Model Answers: Hard

1a

a) rhFVIII can be produced using hamster cells as follows…

Any **four** of the following:

- The gene is cut from the DNA using restriction enzymes/endonucleases **OR** the gene is made into cDNA using reverse transcriptase **OR** the gene is synthesised artificially from nucleotides; [1 mark]
- The gene is amplified / many copies of the gene are made using PCR; [1 mark]
- The gene is transferred into hamster cells using a vector; [1 mark]
- Marker genes are used to identify the hamster cells that have taken up the new gene; [1 mark]
- Hamster cells (containing the new gene) are cultured in a fermenter; [1 mark]
- Factor VIII is extracted **AND** purified; [1 mark]

Reject *references to plasmid vectors.*

[Total: 4 marks]

This question requires you to apply what you know about genetic engineering to the example of rhFVIII production in hamster cells. Most of the process will be the same as the more familiar process of genetically engineering bacteria, but be careful to avoid the use of plasmid vectors. Plasmids are not normally found in animal cells so they will not replicate when the animal cells divide; this means that any new cells produced after the introduction of the plasmids would not contain the new gene.

1b

b) Advantages of producing rhFVIII rather than extracting factor VIII from human blood include…

- Reduced risk of transmitting (blood) disease; [1 mark]
- Increased availability / rate/volume of production **OR** availability not limited by the number of donors; [1 mark]

[Total: 2 marks]

No marks have been allocated here for references to ethical or religious concerns or to immune responses to the proteins. This is because the process still uses hamster cells, so any concerns relating to the use of non-human sources will still apply.

1c

c) i) Post-translational modifications might include…

Any **two** of the following:

- Folding of proteins / development of secondary/tertiary structure / formation of hydrogen/ionic/disulfide bonds; [1 mark]
- Joining of additional polypeptide chains/subunits; [1 mark]
- Addition of (named) non-protein elements / prosthetic groups; [1 mark]
- Break down by proteases; [1 mark]

Named non-protein elements could include haem/iron ions, phosphate, carbohydrate, methyl groups or acetyl groups.

c) ii) It is preferable to produce factor VIII that is identical to that produced by fully developed humans because…

- The protein will not be recognised as foreign / initiate an immune response **OR** the

proteins will not be attacked/disabled/destroyed by the immune system **OR** antibodies will not be produced against the protein; [1 mark]

[Total: 3 marks]

All of the elements of protein structure beyond primary structure can be considered to be post-translational modification, so a good knowledge of secondary, tertiary, and quaternary structures will provide all that you need here.

The risk of introducing non-human proteins into human patients is immune rejection; if the immune system recognises a non-human element in a protein then the proteins will be attacked and destroyed by the immune system, reducing their functionality.

1d

d) The phenotype probabilities for the children of male with haemophilia and a heterozygous woman are…

- (The genotypes of the parents =) X^fY **AND** X^FX^f; [1 mark]
- (The genotypes of the children =) X^FX^f **AND** X^FY **AND** X^fX^f **AND** X^fY; [1 mark]
- (The phenotype probabilities for the children =) 25 % healthy female **AND** 25 % healthy male **AND** 25 % sufferer/haemophiliac female **AND** 25 % sufferer/haemophiliac male; [1 mark]

Accept *correct offspring phenotype probabilities for full marks in the absence of other notes.*

Accept *genotypes of parents and children given within a Punnett square.*

[Total: 3 marks]

Note that being heterozygous / a carrier is not usually an observable trait, so the phenotype of a female carrier will be healthy.

parent phenotypes = male with haemophilia × heterozygous female

parent genotypes = X^fY (only one copy so must be recessive) X^FX^f [1 mark]

Punnett square

	X^F	X^f
X^f	X^FX^f	X^fX^f
Y	X^FY	X^fY

[1 mark]

offspring phenotype probabilities

25% healthy female [1 mark]
25% healthy male
25% sufferer/haemophiliac female
25% sufferer/haemophiliac male

2a

a) i) It is necessary to heat the mixture to 95 °C in order to...

Any **two** of the following:

- Separate the two (DNA) strands / denature the DNA; [1 mark]
- Break the hydrogen bonds (between the base pairs); [1 mark]
- Expose the bases; [1 mark]
- Produce template strands for copying/replication; [1 mark]

a) ii) Primers are included in the mixture because...

Any **two** of the following:

- (Primers) bind/anneal to DNA by complementary base pairing; [1 mark]
- (Primers) attach close/next to the specific section of DNA (to be copied); [1 mark]
- (DNA) polymerase attaches to the double-stranded DNA/dsDNA / to the primer; [1 mark]
- Any reference to forward **AND** reverse primers; [1 mark]

a) iii) The enzyme *Taq* polymerase, rather than any other type of DNA polymerase, is used in PCR because...

Any **two** of the following:

- (*Taq* polymerase) is heat-stable/thermostable / works at high temperatures; [1 mark]
- (*Taq* polymerase) does not need to be added again / replaced for each cycle; [1

mark]

- The process is more efficient/faster (than for other polymerases); [1 mark]

Accept *reverse answers for marking points 1 and 2, e.g. for marking point 1 "other polymerases are not heat stable" and for marking point 2 "other polymerases need replacing regularly"*

[Total: 6 marks]

When learning about the PCR process make sure that you can describe the steps involved and that you can give the **reasons** for each of the stages.

2b

b) i) The term genome can be defined as...

- All of the DNA/genetic material (in a person's cells); [1 mark]

Accept *references to combined nuclear AND mitochondrial DNA.*

b) ii) The type of cell from a blood sample that is suitable for testing for the presence of these faulty alleles and the reason for this is...

- (Named) white blood cell **AND** it contains a nucleus / DNA; [1 mark]

Accept *leucocyte/leukocyte for white blood cell.*

b) iii) Faulty alleles of the *BRCA2* gene can be detected using the microarray as follows.....

Any **four** of the following:

- Probes/reporters are (short) lengths of DNA; [1 mark]
- (Probes are) <u>complementary</u> to the faulty alleles/DNA being tested for; [1 mark]
- Many copies of <u>one</u> type of probe are placed in <u>each</u> cell (of the microarray); [1 mark]
- DNA fragments (being tested) are single-stranded (due to being denatured); [1 mark]
- DNA fragments are labelled (with fluorescent tags); [1 mark]
- (Any) faulty DNA (fragments) / DNA with mutation will hybridise/bind to probes; [1 mark]
- Unbound sample DNA is washed off; [1 mark]
- Laser/UV light detects flurorescence / bound probes / faulty alleles; [1 mark]

Accept *reverse statement for marking point 7, e.g. "bound DNA is not washed off".*

b) iv) Advantages of screening for faulty alleles of the *BRCA2* gene include...

Any **four** of the following:

- Allowing people to make informed choices about lifestyle change; [1 mark]
- Allowing people to make informed choices about the need for preventative treatment; [1 mark]
- Removing worry (if results are negative); [1 mark]
- Preventative treatment can be cheaper than treating the disease; [1 mark]
- Allowing people to make informed choices about having children; [1 mark]
- Can have a positive impact on (cost of) life insurance; [1 mark]

[Total: 10 marks]