

# Automated, high throughput preparation of ProteinChip® Arrays for SELDI-TOF MS profiling

Laurent Jacotot\*, Philippe Vaglio\*, Anthony Gonçalves<sup>1,2,4</sup>, Yves Toiron<sup>1</sup>, Hagay Sobol<sup>3,4</sup>, Jean-Paul Borg<sup>1</sup>, Xavier Saunier<sup>5</sup>, Emmanuel Russo<sup>5</sup>

\*Modul-Bio S.A.S., 232 Boulevard Sainte Marguerite, 13009 Marseille, France; <sup>1</sup>Molecular Pharmacology, <sup>2</sup>Medical Oncology, <sup>3</sup>Genetic Oncology, Cancer Institute of Marseille, Institut Paoli-Calmettes and <sup>4</sup>UMR599 Institut National de la Santé et de la Recherche Médicale (INSERM) <sup>5</sup>University of la Méditerranée, UFR of Médecine, Marseille, France; <sup>5</sup>Tecan France S.A.S, 26 avenue Tony Garnier, 69007 Lyon, France.

The Institut Paoli-Calmettes in Marseille, France, has begun a global search for serum biomarkers with diagnostic, prognostic and/or predictive values in clinical samples from breast cancer patients. As part of the regional anti-cancer center's clinical proteomics program, the researchers are using Tecan's Freedom EVO® 150 platform to automate sample preparation for surface enhanced laser desorption/ionization-time of flight (SELDI-TOF) mass spectrometry (MS) to identify markers of interest.

Comparing protein expression levels in cancer patients' serum with normal serum samples may lead to the identification of important biomarkers that can predict for malignancy of tumors. These biomarkers can also provide valuable information concerning

the likely efficacy of specific anti-cancer treatments, as well as help to identify new molecular targets for innovative therapeutic strategies. Traditionally, biomarkers have been investigated using 1-D or 2-D gel electrophoresis to separate proteins, coupled to mass spectrometry for protein identification<sup>1</sup>. However, these procedures are complicated and require large amounts of material, meaning that large-scale application of these methods has generally been restricted to preclinical and basic science studies.

SELDI-TOF MS allows relatively high throughput protein analysis of very complex biological samples, yet needs only limited preprocessing. The technology combines chromatographic fractionation of the proteome using specific protein chips and TOF MS analysis

that can be applied to various clinical samples, including biological fluids such as serum<sup>2</sup>. Samples are directly bound to Ciphergen ProteinChip® Arrays and analyzed with SELDI-TOF MS profiling, using the ProteinChip® Biomarker system. Preparation of the ProteinChip® Arrays requires equilibration of the ProteinChips® with specific binding buffer, washing, sample spotting, incubation with shaking, washing, a short drying phase and application of the energy absorbing molecule (matrix) application.

In order to make the SELDI-TOF MS analysis more reproducible on a high throughput basis, researchers at the Institut decided to set up complete automation of the pre-analytical steps, i.e. chromatographic capture of proteins on ProteinChip® Arrays. To achieve this, they approached Modul-Bio S.A.S., a software and robotics company based in Marseille, France, that custom-builds flexible robotics systems and LIMS for biological applications, including DNA sequencing, protein interactions or chemical compound screening processes.

Laurent Jacotot, Chief Executive Officer at Modul-Bio, explained, "We custom build to what people need, based on available off-the-shelf systems. For this project, we recommended the Tecan Freedom EVO 150 platform because its programming flexibility meant we could easily add the Ciphergen SELDI-TOF protocol to it. The Institut also wanted to integrate a Teleshake shaker (Variomag) and other equipment with the Tecan's Gemini™ software system, so we developed an application to fully control the speed, motion and sequence of all the devices and movements."

