



# BIOTECHNIA

*A global round up of Biotechnology research*

## Editorial .....

Dear Readers

*It gives me immense pleasure to introduce to you Biotechnia- a newsletter brought to you by the Biotechnology group at the Thapar University campus. Biotechnology seldom needs introduction as applications of organisms for commercial purposes. Today biotechnology is the use of biological systems with engineering perspective to develop products and processes on which the future of mankind would rely on. Biotechnology not only harnesses the core engineering principles but applies them into biological systems which have applications in every field of human activity viz. healthcare, medicine, food, agriculture and environment. Various allied and applied fields have evolved with the combination of biological sciences with engineering sciences like biomedical technology and Instrumentation, Clinical and molecular diagnostics, tissue engineering, biomaterial design and development, computational biology and bioinformatics.*

*The biotechnology group at Thapar Campus have evolved over the years and is continually striving for excellence in research as well as academics which has been duly recognized by the government as well as the industry. The faculty in the department is receiving grants for sponsored research from Department of Biotechnology, Department of Science and Technology, Indian Council for Medical Research, Council for Scientific and Industrial Research, University Grants Commission, All India Council for Technical Education, Board of Research in Nuclear Science (Department of Atomic Energy).*

*The Department of Biotechnology at Thapar University sports a unique position in North India as it is supported by two DST (Department of Science & Technology, GoI) sponsored unit's viz. TIFAC-CORE and STEP-TU. These extended units of the department are equipped with state of the art research facilities for hands on training of students as well as research professionals.*

*The innovative educational programs at the department lays emphasis on inculcating analytical skill sets as well as developing a systems-oriented engineering approach to understand and exploit life sciences for the benefit of mankind. The TIFAC-Centre of Relevance and Excellence (CORE) in Agro & Industrial Biotechnology at Thapar University was established under the MISSION REACH 2020 program envisioned by the former president and distinguished missile scientist Dr. A.P.J. Abdul Kalam. The program was implemented by TIFAC (DST) and CORE at Thapar University was established in the first phase in year 2000 based on the research skills of the faculty and training impetus of the university. Science and Technology Entrepreneurs Park (STEP-TU) at Thapar University is promoted Thapar University and NSTEDB (National Science and Technology Entrepreneurship Development Board), Department of Science and Technology (DST), Govt. of India. Established in 2005, this center focuses on business incubation in multidisciplinary area of Biotechnology viz. Agri-biotechnology, Mushroom Technology, Bio-fertilizers, Plant tissue culture and Food Processing Technology.*

*This issue has articles on Plant tissue culture, epigenomics, pharmacogenomics, news clips of some latest developments in Biotechnology, forthcoming events of the Department of Biotechnology. Your inputs and suggestions are invited to make this e- newsletter as the pulse of biotechnology R&D for the academia as well as the industry.*

**Dr. Sanjai Saxena**

**Vol.1, No.1, 2014**

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Plants, since time immemorial have been used as a valuable natural source of medicine to cure and prevent several diseases. According to WHO, 80% of the world's population depend directly on medicinal plants for their basic preventive and curative healthcare. Over 9000 plants have known medicinal applications in various cultures and countries. Medicinal plants synthesize and accumulate a variety of compounds to combat diseases and form primary line of defense for human beings. Medicinal plants form the resource base for rapidly growing pharmaceutical industry with 25% of the drugs derived from these plants. Thus, there is a global resurgence in trade of herbal medicine. WHO estimated that the present demand of medicinal plants is approximately US \$62 billion per year and demand for medicinal plant based raw material is going high at the rate of 15 to 25% annually and the demand of medicinal plants is likely to increase more than US \$5 trillion by 2050.

The rising demand of plant based drugs is creating a heavy pressure on some high valued medicinal plants. Increasing human activities and rapidly eroding natural ecosystem are threatening their very existence causing a number of species to be either threatened or included in endangered category. Most of the medicinal plants do not produce seeds or their seeds are too small and don't germinate in soil. Likewise majority of plants are not amenable to vegetative propagation thus limiting mass multiplication of desired cultivars. Due to shortage of high quality planting material, cultivation and domestication of medicinal plants is facing great problem. In this context, *in vitro* propagation or micropropagation of medicinal plants holds a significant promise for true- to-type, rapid and mass production of selected elite varieties (Fig.1).

