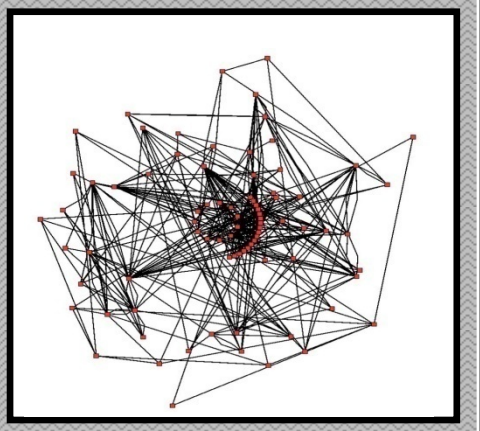

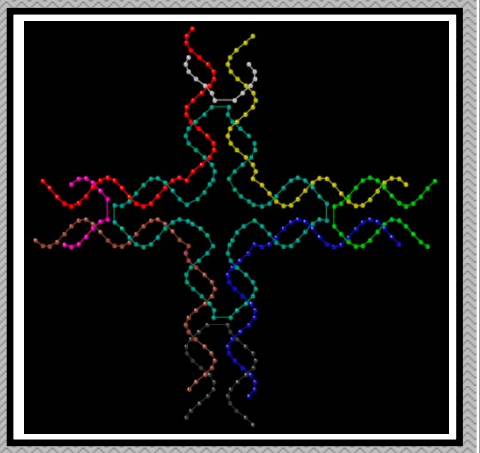

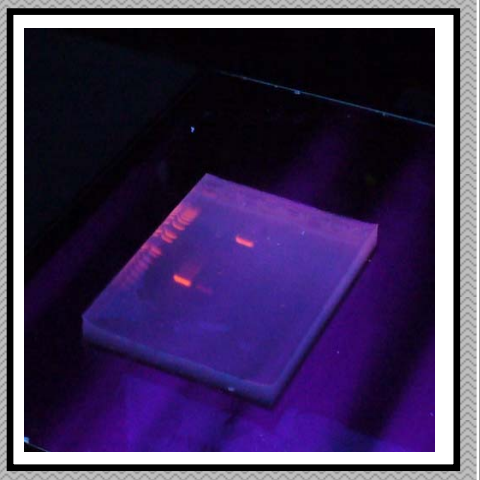

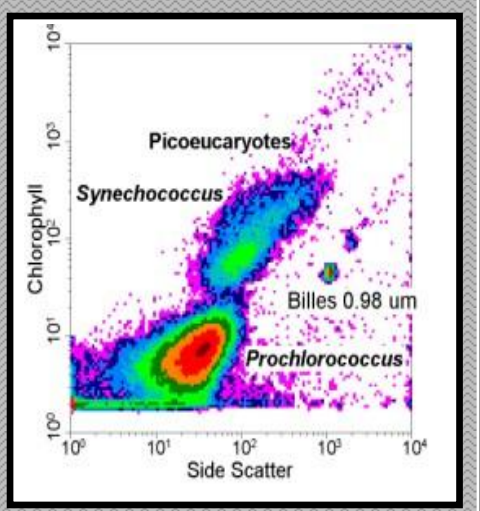

EMHGBN Newsletter

Vol. 2, Issue 7, July 8th, 2008

INSIDE THIS ISSUE:

Eastern Mediterranean Health Genomics and Biotechnology Network (EMHGBN) was created in 2004 with collaboration of representatives of selected centre of excellence in (health related) molecular biology, biotechnology & genomics in the Eastern Mediterranean region by recommendations and efforts of WHO/EMRO.

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Multiple Pulses Improve Electroporation Efficiency in *A. tumefaciens*

An article entitled "Multiple pulses improve electroporation efficiency in A. tumefaciens" aims to determine a method to improve electroporation efficiency in Agrobacterium. The study was carried out by Tariq Mahmood, Tamkina Zar, and S.M. Saqlan Naqvi. Corresponding author of this paper, Pro S.M. Saqlan Naqvi, is working in Department of Biochemistry University of Arid Agriculture Rawalpindi, Pakistan and the paper was published in Electronic Journal of Biotechnology 15 January 2008, Vol. 11, No. 1, ISSN 0717-3458.

