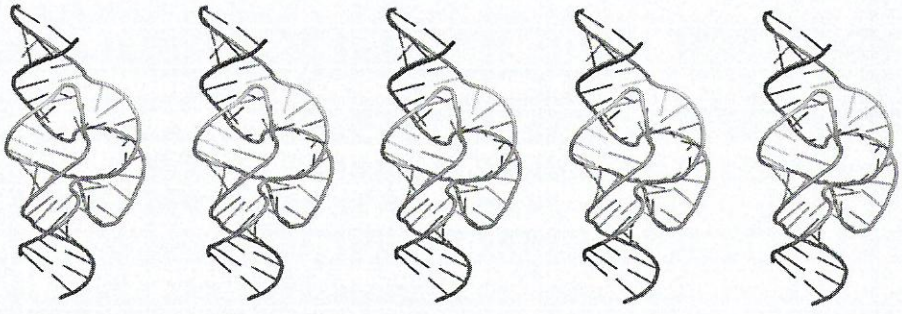
NUCLEIC ACIDS AND PROTEINS



[20]

Structure of a hammerhead ribozyme that cuts RNA

6.1. What do these two abbreviations stand for?

DNA _____

RNA _____

6.2. Discuss the questions with your partner.

1. What do you know about isolating DNA or RNA from cells?
2. What are the requirements to perform this protocol?

6.3. Read the introduction to the text about isolation of DNA and RNA to check your answers to the questions in exercise 6.2.

ISOLATION OF DNA AND RNA – INTRODUCTION

Every gene manipulation experiment requires a source of nucleic acid, in the form of either DNA or RNA. It is therefore important that reliable methods are available for isolating these components from cells. There are three basic requirements: **(1)** opening the cells in the sample to expose the nucleic acids for further processing, **(2)** separation of the nucleic acids from other cell components, and **(3)** recovery of the nucleic acid in purified form. A variety of techniques may be used, ranging from simple procedures with a few steps up to more complex purifications involving several different stages. These days most molecular biology supply companies sell kits that enable purification of nucleic acids from a range of sources.

(adapted from *An Introduction to Genetic Engineering*)

6.4. Check if you know the meanings of the following words.

disruption • viral • lysis • shear • hamper • subsequent • partition • aqueous • precipitate • substantial

6.5. Read the rest of the text about the isolation of DNA and RNA and answer the following questions.

1. Why should opening the cells be done as gently as possible?
2. How can deproteinisation stage be achieved?
3. In what situation should a further purification be performed?
4. What happens to caesium chloride solution containing the DNA preparation during the gradient centrifugation?

ISOLATION OF DNA AND RNA – CONTINUATION

The first step in any isolation protocol is disruption of the starting material, which may be viral, bacterial, plant, or animal. The method used to open cells should be as gentle as possible, preferably utilising enzymatic degradation of cell wall material (if present) and detergent lysis of cell membranes. If more vigorous methods of cell disruption are required (as is the case with some types of plant cell material), there is the danger of shearing large DNA molecules, and this can hamper the production of representative recombinant molecules during subsequent processing.

Following cell disruption, most methods involve a deproteinisation stage. This can be achieved by one or more extractions using phenol or phenol/chloroform mixtures. On the formation of an emulsion and subsequent centrifugation to separate the phases, protein molecules partition into the phenol phase and accumulate at the interface. The nucleic acids remain mostly in the upper aqueous phase and may be precipitated from solution using isopropanol or ethanol. Some techniques do not require the use of phenolic mixtures and are safer and more pleasant to use than phenol-based extraction media.

If a DNA preparation is required, the enzyme ribonuclease (RNase) can be used to digest the RNA in the preparation. If mRNA is needed for cDNA synthesis, a further purification can be performed by affinity chromatography using oligo(dT)-cellulose to bind the poly(A) tails of eukaryotic mRNAs. This gives substantial enrichment for mRNA and enables most of the contaminating DNA, rRNA, and tRNA to be removed.

The technique of gradient centrifugation is often used to prepare DNA, particularly plasmid DNA (pDNA). In this technique a caesium chloride (CsCl) solution containing the DNA preparation is spun at high speed in an ultracentrifuge. Over a long period (up to 48 h in some cases) a density gradient is formed and the pDNA forms a band at one position in the centrifuge tube. The band may be taken off and the CsCl removed by dialysis to give a pure preparation of pDNA. As an alternative to gradient centrifugation, size exclusion chromatography (gel filtration) or similar techniques may be used.

(adapted from *An Introduction to Genetic Engineering*)

Language review: '0' CONDITIONAL

Look at the following sentences taken out of the text:

If *more vigorous methods of cell disruption* ***are required, there is*** *the danger of shearing large DNA molecules...*
If *a DNA preparation* ***is required****, the enzyme ribonuclease (RNase)* ***can be used*** *to digest the RNA in the preparation.*

'0' Conditional is used to show that the condition can be true at any time because it is a fact.
The regular structure of '0' Conditional is: **IF + Present Simple, Present Simple.**
Here the active form **(require, can use)** is changed into passive form **(is required, can be used).**

