

# EMGEN Newsletter

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

### Address:

Biotechnology building, #69, Pasteur Ave., Pasteur Institute of Iran  
Tehran, Iran, 13164

Tel: +98-21-66954324

Fax: +98-21-66465132

E-mail: [emhgbn@gmail.com](mailto:emhgbn@gmail.com), [secretariat@emhgbn.net](mailto:secretariat@emhgbn.net)

Websites: [www.EMGEN.net](http://www.EMGEN.net) and [www.emhgbn.net](http://www.emhgbn.net)

**Prepared by:** Mona Alibolandi, Samaneh Hemmati Shabani, Raziye  
Kebriaee and Shiva Shajari

**Page design:** Mona Alibolandi

**Editor:** Dr. Soroush Sardari

## Genetic studies of “noble cane” for identification and exploitation of genetic markers

*The paper entitled: Genetic studies of “noble cane” for identification and exploitation of genetic markers, which is published in GMR, 9(2):1011-1022 (2010), describes that SSR fingerprints can help sugarcane breeders to clarify the genetic pedigree of commercial sugarcane varieties and evaluate the efficiency of breeding methods. The findings and methods have implications in health sciences. The study was carried out by Seher Nawaz, Farooq A. Khan, Saba Tabasum, M. Zafar Iqbal and Asif Saeed. Corresponding Author of this paper, Dr. Asif Saeed is Assistant professor in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan.*



**Dr. Asif Saeed**

Sugarcane (*Saccharum* sp.) is an important crop in the world followed by sugar beet, but in the Pakistan this crop enjoys a less significant status as compared to other sugarcane growing countries of the world. Consequently, sugarcane lags behind the other crops in utilizing molecular biology tools to unravel the complexity of its genome and to affect the genetic improvement. Many attempts have been made to evolve some outstanding genotypes, which can benefit both the growers and the mill owner, through conventional breeding. The success until now is not appreciating. In *Saccharum* species, high degree of polymorphism and genotype-environment interaction makes it extremely difficult to distinguish the genotypes on the basis of morphological characters. It is due to environmental factors that phenotypic traits may vary from one season to another. Therefore, morphological characters are not reliable markers for systematic and diversity analyses. Molecular markers successfully developed during the last two decades are among the few promising steps that cast some light of optimism into the future of sugarcane improvement using these novel genetic tools. Molecular markers have largely overcome the problems associated with phenotype-based classification. Molecular markers are useful for precise assessment of diversity and phylogenetic relationships among different species and related genera at the DNA level without some of the over simplifying assumptions associated with calculating genetic diversity based on pedigree history. The objective of the present studies is to determine whether polymorphism was sufficient to distinguish sugarcane accessions and to assess the patterns of genetic diversity among

selected group of *Saccharum* species. For this study 40 sugarcane accessions were collected from the germplasm source. The accessions consisted of wild species (*S. barberi*), advance lines and approved varieties (*S. officinarum*). Micro-satellite (SSR) analysis was performed using 50 primers and genetic distance of 40 accessions ranging from 0.60 to 1.11 with an average of 0.85 suggested that the level of genetic diversity among the sugarcane accessions was high. In several other studies, elite sugarcane (*Saccharum* hybrids) germplasm showed genetic diversity as well revealed a broad range (0.324-0.8335) of pair-wise similarity values when tested on 30 or 40 commercial sugarcane cultivars. The complex banding patterns encountered in sugarcane is due to its high level of polyploidy and heterozygosity as compared to other genera. Reports from the International Sugarcane Microsatellite Consortium show the amplification of several fragments per clone with a theoretical maximum of 12 fragments. In the present study, bands were produced in the range of 2-7, which were much lower than the above mentioned studies. In spite of the high polyploidy and heterozygosity of the *Saccharum* genome, few primers amplified a single discrete band across the members of the *Saccharum* complex, suggesting that these allelic regions or primer binding sites are highly conserved and no SSR expansion or contraction has taken place during the evolution of *Saccharum officinarum* and *Saccharum barberi*. Another reason for fewer bands being produced is that the primers range in size from 300-420 bp. A high degree of similarity between *S. officinarum* and *S. barberi* as revealed in the present study has been documented by other marker systems. The proximity between the two species is expected, since *S. barberi* is considered to be the progenitor species of *S. officinarum*. In the present study *S. barberi* clones showed low genetic distance from *S. officinarum* clones as four *barberi* clones do not form distinct clusters but cluster with other *officinarum* clones. AUS-10/72, which belongs to *barberi* and is Australian in origin, along with three accessions, TCP-81, US-173 and US-133, of Brazilian and USA origins, constituted the cluster IIB1a1. The other three *barberi* clones, namely No. 61/77, AUS-10/72 and Katha, fall in the single cluster IA. Although a high range of dissimilarity (0.60-1.119) was estimated among the genotypes, the genetic distances between the *barberi* and *officinarum* clones are not high. These primers sequence vary in different varieties of sugarcane, and this variability may be used to develop molecular markers for mapping sugarcane genes and traits, these sequences being the part of sugarcane genome predicted to be most immediately useful to plant breeder and geneticists. Further this information will be useful for identification of potential germplasm groups and for optimizing hybridization and selection procedures.

## **DNA fingerprinting: a Breakthrough in Human's Identification**

DNA fingerprinting has become an important part of society because beside all its usages, it is a powerful technique for recognizing guilty from innocents. DNA fingerprinting or genetic profiling has become such a permanent part of forensic science that it is hard to say that DNA fingerprinting is invaluable identification technique which has been around for a couple of decades.