There are many approaches available for introducing foreign DNA into a variety of organisms. The most common method for the DNA transformation in microorganism especially the bacterial cells is the electroporation. It is a technique, which employ high intensity pulse to facilitate the entry of exogenous molecules like DNA, RNA, and protein. Applying high intensity electric fields can reversibly permeablize biomembranes to create structural changes, allowing the uptake of foreign material. Researchers are working to improve different parameters for obtaining maximum transformation efficiency; these manipulations include electric field strength, pulse length, DNA quality, cell density etc for different cell types. *Agrobacterium* is one of the reliable and inexpensive methods for plant transformation, but limited work has been reported for improving electroporation efficiency of *A. tumefaciens*.

The transformation efficiency in *Agrobacterium* is very lower than that of *E. coli,* which indicates the need for further studies. Keeping in view the importance and efficiency of this simple plant transformation system, Dr. S.M. Saqlan Naqvi and his co-workers in the Department of Biochemistry, University of Arid Agriculture Rawalpindi, Pakistan, have reported a method to improve electroporation efficiency in *Agrobacterium* in Electronic Journal of Biotechnology.

Two commonly used strains of *Agrobacterium tumefaciens* (LBA4404 and EHA101) were used in this study. Earlier increase in pulse voltage and length beyond a critical limit has been employed, however, in this study another strategy was employed i.e. increase in the number of pulses at constant high voltage and pulse duration. Electro-competent cells of these strains were transformed by pCAMBIA 1301 (11.8 kb) in an electroporator. Transformation was confirmed by PCR and restriction digestion of the isolated plasmid. Cell viability was checked after each electric shock, because irreversible permiabilization of cell membranes can result in cessation of the cytoplasmic

streaming leading to cell death. A major decline in cell survival was observed after first high voltage pulse, similar effect has already been reported rent by many investigators in other bacterial species. Further but slight decrease was observed after second pulse and remained almost stable in response to further shocks in both strains. The highest transformation efficiency observed in LBA4404 after five electric pulses was taken as reference while, transformation efficiency for EHA101 was calculated accordingly. With the increase in the number of electric pulses, transformation efficiency increased gradually in both the strains was taken as reference while, transformation efficiency for EHA101 was calculated accordingly. With the increase in the number of electric pulses, transformation efficiency increased gradually in both the strains.

When compared with single pulse, efficiency increased to about 2.5 times in LBA4404 and five times in EHA101 after five electric pulses. In both these strains the increase was nearly linear. Electric field strength and pulse length of are important factors, which can affect electroporation. Transformation in *Agrobacterium* has already been investigated using variable voltage and pulse duration, but in the present study another aspect i.e. increase in number of pulses at constant high voltage has been analyzed, which results in considerable increase in DNA uptake.

Repair of Infarcted Myocardium by Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Old Myocardial Infarction

An article entitled "Repair of Infarcted Myocardium by Autologous Bone Marrow Mesenchymal Stem Cell Transplantation in Patients with Old Myocardial Infarction" aims to determine the feasibility and effectiveness of transplantation of autologous bone marrow mesenchymal stem cells (BM-MSCs). The study was done by Amir Farhang Zand Parsa, Mandana Mohyeddin Bonab, and Kamran Alimoghaddam. Corresponding author of this paper, Dr. Amir Farhang Zand Parsa, is working in Department of Cardiology, Imam Khomeini Medical Center, Keshavarz Blvd. P.O. Box 14197-33141, Tehran, I.R. Iran and the paper was published in Iranian Journal of Biotechnology, Vol. 5, No. 2, April 2007

The aim of our study was to assess the feasibility of (BM-MSCs) for repairing infarcted region in patients with old myocardial infarction (MI) by intracoronary application through over-the-wire (OTW) balloon during percutaneous transluminal coronary angioplasty (PTCA).

Patients eligibility for inclusion were: 1-history of old MI, 2-age <70 years, 3- presence of severe regional wall motion abnormality and absence of viable myocardium at the infarcted region ,4-absence of severe co-morbidity. From May 2004 to March 2005 implantation of autologous BM-MSCs was performed in five patients with old MI (four male and one female, mean ± SD, age = 48.4±11.28). Time from acute MI to cell therapy was 5.2 ± 3.11 months.

Bone marrow (BM) was obtained from Ilium of each patient under local anaesthesia in a sterile fashion. The BM mononuclear cells were separated by Ficoll method and cultivated in Dulbecco's Modified Eagle's Medium (DMEM) with 10% Fetal Bovine Serum (FBS) and 1% penicillin and streptomycin in eight average vented flasks. Then flasks were incubated at 37˚C. At the end of isolation and cultivation procedure a suspension of pure BM-MSCs with 2-4 x 10^6 cells/ml was prepared for transplantation.

