Denaturing High Performance Liquid Chromatography Encyclopedia Article

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Denaturing High Performance Liquid Chromatography

Denaturing high performance liquid chromotagraphy (DHPLC) is a method of detecting sequence differences between a reference **DNA** and a test DNA (both usually produced by **polymerase chain reaction** amplification, up to 1.5kb in size). It can be used to detect mutations in a **gene** or to type the allele at a single **nucleotide** polymorphisms (SNP). It has been used to identify 160 SNPs on the human **Y chromosome**, and mutations in the whole mitochondrial **genome**, as well as in disease genes such as the breast **cancer** genes BRCA1 and BRCA2. It is an exceptionally accurate and sensitive technique, and is ideally suited for high-throughput analysis of disease genes in patients. However, the automated equipment required is expensive, and is mainly used in **medical genetics**, where there is more money for research.

The reference and test DNA are mixed, then heated to melt (denature) the duplex then cooled to reanneal the duplex. This allows heteroduplexes to form. These are DNA duplexes where one strand is from the reference DNA and the other strand is from the test DNA. If there is a difference between the two DNA sequences, then the two **bases** at that point cannot base pair a DNA mismatch (DNA heteroduplex) is formed. The mixture is then passed through a chromatography column containing alkylated non-porous particles, with a gradient of increasing chemical denaturant (acetonitrile) which causes the DNA to melt. Any heteroduplex passes through the column faster that its homoduplex counterparts, and so any sequence change can be detected by an extra elution peak. Usually for **mutation** detection techniques the DNA must be sequenced to determine the exact position and type of nucleotide change. This is often the case with studies using DHPLC, but it seems that the elution profile from DHPLC varies depending on the type of nucleotide change. Therefore, each mutation has a unique elution profile (signature). It is possible to predict the mutation from the elution profile, and this means that **sequencing** of the DNA is not necessary.