DNA fingerprint of a person is the same for every cell, tissue, and organ and also each person has a unique DNA fingerprint. DNA fingerprint can not be altered by surgery and it is constant during the life.

Due to this, DNA fingerprinting is rapidly becoming the valuable method for distinguishing among human beings.

### **How was DNA fingerprinting discovered?**

Prof Jeffreys and his colleagues were testing to detect the myoglobin gene in grey seals to identify the same gene in the human genome.

During the course of these experiments, Professor Jeffreys found a short sequence of DNA which is repeated many times in many positions in the human genome (minisatellite sequence).

Professor Jeffreys wanted to apply the gene detection methods to study the manual of genes and investigate inherited variation between people.

The wonderful result of his experiments was restriction fragment length polymorphism (RFLP).

They found out that some persons have a nucleotide change (SNP) in a specific site which prevents cutting the DNA at the target site.

"We got our first SNP in 1978," says Professor Jeffreys. "Before that we knew about heritable variation in gene products, such as blood groups, but here we had examples of inherited variation in DNA, the most fundamental level of all."

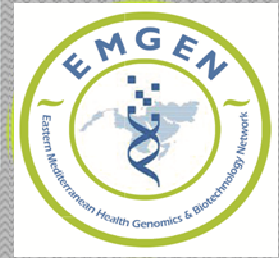
But SNPs were hard to find and assay. Additionally they could not tell much about inherited variation at the DNA level between people. Even they were not able to show genome had changes or not. So Professor Jeffreys decided finding pieces of DNA that would be more variable than SNPs.

The first candidate was tandem repeat DNA where a short sequence of DNA was repeated many times. "Intuitively it seemed that regions of tandemly repeated DNA would be open to mutation processes such as duplication and recombination," says Professor Jeffreys. "They could be highly variable and informative genetic markers."

"The real story of DNA fingerprinting starts at the headquarters of the British Antarctic Survey in Cambridge," says Professor Jeffreys. Tandem repeat DNA in the human genome was hard to understand at first, and the research continued with some dark points but answers found in a completely different project in Professor Jeffreys's lab where was searching for the human copy of the myoglobin gene. Before human, the group decided to look for the myoglobin gene in grey seals because they have lots of myoglobin so produce high amount of mRNA that was important to clone cDNAs.



# Training



"We got the seal myoglobin gene. When we had a look at human myoglobin gene, inside an intron in that gene was tandem repeat DNA (a minisatellite)."

This minisatellite was a golden key. The team could then find more minisatellites and discovered a core sequence which is a piece of DNA that is similar in many different minisatellites. "Using the core sequence we made a probe that should latch onto lots of these minisatellites at the same time." says Professor Jeffreys, "and, to test out the system, we hybridised the probe to a blot with DNA from several different people."

In September 1984, the result was shown with the X-ray in the Leicester University darkroom. "I took one look, thought 'what a complicated mess', then suddenly realized we had patterns," says Professor Jeffreys. "That was a 'eureka'!"

## How is DNA fingerprinting done?

DNA fingerprinting usually is done by performing a Southern Blotting.

**1: Isolating the DNA from the rest of the cellular material.** DNA must be extracted from the cells or tissues of the body. Only a small amount of tissue - like blood, hair, or skin - is sufficient. For example, the amount of DNA found at the root of one hair is usually enough. Isolation can be performed chemically, by using a detergent to extract pure DNA, or mechanically, by a large amount of pressure so that "squeeze out" the DNA.

**2: Cutting the DNA into several pieces of different sizes.** This is done by using restriction enzymes which cut the DNA at specific places. For example EcoR1 which is a bacterial enzyme will cut DNA only when the sequence GAATTC occurs.

**3: Sorting the DNA pieces by size (size fractionation).** The DNA pieces are sorted according to size by a strainer technique called electrophoresis. The DNA pieces are passed through a gel, such as agarose, and an electrical charge which supply enough power to move pieces, with the positive charge at the bottom and the negative charge at the top. Because of negative charge of DNA, the pieces of DNA will be attracted into the bottom of the gel. The smaller pieces are able to move more quickly towards the bottom than the larger pieces so different-sized pieces of DNA will be separated by size, with the smaller pieces at the bottom and the larger pieces at the top.

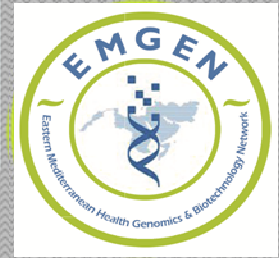
**4: Denaturing the DNA.** All DNA is changed to single-stranded which can be performed by heating or chemically treating the DNA in the gel.

**5: Blotting the DNA.** The gel with the size-fractionated DNA is transferred to a sheet of nitrocellulose paper, and then soaking them overnight to permanently attach the DNA to the sheet. The Southern Blot is now ready to be analyzed.

In order to analyze a Southern Blot, a radioactive genetic probe is used in a hybridization probe with the DNA in question. In case, an X-ray is taken of the Southern Blot after a radioactive probe is bound with the denatured DNA on the paper, only the areas where the radioactive probe binds will be seen on the film. This would let researchers to identify, in a particular person's DNA, the occurrence and frequency of the particular genetic pattern contained in the probe.



