

# RESEARCH TECHNOLOGY SUPPORT FACILITY

## The Research Technology Support Facility

(RTSF) at Michigan State University is supported and managed under the auspices of the Office of the Vice President for Research and Graduate Studies.

RTSF Cores provide technical and analytical support for Biomedical and Agricultural research at Michigan State University in Genomics, Structural Biology, Proteomics, Bioinformatics, and Mass Spectrometry. The RTSF

**Genomics** Core provides single gene to genomic scale DNA sequencing services, DNA fingerprinting and genotyping, quantitative PCR, microarray printing and analysis. The **Macromolecular Structures** Core conducts protein sequencing, HPLC purifications, and synthesizes custom oligonucleotide and peptides. The **Proteomics** Core offers high-

throughput mass spectral protein identification and determination of protein expression patterns. The **Mass Spectrometry** Core uses an array of mass spectral techniques for small molecule identification as well as analyses and for Metabolomic profiling. The **Bioinformatics** Core has staff specialists who assist in the computational analysis and presentation of Genomic, Proteomic and other complex datasets.

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**Access:** The RTSF Cores and their services are accessible to researchers at MSU and academic and research institutions throughout the world. Our mandate is also to provide services to all Michigan Biotech and Pharmaceutical Companies, and we have institutional customers from all over the globe. Information regarding sample preparation and submission, service requests, pricing and billing can be found at our web site:

[www.genomics.msu.edu](http://www.genomics.msu.edu)



**Dr Ian Gray**

*Vice President for Research  
and Graduate Studies*

# Services Offered by the RTSF Facility

## GENOMICS CORE:

**Custom Sequencing:** DNA prepared during routine subcloning or *in vitro* mutagenesis experiments are analyzed using our Custom sequencing service. RTSF uses dye-terminator fluorescent cycle sequencing technology, and sequences are determined on an ABI PRISM® 3130XL Genetic Analyzer. Sequence data and traces are provided via a secure Geospiza Finch web interface. Purified DNA samples may be sequenced using either custom primers or standard primers.



**High-Throughput Sequencing:** High-throughput sequencing is carried out on an Applied Biosystems (ABI) 3730XL capillary sequencer using dye-terminator fluorescent cycle sequencing. Sequence information and traces are available through the Geospiza Finch web interface. High-throughput sequencing projects range from as few as 24 samples to whole genomes. Samples can be submitted as purified template, bacterial cultures, or bacterial and phage libraries. We can plate and pick the clones, isolate and sequence the DNA, and send you the results.

**DNA Fingerprinting and Genotyping (Genescan):** DNA Fingerprinting and Genotyping are available using the ABI PRISM® 3130XL Genetic Analyzer. Such analyses include AFLP (amplified fragment length polymorphism analysis), SSR (simple sequence repeats), and TRFLP (16S microbial identification scans). The ABI GeneScan Analysis System takes advantage of proven multicolor fluorescence technology for sizing and quantitating PCR and other DNA fragments. As in DNA sequencing, GeneScan analyzes four different fluorescent labels visualized as blue, green, yellow, and red. GeneScan Analysis Software automatically identifies all DNA bands and compares the mobility of each band to that of internal standards to calculate the band sizes. Computer workstations containing the ABI GeneScan software are available in the Core for investigator analysis of genotyping data.



**Affymetrix Microarray Analysis:** RTSF is an Affymetrix GeneChip array analysis facility. For GeneChip analysis, biotinylated cRNA samples can either be provided by the researcher or synthesized at RTSF. Gene expression is measured with an Affymetrix high density scanner and the data analyzed with the Affymetrix GeneChip Operating software.

**Custom Microarray Printing/Analysis:** We can amplify your clones and print a custom cDNA array. Alternatively we can design, synthesize and print a custom oligonucleotide array for you. A GeneMachines OmniGrid robot with TeleChem Chipmaker pins is used for printing. Scanning of custom arrays is carried out on our Affymetrix 428 ArrayScanner or our Agilent Microarray Scanner.

Our Bioinformatic Core can assist in data analysis.

**Quantitative PCR:** An ABI Prism 7900HT Sequence Detection System handles real-time quantitation of PCR amplification. This system provides precise quantitative analysis of RNA or DNA levels, and SNP identification. Real time PCR quantitation can be carried with either TAQman primer-probes or the SYBR green protocols on a 384-well format.

