DNA replication

D1.1 · SL and HL

A small DNA trace is the start of an inference, not its conclusion

Connect faithful copying with selective amplification and comparison

Shared D1.1: sequence continuity, semi-conservative replication, helicase/polymerase, PCR, gel electrophoresis and DNA profiling.

AHL: strand direction, leading/lagging synthesis, prokaryotic enzymes and proofreading.

All numerical examples and profiles are invented. The historical nitrogen-labelling experiment is published research.

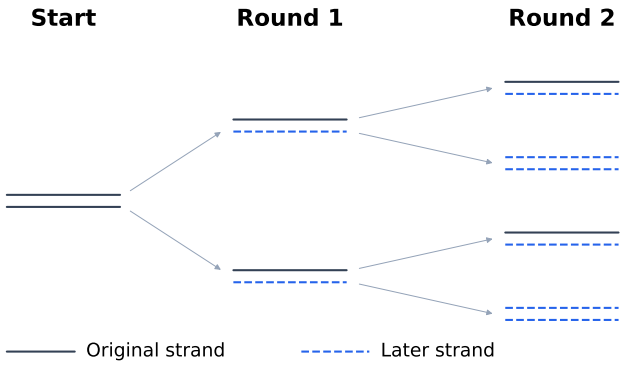
Separate copying, expression and distribution

Process	Product or role
Replication	DNA from DNA; prepares copies for reproduction, growth and tissue replacement
Transcription	RNA from DNA; part of gene expression
Chromosome separation	Distributes DNA copies rather than manufacturing new strands
Accuracy	High fidelity preserves sequence information; errors can still escape correction

Construct a complementary DNA segment

1. **Template.** Aligned bases A–C–G–T–T–A.
2. **Pair each base.** A with T; C with G; G with C; T with A.
3. **New paired segment.** T–G–C–A–A–T. Complementary strands are not identical left-to-right sequences; DNA uses thymine, not uracil.

Track original strands through two complete rounds



Each daughter double helix retains one parental strand and gains one new strand.

Count molecules and original strands separately

1. **After three rounds.** One original molecule gives eight double helices; two contain an original strand.
2. **Molecule fraction.** $2/8 \times 100 = 25\%$ contain original material.
3. **Strand fraction.** Eight double helices have sixteen strands; $2/16 \times 100 = 12.5\%$ are original. No degradation or label exchange assumed.

Experimental measurements test a model's predictions

Meselson and Stahl measured DNA density, not gel fragment length

Bacteria grown with heavy nitrogen were transferred to a light-nitrogen supply. DNA was separated by buoyant density in a caesium chloride gradient.

Intermediate density after one generation, then intermediate and light components, supported conserved parental contributions. Heavy nitrogen was stable, not radioactive.

The 1958 paper discussed molecular interpretation limits. A strand drawing predicts a pattern; it is not direct observation of enzymes.

Semi-conservative replication by numbers



molecules with an original strand: $2/8 = 25\%$

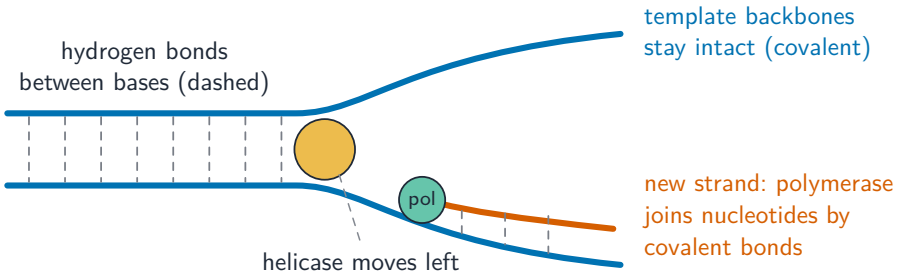
original strands among all strands: $2/16 = 12.5\%$

