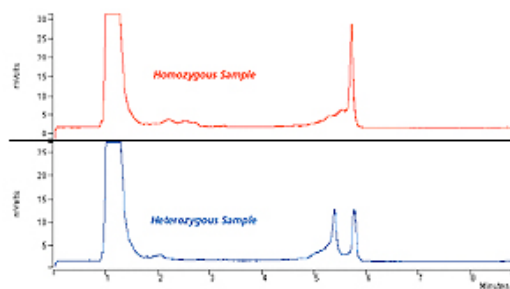




Located at Monash Medical Centre, Clayton, the Gandel Charitable Trust Sequencing Centre was established in 1999. Several complementary services have since been introduced, including a Denaturing High Performance Liquid Chromatography (DHPLC) service in 2004 for researchers requiring analysis of genetic variation. This service is available to researchers and clinicians within the Monash Health Research Precinct (MHRP) as well as external clients from other Institutes.

Instrumentation

The Centre utilises the VARIAN Helix™ System for DHPLC analysis. DHPLC is a highly sensitive method for detecting single nucleotide polymorphisms (SNPs), mutations, and other DNA sequence variations. DNA sequence variant detection by DHPLC involves the resolution of PCR products by ion-pair reverse phase chromatography through a unique DNA separation matrix under partial denaturation conditions. DNA variations are detected as a pattern of peaks that discriminate variant double-stranded DNA from wild type DNA.



Fast, clear results. Detect heterozygous, SNP-positive samples in a real-time trace by DHPLC

Project Applications

- Applications for DHPLC technology include:
- Screening patients for disease causing mutations
 - Linkage analysis in humans and mice
 - Genotyping

DHPLC Service

Researchers requiring DHPLC analysis submit PCR products either in 0.2ml strip tubes or 48/96 well plates. We temperature map your control template that is heterozygous for a mutation and then run each sample at its optimal melting temperature. Ideally, the PCR products submitted should be from 100 to 600 base pairs and have a uniform melting domain. The optimal melting temperature of the product can be estimated using the melting algorithm freely available at <http://insertion.stanford.edu/melt.html>

PCR Template Quality

DHPLC does not require any special primers, reagents or post-amplification purification. PCR quality is important, however, and using a "hot start" approach, careful primer design, using optimal Mg²⁺ concentrations and avoidance of excessive cycle numbers is recommended.

Sample Submission

Please label all sample plates with your full name and date. All samples need to be accompanied by a completed DHPLC request form (download from website). For each sample please provide 5µl of template at a concentration of 10 to 50 ng/ul.

Data Retrieval

DHPLC data is available from the FTP site within 1-2 days of sample receipt and a print-out of the electropherogram is provided for each sample. Files are retained on the FTP for a period of two months. All data is backed-up to a permanent archive.

Pricing (External Clients): DHPLC temperature mapping \$3.50 (GST excl.)
DHPLC sample \$3.50 (GST excl.)

Comprehensive trouble-shooting and technical advice is available to all researchers and we encourage clients to call or email with any enquiries.

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