Molecular techniques

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Tags	

Questions regarding PCR

Why taq polymerase can be used on most organisms

(i)
- Ref to most species of organisms use the same deoxyribonucleotides; **R**: ref to genetic code being universal
- 3D conformation of **active site** of *Taq* polymerase is **complementary** to the **3' OH** group of existing nucleotide;

Why the nucleotide sequence in RNA primer is important in PCR

(ii)
- Ref to specificity e.g. Allows for selective amplification of a segment of DNA / flanking target sequence; [2]
- via **complementary base pairing** to **3' ends** of the template strands;

Comparing PCR and DNA replication

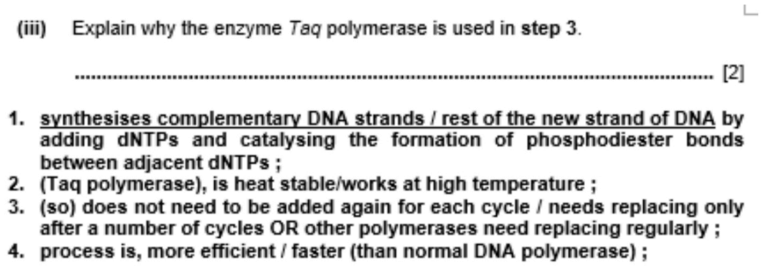
Feature	PCR	DNA replication
Nature of primer	DNA primers provide free 3' OH for polymerase.	RNA primers used.
How primer is synthesized	Primers synthesized artificially with knowledge of the target sequence.	Primers synthesized by RNA primase.
Enzyme for polymerisation	*Taq* polymerase used for polymerisation / catalysis of phosphodiester bonds.	Human DNA polymerase used.
Separation of template strand	Heat to 95°C to denature dsDNA.	Helicase used.
Replication	Amplification of only the target DNA sequence.	Replication of entire DNA molecule.
Location	Occurs artificially in a thermal cycler.	Occurs within nucleus of cells.

Role of primer in PCR

- Primers are short, single stranded DNA sequences that can anneal to target DNA sequence
- They are complementary to the regions flanking gene of interest and thus determine the segment to be amplified

- They provide a free 3′OH group for chain extensions Moderate temperature by DNA polymers so that the gene of interest can be amplified

Role of Taq polymerase in PCR



(iii) Explain why the enzyme *Taq* polymerase is used in **step 3**.

..... [2]

1. synthesises complementary DNA strands / rest of the new strand of DNA by adding dNTPs and catalysing the formation of phosphodiester bonds between adjacent dNTPs ;
2. (Taq polymerase), is heat stable/works at high temperature ;
3. (so) does not need to be added again for each cycle / needs replacing only after a number of cycles OR other polymerases need replacing regularly ;
4. process is, more efficient / faster (than normal DNA polymerase) ;

▼ Notes

- Synthesis complementary DNA strands by adding dNTPs and catalysing formation of phosphodiester bonds between adjacent dNTPs
- Thermostable DNA polymerase, resistant to denaturation at high temperature
- Does not need to be added again for each cycle unlike other polymerase and process more efficient

Pcr process

5. Polymerase chain reaction (PCR) is a molecular technique commonly used in molecular biology.

(a) Describe the aim of PCR.

Produce/amplify a large number of identical copies of DNA from a very small starting .....

..... [1]

(b) Describe what occurs in the

(i) first stage,

1. By heating / increasing temperature to ~95°C, the hydrogen bonds between the complementary base pairs are broken;
2. The target DNA double helix is denatured as the two strands of the target DNA molecules are separated into single-stranded DNA;

..... [2]

(ii) second stage,

1. decreasing temperature to ~55°C;
2. 2 complementary DNA primers anneal/ bind to the 3'ends of the single-stranded target DNA;

... [2]

(iii) third stage of PCR.