The procedure of intracoronary injection of BM-MSC was begun with coronary and left ventricular (LV) angiography to re-evaluate the situation .Then diagnostic catheter was replaced with a guiding catheter. After crossing the infarct related artery occlusion with a long 0.014 inch guide wire and positioning an OTW balloon at the occlusion site the procedure of BM-MSC injection into the infarcted area began with sequential inflation and deflation of OTW balloon. The mean number of $8x10^6$ (ranging 5 to 12 x 10^6) BM-MSCs were injected into the infarcted area. Our procedural success rate was 100%. Selective coronary and LV angiography were performed 6-9 months (mean ± SD of 7 ± 1.4 months) after cell therapy in order to evaluate coronary patency and LV ejection fraction (LVEF).

Clinical and angiographic follow up in our patients revealed significant improvement in their symptoms (dyspnea improved from New York Heart Association class III-IV to I-II and chest discomfort from Canadian Cardiovascular Society class II-IV to I-II) , and appreciable improvement in their LV function (their LVEF increased from mean ± SD of 34% ± 10.83% to 46.25% ± 9.46%, p = 0.051). From this pilot study, it can be concluded that intracoronary transplantation of autologous BM-MSC via catheter based percutaneous transluminal approach (PTCA) in patients with old MI is safe, feasible and effective. The beneficial effect could be attributed to BM-MSCs ability to regenerate myocardium.

Flow Cytometry

What is Flow cytometry?

Flow cytometry is a process for counting, examining cellular properties, and sorting the cells which are suspended in a stream of fluid. By flow cytometry, we can measure physical and multi-colour fluorescence properties of cells flowing in the stream. This process allows quantitative cell analysis at single cell level, at rates of thousands of cells per second. It allows measurement on living cells and even sorting or separation of a sub-population of cells. Flow cytometry uses the principles of light scattering, light excitation and emission of fluorochrome molecules to generate specific multi-parameter data from particles and cells in the size range of 0.5 μm to 40 μm diameter.

A Short History of Flow Cytometry

- Leeuwenhoek (1684) is often cited due to flow cytometry based on microscopy.
- F.T. Gucker (1947) builds the first device for detecting bacteria in a laminar sheath stream of air.
- L. Kamentsky (1965, IBM Labs), and M. Fulwyler (Los Alamos Nat. Lab.) conducted experiments with fluidic switching and electrostatic cell sorters respectively. Both described cell sorters in 1965.
- M. Fulwyler (1965) developed Pulse Height Analyzers to accumulate distributions from a Coulter Counter. This Feature allowed him to apply statistical analysis to samples analyzed by flow.
- L. Herzenberg (1972, Stanford Univ.) built a cell sorter that separated cells stained with fluorescent antibodies. The Herzenberg group coined the term Fluorescence Activated Cell Sorter (FACS)

Flow Cytometers

A flow cytometer has 4 basic components:

1. Fluidics: The purpose of the fluidic system is to transport particles in a fluid stream to the laser beam for interrogation.

The sample is injected into a stream of sheath Fluid. We have two kinds of flow chambers: the flow chamber in a bench-top cytometer and a fluid chamber in a stream-in-air cytometer. The first one is called “a flow cell” and the second one is “a nozzle tip”.

Based on principles of laminar flow, the sample flows in a central core does not mix with the sheath fluid, but it is coaxial with it. The flow of sheath fluid accelerates the particles and focuses them in the center of the core.

To increase both sample pressure and the flow rate, we can increase the width of the sample core. This allows more cells enter to the stream in given moments. Wider sample core causes some cells intercept the laser beam at a less optimal angel. Sometimes, this might be suitable for a kind of research application.

- Higher flow rate is used for qualitative measurements such as immunophenotyping. Since the cells are less in line in the wider core stream, the data are less resolved. But we can acquire the data more quickly.

- Lower flow rate is used for applications in which greater resolution is pretty important such as DNA analysis. By decreasing the width of the sample core, the majority of the cells pass through the center of the laser beam.

1. Optical system: The optical system consists of excitation optics and collection optics. By the terms of excitation optics we mean lasers and lenses. The collection optics consists of a collection lens to collect light from the particle-laser beam interaction and a system of optical mirrors and filters to direct the specified wavelengths of the collected light to designated optical detectors.

