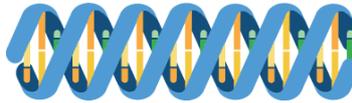


THE STRUCTURE AND REPLICATION OF DNA

DNA is the molecule that holds the instructions for all living things. DNA achieves this *feat* of storing, coding and transferring biological information through its unique structure.

DNA structure

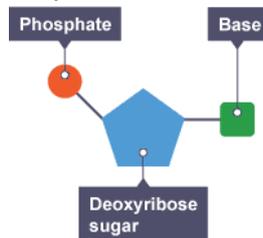


DNA is the molecule that holds the instructions for growth and development in every living thing. Its structure is described as a double-stranded helix held together by complementary base pairs.

DNA as a double stranded helix held together by complementary base pairs.

The basic units of DNA are nucleotides. These nucleotides consist of a deoxyribose sugar, phosphate and base.

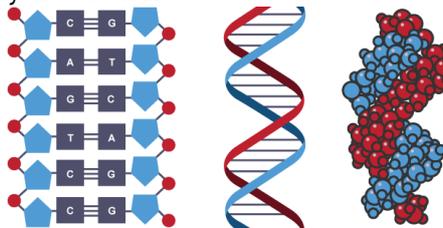
The nucleotides are identical except for the base, which can be an adenine, thymine, guanine or cytosine.



A nucleotide consisting of a deoxyribose sugar, phosphate and base

These basic units are linked together to form **strands** by strong **covalent** bonds between the deoxyribose sugar of one nucleotide and the phosphate of the next nucleotide. These strong bonds form a sugar-phosphate backbone.

The ends of the DNA strand are called the 5' end, pronounced 5 prime ends, at the phosphate end, and the 3' end at the deoxyribose end.



Three versions of the DNA structure showing two strands linked together with the hydrogen bonds.

All cells store their genetic information in the base sequence of DNA. The genotype is determined by the sequence of bases.

Organization of DNA

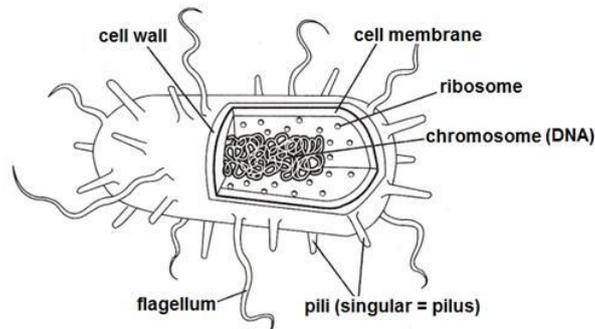
DNA is present in the cells of every living thing. However, the DNA is organized differently in different types of organism.

We can divide cells into two groups based on how they organize their DNA – eukaryotes and prokaryotes.

Prokaryotes

Bacteria are prokaryotes. They do not have a membrane-bound nucleus and their DNA is free in the cytoplasm.

Bacteria have a single circular chromosome in the centre of the cell that holds all the genes needed for that bacterium. Bacteria also have extra circles of DNA called plasmids. These plasmids contain additional genes, such as for antibiotic resistance, which may increase a bacterium's chance of survival. Bacteria can exchange plasmids with other bacteria through hair-like extensions on their surface called pili.

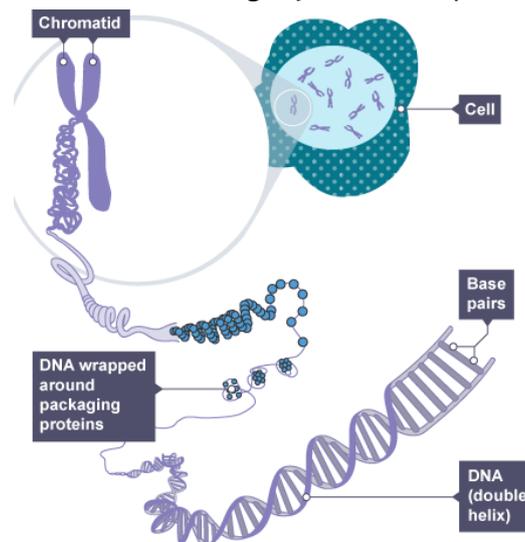


Organization of DNA in prokaryotes

Eukaryotes

Animals, plants and fungi are eukaryotes. They have a membrane-bound nucleus and their chromosomes are linear rather than circular.

The DNA found in the linear chromosomes is tightly **coiled** and packaged around special proteins as shown below.



Eukaryote cell with nucleus, cell and chromosome. The chromosome is magnified to show the chromatic, and within it, the packaging proteins, DNA double helix and base pairs.