Modul-Bio's automated preparation of the ProteinChip® Arrays vastly increased the sample throughput for researchers at the Institut Paoli-Calmettes. The system was specially developed to provide extremely

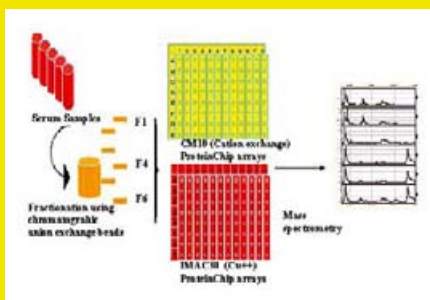
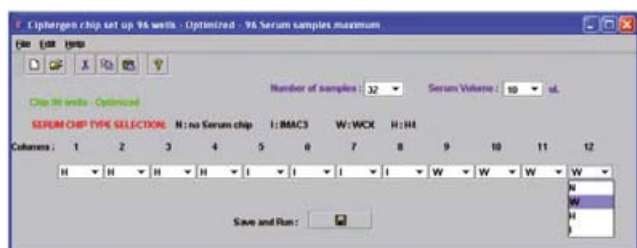


Figure 1: Automating the search for serum biomarkers from breast cancer patients



Figure 2: Tecan's Freedom EVO 150 platform

high-precision liquid volume handling, including liquid level sensing, single or multiple dispensing options and flexibility for different liquid classes. Importantly, automated sample processing generates improved sample quality and data reliability, due to the decrease in possible human error, and makes it easier for laboratories to carry out repetition of data sets. Using the Freedom EVO with eight

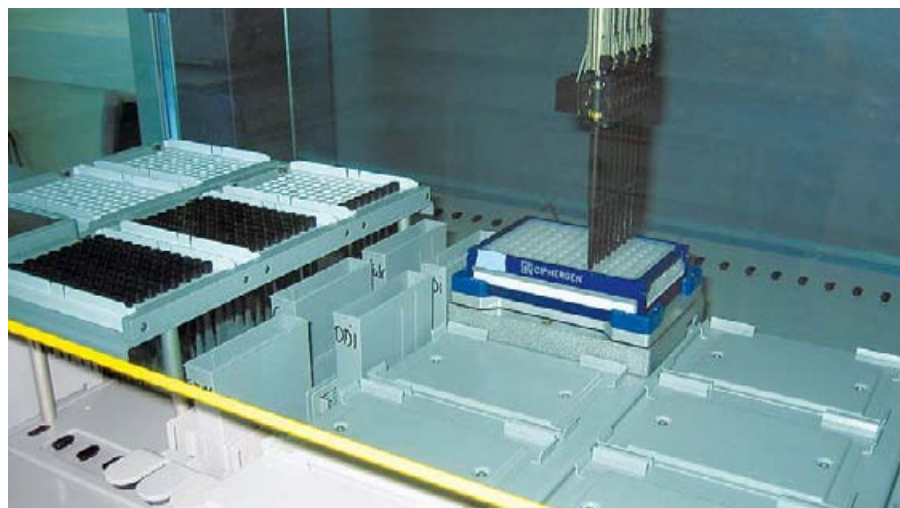


**Figure 3:** Modul-Bio's Swing Java™ interface

pipetting tips and two liquid handling (LiHa) arms, the entire process can be performed within three hours for 96 samples, which equates to 192 or 288 samples each day and the potential to complete 384 samples in a day. Modul-Bio also developed a Swing Java™ interface for this application (Figures 1-3), seamlessly integrated with Gemini, to control the adapted CIPHERGEN protocols on the Freedom EVO (Figure 4). This interface allows the user to choose the number and volume of serum samples to be bound on CIPHERGEN ProteinChips® and the type of ProteinChip® used in each column of the ProteinChip® cassette (standard MTP format). The arrays are available with different chromatographic properties, including hydrophobic, hydrophilic, anion exchange, cation exchange and immobilized-metal affinity surfaces.

Depending on the different type of protein chip, specific protocols with specific buffer are then executed by the Tecan system.

