

The UMDNJ-RWJMS DNA Core Facility, a core facility of The Cancer Institute of New Jersey (CINJ), provides a centralized, high quality, cost-effective facility for DNA analysis.

The DNA Core Facility runs a state-of-the-art, 16-capillary electrophoresis automated ABI 3130XL DNA sequencer, generating 600 to 800 nucleotides of sequence per sample. Sequencing reactions are performed with any primer provided by the user. Reactions are performed by a cycle sequencing protocol with AmpliTaq™ DNA Polymerase using ABI BigDye™ Terminators. Modified protocols are used for difficult or very large templates. Sequencing data, both raw data in a waveform format and called nucleotide sequence, are immediately sent to researchers by email. The high throughput of the ABI 3100 and the use of three staff members for DNA sequencing produces a typical turnaround time of 1-2 days for most sequencing samples. The staff provides expert editing and technical support of sequences.

For DNA fragment analysis, the Facility runs an automated ABI 3100 DNA analyzer. The DNA Facility also has all of the equipment and software necessary for fluorescent DNA fragment analysis studies including, but not limited to, Loss of Heterozygosity (LOH), Microsatellite Instability (MIN), and Single Nucleotide Polymorphism (SNP) assays. The Facility's staff is highly experienced in running these assays, freeing the user to focus on the application of these technologies in their research. The SNP analysis utilizes Applied Biosystem's SNaPshot primer extension kit. With this software and reagents, the ABI 3100 can analyze 160 multiplexed loci in 30 minutes.

In addition, the Core Facility operates an ABI 7900HT Real-Time PCR system. This system can accommodate any real-time PCR application. This new high-throughput system contains robotics enabling multiple 384 or 96 well plates to be loaded automatically. Continuous wavelength detection from 500-660 nm allows the use of multiple fluorophores in a single reaction. The software is designed to perform all necessary calculations including dissociation curve analysis, absolute quantitation, and relative quantitation using standard curve or comparative CT algorithms.

Possible applications include:

- Allelic Discrimination:SNP Analysis using fluorescent 5' nuclease assay
- Measurement of gene expression levels (gene expression quantitation)
- Quantitative PCR
- Array Verification
- Gene Expression
- Analysis of mRNA Expression
- Mutation Detection
- Detection and quantitation of nucleic acid sequences

Using the Applied Biosystems TaqMan Genotyping Assays, large-scale screening of known SNPs can be accomplished in a very short amount of time. These assays contain two TaqMan probes, one for each allele, multiplexed into one reaction well. The 7900HT software rapidly detects end-point fluorescence for each allele and displays the data in tabular and plot formats.

With the DNA Core serving as a centralized location to order synthetic oligonucleotides, researchers can apply DNA sequencing, polymerase chain reactions, site-directed mutagenesis, hybridization, and structural analyses to their studies at a significant cost discounts over what individual laboratories could obtain. Additionally, specialized modified fluorescent oligonucleotides are required for LOH and MIN fragment analysis and SNP studies, cutting edge methods for analysis of molecular mechanisms of many human diseases, as well as probes for Real Time PCR. The technical support provided by the staff for these specialty oligonucleotides greatly speeds the application of these methods to research.

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