HSC | RCF
OMICSRU

HEALTH SCIENCES CENTRE
RESEARCH CORE FACILITY
OMICS RESEARCH UNIT

KUWAIT UNIVERSITY – RCF/OMICSRU NEWS

The newsletter of the HSC RCF/OMICSRU, Kuwait University / Issue No.3 – DEC 2013

Project No. SRUL02/13 **Principal Investigator: Prof. Widad Al-Nakib**

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

Director: Prof. Abu Salim Mustafa

ADVANCING RESEARCH AT THE HEALTH SCIENCES CENTRE, KUWAIT UNIVERSITY

CONTENTS

SUMMARY OF ACHEIVEMENTS IN 2012-13

The Research Core Facility (RCF)/OMICS Research Unit (OMICSRU) at the Health Sciences Centre (HSC) was established to serve primarily the research and teaching needs of the four HSC Faculties (Medicine, Allied Health, Dentistry and Pharmacy) under the dynamic leadership of the Vice Deans of Research. The major aim of the RCF/OMICSRU was to establish and operate a state-of-the-art core facility to conduct front-line basic and clinical research in health sciences, particularly in the areas of Genomics, Proteomics and Cell Biology. Since its establishment, the RCF/OMICSRU has been highly successful and productive in research related to the intended areas, as evident from research publications [n= 86 till date, and 22 papers in 2013, 5 of which were in Q1 journals, *i.e.* among the top 25% in the specialty) in peer-reviewed and indexed journals. Furthermore, to establish and promote research culture at HSC, the staff at RCF/OMICSRU has been actively engaged in conducting teaching/training sessions, workshops and seminars for HSC community (academic staff members, students and technical staff). In addition, RCF/OMICSRU staff has also been helpful in training and teaching of various research methodologies, available at the Unit, to undergraduate and graduate students, through participation in teaching of undergraduate and graduate courses as well as in helping students to conduct their research towards the fulfillment of requirements for undergraduate projects/research ideas, graduate theses (for MSc) and dissertations (for PhD). Moreover, RCF/OMICSRU has been involved in teaching and training of medical graduates perusing postgraduate training (FRCPath and Kuwait Board Residency Programs) and specializing in various disciplines. In addition to being used by faculty members of 18 departments of the four HSC Faculties, RCF/OMICSRU has also been used by researchers from other Faculties of Kuwait University (e.g., Faculty of Science); other institutions in Kuwait (e.g., Kuwait Institute for Scientific Research, Ministry of Health and Dasman, Diabetic Centre, etc.); and institutions out of Kuwait, (e.g. Mansoura University, Egypt; and Arabian Gulf University, Bahrain, etc.).

The year-wise summary, obtained from the RCF annual reports for the last six years (2006/7 to 2012/13, Table 1 on page) shows that the use of RCF has been consistently increasing year by year in all areas of research and teaching mentioned above. In the 18th Poster Day Conference of HSC held in 2013, three of the six awards (two for undergraduate and one for MSc) were given for posters acknowledging RCF/GM01/01/SRUL02/13.

The RCF/OMICSRU staff would like to welcome all researchers (faculty members, staff and students) to utilize the facilities available. To be able to use the RCF facilities, kindly register by logging on to the internet site: http://www.hsc.edu.kw/rcf/LimsAccess.aspx, and follow the instructions.

Thanking you,
With regards,

Prof. Widad Al-Nakib
Principal Investigator of RCF

Prof. Abu Salim Mustafa
RCF Director

Table 1. Year-wise RCF/OMICSRU usage for the last six years

RCF usage with respect to	Year					
	2007/8	2008/9	2009/10	2010/11	2011/12	2012/13
No. of academic staff	26	25	30	37	38	45
No. of projects	13	NA	30	37	39	66
No. of MSc students	4	7	11	10	18	18
No. of PhD students	0	0	0	3	2	5
No. of undergraduates	0	0	0	0	3	3
No. of postgraduates	0	0	0	0	0	8
No. of permanent manpower	4	4	6	6	7	8
No. of major equipment	NA	17	22	26	31	38
No. of seminars/workshops	Nil	Nil	Nil	Nil	1	20
No. of courses taught	Nil	Nil	Nil	Nil	Nil	6
No. of project-staff trained	Nil	Nil	Nil	Nil	Nil	15

NA = not available

The staff of the Research Core Facility (RCF) would like to invite you to use ABI 7900 HT Real-Time PCR System for your research (funded projects of staff and students).