**Additional Genomic Services:** Other services provided by the Genomics core include DNA purification, PCR product purification, and robotic sample handling.



## Equipment

- Applied Biosystems PRISM 3730XL Genetic Analyzer
- Two Applied Biosystems PRISM 3130XL Genetic Analyzers
- Fourteen Perkin-Elmer 9700 Thermocyclers
- Qiagen 3000 robot for DNA/PCR fragment purification and rerecking/liquid handling
- GeneMachines RevPrep Orbit DNA purification robot
- Beckman FX and Biomek 2000 robots
- Autogen 850α DNA purification robot
- Applied Biosystems 7900HT Sequence detection system
- Affymetrix hybridization oven model 640, Affymetrix GeneChip fluidics Station model 400, GC3000 High Density Scanner
- GeneMachines OmniGrid Spotting Robot
- Affymetrix 428 microarray scanner
- Agilent Microarray Scanner G2505B
- Agilent Bioanalyzer 2100
- Applied Biosystems 6100 PrepStation
- BioRad GelDoc



Biomek Photo courtesy of Beckman Coulter

## Personnel Phone 517-432-9814

Jeff Landgraf, Ph.D.: Manager, Custom microarray design, spotting and analysis (Ext. 127), [landgra1@msu.edu](mailto:landgra1@msu.edu)

Therese Best: Custom sequencing (Ext. 125), [best@msu.edu](mailto:best@msu.edu)

Shari Tjugum-Holland: High-throughput sequencing, DNA Fingerprinting/genotyping (Ext. 126), [tjugumh1@msu.edu](mailto:tjugumh1@msu.edu)

Annette Thelen, Ph.D.: Affymetrix GeneChip Arrays, Quantitative PCR (Ext. 128), [thelena@msu.edu](mailto:thelena@msu.edu)

Also contact Genomics Core by Fax at 517-432-5404, or by email at [gtsf@msu.edu](mailto:gtsf@msu.edu)



## BIOINFORMATICS CORE:

The Bioinformatics Core was established to provide expert help for researchers who have an integral need for computational analysis for advanced computational analysis or web design. Thus, the Bioinformatics Core provides contract services for the computational analysis of sequence and microarray data, and for the development of Web resources to display such information. In addition, the staff of the Bioinformatics Core is available for expert consultation for researchers who want to design, develop or use computational resources and software on their own. The Bioinformatics Core supports and maintains several Sun Microsystems servers that provide sequence and microarray analysis tools for the Michigan State University research community <http://www.genomics.msu.edu/bioinformatics/Bioinformatics.html>.

The most common types of analyses offered by the Bioinformatics Core include sequence analysis of cDNA libraries, and analysis of spotted and GeneChip microarray data.

**Custom Web Page Services:** Many researchers require web sites to present their experimental results to the scientific community. The Bioinformatics group has considerable experience in designing and hosting database-backed web sites that allow interaction, querying and analysis of the underlying data. An example of a popular web site developed by the Bioinformatics Core is: <http://genomics.msu.edu/fruitdb/index.html>.

**cDNA Library Analysis:** The analysis pipeline developed by the Bioinformatics Core for analysis of cDNA sequence data involves removal of vector and low quality sequence data, and then assembly into clusters of cDNAs derived from a common mRNA. Cluster allows a relative estimate of the expression of a given gene in the tissue or cell type from which the cDNA library was constructed. The clusters are then annotated according to protein function using identities obtained by comparison with known protein sequences using BLAST. Further annotation may be obtained using programs that predict subcellular location or detect the presence of small sequence motifs. The final results are presented in Web-based form that allows the user to sort the data to determine specific expression profiles in libraries obtained from different tissues or isolated from the same tissues following differential treatments. Hyperlinks to web services at MIPS, TIGR and NCBI are provided to enhance the annotation. Graphical comparisons of gene frequency between different libraries are also possible.

**Microarray Analysis:** The Bioinformatics Core also maintains and develops software to analyze custom or Affymetrix GeneChips microarray data. For these studies, data is first normalized and then analyzed statistically with the appropriate replicate samples to determine changes in gene expression and to identify genes with similar expression profiles. The Bioinformatics Core can provide instruction in the use of programs such as Spotfire, Genespring, R, Affymetrix Data Mining Tools, and other programs in development that allow investigators to obtain statistically significant results from experimental array data.