6.6. Use the prompts to write the sentences in '0' Conditional. Use the passive form wherever possible.

1. The success / can / achieve / if / everything / prepare /.

_____

2. If / there / an emergency / these steps / should / follow /.

_____

3. If / the fault / find / the workers / reprimand /.

_____

4. New laboratory glassware / ought to / order / if / the old one / can / no longer / use /.

_____

5. If / any unforeseen circumstances / emerge / the public / need / inform /.

_____

6.7. Correct the collocations from the text: *Isolation of DNA and RNA* by rearranging the other words.

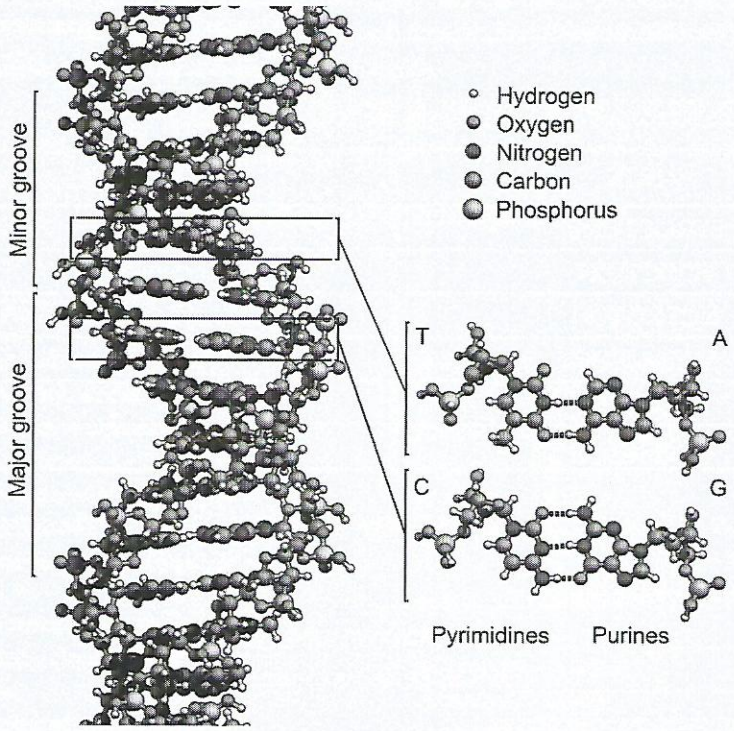
a) reliable acid
b) cell methods
c) nucleic components
d) molecular protocol
e) isolation biology

6.8. Use the correct collocations in your own sentences.

6.9. In the text: *Isolation of DNA and RNA* find the verbs which mean:

a) to make visible –
b) to make possible –
c) to prevent the movement or progress –
d) to include, contain as a part –
e) to continue in the same state or condition –
f) to need something –
g) to apply –

6.10. Using '0' Conditional with Passive Voice whenever possible, write your own seven sentences with the verbs from exercise 6.9.



[21]

The structure of the DNA double helix

6.11. Listen to some information about DNA and answer the following questions.

1. What is the role of proteins?
2. What does it mean that DNA acts as a molecular code for making proteins?
3. If we think of our body as a house, what can proteins be compared to?

6.12. Without reading the text below, how would you explain the term 'protein engineering'?

6.13. Read the text below and check your answer to the question in exercise 6.12.

Protein engineering

One of the most exciting applications of gene manipulation lies in the field of protein engineering. This involves altering the structure of proteins *via* alterations to the gene sequence and has become possible because of the availability of a range of techniques, as well as a deeper understanding of the structural and functional characteristics of proteins. This has enabled workers to pinpoint the essential amino acid residues in a protein sequence; thus, alterations can be carried out at these positions and their effects studied. The desired effect might be alteration of the catalytic activity of an enzyme by modification of the residues round the active site, an improvement in the nutritional status of a storage

protein, or an improvement in the stability of a protein used in industry or medicine. Proteins that have been engineered by the incorporation of mutational changes have become known as muteins. There are two types of approach that can be used to engineer proteins. These are sometimes called rational design and directed evolution.

(adapted from *An Introduction to Genetic Engineering*)

6.14. Provide the adjectives corresponding to the following nouns.

NOUN	ADJECTIVE
STRUCTURE	
FUNCTION	
NUTRITION	
MUTATION	
ESSENCE	
CATALYST	

6.15. Use either a noun or an adjective from exercise 6.14. to fill the gaps in these sentences.

1. You need the right kind of _____ to look and feel well.
2. There is _____ work to be done to proceed with the plan.
3. The main _____ of this device is to measure the altitude.
4. The result of the survey served as a _____ for the change in the system.
5. The lecture was about the _____ differences between DNA and RNA.

Language review: PRESENT PERFECT vs PAST SIMPLE

Look at these examples of **PRESENT PERFECT** (subject + HAVE / HAS + Past Participle) from the text:

*...and **has become** possible because....*
*This **has enabled** workers to pinpoint.*
*Proteins **have become known** by...*

PRESENT PERFECT is used here because the results or consequences of the action are more important than the time when the action took place.

PAST SIMPLE is used when we state when the action happened and do not emphasize the result.

*It **became** possible two years ago.*
*Last week this **enabled** the workers to pinpoint...*
*Proteins that **were engineered** last month...*

6.16. Put the verbs in brackets in either Present Perfect or Past Simple tense.

1. In our yesterday's experiment we _____ (use) solutions of nucleic acids.
2. The announcement of the results _____ (lead) to great excitement at our university.
3. _____ (teachers, enable) you to retake the exam later this month?
4. In the past people _____ (not, be, aware) of the existence of most scientific formulas.
5. _____ (they, publish) any of their reports so far?

GLOSSARY

alter – zmieniać, modyfikować
aqueous – wodny, wodnisty
caesium chloride – chlorek cezu
catalyst – katalizator
disruption – rozerwanie
enable – umożliwiać
gradient centrifugation – wirowanie w gradiencie (stężeń)
hamper – utrudniać, powstrzymywać
improvement – ulepszenie
kit – zestaw
lysis – liza (rozpad, rozkład)
nucleic acid – kwas nukleinowy
nutrition – odżywianie
partition – podział
pinpoint – wskazywać
precipitate – wytrącać
purify – oczyszczać
residue – osad
shear – obcinać, ścinać
spin – wirować
subsequent – następny
substantial – istotny
viral – wirusowy