Interestingly, circular chromosomes are also found in mitochondria and chloroplasts. They both use their own DNA to make some proteins needed for their function. This gives evidence for the theory that mitochondria and chloroplasts originated from prokaryotic cells that were **engulfed** by a larger cell.

The need for DNA replication

DNA replication is the process by which a cell makes an identical copy of its DNA. This process is performed at the beginning of every cell division so that when the cell divides, each daughter cell will inherit an identical copy of the DNA.

Requirements for DNA replication

- ☞ Original DNA **template** - DNA is a double helix made of two complementary strands. Each strand can be used as a template to create a new DNA molecule.
- ☞ Free DNA nucleotides – needed to form the new strands.
- ☞ DNA polymerase – an enzyme that adds new nucleotides to a growing strand of DNA.
- ☞ Primers – needed to start the process because DNA polymerase can only add nucleotides to an existing strand of DNA.

DNA replication

Stage one

The DNA is unwound and unzipped. The helix structure is unwound. Special molecules break the weak hydrogen bonds between bases, which are holding the two strands together. This process occurs at several locations on a DNA molecule.

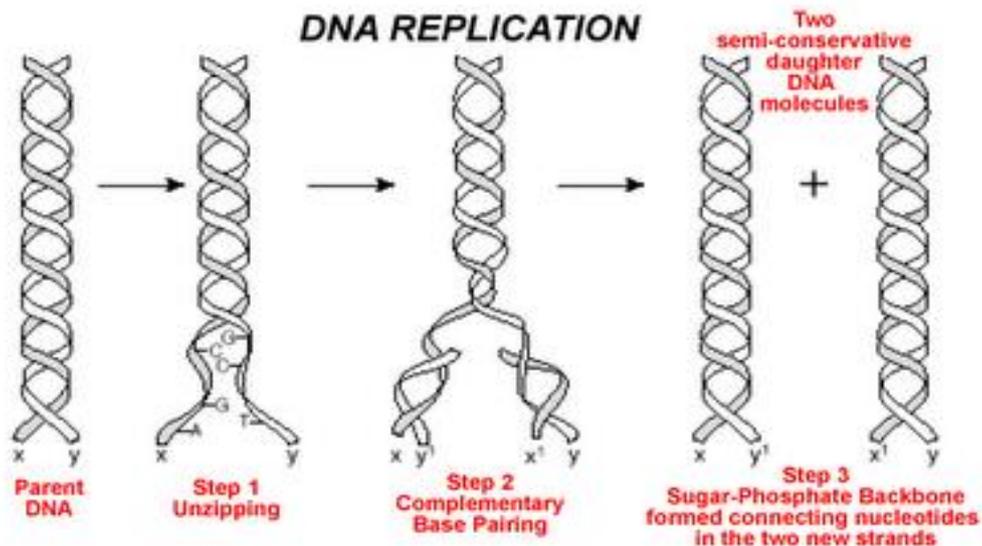
Stage two

DNA polymerase adds DNA nucleotides in a 5' to 3' direction. Complementary DNA nucleotides are added to the now exposed bases on both strands. Adenine pairs with thymine, thymine with adenine, cytosine with guanine and guanine with cytosine. A primer is needed to start replication. Leading strand is synthesized continuously. DNA polymerase adds nucleotides to the deoxyribose (3') ended strand in a 5' to 3' direction.

Lagging strand is synthesized in fragments. Nucleotides cannot be added to the phosphate (5') end because DNA polymerase can only add DNA nucleotides in a 5' to 3' direction. The lagging strand is therefore synthesized in fragments. The fragments are then sealed together by an enzyme called ligase.

Stage three

The two new strands twist to form a double helix. Each is identical to the original strand.



Polymerase chain reaction

The Polymerase Chain Reaction (PCR) is a technique for the amplification of DNA in vitro (this describes experiments with cells outside their normal environment).

This technique allows scientists to easily and cheaply turn a single strand of DNA into millions of copies which can then be used for analysis.

The analysis of DNA is used in the Human Genome Project, paternity testing, diagnosis of genetic disorders and the detection of infection.

Requirements for PCR

- ☞ DNA – the original strand of DNA which needs amplified.
- ☞ Complimentary primers – primers are short complementary sequences of RNA needed to start DNA synthesis.
- ☞ Thermal cycler – equipment that varies the temperature of the reaction.
- ☞ Heat-tolerant polymerase – an enzyme which will add nucleotides to the growing strand and which is not denatured by the high temperatures used in the reaction.
- ☞ Supply of nucleotides – to synthesize the new strands of DNA.

