DHPLC Submission

DHPLC Service

For this service, clients submit PCR products either in 0.2ml strip tubes or 48/96 well plates depending on the number of samples submitted. We temperature map your control template and then run each sample at its optimal melting temperature. Data is then available on CD or from the FTP site and a print-out of each trace is supplied for each sample.

PCR Template Size and Melting Temperature

Ideally, the 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 the website
http:// insertion.stanford.edu/melt.html
Note: The program often under estimates the optimal melting temperature hence the optimal melting temperature needs to be determined by temperature mapping of a control template prior to running samples.

DHPLC Request Form

A completed DHPLC Request forms needs to accompany all samples submitted. Available to down-load from the website, please record sample names (< 10 characters long) and the estimated melting temperature of your templates.

PCR Template Quality

DHPLC does not require any special primers, reagents or post amplification purification. However, PCR quality is important and using a “hot start” approach, careful primer design, using the optimal Mg^{2+} concentration and avoidance of excessive cycle numbers is recommended.

Some polymerase buffers contain components that may reduce the column performance. Buffers containing the following components should not be used:
Unidentified proprietary stabilisers or enhancers
Bovine Serum Albumin
Autoclaved water
Mineral oil
Formamide
Proteinase K

The following components should be kept to the minimum, <1% final concentration:
PEG
Detergents such as tritonx100, NP40, Tween20, SDS/SLS

The recommended maximum final concentration of glycerol is 2%, DMSO is 10% and Betaine 1.25 to 2.5 M.

Quality Control

Validation of the system's performance is continually monitored. Separation efficiency is checked prior to every run using puc Hae*III* and actual column oven temperature is checked regularly using DYS271 Mutation Standard.

Recommended DHPLC review articles

Xiao W, Oefner PJ: **Denaturing high-performance liquid chromatography: A review**. *Hum Mutat* (2001) 17:439-474

Lillebarg SL: **In-depth mutation and SNP discovery using DHPLC gene scanning**. *Current Opinion in Drug Discovery & Development* (2003) 6(2):237-252