## Equipment

- Sun Microsystems 3500 enterprise server
- Sun Microsystems 250 workstation
- 24 node UNIX Cluster
- GCG Sequence analysis software
- Stackpack EST clustering software
- Four processor, 16 Gb Opteron workstation

## Personnel

Curtis Wilkerson, Ph.D.:	Bioinformatics Manager	517-432-9210, <a href="mailto:wilker13@msu.edu">wilker13@msu.edu</a>
Matthew Larson:	Data Base Administrator	517-353-7122, <a href="mailto:l Larsonm@msu.edu">l Larsonm@msu.edu</a>
Kevin Carr:	Bioinformatics Specialist	517-353-6794, <a href="mailto:carrk@msu.edu">carrk@msu.edu</a>
Andrew Bomhof:	Systems Administrator	517-353-7236, <a href="mailto:bomhof@msu.edu">bomhof@msu.edu</a>

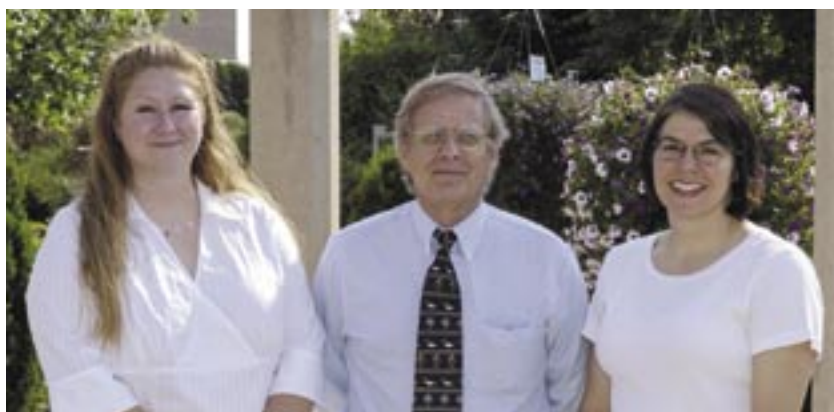
## MACROMOLECULAR STRUCTURE CORE:

The Macromolecular Structure Core (MSC) synthesizes oligonucleotide and peptides, and analyzes proteins, peptides, carbohydrates, and other small molecules. DNA synthesis is available with standard or modified bases, including 5'- and 3'-fluorescently labeled or biotinylated oligonucleotides. Peptide synthesis and peptide conjugations to carrier proteins are available. The MSC offers N-terminal sequencing using both high-throughput and high sensitivity N-terminal sequencers. C-terminal sequencing is performed by carboxypeptidase digestions followed by amino acid analysis or by MALDI-TOF. The MSC has a wide variety of HPLC equipment from capillary to preparative separations that employ UV absorbance, fluorescent excitation, or electrochemical detection. Mass mapping is performed by capillary or nano LC-MS. Molecular weight determinations of biomolecules are performed by MALDI-TOF or PAGE. Electroelutions or electrotransfers are available on PAGE samples. Protein-protein and other biomolecule interactions can be quantitated by surface plasmon spectrometry on a BIACORE 2000. Molecular weight determinations or the thermodynamics of self associating systems may be studied on a Beckman Instruments XLI analytical ultracentrifuge.



### Equipment

- Two Applied Biosystems Procise cLC 494 Protein/Peptide Sequencers
- Applied Biosystems Procise HT 494 Protein/Peptide Sequencer
- Applied Biosystems 3900 Oligonucleotide Synthesizer
- Applied Biosystems 3948 Oligonucleotide Synthesizer
- Two Applied Biosystems 394 Oligonucleotide Synthesizers
- Beckman Instruments XLI Analytical Ultracentrifuge
- BiaCore 2000 Surface Plasmon Resonance Spectrometer
- Two Dionex P680 HPLC Equipped with PDA and Fluorescence
- GeneMachines Polyplex HT Oligonucleotide Synthesizer
- Four Waters Analytical HPLCs Equipped with PDA, UV, and Electrochemical Detectors
- Two Waters Preparative HPLCs Equipped with PDA and UV Detectors
- Molecular Dynamics Phosphoimager
- Waters 2690 based Amino Acid Analyzer
- Applied Biosystems 173 Capillary HPLC
- Applied Biosystems 140C Microbore HPLC
- Applied Biosystems 432 Peptide Synthesizer
- BMG Technologies FLUOROstar OPTIMA
- Hitachi L8800 Amino Acid Analyzer



