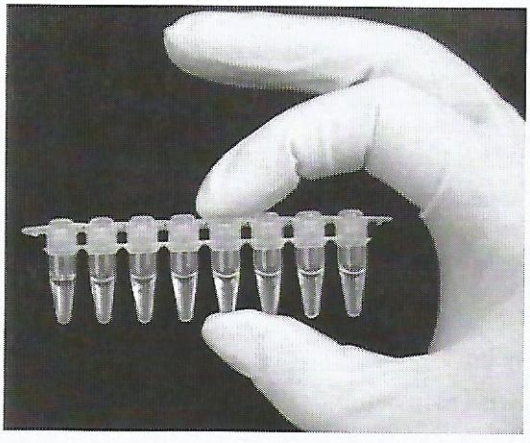
PCR AND NUCLEIC ACID HYBRIDISATION



[22]

A strip of eight PCR tubes

7.1. What does the abbreviation PCR stand for?

7.2. Can you explain the use/uses of PCR?

7.3. Read the text and check your answers.

Since its introduction in the mid 1980s, the polymerase chain reaction (PCR) has had a major impact on recombinant DNA technology. PCR facilitates the amplification of virtually any fragment of DNA from about 0.2 to 40 kbp in size. Because the amplification reaction is cyclical and the concentration of DNA doubles at each cycle, the total amount of DNA in the reaction increases exponentially; the theoretical yield from each original template molecule is about 10^6 molecules after 20 cycles, and about 10^9 molecules after 30 cycles. PCR requires a thermostable DNA polymerase, template DNA, a pair of oligonucleotide primers and a complete set of deoxynucleotide triphosphates (i.e. dATP, dCTP, dGTP and dTTP) substrates.

Oligonucleotide primers for PCR are synthesized chemically to be complementary to sequences which flank the region to be amplified and are usually about 20 nucleotides in length. The primers are designed to bind (anneal) specifically to the opposite strands of the

template molecule, in such a way that their 3′ ends face the region to be amplified. It is the specificity of the primer annealing reaction which ensures that the PCR amplifies the appropriate region of the template DNA. A key feature of the PCR is that the entire DNA amplification reaction is carried out in a single tube containing enzyme, template, primers and substrates. Each cycle of amplification therefore involves annealing, extension and denaturation reactions, each brought about at different temperatures. Since the dissociation reaction may occur at temperatures as high as 95°C, and there may be as many as 35 cycles in a single PCR, a highly thermostable DNA polymerase is a basic requirement for PCR. *Taq* polymerase, isolated from the hot-spring archaea *Thermus aquaticus,* was the first thermostable DNA polymerase to be employed in PCR.

The first step in the polymerase chain reaction is the denaturation of the double-stranded DNA template by heating to about 95°C. The reaction mixture is then cooled to allow the oligonucleotide primers to anneal to the resulting single-stranded templates. The temperature at which annealing occurs is dependent on the length and G+C content of the primer sequences, but is usually designed to be in the range 50–65°C. After the annealing step the temperature is raised to about 70°C, the optimum temperature for the synthesis of the complementary strand by the thermostable DNA polymerase. The cycle of denaturation, annealing and synthesis is repeated 20–35 times in a typical PCR.

(adapted from *Basic Biotechnology*)

7.4. Read the text again and answer the following questions.

1. Why does the amount of DNA grow during PCR reaction?
2. What is necessary for PCR to take place?
3. What reactions are involved in DNA amplification?
4. What is the first step in PCR?
5. Why is the temperature raised to about 70°C after the annealing step?

7.5. Use the verbs in these sentences. Use appropriate tenses.

facilitate • flank • synthesise • bind • amplify

1. Electric guitars are _____ through loudspeakers.
2. The platform will probably _____ the communication between students and teachers.
3. The problems they encountered _____ the team much closer together.
4. The professor entered the hall _____ by his assistants.
5. There are many vitamins that the body cannot _____ itself.

7.6. Fill the gaps in the sentences with the nouns. All the nouns come from the text about PCR. The number of dashes corresponds to the number of letters.

