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ABSTRACTS

Technology developments and applications

461: Open source drug discovery for infectious diseases—a case application on Tuberculosis (Phase I)

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The Human Genome sequence created stir in the scientific community with the promise to make a remarkable difference to healthcare. With Mycobacterium tuberculosis genome sequenced 10 years ago we still lack an effective therapy for tuberculosis. If we have not been able to solve the TB problem, how can we solve the problems of other complex human diseases? For life-style diseases, pharmaceutical companies actively scout advances in basic research in search of new and potentially lucrative drug targets. For TB, this is not the case. Early stage drug discovery is the key bottleneck in the pipeline to novel drugs for tuberculosis. Most public-funded institutions are involved in a lot of biomedical research but the journey from lead molecule to market is a 'close-door' activity which increases the drug discovery cost to a great extent. Open Source Drug Discovery (OSDD) is a CSIR lead initiative with the vision to provide affordable healthcare to the developing world by providing a global platform where the best minds can collaborate and collectively endeavor to solve the complex problems associated with discovering novel therapies for the diseases of the third world countries. Taking cue from the successes of 'Open Source approach' in IT and 'Human Genome Project', there is an urgent call for such a programme in healthcare. The OSDD concept aims to bring in the power of genomics, computational technologies and participation of young and brilliant talent from Universities and industrial partners. In this context, the development of OSDD portal is a key deliverable of the project. It will be the source of interaction among scientists and their peers, of storing data and retrieval, of analysis of biological systems through algorithms to name a few. Research problems demanding innovations will be posted. Users both from within and from outside the project community will be allowed to participate and appropriate prizes will be announced for the best solutions. The project 'Open Source Drug Discovery' conception envisions the participation of scientists and students in growing numbers as the momentum of the Open Source movement grows. Private companies are becoming conscious of their social responsibility and many would like to join such initiatives. OSDD project is committed to provide world class portal in Open Source mode to build and coalesce user community including tapping intelligent minds for affordable drug development for M. tuberculosis and will be presented in detail.

462: Testing SNP markers for human identification application

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Developing a detection system for degraded DNA samples presents a big challenge for the field of DNA-based human identification. Currently commercial human DNA identification methods are based on the use of short tandem repeat (STR) DNA markers. Applied Biosystems' AmpF/STR Identifier and Promega's PowerPlex 16 systems for human identification generate the STR PCR fragments up to 370 base pairs and 470 base pairs, respectively. When DNA samples are degraded, however, neither kit is able to generate the PCR products required for the identification. We investigated the use of single nucleotide polymorphism (SNP) markers for human identification. In the SNP approach much shorter DNA fragment are required, this will allow the system to work on degraded DNA samples. We select SNP markers with allele frequencies ranging from 0.35 to 0.5 among the Asian, African and Caucasian populations and test these markers on DNA samples from these populations. We calculated that 50 SNP markers can provide discrimination power of about one thousand trillion, so the odds that two individuals will have the same 50 SNP DNA profiles are about one in one thousand trillion. The discrimination power from 50 SNP is much higher than the current commercial products. Another advantage of SNP technology is that the SNP probes can be put on DNA chip or array platform. We test SNP markers on the DNA samples from Asian, African and Caucasian populations and are looking for the SNP markers which have desired allele frequencies cross all the human populations. Some SNP markers showed desired allele frequencies among all the population and some SNP markers only for certain population. This information is valuable for the SNP Human Identification technology development.

463: Enhanced workflow for sequencing PCR products by capillary electrophoresis

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Since the introduction of Sanger dideoxy sequencing, significant efforts have been directed toward increasing throughput by streamlining workflow. We describe here further enhancements for a PCR product resequencing workflow capable of reducing the total time, from beginning PCR reactions through completion of basecalling, to ~4 h. The workflow shown employs a new AmpliTaq Gold® Fast PCR Master Mix in conjunction with modified thermal cycler conditions to substantially reduce the time required for PCR amplification. Process time is further reduced through optimization of cycle sequencing conditions (BigDye® Terminator v1.1 Cycle Sequencing Kits). We have coupled these improvements with an efficient sequencing reaction cleanup protocol and decreased Capillary Electrophoresis (CE) run time using MicroAmp® Fast 96-Well Reaction Plates on an Applied Biosystems 3130 xl Genetic Analyzer. Overall data quality compares favorably with data obtained using previously documented methods. The increased efficiency and generation of high-quality results is vital to numerous research applications in reducing time for screening and validation.

