

Advanced Molecular Biological Techniques

The Polymerase Chain Reaction (PCR)

- The goal of PCR is to make copies of the DNA sequences without inserting it into a plasmid
- Instead of using the enzymes DNA helicase and gyrase, in this reaction the DNA is unwound using heat (94 - 96°C breaks H-bonds), and the single strands serve as a template to build complementary strands
- nucleotides that can base-pair with the ends are made and mixed with the DNA sample; as the mixture is cooled (50 - 60°C) the nucleotides anneal (bind to the ends of the DNA strand)
- these short sequences serve as primers (DNA primers) are that are complementary to the opposing 3'-5' end, making them 5'-3' primers
- One of the primers is known as the forward primer and the other as the reverse primer because they work in opposite directions
- Taq polymerase builds the complementary strand from nucleotides that have been added to the solution.
- the DNA strand will form at about 72°C
- ordinary DNA polymerase III denatures at temperature above 37°C so it can not be used in building the new strand.
- the procedure is repeated over and over again to amplify the sequence.
- more than 1 billion copies can be made after 30 cycles.
- see Fig. 1 on pg. 297
- variable-length strands (strands of unequal length) form after the first cycle.
- constant-length strands (strands of equal length) form after the second cycle.
- PCR is useful in forensic criminal investigations, medical diagnosis, and genetic research (basically any time you only have a small piece of DNA and you want to test it)

Restriction Fragment Length Polymorphism Analysis

- polymorphism is the difference in DNA sequence, coding or non-coding, that can be detected between individuals.
- Organisms of the same species are polymorphic unless the individuals are identical twins.
- Polymorphisms are compared in coding regions to identify individuals with specific mutations (ex/ sickle cell anemia)
- Polymorphisms in non-coding regions are often found in microsatellites (VNTR) that can be used to identify people in forensic investigations
- Every person has slight variations in their genetic code, and when their DNA is digested by a restriction enzyme, it is cut to different lengths
- This process is called restriction fragment length polymorphisms (RFLPs)
- If that digested DNA is then run on a gel, the fragments are carried to a different point depending on their lengths, this appears as a smear because there are so many fragments reveals a pattern that is unique to all individuals (except for identical twins)
- the gel is placed against a nylon membrane and placed in a solution containing radioactive complementary probes allowing for the single-stranded DNA to migrate onto the nylon from the gel via an electrical current (known as Southern blotting).

- the nylon membrane is placed beside a x-ray film (autoradiogram) for 2 to 3 weeks, where the radioactive probes burn an image onto the x-ray film, which is then developed resulting in a banding pattern that is unique to that individual (except for identical twins)
- see Fig. 3 on pg. 7

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DNA Sequencing

- Completed in 2003, the Human Genome Project was designed to decipher the genetic sequence of all 46 chromosomes.
- It used computer technology to read the DNA sequence, made possible by laboratory techniques developed over the last 25 years
- Sanger dideoxy method used the process of DNA replication to sequence the DNA by using dideoxy nucleoside triphosphates.
- A short single stranded radioactive primer is added to the end of the DNA template then placed into four reaction tubes.
- Each tube contains DNA polymerase and free nucleotides (in the form of deoxynucleoside triphosphates - dATP, dTTP, dGTP and dCTP).
- see Fig. 4 on pg. 301.
- in each tube one type of radioactively labelled dideoxy analogue (ddATP in tt. 1, ddTTP in tt. 2, ddGTP in tt. 3, and ddCTP in tt. 4) is added.
- a dideoxy analogue is a deoxynucleoside triphosphate whose deoxyribose sugar is missing the -OH group on Carbon 3
- see Fig. 5 on pg. 302
- DNA polymerase creates the bond between the -OH on Carbon 3 to the incoming phosphate.
- if the -OH group is missing (the dideoxy analogue has bound to the chain) the process ceases, leaving a fragment of DNA at that point
- this is also known as the chain termination technique.
- Since only some of the nucleosides available in each test tube are the dideoxy analogues, different lengths of DNA can be created.
- the contents of all four tubes can be separated out through electrophoresis.
- the fragments created can then be read out in ascending order.
- see Fig. 6 on pg. 302.
- the Human Genome project used this technique but the ddNTP are fluorescently tagged with different colours, so the computer can read the sequence and position of the tags, instead of relying on a human to do so

APPLICATIONS

Read Pgs. 305 - 309 and make notes on the various applications: Medical, Agricultural, and Forensics.

Seatwork

Pg. 302 # 16 - 22 Pg. 309 # 1 - 4, 8