Most flow cytometers have more than one light source:

- Laser: High power water-cooled lasers like Argon, Krypton, Dye laser; low power air-cooled lasers like Argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV); diode lasers like blue, green, red or violet.

- Arc-lamps: mercury and xenon lamps.

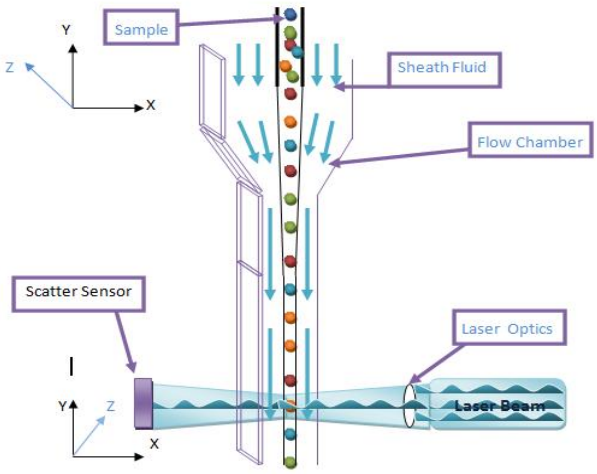


Figure 1- A Schematic picture of a typical flow cytometer

2. Data acquisition: Each measurement from each detector is referred to as a "parameter". Data are acquired as a "list" of the values for each "parameter" (variable) for each "event" (cell). Acquired data is plotted by the softwares and displayed as histograms or dot plots. An electronic window called a gate allows the user to identify 'events' of interest

3. Fluorochromes: According to different applications and different targets fluorchromes can change. The following picture shows the different targets of fluorochromes in a cell.

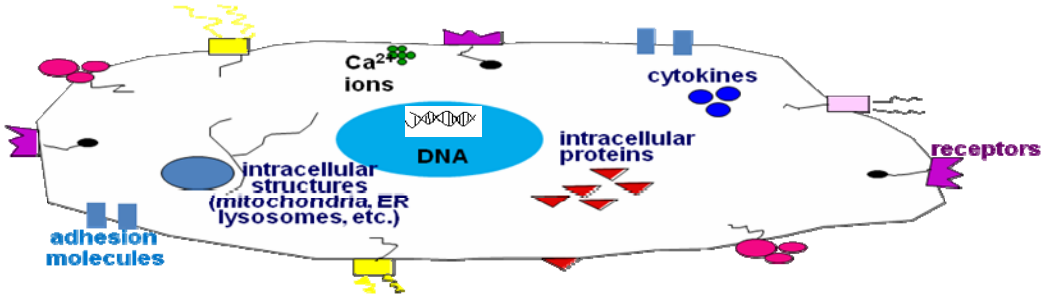


Figure 2- Targets for fluorochromes

The following table is a selection of most frequently used fluorochromes with the properties.

Fluorochromes	Excitation	Emission	Application
Indo-I(Unbound)	335	490	Calcium Flux
Indo-I(Bound to Ca)	335	405	Calcium Flux
Hoechest	350	470	DNA analysis
Alexa350	350	442	Phenotyping
Green Fluorsent Protein(GFP)	488	510	Report Molecule
YO-PRO-I	488	510	Apoptosis Analysis
Fluorescein diacetate	488	530	Cell viability
SNARF-I	488	530-640	pH measurement
dsRED	488	588	Reporter molecule
Rhodamine 123	515	525	Membrane potential
LDS-751	543	712	Nucleated cell detection
CMXRos (Mitotracker Red)	560	610	Mitochondrial membrane potential

From Flourscence to Computer Display

Scattered and emitted light from cells and other particles are converted to electrical pulses by optical detector. Confocal lenses focused at the intersection point of cells and light source, pick up Parallel light waveforms (collimated light). By the usage of optical filters, light is sent to different detectors. The most common type of detector in flow cytometry is the photomultiplier tube (PMT).

The electrical pulses originating from light detected by the PMTs are then processed by a series of linear and log amplifiers. Logarithmic amplification is used to measure fluorescence in cells.

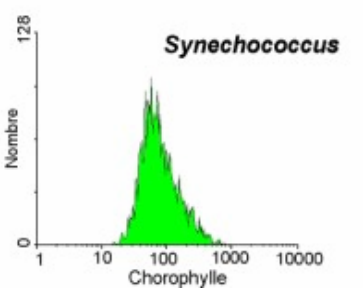
After amplification of various signals, they are processed by an Analog to Digital Converter (ADC) which allows for events to be plotted on a graphical scale (histograms).