Applications of ABI 7900 HT Fast Real -Time PCR System

1. Absolute Quantitation
2. Relative Quantitation
3. SNP Genotyping
4. Qualitative (Plus/minus) assay

The Applied Biosystem 7900HT Fast Real-Time PCR System accommodates higher density plates without compromising speed, resolution, or robust performance. A laser scans and excites the fluorescent dyes in each of the wells; a spectrograph and charge-coupled device (CCD) camera spectrally resolves and collects the fluorescence emission from each sample. The machine is a complement to the ABI 7500 Fast Real-Time PCR system and hence more slots are now available for performing Real-Time PCR experiments at RCF.

RCF STAFF & THEIR SPECIALIZATIONS

Dr. FATMA SHABAN

Ph.D Immunology

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Specialized in Tissue & Cell Culturing, Recombinant DNA Techniques, Epitope Mapping & Immunological Techniques

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Specialized in Real Time PCR Oligonucleotides Synthesis & Biorobots

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M.Sc. Biotechnology

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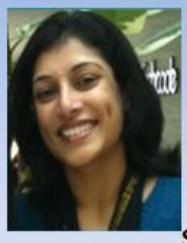
Specialized in Confocal Microscopy, Immunological Assays & Spectrophotometry



Ms. SUNITHA PRAMOD

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Specialized in Flow Cytometry Live Cell Imaging, Laser Microdisection & Tissue Culturing

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Specialized in IVF, Karyotyping Nucleic Acid Extraction, PCR

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B.Tech, M.Sc. Pharmacology &Biotechnology

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riyasengland001@gmail.com
24636058

Specialized in DNA Sequencing & Mutation Detection

MAJOR INSTRUMENTS & TECHNOLOGIES AVAILABLE AT RCF

GENOMICS

-3400 DNA Synthesizer: Primer synthesis

-WAVE 4500 System: DHPLC-high throughput mutation detection system

-ABI 3130 Genetic Analyzer: DNA sequencing & Fragment analysis

-CEQ™8000 Genetic Analysis System: DNA sequencing & Fragment analysis

- Illumina MiSeq: Next Generation Sequencer

-ABI 7500: Real-Time PCR System

-ABI 7900HT:Real-Time PCR System

-Affymetrix GeneChip: DNA Microarray System

-Agilent DNA Microarray: CGH & Gene Expression

-UltraLum Omega 16vS: Gel Documentationsystem

- UVP - Biospectrum®AC: Imaging System

- BioradExperion™: Automated Electrophoresis

- Agilent 2100 Bioanalyzer: Electrophoresis on a chip

- Thermal Cyclers: PCR

- Biorobot M48 & Universal: High throughput nucleic acid extraction& sample preparation

FLOW CYTOMETRY& TISSUE CULTURE:

- Cytomics FC 500: Flowcytometery

- Vi-Cell Series Cell Viability Analyzers

- GC 1000-Gamma Cell 1000 Elite: Irradiation of cells

- Tissue Culture Facility: 4 Laminar Flow Hoods + 6 CO_2 incubators

- Thermo CryoPlus 3: Storage of cells

PROTEOMICS

-ABI 4800 MALDI TOF/TOF Analyzer: Mass Spectrometry

-ProteomeLab™ PF 2D: Protein Fractionation System

- ProteomeLab™ PF 800: Protein Characterization System

- Fluoroskan: Fluorescence Reader

- Multiskan: Spectrophotometer

- Appliskan: Luminescence, fluorescence and absorbance reader

- Western Blotting

- BioTek Epoch: Low-volume (2 µl) spectrophotometer for proteins, RNA/DNA

- ELISA Washer and Reader

- Dark Room

CELL BIOLOGY& MICROSCOPY

- LSM 510 Meta: Confocal Microscopy

-Culture Cell Imaging System

- Invitro Fertilization System

- Cell Observer: Complete System for Live Cell Imaging

- PALM Microbeam: Laser micro-dissection

- Axio Imager: Fluorescence Microscopy

- Optima L-100:Ultracentrifuge

-Automated Karyotyping System: Multicolor FISH

- Axiovert: Phase Contrast Microscopy

RCF UTILIZATION

From April 1, 2012 to March 31, 2013:

Number of projects– 66

Number of samples – 15,938

Number of researchers – 45

Number of MSc students – 18

Number of PhD students – 5

Number of undergraduates– 3

Number of requests -

Faculty	Number of requests
Allied Health, HSC	189
Dentistry, HSC	85
Genetic Center, MOH	3
Medicine, HSC	769
Pharmacy, HSC	194
Science, KU	22
Total	**1,262**

"We are ready to welcome you at the RCF"

To reserve RCF instruments, read the details in the next column

For any enquiries or complains or for arranging a visit to the RCF, please contact the RCF Director by e-mail at:

abusalim@hsc.edu.kwor by dialing the Phone nos. Extension: **36426 /36505 or mobile no. 66529609**

RCF uses the Laboratory Information Management System (LIMS) for its operation.

Kindly follow these guidelines to ensure the smooth functioning of the RCF and to be able to serve you better.

1. **The person interested should visit the RCF website** http://www.hsc.edu.kw/rcf/ and click at Access RCF for Equipment Use to book an instrument.

2. **Generate the LIMS request form by filling in the required details. It is mandatory to fill-in the PI's name as well as the student's name (in case of student usage) in the LIMS form.**

3. **The LIMS form should be duly signed and sealed by the PI.**

4. **The LIMS form should be brought to the RCF along with the sample(s). In the absence of LIMS form the sample(s) will not be accepted.**

5. **After the processing is completed, the user will receive a confirmation e-mail, so that he/she can come and collect the results.**

6. **The results are provided in CDs, so be sure to bring a CD with you for copying your results (External Hard Disks are not allowed.**

7. **The results in RCF database are stored for a maximum period of one month, where applicable. Please collect your results within one month, otherwise they will be removed from the data base.**

For more information on how to fill the LIMS forms, contact any of the RCF Staff

PUBLICATIONS

Since its establishment, RCF has helped to publish 86 papers in scientific journals. A year-wise summary of the number of papers published is given below.

Year	Number of papers
2006	5
2007	6
2008	5
2009	5
2010	10
2011	22
2012	11
2013	22
TOTAL	**86**

PUBLICATIONS IN 2013:

1. Al- Sabah S, Alasfar F, Al-Khaledi G, Dinesh R, Al- Sabah M, Abul H. Incretin response to a standard test meal in a rat model of sleeve gastrectomy with diet-induced obesity. Obes Surg 2013, DOI 10.1007/s11695-013-1056-2.
2. Mustafa AS. Diagnostic and vaccine potentials of ESAT-6 family proteins encoded by *M .tuberculosis* genomic regions absent in *M. bovis* BCG. J Mycobac Dis 2013; 3:2.
3. El Salhy M, Honkala S, Soderling E, Varghese A, Honkala E, Relationship between daily habits, *Streptococcus mutans*, and caries among schoolboys. J Dentistry 2013; 4:1.
4. Ezzeddine R, Al-Banaw A, Tovmasyan A, Craik JD, Batinic-Haberle I, Benov LT, Effect of molecular characteristics on cellular uptake, subcellular localization, and phototoxicity of Zn(II)N – alkypyridylporphyrins. J Biol Chem 2013; 288:51.
5. Tovmasyan A, Weitner T, Sheng H, Lu M M, Rajic Z, Warner DS, Spasojevic I, Reboucas JS, Benov L, Batinic-Haberle I. Differetial coordination demands in Fe versus Mn water-soluble cationic metalloporphyrins translate into remarkably different aqueous redox chemistry and biology. Inorganic Chemistry 2013; 52:5677-5691.
6. Tovmasyan A, Reboucas JS, Benov L. Simple biological systems for assessing the activity of superoxide dismutase mimics. Forum Review Article.2013; 5576.
7. Shaban K, Amoudy HA, Mustafa AS. Cellular immune responses to recombinant *Mycobacterium bovis* BCG constructs expressing major antigens of region of difference 1 of *Mycobacterium tuberculosis*. Clin Vaccine Immunol 2013; 20:8.

