2017 Molecular Techniques STQ

2017 / H2 / ACJC PRELIM / P2 Q2 (CND, Stem Cells. Incl)

2 Recently, scientists discovered the presence of a population of bone-marrow derived stem cells that have the ability to form heart muscles cells when transferred to the heart. The stem cells were removed from the bone marrow and cultured so that they divided by mitosis. It was proposed that these stem cells resembled embryonic stem cells.

(a) (i) Describe **two** similarities between these bone-marrow derived stem cells and embryonic stem cells.

---

[2]

(ii) Describe how the rate of mitosis is controlled.

---

[4]

(iii) State an advantage of using bone marrow derived stem cells rather than heart stem cells for the treatment of heart diseases.

---

[1]

(c) Troponin is a protein that is integral to muscle contraction in heart muscles. **Fig. 2.1** shows part of its DNA sequence. The entire sequence is 63 base pairs.

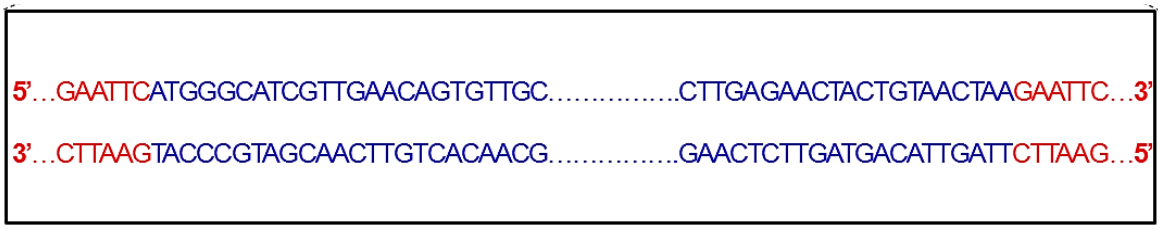

Fig. 2.1

PCR can be used to confirm presence of troponin DNA sequence. The following pair of primers are used.

Primer	Primer sequence
1	5' AATTCATGGGCATCG 3'
2	5' GAATTCTTAGTTACA 3'

(i) In the boxed area in Fig. 2.1, circle and label the DNA sequences where Primers 1 and 2 will anneal. [1]

(ii) Explain how results of gel electrophoresis of the PCR products are able to show that troponin DNA has been successfully amplified.

[1]

(iii) Besides the use of PCR, nucleic acid hybridisation can also be used to determine presence of troponin DNA.

Outline how nucleic acid hybridisation can be used to identify troponin DNA.

[4]

[Total: 13 marks]

2017 / H2 / ACJC PRELIM / P2 Q4 (Mutations Incl)

2 Sickle cell anaemia is a recessive genetic disease caused by a mutation that commonly occurs in the DNA, resulting in hydrophobic valine replacing hydrophilic glutamic acid at the 6th amino acid position of the β chain.

(a) State the type of mutation that commonly occurs to result in sickle cell anaemia. [1]

_____

(b) Describe the effects of this change in amino acids on the red blood cells of an individual with the disease. [4]

_____

_____

_____

_____

_____

_____

_____

_____

To detect if individuals are afflicted with sickle cell anaemia, restriction fragment length polymorphism (RFLP) analysis can be carried out using gel electrophoresis and Southern Blotting. Restriction enzymes are used to digest the DNA before RFLP analysis and the mutation removes a recognition site of the restriction enzyme *MstII,* as shown in Fig. 4.1. The enzyme's recognition sites on the normal allele and the mutant allele are shown by arrows.

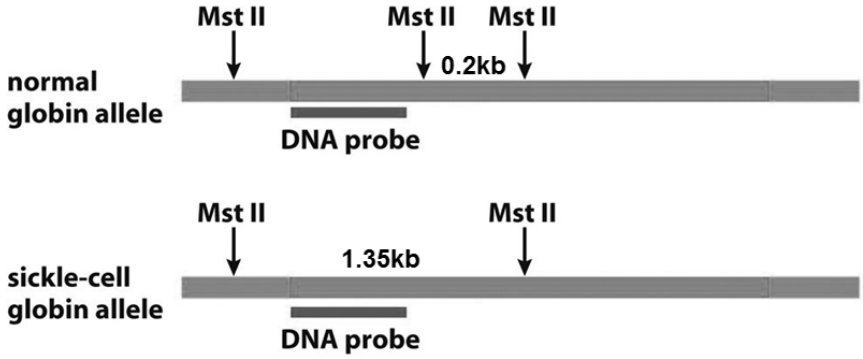

Fig. 4.1

(c) Draw, on Fig. 4.2, the expected band patterns produced by DNA from individuals with sickle cell anaemia. [1]

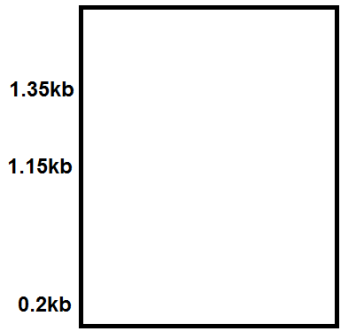

Fig. 4.2

(d) Suggest why it is necessary to carry out Southern Blotting after gel electrophoresis. [2]

_____

_____

_____

_____

(e) Outline the role of the DNA probe in Southern Blotting. [1]

_____

_____

[Total: 9]

2017 / H2 / SAJC PRELIM / P2 Q3 (Cancer, CGE Incl)

QUESTION 3

Epidermal growth factor (EGF) is released by cells, and is picked up either by the cell itself or by neighbouring cells. It regulates the production of a number of proteins in target cells. Protein produced and its effect depends on the type of target cell.