2 of 16 strands are original: 12.5%

Keep the two kinds of bonds and the enzyme roles distinct

Feature	Explanation
Helicase	Unwinds and separates paired strands by disrupting hydrogen bonds
Templates	Intact covalent backbones retain the ordered base sequences
DNA polymerase	Builds new DNA using template and free DNA nucleotides
New backbone	Covalent bonds join adjacent nucleotides; base pairing links complementary strands

Helicase and DNA polymerase

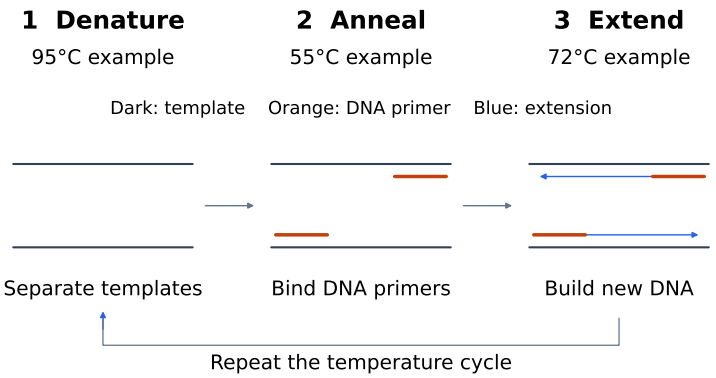


DNA polymerase builds the new strand by base pairing

Assemble the materials for a selected target

Material	**Role**
Template DNA	Contains the region to copy
Two DNA primers	Pair with opposite templates at flanking sites; help determine the target
Free DNA nucleotides	Building material for extension
Taq and buffer/ions	Thermostable enzyme and suitable reaction conditions

A temperature cycle separates, primes and extends



PCR uses heating to separate templates and polymerase to build new DNA.

Explain each temperature rather than recalling three numbers

1. **95°C example.** Denaturation separates paired templates; helicase is not needed for this step.
2. **55°C example.** Annealing permits DNA primers to pair with suitable matching sites.
3. **72°C example.** Taq extends from bound primers. Settings depend on primers, enzyme and product; these are illustrative, not universal.

Thermostability and primer specificity solve different problems

Surviving the hot stage does not mean extension occurs there

Taq tolerates repeated heating, so enzyme need not be added after every cycle. Its main extension stage differs from denaturation.

Two primer sequences select a region rather than copying every gene automatically. Primers are starting points; free nucleotides supply building material.

PCR amplifies DNA. It does not itself sort fragment sizes or establish a person's identity.

State the ideal amplification model before using powers

1. **Model.** $N = N_0 \times 2^n$: five defined targets, complete doubling each cycle.
2. **Ten cycles.** $N = 5 \times 2^{10} = 5120$ targets.
3. **Five more cycles.** Multiplication factor $2^5 = 32$, not five additional molecules.

An ideal doubling model is not a guaranteed laboratory yield

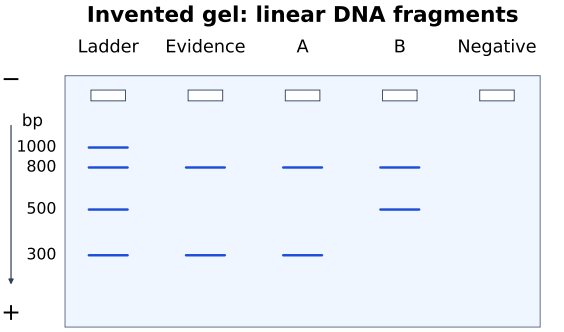
Account for both early products and late reaction limits

In early cycles from long genomic templates, not every new strand has both ends defined by primer sites.

Incomplete reactions and wrong priming can reduce intended amplification or create other products.

Reagent limitation and accumulated products prevent unlimited doubling. The stated equation models defined targets under ideal conditions.

Read the wells, electrodes and size ladder first



Smaller fragments travel farther toward the positive electrode

Smaller linear fragments travel farther toward the positive electrode.

