Bio Factsheet

Number 67

Modern Techniques in Biology: Genetics

The techniques and applications of DNA finger printing and the polymerase chain reaction (PCR) are on most of the latest A-level Biology syllabuses. This factsheet will describe these techniques and their main applications.

The Polymerase Chain Reaction

What it is: The polymerase chain reaction (PCR) is a method of specifically amplifying a chosen region of DNA (sometimes referred to as a 'target'). Initially, there may be only a few copies of the chosen base sequence of interest, but after PCR there will be millions of copies of this sequence.

What PCR is used for: By using PCR, it is possible to determine whether a given sequence is present or absent in a DNA sample. The method is sensitive enough to detect the presence or absence of single base changes (point mutations). Some diseases (e.g. cystic fibrosis) are the result of such changes and consequently PCR can be used for disease diagnosis. PCR is used in forensic science, for instance, the DNA present in hair follicles or spots of blood can be amplified and this can lead to the identification or elimination of individuals (see DNA fingerprinting). PCR is also used to synthesize large amounts of specific genes for use in gene therapy, for example, in the treatment of cystic fibrosis or alpha-1 antitrypsin deficiency. PCR is also used to amplify DNA of different species when DNA fingerprinting is used to determine taxonomic (evolutionary) relationships between species.

> ***Exam hint:** Examination questions commonly ask candidates to recall a use for the polymerase chain reaction!*

The principal of PCR technique

To perform PCR it is first necessary to identify the DNA target. The target is a known sequence of bases within the DNA sample. A primer is then designed that binds specifically to this region, through complementary base pairing. Primers are short sequences of nucleotides, generally between 20 and 30 bases long that are chemically synthesised. A single reaction uses two different primers (one pair). In addition to the primer that binds to the target region of the DNA, a second primer is necessary; it binds to the other complementary strand of DNA at a place that determines the length of the product, as newly-synthesised DNA is created between the two primers. The length of the product includes the length of the primers. As an example, if two primers, each 20 bases long, bind to DNA 100 base pairs (bp) apart, the product that they will generate will be 140 base pairs (bp) long.

> ***Remember** – PCR produces a DNA copy from a DNA template. This is in contrast to transcription which produces a messenger RNA molecule from a DNA template.*

Reagents that are required

Each reaction must contain all of the reagents necessary to synthesise DNA. These are:

- DNA sample: the DNA from which all of the reaction products are subsequently generated. This is referred to as the 'original template'.
- Primers: to direct the synthesis of a given region of DNA.
- Deoxynucleotide triphosphates (dNTPs): contain the bases that comprise the DNA
- Buffer
- *Taq* DNA polymerase: a temperature stable (thermostable) DNA polymerase which catalyses the assembly of the dNTPs into DNA.

> *Taq* stands for Thermophilus aquatilis, this is a bacterium that lives in hot springs from which this polymerase is obtained.

The mechanism of the reaction

PCR is basically a series of three-stage cycles during which DNA is synthesised. Products from one cycle can act as templates for further cycles. Three different temperatures are used, one for each stage of the cycle. The three stages are:

