observed is primarily due to the deletion of the target gene or is a secondary consequence of the deletion of the target gene on a downstream pathway

QUESTION 6: Biofuels **[15]**

6.1. Describe (using both schematic and wording) in detail how biodiesel is produced. Is there room for improvement? (5)

By transesterification of a triglyceride (canola/rapeseed or soybean oil) with an alcohol (usually methanol) in the presence of a catalyst (usually methoxide) which generates glycerol and FAME (fatty acid methyl ester). This process is a mature, low-cost, thermochemical conversion, and thus little room for improvement using an enzyme system. Only opportunity for improvement lies in selection of alternative crops with higher yield in oil and optimal unsaturation. Also, for biodiesel business to expand, an alternative use for glycerol (byproduct) must be developed.

6.2. Various feedstocks are used to produce biofuels. Using corn as a feedstock, explain in detail the two processes employed for corn processing. (10 X ½ = 5)

Dry milling Dry milling simpler than wet milling
• Corn kernels milled with hammer mill or grinder
• Addition of water and heat to liquefy corn starch; thermostable α-amylase
Partially hydrolyzed starch maltodextrins & free Glc
• Cooling to 50-60ºC, pH 5; addition of glucoamylase
cleavage of single glucose units
• Critical parameter is maximizing Glc generation & minimizing reversion sugars
• Corn slurry-enzyme mix cooled to 40ºC & addition of S. cerevisiae
Fermentation over a few days; Glc to ethanol (9%).
While dry milling is less capital intensive, it also yields less ethanol per bushel of corn than wet milling.

Wet milling Steep corn with water & sulfur dioxide to swell corn
40-60 hrs with lactic-acid fermentation by *Lactobacillus*
• Separation of corn pericarp, starch, germ & soluble portion
• Series of separation processes to yield highly purified corn starch & corn oil
• Highly purified corn starch is largest product by weight
• Starch can be hydrolyzed (acid)
• Starch conversion at high T with α-amylase to high MW oligomers
• Maltodextrin coverted to >95% Glc, higher purity than dry mill process
• HFCS produced from xylose/glucose isomerase
Purified Glc is versatile & C source for many fermentative processes
Higher value products e.g. antibodies, amino acids, vitamins

6.3. Biotechnology is the exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms to produce desired products. Using any two genetic manipulation approaches, explain how

you will enhance both cellulose break down and sugar fermentation. Use 1 approach for cellulose and 1 approach for sugar fermentation (5)

6 biotechnological approaches are discussed in class (with reference articles) namely:

Directed evolution, DNA shuffling, Metabolic engineering, Classical mutation and selection or plant breeding, Recombinant DNA, System biology.

Students should use 1 approach for each process and explain how these can be using to enhance the processing.

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 in July 2018:

7.1.1. Assess and justify whether you can be confident that any of the targeted proteins were indeed present in cultured bacteria. Point out the evidence clearly. (2)

The elution lanes (4,5,9,10) all have the same single band. The supernatant has a reasonably strong band for the same Mw.

7.1.2. During the practical, you discovered the NaCl concentration in all buffers was 4x too high (2M instead of 500mM). How did this affect the results discussed in 7.1.1? (2)

Bands in elution lanes are very weak; the protein probably didn't really bind to the column well, and would have been eluted with the washes (lane 3)

7.1.3. Discuss 3 ways in which the above gel fails to yield the information it was meant to provide, and how you would fix this if you repeated the experiment. (3)

Protein runs too fast – use higher % acrylamide, or use tricine buffer. Marker lane is squished – don't use the edge lanes. Bands are too weak – load more protein. Bands are too weak – boil the samples (remember the students forgot to boil the samples).

7.2. Discuss why crystals are necessary for 3D structure determination using X-rays. (2)

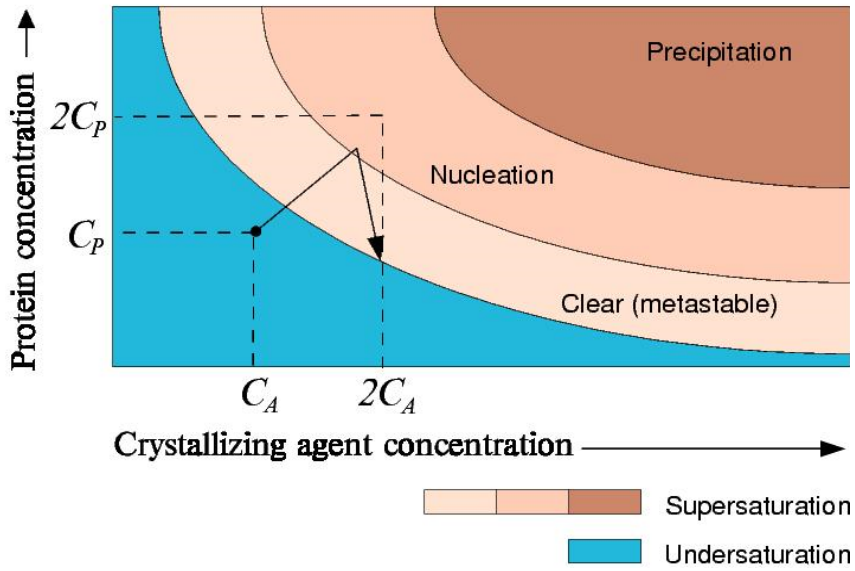
Crystals amplify the signal. Need diffraction because cannot focus X-rays like light.

7.3. Discuss how entropy drives both micelle formation and therefore protein folding. (3)

Proteins and micelles turn their hydrophobic parts inward, away from water. To solubilise hydrophobic groups, water must form ordered clathrates – low entropy state. Removing these groups from the water releases the water giving the molecules more degrees of freedom – a higher-entropy state.

7.4. Describe a general strategy for achieving larger crystals simply by changing concentrations of crystallization components, and explain the principle; sketch a schematic phase diagram if it simplifies your answer. (3)

Increase protein concentration, decrease precipitant concentration. It works because the systems spends more time in the metastable zone. 3rd point for either describing the phase diagram correctly, or drawing something like this.



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