Explain what the visible gel can and cannot show

Observation	**Interpretation**
Negative DNA backbone	Migration toward the positive electrode
Gel sieve	Smaller comparable linear fragments travel farther in the same run
Size ladder	Known fragment lengths support size estimates
A visible band	Many similarly sized fragments; neither one molecule nor a complete sequence

Compare the complete illustrated pattern

1. **Evidence.** Bands correspond to 800 and 300 base pairs.
2. **References.** A: 800/300; B: 800/500. A matches these illustrated sizes; B differs.
3. **Limits.** One shared 800-base-pair band is insufficient. Equal size does not guarantee identical sequence or unique identity.

Distance is not a universally linear base-pair ruler

Compare like DNA forms under the same run conditions

Estimate size with the ladder and an appropriate calibration, often involving logarithm of fragment length.

Do not infer that twice the migration distance means half the base pairs.

Supercoiled, nicked circular and linear DNA can migrate differently. This example compares linear fragments, not every DNA conformation.

Interpret a blank sample only after checking the controls

1. **Positive control fails.** The method has not demonstrated successful amplification/detection; sample blanks are not safely interpretable as valid negatives.
2. **Negative control has product.** Suspect contamination or unintended amplification rather than an extra matching person.
3. **Response.** Investigate handling, reagents and procedure and repeat appropriately before accepting the profiles. Clean controls do not exclude every error.

Use amplification and separation as steps toward comparison

A marker is selected inherited variation, not an entire identity

At a simple autosomal marker, one allele normally comes from each biological parent.

PCR copies selected marker regions; separation resolves product sizes; analysis compares alleles across markers.

Many suitable variable markers improve discrimination. Same size alone does not mean the same complete sequence or a uniquely identified person.

Test both markers in an invented paternity example

Person	**Marker 1; marker 2**
Mother	12, 14; 7, 9
Child	14, 16; 9, 12
Candidate A	16, 18; 10, 12
Candidate B	20, 22; 10, 12

Identify the required paternal contribution before deciding

1. **Marker 1.** Mother can supply 14; the other parent must supply 16 under the simple model.
2. **Marker 2.** Mother can supply 9; the other parent must supply 12.
3. **Inference.** A is consistent at both markers. B lacks 16 and is inconsistent, assuming correct samples/measurements and no relevant mutation. A is not thereby proven to be the father.

More reliable markers reduce a chance match

Do not invent a probability from the number of bands alone

Agreement must extend across more sufficiently independent inherited markers. Related people can share more alleles than unrelated people.

Validated statistics, quality controls, mixtures and possible mutation affect the inference.

Repeating a contaminated sample is not independent corroboration; ordinary DNA profiles also cannot uniquely distinguish identical twins.

A contributor inference is not proof of an offence

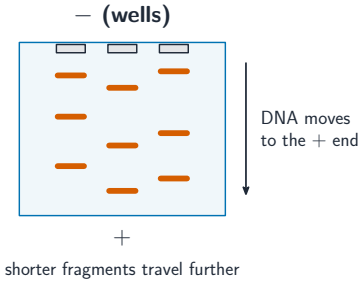
Interpret a forensic profile with the other evidence

A match may support that a person contributed DNA to a trace; it does not establish when or how the DNA arrived.

Transfer, contamination, mixtures, degradation and sample handling can alter interpretation.

Use documented provenance, appropriate controls and other case evidence. Do not equate a matching pattern with guilt.

Gel electrophoresis

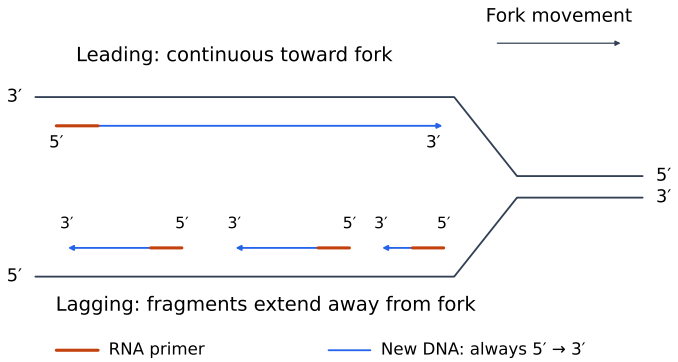


Shorter fragments travel further

Orient the template before constructing the complement

1. **Template.** $3'$-ACGTTA-$5'$.
2. **Aligned daughter.** $5'$-TGCAAT-$3'$: complementary and antiparallel.
3. **Chemistry.** The incoming nucleotide's $5'$ phosphate joins the growing strand's free $3'$ end. Every new DNA segment grows $5'$ to $3'$.

