

CHECKLIST OF KEY CONCEPTS

Basic steps

1. The ability of making recombinant DNA molecules generates a range of applications. These include sequencing genes, transfer of genes from one source to another and the production of proteins using bacteria. In addition it allows the study of evolutionary relationships and individualisation for forensic purposes.
2. The development of efficient means of storing, analysing and recovering data is an essential element of the process in many situations.

Key techniques

3. The identification and isolation of restriction endonucleases that cut DNA at highly defined sequences was a key step in the development of the technology.
4. The presence of a known sequence, typically of about 20 nucleotides in length, can be ascertained by hybridization to a labelled probe.
5. DNA sequencing is carried out by the dideoxy method. Originally this was a manual procedure but automated methods have largely replaced manual sequencing. These are very high throughput procedures and allow the sequencing of whole genomes, including the human genome.
6. The polymerase chain reaction (PCR) revolutionised many aspects of DNA technology because of its capacity to generate large number of copies of defined regions of DNA.

Cloning of DNA

7. Pieces of DNA can be combined either by utilising restriction endonucleases that generate sticky ends by blunt end ligation. This allows the insertion of defined pieces of DNA into bacterial plasmids, followed by the unlimited replication of the plasmid in a suitable bacterial host.
8. When DNA fragments of longer than 10 kb need to be inserted into a host bacterial plasmids cannot be used. Bacteriophage λ , yeast artificial chromosomes and bacterial artificial chromosomes offer alternatives.
9. mRNA can be back copied to form DNA and a cDNA library generated from any source. A single mRNA can then be isolated if a suitable screening procedure is available.

Applications of DNA technology

10. By using PCR and synthetic primers with a single base change it is possible to generate genes with a point mutation.
11. Within the human population there exist regions of short tandem DNA repeats. The number of repeats varies from one individual to another and by the use of suitable PCR primers it is possible to identify the source of a given DNA sample with an extremely high degree of confidence.
12. Using a similar approach the presence or absence of genetically inherited diseases can be checked for any individual.

13. Once a gene has been identified in an animal species it can be 'knocked out' to investigate its role. This requires the use of embryonic stem cells.
14. Embryonic stem cells can divide indefinitely. The gene of interest can be introduced into such cells so that only cells that have lost the gene can survive under a specific set of conditions and those cells can then be introduced into a blastula (normally mice are used) and progeny mice selected with the desired characteristic.
15. Another technique that has been used to inactivate individual genes is the use of RNA interference (RNAi). Short pieces of RNA (21-23 bases long) that are complementary to a region in mRNA can inactivate it with the assistance of specific proteins.

Recent new techniques

16. Microarray technology, in which thousands of different sequences are spotted onto a single chip, combined with hybridization, now allows the investigation of which genes are turned on under a range of different conditions.
17. Genes can be inserted into cells artificially, raising the possibility that genetic diseases may be curable in this way.
18. Vast databases of DNA sequences are now available. Their analysis has given rise to a whole new science called bioinformatics.