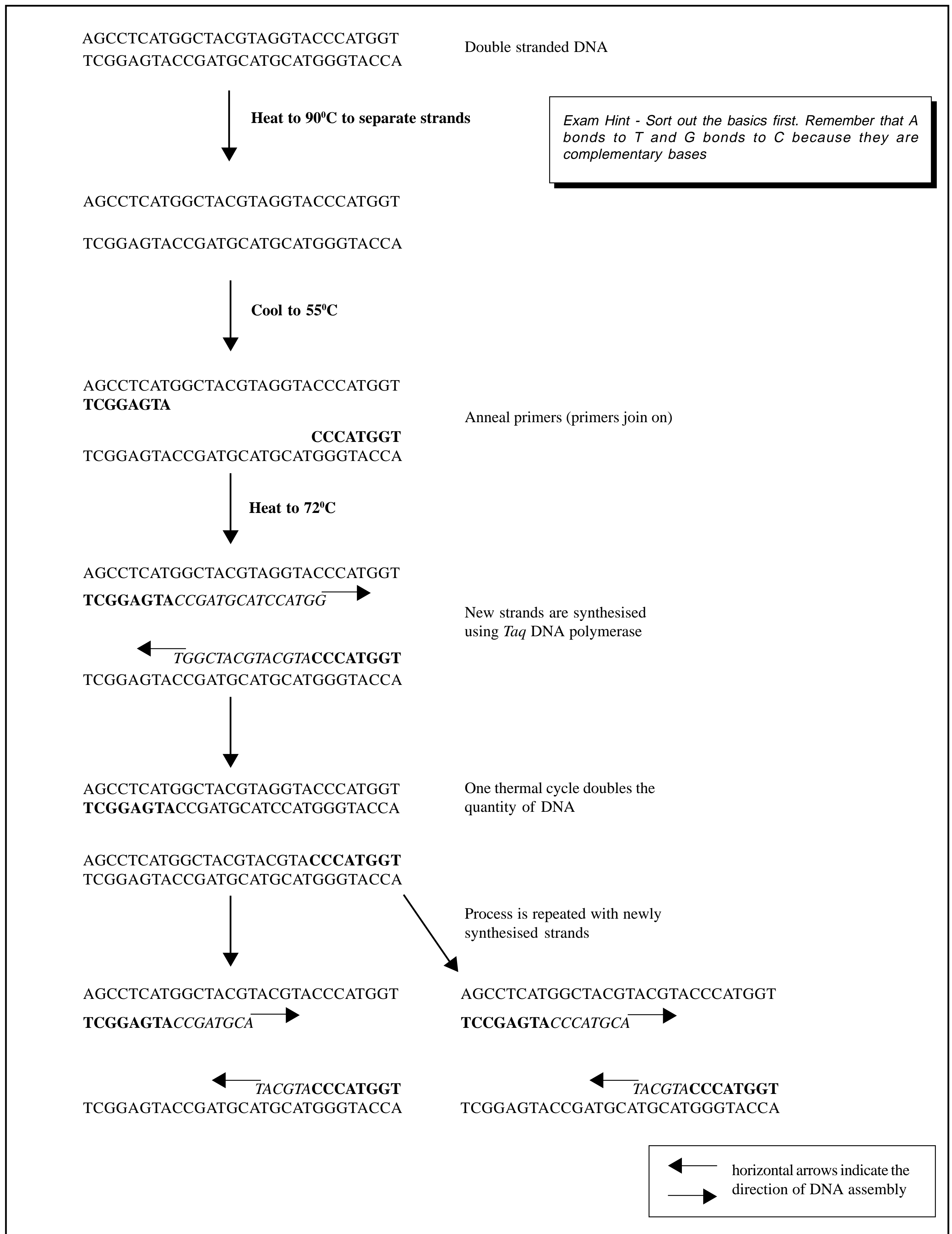
- **Denaturation** (around 95^{0}C). At this temperature, the hydrogen bonds between the two strands of DNA are broken and the strands separate.

- **Annealing** (55 - 70^{0}C). Here the primers bind specifically to their complementary sequence in the single-stranded DNA.

- **Extension** (72^{0}C). Here, at the optimum temperature for the enzyme, the *Taq* DNA polymerase extends the primers from their 3' termini, creating new DNA, using the original single-stranded DNA onto which the primers have bound as a template on which to create a new strand (with complementary base pairing and the formation of covalent bonds between the dNTPs).

The three-stage cycle is repeated, generally 30 to 40 times. To do this, all of the reagents are pipetted into a tube which is then placed into a thermal cycler. This is a machine in which a metal block is programmed to rapidly and accurately produce the different temperatures required for PCR. At the end of the temperature cycling, enough product has been generated to be seen on an agarose gel (described below). Note that, until other factors become limiting, the amplification is exponential, with the amount of product doubling after each cycle. The mechanism of the polymerase chain reaction is illustrated in Fig 1.

Fig 1. Mechanism of PCR



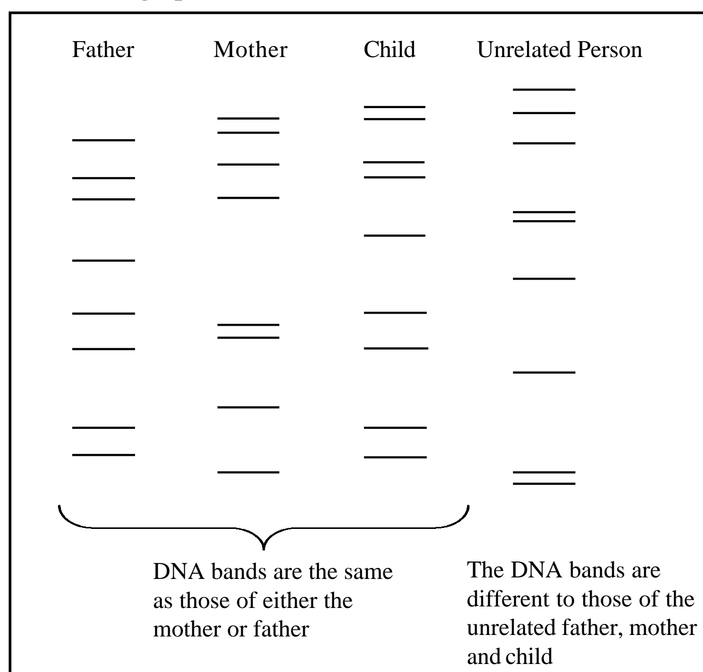
How the reaction products are analysed
PCR products can be visualised by **gel electrophoresis**. Agarose gels are used for this. These separate DNA molecules on the basis of their **size**, with larger molecules moving through the pores of the gel more slowly than smaller ones. This movement of the DNA is induced by applying an electric current across the gel, which is submerged in buffer in a gel tank. DNA is negatively charged and moves towards a positive electrode. After the DNA has been separated, it is visualised by staining with a dye that binds to it and fluoresces under ultraviolet light. The most commonly used dye for this purpose is called ethidium bromide.