### Personnel Phone 517-355-4755

Joseph Leykam: Manager

Colleen Curry: DNA Synthesis,  
Oligo Design

Stacy Trzos: Protein Sequencing,  
Peptide Synthesis,  
Mass Spectrometry

Also contact Macromolecular Structure  
Core by Fax at 517-353-8638, or by  
email at [msssf@msu.edu](mailto:msssf@msu.edu)

## PROTEOMICS CORE:

The RTSF Proteomics core provides high-throughput protein identification, global protein expression analysis and protein-protein interaction mapping. The facility also offers gel electrophoresis (1-D and 2-D), automated imaging and spot extraction, multidimensional protein identification technology (MudPIT), and quantitative proteomics using  $^{18}\text{O}$  and iTRAQ. Our high-throughput nano-scale ESI-LC-MS/MS instruments collect parent and product ion mass spectra of complex peptide mixtures at or below the femtomole level. These product ion spectra can then be searched against various



nucleic acid and protein databases to rapidly determine protein identities and post translational modifications and the results can be securely disseminated to clients using an in house relational database system. For more information please visit [www.proteomics.msu.edu](http://www.proteomics.msu.edu)

## Equipment

- ThermoElectron LTQ-FT with the Proteome X 2.0 HPLC system
- Waters Q-ToF Ultima API LC-MS/MS, with a Waters CapLC capillary HPLC
- ThermoElectron LTQ, with a Micron Paradyne Capillary HPLC
- Micromass MassPrep XL robot
- BioRad Proteome Works spot cutter robot with a FX Proplus Imaging System

**Personnel** Phone: 517-353-4622

Curtis Wilkerson, Ph.D.: Manager  
wilker13@msu.edu

Douglas Whitten: Staff Scientist  
whitten3@msu.edu



## ANALYTICAL CORE: THE MASS SPECTROMETRY FACILITY

The Mass Spectrometry Facility provides a wide range of low- and high-resolution mass analysis of small molecules for structural analysis, and high throughput analysis of physiological samples for quantitative profiling of small molecule metabolite levels in physiological fluids and tissues (Metabolomics). With the addition of new instrumental capabilities during 2005, the Facility will encourage researchers to learn how to perform many mass spectrometric analyses in an “open access” environment. The more traditional approach, which relies on trained facility staff to perform analyses, will continue to be available as needed. The Facility will emphasize analysis of “small” molecules such as synthetic and natural products and metabolites, whereas more extensive capabilities for protein identification and quantification and peptide sequencing are available through the Proteomics Facility.

**Mass Spectrometry for Structure Determination and Confirmation** Open access high-resolution mass spectra can be obtained using a Waters QToF Ultima using electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) in either positive or negative ion modes. Samples are introduced using either flow injection analysis (FIA) or via LC/MS. This instrument also allows for high-resolution MS/MS analyses under data system control. MALDI analyses can also be performed in an open access environment on one of two MALDI-TOF spectrometers. More traditional accurate mass determinations for purified chemicals are available using fast atom bombardment (FAB) using the Facility’s JEOL HX110 double focusing magnetic sector mass spectrometer, which often affords high-resolution mass measurements accurate to 4 decimal places for confirmation of elemental formulas. Analysis of purified volatile samples is also available via direct inlet electron impact (DI EI) mass spectrometry to obtain confirmation of empirical formula composition from high-resolution accurate mass analysis.

**Metabolomic Analyses** Quantitative profiling of metabolites and more global metabolome analyses of biological tissues or fluids can be performed using GC/MS, LC/MS, or LC/MS/MS. The NIST spectra library database can be employed to help with the identification of metabolites from GC/MS data. Members of the facility staff are available for consultation regarding derivatization and other aspects of analytical method development. One Agilent GC/MS system is dedicated to electron ionization (EI) mode exclusively and the other can use chemical ionization (CI) using methane gas in either positive or negative modes.

Liquid chromatography mass spectrometry (LC/MS) allows for the analysis of nonvolatile molecules and mixtures of molecules and is equipped with a variety of electrospray (ESI), nanospray (NSI) and atmospheric pressure chemical ionization (APCI) sources. The triple quadrupole LC/MS/MS instrument is capable of operating in various scan modes (precursor, product, or neutral loss scans) for selective detection of specific compound groups.