An efficient and reproducible protocol was established in our laboratory for the mass production of two important medicinal plants namely *Tylophora indica* and *Stevia rebaudiana* under *in vitro* conditions (Fig.2,

Fig.3). *Tylophora indica* (Asclepiadaceae) commonly known as "Antmul" is traditionally used as a folk remedy in treatment of bronchial asthma, bronchitis, dysentery, whooping cough, rheumatism, allergies and inflammation.

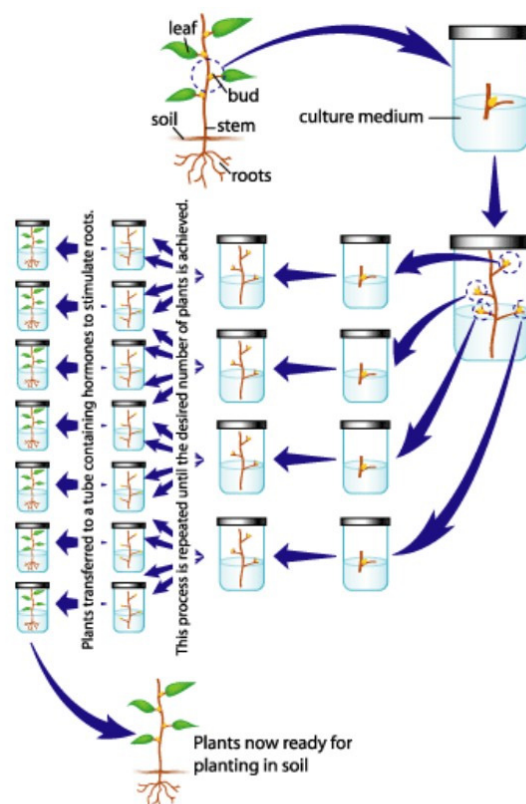


Fig. 1 Micropropagation Technology

Source: [forum.grasscity.com/advanced-growing-techniques/998333-tissue-culture-micropropagation.html](http://forum.grasscity.com/advanced-growing-techniques/998333-tissue-culture-micropropagation.html)

*Stevia rebaudiana* Bertonni (Asteraceae) popularly known as 'Sweet leaf' is as contraceptive, anti-hypersensitive agent, anti-cancer agent, also possess hypotensive and hypoglycaemic activity. The



Fig.2 Multiple shoot proliferation in *T. indica*



Fig.3 Six (06) months-old hardened plants of *Tylophora indica*

leaves of *Stevia* are the source of diterpene glycosides, among these stevioside is a high intensity, non-calorific, sweetener being 300 times sweeter than sucrose.



Fig.4 Numerous shoots formed from leaf explant in *S. rebaudiana*



Fig. 5 Three months old acclimatized plants of *S.rebaudiana*

All the three techniques of micropropagation namely forced axillary branching, *de novo* adventitious shoot formation both directly from the explants and indirectly from the callus and somatic embryogenesis were successfully employed for the mass multiplication of these two plant species. The regenerated shoots were rooted on a separate root inducing media and the rooted plantlets of *S. rebaudiana* (Fig.4 and Fig.5) and *T. indica* were transferred successfully to the field conditions through successive hardening stages showing 80% and 90% survival rates respectively with no phenotypic variations.

## Epigenomics

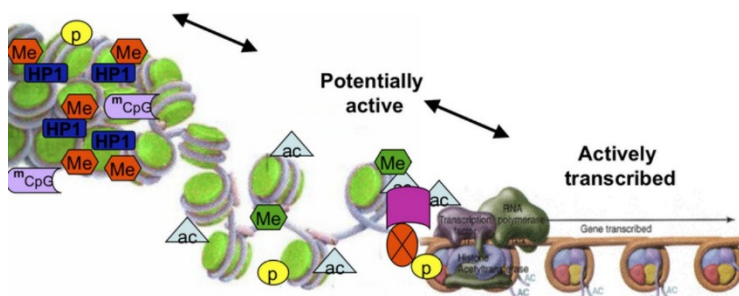
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In the post genomics era, epigenomics is an emerging field that encompasses determination of epigenetic modifications in the entire genome. Epigenetic modifications alter the expression of genetic information without any change in the DNA sequence. The two major epigenetic modifications are DNA methylation and histone modifications. Epigenomics began with a pilot project in which human major histocompatibility complex region of human chromosome 6 was analyzed for DNA methylation patterns in several different cell types. Similar work studied the methylation pattern of gene promoters in human chromosome 21. Recently, genome wide DNA methylation analysis has been performed in mouse primordial germ cells.