8. Mustafa AS. *In silico* analysis and experimental validation of *Mycobacterium tuberculosis* specific proteins and peptides of *Mycobacterium tuberculosis* for immunological diagnosis and vaccine development. Med Prin Pract.2013; 22:43.
9. Mustafa AS. Diagnostic and vaccine potentials of ESAT-6 family proteins encoded by *M. tuberculosis* genomic regions absent in *M. bovis* BCG. J Mycobac Dis 2013; 3:2.
10. Hanif SNM, Mustafa AS. TB DNA vaccines: review and advances. vaccines and vaccine technologies. Omics Group eBooks.
11. Ahmad S, Dalwai A, Al-Nakib W. Frequency of enterovirus detection in blood samples of neonates admitted to hospital with sepsis-like illness in Kuwait. Med Virol 2013; 85 : 1280-5.
12. Khajah MA, Almohri I, Mathew PM, Luqmani YA. Extracellular alkaline pH leads to increased metastatic potential of estrogen receptor silenced endocrine resistant breast cancer cells. PLoS One 2013; 8 :e76327.
13. El-Hashim AZ, Jaffal SM, Al-Rashidi FT, Luqmani YA, Akhtar S. Nerve growth factor enhances cough via a central mechanism of action. Pharmacol Res 2013; 74:68-77.
14. Bitar MS, Abdel-Halim SM, Al-Mulla F. Caveolin-1/PTRF upregulation constitutes a mechanism for mediating p53-induced cellular senescence: implications for evidence-based therapy of delayed wound healing in diabetes. Am J Physiol Endocrinol Metab 2013; 305 :E951-63.
15. Parvathy SS, Masocha W. Matrix metalloproteinase inhibitor COL-3 prevents the development of paclitaxel-induced hyperalgesia in mice. Med Prin Pract 2013; 22:35-41.
16. Al-Awadhi R, Chehada W, Al-Jassar W, AL-Harmi J, Al-Saleh E, Kapila K. Viral load of human pappiloma virus in women with normal and abnormal cervical cytology in Kuwait J Infect DevCtries 2013; 7: 130-136.
17. Al-Saeedi FJ, Mathew PM, Luqmani YA. Assessment of tracer 99mTc(V)-DMSA uptake as a measure of tumor cell proliferation *in vitro*. PLoS ONE 2013; e54361.
18. Albert MJ, Mustafa AS, Islam A, Haridas S. Oral immunization with Cholera toxin provides protection against *Campylobacter jejuni* in an adult mouse intestinal colonization model. mBio 2013; 4:e00246-13.
19. Mouihate A, Al-Bader MD. Glucocorticoid-induced fetal brain growth restriction is associated with p73 gene activation. J Neurosci Res 2013; 91:95-104.
20. Edan RA, Luqmani YA, Masocha W. COL-3. A chemically modified tetracycline, inhibits lipopolysaccharide-induced microglia activation and cytokine expression in the brain. PLoS One 2013; 8:e57827
21. El Farran CA, Sekar A, Balakrishnan A, Shanmugam S, Arumugam P, Gopalswamy J. Prevalence of biofilm-producing *Staphylococcus epidermidis* in the healthy skin of individuals in Tamil Nadu, India. Ind J Med Microbiol 2013; 31:19-23.
22. Al-Awadhi R, Chehadeh W, Al-Jassar W, Al-Harmi J, Al-Saleh E, Kapila K. Phylogenetic analysis of partial L1 gene of 10 human papillomavirus types isolated most commonly from women with normal and abnormal cervical cytology in Kuwait. Arch Virol 2013; 158:1687-99.

WORKSHOPS CONDUCTED BY THE RCF IN 2012-2013

RCF Workshop 1 – March 14, 2012

Topic: Latest Advances in Microarray Applications and DNA Target Enrichment Technology.

Speaker: Dr. YannFilaudeau , Agilent Technologies Life Science and Applied Genomics Division.