The output data in flow cytometry are stored in the form of FCS 2.0 or 3.0 standard in computer.

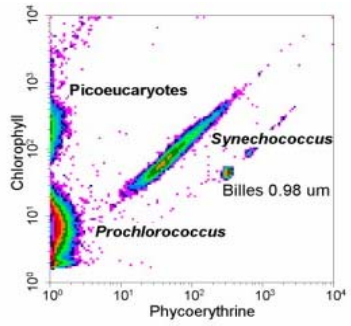
Histograms

We have two kinds of histograms in flow cytometry.

1. One-parameter histograms: for generating this graphical scale, the ADC assigns a channel number based on the pulse height for individual events. Therefore, brighter specific fluorescence events will yield a higher pulse height; so a higher channel number will be displayed in histogram.



2. Two-parameter histograms: This is similar to a topographical map.



Gating

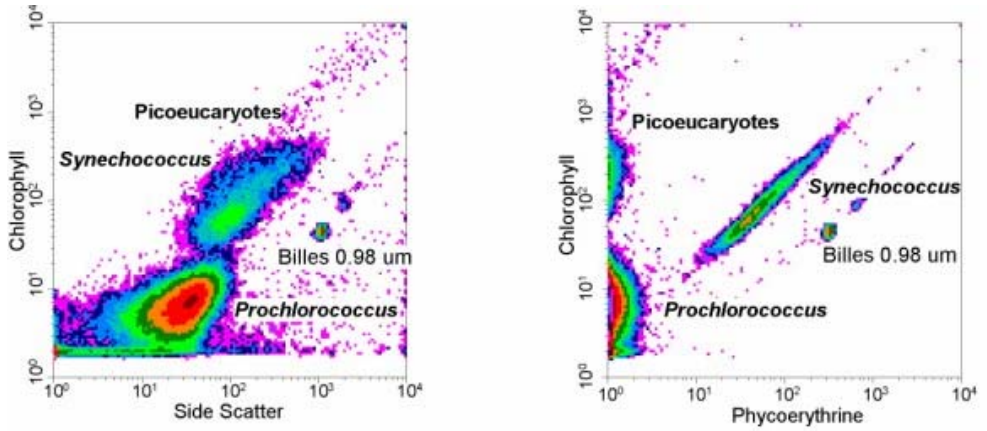
A gate is a numerical or graphical boundary that can be used to define the characteristics of particles to include for further analysis. A gate can be used to restrict the analysis to a specific population within the sample.

Listmode data files

A Listmode file consists of a complete listing of all events according to all parameters gained, as specified in the acquisition protocol. This file follows a FCS 3.0 standard. Raw listmode data files can be opened or replayed in any flow cytometer analyzer software. It is evident that protocols serve as template. It allows you to collect specified parameters (e.g. FLS, FL1, FL2, etc), and the data is Gated, contains all the regions from which your statistics will be generated. Protocols, also, contain other information which serves (zamani ke "serves" mishe khode word ham eshkal migire) as direct interface between computer workstation and the cytometer.

Such information can be high voltage settings for the PMTs, gains for amplification of linear parameters, fluorescence compensation, sample flow rates and etc.

One of important things which should be remembered is that one can only adjust regions, gating, and parameters to be displayed. Settings such as amplification, fluorescence compensation, etc cannot be modified. At last, when you use software such as FlowJo, WINMIDI, and/or ExPo for opening your listmode files, you will have to specify display parameters (parameters for displaying data), and create regions and gating according to the protocol used for collecting the data. The following picture is a part of a listmode file.



Applications in research and diagnostics

- Characterization of cells: phenotyping analysis, proliferations, viability assessment, cell cycle analysis
- Detection measurement of soluble molecules
- Separation (sorting) of specific sub-populations

In next Issue we will discuss about the mentioned applications and Fluorscence-activated cell sorting in details.

Ref: http://probes.invitrogen.com/resources/education/tutorials/4Intro_Flow/player.html
http://www.stemcell.umn.edu/img/assets/10061/Intro_to_Flow_Cytometry_Learning_Guide.pdf
http://biology.berkeley.edu/crl/pdfs/FlowBasics2.ppt
http://www.tifr.res.in/~aset/full_text/Flow%20ASET%202006%20for%20arch_280706.ppt

NIBGE: National Institute for Biotechnology and Genetic Engineering

The National Institute for Biotechnology & Genetic Engineering (NIBGE), Faisalabad, Pakistan, has been established in 1994. As Dr. Zafar M. Khalid, Director of NIBGE, says " It has established itself as Centre of Excellence in Biotechnology by upgrading its infra-structure and improving the quality of scientific manpower."

