DP Biology – D1.1

DNA replication

- How do cells make new DNA?
- What biotechnology has understanding DNA replication made possible?

SL statements D1.1.1–5. Textbook pp. 602–610.

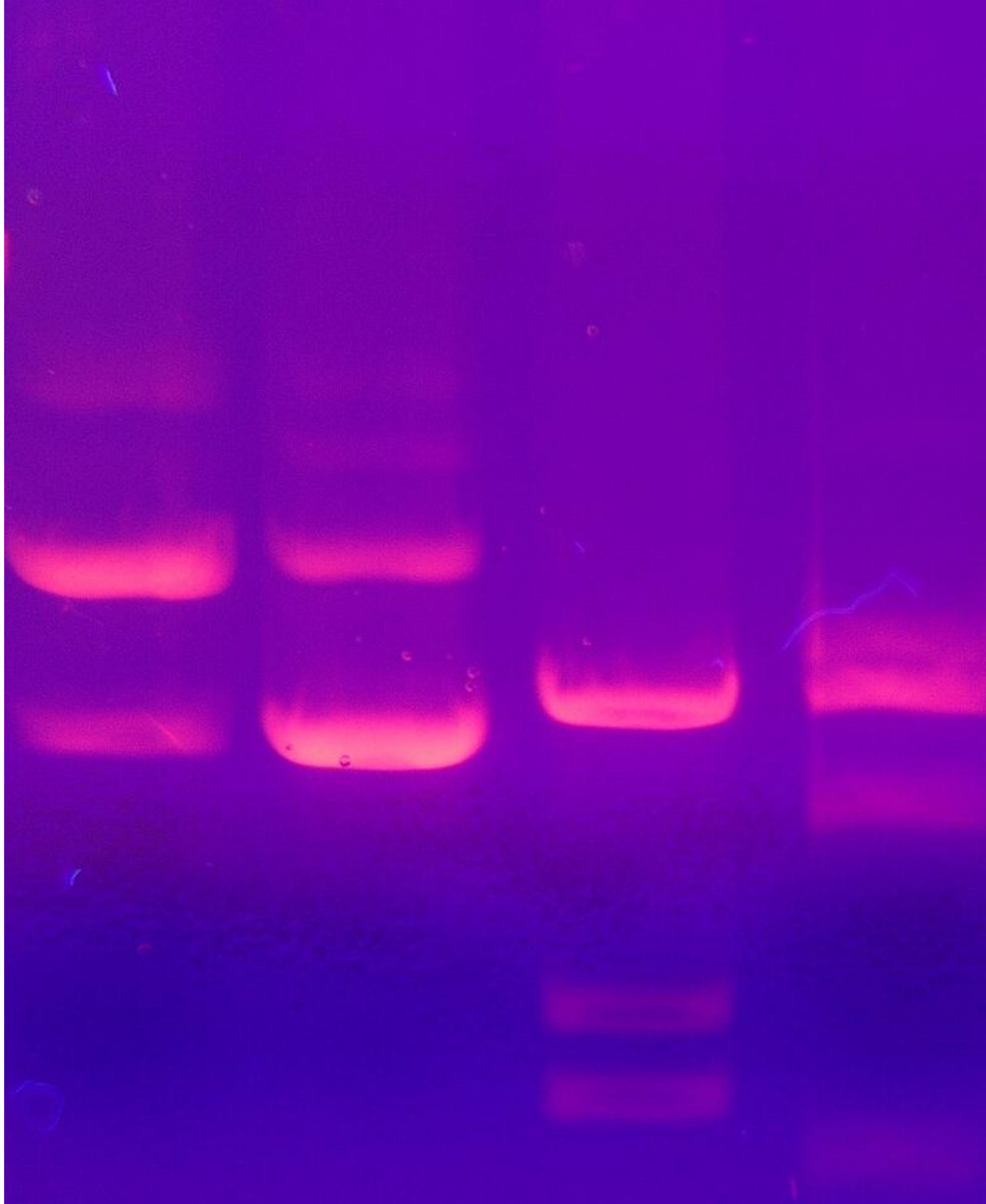


Image: DNA fragments on an electrophoresis gel. Mnolf, CC BY-SA 3.0.