Notably, all the CpGs in the genome are not methylated and these patterns of DNA methylation are largely inherited by daughter DNA molecules after DNA replication. There are certain GC rich sequences in the genomes that are rich in CpGs and are usually not methylated. Such regions of DNA are called CpG islands and are often found in the 5' regions of housekeeping genes. DNA methylation leads to gene silencing and thus plays important role in tissue specific gene regulation and embryonic development. It is also responsible for gene imprinting and X chromosome inactivation. Aberrant DNA methylation has been correlated with cancer. Keeping in view these implications, it is indeed important to study DNA methylation status of entire genome in different cell types and at different developmental stages for insight into the role played in spatio-temporal regulation of different stages. This has led to advent of epigenomics.



Source: UC Davis Genome Centre, Epigenomics Focus Group

In eukaryotes, DNA methylation occurs at position 5 of cytosine base in CpG dinucleotide sequence context. DNA methylation is catalyzed by members of DNA methyltransferase enzyme family.

DNA methylation can be detected by several methods including the use of methyl sensitive restriction enzymes but bisulfite sequencing is the best method for comprehensive and detailed analysis. Bisulfite sequencing is based on chemical reaction between Sodium bisulfite and DNA that results in conversion of cytosine's into uracil's except for the methylated cytosine's. The converted DNA is amplified by Taq DNA polymerase and sequenced using specially designed



primers. Comparison of the sequencing results with unconverted DNA sequence reveal methylated cytosines in the DNA. Modification of this technique, whole genome shotgun bisulfite sequencing has been used in the epigenome analysis.

Practically each cell of multicellular organisms contain identical genetic information, yet we find several different cell types varying in structure as well as functions. This variation is caused by presence of different epigenomes (different methylation patterns) leading to differential gene expression in the cells. Thus transcriptomes and proteomes are tightly associated

with epigenomes. Characterization of distinct methylation patterns can be associated with complex diseases such as cancer and it can provide biomarkers for detection of different classes of cancer. Not only in advancing fundamental research, epigenomics is also a promising field in for developing diagnostics and therapeutics application.

*(The picture used in this article has been sourced from UC Davis Genome Centre, Epigenomics Focus Group. It has been purely used for awareness purpose in academics and for information to readers with no commercial intensions or involvement)*

## The Role of Pharmagenomics in Personalized Medicine

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Pharmacogenomics is the study of how an individual's genetic inheritance affects the body's response to drugs. The term is coined from the words genomics and pharmacology and is thus the amalgamation of pharmaceuticals and genetics. Thus, pharmacogenetics is the intersection of the fields of pharmacology and genetics. The field of pharmacogenomics has the potential that drugs might one day be tailor-made for individuals and adapted to each person's own genetic makeup. A person's diet, age, sex, lifestyle and overall health all can influence a person's response to medicines, but understanding an individual's genetic makeup is the key for the development of creating personalized drugs with greater efficacy and safety [1]. The way a person responds to a drug (this includes both positive and negative reactions) is a complex mechanism that is influenced by many different genes. Pharmacogenomics has used different approaches of new generation technologies that has led to the understanding of disease susceptibility and pathophysiology, providing enormous potential for novel therapeutic strategies. Pharmacogenomic research has gained huge importance with advances in molecular genetics and genomic sequences and thus permits rapid screening for specific polymorphisms as well as to identify the genetic sequences of target genes such as

those coding for enzymes, ion channels and other types of receptor involved in drug response. Individual variation in response to drug is a common clinical problem. Variations ranges from failure to respond to the drug to adverse drug interaction and drug-drug



Source: National Cancer Institute (NCI), NIH, USA

interaction when more than two drugs are taken concomitantly. It is now clear that these individual variations are inherited and are due to genetic variation from individual to individual [2].

