ABDULMOHSEN ABDULRAZZAQ HEALTH SCIENCES CENTRE

KUWAIT UNIVERSITY – RCF NEWS

The newsletter of the HSC Research Core Facility, Kuwait University / Issue No.1 – February 2012

Project Nos. GM01/01 & GM01/05 **Principal Investigator: Prof. Widad Al-Nakib**

Director: Prof. Abu Salim Mustafa

Co-Investigators: Prof. Eino Honkala, Prof. Samuel Kombian, Prof. Chacko Mathew, Prof. Abu Salim Mustafa

It gives us a great pleasure to welcome you to the first issue of the Research Core Facility (RCF) newsletter. In the recent years, technological advancements in biomedical research have necessitated to build specialized laboratories that can be accessed and utilized by researchers of varied disciplines. These laboratories should facilitate in-depth study of disease processes at cellular and molecular levels. The RCF fulfills such a necessity at the Health Sciences Centre (HSC), Kuwait. It comprises of an integrated set of multipurpose laboratories, which are housing the most modern and state-of-the-art instruments required for health-care related research work. The details of these laboratories and equipment are given on page 2. The instruments in the RCF are operated by a dedicated team of suitably qualified and trained technical staff.

Although, the RCF is meant for researchers in the broad area of biomedical research, it is especially useful for researchers in the field of human health, e.g., to identify and evaluate prognostic, diagnostic and therapeutic markers etc., who are working at the HSC and other health-related institutions in Kuwait. Furthermore, collaborative research with researchers/institutions out of Kuwait is also encouraged.

However, well-equipped laboratories and most-modern equipment do not guarantee top-notch research to benefit our community and humanity. These must be coupled with enthusiasm to use appropriately. Through this newsletter, we encourage you to come and visit the RCF and discuss with us the ways you can use this facility towards your own benefit and for the benefit of people in Kuwait and abroad. Hoping to see you soon at the RCF.

Prof. Widad Al-Nakib
Principal Investigator of RCF

Prof. Abu Salim Mustafa
Director of RCF

We invite you to visit the Research Core Facility and see for yourself the endless possibilities that you have here for your project work.

For arranging a visit contact **Prof. Abu Salim Mustafa**, the Director of RCF: abusalim@HSC.EDU.KW or by dialing Phone Extension: **6426** or **6505**

RCF STAFF & THEIR SPECIALIZATIONS

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INSTRUMENTS & TECHNOLOGIES AVAILABLE AT RCF

GENOMICS

- 3400 DNA Synthesizer – Primer Synthesis
- WAVE 4500 System – DHPLC-high through put mutation detection system
- ABI 3130 Genetic Analyzer – DNA sequencing & Fragment analysis
- CEQ™8000 Genetic Analysis System – DNA sequencing & Fragment analysis
- ABI 7500 Real – Time PCR System
- ABI 7900HT Low Density Array Analyzer - QRT-PCR
- Affymetrix – GeneChip Microarray System
- Automated Karyotyping System – Multicolor FISH
- Agilent DNA Microarray for CGH & Gene Expression Analyzer
- Ultra Lum Omega 16vS – Gel Documentation system

PROTEOMICS

- ABI 4800 MALDI TOF/TOF Analyzer – Mass Spectrometry
- ProteomeLab™ PF 2D – Protein Fractionation System
- ProteomeLab™ PF 800 – Protein Characterization System

FLOW CYTOMETRY:

- Flowcytometer Cytomics FC 500
- Vi-Cell Series Cell Viability Analyzers
- Cell Lab Quanta – Flowcytometry
- EPICS Elite Flow Sorter – Analyze and sort up to 5 colors simultaneously

CELL BIOLOGY

- LSM 510 Meta – Confocal Microscopy
- Culture Cell Imaging System
- In vitro Fertilization System
- Cell Observer – Complete System for Live Cell Imaging
- Palm Robo – Laser micro-dissection
- Axio Imager – Fluorescence Microscopy

For more information about the instruments and the technologies visit:

http://www.hsc.edu.kw/vpo/rcf/

or you are most welcome to arrange a visit to the Research Core Facility.