1. Pairs of _ _ _ m _ _ _ should have similar melting temperatures since annealing in a PCR occurs for both simultaneously.
2. A _ _ _ _ _ e _ _ _ _ is an enzyme that catalyzes the formation of new DNA and RNA from an existing strand of DNA or RNA.

3. DNA is usually a double-helix and has two s _ _ _ _ _ _ running in opposite directions.
4. The _ _ _ a _ _ _ _ _ _ _ _ of nucleic acids such as DNA due to high temperatures is the separation of a double strand into two single strands.
5. A t _ _ _ _ _ _ _ is a nucleic acid molecule that acts as a pattern for the sequence of assembly of a protein, nucleic acid, or other large molecule.

Language review

Look at these two examples from the text:

The primers ***are designed to bind*** *(anneal) specifically to the opposite strands of the template molecule...*

TO BE DESIGNED TO DO SOMETHING / TO DESIGN SOMETHING TO DO SOMETHING – to be intended for a particular purpose.

The reaction mixture is then cooled ***to allow the oligonucleotide primers to anneal*** *to the resulting single-stranded templates.*

TO ALLOW SOMETHING TO DO SOMETHING

to give something the time or opportunity to do something.

7.7. Order the words to makes sentences. Make sure you use the structures from the Language review box correctly.

1) skills / these / designed / your / improve / exercises / are / to /.

_____

2) proceed / this / allows / to / experiment / process / the / with /.

_____

3) equipment / to / down / allow / the / continue / cool / you / before /.

_____

4) fulfill / introduced / the / of / expectations / students / was / change / this / to/ the /.

_____

5) time / should / allow / project / we / how / finish / much / the / to / ?

_____

6) has / to / implemented / programme / who / the / support / newcomers / the / ?

_____

7.8. What do you know about nucleic acid hybridisation?

7.9. Read the first paragraph of the text 'Nucleic acid probes and hybridisation' and check your answer to the question in exercise 7.8.

Nucleic acid probes are used to detect specific target DNA molecules. The soluble probe binds (i.e. hybridises) to the target DNA that is immobilised onto a nylon or nitrocellulose membrane. Hybridisation is used for a variety of biotechnological applications including the detection of cloned DNA, analysis of genetic organization and

the diagnosis of genetic diseases. Although nucleic acid hybridisation techniques are used in a wide variety of contexts, the same basic principles apply. Nucleic acid hybridisation exploits the ability of single-stranded probe nucleic acid (DNA or RNA) to anneal to complementary single-stranded target sequences (DNA or RNA) within a population of non-complementary nucleic acid molecules.

(adapted from *Basic Biotechnology*)

Language review: 'USE'

Look at these two extracts from the text above:

TO BE USED TO DO SOMETHING
Nucleic acid probes are used to detect specific target DNA molecules.

TO BE USED FOR SOMETHING
Hybridisation is used for a variety of biotechnological applications...
Other structures with USE:

TO USE SOMETHING FOR DOING SOMETHING
They used animals for conducting scientific experiments.

TO USE SOMETHING AS SOMETHING
She uses the room as her study.

7.10. Complete the sentences. Use the structures from the Language review box.

1. These tests are used _____ selection purposes.
2. Do you think this thesis should be used _____ the starting point for the discussion?
3. These machines shouldn't be used _____ producing such drugs.
4. Genetic engineering can be used _____ modify the genetic compositions of plants, animals, and microorganisms.
5. In biology, hybridisation is usually used _____ a term in agriculture or in plant production where new hardy and disease-resistant crops are formed.

7.11. Listen and take notes under the following headings.

— What is FISH?
— How does FISH work?
— What is FISH used for?

Read the audio script to check your answers.

GLOSSARY

amplification – wzmocnienie
anneal (bind) – przyłączyć, spleść
archaea – archeon (pierwotny, bezjądrowy organizm jednokomórkowy)
bind – związać
complementary – komplementarny
denaturation – denaturacja
dissociation – dysocjacja, odłączenie
exploit – wykorzystywać
expotentially – ekspotencjalnie

facilitate – ułatwić, udostępnić
flank – otaczać
impact – siła uderzenia, wpływ
nucleic acid – kwas nukleinowy
primer – starter, primer
probe – próbnik
strand – nić
substrate – substrat
template – matryca
yield – uzysk, wynik