The role of an individual's genetics plays an important factor in the overall impact of the person's reaction to a specific drug. These individual variations

may be because of differences in the drug targets or differences in the enzymes that metabolize drugs. Differences in drug targets can lead to differences in drug efficacy but differences in enzymes lead to differences in either efficacy or toxicity [3]. The Human Genome project has played an important role in the identification of the molecular composition of the enzymes so that we can study correlation between genotypic and phenotypic variability. These discoveries shall help us to detect the individuals who will experience the adverse reactions to medicines. This has become possible with the identification of SNPs (Single Nucleotide Polymorphisms). The variations in drug responses due to SNP play an important role. Polymorphisms in any one gene including those coding for drug transporters, drug receptors, and cell signaling pathways can be important determinant of clinical response. Now for more than 20 human metabolizing enzymes, polymorphisms have been identified. Important examples are polymorphisms in cytochrome P450 enzyme [4]. The Cytochrome P450s are multigene family of enzymes found predominantly in liver that are responsible for the metabolic elimination of most of the drug in medicine. Genetically determined variability in the level of expression or function of these enzymes has a profound effect on the drug efficiency. In “poor” metaboliser the genes encoding specific cytochrome P450s often contain inactivating mutations, which result in a complete lack of active enzyme and a severely compromised ability to metabolize drugs [5].

Recently the US Food and Drug Administration (FDA) has approved Ivacaftor a drug for the treatment of a subset of cystic fibrosis patients. This drug is tailored made for those cystic fibrosis patients carrying the G551D genetic variant in the cystic fibrosis transmembrane regulator (CFTR) gene, which encodes a protein that regulates chloride and water transport in the body and is defective in the disease. Ivacaftor targets the CFTR protein, increases its activity, and consequently improves lung function [6].

Thus, SNP's may lead to personalized medicines for a wide variety of disease. Pharmacogenomics thus aims to improve drug safety and efficacy treatment to fit individual patient profile *i.e.*

personalized medicine or the right medicine for the right person. SNP's provides pathway towards a rational designing of tailor made medicine. In addition, knowledge of polymorphisms can provide important information on the correct doses of drugs. The fact that genetically determined diversity causes drugs to be ineffective in certain people can be exploited in testing and development of new drugs. Performance of SNP analysis at the early stage of clinical trials can permit selection of patients who are biochemically equipped to respond to the investigational substance. Suitable participants can then be identified by means of genetic tests that provide information on metabolic status, drug target polymorphism and the presence of specific diseases. Even small groups of subjects selected in this way can yield statistically significant results, thus reducing total cost of clinical trial.

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*(The picture used in this article has been sourced from National Cancer Institute (NCI), NIH, USA and has been purely used for awareness purpose in academics and for information to readers with no commercial intensions or involvement)*

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A number of small entrepreneurs in Punjab have begun working at the grassroots level to reduce their carbon footprint and ensure sustainable development. And Science and Technology Entrepreneurs' Park (STEP) in Agro and Industrial Biotechnology at Thapar University, Patiala is providing them with handholding services through commercialisation and transfer of technology.

Focusing on agri-biotechnology, bio-fertilisers, bio-pesticides, mushroom cultivation, plant tissue culture, food processing technology, soy processing and spirulina production technology, STEP has successfully incubated 25 companies, of which 10 have started their own ventures, thereby providing employment to more than 100 people.



After attending a mentorship programme at STEP, Sandeep Garg, 48, a sub-divisional officer with Punjab's public health department, resigned his government job and set up Ecolabs - a solid waste-management company - in Mohali. In collaboration with STEP, Garg has developed a strain of bacteria to recycle organic waste into bio-compost.

"The process can be of immense use in producing organic manure with the help of organic waste generated by restaurants and hotels. We will soon do the commercial launch and explore its use by municipal corporations on a larger scale," Garg said.

Rajiv Aneja's journey has been similar to that of Garg. He gave up the option of working in the United States and set up a venture called ABCA Biosolutions, headquartered at Chandigarh, to work in the field of algal biodiesel. "The cultivation of algae as a substitute for corn and jatropha bio-diesel is very eco-friendly and cost-effective. It has been proven in the US that corn yields 450 gallons of oil per acre per year, whereas algae yields 9,000 gallons per acre per year of oil. The yield of algae may vary in different parts of India but it would definitely be much higher than other crops. This would save on land requirement, cost of irrigation and ensure a higher income for farmers," said Aneja.

Aneja, 32, did his doctorate at Thapar University and went to the US in 2009 for three years on a Bridge and Edge grant from the US government to study business management in the life sciences. "At the present pace of consumption, our natural resources will not last for more than three decades, so it is high time we seriously work towards sustainable development," he said.

