

Research Technology Support Facility Resource Document

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 Bioinformatics, Flow Cytometry, Genomics, Mass Spectrometry, and Proteomics. The RTSF Genomics Core provides single gene to genomic scale DNA sequencing services, DNA fingerprinting and genotyping, quantitative PCR, microarray printing and analysis. 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. The Flow Cytometry Core offers two multiple laser based optical flow cytometers that analyze cell populations for multiple characteristics simultaneously.

Full information about the facility and its resources can be obtained at: <http://rtsf.cns.msu.edu/>.

Bioinformatics Core: RTSF has a staff of three Bioinformatics specialists who support the work of the other cores, and distribute and maintain bioinformatics and sequence analysis software. These individuals are also available on a fee for service basis for computation analysis, design and maintenance of relational databases, websites, and program development. Curtis Wilkerson heads the core, Matt Larson is our relational database specialist, and Kevin Carr is our systems administrator, security specialist and newest bioinformatics specialist.

Flow Cytometry Core: This facility has available two flow cytometers – an Influx cell sorter and an LSR II for cell analysis only. The Influx is capable of sorting cells from bacteria to plant protoplasts in size from low to high speeds as dictated by the cells being sorted. Single to 6 way collection of sorted cells is available including collection to a variety of culture plates and tubes. The Influx is a BSL2⁺ capable sorter installed in a Baker hood with the Influx having additional aerosol collection safety equipment. The machine is equipped with 5 lasers. A 561 laser is used to excite PE and its conjugates (PE, PE-Texas Red, PE-Cy5 or Cy5.5, and PE-Cy7) as well as mCherry, mTomato, and others. A blue laser (488nm) is used to excite FITC, PI, PerCP-Cy5.5 with a filter set for GFP/YFP. A red laser (640 nm) is used to excite APC, Alexa 700 and APC-Cy7. A violet laser (405nm) is used to excite cyan proteins or Pacific Blue, and Pacific Orange. A uv laser (355nm) allows use of indo-1 calcium flux, Hoechst 342 red/blue stem cell side population and DAPI for cell cycle analysis. Data acquisition is with Sortware.

The LSR II is equipped with 3 lasers. The blue laser has 4 detectors capable of detecting FITC, PE, PE-Cy7 and one of the following (PE-TxRed, PE-Cy5, PerCpCy5.5). It has a red laser with three detectors for 660 nm, 700 nm and 780 nm fluorescence. It also has a violet laser (405) with two detectors which are useful for violet excited cyan protein or Pacific blue and Pacific orange. Data acquisition is with DIVA software. Both machines use FCS3.0 data file format for export to post acquisition analysis.

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Software for data analysis is available for cell cycle analysis (Modfit), and phenotyping (WinList 6 and FlowJo). A large variety of methods for the study of plant and animal cells may be executed on these machines. Apoptotic cell death, cell proliferation, cell activation and signaling, cell integrity, cell and organelle function, and presence or absence of plasma membrane, cytoplasmic or nuclear markers for up to 9 or 15 markers can be done simultaneously, LSR II or Influx respectively. Studies of cellular DNA can be done to determine DNA content and presence or absence of aneuploidy and polyploidy.

Genomics Core: The Genomics core provides small scale to genome size high throughput DNA sequencing. The core also provides infrastructure for SNP profiling, fragment analysis genotyping, Quantitative Real Time PCR, and a number of microarray platforms for gene expression, aCGH, and methylation analysis. A partial list of equipment within the core includes three “Next-Gen” sequencing platforms: a Roche/454 GS FLX sequencer; an Illumina HiSeq2000 and an Illumina GAIIX; an ABI 3730xl high through-put 96 capillary DNA sequencer; an ABI 3130xl 16 capillary DNA analyzer; two Biomek FX and a BioMek 2000 robot for liquid handling, plasmid preparation, cherry picking and plasmid purification; an Autogen PI-50a DNA Isolation System for purification of genomic DNA from plant, fungi, and mammalian tissue; fourteen PE9700 thermocyclers; an Agilent Bioanalyzer for RNA and DNA evaluation; an ABI 7900HT Sequence Detection System for Q-PCR and SNP analysis; an Illumina BeadXpress reader for multiplex SNP profiling, expression analysis, and protein quantitation; a GeneMachines Omnigrid 100 array printer for custom microarray production; An Agilent G2505B microarray scanner; and a complete Affymetrix GeneChip system with 7G scanner. Kevin Carr is the bioinformatics specialist who manages next generation sequencing data analysis.

Mass Spectrometry Core: The RTSF Mass Spectrometry Core is an open access resource where MSU researchers and facility staff perform analyses of small molecules, intact macromolecules, peptides, and metabolomic analyses using 12 mass spectrometers. Analyses typically involve compound identification or quantitative analysis. Students and other researchers are encouraged to become certified users of the instruments, and the facility staff offers frequent training in the theory and operation of facility instruments. Trained instrument users enjoy 24/7 open access to instruments; three staff experts perform analyses and provide consulting regarding method development and data interpretation. The facility houses six LC/MS/MS instruments including Waters Xevo TQ-S and Quattro Premier XE UPLC/MS/MS, Quattro micro, QToF Ultima, AB/Sciex QTRAP 3200, and Thermo LCQ Deca XP+ LC/MS/MS instruments. The complement of LC/MS/MS instruments offers analyses using electrospray, atmospheric pressure chemical ionization (APCI), or atmospheric pressure photoionization (APPI). The QToF instrument provides accurate mass (high resolution) analyses and analyses of peptides and intact macromolecules. Five GC/MS instruments are also available including three Agilent (5973 and 5975) and one Thermo DSQ-II quadrupole GC/MS systems and a Waters GCT Premier GC/TOF high resolution MS instrument. MALDI analyses of materials ranging from small molecules, peptides, oligonucleotides, metabolites, and polymers can be performed on a recently upgraded Shimadzu AXIMA-cfr plus instrument equipped with MALDI imaging capability. Metabolomic and metabolite profiling analyses can be performed using a variety of

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instrument platforms, and are supported with data processing and statistical analysis software. Methods are currently running for high-throughput LC/MS/MS profiling of amino acids, phytohormones, signaling oxylipins, structural lipids, and organic acid metabolites. The facility performs analyses of samples from more than 100 institutions from across North America.

Proteomics Core: The Proteomics Core has at its disposal three state of the art mass spectrometers (LC-MS); two Thermo linear ion-trap (LTQ) mass spectrometers and one Thermo Fourier Transform Ion cyclotron resonance linear ion-trap (LTQ-FT) mass spectrometer capable of parts per billion accuracy. All collect data at speeds 10-20 times that of previous available instruments. All of the mass spectrometers are interfaced to Waters nano-acquity ultra performance liquid chromatography instruments. These UPLCs provide enhanced peptide separation which allows us to process much more complex mixtures of proteins. We also have a full complement of 1 and 2D gel boxes specifically for the separation of protein samples to be analyzed by LC-MS, as well as a BioRad Proteome Works spot cutter robot/BX Proplus Imaging System for cutting out gel slices. We have several laminar flow hoods in a positive pressure room to minimize sample contamination during sample processing. We also have a 20 processor computer cluster to processes large proteomic datasets. The programs Sequest, Mascot, and X! Tandem are available to match spectra to peptide sequences and the program Scaffold is available to provide additional statistical validation of the data as well as allow users to access their data in an efficient manner.