PCR: copy a selected region

The polymerase chain reaction amplifies a chosen DNA region in vitro, producing many copies from a small sample. It copies DNA; it does not separate fragments or identify a person by itself.

1. SUPPLY THE COPYING SYSTEM

Template DNA supplies the sequence. Two primers bind on opposite strands, flanking the target and pointing towards it. Free DNA nucleotides supply building blocks. Thermostable Taq polymerase extends the primers; a suitable buffer provides the required chemical conditions.

2. REPEAT THREE TEMPERATURE STAGES

A. Denaturation About 95°C	Heat separates the strands by disrupting hydrogen bonds between bases; the backbones remain intact.
B. Annealing About 50-65°C	Cooling lets primers pair with complementary template sequences. The appropriate temperature depends on the primers.
C. Extension About 72°C	Taq adds complementary nucleotides to each primer's 3' end, synthesising new DNA in the 5' to 3' direction.

Taq tolerates repeated high-temperature stages, so fresh enzyme is not needed after every cycle. Newly made DNA becomes template in later cycles.

3. UNDERSTAND THE IDEAL DOUBLING MODEL

Ideal model: $N = N_0 \times 2^n$. N_0 is the starting copy count; n is the cycle count. One starting copy gives 1,024 after 10 perfect doublings. Real amplification is less efficient and eventually levels off.

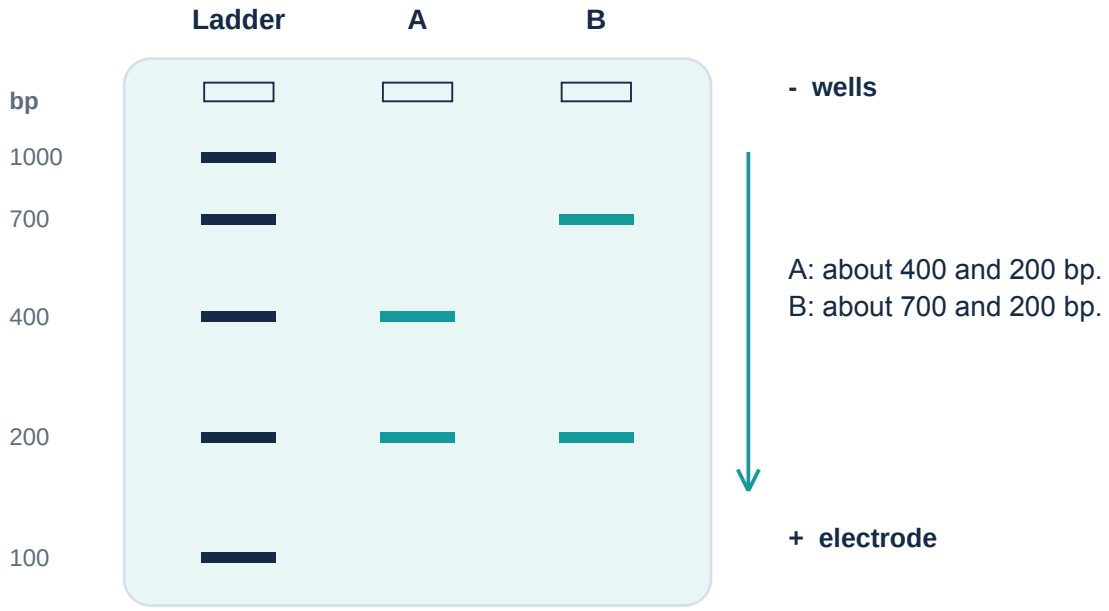
PCR purpose and amplification: NHGRI fact sheet.
PCR components and temperature stages: Addgene.

Cambridge Biology 9700: 19.1.8-11, p. 47; profiling follows website outline.

Separate DNA by fragment length

DNA has a negatively charged phosphate backbone. In an electric field, linear DNA fragments move through the gel towards the positive electrode. Shorter fragments generally travel farther in the same run.