1. Heating to ~72 °C, the optimum temperature of Taq polymerase;
2. Catalyses the formation of phosphodiester bond between adjacent deoxyribonucleotides to synthesise new DNA strands via complementary base pairing in the 5' to 3' direction;

... [2]

Advantages of PCR

- It is sensitive as only a minute amount of source DNA is required to amplify a large amount of DNA products
- By using specific primers, PCR can selectively amplify a particular segment of DNA
- PCR is fully automated within the thermocycler
- It can amplify millions of targeted DNA segment rapidly and efficiently

Limitations of PCR

- Taq polymerase lacks proofreading ability as errors occuring early in PCR reaction will get compounded with each replication cycle
- Success of PCR requires knowledge of sequences flanking target region to be amplified

- DNA fragments to be amplified are limited to about 3kb. Further increases in length of target sequences decrease efficiency of amplification
- Minute amounts of contaminant DNA may result in unwanted DNA sequences being amplified to significant amounts

Why are three temperatures needed during one PCR cycle

- Heating to Highest temperature (95 degree Celsius) causes hydrogen bonds between complementary bases of each strand to break, causing denaturation of double stranded DNA into single stranded DNA, exposing bases doe complementary base pairing
- Lowest temperature (55 degree celcius) allows primer to anneal specifically to regions flanking the target DNA sequence via complementary base pairing, where primers determine the segment to be amplified and provide a free 3OH group for chain extensions
- Moderate temperature of 70 degree celcius is the optimum temperature of Taq polymerase which performs the synthesis of complementary DNA strand
    - Chain extensions occur from 3′ end of primer which provides free 3′ OH required by polymerase

Describe how PCR is carried out to amplify only the region of DNA that is interested

- Design and use primers with nucleotide sequences Complementary to regions flanking the target gene
- Ensure that the nucleotide sequence on the primer is not complementary to other regions of DNA
- After denaturation of DNA double helix, cool PCR mixture to 64 degree celcius in the presence of excess primer

Questions regarding southern blotting and nuclei acid hybridisation

Describing southern blotting and nuclei acid hybridisation after gel electrophoresis

- [blotting] DNA fragments are transferred from gel to nitrocellulose / nylon /DNA-binding membrane using capillary action / as transfer buffer is absorbed by the layers of paper towels / absorbent papers
- [denaturation] Transfer buffer contains alkali solution / sodium hydroxide that denatures the DNA as it is being transferred
- [hybridisation] Radioactive probes are added to the nitrocellulose / nylon /DNA-binding membrane for a period of time, allowing them to bind to 200bp / 1200bp / 1400bp fragment before unbound probes are washed off;
- [visualisation] Washed membrane is exposed to X-ray film to detect the presence of radioactivity / Autoradiography is used to expose the bands that the radioactive probes has bound to; (reject: use of UV light)

How to know which probe is used and why

- Observe band pattern and see which band pattern comes up
    - Only the __bp fragments shown but not the __bp which can be produced upon restriction digest
- This is because probe is complementary in equence to the regions that are found on the __bp fragment

Questions regarding gel electrophoresis

Finding the presence of specific gene (gel electrophoresis)

- Separate PCR products by performing gel electrophoresis which separates DNA fragments using current
- **Meshwork of Agarose polysaccharides impede movement of longer fragments more than shorter fragments causing them to migrate slower than shorter fragments and end up nearer to the well (included point in most gel electrophoresis questions)**
- For visualisation, stain gel with ethidium bromide followed by visualisation under UV light
- If fragment that corresponds to gene not seen, gene is absent

Outline main principles that allow gel electrophoresis to separate DNA fragments

- Dense loading buffer is mixed with DNA sample to help it sink to the bottom of nearest negative electrode
- Negatively charged DNA migrates out of well towards direction of positive electrode when subjected to an electric field
- Meshwork of Agragrose polysaccharides impede movement of longer fragments more than shorter fragments causing them to migrate slower than shorter fragments and end up nearer to the well

why is a DNA marker used

- contsins a mixture of DNA fragments of known sizes
- Acts as a standard that the fragments of unknown sizes can be compared with to estimate fragment size