The pace of development in biotechnology over the last few years has been astonishing. Increasing number of transgenic crops, completion of Arabidopsis and rice genome along with human genome are some of our goals. Health biotechnology has also moved on from drug discovery pursuits to other areas. Stem cell research holds a great promise for future use of this technology in disease therapy. NIBGE has been alive to all these developments and has developed its research programmes. One of the goals while establishing NIBGE was to develop 'receiving units' for the new emerging technologies. So, NIBGE has developed infrastructure which is at par with international standards and has been able to establish several links with various biotechnology laboratories in developed countries. NIBGE has become internationally a lead centre for research on cotton leaf curl virus by deciphering the virus genetic code and documenting the genetic diversity existing in the field. Similarly establishment of a Plant Genomic Laboratory in collaboration with PARC (Pakistan Agricultural Research Council) is a step in right direction.

NIBGE has emerged as a focal point for all national and international activities related to biotechnology in the country. Human resource development is the basis for development in this area. NIBGE is contributing effectively by conducting M.Phil Biotechnology programmed in collaboration with the Quaid-e-Azam University, Islamabad, Pakistan. This is now being extended to Ph.D. programme. NIBGE has also taken a lead role in the finalization of Biosafety Guidelines for Genetically Modified Organism (GMO). Therefore, a series of training courses for capacity building in Environmental Impact Assessment will be held during the current year.

Bioinformatics is fast developing as a discipline and NIBGE has already initiated a Bioinformatics Cell, with the help of computer specialists in simulation and modeling. Other organizations of PAEC (Pakistan Atomic Energy Commission) are instrumental in providing help and assistance in this regard.

In the present scenario of WTO and various IPR constraints, development and commercialization of biotechnology has become complex and needs special skills. Through the commercial biotech company, PIBS, established by NIBGE, efforts will be made to resolve such issues and to enter into joint venture agreements with international private sector biotech companies which will eventually help in the transfer of technology.

NIBGE Research Divisions

NIBGE has following divisions to provide international standards: Plant biotechnology, plant microbiology, health biotechnology, industrial biotechnology, bioprocess technology, environment biotechnology, biotech interdisciplinary.

Health Biotechnology Division

To enhance the health and high quality among Pakistanis, this center provides diagnostic service, teaching and conducting research in laboratory medicine using cutting edge biotechnological approach.

Specific Objectives

- Molecular epidemiology of viral hepatitis, typhoid, enterobacter pathogens and tuberculosis
- Identification, characterization of biologically immunogenic protein antigens for development of modern vaccine and diagnostic kits
- Finding protein-based biomarkers for disease diagnosis.
- Linkage analysis of monogenic genetic disorders
- PCR-DNA based molecular diagnostic services (commercial aspect)

Historical Prospective

The health related work was initiated in PAEC for NIBGE at Biomedical Division at INMOL, Lahore soon after the approval of PC1 in 1988. Later, with the establishment of NIBGE at NIAB Campus, Faisalabad in 1991, the Health related work was initiated in Basic Biology Division. With the passage of time and in the interest of NIBGE, the name of this division was changed to Basic Biology and Molecular Medicine Division and finally to Health Biotechnology Division (HBD). A separate new block of HBD was established in 1999.

Facilities Available for Research and Diagnostics

HBD consists of a two-storied building for biomedical research and molecular diagnostics at NIBGE premises. This building has a separate PCR/DNA diagnostic section; cytogenetics; human genetics laboratory; human tissue culture laboratory; bacterial culture laboratory; typhoid and enterobacter research laboratory; DNA cloning/analysis laboratory; metabolomics laboratory; DNA/ protein analysis laboratory and a walk-in cold room.