Aneja is getting queries from large corporate players in the transport sector and farmers in southern India, and has high hopes about the future of the project. "The initial cost of bio-diesel may be higher than the prevailing price of fossil fuel but in the long run the costs would be compressed," he said.

Even a small progressive farmer like Satnam Singh of Samana Mandi is harnessing soy-technology. Singh, 45, has installed a low fat-high protein soya milk and cheese plant with a capacity of 2,200 milk packets and 800 paneer packets per day. The by-products of soya processing are also consumable. The liquid residue of cheese is used in making jal-jeera, a beverage, and the solid residue is used as fodder for animals. So the project has a low carbon footprint.

The support of scientists at STEP helped Singh to diversify from wheat and paddy cultivation, and he

now sells his soya products under the brand name 'Anmol Soy' in Punjab and Chandigarh. STEP was set up in April 2005, as a joint venture between the National Science and Technology Entrepreneurship Development Board (NSTEDB), the Union government's department of science and technology (DST), and Thapar University.

It got government funding in the initial years but is now self-financed. STEP's executive director, Dinesh

Goel, said it has conducted 74 entrepreneurship development programmes and 16 farmers' meets since its inception, in the process providing hands-on training to hundreds of people, including students of various reputed institutes, teachers, researchers, farmers, NGOs, and entrepreneurs. After exiting STEP, they remain available for consultancy and other services.

The core facilities that STEP provides the SMEs are business incubation, entrepreneurship education, consultancy, training and mentoring.

## News Snippets

### Colored silk production by Silkworms on special diet

The National Chemical Laboratory (NCL) jointly with Central Sericultural Research and Training Institute in Mysore have developed a green method which directly produced colored silk. The technique generally involves feeding the silkworm's mulberry leaves dipped in different dyes. The dye is transported along the biochemical pathways of the silkworm to produce a colored cocoon and colored silk fiber. The dye is transported along the biochemical pathways of the silkworm to produce a colored cocoon and colored silk fiber. This idea is not new and has been tested using an expensive fluorescent dye Rhodamine, however the Indian Scientists have exploited "azo" dyes which are expensive and have been heavily exploited by the textile industry. The researchers say their work was prompted by the fact that for large-scale commercial synthesis of colored silk, the usage of common cheap textile dyes is necessary. The scientist evaluated seven dyes out of which three have been successfully incorporated into caterpillar's silk without harming the silkworm or affecting its growth.

(Source: Nature News India, K.S. Jayaraman, doi: 10.1038 / nindia.2013.163)

### Neurogenesis by curcumin nanoparticles

A joint research of Scientists from CSIR- Indian Institute of Toxicology Research (IITR), Lucknow; Academy of Scientific and Innovative Research and CSIR-Institute of Genomics & Integrative Biology, New Delhi, India have revealed that curcumin laced nanoparticles can boost the process of making new neurons (neurogenesis) which could eventually be helpful in treatment of several neurodegenerative disorders like Alzheimer's disease. Researchers have previously established that by targeting the endogenous neural stem cells to induce neurogenesis, it is possible to influence the brain's self-regenerative

capacity. With this knowledge researchers have found curcumin a neuroprotective agent with poor brain bioavailability can be delivered using encapsulated nanoparticles can induce proliferation of the neural stem cells. The researchers have created nanoparticles with curcumin and poly (lactic- 115 co-glycolic acid) (or PLGA) to induce neurogenesis. The nanoparticles significantly increase expression of genes involved in cell proliferation (reelin, nestin, and Pax6) and neuronal differentiation (neurogenin, neuroD1, neuregulin, neuroligin, and Stat3). The curcumin nanoparticles increased neuronal differentiation by activating the Wnt/ $\beta$ -catenin pathway, involved in regulation of neurogenesis. These nanoparticles reversed learning and memory impairments in an amyloid beta induced rat model of AD-like phenotypes, by inducing neurogenesis.

(Source: Nature News India, K.S. Jayaraman, doi: 10.1038 / nindia.2013.167).