Workshop Topics Included:

1. An Overview of Agilent Microarray Platform and Update on recent Clinical and Research Applications: CGH+SNP, Expression & Exon, Methylation
2. Focused on the next generation sequencing systems on DNA that matters

RCF Workshop 2 – April 3, 2012

Topic: Advanced Research Applications for Multimode Microplate Readers

Speaker: Dr. Steven Fisher, BioTek Instrument, USA

Workshop Topics Included:

1. An Introduction to Microplate instruments
2. Recent advances in the Multimode microplate instrumentation
3. The various applications for Multimode microplate readers

RCF Workshop 3 – May 13, 2012

Topic: Affymetrix Next Generation Clinical and Research Microarray Solutions

Speakers:

Dr. Maher Derbal - Marketing Manager, Affymetrix Middle East,Asia and Africa. Dr. David Webber – Vice President, Affymetrix, USA

Workshop Topics Included:

1. An Overview of Affymetrix Microarray Platform
2. An Update on the recent Clinical and Research Applications

RCF Workshop 4 – May 16&17, 2012

Topic: Real-Time PCR in Health Sciences: ABI System 7500.

Speaker: Dr. Zyju D Pillai ,Manager-Application Support, Technical Services Co. WLL, Al Essa Life Science, Kuwait

Workshop Topics Included:

1. Introduction to Real-Time PCR-Technology and its applications
2. Demonstration and Data Analysis
3. Salmonella Detection
4. Genotype analysis

RCF Workshop 5 – May 31, 2012

Topic: High Stability PCR and Real –Time PCR Assays using reagents from Solis BioDyne

Speaker: Dr. Ajmal Khan, United Laboratories, Kuwait.

Workshop Topics Included:

1. Introduction to room temperature stable PCR and Real-Time PCR reagents from Solis BioDyne
2. Demonstration of Real-Time PCR using Solis BioDyne reagents& interpretation of results

"RCF is ready for DNA sequencing requests"

To book the equipment, please visit the RCF website http://www.hsc.edu.kw/rcf/LimsAccess.aspx

For enquiries please contact the Director at:abusalim@HSC.EDU.KWorby dialing at**36426 /36505**

RCF Workshop 6 – September 4, 2012

Topic: Grant Writing for Kuwait University

Speaker: Prof. Abu Salim Mustafa, RCF Director, Research Core Facility

Workshop Topics Included:

1. Grant Support System at Kuwait University which includes Administration, Funding Sources and Types of Grants
2. Writing a Grant Proposal for Kuwait University
3. The procedures for Grant Submission, Review and Approval

RCF Workshop 7 – September 5, 2012

Topic: Grant Implementation for Kuwait University

Speaker: Prof. Abu Salim Mustafa, RCF Director, Research Core Facility

Workshop Topics Included:

1. An Introduction to RCF in Grant Implementation
2. All Procedures for Grant Implementation, Including Budget Expenditure for Manpower, Running Cost, Equipment, Scientific Mission, and Visiting experts
3. Project Reports and Productivity
4. Research Incentives and Awards

RCF Workshop 8 – September 23, 2012

Topic: My Sample; My Study; Introducing Illumina's desktop sequencing system

Speaker :Dr. Laura Ingram, Application Scientist, Appliance Global, Middle Eastern Region

Workshop Topics Included:

1. An Introduction to MiSeq, the latest next generation sequencing platform by Illumina
2. A brief Overview of the Sequencing workflow and various sample preparation techniques
3. Discussed the range of applications enabled on the MiSeq

RCF Workshop 9 – October 1, 2012

Topic: Pharmacological Screening of Malaysian Plants for Anti-inflammatory Activity: Actions and Cellular Mechanisms of *17-O-Acetylacuminolide*

Speaker: Dr. Mouna Achoui, Department of Pharmacology, University of Malaya, Kuala Lumpur, Malaysia

Seminar Overview :

Due to the resistance of diseases, caused by persistent inflammation, to conventional treatments there is a pressing need for the development of novel anti-inflammatory drugs. In the study 17-O-acetylacuminolide (AA) from *N. foetida* was isolated and identified as having positive anti-inflammatory activity. AA was further examined for its anti-inflammatory activity in different cell lines as well as in vivo. Moreover, the effects of this compound on a number of important regulatory kinases and transcription factors were investigated in order to elucidate its mechanism of action. The outcomes of this study support the anti-inflammatory effects of 17-O-acetylacuminolide and its potential as a drug lead for an anti-inflammatory drug development.

RCF Workshop 10 – November 26, 2012

Topic: Assessment of water samples in Kuwait for chemical & Microbial pollutants.