464: ERα-bound chromatin interactions are prevalent and functional in the human breast cancer genome

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The three-dimensional structures of genomes in vivo are thought to be functional, but our understanding of chromatin interactions is hampered by a lack of genome-wide methods capable of detecting de novo interactions. Here we developed and validated the ChIA-PET method, a genome-wide, high-throughput, unbiased, and de novo approach for detecting chromatin interactions. Using ChIA-PET, we characterized ERα-mediated interactions in estrogen-treated human breast adenocarcinoma cells (MCF-7), generating the first whole genome interactome map. We detected an unprecedented number of interactions between ERα binding sites, suggesting that ERα-bound interactions are a primary mechanism by which ERα functions. Uncovering these interactions on a genome-wide scale would have been highly laborious and expensive with previous methods. With this resource, we showed that ERα-bound interactions are primarily intrachromosomal, complex, and functional. Specifically, we found that ERα-bound interactions are associated with gene activation, PolII marks, H3K4me3 marks, and FoxA1 binding. Our data offers a parsimonious mechanism that allows for the maximal sharing of transcription factors by bringing genomically distant transcription factor binding sites in 'cis' to function in close spatial proximity, increasing the number of Estrogen Response Elements in interaction complexes, and increasing the local concentration of factors involved in transcription such as PolII and inducing H3K4me3 marks, thus allowing transcription to proceed. We anticipate that the ChIA-PET method will be a starting point for detailed, whole genome investigations into chromatin interactions, and the extensive set of chromatin interactions found here will be a rich resource for studies on chromatin interactions and ERα biology.

465: Novel biomarkers in early stages of lung adenocarcinoma identified with oligonucleotide based DNA microarrays

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Expression profiling analysis of human cancers is a promising approach for obtaining precise molecular classification of cancers, for developing stratification tools for therapeutic regimes, and for predicting survival and disease recurrence. We prepared a 14,640 spot microrarrays for the expression monitoring of 3,048 genes involved in tumour development and apoptosis of cancer. The study included 45 patients with operable lung adenocarcinoma. The microarrays were prepared with 70-mer oligonucleotide sets AROS Human Apoptosis Subset v2.0 in Human Cancer Subset V3.0 (Operon) by spotting them to Nexterion Slide E using an MG1000 spotter. Forty genes (32 up-regulated and 8 down-regulated) with differential expression between N2 and N0 stage lung adenocarcinoma were identified. Selected up-regulated (SPP1, CLEC3B, TTYH1, RAI1, GATA2, RBL2, WNT2, KRT19, CRABP2, PMP22, MVP, HOXA4, IGLL1, MAS1 and CARD10) and down-regulated (CALM3, CD52, HBA2, CAV1, PRLR, VIPR1, NFKBIA, CYP4B1, VIM and S100A4) genes were further investigated with real time PCR for expression and copy number alterations. Losses and/or gains were characteristic of four genes, indicating that altered expression of those genes is probably due to copy number variations. Interestingly, a reverse correlation between SPP1 and VIPR1 expression was also indicated. Down-regulation of the VIPR1 gene in lung ADs and SCLCs corroborates its proposed tumour suppressor role. The lack of association between changes in expression or copy number of the VIPR1 gene with histological data suggests that changes in both expression and copy number are likely to occur early in AD development. Identification of the differentially expressed genes in lung cancer may provide better molecular markers for early diagnosis and identification of novel intervention sites for anti-cancer therapy.

466: Development of a high-throughput pyrosequencer with integrated sample preparation

