

DNA replication What is DNA replication?	DNA replication Why is DNA replication needed in multicellular organisms?
DNA replication Semi-conservative replication	DNA replication How does complementary base pairing make replication accurate?
DNA replication Role of helicase	DNA replication General role of DNA polymerase
DNA replication The three PCR stages and temperatures	DNA replication What are primers in PCR?

For reproduction, and for growth and tissue replacement, since every new cell needs a full copy of the genome.

The production of exact copies of DNA with identical base sequences.

Each template base determines the base added (A-T, C-G), so the new strand's sequence is exactly complementary to the template.

Each new DNA molecule contains one strand from the original molecule and one newly synthesised strand.

Links free DNA nucleotides, paired to the template by complementary base pairing, into a new strand.

Unwinds the double helix and breaks hydrogen bonds between bases, separating the strands.

Short single-stranded DNA sequences that bind to the ends of the target sequence and give DNA polymerase a starting point.

Denaturation about 95 °C (strands separate); annealing about 55 °C (primers bind); extension about 72 °C (Taq polymerase builds new strands).

DNA replication

Why is Taq polymerase used in PCR?

DNA replication

Basis of separation in gel electrophoresis

DNA replication

What does DNA profiling compare?

DNA replication

Why does using more markers improve DNA profiling?

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Paternity test rule

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To which end of a strand do DNA polymerases add nucleotides?

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Leading strand vs lagging strand

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Okazaki fragments

Negatively charged DNA moves towards the positive electrode; smaller fragments move faster and travel further through the gel.

It is from the hot-spring bacterium *Thermus aquaticus*, so it is not denatured at 95 °C and works best at about 72 °C.

It reduces the probability of a false match between two different people, increasing reliability.

Lengths of short tandem repeat (STR) regions, amplified by PCR and separated by gel electrophoresis.

The 3' end: the 5' phosphate of the new nucleotide bonds to the 3' -OH, so strands grow 5' to 3'.

Every band in the child's profile must be present in either the mother's or the father's profile.

Short sections of DNA made discontinuously on the lagging strand, later joined by DNA ligase.

Leading: continuous, one RNA primer.
Lagging: discontinuous, as Okazaki fragments, each needing a primer.

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DNA primase and DNA polymerase III

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DNA polymerase I and DNA ligase

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DNA proofreading

Protein synthesis

Transcription

Protein synthesis

Base pairing in transcription

Protein synthesis

Why must DNA templates be stable?

Protein synthesis

Why is transcription a key stage of gene expression?

Protein synthesis

Translation

Polymerase I removes RNA primers and replaces them with DNA; ligase seals gaps in the sugar-phosphate backbone.

Primase makes RNA primers; DNA polymerase III adds DNA nucleotides to the primer's 3' end.

The synthesis of RNA using a DNA template, carried out by RNA polymerase.

DNA polymerase III removes a nucleotide with a mismatched base from the 3' terminal and replaces it with a correctly matched one.

In non-dividing somatic cells the same sequences are transcribed for the life of the cell, so transcription must not change them.

Template A pairs with RNA U; T with A; C with G; G with C. Held by hydrogen bonds.

The synthesis of a polypeptide from mRNA: the mRNA base sequence is translated into an amino acid sequence.

It is the first stage, so genes can be switched on or off by controlling transcription.

Protein synthesis

Where do mRNA and tRNA bind on the ribosome?

Protein synthesis

Codon vs anticodon

Protein synthesis

How does the polypeptide grow during elongation?

Protein synthesis

Why is the genetic code a triplet code?

Protein synthesis

Degeneracy and universality

Protein synthesis

Start codon and stop codons

Protein synthesis

Sickle-cell mutation

Protein synthesis

Effect of haemoglobin S

Codon: three bases on mRNA coding for one amino acid. Anticodon: complementary three bases on tRNA.

mRNA binds to the small subunit; two tRNAs can bind simultaneously to the large subunit.

4 bases and 20 amino acids: pairs give 16 combinations (too few); triplets give 64.