Separate fork movement from new-strand growth



Leading and lagging segments both grow in the 5′ to 3′ direction.

Explain why one template needs repeated starts

1. **Leading.** The 3′-to-5′ template toward this fork allows continuous daughter synthesis toward the fork after initiation.
2. **Lagging.** The opposite template needs short 5′-to-3′ Okazaki fragments extending away from the advancing fork.
3. **Priming.** Primase initiates leading synthesis once in this model and supplies another RNA primer for each lagging fragment. Processing and ligation eventually produce a continuous daughter strand.

Assign each step to the appropriate prokaryotic enzyme

Enzyme	**Role**
Primase	Makes RNA primers
DNA polymerase III	Extends new DNA from the starting ends
DNA polymerase I	Removes RNA primer material and replaces it with DNA
DNA ligase	Seals remaining nicks between adjacent DNA sections

Cellular replication and PCR use different components

Keep the model's organism and primer chemistry explicit

The polymerase I/III names here describe the prokaryotic system, not universal names for eukaryotic replication.

Cells use RNA primers and helicase. Standard PCR uses supplied DNA primers and thermal separation.

Ligase seals a backbone nick; it does not supply all missing nucleotides or reunite the parental strands instead of making daughters.

Correct the new strand rather than changing the template

1. **Error.** Template C has an incorrectly added terminal A in the new strand.
2. **Proofread.** Polymerase III removes that mismatched nucleotide from the growing $3'$ terminal and replaces it with G.
3. **Continue.** Synthesis resumes $5'$ to $3'$. Removal is not ligation or helicase action; proofreading lowers error frequency without eliminating every mutation.

Attempt the complete handout practice before checking

Name the product, denominator, control and inference

Explain continuity, complementary pairing and strand inheritance; distinguish helicase from polymerase.

Use an ideal PCR calculation, interpret gel sizes/controls, and evaluate the invented paternity and forensic examples.

AHL: orient sequences and fork synthesis; explain enzyme roles and terminal proofreading.

Check the practice counts and amplification

1. **Five replication rounds.** $2^5 = 32$ molecules; two original-containing; $2/32 = 6.25\%$.
2. **Individual strands.** $2 \times 32 = 64$ strands; $2/64 = 3.125\%$ original.
3. **PCR.** $2 \times 2^{12} = 8192$ targets; five more ideal cycles multiply by 32. State complete doubling and no label exchange/destruction.

Check the practice pattern and inheritance reasoning

1. **Gel.** A matches 800/300; B differs at 500. DNA migrates positive; shorter comparable fragments travel farther.
2. **Paternity.** Required paternal alleles 16 and 12. B lacks 16; A is consistent, not proven unique.
3. **Inference.** More suitable markers reduce chance agreement. A forensic match does not establish time, transfer mechanism or guilt; controls and independent evidence matter.

AHL practice: orientation and terminal correction

1. **Sequence.** $5'$-CTGAAT-$3'$, or reversed $3'$-TAAGTC-$5'$.
2. **Correction.** Template G with newly added terminal T: remove T at the new strand's $3'$ end and replace with C.
3. **Fork.** Leading rightwards and lagging fragments leftwards for the given fork; all new synthesis $5'$ to $3'$. Primase starts, III extends, I replaces primers, ligase seals nicks.

Check yourself

Track the DNA and qualify the conclusion

Count double helices separately from individual strands. A new strand that becomes a later template is not an original labelled strand.

PCR amplifies, a gel separates, and profile analysis compares. A matching size is not a complete sequence or proof of guilt.

Use the actual controls and assumptions. Distinguish a useful comparison from a stronger claim that needs independent evidence.