### Instrumentation and Capabilities

- JEOL HX110: Double focusing magnetic sector – FAB; high and low resolution, MS/MS
- JEOL AX 505H Double focusing magnetic sector – EI/CI; GC/MS, Direct inlet high and low resolution
- Waters QToF Ultima: open access quadrupole-time of flight – ESI/APCI; high-resolution FIA or LC/MS; MS/MS
- Waters Quattro triple quadrupole LC/MS/MS system: ESI/APCI, metabolite profiling
- PerSeptive Biosystems Voyager-DE STR MALDI-TOF
- ThermoFinnigan LCQ DecaXP ESI/APCI/NSI; FIA, LC/MS, and multistage MS/MS.
- Two Agilent 5973N Inert quadrupole GC/MS with autosamplers (EI/CI/negative ion CI)

**Personnel** Phone: 517-353-0612; Fax 517-353-6342

A. Daniel Jones: Manager; metabolomics, MALDI, LC/MS and GC/MS; open access techniques (djones@chemistry.msu.edu)

Bev Chamberlin: Staff Scientist; FAB, GC/MS, high-resolution analyses (chambe16@msu.edu)

Lijun Chen: Staff Scientist; FAB, GC/MS, high-resolution, LC/MS (chenlij1@msu.edu)



## TO REACH RTSF:

**Genomics Core:** S18 Plant Biology Building, Wilson Rd., MSU

**Bioinformatics Core:** 202 Biochemistry Building, Wilson Rd., MSU

**Macromolecular Structures Core:** 223 Biochemistry Building,  
Wilson Rd., MSU

**Proteomics Core:** 3B Biochemistry Building, Wilson Rd., MSU

**Mass Spectrometry Core:** 11 Biochemistry Building, Wilson Rd., MSU



## TO CONTACT RTSF STAFF:

### ADMINISTRATIVE STAFF

Joseph Leykam: Director, 517-355-4755, jleykam@msu.edu, fax 517-353-8638

David DeWitt: Director, 517-353-5284, dewitt@msu.edu, fax 517-355-6758

Phyllis Sherwood: Business Manager, 517-353-0854, sherwo22@msu.edu, fax 517-355-6758

**GENOMICS CORE PERSONNEL:** (Phone: 517-432-9814, Fax: 517-432-5404, or gtsf@msu.edu)

Jeff Landgraf, Ph.D: Manager, Custom microarray design, spotting and analysis (Ext. 127), landgra1@msu.edu

Therese Best: Custom sequencing (Ext. 125), best@msu.edu

Shari Tjugum-Holland: High-throughput sequencing, DNA Fingerprinting/genotyping (Ext. 126),  
tjugumh1@msu.edu

Annette Thelen, Ph.D: Affymetrix GeneChipArrays , Quantitative PCR (Ext. 128), thelena@msu.edu

### BIOINFORMATICS CORE PERSONNEL:

Curtis Wilkerson, Ph.D.: Manager, 517-432-9210, wilker13@msu.edu

Matthew Larson: Data Base Administrator, 517-353-7122, larsonm@msu.edu

Kevin Carr: Bioinformatics Specialist, 517-353-6794, carrk@msu.edu

Andrew Bomhof: Systems Administrator, 517-353-7236, bomhof@msu.edu

**MOLECULAR STRUCTURES CORE PERSONNEL:** (Phone: 517-355-4755, Fax: 517-353-8638, or msssf@msu.edu)

Joseph Leykam: Manager, jleykam@msu.edu

Colleen Curry: DNA Synthesis, Oligo Design

Stacy Trzos: Protein Sequencing, Peptide Synthesis, Mass Spectrometry

**PROTEOMICS CORE PERSONNEL:** (Phone 517-353-4622)

Curtis Wilkerson, Ph.D.: Manager wilker13@msu.edu

Douglas Whitten: Staff Scientist whitten3@msu.edu

**MASS SPECTROMETRY CORE PERSONNEL:** (Phone: 517-353-0612, Fax: 51-353-6342)

A. Daniel Jones: Manager;  
metabolomics, MALDI, LC/MS  
and GC/MS; open access techniques  
(djones@chemistry.msu.edu)

Bev Chamberlin: Staff Scientist;  
FAB, GC/MS, high-resolution  
analyses (chambe16@msu.edu)

Lijun Chen: Staff Scientist; FAB,  
GC/MS, high-resolution, LC/MS  
(chenlij1@msu.edu)