This set-up has been used at the Institut Paoli-Calmettes to investigate serum samples from two different groups of patients. In the first study, samples from high-risk, early breast cancer patients were analyzed for serum biomarkers that could predict for metastatic relapse or metastasis-free survival. The second study investigated samples from patients certified with and without BRCA1 mutations, which have been implicated in a large number of hereditary breast cancers, in order to generate plasma protein biomarkers that could be associated with the presence of the mutation<sup>3</sup>.



**Figure 4:** Tecan's Freedom EVO 150 platform and CIPHERGEN ProteinChips® cassette

SELDI-TOF MS techniques make it possible to analyze biomarkers from small sample volumes requiring only limited pre-processing phases, and the availability of tools such as Tecan's Freedom EVO platform and CIPHERGEN's ProteinChip® Arrays and Biomarker System make large-scale analyses much easier. Coupled to appropriate bioinformatic tools, SELDI-TOF MS has shown to be a very promising method for probing serum to identify protein patterns and/or biomarkers related to various stages and types of solid tumors<sup>4,9</sup>, which could serve as early diagnostic markers.

*JAVA™ is a trademark of Sun Microsystems, Inc. in the United States and other countries*

*ProteinChip® is a registered trademark of CIPHERGEN Biosystems Inc., Fremont, CA, USA*

## References

- Hanash SM. (2000) Biomedical applications of two-dimensional electrophoresis using immobilized pH gradients: current status. *Electrophoresis* 21: 1202-1209.
- Fung ET, Thulasiraman V, Weinberger SR, Dalmaso EA. (2001) Protein biochips for differential profiling. *Curr Opin Biotechnol* 12: 65-69.
- Gonçalves A, Esterni B, Bertucci F, Sauvan R, Chabannon C, Cubizolles M, Bardou VJ, Houvenaegel G, Jacquemier J, Granjeaud S, Meng X-Y, Fung ET, Birnbaum D, Maraninchi D, Viens P, Borg J-P. (2005) Post-operative serum proteomic profiles may predict metastatic relapse in high-risk primary breast cancer patients receiving adjuvant chemotherapy. *Oncogene* (in press)
- Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, Mills GB, Simone C, Fishman DA, Kohn EC, Liotta LA. (2002) Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 359: 572-577.
- Adam BL, Qu Y, Davis JW, Ward MD, Clements MA, Cazares LH, Semmes OJ, Schellhammer PF, Yasui Y, Feng Z, Wright GL Jr. (2002) Serum protein fingerprinting coupled with a pattern-matching algorithm distinguishes prostate cancer from benign prostate hyperplasia and healthy men. *Cancer Res* 62: 3609-3614.
- Petricoin EF III, Ornstein DK, Pawletz CP, Ardekani A, Hackett PS, Hitt BA, Velasco A, Trucco C, Wiegand L, Wood K, Simone CB, Levine PJ, Linehan WM, Emmert-Buck MR, Steinberg SM, Kohn EC, Liotta LA. (2002) Serum proteomic patterns for detection of prostate cancer. *J Natl Cancer Inst* 94: 1576-1578.
- Koopmann J, Zhang Z, White N, Rosenzweig J, Fedarko N, Jagannath S, Canto MI, Yeo CJ, Chan DW, Goggins M. (2004) Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionization mass spectrometry. *Clin Cancer Res* 10: 860-868.
- Wadsworth JT, Somers KD, Cazares LH, Malik G, Adam BL, Stack BC Jr, Wright GL Jr, Semmes OJ. (2004) Serum protein profiles to identify head and neck cancer. *Clin Cancer Res* 10: 1625-1632.
- Zhang Z, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, Rosenzweig JM, Cameron B, Wang YY, Meng X-Y, Berchuck A, van Haaften-Day C, Hacker NF, de Bruijn HWA, van der Zee AGJ, Jacobs IJ, Fung ET, Chan DW. (2004) Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. *Cancer Res* 64: 5882-5890.