

## **CENTRAL INSTRUMENTATION FACILITY**

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**Real Time PCR** (7000 Sequence Detection System; Applied Biosystems, Foster City, CA 94404, USA)

Real time PCR is a widely used technique that detects and quantitates nucleic acid sequences mainly targeting DNA, cDNA and RNA. It is commonly used for both diagnostic and basic research. In diagnostic, this technique is deployed as a tool to detect newly emerging diseases, such as flu in diagnostic tests. In research, the technology may be used in determining as to how the genetic expression of a particular gene changes over time and to test for differences in gene copy number.

It has variety of applications:

- a) Quantification of gene expression
- b) Viral quantitation
- c) Array verification
- d) SNP genotyping
- e) Mutation scanning
- f) Copy number variation
- g) Pathogen detection

**Denaturing High Performance Liquid Chromatography (dHPLC)**, (Wave System 4500; Transgenomic, Emmet Street Omaha, NE. USA)

Denaturing High Pressure Liquid Chromatography (DHPLC) is a technique, which uses heteroduplex formation between wild-type and mutated DNA strands to identify mutations. Heteroduplex molecules are separated from homoduplex molecules by ion-pair, reverse-phase liquid chromatography on a special column matrix with partial heat denaturation of the DNA strands. DHPLC is a very useful method for the screening of a large number of samples for mutations. So far, it has mainly been used for the analysis of germline mutations in various inherited diseases.

**Laser Capture Microdissection (LCM)**, (Leica AS LMD; Leica Microsystems, Wetzlar, Germany)

Laser capture microdissection is a method for isolating specific cells of interest from microscopic regions of tissue that has been sectioned. LCM is a useful method of collecting selected cells for DNA, RNA and/or protein analyses. LCM can be performed on a variety of tissue samples including blood smears, cytological preparations, cell cultures and aliquots of solid tissue.

**Tissue Microarray (TMA)**, (Minicore; Alphelys Impasse Paul Langevin, Plaisir, France)

Tissue microarrays are produced by a method of re-locating tissue from conventional histologic paraffin blocks such that tissue from multiple patients or blocks can be seen on the same slide. This is done by using a needle to biopsy a standard histologic section and placing the core into an array on a recipient paraffin block. Tissue microarrays are particularly useful in analysis of cancer samples.

**DNA Sequencer** (3130 XL Genetic Analyzer; Applied Biosystems, Foster City, CA 94404, USA)

DNA sequencing is a technique to determine the exact order of the base pairs in a segment of DNA. The instrument is mainly used for DNA sequencing and also offers fragment analysis for microsatellites and SNP's.

It has wide applications:

- a) *De novo* sequencing
- b) Resequencing
- c) Comparative sequencing
- d) Mutation/heterozygote detection
- e) SAGE
- f) SNP validation and screening
- g) Genotyping
- h) Microsatellite analysis
- i) AFLP analysis
- j) LOH
- k) Conformational analysis

**Bioanalyzer** (Agilent 2100 Bioanalyzer; Agilent Technologies, Waldbronn, Germany)

Bioanalyzer is a microfluidics-based platform for sizing, quantification and quality control of DNA, RNA, proteins and cells on a single platform. The individual samples are analyzed and a pseudo gel image is created and bands are sized and quantified.

It is widely used for determining the quality of RNA before running microarray or real-time quantitative pcr experiments.

It has multiple applications:

- a) RNA sample QC prior microarray or qPCR experiments
- b) DNA analysis of PCR/mPCR and RT-PCR products
- c) Protein expression analysis
- d) Protein purity during purification
- e) Cell-based analysis in gene silencing or apoptosis