Objectives for each class

Class	Statements	By the end of the class, you can...
1	D1.1.1–3	Explain why DNA is replicated, why replication is semi-conservative and accurate, and what helicase and DNA polymerase do
2	D1.1.4	Explain how PCR copies DNA and how gel electrophoresis separates fragments
3	D1.1.5	Use a DNA profile to reach a conclusion in forensic and paternity cases

Overview of this chapter

Part	Statements	What it covers
Why copy DNA	D1.1.1	Reproduction, growth and tissue replacement
How copies stay exact	D1.1.2	Semi-conservative replication and base pairing
The enzymes	D1.1.3	Helicase and DNA polymerase
Tools in the lab	D1.1.4	PCR and gel electrophoresis
Uses	D1.1.5	Forensics, paternity and diagnosis

Why cells copy their DNA

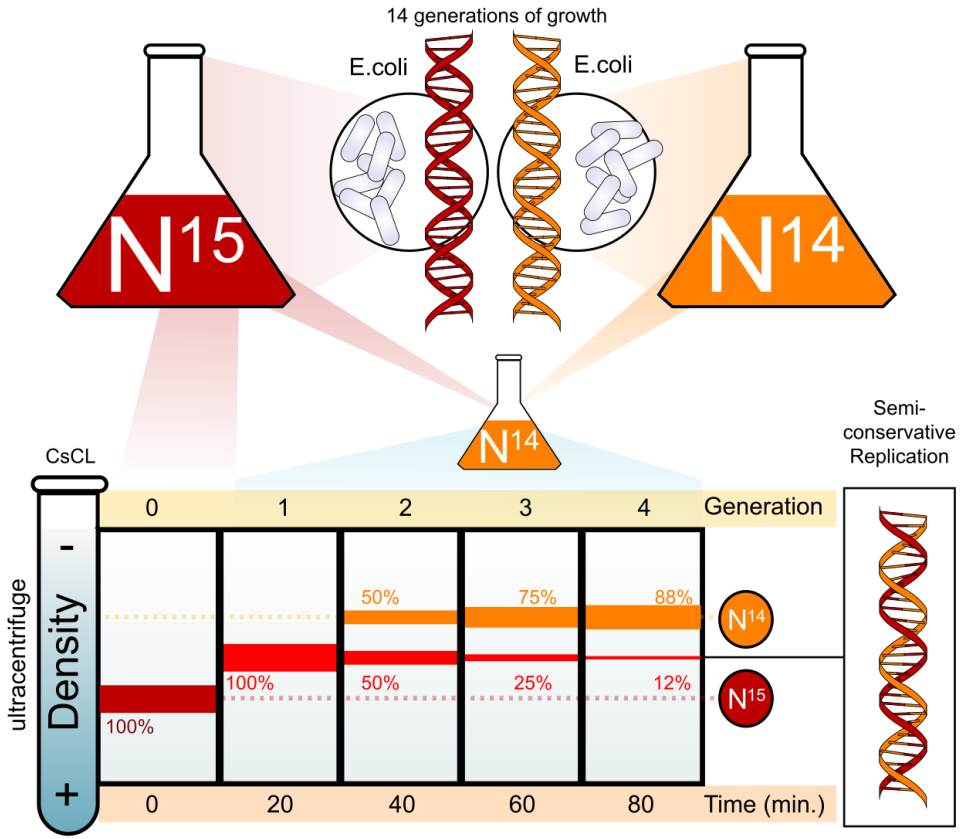
- **DNA replication** copies DNA exactly, so that the base sequence stays the same
- It happens before a cell divides, in the S phase of interphase

Need	Why copies are required
Reproduction	Offspring need copies of the base sequences of their parents, whether reproduction is sexual or asexual
Growth	Multicellular organisms grow by adding cells, and each new cell needs a full set of DNA
Tissue replacement	Lost or damaged cells, such as skin cells, are replaced by new cells with the same genes

Common mistake

Replication is not the same as cell division. DNA is copied first, and the cell divides later.