Fig. 3 shows how EGF regulates 3 genes.

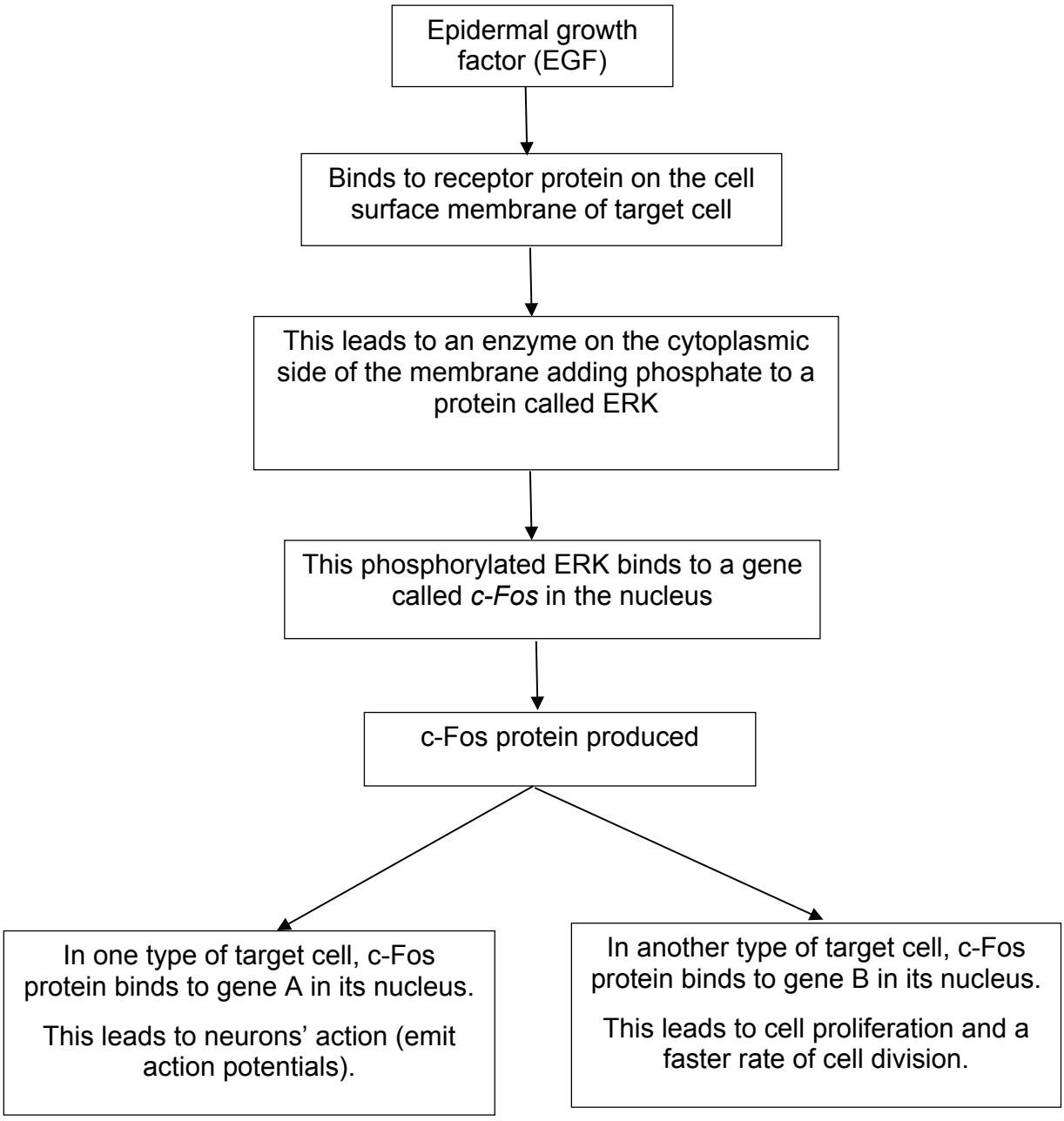

Fig. 3

(a) Name the **two** transcription factors in Fig. 3.

……

……..[1]

(b) The *c-Fos* gene can be a proto-oncogene.

Use the information in Fig. 3 to explain how a mutation of the *c-Fos* gene can result in the formation of a tumour.

……

……

……

……

……

……..[3]

(c) Gene B has been associated with a significant number of human cancers. Scientists used polymerase chain reaction (PCR) to make multiple copies of gene B extracted from a patient's cancer tissue sample.

The reaction mixture includes the sample of DNA to be copied plus the following ingredients:

- DNA primers
- buffer solution
- heat-stable DNA polymerase (Taq polymerase)
- deoxyribonucleoside triphosphates (deoxyATP, deoxyTTP, deoxyCTP and deoxyGTP)

(i) Suggest why a buffer needs to be present in the reaction mixture.

……

…….[1]

(ii) The deoxyribonucleoside triphosphates that are added to the reaction mixture are the monomers used for making the new DNA strands.

Suggest **one further** reason for adding the deoxyribonucleoside triphosphates to the reaction mixture.

……

……[1]

(iii) In the first stage of PCR, the mixture is heated to a temperature of around 90°C to denature the DNA. Suggest why high temperatures are needed to separate the two DNA strands.

……

……

……

……[2]

(iv) At the end of several cycles of PCR, many copies of the DNA sample in the reaction mixture will have been made. The DNA samples are then separated out to produce a DNA banding pattern.

State the technique used to separate out the DNA samples **and** describe how this technique works.

……

……

……

……

……

……

……[4]

[Q3: 12 marks]