

Assay Development, RNA Services, Target Gene Expression, Quantitative RT-PCR	
Service Title	High Resolution Melting
Workflow Code	service
Short Service Description	High resolution melt (HRM) analysis is used to characterize DNA samples according to their dissociation behaviour as they transition from double stranded DNA (dsDNA) to single stranded DNA (ssDNA) with increasing temperature. The Roche Lightcycler 480 instrument collects fluorescent signals with much greater optical and thermal precision than previous methods to create new application possibilities.
Base Price (Price per unit)	Price on enquiry
Turn Around Time	\Application specific
Detailed Description and background	
<p>High Resolution Melting (HRM) is a post-PCR method, analyzing genetic variations (SNPs, mutations, methylations) in PCR amplicons. It goes beyond the power of classical melting curve analysis by studying the thermal denaturation of a double-stranded DNA in much more detail and with much higher information yield than ever before. HRM characterizes nucleic acid samples based on their disassociation (melting) behaviour. Samples can be discriminated according to their sequence, length, GC content or strand complementary. The most important High Resolution Melting application is gene scanning (the search for the presence of unknown variations in PCR amplicons prior to or as an alternative to sequencing). Mutations in PCR products are detectable by High Resolution Melting because they change the shape of DNA melting curves. The more recently introduced LightCycler 480 (Roche Molecular Systems) is capable of HRM and thermal cycling. The LightCycler 480 is a block-based instrument design and it has better thermal uniformity than other block-based instruments, it nevertheless does exhibit measurable thermal and optical non-uniformity</p>	
Service Details	
<p>Gene Scanning by High Resolution Melting Curve Analysis generally requires the use of a special generic DNA dye that works at high, saturating concentrations without inhibiting PCR and therefore leads to homogeneous staining of homo-or heteroduplex DNA. An instrument with suitable excitation/emission wavelengths, high data acquisition rates, and outstanding temperature homogeneity. A software algorithm that analyzes the shape of the melting curves and groups those that are similar. In a Gene Scanning experiment, sample DNA is first amplified via real-time PCR in the presence of a proprietary saturating DNA dye. A melting curve is then performed using high data acquisition rates, and data are finally analyzed using a Gene Scanning Software, by three basic steps: 1.)Normalization: the pre-melt (initial fluorescence) and post-melt (final fluorescence) signals of all samples are set to uniform, relative values from 100% to 0%. 2.)Temperature shifting: the temperature axis of the normalized melting curves is shifted to the point where the entire double-stranded DNA is completely denatured. Samples with heterozygous SNPs can then be easily be distinguished from the wild type by the different shapes of their melting curves. 3.) Difference Plot: the differences in melting curve shape are further analyzed by subtracting the curves from a reference curve. This helps cluster samples automatically into groups that have similar melting curves (e.g., those who are heterozygote as opposed to homozygotes).</p>	
Service Deliverable	
Report includes the QC quality gate results, the raw data and an analysis report. Bioinformatic analysis and interpretation of data is optional.	
Sample/Info Submission Info	
Samples are run in 96 well plates and monitored by third generation saturation dyes, such as BEBO, Chromofy etc.	
Pricing Details	Price on request
Key Words	gene expression. qPCR RT-PCR. Genomic DNA. Pathway analysis. cDNA



CPGR

CENTRE FOR PROTEOMIC
& GENOMIC RESEARCH

P. O. Box 81
Observatory, 7935
South Africa
Tel: +27 21 447 5669
Fax: +27 21 447 3228
Vat Reg No: 4380228983

Sample Shipping Address	Institute of Infectious Disease and Molecular Medicine, UCT, Faculty of Health Sciences, Wernher and Beit Building, Level 2, Lab S2.09, Anzio Road, Observatory, Cape Town 7925, South Africa
Related services	Bioinformatics. Sample Preparation