Replication is semi-conservative



The Meselson-Stahl experiment. LadyofHats, public domain.

- The two strands of the double helix separate
- **Each strand acts as a template** for a new strand
- Each new molecule has **one old strand and one new strand**
- This is why it is called **semi-conservative**

Evidence

Meselson and Stahl grew bacteria on heavy nitrogen, then on normal nitrogen. The DNA densities they found fit only this model.

Activity

Predict the DNA bands after one and after two generations if replication were fully conservative instead.

Base pairing makes copying accurate

D1.1.2

Strand	Base sequence
Template	A T G A C C G C T
New strand	T A C T G G C G A

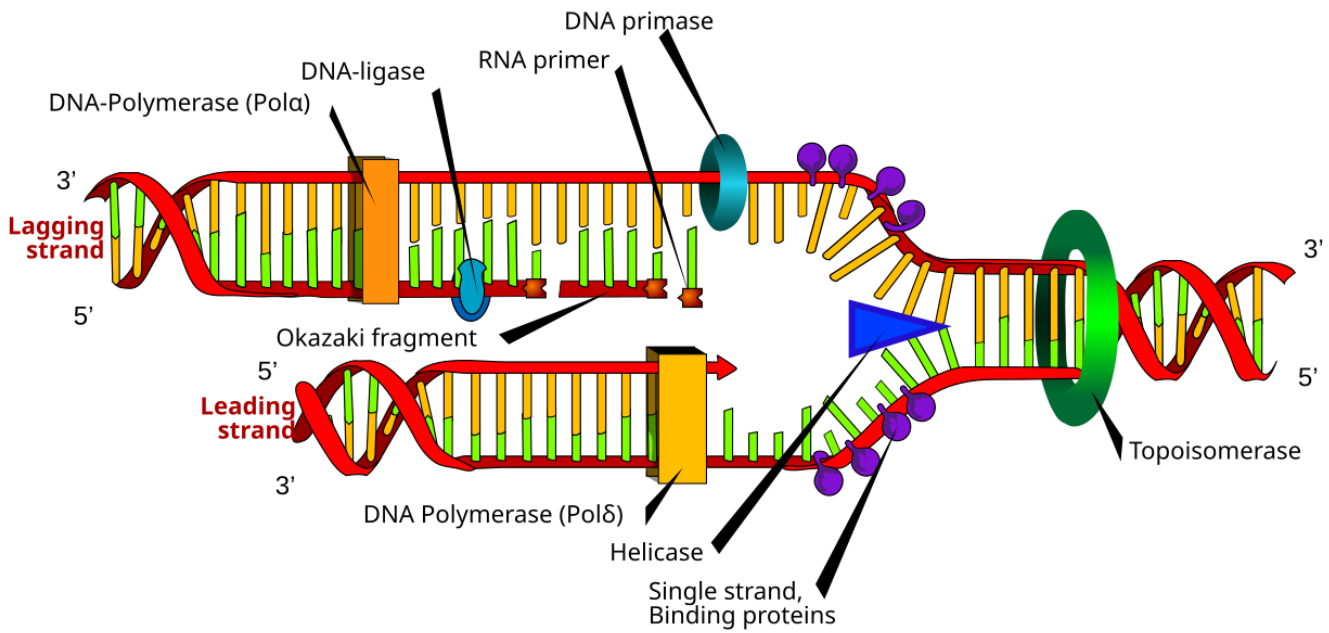
- **Adenine** pairs only with **thymine**; **cytosine** pairs only with **guanine**
- Only complementary bases form hydrogen bonds in the correct position
- So the base on the template decides which nucleotide joins next, and both new molecules match the original

Key idea

Hydrogen bonds between complementary bases are what makes the copying so accurate. Mistakes are rare, but when they do happen they are the source of mutations (D1.3).