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The Human Genome Project was essentially accomplished by a reduction in the cost of DNA sequencing by three orders of magnitude. To reduce the cost further and achieve the goal of 1,000 dollars per genome, a highly integrated platform with a new implementation of a robust sequencing technology with a proven reputation is needed. Depelopment of a flexible Superscalar Pyrosequencer to generate sequence data on mammalian genome in a single run with 20× coverage is underway. Pyrosequencing, a sequencing-by-synthesis method for DNA sequencing, is emerging as a popular platform for DNA analysis. It relies on the bioluminometric real-time detection of inorganic pyrophosphate (PPi) released on successful incorporation of nucleotides during DNA synthesis. Here, 1 pmol of DNA template can generate more than 6 billion photons at a wavelength of 560 nm, which can be easily detected. An inexpensive lab-on-a-chip platform integrated with CMOS image sensors is leveraged to perform pyrosequencing. This will enable any lab to perform de novo genome sequencing. To reach this goal, significant improvements have been made in the chemistry, microfluidic device fabrication, base-calling, assembly, and detection parts. We will present results from the first generation of our device, where the Pyrosequencing analysis will take place inside nanofluidic fiberoptic faceplates. First, a picolitre sized 50 micron wells are fabricated by SU-8 photolithography on fiber-optic faceplates. The DNA fragments and enzymes are immobilized on 30 micron sepharose beads and 2 micron paramagnetic beads, respectively. The beads are centrifuged into the SU-8 wells resulting only one DNA bead per well. The final device is integrated by pitch-matching wells on picotiterplate to pixels of an inexpensive and custom designed CMOS image sensor. A microfluidic flow-system consisting of a valve selector and peristaltic pump is designed to direct the reagent from one of the seven input reservoirs to the inlet of the device. The 50 mm × 2 mm region of the device is capable to sequence more than one million bases. To automate the whole system, a new scheme for template preparation will also be presented. The end result of this joint effort project will be an integrated Pyrosequencing machine enabling sequencing of 3 Gigabase of de novo genome in a single run for <1,000 dollars.

467: The evolution of a high-capacity, multi-user sample management network

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GenVault develops technology to simplify and reduce the cost of biological sample management. They were the first to conceive of high-density archives based on room temperature storage and the automated recovery of discrete bits of air-dried specimen material. A number of products are now based on that dry-state focus: including manual and automated hardware devices to organize room-temperature sample handling for a few hundred up to a few million specimens; a robust sample management software system; a chemical bar code that can be used to tag each air-dried sample internally; dry-state biological sample storage on treated paper disks in a 384 well plate format; a processing chemistry that enables the complete release of native DNA from dried tissue stored on such paper or other porous material; and finally, the development of non-paper, dry state storage media based on ceramics and porous, sponge-like elastomers. Those new storage matrices greatly extend the mass, volume and the type of bio-sample that can be stored in the air-dried state. Those various dry-state storage technologies will be briefly reviewed, individually. However, more importantly, GenVault has found a way to integrate those several dry-state sample storage technologies into a system that links both the (new) dry-state sample management approaches with (the best existing) methods of refrigerated sample management. That integrating technology, referred to as the Dynamic Archive System, allows DNA and protein (in both the refrigerated and dry-state) to be collected and managed in parallel, with information technology to coordinate sample acquisition and sample sharing among multiple sites. A timely example, for the largest-scale extension of that Dynamic Archive technology, will be discussed here, from the nation of Canada, who have recently announced completion of a multimillion-dollar national genetic archive located in Quebec. This 'Chicoutimi Biobank' allows highly regulated sample collection, storage and retrieval from sites throughout Quebec, to be shared internationally among the Public Population Project in Genomics (P3G) consortium, and with enough capacity to support several decades of robust sample collection and sharing. It is proposed that, with minimal site-specific modification, such Dynamic Archives could serve as a flexible infrastructure standard for population-scale genetics and proteomics, among large (and small) biobanks currently being developed throughout North America, Europe and Asia.

468: Human whole genome sequencing by high-throughput, short read oligonucleotide ligation

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The advent of new ultra-high throughput sequencing methods allows contemplation of resequencing thousands of human genomes to extensively survey common and rare forms of genetic variation that could have implications in health and disease. We sequenced the genome of an individual of African origin with the Applied Biosystems SOLiDTM system. The method allows sequencing of single or paired ends 25–50 bp reads of 108–109 templates on a single array containing beads with clonally amplified templates. We collected over a billion reads in 7 runs, amounting to a total of 32 Gbp of sequence, and obtained an average 12× haploid sequence coverage and 130× clone coverage. After aligning those reads to the hg18 reference assembly, over 95% of the genome is covered and 99.45% of the genome is spanned by a paired end clone. A novel error correction technique improves the accuracy of the aligned reads to >99.95%. With such accuracy, our results suggest that heterozygote identification can be efficiently achieved at 10–15× coverage levels, by comparison with known HapMap genotypes. We detected over 2 million SNPs, of which 81.4% are in dbSNP. We categorize the genes using the Panther ontology, and annotate the damaging potential of non-synonymous SNPs using PolyPhen. We discovered that transcription factors, ligases, growth factors, receptors, and RNA helicases are the molecular functions most under-represented for damaging mutations, and GPCR genes involved in Olfaction, and genes for Immunity and defense are the biological functions most over-represented for damaging mutations. Olfaction and Immunity have previously been observed to be under recent positive selection in

