

Biochemistry and Biotechnology Facility

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The "Biotech Facility" makes available to Fox Chase investigators a group of state-of-the-art methods for the identification, characterization, and utilization of proteins, peptides and genes. The facility encompasses DNA synthesis, Real-Time PCR, mass spectrometry, proteomics, mutation detection, and HPLC support for purification and analysis. The benefits to the users include the readiness of equipment and expertise in the facilities, the savings in space, training, and unnecessary equipment duplication. The rapid response and the ability to cross-reference with the experience of other similar projects done in the facilities at Fox Chase are primary dividends. The Biotech Facility web site address is <http://web-apps.fccc.edu/fccc/yeung/index.html>. Users will be able to follow the daily progress of their projects in this facility through the Ethernet.

The DNA Synthesis Facility provides Fox Chase researchers with quality oligonucleotides in a timely manner. Quantitation, FPLC analysis, and interpretation of the final products are provided to the user. Special care is taken in the synthesis of both the routine and the custom oligonucleotides. Every oligonucleotide is analyzed prior to delivery. Delivery is typically within 24 hours of order. To our knowledge, the quality of our oligonucleotides has not been a variable in any of the experiments in our institution over the last 19 years. Most commercially available DNA modifications can be synthesized in the facility. This facility provides assistance in gene synthesis, including the conversion of codon usage to the *E. coli* codon usage to minimize translational errors. Current users of the facility include the research staffs of 56 Fox Chase laboratories. As member of the Nucleic Acids Research Group of the Association of Biomolecular Resources Facilities (ABRF) that includes 111 international DNA synthesis core facilities, A. Yeung brings the latest innovations on DNA and protein technologies to the users of the facility.

Real-Time PCR Facility provides full-service quantitative qRT-PCR support as well as operation by the users. Assays are performed using an Applied Biosystems 7900HT Real-Time PCR instrument and a Smartcycler with 48 independently programmable PCR wells suitable for the Sybr Green assays, Taqman assays, and FRET (Fluorescence Resonance Energy Transfer) assays. An Agilent Bioanalyzer allows rapid and sensitive determination of the integrity of RNA preparations prior to being used as templates for Real-Time PCR experiments. We can achieve a sensitivity range of six logs of template concentrations, down to one mRNA molecule of a given gene per cell, and a tight standard deviation of about +/- 7% error among replicate assays.

For gene expression studies, the users only have to provide the RNA sample and the gene accession numbers. We do literature and database searches to verify whether alternate transcripts are known to exist and design appropriate assays. Our services include: the validation of DNA array gene expression data, quantitation of gene expression of low abundance messages, determination of the zygosity of transgenic mice, quantitative analysis of alternative spliced transcripts, quantification of DNA in immunoprecipitated histone fractions (ChIP assays), and validation of the effectiveness of siRNA transcription silencing.

The Mass Spectrometry Facility assists researchers in experiments where accurate measurements of mass are important. Applications include: 1) *Intact protein mass measurements* verify fidelity of recombinant protein reagents; 2) *site-directed mutagenesis*; 3) *determine sites of proteolytic processing*; and

4) success of chemical derivatization, all of which are part of a comprehensive characterization of post-translational modifications of a given protein. *Peptide mass mapping and LC-MSMS analysis following gel purifications and endoprotease digestion* are used to identify unknown proteins and provide detailed maps of the sites of post-translational modification. *Small molecule structure and quantitative determination* are accomplished by MSMS and LC-MS analyses. *Enzymatic ladder sequencing of peptides and oligonucleotides* provides detailed sequence information on purified biopolymers. *De novo* peptide sequencing is performed by expert interpretation of MSMS data for proteins not found in any sequence database. Instruments include a Bruker Reflex IV, an Applied Biosystems QSTAR, and a Finnegan LCQ Classic.

The Proteomics Facility supports the study of biology through simultaneous analysis of large numbers of proteins. Protein resolution provided include high-resolution 2D gels, 2D-HPLC chromatography-MS, HPLC-1D gel LC/MS/MS, and serum peptide proteomics. It is equipped with highly-trained proteomics personnel and state-of-the-art equipment to perform thoughtful medium throughput proteomics studies. We use two-dimensional PAGE to compare thousands of cellular proteins from different cell types and identify up to two thousand protein spots per sample. About half of the proteins are protein isoforms. Proteins in spots of interest are identified with mass spectrometry at up to 384 spots per day. Both pilot scale and investigational scale service packages are provided. This global approach is different from the targeted protein complex analysis services offered by the Mass Spectrometry Facility.

Enzymatic Mutation Detection of the presence and the location of a mutation in a polymerase chain reaction (PCR) product is accomplished by using the CEL I mismatch endonuclease discovered in A. Yeung's laboratory. This is a reliable high throughput method that can scan for mutations in target genes. For large number of DNA samples to be screened, or for DNA samples containing significant amounts of normal DNA alleles. It can detect mutations missed by direct DNA sequencing. It has been developed into a method of genomic mutation scanning, as well as facilitated the discovery of knock-out mutants, in plants, zebra fish, and mice. The CEL nuclease is now marketed by Transgenomic Inc. under the trade name of SURVEYOR.

The HPLC/FPLC/Smart Facility provides the following services on demand:

- High resolution peptide mapping by reversed phase HPLC and MALDI-TOF mass spectrometry;
- Analytical and semi-preparative HPLC/FPLC/SMART systems services to assess the purity of proteins and for end-stage purification before structural analyses such as protein sequencing. This application is coordinated with the use of MALDI-TOF mass spectrometry;
- Semi-preparative HPLC to purify synthetic oligonucleotides by reversed-phase chromatography with or without anion-exchange chromatography;
- HPLC analysis of nucleotide metabolites and drugs;
- Purification of synthetic or proteolytically derived peptides.

* Personnel left Fox Chase