# Training



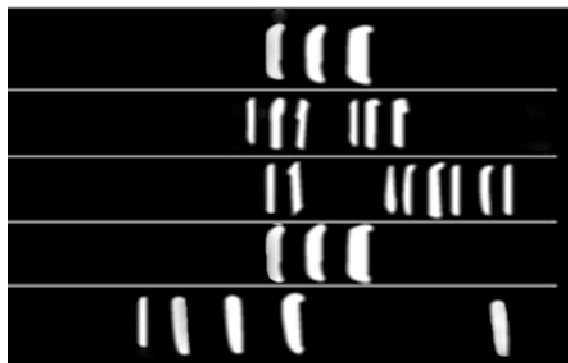
## Applications of DNA Fingerprinting

### Forensics and Criminal Identification:

Alec Jeffreys was looking for ways to prevent of hereditary diseases and genetic disorders. At that time also he was inquired by the police to help investigate a rape case, and he successfully recognized the rapist from his DNA and it was the first use of DNA fingerprinting in Forensics sciences which is now the strongest application of DNA fingerprinting. Very soon, DNA profiling was being used by forensic scientists around the world.

DNA fingerprinting allows detective to identify criminals from any semen, hair blood, or even dead skin that they may leave at the scene of the crime. A DNA fingerprint allows police to distinguish innocent from guilty. The exactness of DNA testing is known as crucial evidence in many police investigations. This cannot be denied that tow strange people have never found with the same fingerprint but some researchers say that some certain patterns of DNA are more common in certain racial and a match could be more than thing that currently thought.

In a growing number of crime police are trying to investigate a criminal by testing a large number (sometimes tens of people who were near the scene of the crime), this rouses the risk of a failure of justice if DNA testing is wrongly reliable. However, generally DNA examining is a trustful technique which could convict many criminals and confirm many innocent suspects.



In the example DNA which is collected at the scene of a crime is compared with DNA samples that were collected from 4 suspects. The DNA has been chopped into smaller pieces and run on a gel. The pattern of suspect C is matched.

In many countries, all criminals or just convicted of serious offences have their DNA pattern taken and kept with their customary fingerprints. It has been suggested to perform a nationwide overview and recording data of the DNA of the population. This could be a very effective prevention of committing crimes.

### Paternity and Maternity:

Each strand of DNA has sequences which include genetic information that shapes development of organisms (exons) and sequences which seem provide no genetic information (introns). Although introns seem not functional, researches have been shown that they have reiterate sequences. These sequences which called Variable Number Tandem Repeats (VNTRs) can have within twenty to one hundred base pairs in anywhere of strand.

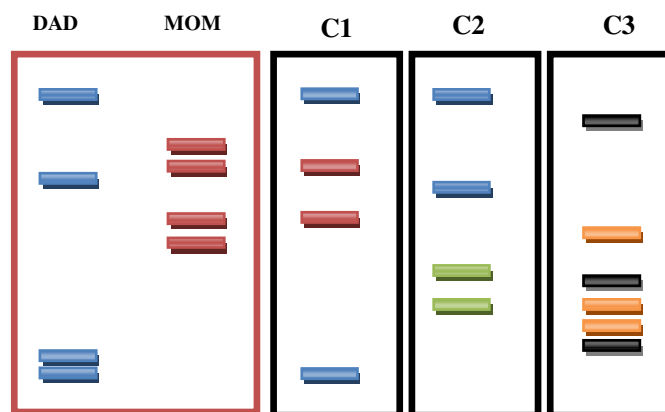


# Training



VNTRs can be utilized to show paternity and maternity because people inherit their VNTRs from their parents. Patterns of VNTR are so individual in a way that a parental VNTR pattern can be rebuilt if only the VNTR patterns of children are clear.

To confirm if a person has a specific VNTR, a Southern Blot will be done and then the Southern Blot will probe with in a hybridization reaction beside a VNTR in question in radioactive version. Parent-child pattern analysis has been used to explain father-identification cases such as more complicated cases of confirming legal nationality, adoption or biological parenthood.



VNTRs are related to the genetic information which is given by parents. People may have VNTRs inherited from their mother or father, or both. This scheme is the VNTR patterns for mother, father, and their children: C1 (biological child), C2 (step father's child) and C3 (adopted child, not biologically related).

## Inherited Disorders:

DNA fingerprinting is used to detect inherited disease in both prenatal and newborn babies widely. These disorders are included cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia and many other disorders.

Diagnose of such disorders in early make the medical staff ready to prepare themselves and the parents for sufficient treatment of the child. Genetic counselors use DNA fingerprint information to help parents understand the possibility of having an influenced child or use this information in their decisions for future pregnancy.

## Cures for Inherited Disorders:

Researches which are about inherited disorders on the chromosomes basically depend on the information that DNA fingerprints are contain. By studying the DNA fingerprints of families who are relatives and have a history of some inherited diseases or by comparing large groups of people with and without the disorder, it is help to detect DNA models which are associated with the certain disease. This idea is a crucial first step in sketching a final genetic cure for inherited diseases.



# Training



## Personal Identification:

Because all organs and tissues of an individual have the same DNA fingerprint, in some countries such as U.S. armed services have started to collect DNA fingerprints of all staff for later use for example in cases that they need to identify someone or detection persons' identification who were lost in mission. In near future the DNA fingerprint method will be over all dog tags, dental records, and blood typing strategies which are currently in use.

However, the idea of using DNA fingerprints as a genetic label to identify peoples' identity has been interested. This does not seem to occur in the near future. Because this technology needs to extract, record and then explain millions of certain VNTR models that are both precious and unpractical.