human populations; thus we recapitulate evidence of natural selection in a single human sample. About 90,000 small insertions and deletions (<12 bp) were identified with the use of paired end reads. Indels are significantly underrepresented in translated exons. The analysis of the distance and orientation of the paired end reads allowed the identification of thousands of putative insertions and deletions ranging from 50 bp to several Kb. We predict 50 inversions (25% previously reported in other samples) and four gene fusions resulting from deletions (two previously reported). Depth of coverage analysis allows the inference of copy-number variants. Our results provide guidance for future studies to discover sequence and structural variants in human populations and cancer with short-read next generation sequencing.

469: Evaluation of factors affecting the success of multiplex PCR

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A set of 200 genomic PCR primers was tested in multiplex experiments. All primers were working flawlessly in single-plex experiments, as determined by gel electrophoresis. In multiplex environment about 5% of 2-plex experiments and 25% of 4-plex experiments failed to produce at least one of expected PCR products. By using statistical model we determined the factors that have correlation with the degradation of the quality of multiplex PCR. The most important factor was the number of possible alternate genomic binding sites of primers, as determined by nearest-neighbour thermodynamic model. The same factor is also correlated with the degradation of the quality of single-plex PCR, but the effect is much stronger in multiplex conditions. We also found, that the hybridization affinity between primers of different PCR, calculated by nearest-neighbour thermodynamic model, is correlated with the degradation of the quality of multiplex PCR, although the effect is much lower than the effect of genomic hybridization. These results can be used in the design process of primers for multiplex PCR. Lower quality primers can be eliminated already in design phase. Additional testing for unwanted hybridization between different PCR primer pairs can be used as criterion in designing multiplex groups.

470: The potential of RNA interference—an overview

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The recent discoveries of RNA and RNAi have revolutionized our understanding of gene regulation. RNAi, with its high specificity, limited side effects, can be used as a tool to control the expression of specific genes and has potential as a therapeutic strategy to reduce the expression of unwanted genes. Being an area of intense, upfront research, it is proving to be useful to analyze quickly the functions of a number of genes and even mutations in a wide variety of organisms. RNAi has established a novel paradigm with far-reaching consequences in the field of regulation of transcription, including the study of many common and uncommon genetic disorders. The major challenge in turning RNAi into an effective therapeutic strategy is the delivery of its agents like synthetic double stranded RNAs to the target cells in mammalian body, which induces a set of different antiviral responses with the production of interferons that limits the ability of a virus to replicate further to carry out the mechanism of silencing. This challenging task of RNAi delivery can be dealt with methodology like viral vectors - Retrovirus, Lentivirus, Adenovirus, Adeno-Associated-Virus (AAV), and Baculovirus or by SNALP—stable nucleic acid-lipid particles or by particulate carriers like polyethyleneimine (PEI) or even by some modified bacterial carriers. Once this challenge is met then it will accelerate discoveries and deepen our existing knowledge on the whole genome. RNAi may facilitate drug screening and development by identifying genes that can confer drug resistance, providing information about the modes of action of novel compounds. It will be possible to find targets for various diseases ranging from oncogenes to growth factors and single nucleotide polymorphisms (SNPs), Hepatitis B, C virus and even HIV virus have also been important targets for potential RNAi therapy. The potency and potential therapeutic utility of RNAi is pumping renewed vigour into delivery-related research and this technology is expected to be the most soughtful treatment for the upcoming generations.