Speaker:Dr. Mouna Achoui

Workshop Topics Included:

1. Introduction to types of water pollutants.
2. Overview of water sample collection procedures.
3. Overview of methods for the identification of microbial & chemical pollutants.

RCF Workshop 11 –February 20, 2013

Topic: Artificial Nanoscale Adaptive Immune Response against HIV-1:Bio-Nano Design & Architecture of Biomimetic Immunological Systems

Speaker: Mr. YousefAleneze, Loyola University, Chicago, USA

Workshop Topics Included:

Mr. Aleneze's research is centered around destroying viruses and HIV-infected cells using chemically engineered compounds. The seminar included the ambitions he wants to develop a cost effective, affordable, non-toxic and functional HIV cure from computational therapeutic small molecule design to the translational sciences where the final product could be considered very potent against HIV. He believes that his research concept bridges the latest findings of bio-nanotechnology and weak vulnerable regions in the HIV structure that are prone to viral destruction. In other words ,the research concept has potential to discover new frontiers in manipulating low cost nanotechnology to destroy the virus completely.

RCF Workshop 12 – March 5, 6 & 7, 2013

Topic: Studying Expression Profiles of Complex Diseases using Affymetrix

Speaker: Mr. Muad Abu Tayeh, Affymetrix Application Specialist, Gulf Scientific Corporation, Riyadh, Saudi Arabia

Workshop Topics Included:

The Workshop program included a seminar followed by demonstration in the lab. The seminar had a total of 12 attendees. Mr. Muad gave a talk about the Affymetrix and the various applications mainly concentrating on studying the expression profiles of complex diseases. The attendees included faculty members, graduate students and research staff. The hand on training was restricted to 5 people from various departments of the Faculty of Medicine and Allied Health Sciences.

RCF Workshop 13 – March 18-20 , 2013

Topic: Molecular Data Analysis & Computational Biology

Speakers:

Mr. Chadi EL Farran, Research Assistant, Research Core Facility

Dr. Nazima Habibi, Research Associate, Research Core Facility

Mr. Mohammed Asadullah, Dept. of Microbiology, Faculty of Medicine

Workshop Topics Included:

The workshop was conducted as two parallel sessions on three days. The attendees were given background information and hands-on training with the commonly used bioinformatics' tools to analyze nucleic acid and protein sequences. The workshop included various topics such as introduction to the main online databases and how to retrieve the data, BLAST, Primer designing, Restriction mapping, ORF FINDER, Motif finding tools and about the Protein data bank.

RCF Workshop 14 – March 21 , 2013

Topic: Digital TMA(Tissue Microarray) and Slide Scanning workflow using virtual microscopy

Speaker:Mr.HankoCsaba,Hungary

Workshop Topics Included:

1. Introduction, application and performance of TMA
2. Introduction, application and performance of slide scanning workflow using virtual microscopy

SEMINARS CONDUCTED BY RCF STAFF IN 2012

RCF SEMINAR 1 - September 9, 2012

Topic: ABI 3130 Genetic Analyzer

Speaker: Mr. Chadi EL Farran

Overview:

- Parts of the instrument
- Reagents and consumables required to maintain the instrument
- Applications: DNA sequencing and fragment analysis with emphasis on the principles

RCF SEMINAR 2 - September 16, 2012

Topic: Carl Zeiss - Meta System

Speaker: Ms. Manar El-Borsaly

Overview:

- Parts of the microscope
- Software used for data acquisition and interpretation
- Applications: FISH, Karyotyping, MFISH, CGH (Comparative Genomic Hybridization)

RCF SEMINAR 3 - September 30, 2012

Topic: PF2D Beckman Coulter for 2D analysis of Proteins

Speaker: Mrs. Betty Teena Thomas

Overview:

- Introduction to1D-SDS PAGE and Western blotting and their applications
- The principle of protein fractionation using PF2D
- Software used for interpretation of results
- Applications & Advantages of PF2D

RCF SEMINAR 4 - October 7, 2012

Topic: Confocal Laser Scanning Microscope

Speaker: Mrs. Jucy Gabriel

Overview:

- Parts of the microscope
- Principle and parts of conventional fluorescence microscope
- Principle of Confocal Microscopy
- Advantages of Confocal Microscopy.
- The applications of Confocal Microscopy
- Good sample preparation practices