## Problems with DNA fingerprinting

Nothing about DNA fingerprinting is completely sure, the same as anything else in the scientific world. Since DNA fingerprint has been known as a sense like a fingerprint, the VNTR pattern for a person is entirely and completely singular to that person. In fact, all that a DNA fingerprinting can do is increased a probability that the person in question is truly the person to whom the VNTR pattern belongs (of a child, criminals or whatever else).

### 1- High Probability

The probability of a DNA fingerprint belonging to a person needs a high reasonability especially in criminal cases where the just evidences help a suspect to confirm her or his guilt or innocence. Using certain unusual VNTRs or union of VNTRs to exist the VNTR pattern arise the probability that the two DNA samples match or connect truly.

### 2- Determining Probability

#### A. Population Genetics

VNTRs are known as a conclusion of genetic inheritance while they are not spread through all of human population. So a donated VNTR cannot have a firm probability because it is so much depending on human being's genetic background. The difference in level of probabilities is specifically clear between different racial. Some VNTRs that happen very frequently in Hispanics will seldom happen in Caucasians or African-Americans.

#### B. Technical Difficulties

Some errors among the hybridization and probing process must also be affected on the probability and mostly this error is not satisfactory. Most people believe that an innocent person should not be in jail, a guilty person let be free, or a biological mother refuse her legal right to her child custody just because a technician in lab did not manage accuracy of an experiment. When the DNA sample available is a little, this is a special occasion which needs an important attention because there is not much room for error. Especially if the analysis of the DNA sample will be based on amplification of the primitive sample because if the incorrect DNA will be amplified the consequences can be seriously harmful.

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## Tiny Molecule Found to Boost Production of Brain Cells, Protects New Cells from Dying

Researchers from University of Texas have discovered a compound which keeps brain cells and boost learning memory in perfect condition. This study appears in the July 9, 2010, issue of the journal *Cell*.

Over a three-year period, the research team led by Dr. McKnight and Dr. Andrew Pieper, assistant professor of psychiatry and biochemistry at UT Southwestern, investigated 1,000 individual molecules.

They were trying to find out which molecules could enhance the production of neurons in the adult mouse hippocampus (hippocampus is a region of the brain which has important role in learning).

Finally, they found that one of the compounds, called P7C3, could protect newborn neurons from dying. The researchers then administered P7C3 to “knockout” mice lacking a gene that controls the generation of new neurons in the hippocampus. Humans who lack this gene have a variety of learning disabilities, and the knockout mice show related abnormalities as well as a poorly formed hippocampus. When the knockout mouse received P7C3, however, normal structure and function of the hippocampus were restored.

In elderly rats, P7C3 increased both the birth and survival of new neurons, and the memory and learning capability. The researchers are now evaluating the potential of P7C3 in protecting cells from dying in other neurodegenerative disease.

Dr. McKnight was one of the first 12 recipients of the NIH Director’s Pioneer Award, which is designed to allow researchers to do risky experiments that have the potential for producing highly innovative results. “When I received the award, I thought ‘I’m not going to waste it on something safe. That’s what the NIH expected of me and my team,’” Dr. McKnight said. “I’d like to give the NIH credit for betting on ‘cowboy’ science. If this pans out, it will be the most useful contribution of my career.” Dr. Francis Collins, director of the NIH, said Dr. McKnight’s results precisely fit the award’s purpose. “The NIH Director’s Pioneer Award gives highly innovative investigators the freedom to pursue bold new avenues of research. Such approaches can yield substantial payoffs, as in the case of the exciting clinical implications of Prof. McKnight’s basic neurobiological research discovery,” Dr. Collins said.

**Source:** <http://www.biotechdaily.com>, posted on 11 Aug., 2010

## A New Role for Insulin in Cell Survival, Cell Metabolism and Stress Response

Researchers from Buck Institute have found out insulin affect cell metabolism and cell survival. Scientists have known about insulin signal pathway, which is involved in aging, diabetes and stress response but Researchers from Buck Institute showed insulin is active at a deeper level of cell activity. This study was published in the September 8<sup>th</sup> issue of *Cell Metabolism*.

Insulin is extremely entailed in many cell functions. Gordon Lithgow, PhD, from Buck Institute faculty says, people have known about insulin role in transcription, where DNA produces RNA but our new research in the nematode worm *C. elegans*, showed that insulin is also have important role at the translation level, where RNA synthesized Protein.

This discovery opens a host of opportunities. Nowadays scientists are trying to understand why older people are candidate for disease. Lithgow said we want to know why diabetes is associated with aging, because here we have an insulin signaling pathway involved in aging, diabetes and stress response. This gives us more precise avenues to explore how we might intervene in disease.

Researcher demonstrated that increased tolerant to stress due to lower insulin signaling is not associated to stress induced responses at transcription level instead it requires active protein translation.

Lithgow says the research fits in with work being done in the Buck Institute laboratories of Brian Kennedy and Pankaj Kapahi, all of which point to the importance of translation. Lithgow, who directs the Institute's Geroscience program, says the research will lead to new collaborations.

Obtained results highlight the significant role of protein homeostasis in maintaining metabolic equilibrium. What proteins are made within the cell? When are they made? How and when are they gotten rid of? What happens when they are damaged? Lithgow believes protein homeostasis is vital for healthy aging and is intrinsically involved in diseases such as Parkinson's and Alzheimer's where protein homeostasis seems to get muddled up. At the end Lithgow said “now we need to connect with what is known about insulin signaling in diabetes with various disease states; we need to know how this part of cell metabolism is related to the aging and disease.”