471: A new assay in functional genomics: monitoring cell differentiation in live cell chips by electric cell-substrate impedance sensing

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There is a great demand for cell-based in vitro screening systems for drug target validation and generation of resources for cell therapies. Our assay is focused on the formation of contacts between cells and between cells and the extracellular matrix, which is important for cell differentiation processes relevant in cancer therapy and tissue repair. Cellular interactions are measured with a method called electric cell-substrate impedance sensing (ECIS). By applying alternating current and measuring the voltage across an electrode, the impedance can be calculated and broken down into a series resistance and capacitance. Insulating membranes of spreading cells block and constrain the current flow, resulting in measured variations in the impedance. We showed the applicability of this system with different cell types and chip designs (Maercker et al. Eng Life Sci (2008) 8, 1–8). Mesenchymal stem cells (MSCs), isolated from bone marrow, were transferred to a specialized multi-well live cell chip with integrated electrodes which allowed us to measure cellular interactions by ECIS. The attachment of MSCs was tested after in vitro induction of differentiation into bone or fat tissue. Within 24 h, we were now able to distinguish different differentiation stages, which also were visualized by light microscopy in parallel. Specific impedance profiles already were visible within the first 2 h after starting growth in differentiation medium. In a "wound healing assay" in the live cell chips, adipogenic cells showed a reduced migration activity compared to non-differentiated MSCs. With HaCaT keratinocytes, we tested cell attachment on the level of gene expression. After induction (TetOn system) of the expression of nidogen, a protein critical for extracellular matrix

formation, cells showed stronger adhesion than without nidogen expression, which was documented by characteristic impedance spectra. Compatible RNAi silencing experiments are under way. With our measurements we are able to discriminate between cell types, cell densities, and differentiation status. It even is possible to describe the function of single adhesion molecules. Therefore, this noninvasive cell-based assay most probably not only will help us to improve biological resources for advanced cell therapies. Simulation of the homing process of stem cells in an in vitro assay also is a promising setup for large-scale gain-of-function or loss-of-function screenings in functional genomics.

472: Keeping pace with the innovation: an easily adaptable technique for rapidly generating humanized animal models in the era of human genome

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The phenomenal increase in the amount of information about human genome is having a huge impact on biomedical research. Transgenic mice have become invaluable resource and it serves as the most powerful tool for analysing gene function and regulation in vivo. Through research utilizing the transgenic mouse or non-human primate as a model for human disease, investigators can translate basic biological phenomena into a human health perspective. A rapid procedure for making transgenic mice is also needed for screening thousands of human genes whose functions are yet unknown.

Pronuclear DNA microinjection in the oocyte is the most frequently used technique for generating transgenic animals. This and other techniques for making transgenic animals are fairly complicated, requiring trained personnel, costly infrastructure and a large number of zygotes harvested from animals that are then killed. Due to technical difficulties in obtaining substantial number of embryos from non-human primates and limitations in repeated surgical intervention in them, it is practically difficult to make much needed models of transgenic primates necessary to study several diseases specific to primates. All these drawbacks generate a need for developing a handy and cost effective technique for producing transgenic animals preferably with minimum or no loss of animal lives. We have developed a reproducible non-terminal technique for inserting genes in testicular spermatogonial cells through in vivo electroporation of the testis. The optimal result was obtained by injecting linearized DNA into the testis of male mice, followed by electroporation using 8 square 40 V electric pulses in alternating direction with a time constant of 0.05 s and an inter pulse interval of $\sim$1 s. The transgenesis was confirmed by PCR, Southern blot analysis and immunohistochemical studies of the progeny. More than 90% of males electroporated with any one of the four different constructs successfully sired transgenic pups. Such electroporated males provide a valuable resource for continuous production of transgenic founders. This method requires neither assisted reproductive techniques nor sophisticated laboratory setup and highly trained personnel. This ethically superior (deathless) and easily adaptable time saving procedure opens avenues for developing several transgenic mice in short span of time and broadens the scope of making transgenic sub-human primates. [This study was funded by Department of Biotechnology, India].

473: Replacing PCR with COLD-PCR enriches variant DNA sequences and redefines the sensitivity of genetic testing