### Naturally produced compound rewinds age related demise in Mice

The heart of this finding is based on the communication between mitochondrion and the nucleus. As the communication between the nucleus and the mitochondrion breaks down the process of aging accelerates. "The aging process we discovered is like a married couple—when they are young, they communicate well, but over time, living in close quarters for many years, communication breaks down," said Harvard Medical School Professor of Genetics, David Sinclair, senior author on the study. "And just like with a couple, restoring communication solved the problem. This study was jointly carried out by Harvard Medical School, the National Institute on Aging, and the University of New South Wales, Sydney, Australia. Sinclair and his group had been working on a group of genes called sirtuins while focusing on aging. One of these



genes, SIRT1, was activated by the compound resveratrol, which is found in grapes, red wine and certain nuts. Ana Gomes, a postdoctoral scientist in the Sinclair lab, had been studying mice in which this SIRT1 gene had been removed. While they accurately predicted that these mice would show signs of aging, including mitochondrial dysfunction, the researchers were surprised to find that most mitochondrial proteins coming from the cell's nucleus were at normal levels; only those encoded by the mitochondrial genome were reduced. As Gomes and her colleagues investigated potential causes for this, they discovered an intricate cascade of events that begins with a chemical called NAD and concludes with a key molecule that shuttles information and coordinates activities between the cell's nuclear genome and the mitochondrial genome. Cells stay healthy as long as coordination between the genomes remains fluid. SIRT1's role is intermediary, akin to a security guard; it assures that a meddlesome molecule called Hypoxia Inducible Factor (HIF)-1 does not interfere with communication. For reasons still unclear, as we age, levels of the initial chemical NAD decline. Without sufficient NAD, SIRT1 loses its ability to keep tabs on HIF-1. Levels of HIF-1 escalate and begin wreaking havoc on the otherwise smooth cross-genome communication. Over time, the research team found, this loss of communication reduces the cell's ability to make energy, and signs of aging and disease become apparent. Gomes found that by administering an endogenous compound that cells transform into NAD, she could repair the broken network

and rapidly restore communication and mitochondrial function. If the compound was given early enough—prior to excessive mutation accumulation—within days, some aspects of the aging process could be reversed.

(Source: *Florida Biotechnology News*, 22 December 2013)

#### Milk: a new drug delivery agent

Sanaz Haratifar and Milena Corredig, of the Department of Food Science and Department of Human Health and Nutritional Sciences of the University of Guelph, Ontario, Canada have carried out a new study of delivering tea polyphenols through milk. The major extractable polyphenol from green tea which is also the most active one is Epigallocatechin gallate (EGCG). The tea polyphenols have been found to inhibit tumor formation, reduce cancer cell proliferation apart from increasing apoptosis and inhibiting angiogenesis. For several reasons, tea catechins have poor bioavailability and the goal of the current study was to encapsulate EGCG in casein (milk protein) molecular aggregates, known as micelles, to maintain and enhance catechin bioavailability. The EGCG diluted in milk was effective against human colorectal cancer cell line HT-29 in a dose dependent fashion. Dr. Haratifar commented "This study showed that the binding of EGCG to the casein micelles did not affect the bioefficacy of EGCG and cell uptake at concentrations higher than 0.03 mg of EGCG/mL of skim milk."

(Source: S. Haratifar, K.A. Meckling, M. Corredig. *Antiproliferative activity of tea catechins associated with casein micelles, using HT29 colon cancer cells*. *Journal of Dairy Science*, 2013; DOI: 10.3168/jds.2013-7263

Forthcoming Events @ Thapar University

MOLECULAR MEDICINE AND DIAGNOSTICS WORKSHOP  
29<sup>th</sup> January 2014

TIFAC-CORE in Agro and Industrial Biotechnology along with Department of Biotechnology (DBT) is jointly organizing a one day workshop on Molecular Medicine and Diagnostics on 29<sup>th</sup> January 2014. The industrialization of molecular biology assay tools has made it practical to use them in clinics. Miniaturization into a single handheld device can bring medical diagnostics into the clinic and into the office or home. The workshop is based on the recent developments in molecular medicine and their applications in diagnosis of diseases. The speakers in this workshop are from the industry as well as academia and shall highlight the development in respective areas of research specializations which are having clinical applications.

For further information on this workshop please keep visiting [www.thapar.edu](http://www.thapar.edu).

Dear Readers,

I hope that you must have found this issue of "Biotechnia" informative and interesting. Your comments are always appreciated to improve the newsletter brought to you by the Biotechnology group @Thapar University, Patiala.

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