Other Buck Institute researchers involved in the study include Aric N. Rogers, Silvestre Alavez, Alan E. Hubbard, Simon Melov and Pankaj Kapahi. Contributors include Gawain McColl and Ashley I. Bush from the Mental Health Research Institute, Parkville, Victoria, Australia; and Christopher D. Link, Institute for Behavioral Genetics, University of Colorado, Boulder. The research was supported by grants from The Glenn Foundation for Medical Research, The Herbert Simon Foundation, American Foundation for Aging Research, The Larry L. Hillblom Foundation, and the National Institutes of Health.

**Source:** <http://www.sciencedaily.com>, posted on 8 Sep., 2010

## Insect Brains are Rich Stores of New Antibiotics

Researchers from the University of Nottingham have discovered powerful antibiotics in the brains of cockroaches and locusts. It seems Cockroaches can be more of a health benefit than a health hazard.

Simon Lee, a postgraduate researcher who is presenting his work at the Society for General Microbiology's autumn meeting in Nottingham said "we identified nine different molecules in the insect tissues which had antimicrobial effects."

These substances could be a great alternative and a novel antibiotic for multi-drug resistant bacterial infections. The group found that the tissues of the brain and nervous system of the insects were able to kill more than 90% of Meticillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*, and also these substances are not cytotoxic for human cells.

Studying the specific properties of the antibacterial molecules is going on in the laboratory of University of Nottingham. They are trying to develop these antimicrobial substances for treatment of *E. coli* and MRSA infections that are resistant to current antibiotics. On the other hand current drugs may be effective but have serious side effects; due to this, these substances could be a potential alternative to current drugs.

The pharmaceutical industry is generating fewer and fewer new antibiotics due to lack of financial incentives, meaning that alternative sources of new drugs are much needed. Mr Lee said "Insects often live in unhygienic environments where they encounter many different types of bacteria. It is therefore logical that they have developed ways of protecting themselves against micro-organisms."



*Cockroach. Cockroaches could be more of a health benefit than a health hazard according to scientists from the University of Nottingham, who have discovered powerful antibiotic properties in the brains of cock-*

**Source:** <http://www.sciencedaily.com>, posted on 7 Sep., 2010

## NMR Spectroscopy

**Nuclear magnetic resonance spectroscopy**, most commonly known as **NMR spectroscopy**, is the name of a technique which describes the magnetic properties of certain nuclei. The most important applications of this technique are proton NMR and carbon-13 NMR spectroscopy. In general, NMR can apply for any nucleus possessing spin. Various types of information can be achieved by an NMR spectrum.

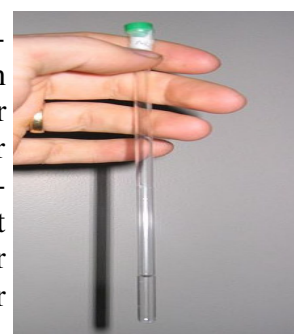
NMR spectrum provides information on the number and type of chemical identities in a molecule. However, NMR can be given lots of information about molecules. The effect of NMR spectroscopy on the natural sciences has been considerable. NMR can be used to study mixtures of analytes, to investigate dynamic effects such as change in temperature and reaction mechanisms. NMR is an invaluable tool in studying structures of protein and nucleic acid and their function. It can be used for many types of samples, both in the solution and the solid state.



A 900MHz NMR instrument with a 21.2 T magnet at HWB-NMR, Bir-

### Basic NMR techniques

When a molecule placed in a magnetic field, nuclei (such as  $^1\text{H}$  or  $^{13}\text{C}$ ) absorb at a frequency characteristic of the isotope. The resonant frequency, energy of the absorption and the intensity of the signal are proportional to the power of the magnetic field. For instance, in a 21 tesla magnetic field, protons resonate at 900 MHz. It is usual to refer to a 21 T magnet as a 900 MHz magnet, although different nuclei resonate at a different frequency at this field strength. In the Earth's magnetic field the nuclei resonate at audio frequencies. This effect is applied in Earth's field NMR spectrometers and other instruments. Because these instruments are simple and cheap, they are often used for teaching and field work.



NMR sample

### Chemical shift

Depending on the local chemical environment, different protons of a molecule resonate at different frequencies. Since both this frequency shift and the primary resonant frequency are directly proportional to the strength of the magnetic field, the shift is converted into a *field-independent* dimensionless value known as the chemical shift.



# Application



The chemical shift is described as a relative measure from reference resonance frequency. (For the nuclei  $^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{29}\text{Si}$ , TMS (tetramethylsilane) is generally used as a reference.) This dissimilarity between the frequency of the signal and the frequency of the reference is divided by frequency of the reference signal to obtain the chemical shift. The frequency shifts are very small in comparison to the fundamental NMR frequency. A typical frequency shift might be 100 Hz, compared to a fundamental NMR frequency of 100 MHz, so the chemical shift is usually reported in parts per million (ppm).<sup>[1]</sup> For being able to detect such small frequency differences it is necessary, that the external magnetic field varies much less throughout the sample volume. High resolution NMR spectrometers use to adjust the homogeneity of the magnetic field to parts per billion (ppb) in a volume of a few cubic centimeters.