A peptide bond links the next amino acid to the chain, then the ribosome moves one codon along the mRNA.

Start: AUG (methionine). Stop: UAA, UAG, UGA.

Degeneracy: more than one codon per amino acid. Universality: the same codons code for the same amino acids in almost all organisms.

At low oxygen it forms long fibres that distort red blood cells into rigid sickle shapes.

Base substitution in the haemoglobin beta-chain gene: codon GAG → GUG, glutamic acid → valine at the sixth amino acid.

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Direction of transcription and translation

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The promoter

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Examples of non-coding DNA

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Post-transcriptional modification

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Alternative splicing

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Steps of initiation of translation

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A, P and E sites

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Pre-proinsulin to insulin

A DNA sequence before a gene where transcription factors bind, allowing RNA polymerase to bind and start transcription.

Both 5' to 3': RNA grows at its 3' end; ribosomes move from the 5' end to the 3' end of mRNA.

Introns removed and exons spliced together; 5' cap and 3' polyA tail added to stabilise the mRNA.

Regulators of gene expression, introns, telomeres, and genes for rRNA and tRNA.

Small subunit binds 5' end of mRNA, moves to AUG; initiator tRNA (Met) pairs; large subunit attaches; next tRNA binds.

Splicing different combinations of exons so one gene codes for different polypeptides.

Signal sequence removed → proinsulin (folds, disulfide bonds form); C-peptide removed → insulin of A and B chains.

A: incoming tRNA with amino acid. P: tRNA holding the growing polypeptide. E: exit of tRNA without amino acid.

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Role of proteasomes

Mutation and gene editing

Gene mutation

Mutation and gene editing

**Single-nucleotide polymorphism
(SNP)**

Mutation and gene editing

**Why may a base substitution have no
effect on a polypeptide?**

Mutation and gene editing

Frameshift

Mutation and gene editing

Causes of gene mutation

Mutation and gene editing

Examples of mutagens

Mutation and gene editing

Randomness in mutation

A structural change to a gene at the molecular level: a substitution, insertion or deletion of bases.

Break down unwanted or faulty proteins so amino acids are recycled; a functional proteome needs constant breakdown and synthesis.

The genetic code is degenerate, so the new codon may code for the same amino acid.

A single-base difference between individuals, resulting from a base substitution mutation.

Mutagens (chemicals and radiation) and errors in DNA replication or repair.

Insertion or deletion of bases (not a multiple of three) shifts the reading frame, changing every following codon.

Mutations can occur anywhere in the genome (some bases more often); no natural mechanism deliberately changes a base to change a trait.

Chemical: benzo[a]pyrene, nitrosamines (tobacco smoke), mustard gas. Radiation: UV, X-rays, gamma rays.

Mutation and gene editing

Germ-cell vs somatic-cell mutation

Mutation and gene editing

Why is mutation essential for evolution?

Mutation and gene editing

Risk of commercial genetic tests

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Gene knockout

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Knockout libraries

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Roles of guide RNA and Cas9

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A successful use of CRISPR-Cas9

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Why is international regulation of CRISPR needed?

It is the original source of all genetic variation (new alleles) on which natural selection acts.

Germ cell: can be inherited by offspring.
Somatic cell: not inherited, but can cause cancer.

Investigating a gene's function by making it inoperative and comparing the organism's phenotype with wild type.

Without expert interpretation, information on future disease risk could be misunderstood.

Guide RNA pairs with the target DNA sequence; Cas9 cuts both DNA strands at that site.

Collections of model organisms (e.g. yeast, mice), each with a different gene knocked out, available to researchers.

Some uses (e.g. germline editing) raise ethical issues, and countries have different regulatory systems.

Editing patients' blood stem cells so they make fetal haemoglobin, treating sickle-cell disease (approved 2023).

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Conserved vs highly conserved sequences

Mutation and gene editing **HL only**

Two hypotheses for conserved sequences

Functional requirements of the gene product (selection removes changes) and slower rates of mutation.

Conserved: similar across a species or group. Highly conserved: similar over long periods of evolution.