RCF UTILIZATION

During 2008-2010:

The number of projects which used the facility – 46

The number of samples run in the facility – 38698

The number of researchers who used the facility – 49

The number of MSc students who used the facility – 16

The number of PhD students who used the facility – 5

Faculty	Number of requests
Allied Health	146
Amiri Hospital	1
Dentistry	124
Genetic Center	27
Medicine	1148
Pharmacy	94
Research Core Facility	157
Science	2
Others	2
Total	**1701**

In 2011:

The number of projects which used the facility – 52

The number of samples run in the facility – 21803

The number of researchers who used the facility – 40

The number of MSc students who used the facility – 11

The number of PhD students who used the facility – 2

Faculty	Number of requests
Allied Health	105
Amiri Hospital	4
Dentistry	104
Environmental Chemistry	1
Genetic Center	13
Medicine	675
Pharmacy	186
Total	**1088**

PUBLICATIONS

Forty-nine papers, which acknowledged RCF have been published.

Year	Number of papers
2006	5
2007	7
2008	5
2009	5
2010	16
2011	11
Total	**49**

LATEST PUBLICATION:

Sanaa Al Saleh, Fahd Al Mulla, Yunus A. Luqmani – PLoS ONE - 6(6): e20610

Estrogen receptor silencing induces epithelial to mesenchymal transition in human breast cancer cells.

We propose the hypothesis that loss of estrogen receptor function which leads to endocrine resistance in breast cancer, also results in trans-differentiation from an epithelial to a mesenchymal phenotype that is responsible for increased aggressiveness and metastatic propensity. siRNA mediated silencing of the estrogen receptor in MCF7 breast cancer cells resulted in estrogen/tamoxifen resistant cells (pII) with altered morphology, increased motility with rearrangement and switch from a keratin/actin to a vimentin based cytoskeleton, and ability to invade simulated components of the extracellular matrix. Phenotypic profiling using an Affymetrix Human Genome U133 plus 2.0 GeneChip indicated geometric fold changes ≥3 in approximately 2500 identifiable unique sequences, with about 1270 of these being up-regulated in pII cells. Changes were associated with genes whose products are involved in cell motility, loss of cellular adhesion and interaction with the extracellular matrix. Selective analysis of the data also showed a shift from luminal to basal cell markers and increased expression of a wide spectrum of genes normally associated with mesenchymal characteristics, with consequent loss of epithelial specific markers. Over-expression of several peptide growth factors and their receptors are indicative of an increased contribution to the higher proliferative rates of pII cells as well as aiding their potential for metastatic activity. Signaling molecules that have been identified as key transcriptional drivers of epithelial to mesenchymal transition were also found to be elevated in pII cells. These data support our hypothesis that induced loss of estrogen receptor in previously estrogen/antiestrogen sensitive cells is a trigger for the concomitant loss of endocrine dependence and onset of a series of possibly parallel events that changes the cell from an epithelial to a mesenchymal type. Inhibition of this transition through targeting of specific mediators may offer a useful supplementary strategy to circumvent the effects of loss of endocrine sensitivity.

"We are ready to receive DNA sequencing requests"