By understanding different chemical environments, the chemical shift can be used to obtain some structural information about the sample's molecules. The conversion of the raw data to this information is called *assigning* the spectrum. For instance, for the  $^1\text{H}$ -NMR spectrum of ethanol ( $\text{CH}_3\text{CH}_2\text{OH}$ ), three specific signals at three specific chemical shifts would expect: one for the  $\text{CH}_3$  group, one for the  $\text{CH}_2$  group and one for the  $\text{OH}$  group. A typical  $\text{CH}_3$  group has a shift around 1 ppm, a  $\text{CH}_2$  attached to an OH has a shift of around 4 ppm and an OH has a shift around 2–3 ppm depending on the solvent.

Due to molecular movement at room temperature, the three methyl protons average out during the NMR experiment (which takes a few ms). These protons become degenerate and form a peak at the same chemical shift. The peaks sizes also indicate chemical structure. In the above example, the proton spectrum of ethanol, the  $\text{CH}_3$  peak would be three times as large as the OH. Similarly the  $\text{CH}_2$  peak would be twice the size of the OH peak but only 2/3 the size of the  $\text{CH}_3$  peak.

New software has advantage for analysis of the size of peaks to evaluate how many protons give ascend to the peak. This is known as integration, a mathematical process which estimates the area under a curve. The analyst must assimilate the peak and not asses its height because the peaks also have *width*, and thus its size is dependent on its area not its height. However, it should be mentioned that the number of protons, or any other observed nucleus, is only proportional to the power of the NMR signal, in the simplest one-dimensional NMR experiments. In more accurate experiments, for instance, experiments typically used to obtain carbon-13 NMR spectra; the integral of the signals depends on the relaxation rate of the nucleus, and its scalar and dipolar coupling constants. These factors are poorly known, therefore, the integral of the NMR signal is difficult to analyze in more complicated NMR experiments.

## Two-Dimensional NMR

In one-dimensional pulsed Fourier transform NMR the signal is verified as a function of one time variable and then Fourier transformed to give a spectrum which is a function of one frequency variable. In two-dimensional NMR the signal is recorded as a function of two time variables,  $t_1$  and  $t_2$ , and the resulting data Fourier transformed twice to yield a spectrum which is a function of two frequency variables. Two-dimensional NMR spectra supply more information about a molecule than one-dimensional NMR spectra and are particularly helpful in determining the structure of a molecule, particularly for molecules that are too complicated to work with using one-dimensional NMR. Various types of two-dimensional NMR include Correlation spectroscopy (COSY), J-spectroscopy, exchange spectroscopy (EXSY), Nuclear Overhauser effect spectroscopy (NOESY), total correlation spectroscopy (TOCSY) and heteronuclear correlation experiments, such as HSQC, HMQC, and HMBC.





# Application



## Solid-state nuclear magnetic resonance

Structural analysis of molecules or macromolecules and their dynamics have inherent advantages and disadvantages. In X-ray crystallography, we don't have any limitation about the size of macromolecule, but it requires making macromolecule crystals with high quality. Solution-state NMR only requires that the molecule be soluble at convenient concentration, but it is difficult to analyze biomolecules over 30 kDa.

Solid-state NMR (ssNMR) does not need that the sample in soluble or crystal form and this method can be applied to study molecules larger than 100 kD. However, ssNMR has set of limitations which scientists try to overcome. In solution NMR, the molecules in the sample tumble randomly at rates fast enough to average out anisotropic chemical shifts and couplings. The advantage of this natural isotropy (same in all directions) is that the NMR spectrum appears as a group of narrow, clear lines with sharp transitions. The disadvantage of this is that orientation-dependent (anisotropic) data are not present. In solution state NMR, some of this information can be recovered by orienting the molecules partially, for instance by adding phage particles that line up in the magnetic field. In solids, all of the anisotropic features are present and potentially limit the features observable in NMR spectra of biological macromolecules. However, NMR spectroscopists have methods of suppressing and controlling anisotropic interactions.

## NMR spectroscopy applied to proteins

Same as X-ray crystallography, NMR spectroscopy was used to obtain high resolution 3-dimensional structures of the protein. NMR is applied for relatively small proteins, usually smaller than 35 kDa.

NMR spectroscopy is often the only way to obtain high resolution structural information about unknown proteins. Due to the large number of atoms present in a protein molecule in comparison with a small organic compound, the basic 1D spectra become crowded and data analysis become untenable. Therefore, multidimensional (2, 3 or 4D) experiments have been developed to overcome this problem. To facilitate these experiments, it is desirable to isotopically label the protein with  $^{13}\text{C}$  and  $^{15}\text{N}$  because the predominant naturally occurring isotope  $^{12}\text{C}$  is not NMR-active, whereas the nuclear quadrupole moment of the predominant naturally occurring  $^{14}\text{N}$  isotope prevents high resolution information to be obtained from this nitrogen isotope. The most important method used for structure determination of proteins applies NOE experiments to measure distances between pairs of atoms within the molecule. Finally, the obtained distances are used to make a 3D structure of the molecule by solving a distance geometry problem.

### Source:

1-[http://en.wikipedia.org/wiki/NMR\\_spectroscopy](http://en.wikipedia.org/wiki/NMR_spectroscopy)

2- Bimolecular solid state NMR: Advances in Structural Methodology and Applications to Peptide and Protein Fibrils. Tycko, R. *Annual Review of Physical Chemistry* (2001)

3-Two-Dimensional NMR Methods for Establishing Molecular Connectivity, Martin, G.E; Zekter, A.S., VCH Publishers, Inc: New York, 1988 (p.59).