Electrophoresis is necessary to separate PCR products from the original template and also to separate marker DNA which is also loaded onto the gel. The marker DNA consists of a series of fragments of known size, against which the size of PCR product can be compared.
As PCR results in the amplification of product DNA of a specific size, the amount of product on the gel is much greater than the amount of original template DNA and a strong band corresponding to the product is clearly seen.

Reverse transcriptase polymerase chain reaction (RT-PCR)
PCR can also be used to identify mRNA targets, by a process known as the Reverse Transcriptase Polymerase Chain Reaction. Here, in an initial stage that is not present when DNA targets are amplified, the primers bind to RNA and reversetranscriptase is used to make a cDNA copy of the target. Once the DNA copy has been made, the reaction can proceed as outlined above.

DNA Fingerprinting
A DNA fingerprint is a DNA-based pattern composed of series of bands of different sizes. Any given individual is likely to possess a unique combination of bands, hence the term 'fingerprint'. Approximately half of these bands are inherited from an individual's mother and approximately half from their father, as illustrated in Fig 2.

Fig 2. DNA fingerprints of a father, mother and child showing that the child inherits approximately half of the DNA bands from its father and half from its mother. An unrelated person has a very different fingerprint.



Uses of DNA fingerprinting
The chances of two individuals producing identical fingerprints is vanishingly small. Consequently, DNA fingerprinting can be used to identify individuals. It can also be used to determine familial relationships. Although much of the usefulness of the method has been concerned with human investigations, as fingerprints can be generated for other species, the technique has also been adopted by biologists in studying problems related to behaviour, population biology, taxonomy and conservation.

The theoretical basis of DNA fingerprinting
DNA fingerprinting detects the presence, throughout the genome, of DNA sequences termed **Variable Number Tandem Repeats (VNTRs).** Between individuals, VNTRs vary in both their precise sequence and exact position, in that the individuals DNAs may be different. It is this variation that causes DNA fingerprints to be different.

The nature of VNTRs
Genomes do not just contain unique sequences of single copy DNA. They also contain extensive regions in which repetitive sequences are arranged end to end, in tandem. This is **satellite DNA.** The number of bases that comprise these repeated sequences varies. **Minisatellite DNA** contains repetitive sequences between approximately 9 and 70 bp long, whereas in **microsatellite DNA** they are generally less than 4bp long. At any given position **(locus)** in the genome, the number of repeats in the tandem arrangement can vary. Consequently, these sequences can be referred to as **Variable Number Tandem Repeats (VNTRs).**

The DNA fingerprinting procedure
To produce a DNA fingerprint several steps have to be performed, as follows:
Initially, the DNA has to be extracted from a biological sample, leaving behind any protein, carbohydrate or other materials that may also be present. The extracted DNA is then cut into a series of fragments by enzymes known as **restriction enzymes.** These recognise specific sequences in the DNA **(restriction sites),** which are generally motifs (groups) of 4 - 6 bp. When DNA is incubated with a restriction enzyme (a **restriction digest)** the strands are cut at specific points in the vicinity of the restriction site. The pieces of DNA produced as a result of this are **restriction fragments.** A large number of fragments of different sizes are generated as a result of the restriction digest, and these are then separated by gel electrophoresis.