HBD at NIBGE has research and diagnostic facilities to carry out research in areas of infectious diseases (HBV/ HCV, tuberculosis, enteropathogens), genetic diseases (β-thalassemia, leukemia and other monogenic disorders); modern vaccines (conjugate vaccines, recombinant and therapeutic HBV vaccines); biologicals (Streptokinase, tissue plasminogen activator and growth hormone) and diagnostic kits development (PCR diagnostics/ Immuno-chromatographic strips). This division is equipped with DNA sequencer; conventional PCR; real time PCR; gradient PCR; high speed refrigerated centrifuge; microfuges; incubators; shaking water baths; thermal block; freezers; refrigerators; cold cabinets; gel documentation systems; refrigerated shaker; CV chromoscan work station , safety cabinet (class II), fume hood and other routine instruments in molecular / biochemistry laboratory. The laboratories in HBD are fully equipped with trained scientists and experts in their specific areas.

Focus of Research and Diagnostics

HBD at NIBGE focuses its efforts on the early detection of infectious and genetic diseases prevalent in Pakistan. The division forges tools that are preferably applicable where sophisticated technology support is lacking.

Research aims at the application of these tools for diagnosis of diseases in clinical setting and for the epidemiological investigations. In the past ten years, this division has made good national and international collaborations with R&D institutions, pharmaceutical industries in its efforts to develop and evaluate methods for the detection of infectious and genetic diseases and production of recombinant therapeutic agents and vaccines.

Molecular Diagnostics

The first PCR-based diagnostic test was made available for public in 1995 after research and validation on Pakistani population and comparing results with conventional techniques. A number of seminars were delivered to the medical community to introduce the molecular tests for early diagnosis of diseases prevalent in Pakistan. The tests being performed in this division are as below:

- DNA/ PCR based test for the detection of tuberculosis, hepatitis B,hepatitis C, typhoid, bcr-abl translocation, detection of mutaions in β-thalassemia and male infertility in clinical samples.
- Detection of chromosamal abnormalities by karyotyping.

The diagnostic facilities at NIBGE have been the life-line of research carried out in health biotechnology division. Until Now, almost all of the publications are based on the clinical samples collected here. It has also provided revenue / funds whenever a research group needs them. And this is continuing to serve this purpose

Teaching

The scientists in HBD at NIBGE are also involved in teaching and research activities leading to M.Phil / PhD programmes of Quaid-e-Azam University, Islamabad at NIBGE Campus. Currently, three M.Phil and twenty-six PhD students working are working at HBD under the supervision of Scientists declared as PhD supervisors by Higher Education Commission.

Future Goals

The future goals of Heath Biotechnology Division are broadly to work in the biomedical research and diagnosis by exploiting biotechnology methods. Some of the area is:

- Inexpensive and rapid disease diagnosis using molecular biology methods for the infectious diseases (tuberculosis, typhoid, hepatitis B &C)
- Development of Recombinant and therapeutic vaccines plus biological and diagnostic kits to prevent them.
- Prenatal diagnosis and genetic counseling for chromosomal abnormalities and other genetic disorders.
- Identification of genomic, proteomic, metabolomic markers for the genetic (microcephaly, skin disorders, synpolydectyly) and metabolic disorders (diabetes, heart diseases) for their early diagnosis, disease management and identifying molecular drug targets for their therapeutic intervention.

Research Groups	***Research Areas***
Viral Hepatitis (B&C) Epidemiology	Genotyping, Clinical and molecular
Human Enteric Pathogens (Salmonella typhi, Salmonella paratyphi A & B, Pathogenic E. coli, Shigella, Rota Virus)	Virulence & Drug resistance
Tuberclosis	Drug resistance, Molecular epidemiology
Leukemia	Fusion genes , Drug resistance, RNA interference based therapy
Monogenic Genetic Disorders	Mutation Detection, Linkage Analysis
Modern Vaccines & Biologicals Development	Recombinant Hepatitis B Vaccine, ConjugateTyphoid Vaccine , tPA& Streptokinase

[Ref: http://www.nibge.org **]**

Anouncement

The 2nd International Student Conference of Biotechnology
15-17 November, 2008, Tehran, Iran

The 2nd international student conference of biotechnology is going to be organized by the Student Scientific Society of Biotechnology of Tehran University, with the goal of bringing young scholars together, creating a space to present their research results and to encourage the idea of academic research in the field of biotechnology.

Participants are required make registration through “Registration and Costs” pathway, and then log in the site by typing your user ID and password. After login process, “Invoice” for payment and “Send/edit Articles” for submitting abstract links will be accessible.

