TSSM
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Biology
Teach Yourself Series
Topic 12: Molecular Biology (Unit 4)

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Molecular Biology

Molecular biology involves the study of biological molecules. It is the study of the production, structure and function of all types of biological molecules. It also incorporates the techniques used to manipulate these molecules. In Unit 4 the techniques involved in the molecular biology field of gene technology are considered in detail. Rational drug design is also considered.

Gene technology is a fast growing field. We currently we have the ability to work with and manipulate DNA in a number of ways, including:

- DNA can be removed from one cell and spliced into another.
- The rate of expression of a gene can be altered
- A gene may be removed or turned off.
- A piece of DNA can be cut from a larger piece
- The size of molecules can be established
- Genes can be cloned
- Many copies of DNA can be made from small numbers of copies
- DNA can be sequenced

Restriction enzymes

As it appears in Unit 4

Restriction enzymes (also called endonucleases) act as molecular scissors. They recognise, bind to and cut specific DNA sequences. These are called recognition sites, and they are DNA segments of 4 to 8 base pairs. When a single strand is cut two separate sections of DNA are produced. These sections may have either blunt ends or sticky ends. Sticky ends have exposed bases at the ends which can bind to other pieces of DNA with complementary sticky ends. This means that they can be easily joined. Blunt ends do not have exposed bases can be made to join any other blunt end. The choice to produce blunt or sticky ends depends on what the researcher wishes to achieve.

The table below shows a few examples of restriction enzymes, their recognition sequence and the fragments that are produced. Each enzyme has a different recognition sequence.

ENZYME	SOURCE	RECOGNITION SEQUENCE	FRAGMENTS PRODUCED
EcoRI	*Escherichia coli*	5' GAATTC 3' CTTAAG	5' G AATTC 3' 3' CTTAA G 5'
BamHI	*Bacillus amyloliquefaciens*	5' GGATCC 3' CCTAGG	5' G GATCC 3' 3' CCTAG G 5'
HindIII	*Haemophilus influenzae*	5' AAGCTT 3' TTCGAA	5' A AGCTT 3' 3' TTCGA A 5'
Sau3A	*Staphylococcus aureus*	5' GATC 3' CTAG	5' GA TC 3' 3' CT AG 5'
Pov11	*Proteus vulgaris*	5' CAGCTG 3' GTCGAC	5' CAG CTG 3' 3' GTC GAC 5'
Alu1	*Arthrobacter luteus*	5' AGCT 3' TCGA	5' AG CT 3' 3' TC GA 5'

The restriction enzyme EcoRI recognizes a DNA strand with the leading sequence GAATTC. As can be seen, when used it will create the production of two strands with sticky ends:

Sticky end

PvuI I produces blunt ends:

Review Questions

1. Identify the term used to identify such substances as SauA I. _____

2. Identify the kinds of ends that will be produced by the following restriction enzymes:

i. PvuI I _____

ii. AluI _____

iii. HindIII _____

3. The leading strand of a segment of DNA contains the sequence GAATTG. Discuss the effect that this alteration will have on the sticky ends produced by DNA enzymes.

_____

_____

_____

Restriction enzymes can be used to cut a desired gene from a larger segment of DNA. It is essential that the recognition sites are located surrounding the gene, but not within it.

Review Question

4. The information below shows the sequence of a leading strand of DNA (the lagging strand not shown would be complementary). The section shown in bold is the desired gene.

5'GTCAGGATTGACCCCTCCCAAGCTTCTATGTTTCAGCTCTCCAAATC**GAATTCGGAGG**

CGATTATCGTCGAAATTCTGGAGCTAGGGAGTTTCAGTTTCAGTTTTA

TTAGAATTAGACAGCTGCATCAAGCTTCTTGGAAGAGATTTCAGTAAGCTTAT

Identify which enzyme would be the most appropriate to use to cut out the desired gene. Provide two reasons for your choice.

5. Explain why sticky ends are sometimes more desirable than blunt ends.

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Solutions to Review Questions

1. Restriction enzyme or endonuclease

2. The answers are as follows:
- **i.** Blunt ends
- **ii.** Blunt ends
- **iii.** Sticky ends

3. EcoRI will no longer recognise the DNA sequence and will not bind to it or cut it.

4. Hind III. This is the most appropriate enzyme as it produces sticky ends and because it will cut around the desired gene rather than inside the gene.

5. Sticky ends are more desirable because the complementary bases they possess are able to seal the ends more effectively.

6. Annealing occurs when 2 complementary sticky ends expose bases. Hydrogen bonds reform between the complementary bases. DNA ligase then reforms bonds in the sugar-phosphate back bone strengthening the join between the 2 fragments.

7. The diagram should look like this:

8. The fragments in lane 1 are approximately 420 and 8000bp. The fragments in lane 2 are approximately 250 and 9000bp.

9. Electrophoresis separates DNA fragments based on their size. The mutated copy of the gene has more repeats than the normal gene. If electrophoresis were performed the mutated copy would remain closer to the origin than the normal copy because larger fragments do not travel through a gel as far as smaller fragments.

10. Person 3 is most likely to have committed the crime. The genetic fingerprint of person 3 matches the genetic fingerprint from the crime scene.

11. The bar in lane 1 is 5.0kb long so this must be the plasmid vector without the gene.

The bar in lane 2 is 5.8kb long so this must be the plasmid plus the desired gene.
The bar in lane 3 is 0.8kb long so this must be the desired gene.
The bar in lane 4 is 1.6kb long so this must be 2 copies of the gene that have annealed to each other.

12. The answers are as follows:

i. All of the bacteria will grow because there is nothing in the agar that will kill them.

ii. None of the bacteria will grow. The bacteria do not have the recombinant plasmid which means that they are sensitive to the ampicillin in the agar plate.

iii. All of the bacteria will grow whether they have taken up the recombinant plasmid or not because there are no antibiotics in the agar.

iv. Only the bacteria that have taken up the recombinant plasmid will grow so there will be discrete colonies growing on the agar plate. The bacteria that did not take up the recombinant plasmid will be killed off by the ampicillin in the agar.

13. When performing PCR, the DNA strands are separated by raising the temperature over 90°.

14. The DNA template.

15. Process 1 is the denaturation stage. The temperature is raised to approximately 92° separating the double stranded template into 2 single strands.

16. Approximately 92°. This temperature is sufficient to denature any enzymes present in the PCR tube.

17. A primer.

18. Complementary and antiparallel.

19. Annealing stage. The primers bind to the ends of the template strands.

20. Extension stage. DNA polymerase binds to the primers and assembles a new complementary strand in a 5' to 3' direction.

21. The two strands of DNA are antiparallel. Since DNA synthesis can only occur in a 5' to 3' direction the two strands synthesise in opposite directions.

22. DNA probes are used to locate a specific DNA sequence.

23. The labeling allows the sequence to be detected.

24. Reverse transcriptase is used to produce DNA from an RNA template.

25. Microarrays can be used to detect the presence of a specific DNA sequence or to calculate the activity of a gene.