Helicase and DNA polymerase

D1.1.3



- Separation produces a **replication fork**
- Free nucleotides pair with exposed bases
- Polymerase forms **covalent bonds** between neighbouring nucleotides
- ATP is needed for unwinding

A replication fork. LadyofHats, public domain. HL covers the other enzymes shown.

Enzyme	Role at SL
Helicase	Unwinds the double helix and separates the strands by breaking hydrogen bonds between bases
DNA polymerase	Links free nucleotides into a new strand, using the old strand as a template

PCR copies a chosen section of DNA

D1.1.4



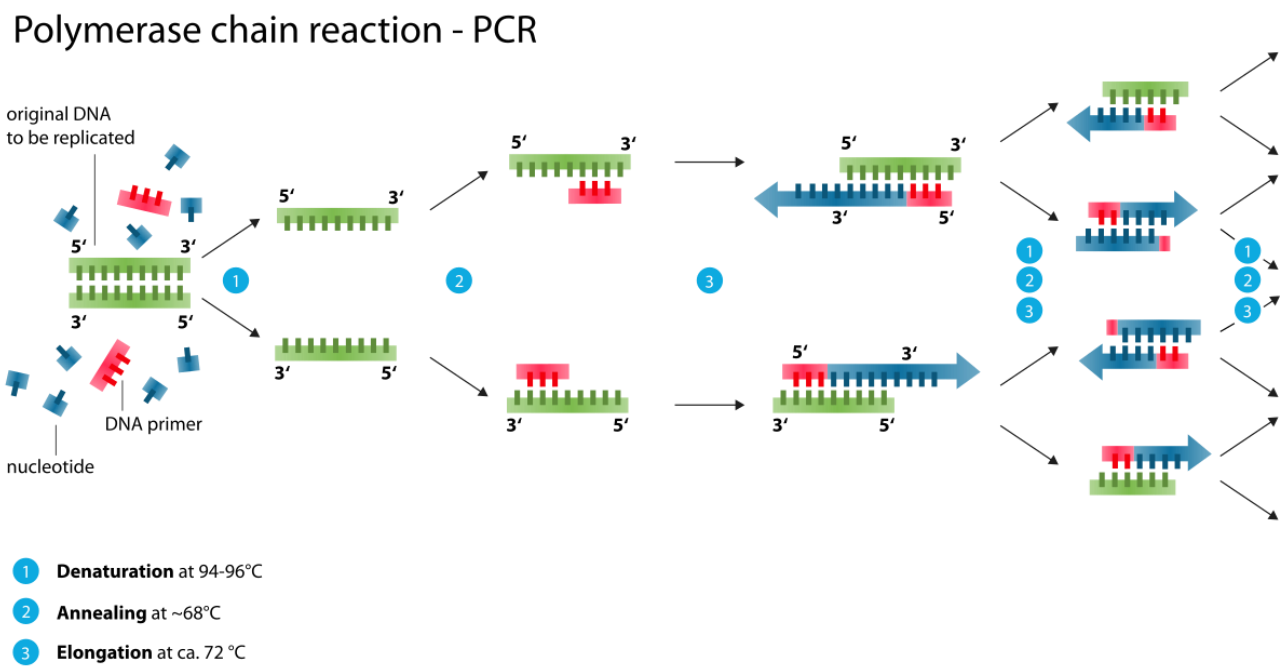
A thermal cycler. Juan Carlos Fonseca Mata, CC BY-SA 4.0.

Ingredient	Why it is needed
DNA sample	Holds the section to be copied
Primers	Short single strands of DNA, which mark the start of the section
Nucleotides	The building blocks for new strands
Taq polymerase	Builds the new strands, and survives high temperatures

Key idea

PCR amplifies a tiny sample. It is automated, and a thermal cycler changes the temperature in each cycle.

One cycle of PCR



Step	Temp	What happens
Denaturation	95°C	Strands separate
Annealing	40°C	Primers attach
Elongation	72°C	Taq builds strands

Steps in a PCR cycle. Enzoklop, CC BY-SA 3.0.