The PCR process

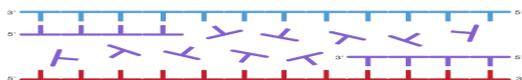
1. DNA heated - to **denature** the DNA and separate the two strands.
2. DNA cooled - in preparation for adding primers.
3. Complimentary primers added - which are complementary to the target sequences at the two ends of the region to be amplified.
4. Heat-tolerant DNA polymerase added - which replicates the region of DNA to be amplified. Two strands are formed.
5. Repeated cycles are carried out - which amplify this region of DNA from a single strand to millions of copies.



DNA is separated by heating to 95°C



At approximately 60°C primers bind to complementary bases at the edges of the area of interest



At 72°C heat-tolerant DNA polymerase extends the single stranded DNA molecule, creating new double standard DNA



The process is repeated for 25-35 cycles to amplify the DNA sample

The thermal cycler allows this process to be automated. The reaction mixture is added, and then repeated cycles of heating and cooling cause the DNA to be continually denatured and replicated.

The Structure and Replication of DNA

READING COMPREHENSION

Tick the right answer

- 1) What is the name for the basic units which make up strands of DNA?
 - a. Phosphates
 - b. Nucleotides
 - c. RNAs
- 2) What are the DNA base pairing rules?
 - a. Adenine to Guanine, Cytosine to Thymine
 - b. Adenine to Cytosine, Thymine to Guanine
 - c. Adenine to Thymine, Cytosine to Guanine
- 3) The two strands of DNA are held together by which kind of bonds?
 - a. Peptide
 - b. Covalent
 - c. Hydrogen bonds
- 4) What are DNA nucleotides made up of?
 - a. A deoxyribose sugar, phosphate and base
 - b. A ribose sugar, phosphate and base
 - c. A deoxyribose sugar, phosphate and ATP
- 5) DNA is found as linear chromosomes, tightly coiled and packaged with associated proteins in which type of organism?
 - a. Eukaryotes
 - b. Prokaryotes
 - c. Both eukaryotes and prokaryotes
- 6) Which of these enzymes are not needed for DNA replication?
 - a. Ligase
 - b. Restriction endonucleases
 - c. DNA Polymerase
- 7) Why can both strands of DNA not replicated continuously?
 - a. DNA nucleotides can only be added in a 5' to 3' direction
 - b. The DNA polymerase can only act on one strand at a time
 - c. There is not enough space for both strands to be copied
- 8) In DNA replication, the fragments of DNA which have been replicated discontinuously are joined together using what?
 - a. Ligase
 - b. DNA Polymerase
 - c. Restriction endonucleases
- 9) PCR is a technique used for what?
 - a. The amplification of DNA in vitro
 - b. The amplification of DNA in vivo
 - c. Sequencing the nucleotide bases of DNA
- 10) What are primers?
 - a. Single stranded random sequences of nucleotides
 - b. Single stranded sequences of nucleotides which are complementary to a target sequence
 - c. Double stranded sequences of nucleotides which are complementary to a target sequence

Vocabulary

Match the words in A with their definitions in B

| A | B | C |
|-------------|---|---------|
| 1) Feat | a) Something that is used as an example of how to do, make, or achieve something | 1. +... |
| 2) Strand | b) A remarkable deed. | 2. +... |
| 3) Covalent | c) To overwhelm or envelop completely. | 3. +... |
| 4) Coiled | d) To modify the molecular structure of s.th especially by heat, acid, or ultraviolet radiation so as to destroy or diminish some of the original properties and especially the specific biological activity. | 4. +... |
| 5) Engulfed | e) An elongated or twisted body resembling a rope. | 5. +... |
| 6) Template | f) To move in a circular or spiral course. | 6. +... |
| 7) Denature | g) A chemical bond formed between atoms by the sharing of electrons. | 7. +... |

GRAMMAR

Question Tags

We use tags in spoken English but not in formal written English.

They are not really questions but are a way of asking the other person to make a comment and so keep the conversation open.

Making a tag is very mechanical. To make a tag, use the first auxiliary. If there is no auxiliary, use do, does or did. With a positive sentence, make a negative tag and with a negative sentence, make a positive tag.

A question tag is a small question at the end of a statement.
Question tags are used when asking for agreement or confirmation.

a positive statement + a negative question tag

You ⁺are a student, ⁻aren't you?

A subject pronoun comes after an auxiliary or a form of the verb To Be

a negative statement + a positive question tag

Mary ⁻isn't a teacher, ⁺is she?

A subject pronoun is used to replace the noun or noun phrase

Intonation and Meaning

The intonation of a question tag shows the exact meaning of it.