4- [http://www.bmrbl.wisc.edu/solid\\_state/](http://www.bmrbl.wisc.edu/solid_state/)



# Weblink



[www.biospace.com](http://www.biospace.com)

**BioSpace** is the leading online community for industry news and careers for life science professionals. For over 23 years, BioSpace has provided quality recruitment and job seeking opportunities for professionals in the biotechnology and pharmaceutical industries. In addition, BioSpace has accelerated communication and discovery among business and scientific leaders in the biopharmaceutical market.

**BioSpace.com** offers a multi-faceted venue for industry professionals to come together on the Web. This site presents:

- 1- The online job board
- 2-Daily biotech news feeds
- 3-connect recruiters job seekers

The screenshot shows the BioSpace website homepage. At the top left is the BioSpace logo with the tagline 'Life • Science • Community'. To its right is a search bar with the text 'Search the Site' and a dropdown menu set to 'News'. Further right is a 'Community Login' button with a user icon and the text 'What is this?'. Below the header is a navigation bar with tabs for 'Biotech/Pharma', 'Med Device/Diag', 'Clinical Research', and 'Academic/BioMed'. To the right of these tabs are links for 'Employers', 'Post Job', 'Search Resumes', and 'Login'. Below the navigation bar is a row of links: 'HOME', 'CAREER NETWORK', 'NEWS', 'HOTBEDS', 'EVENT CENTER', 'CAREER FAIRS', 'COMPANY PROFILES', 'BIOPHARM INSIGHT', and 'ABOUT US'. The main content area is divided into several sections. On the left is a 'NEWSLETTERS' section with links for 'Free Newsletters', 'Archive', and 'My eNewsletters'. Below that is a 'NEWS' section with links for 'News by Subject', 'News by Disease', 'News by Date', 'PLoS', 'Search News', 'Post Your News', and 'JoVE'. Further down is a 'CAREER NETWORK' section with links for 'Job Seeker Login', 'Most Recent Jobs', 'Search Jobs', 'Post Resume', 'Career Fairs', 'Career Resources', and 'For Employers'. At the bottom left is a 'COMMUNITY' section. The central part of the page features a large banner for 'The NEW 3500 Series Genetic Analyzer' with the tagline 'It becomes you.' and an image of a human eye. To the right of the banner is a 'Job Search' section with a search bar, a dropdown menu for location, and a 'Search' button. Below the job search is a 'Featured Stories' section with links for 'BioPharm Executive: Lilly, By Any Other Name...', 'Vitamin D Linked to Cancer, Autoimmune Disease Genes, Oxford University Study', and 'Roche (RHHBY) Enters \$1.1B Drug Deal With US's Alleron Therapeutics'. To the right of the featured stories is a green box for '800 Life Science Companies can't be wrong' with the text 'Trust the lab 800 others have for your small and large molecule product development' and the Lancaster Laboratories logo. At the bottom right is an orange box for 'ONLINE LAB AUCTION' with the text 'AUG 31 :: 9AM ET'. The footer of the page includes a small logo on the left and the text 'Internet | Protected Mode: On' on the right.

# Announcement

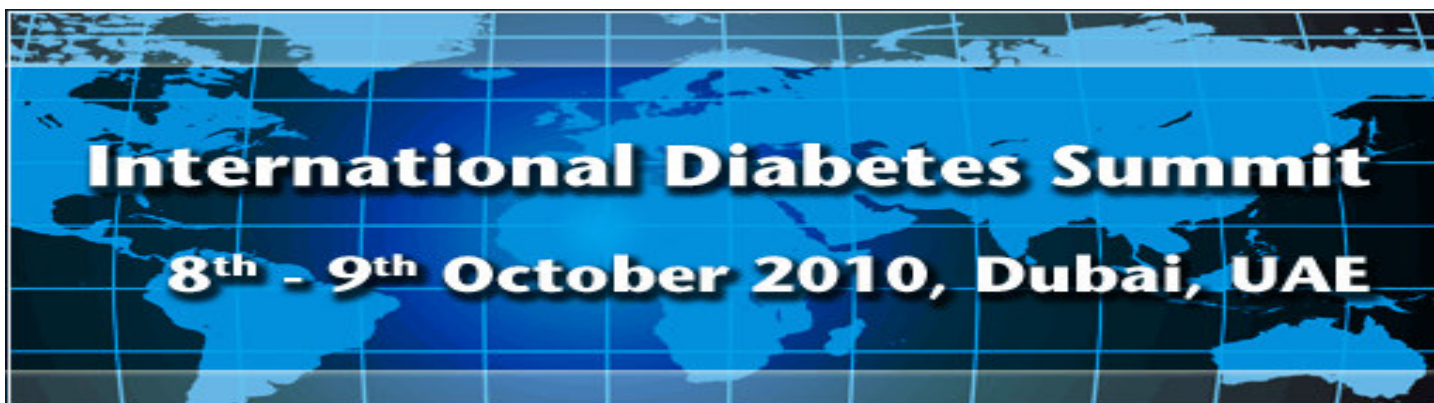


## International Conference on Antimicrobial Research



The preliminary scientific program will be available by 22 September. The definitive scientific program will be uploaded on the conference website by mid-October. The conference (scientific program) will start on Wednesday 3 November at 11:00 and it will finish on Friday 5 November around 19:00. The registration desk will be opened from Wednesday 3 November at 9:00 for distribution of the conference materials (but they can be picked up at anytime during the conference).

**For more information:** <http://www.formatex.org/icar2010/>



This conference is accredited by Ministry of Health, UAE for **10 CME credit points** and by Council of Nursing and Nursing Specialization for Cooperation Council States by **10 CNE credit points**.