Worked example

Each cycle doubles the DNA. Starting from one molecule: 2 after one cycle, 4 after two, 8 after three. After 30 cycles there are over 1 billion copies, in a couple of hours.

Why Taq polymerase is used



A hot spring in Yellowstone. Jim Peaco, National Park Service, public domain.

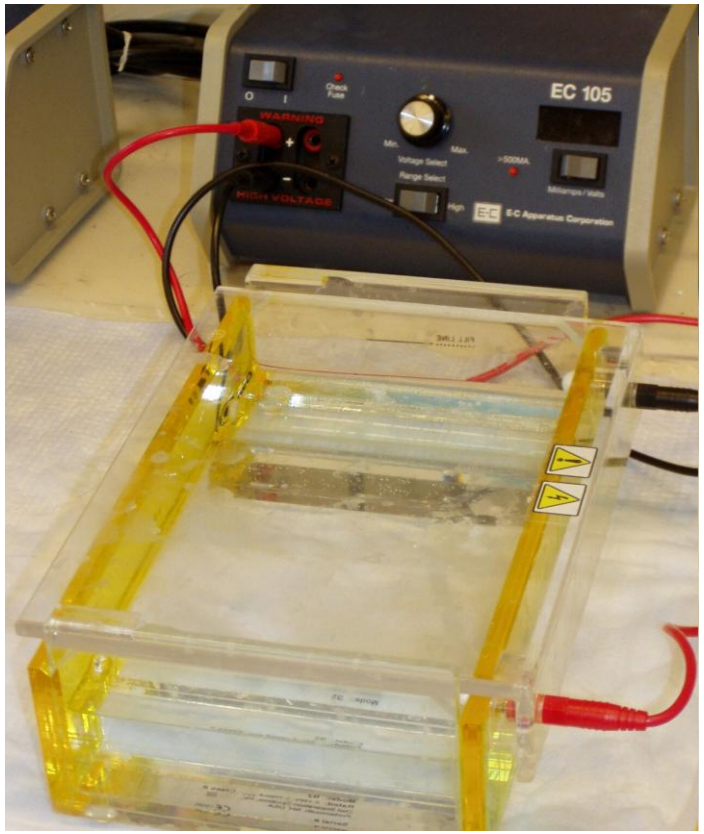
- Taq comes from **Thermus aquaticus**, a bacterium found in hot springs
- Its enzymes must work at high temperatures
- It is not denatured at 95°C, so it survives every cycle
- Most other polymerases would be destroyed in the first cycle

Link

An adaptation that evolved for life in a hot spring became a key tool in biotechnology. This is why studying unusual organisms can be useful.

Gel electrophoresis separates fragments

D1.1.4



Loading and running a gel. Jeffrey M. Vinocur, CC BY 2.5.

- DNA samples are placed in **wells** at one end of a gel
- An electric current is applied across the gel
- DNA has **negatively charged** phosphate groups, so it moves towards the **positive** electrode
- The gel acts as a **molecular sieve**: small fragments travel furthest
- A **DNA ladder** contains fragments of known length, for comparison

Common mistake

Fragments separate by length. They all carry negative charge, so charge is what makes them move and not what separates them.

Reading a gel



Schematic gel. Wells are at the top; the positive electrode is at the bottom.

- Each band contains many fragments of the same length
- Bands near the wells are the longest fragments
- Compare with the ladder to estimate length

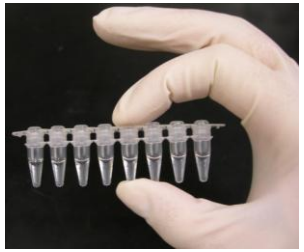
Question

Which sample has the shortest fragment? Which sample shares a band with each of the other two?

What PCR and electrophoresis are used for

D1.1.5

Application	How the tools help
Forensic investigation	Tiny samples from a crime scene are amplified, then compared with samples from suspects
Paternity testing	Fragments in a child are compared with those of the mother and the possible father
Diagnosis of infection	PCR detects the genetic material of a pathogen; for a coronavirus, RNA is first copied into DNA
Research	Copying a gene gives enough DNA to study or sequence



PCR tubes hold the very small volumes used in each reaction. Image: Madprime, CC0. The range of uses shows why this technique changed biology so quickly.