RCF SEMINAR 5 - October 14, 2012

Topic: Flowcytometer - Cytomics FC 500

Speaker: Mrs. Sunitha Pramod

Overview:

- Principle of flow Cytometry
- Specifications of the instrument
- Types of data output from flowcytometry
- Principle of gating
- Applications of flowcytometry with emphasis on the principles

RCF SEMINAR 6 - October 21, 2012

Topic: 3400 DNA Synthesizer

Speaker: Mrs. Faiza Rasheed

Overview:

- Specification of 3400 DNA synthesizer specifications
- The principle of chemical synthesis of oligonucleotides
- The various types of oligonucleotides that can be synthesized &applications of each

WHAT'S NEW

BIOINFORMATICS SERVICES AT RCF:

The RCF facilitates the services for Primer Designing, Protein Structure Prediction, and DNA Sequence Assembly through available online softwares.

Recently, the RCF has acquired the GeneSpring GX software for gene expression analysis, and Real-Time StatMiner software for the analysis of data generated from Real-Time PCR experiments.

In the near future, RCF will have the Protein Pilot Software for proteomics and the Microbial identification software for Metagenomics analysis.

The RCF will also acquire a Bioinformatics Workstation for the downstream analysis of DNA sequence data generated by using Next Generation Sequencers.

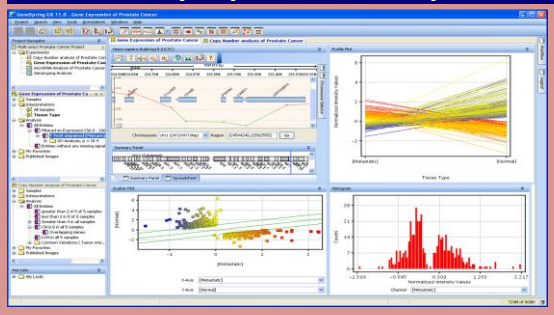
Contact the RCF director/staff, in case, you need any advice related to the usage of bioinformatics tools.

The Epoch Biotek low-volume (2 µl) spectrophotometer is the latest addition to the family of RCF machines.

It has a range of 200 nm to 999 nm wavelength and a reading capacity of 6- to 384-well microplates, controlled with the Gen5 Data Analysis software interface.

It has an automated path length correction for direct quantification which makes the quantification of DNA, RNA and protein samples simple and cost effective.

You can analyze your Microarray data at RCF:



Agilent GeneSpring v 12.5 is now available at RCF for data analysis from a variety of microarray platforms such as Affymetrix, Illumina and Agilent.

All are welcome to use the software.

Agilent's GeneSpring GX software provides powerful, accessible statistical tools for fast visualization and analysis of microarrays - expression arrays, miRNA, exon arrays and genomics copy number data, Pathway Analysis and Visualizations.

NEXT GENERATION SEQUENCING: AN INTRODUCTION (By Dr. Mouna Achoui)

DNA sequencing determines the order of the nucleotide bases adenine, guanine, cytosine and thymine in a DNA strand. It is a multistage process that utilizes various technologies to read the nucleic acid sequence. DNA sequencing is essential for various aspects of biological research such as molecular cloning, personalized medicine and forensic biology. DNA sequencing technologies have come a long way since the first full genome of the bacteriophage φX174 was sequenced in 1977. The technologies have become faster, cheaper and much easier to use. Based on their novelty, sequencing methods could be categorized into three groups:

1. Basic methods such as the Sanger sequencing (chain-termination method)
2. Next Generation Sequencing (NGS) (Amplified Single Molecule Sequencing)
3. Third Generation Sequencing (Single Molecule Sequencing)

As the title implies, NGS is of particular interest here. NGS or high-throughput nucleotide sequencing techniques increase the range, complexity, sensitivity, and accuracy of results by greatly increasing the scale of operations, and thus the number of nucleotides, and the number of copies of each nucleotide sequenced. The sequencing may be done by analysis of the synthesis products, the ligation products, or hybridization to preexisting sequences. Applications of NG Sequencers include de novo sequencing, mate-pair, whole genome or target-region resequencing, small RNA, transcriptome, RNA seq, epigenomics, and metagenomics. There are various platforms for NGS, these include 454 Sequencing from Roche; Genome analyzer (GA), HiSeq and MiSeq from Illumina; as well as SOLiD systems and Ion torrent from Life Technologies. Prior to sequencing, a sample's library needs to be prepared and amplified by either Emulsion PCR [for Semiconductor sequencing (Ion Torrent), Pyrosequencing (454), and Sequencing by ligation (SOLiD)] or Bridge PCR for Reversible terminator sequencing (Illumina). A brief description of the various sequencing technologies are outlined below.

