

Feeding the SRMAtlas project

A Freedom EVO® 150 workstation is being used to great effect by the proteomics group at the Institute for Systems Biology (ISB), Seattle, to generate samples for analysis by mass spectrometry, including for the world-renowned Selected Reaction Monitoring Atlas (SRMAtlas) project. The system is equipped with a variety of modules to meet the requirements of a range of different projects, and provides the reproducibility and throughput to enable these studies to be run entirely in house.

The multidisciplinary ISB in Seattle, USA, was established in 2000, and is involved in collaborative studies on model organisms – such as halobacterium and yeast – monitoring the genetic, translational and proteomic effects of biological perturbations on the functioning of systems within the organism. The proteomics department at the ISB, led by Dr Robert Moritz, comprises a team of investigators and computational biologists working together on multiple projects and collaborations. Research Associate Doug Spicer joined the team to run automation in the department, and took over a Freedom EVO 150 workstation to develop and routinely run automated protocols for four separate projects. These include automated methods for immunoprecipitations, phosphokinase enrichment and in-gel protein digests with trypsin, plus assay development for the human SRMAtlas, the collaborative project between ISB and the Swiss Federal Institute of Technology (ETH) Zürich to develop a resource for quantification of all 20,000 human proteins for deployment in human tissues, cell lines and blood.

Doug explained: “The system was purchased two years ago, originally to perform reproducible plasma glyco-capture to generate samples for mass spectrometry. The platform is capable of rapidly processing hundreds of crude peptide samples to create multiplexed assays, generating sufficient samples in one day to ‘feed’ three mass spectrometers over a three to four day period, producing data for the SRMAtlas project. It is an excellent, robust and flexible system fitted with a range of modules to meet the varying specific needs of each of our projects.”



The ISB team with the Freedom EVO. Left to right: Meg Kapousouz, Doug Spicer, Sarah Li

The ISB system can perform both single- and multi-channel pipetting using an eight-channel Liquid Handling Arm, and the Robotic Manipulator Arm interfaces with a variety of modules to maximize flexibility. These include a Te-Shake™ module for immunoprecipitation and phosphokinase enrichment protocols, and a Te-VacS™ vacuum manifold for in-gel digests and the SRMAtlas project. Doug added: “Separation of the fragments from in-gel protein tryptic digests is performed using a vacuum manifold plate with micro-holes drilled in the bottom of its wells. To avoid occlusion of these holes – which would prevent the reagents from draining through the plate into the vacuum manifold – hexagonal beads are placed in the wells of the microplate to act as

a filter and the gel fragments are placed on top. This allows the plates to drain correctly at a lower vacuum manifold pressure, and enables us to achieve good recovery of fragments from protein digests.”

As differing protocols require either a static or shaking incubator, a third-party thermal mixer was integrated onto the workstation, with assistance from the Tecan applications team. The system’s software has a liquid class feature that helps to ensure effective, accurate handling of different liquid types – such as detergent, DMSO and acetonitrile – within the same protocol.

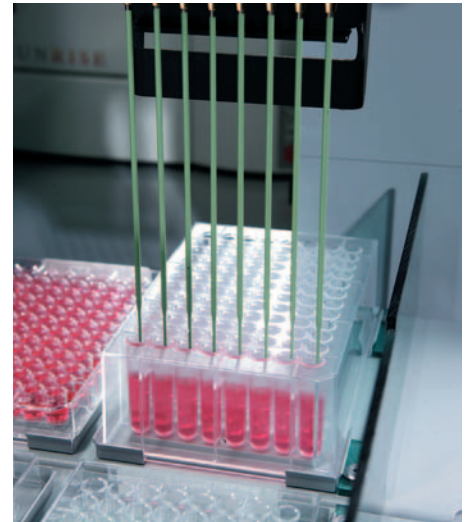
He continued: “Having the ability to establish different ways of pipetting very

different liquids has proven very helpful in overcoming the problems associated with handling these solutions precisely. Most significantly, although we are using fixed pipette tips, there are no problems with cross contamination and we are achieving high consistency and reproducibility between runs, which is essential in proteomics. Sample throughput and accuracy of pipetting are critical in the human SRMATlas project, ensuring consistent representation of each peptide in one pool taken from all samples in one 96-well plate. These 96-well plates contain peptides characteristic of the original proteins, and over 1,700 96-well plates represent the whole of the human SRMATlas, a compendium of the human proteome. Our throughput ranges from 20 to 24 plates daily, and the Freedom EVO's volumetric accuracy makes consistent representation of each peptide in the pool possible – an advantage over manual protocols. This has enabled us to carry out the project entirely in house."

Doug concluded: "I really appreciate the time and effort Tecan invests in developing its systems. We have a very good rapport with the application specialists and service engineers, and feel they really take on board the importance of refining the liquid handling features that are critical in most projects. If ever I find I can't put together an appropriate automated protocol using my own experience, I know that Tecan will give me the help I need."

To find out more on Tecan's proteomics solutions, visit www.tecan.com/proteindigest

To learn more about the Institute for Systems Biology, Seattle, USA, go to www.systemsbiology.org



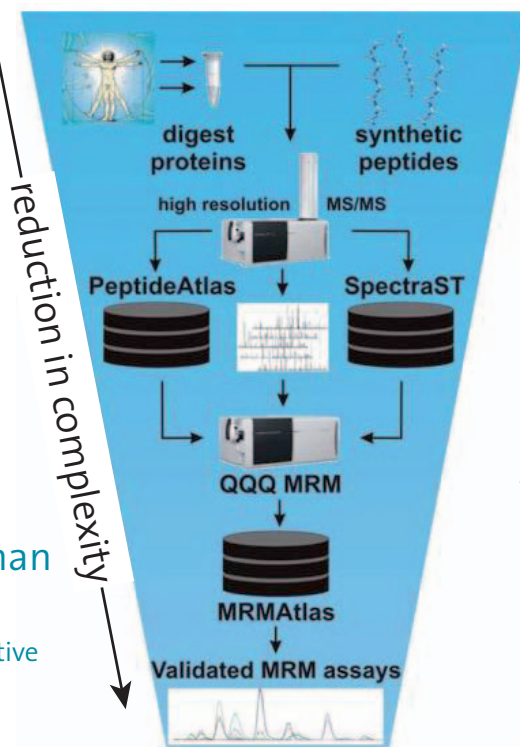
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Developments at ISB – human SRMATlas

Human proteins
(from natural source or synthetic)

Develop human PeptideAtlas
(from tryptic digests or synthetic peptides)

Develop human SRMATlas
(verified quantitative assays)



Synthetic 'proteotypic' peptide
(from cheap synthesis)

Develop optimized transitions from PeptideAtlas