If the intonation of the question tag goes **up**, it means you are not sure and you want to know the answer.

John **doesn't** speak Spanish, **does** he?

If the intonation of the question tag goes **down**, it means you are checking / confirming information or making conversation.

John **doesn't** speak Spanish, **does** he?

Positive Question Tags

Question tags are used when asking for agreement or confirmation.

a **negative statement** + a **positive question tag**

Negative Statement

| | | | |
|------|-----------|----------------------|-------------|
| You | aren't | a teacher, | are you? |
| He | isn't | crazy, | is he? |
| He | wasn't | relaxed, | was he? |
| They | weren't | late, | were they? |
| You | don't | French, | do you? |
| You | didn't | study for the test, | did you? |
| You | haven't | been here all week, | have you? |
| You | hadn't | done it before then, | had you? |
| You | won't | fail the exam, | will you? |
| You | can't | drive a car, | can you? |
| You | couldn't | do it for me, | could you? |
| We | mustn't | say anything, | must we? |
| You | shouldn't | be so busy, | should you? |
| You | wouldn't | stop me, | would you? |

Positive Tag

Statements using *barely*, *hardly*, *neither*, *no*, *nobody*, *none*, *nothing* and *seldom* are treated as negative statements.

| | | | |
|---------|------|-----------------|-----------|
| Nobody | went | to the meeting, | did they? |
| Nothing | is | ready, | is it? |

Negative Question Tags

Question tags are used when asking for agreement or confirmation.

a **positive statement** + a **negative question tag**

Positive Statement

| | | | |
|------|---------|-------------------------|----------------|
| You | are | a student, | aren't you? |
| He | is | very busy, | isn't he? |
| He | was | happy, | wasn't he? |
| They | were | surprised, | weren't they? |
| You | speak | English, | don't you? |
| He | studies | Spanish, | doesn't he? |
| You | studied | for the test, | didn't you? |
| You | have | studied all week, | haven't you? |
| You | had | arrived before he left, | hadn't you? |
| You | will | pass the exam, | won't you? |
| You | can | speak two languages, | can't you? |
| You | could | do it for me, | couldn't you? |
| We | must | be patient, | mustn't we? |
| You | should | go now, | shouldn't you? |
| You | would | like a new job, | wouldn't you? |

Negative Tag

Negative auxiliaries and verbs in tags are usually in their contracted form (= n't)

Exceptions

| | | | |
|---|-------|----------|-----------|
| I | am | late, | aren't I? |
| | Let's | go home, | shall we? |

BE CAREFUL

Exercise 1: Tick (✓) the correct question tags to complete the questions.

- She's an accountant, _____?
 (A) aren't she (B) isn't she (C) she isn't (D) doesn't she
- I'm here, _____?
 (A) aren't we (B) aren't I (C) am I not (D) doesn't I
- You and I are very busy right now, _____?
 (A) aren't you (B) isn't she (C) she isn't (D) aren't we
- It isn't very windy today, _____?
 (A) hasn't it (B) isn't it (C) is it (D) doesn't it

Exercise 2: These are sentences from real conversations. Put in the question tags.

- I'm cooking tonight, _____?
- He's not a baby, _____?
- It'll be all right, _____?
- Laura's still got short hair, _____?
- She doesn't feel well, _____?
- They left early, _____?
- She didn't feel well yesterday, _____?
- They've left early, _____?

Exercise 3: Match the beginning of the sentences and the question tags.

| Tag Question | Question Tag | Answer |
|-----------------------------------|-----------------|---------|
| 1. They are away for a few weeks, | a. was it? | 1. +... |
| 2. It wasn't your turn, | b. don't you? | 2. +... |
| 3. You're not serious, | c. doesn't she? | 3. +... |
| 4. She isn't Italian, | d. will you? | 4. +... |
| 5. She needs some help, | e. is she? | 5. +... |
| 6. It's warm, | f. aren't they? | 6. +... |
| 7. There is a problem, | g. isn't it? | 7. +... |
| 8. You come here often, | h. isn't there? | 8. +... |
| 9. Don't forget, | i. are you? | 9. +... |

Exercise 4: Find and correct the mistakes in each sentence.

- You're Australian, aren't they? _____
- You aren't English, aren't you? _____
- Mary smokes, didn't she? _____
- Sharks don't like vegetables, don't they? _____
- Moles can't see, can it? _____
- You'll be there, won't she? _____
- David won't come, has he? _____
- Bob's got two cats, isn't he? _____
- The music isn't very good, has it? _____
- All the lessons start at 8.00, doesn't they? _____