To book your orders or for your enquiries please contact the Director by e-mail at:

abusalim@HSC.EDU.KW

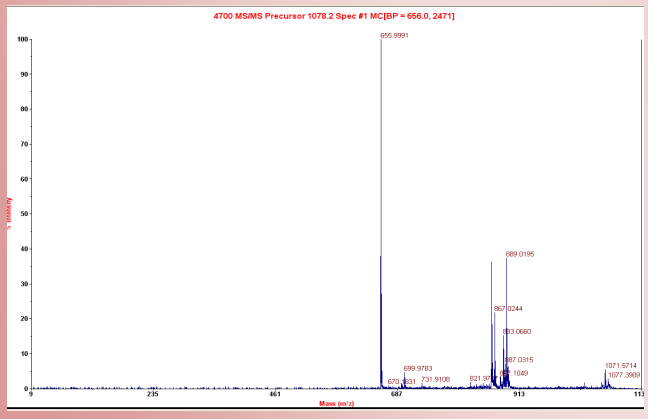
WHAT'S NEW

From left: Anees Fathima and Betty Thomas, both are proteomics specialists, infront of the 4800 MALDI TOF/TOF Analyzer.

We are now optimizing the 4800 MALDI TOF/TOF proteomics manufactured by Applied Biosystems. This mass spectral instrument can perform high sensitivity and fast sequencing of peptides in complex biological mixtures.

The machine uses a focused laser beam which evaporates the compounds from the sample. The resulting ions are injected into a tube (1 -2 m in length), accelerated and allowed to drift towards a detector. Their time-of-flight (from their ionization till they hit the detector) is proportional to their molecular weight.

"Another milestone in RCF. This machine can produce results so powerful that every scientist is going to consider this technology for his/her research work."

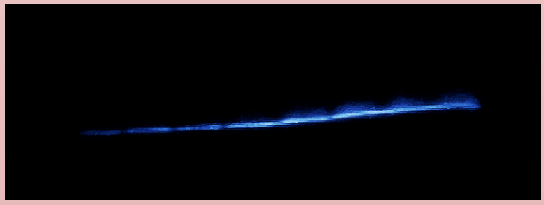


Spectrum obtained after MS/MS analysis. Each peak represents the

From left: Betty Thomas and Manar El-Borsaly optimizing the UVP method for detection of bands on western blots.

A new method for the detection and quantification of bands in Western blotting is being optimized in RCF. This method involves using Biospectrum®AC imaging system manufactured by Ultra Violet Product (UVP).

"Blots can be detected immediately after adding the chemiluminescent reagent. Hence, faster results with cleaner lab coats." said Betty Thomas, an expert on western blotting in RCF. Indeed, this method once optimized will cut down both the cost and time required to do western blotting as the routine western blotting technique performed earlier involved using luminal reagent, transferring membranes to cassettes, and developing them using a developing solution. "Easier, faster and much cleaner western blotting." said Manar El-Borsaly, another expert on Western blotting.



UVP image of a Western blot obtained using the new method.

Latest addition to Man Power:

Mr. Vishnu Ramasubramanian an expert in DNA microarray will soon join the RCF family. His duties will include the optimization of Microarray technology (Genomics & Proteomic) and maintaining the equipment in order to ensure reliable results.

USEFUL PROTOCOL

In each issue of RCF news we are going to discuss about one technique which is performed in the facility. Flow Cytometry technology is discussed in this issue. The machine we use here is “Cytomics FC 500” manufactured by Beckman Coulter.

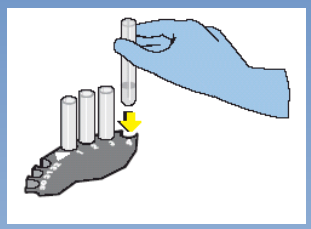
The Cytomics FC 500 is a system for the qualitative and quantitative measurement of biological and physical properties (such as cell surface markers, apoptosis and cell cycle) of cells and other particles. These properties are measured when the cells pass through one or two laser beams in single-file.

The instrument can simultaneously measure forward scatter, side scatter and five fluorescent dyes using two lasers at 488 nm and either 635 nm (Solid-state laser) or 633 nm (HeNe laser). Therefore, the instrument can perform correlated multi-parameter analysis of individual cells.“The technique is so powerful and can be routinely used in the diagnosis of health disorders and in many other applications in both research and clinical practice. The simplicity and speed are added bonuses.” said Sunitha Pramod, an expert on flow Cytometry at RCF.