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The Polymerase Chain Reaction (PCR) has become the cornerstone of molecular diagnosis, with almost every genetic test aiming to identify DNA sequence variation incorporating PCR. A commonly encountered problem is that variant DNA sequences exist in the presence of a large majority of wild type alleles such as when DNA is obtained from heterogeneous cancer biopsies or when fetal alleles are sought in maternal blood. As a result, downstream assays are severely limited in their ability to identify subtle genetic changes that can have profound impact in clinical decision-making and outcome. We describe Co-amplification at Lower Denaturation temperature (COLD-PCR), a novel form of PCR that amplifies minority alleles selectively from mixtures of wild-type and mutation-containing sequences irrespective of the mutation type or position on the sequence (Technical Report, Nature Medicine, May 2008 issue). In COLD-PCR, an intermediate annealing temperature is used during PCR-cycling to allow cross-hybridization of mutant and wild type alleles; hetero-duplexes, which melt at lower temperatures than homo-duplexes, are then selectively denatured and amplified at Critical Denaturation Temperature (Tc), while homo-duplexes remain double-stranded and do not amplify efficiently. To validate COLD-PCR, we used serial dilutions of DNA from tumor-derived cell lines and genomic DNA from a series of colon and lung cancer surgical specimens, and plasma-circulating DNA collected under IRB approval were utilized for validation on clinical specimens. By replacing regular PCR with COLD-PCR prior to application of a range of assays (Sanger sequencing; Pyrosequencing; MALDI-TOF; dHPLC; RFLP; and Taqman) we improved mutation detection sensitivity up to 100-fold and identified several additional TP53/KRAS/EGFR mutations in heterogeneous cancer samples. About 4 of 43 surgical samples and 3 of 10 plasma samples tested contained clinically important mutations that were not detected by any of the methods tested when preceded by regular-PCR, but they were detectable following COLD-PCR. Replacement of regular PCR with COLD-PCR provides a universal boost to all mutation detection technologies and enables them to be used with the required confidence in routine screening of cancer specimens for somatic mutations, including low-level mutation screening of surgical/FFPE tumor samples or bodily fluids. COLD-PCR is equally applicable to fields other than cancer, such as pre-natal diagnosis, in infectious diseases and in epigenetics.

474: A new alternative for two dimensional gel electrophoresis: OFFGEL electrophoresis combined with high sensitivity protein sizing on microchips

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In the field of biomarker discovery there is still the need for easy to use and robust instrumentation that allow reproducible fractionation of complex protein mixtures with high resolution and highly sensitive

protein detection. Here we present a combination of methods that separate proteins in analogy to 2 dimensional gel electrophoresis according to their isoelectric point (pI) and molecular weight. For the first dimension, OFFGEL electrophoresis was implemented by using an in-liquid pI fractionation device. This method takes advantage of the of the high resolving power of gel-immobilized pH gradients but delivers sample in contrast to conventional isoelectric focusing in liquid phase. For the second dimension, a new method for high sensitivity analytical protein sizing on microchips was employed. This technique offers a sensitivity equivalent or better than silver staining but a linear dynamic range across four orders of magnitude.

475: Anti-Leishmanial Efficacy study of a new Liposomal Amphotericin B preparation in mouse model using Quantitative Real Time PCR

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In the present study we, for the first time, standardized a quantitative real time PCR (qRT-PCR) for evaluating the efficacy of new stable ionic amphiphilic formulation of amphotericin B against visceral leishmaniasis infection in mice model. BALB/c mice were infected via tail vein with *Leishmania donovani* (MHOM/IN/KE16/1998) promastigotes and after establishment of visceral infection animals were treated with new liposomal amphotericin B formulation. Using quantitative Real Time PCR with primers highly specific for the ITS region of *L. donovani* we observed the reduction in parasite load from liver and spleen. This is much more rapid than micro titration assays or even a conventional PCR. With this technique we were able to quantify parasite load of even 0.1 parasites per reaction. A dose of 5.0 and 7.5 mg/kg body weight the drug was able to completely clear the infection from infected mice.

Observations reveal that there is reduction in parasite load by 2 log unit in liver and spleen compared to untreated, 1 week after last drug dose was given and finally resulting in complete clearance of parasite. Leishmania DNA levels vary during and after treatment and their quantitation using qRT-PCR seems to be useful in real time analysis of the efficacy of drug.

476: Polymorphism of xenobiotics metabolic genes and risk prediction: A potential tool for cancer prevention

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It has been known that most carcinogens require metabolic activation in the human body for the carcinogenic effects. There are two major enzyme systems that metabolize potential carcinogens, either synthetic or naturally occurring in human body, which has been classified as phase I and phase II. Generally, phase I enzymes can activate the carcinogen directly and produce more active metabolites. Phase II enzymes can detoxify and process the activated metabolites for final breakdown or excretion. Therefore, the genetic polymorphism at the loci encoding Phase I enzymes, such as CYP1A1 and NQO1, and phase II enzyme GSTM 1 may result in inter-individual variation in carcinogen metabolism and are related with the origin of high risk genetic traits or risk population. That's why it is considered that the monitoring of carcinogen activating and deactivating gene polymorphism can be used in cancer prevention and therapy. In present article we are presenting the present state of knowledge in the field of genetic susceptibility and risk prediction.