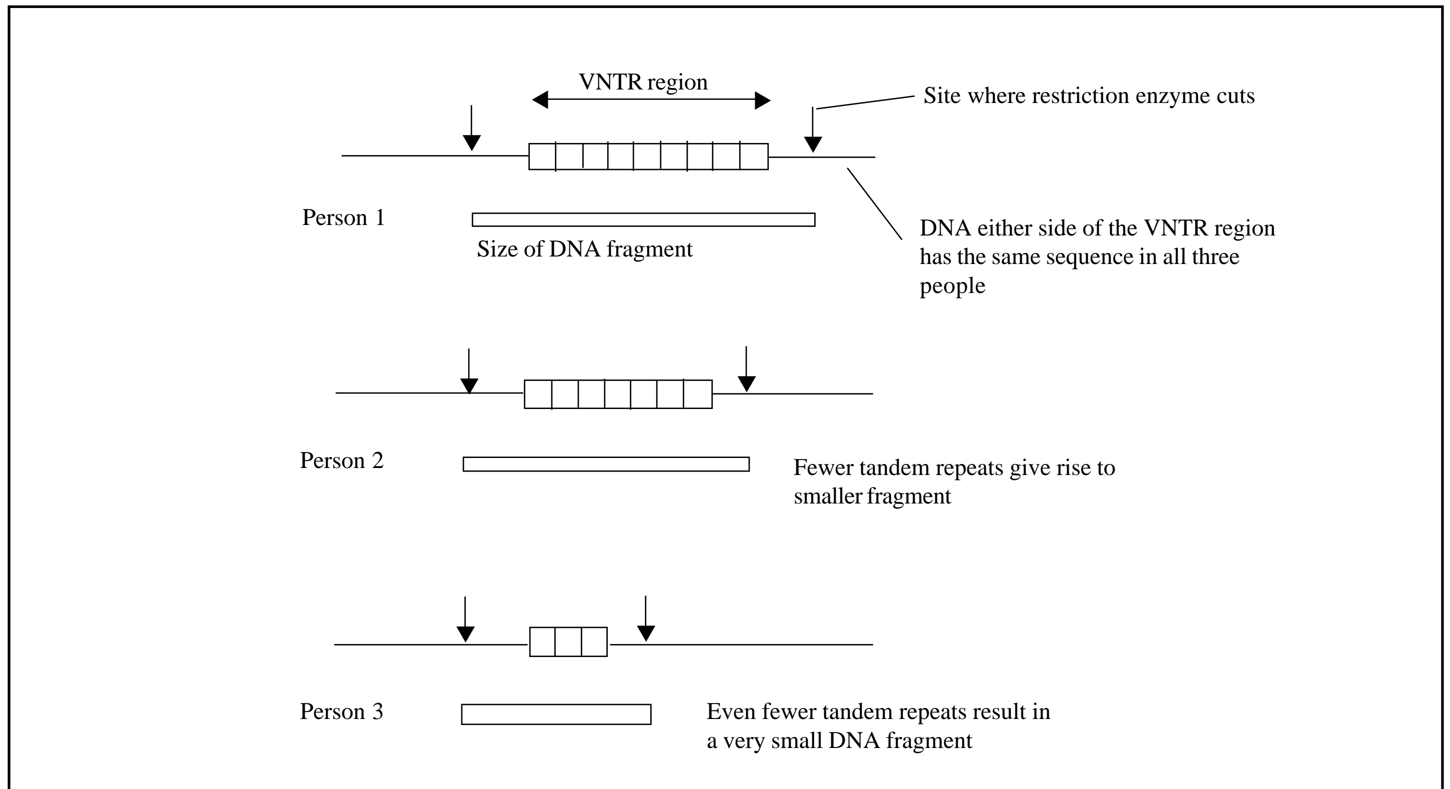
The DNA is then transferred to a charged membrane by **Southern blotting.** To perform a Southern blot, a positively-charged membrane is placed on top of the gel and the negatively charged DNA fragments are transferred from the gel to the membrane. They are then irreversibly bound to the membrane (or **blot** as it is then called), whilst maintaining their relative positions on the gel. Following this, the blot is subjected to **hybridisation (see** below) using a **labelled probe.** A probe is a short piece of DNA containing a known sequence. For DNA fingerprinting, the sequence is the repetitive unit of the VNTR under study. The probe can synthesised chemically and when this is done, a small number of radioactive bases can be incorporated into the DNA, in a process known as **radio-labelling.** Through complementary base pairing, the probe will then bind onto the blot at any position where DNA of the appropriate sequence is found. This is **hybridisation.** Finally, through **autoradiography,** the position of the bound probe on the blot can be detected. In autoradiography a photographic film is placed against the blot and the radioactivity in the probe causes the film to be exposed.

Why fingerprints vary between individuals

DNA fingerprinting reveals VNTRS. VNTRs are scattered throughout the genome. When they are identified using DNA fingerprinting, they are present in a fragment of DNA. The fragment will generally contain the VNTR plus additional DNA (see Fig 3). Differences in the sizes of the fragments containing the VNTRs results in the fragments running at different speeds when they are subjected to gel electrophoresis and this ultimately results in the fragments being in different positions on the blot.