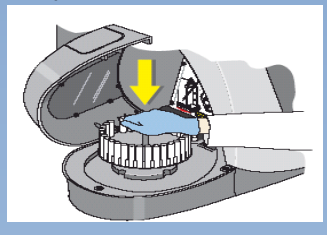
Sunitha Pramod, flow Cytometry specialist in RCF, is working on apoptosis using the Cytomics FC 500.

STANDARD OPERATING PROTOCOL OF FLOW CYTOMETRY AT RCF:

- Check if the waste container is empty, sheath container and cleaning agent container are filled.
- Turn on the PC (to which the machine is connected)
- Log in windows and open the CXP software to turn on the machine (allow the machine to warm up for 40 minutes before performing any test).
- Select a protocol if needed and known.
- Click on view in the tool bar options and select **Resource Explorer** and **Acquisition manager**.
- Prepare the sample according to the reagent package inserted.
- Place the sample tubes in a carousel.



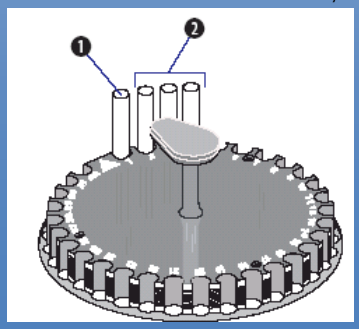
- Open the Multi Carousel Loader (MCL) cover and place the carousel in the MCL then close the cover.



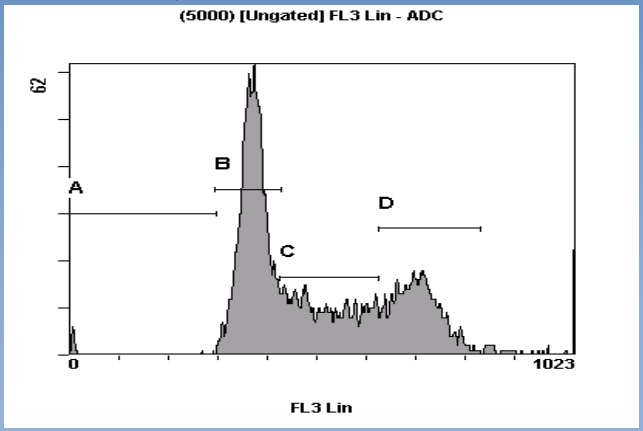
- Click on the protocol panel in the Resource Explorer and select the required protocol.
- Drag and drop the selected protocol from the Resource Explorer to the Acquisition manager.
- Enter the carousel ID number (it will be seen on the carousel) in the Acquisition manager.

Carousel No.	Location
56	1

- Close the MCL Tube Access door and click  .
- During the sample cycle the following series of Cytometer status messages appears: Awaiting Sample, Preparing Sample, Acquiring, Stopping.
- The instrument has to be cleaned before shutting it down.
- The instrument is cleaned using the cleaning solution (1 part of high quality bleach and 9 parts of distilled water or IsoFlow sheath fluid).
- 2 ml of the cleaning solution is placed in a test tube which is placed into carousel position 1 and three freshly prepared tubes, each containing about 2 ml of distilled water or IsoFlow sheath fluid, into positions 2, 3 and 4 of the carousel.



- Select the cleaning panel by clicking on  and selecting Cleanse.PNL.
- Run the protocol the same way done for samples.
- To switch off the cytometer make it first idle by clicking on  .
- Close all open windows.
- Click on  "FC OFF" (located in start menu) to turn off the machine.



A single parameter histogram of HEK cells stained with PI. Such histograms are used for cell cycle analysis.A, Apoptotic cells.B, G0/G1 pase.C, S-phase. D, cells in G2-phase

Editor: Chadi Abdul Kader EL Farran

Photography: Chadi Abdul Kader EL Farran, Sunitha Pramod