Why DNA differs between people

D1.1.5

- Most human DNA does not code for proteins
- In some non-coding regions a short sequence repeats many times: a **short tandem repeat (STR)**
- The **number of repeats** varies between individuals, so fragment length varies too
- An individual has two copies of each locus, one inherited from each parent

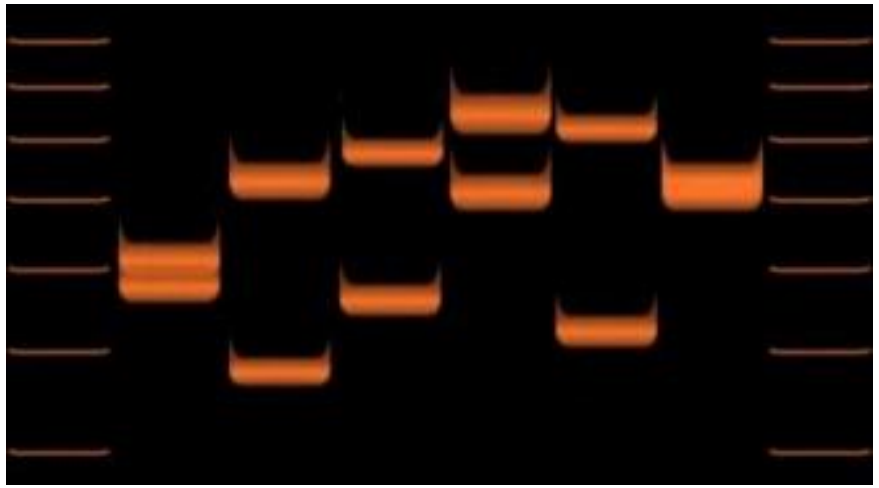
Person	Sequence at one locus	Repeats
P	GATA GATA GATA GATA	4
Q	GATA GATA GATA GATA GATA GATA GATA	7

Key idea

PCR copies these loci, and electrophoresis separates them by length. Many loci are compared, so it is very unlikely that two unrelated people match at all of them.

Forensic investigation: reach a conclusion

D1.1.5



A DNA profile from one locus. PaleWhaleGail, CC BY-SA 3.0.

Sample	Band 1	Band 2	Band 3
Crime scene	Yes	Yes	Yes
Suspect 1	Yes	No	Yes
Suspect 2	Yes	Yes	Yes
Suspect 3	No	Yes	No

- A profile from a crime scene is compared with those of suspects and of the victim
- All bands must match for the samples to come from the same person

Common mistake

A match is strong evidence, but it is not proof of guilt. It shows that a person's DNA was at the scene, and not how or when it got there. Results are also a matter of probability.

Paternity: half from each parent

D1.1.5

Person	Band 1	Band 2	Band 3	Band 4
Mother	Yes		Yes	
Child	Yes	Yes		
Man X		Yes		Yes
Man Y			Yes	Yes

- Every band in the child must come from the mother or the father
- Remove the bands explained by the mother. The father must have the remaining bands

Worked example

The child's band 1 comes from the mother. Band 2 is not in the mother, so it comes from the father. Man X has band 2 and man Y does not, so man Y is excluded.

Check that you can explain

1. Three reasons why DNA is replicated
2. What semi-conservative means
3. Why base pairing makes copying accurate
4. What helicase and DNA polymerase do
5. The steps and temperatures of PCR
6. Why Taq polymerase is used
7. How a gel separates fragments
8. How to read a forensic or paternity profile

Exit activity: 5 minutes

On your own and without notes. Hand this in as you leave.

1. A template strand reads A T T G C A. Write the complementary strand.

2. Name the enzyme that breaks hydrogen bonds between strands, and the enzyme that links nucleotides together.

3. Explain why fragments of DNA move through a gel, and why they separate.

Image credits

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