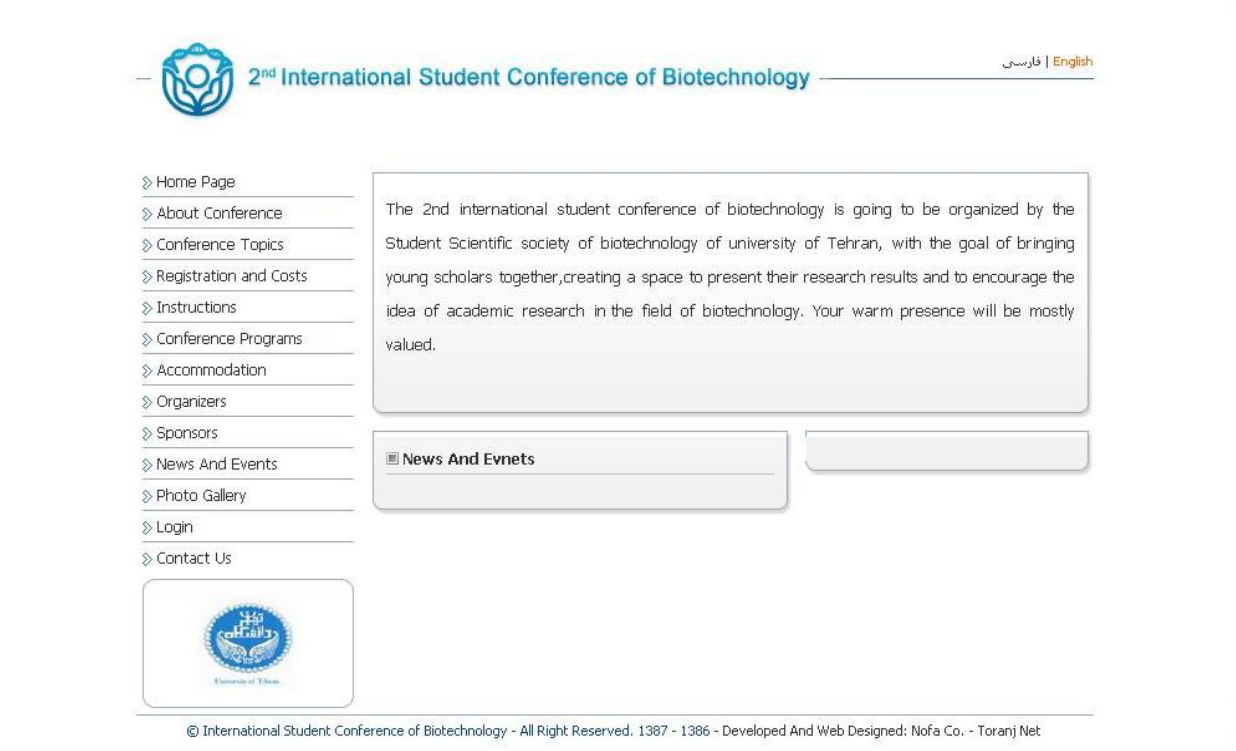
The Paper Submission will close on 21 September 2008. Registration closes on 8 October 2008.

Web address: http://biotech.ut.ac.ir/

Secretariat:

Tel: +98 21 66491622

Fax: +98 21 22840752



Cover Pictures Description (up to down)

Title: Metabolic network showing interactions between enzymes and metabolites in the *Arabidopsis thaliana* citric acid cycle.
Description: Metabolic network showing the links between enzymes and metabolites that interact with the Arabidopsis TCA cycle KEGG classification M00009. Enzymes and metabolites are the nodes (red), interactions are the lines. In total, 43 enzymes and 40 metabolites are shown. Created on Cytoscape using data from VirtualPlant 0.9.
Source: http://en.wikipedia.org/wiki/Metabolic_network_modelling

Title: Self-Assembled DNA Nanostructures
Description: Each tile consists of nine DNA oligonucleotides as shown. An atomic force microscope image of a self-assembled DNA nanogrid. Individual DNA tiles self-assemble into a highly ordered periodic two-dimensional DNA nanogrid.
Source: http://en.wikipedia.org/wiki/Image:DNA_nanostructures.png

Title: Fluorescnece Electrophoresis
Description: Agarose gel following Agarose Gel Electrophoresis on UV light box Gel from a research project on hepatitis B virus Regulatory Genomics
Source: http://en.wikipedia.org/wiki/Image:AgarosegelUV.jpg

Title: Two-Parameter Histogram in Flow Cytometry
Description: You can find this article in the training section.
Source: http://en.wikipedia.org/wiki/Image:Picoplancton_cytometrie.jpg