What is being seen in the DNA fingerprint is a series of fragments of different sizes each containing a VNTR. This is known as **restriction fragment length polymorphism (RFLP).** Individuals differ in the precise makeup of their genomes. Mutations may create or delete restriction sites, and insertions and deletions may occur at other positions in the DNA. Such changes will result in the restriction fragments produced from one individual differing in length from those produced by another and this is reflected in their DNA fingerprint.

Fig 3. Diagram showing how VNTRs in three people give rise to DNA fragments of different sizes when DNA is cut with restriction enzymes



Practice Questions

1. The diagram shows the results of a DNA fingerprint analysis using a microscopic blood sample found at the scene of a crime. The DNA profile of the blood is shown on the left and labelled' scene of crime'. DNA profiles produced using blood samples from four suspects are shown on the right.

(a) (i) Which suspect has been incriminated by the DNA analysis? 1
(ii) Give a reason for your answer. 1

(b) Briefly describe the part played by each of the following in the production of the DNA profiles:
(i) polymerase chain reaction. 1
(ii) restriction endonucleases. 2
(iii) gel electrophoresis. 2
(iv) radioactive DNA probes. 3

Total 10

2. (a) Part of a DNA molecule is shown below. Write the sequence of bases of the complementary strand produced by the polymerase chain reaction. 2

ATTGCGSTAGGTAT

(b) State the difference between PCR and transcription. 2

(c) What is the function of the enzyme DNA polymerase in PCR? 1

(d) The enzyme DNA polymerase used in PCR was originally obtained from bacteria found in hot springs. Explain why DNA polymerase obtained from human cells would not be suitable for use in PCR. 3

Total 8

3. Biologists have traditionally recognised three different subspecies of chimpanzee based on physical characteristics, genetics and geography. *Pan troglodytes troglodytes* lives in Central Africa, *Pan troglodyte schweinfurthei* lives in East Africa and *Pan troglodyte verus* lives in West Africa. Recently, a small new population of chimpanzees living in southeastern Nigeria has come to the attention of biologists. It is suspected that this small population make up a new subspecies. Genetic analysis of animals for taxonomic purposes is usually carried out on mitochondrial DNA.

   (a) (i) Name a technique which could be used to compare the mitochondrial DNA of the different chimpanzee subspecies. 1

   (ii) Briefly describe the technique you have named. 5

   (iii) What results would you expect to see if thenigerian chimpanzees were a distinct subspecies? 2

   (b) Name two other applications of the technique you have described. 2

Total 10

Answers

1. (a) (i) suspect 3; 1

   (ii) because the bands match closely to the DNA at the scene of the crime/other individuals' bands don't match. 1

   (b) (i) increase quantity/number of copies of DNA (under investigation)/amplification of DNA; 1

   (ii) used to cut DNA into fragments;
   if use the same restriction endonuclease, samples from all suspects are cut at the same/similar places; 2

   (iii) separates cut fragments of DNA;
   according to size; 2

   (iv) probes are DNa strands with complementary sequences to cut fragments;
   labelled using radioactive tracer/phosphorus-32;
   probes bind to the complementary sequences;
   fragments/radiation detected by autoradiography/using X-ray film;
   specific sequences show up as dark bands; max 3

Total 10

2. (a) TAACGCCATCCATA;; (delete 1 mark for each mistake) 2

   (b) the PCR produces a DNA copy of a DNA template, transcription produces mRNA;
   the complementary strand produced by transcription has base U rather than T; 2

   (c) produces a DNA copy of a DNA template; 1

   (d) the PCR involves high temperatures/ temperatures as high as 90°C;
   DNA polymerase from human cells would be denatured at this temperature;
   DNA polymerase from hot spring bacteria would still act at 90°C; 3

Total 8

3. (a) (i) DNA fingerprinting; 1

   (ii) collect chimpanzee mitochondria by cell disruption/ (ultra)centrifugation;
   extract mitochondrial DNA and split with restriction endonucleases;
   separate DNA fragments by gel electrophoresis;
   blot onto nylon membrane/Southem blotting;
   treat with radioactive/fluorescent gene probe to recognise and label specific base sequences;
   overlay with X-ray film to detect labelled sequences; max 5

   (iii) labelled areas of DNA show up as dark bands;
   if (many) bands do not match those of other subspecies/are different from other subspecies, then they are probably an independent subspecies; 2

   (b) forensic science/murder/rape;
   paternity disputes;
   confirming animal pedigrees;
   identification of human remains;
   locating genes causing inherited diseases;
   locating genes for animal/plant breeding; max 2

Total 10

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