1. READ THE LADDER AND THE DIRECTION



Illustrative linear-DNA gel. A DNA ladder supplies known fragment lengths; bp means base pairs. Each band contains many fragments of similar length.

2. SEPARATION DOES NOT REVEAL THE BASE SEQUENCE

A stain or fluorescent label makes DNA visible. Compare band positions with the ladder to estimate length. Bands at the same position have similar lengths, but need not have identical base sequences.

3. USE CONTROLS WHEN CHECKING PCR PRODUCTS

A no-template control should not show the target band; a target-sized band can indicate contamination. A known positive control checks that the assay can work. A missing sample band alone does not prove the target DNA is absent.

DNA movement, fragment length and ladder: Addgene, Gel Electrophoresis.
PCR controls and interpretation: QIAGEN, PCR controls.

Cambridge Biology 9700: 19.1.8-11, p. 47; profiling follows website outline.

Compare DNA profiles carefully

DNA profiling compares variable regions, often short tandem repeats (STRs). Different repeat numbers can produce different fragment lengths. PCR can copy the chosen regions before their lengths are measured.

1. COMPARE ALLELES AT THE SAME LOCUS

Simplified, complete single-source example. Numbers are repeat counts, not base-pair lengths. Each pair represents two autosomal alleles.

STR locus	Questioned	Person A	Person B
Locus 1	12, 15	12, 15	12, 14
Locus 2	8, 10	8, 10	8, 10
Locus 3	16, 16	16, 16	15, 16

A is consistent at all three loci. B differs at loci 1 and 3, so B is excluded as the sole source under these assumptions. Three matching loci are not enough to establish a unique identity.

2. A MATCH IS EVIDENCE, NOT CERTAINTY

Assess many independent loci and the rarity of the combined profile. Relatives may share alleles; identical twins usually share standard STR profiles. Contamination, degraded DNA and mixtures can complicate interpretation. A match alone does not show how or when DNA arrived.

3. KEEP INHERITANCE AND MEASUREMENT DISTINCT

At a typical autosomal STR locus, a child inherits one allele from each parent. One visible allele length can mean both copies have the same repeat count. Modern profiling often uses capillary electrophoresis with peaks; classroom gel-band diagrams are simplified representations.

STR variation and identity testing: NIST STRBase.
Matching, mixtures and limitations: NIST DNA Mixtures explainer.

Cambridge Biology 9700: 19.1.8-11, p. 47; profiling follows website outline.

From samples to shared data

Further 19.1 syllabus links: microarrays compare many known sequences in parallel, while biological databases make sequence and structure information searchable and reusable.

1. MICROARRAYS RELY ON COMPLEMENTARY PAIRING

A chip carries many spots of known, single-stranded DNA probes. Labelled sample nucleic acids bind to complementary probes by hybridisation. A scanner detects the label at each position.

A. Genomic analysis

Labelled genomic DNA can reveal whether known sequences or variants are present, depending on the array design. An array tests its existing probes; it does not read every unknown base.

B. Gene-expression analysis

Extract mRNA and make labelled cDNA. Hybridise it to the array; signal intensity, after suitable controls and normalisation, estimates relative transcript abundance. More signal is not direct proof of more active protein.

2. DATABASES CONNECT SEQUENCE, STRUCTURE AND FUNCTION

DNA / genome data	GenBank stores nucleotide sequences and annotations. Compare sequences to identify similar genes or investigate ancestry.
Protein sequences	UniProt links amino acid sequences with functional information. Similarity can suggest, but does not prove, a shared function.
Protein structures	The Protein Data Bank stores 3D structural data. Structures help explain molecular interactions and generate hypotheses.

Shared records reduce repeated work and support global comparisons. Check record quality, evidence and updates before drawing conclusions.

Genomic microarray principle: NHGRI, DNA Microarray Technology.
Expression arrays: EMBL-EBI, Functional Genomics II.
Database names above link to GenBank, UniProt and RCSB PDB.

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