477: Novel PEI based nanoparticles for delivery of nucleic acids

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Gene delivery mediated by polyethyleneimine (PEI) has been widely investigated as a potential non-viral delivery carrier both in vitro and in vivo. The application of PEI as a gene carrier, however, is hampered by its inherent charge associated toxicity in the cells. In an attempt to circumvent the toxicity of PEI and yet maintain the high transfection efficiency, novel nanoparticles of PEI crosslinked with hexametaphosphate (P-HMP) were designed and explored in vitro and in vivo. The size of nanoparticles was found to be less than 200 nm as deduced by DLS, AFM and TEM studies. Also, all the nanoparticles carried a positive zeta potential which decreased when complexed to nucleic acids. The efficacy of nanoparticles to impart protection to complexed DNA was assessed by treatment with DNase over a period of 2 h. It was observed that the complexed DNA was conferred a good protection even after 2 h of treatment with DNase I. Uptake studies of P-HMP (7.7%) nanoparticles at different time intervals, based on confocal laser scanning microscopy imaging indicate the internalization and presence of nanoparticles in the nucleus of cells after 2 h. The in vitro transfection efficiency of P-HMP nanoparticles was evaluated in different cell lines and compared with commercial reagents, Geneporter 2^{TM}, Fugene^{TM} and Superfect^{TM}. The transfection efficiency of P-HMP (7.7%) was observed to be nearly 1.3–6.4-fold higher as compared to commercial reagents. P-HMP (7.7%) nanoparticles also efficiently delivered GFP specific siRNA, resulting in >80% suppression in gene expression in COS-1 cells. the cell viability profiles of P-HMP nanoparticles/DNA complexes showed more than 80% cell viability in various mammalian cell lines. The biodistribution study of labeled P-HMP (7.7%)/DNA complex was carried out in Balb/c mice. It was found that the labeled P-HMP (7.7%)/DNA complexes accumulated in almost all the tissues. Highest retention of the labeled formulation was found to be in liver. These results signify the application of P-HMP nanoparticles as a potent delivery agent both in vitro and in vivo.

478: Characterization of whole blood gene expression profiles in sickle cell disease with enhanced transcript detection using globin mRNA reduction

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Room temperature whole blood mRNA stabilization procedures, such as the PAX gene system, are critical for the application of transcriptional analysis to population based clinical studies. Global transcriptome analysis of whole blood RNA using microarrays has been proven to be challenging due to the high abundance of globin transcripts that constitute 70% of whole blood mRNA in the blood.

This is a particular problem in patients with sickle cell disease, secondary to the high abundance of globin-expressing nucleated red blood cells and reticulocytes in the circulation. In order to more accurately measure the steady state whole blood transcriptome in sickle cell patients we evaluated the efficacy of reducing globin transcripts in paxgene stabilized RNA samples for genome-wide transcriptome analyses using oligonucleotide arrays. We demonstrate here by both microarrays and Q-PCR that the globin mRNA depletion method resulted in 55–65-fold reduction in globin transcripts in whole blood collected from healthy volunteers and sickle cell disease patients. This led to a dramatic improvement in microarray data quality with increased detection rate of expressed genes and improved overlap with the expression signatures of isolated peripheral blood mononuclear (PBMC) preparations. The differentially modulated genes from the globin depleted samples had a higher correlation coefficient to the 116 genes identified to be significantly altered in our previous study on sickle cell disease using PBMC preparations. Additionally, the analysis of differences between the whole blood transcriptome and PBMC transcriptome reveals important erythrocyte genes that participate in sickle cell pathogenesis and compensation. The combination of globin mRNA reduction after whole-blood RNA stabilization represents a robust clinical research methodology for the discovery of biomarkers for hematologic diseases and in multicenter clinical trials investigating a wide range of nonhematologic disorders where fractionation of cell types is impracticable.