**For more information:** <http://www.fleminggulf.com/life-science/middle-east/international-diabetes-summit>



# Announcement



## 3<sup>rd</sup> International Conference on Biotechnology for the Wellness Industry (ICBWI 2010)



This conference serves to provide a platform for people associated with the wellness industry to disseminate knowledge and share ideas. The conference aims to bring academicians, scientists, traditional practitioners, conventional health providers, complementary health providers, decision makers and policy makers forward for sharing of views and experiences.

**For more information:** <http://www.cepp.utm.my/icbwi2010>

## International Symposium of Drug Analysis 2010



Drug analysis 2010 will be held 21-24 Sep, 2010 in Antwerp, Belgium in the University Campus of Antwerp.

**For more information:** <http://www.druganalysis.org/>





# Announcement



The Biotechnology/Bimolecular chemistry program intends to hold **The 1st International Biotechnology Innovation Conference** (a Three days conference), it will take place November 21st: 23rd, 2010. The opening ceremony will be held in the main celebration hall, and the rest of the scientific events will take place in the Cairo university conference center, while the social and entertainment events will be done outside of the campus.

International distinguished scientists will be invited to attend the conference and referee the inventions participating in the conference according to their specialization. Other young scientists (18-35 years old) from other universities and disciplines, locally and internationally, will be invited to present their own inventions and innovations in the different scientific fields.

For more information: [www.ibic-egypt.com](http://www.ibic-egypt.com)

**IBIC-Egypt**

## Recently Published Books

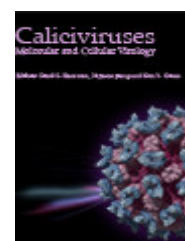
### *1- Caliciviruses: Molecular and Cellular Virology*

Edited by: Grant S. Hansman, Xi Jason Jiang and Kim Y. Green

ISBN: 978-1-904455-63-9

Publisher: Caister Academic Press

Publication Date: April 2010



### *2-Nanotechnology in Water Treatment Applications*

Edited by: T. Eugene Cloete, Michele de Kwaadsteniet, Marelize Botes

ISBN: 978-1-904455-66-0

Publisher: Caister Academic Press

Publication Date: June 2010

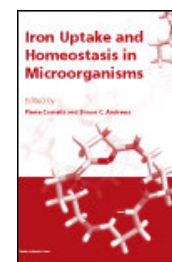


### *3- Iron Uptake and Homeostasis in Microorganisms*

Edited by: Pierre Cornelis and Simon C. Andrews *Laboratory of Microbial Interactions, Vrije Universiteit Brussel, Belgium and School of Biological Sciences, University of Reading, UK (respectively)*

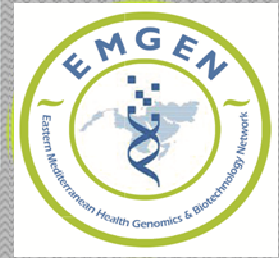
Publisher: Caister Academic Press

Publication date: June 2010





# Cover Picture



## **Title:** Reverse genetics

Mosses are small, soft plants that are typically 1–10 cm tall, though some species are much larger. *Physcomitrella patens* is a moss (Bryophyta) used as a model organism for studies on plant evolution, development and physiology. Mosses share fundamental genetic and physiological processes with vascular plants, although the two lineages diverged early in land plant evolution. *Physcomitrella patens* is one of a few known multicellular organisms with highly efficient homologous recombination. Basically, this means that researchers are able to target an exogenous DNA sequence to a specific genomic position (a technique called gene targeting) to create knockout mosses. This approach is called reverse genetics and it is a powerful and sensitive tool to study the function of genes and, when combined with studies in plants like *Arabidopsis thaliana*, can help to unravel major molecular trends of plant evolution.

Source: [http://en.wikipedia.org/wiki/Physcomitrella\\_patens](http://en.wikipedia.org/wiki/Physcomitrella_patens)

## **Title:** Photobioreactor

A photobioreactor is a bioreactor which incorporates some type of light source to provide photonic energy input into the reactor. Also, an open pond could be seen as photobioreactor, but mostly the term photobioreactor only refers to closed systems, systems closed to the environment having no direct exchange of gases and contaminants with the environment. Nowadays, some extremophiles (organisms that can grow under extreme conditions) are grown into open ponds. However, many other microalgae are promising for the production of an enormous variety of compounds. To cultivate also these algae and their products, monocultures have to be maintained and for that, enclosed photobioreactors have to be used. A photobioreactor can be described as an enclosed, illuminated culture vessel designed for controlled biomass production of phototrophic liquid cell suspension cultures.

Source: <http://en.wikipedia.org/wiki/Photobioreactor>

## **Title:** Actinobacteria

*Actinomyces*, is a genus of the actinobacteria class of bacteria. They are all Gram-positive and are usually described as looking like "sulfur granules." They can be either anaerobic or facultatively anaerobic. *Actinomyces* species do not form endospores, and, while individual bacteria are rod-shaped, morphologically *Actinomyces* colonies form fungus-like branched networks of hyphae. *Actinomyces* are known for causing disease in humans, and for the important role they play in soil ecology. As such, their presence is important in the formation of compost. Many *Actinomyces* species are opportunistic pathogens of humans and other mammals, particularly in the oral cavity. Many antibiotics are also produced from actinomycetes such as *saccharopolyspora erythraea*, which produces erythromycin.

Source: <http://en.wikipedia.org/wiki/Actinobacteria>