Roche 454: This sequencer was the first commercially launched next generation system. It uses pyrosequencing technology which relies on the detection of pyrophosphate released during nucleotide incorporation. On a picotiter plate, one of the dNTPs (dATP, dGTP, dCTP, dTTP) will complement the bases of the template strand with the help of ATP sulfurylase, luciferase, luciferin, DNA polymerase, and adenosine 5′ phosphosulfate (APS). For matched bases, pyrophosphate (PPi) will be released and the ATP transformed from PPi drives the luciferin into oxyluciferin and generates visible light. At the same time, the unmatched bases are degraded by apyrase. Addition of dNTPs is performed sequentially and as the process continues, the complementary DNA strand is built up and the nucleotide sequence is determined from the signal peaks in the Pyrogram trace.

- **SOLiD:** The sequencer adopts the technology of two-base sequencing based on ligation sequencing. On a flowcell, the libraries can be sequenced by 8 base-probe ligation which contains ligation site (the first base), cleavage site (the fifth base), and 4 different fluorescent dyes (linked to the last base). The fluorescent signal will be recorded if the probes complementary to the template strand bind the target, and vanished by the cleavage of the probes' last 3 bases. And the sequence of the fragment can be deduced after 5 rounds of sequencing using ladder primer sets.

- **Illumina:** The sequencer adopts the technology of sequencing by synthesis (SBS). Four kinds of nucleotides (ddATP, ddGTP, ddCTP, ddTTP) which contain different cleavable fluorescent dyes and a removable blocking group would complement the template one base at a time, and the signal could be captured by a (charge-coupled device) CCD. Platforms which employ the sequencing by synthesis chemistry include the HiSeq, MiSeq and the Genetic Analyzer (GAIIx). Upgrades have been made to the mechanics that allow the HiSeq 2000 to generate twice as much data in less time than the GAIIx, and the MiSeq is designed for the fastest turnaround. Below is a table comparing between the different Illumina platforms. The different specifications allow researchers to choose the instrument which best meets their needs.

HiSeq 2500	**HiSeq 2000**	**MiSeq**
• 150 million reads per lane • 2x150 read lengths • 40 hour run time • 2 lanes per flowcell • 2 flowcells simultaneously	• 180 million reads per lane • 2x100 read lengths • 14 day run time • 8 lanes per flowcell • 2 flowcells simultaneously	• 7.5 million reads per lane • 2x250 read lengths • 27 hour run time • 1 lane per flowcell • 1 flowcell

Of particular interest here is the MiSeq platform, it integrates the functions of cluster generation, SBS, and data analysis in a single instrument and can go from sample to answer (analyzed data) within a single day (as few as 8 hours). The Nextera, TruSeq, and Illumina's reversible terminator-based sequencing by synthesis chemistry are used by MiSeq. **The MiSeq platform** is currently available at the **Research Core Facility** and can be utilized for efficient sequence assembly, de novo sequencing, large-scale structural variation detection, and more. The combination of short inserts and longer reads supported by MiSeq increase the ability to fully characterize any genome. Moreover, a wide array of available sample preparation methods serve to enable diverse applications, including whole-genome and candidate region re-sequencing, transcriptome analysis, small RNA discovery, methylation profiling, and genome-wide protein-nucleic acid interaction analysis.

- **Ion Personal Genome Machine (PGM) (Ion Torrent):** This sequencer uses semiconductor sequencing technology. When a nucleotide is incorporated into the DNA molecule by the polymerase, a proton is released. By detecting the change in pH, PGM recognizes whether the nucleotide is added or not. The chip is flooded with one nucleotide after another which will result in no voltage if it is not the correct nucleotide or a double voltage if two nucleotides are added simultaneously. PGM is the first commercial sequencing machine that does not require fluorescence and camera scanning, resulting in higher speed, lower cost, and a smaller instrument size.

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