479: A novel photoreactive heterobifunctional reagent, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine (MHAHD) for the preparation of oligonucleotide microarrays on modified glass surfaces

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A new heterobifunctional reagent, 1-*N*-(maleimidohexanoyl)-6-*N*-(anthraquinon-2-oyl) hexanediamine (MHAHD), has been developed, useful for making bioconjugates and immobilization of biomolecules, viz., oligonucleotides, peptides, proteins, etc., on a variety of carbon-containing solid surfaces under the influence of light and microwaves. Its maleimide moiety reacts with mercaptoalkyl functions present in biomolecules under microwaves, and the anthraquinone structure reacts with a variety of modified glass surfaces under ultraviolet irradiation (365 nm). The reagent has been used in two ways. First, the reagent, MHAHD, was first brought in contact with the modified glass support and exposed to long wavelength ultraviolet light (365 nm), thereby generating active maleimidoalkyl functions on the surface, which later react with appropriate mercaptoalkyl-containing biomolecules to fix them on the supports under microwaves in just 15 min. In another route, the proposed reagent was allowed to react first with mercaptoalkylated oligonucleotides to form the appropriate biomolecule-anthraquinone conjugate, which was then brought in contact with modified glass microslides, and exposed to ultraviolet light (365 nm), resulting in immobilization of the conjugates on the support. Both of the routes work satisfactorily and we could successfully immobilize a number of modified oligonucleotides on a modified glass surface. The oligonucleotide arrays produced by both the routes were analyzed by hybridization experiments (hybridization efficiency 30.13%) and subsequently used for the discrimination of base mismatches. The constructed microarrays were found to possess good thermal stability (only 4.5% loss of fluorescence intensity observed after ten cycles).

480: MetaBioME: comprehensive metagenomic BioMining engine to search novel biocatalysts in metagenomic datasets

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Microbial enzymes or biocatalysts offer ecologically friendly or 'green' solutions for cost-effective implementation of biochemical processes which produces a large variety of chemical substances used in areas like industry, biotechnology, agriculture, pharmaceutics, etc. However, only a few enzymes are currently employed for such applications due to the limited number of sequenced microbes, which is principally limited by the fact that most (>98%) of the microbes cannot be cultured, a necessary step for their sequencing by traditional methods. This yet unculturable majority of microbes conceal an enormous treasure of unknown biological functions locked in their hereunto unidentified genes, proteins and biochemical pathways which can enrich life sciences, industry, biotechnology and human health. In this scenario, metagenomics has emerged as a powerful culture-independent approach for exploring the complexity of microbial genomes in their natural environments. Therefore, to harness the potential of metagenomic information, we used our in-house developed computational resource 'iMetaSys' to carry out the comprehensive mining of ten diverse publicly available metagenomic datasets. Using available information, we curated a dataset of $\sim$500 known biocatalysts and classified them into nine broad application categories namely Agriculture, Biotechnology, Energy, Industry, Medicare, Environment, Nutrition, Enzymatic Analysis and General Applications. We mined the metagenomic datasets for these biocatalysts and identified several novel biocatalysts which could be used as alternatives to the existing ones. Using this strategy, we developed the comprehensive Metagenomic BioMining Engine (MetaBioME) platform to facilitate the computational identification and validation of commercially useful novel biocatalysts from metagenomic datasets and to carry out more advanced analyses. The main features of MetaBioME and a summary of the work will be presented.

481: 'Universal' control assay selection and evaluation using comparative genomics approach for copy number variation detection

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The importance of copy number variations (CNVs) in human genome has been demonstrated in recent publications, and various technologies, including TaqMan® real-time PCR assays, have been developed for detecting copy number variations. One of the challenges for accurately determining the gene copy number in a given sample is to use an appropriate control or reference assay for normalization in TaqMan® copy number assays. The quality of control assay will significantly affect the accuracy of copy number calling. In order to minimize the variability of control assay across populations and species, the target sequences of control/reference assay should possess the following properties: (1) exact two copies in diploid genome; (2) extremely conserved in reference genome and other mammalian genomes; (3) not located in regions with highly genome

rearrangement activities such as CNVs, indels, inversions, and segmental duplications, or with abundant genetic variations such as SNPs; (4) not susceptible to the diseases; (5) optimal sequence thermodynamics and compositions for TaqMan® assay design. To achieve such goals, we use a comparative genomics approach to select 42 candidate target sequences with high quality from multiple species including human, chimpanzee, rhesus monkey, mouse, and rat for control/reference assay design. Those newly defined control/reference assays have been tested in various samples, and their performances have been carefully evaluated. The top five control assays are suggested as 'universal' for copy number detection in multiple species using TaqMan® real-time PCR technology.