OMICs Varadero 2014

International Meeting on OMICs and Bioinformatics

Scientific Program

October 28th- 31st 2014

Memories Varadero Hotel, Cuba

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Welcome to OMICs Varadero 2014.

After the successful experience with Bioinformatics 2012 and Bioinformatics/OMICs 2013, OMICs 2014 was organized with a wider scope. The Meeting will cover topics such as Bioinformatics and statistical methods for genomic and proteomic research, Technologies for OMICS data generation, Mass spectra data processing and computational proteomics, Next Generation Sequencing: RNASeq, ChipSeq, DNA Methylation, Data Management and analysis, MetaGenomics; Personalized Medicine: Selection of drug-response markers, translational biomarkers, Cancer Genomics and Proteomics, Epigenetics, Integrative OMICS data analysis and System Biology based bioinformatics software development.

Scientific Program: Oral presentations

October, Tuesday 28th

Plenary Lectures

17:00 - 17:45	Cuban Biotechnology and the Center for Genetic Engineering and Biotechnology**.** **Dr. Gerardo Guillen Nieto** (Cuba)
17:50 - 18:35	Prospects and Opportunities for Genomic Medicine in Latin America. **Dr. King Jordan** (USA)

Wednesday, October 29th

Genomics

Chairpersons:	*Dr King Jordan, Dr Julio R Fernandez*
09:00 - 09:30	Admixture, selection and immunity in Latino genomes - **Dr King Jordan** (USA)
09:35 – 10:05	DNA methylation alterations in ovarian cancer: impact on cancer initiation, progression and response to therapy - **Dr Dimcho Bachvarov** (Canada)
10:10 - 10:40	OptiType: precision HLA typing from next-generation sequencing data - **Dr Oliver Kohlbacher** (Germany)
10:40 – 10:55	 - Poster viewing
10:55 - 11:25	Rs12979860 and rs8099917 polymorphisms of IL28b: simultaneous genotyping in Cuban health donors and HCV genotype 1 patients treated with CIGB230 in combination with INF-α plus ribavirin - **Dr Daniel Palenzuela** (Cuba)
11:30 - 12:00	Transcriptome analysis of tropical plants facilitated by next generation sequencing - **Dr Leonardo Mariño-Ramírez** (USA)
12:05 – 12:35	COMMD1 is a target for a cytotoxic peptide CIGB-552 - **Dr Julio R Fernandez** (Cuba)

Thursday, October 30th

Genomics – Bioinformatics

Chairpersons:	*Dr Leonardo Mariño-Ramírez – Dr Ricardo Bringas*
09:00 - 09:30	Highly sensitive feature detection in metabolomics and its application to high-througput nontargeted profiling - **Oliver Kohlbacher** (Germany)
09:35 – 10:05	National Center for Biotechnology Information (NCBI) services and databases for human variation and disease analysis - **Dr Leonardo Mariño-Ramírez** (USA)
10:10 - 10:40	BisoGenet 3.0: Building Biological networks in the Big-Data era - **Dr Ricardo Bringas** (Cuba)
10:45 - 11:15	Applications of Pyrosequencing Technology in Personalized Medicine - **Dr Enrique Sánchez** (Mexico)
11:15 - 11:30	 - Poster viewing
11:30 - 12:00	Marine Conus Toxins, A Strategy from Nature to Engineering Proteins from Big to Small Size - **Dr Alexei Fedórovish** Licea (Mexico)
12:05 – 12:30	Bioinformatics analysis of a Sweet potato RNASeq experiment: from sequences to pathways - **Dr Maria Elena Ochagavia** (Cuba)
12:35 – 13:00	Qiagen Solution for Personalized Medicine by Real time PCR - **Dr Enrique Sánchez** (Mexico)

Friday, October 31st

Proteomics

Chairpersons:	*Dr Bruno Domon – Dr Vladimir Besada*
08:30 – 08:55	Advances in Quantitative Proteomics - **Dr Bruno Domon** (Luxemburg)
09:00 - 09:25	Charge state selective separation of tryptic peptides for enhanced proteomic studies – **Dr Vladimir Besada** (Cuba)
09:30 - 09:55	Design of beta-hairpin peptides as entry inhibitors of Dengue virus **- Dr Glay Chinea** (Cuba)
10:00 - 10:25	Harmonization of Proteomics Workflows and Data: Integration of Results - **Dr Bruno Domon** (Luxemburg)
10:30 - 10:55	Structural characterization of sulfated N-glycans in a serine protease from the neotropical moth Hylesia metabus (Cramer [1775])(Lepidoptera, Saturniidae) - **Dr Gleysin Cabrera** (Cuba)
11:00 - 11:25	CIGB-300: A novel peptide-based CK2 inhibitor for cancer therapy. Proteomic tools for mechanism elucidation - **Dr Yasser Perera** (Cuba)

Poster presentations

Author	Country	Title
Raquel Quatrini	Chile	Comprehensive Characterization of the Pangenome of *Acidithiobacillus caldus*
Dubravko Pavokovic	Croatia	Expression and identification of intrinsically disordered proteins in *in vitro* sugar beet cell line during abiotic stress
Osvlado Yantorno	Argentina	Comparative transcriptional and translational analysis of Bordetella pertussis Tohama I reference strain and clinical isolates growing in biofilm
Yuliet Mazola	Cuba	Integrating Bioinformatics Tools to Handle Glycosylation
Amaury Pupo	Chile	Understanding the structural basis of permeation and selectivity in Hv1 proton channel
Cecilia Fernandez	Argentina	
Carlos Manuel Aleaga	Cuba	"Protium" a Platform for Analysis of Mass Spectrometric Data
Pamela Villegas	Chile	Biosintesis y caracterización de polihidroxialcanoatos producidos por *Burkholderia xenovorans* LB400
Mehmet Burçin Mutlu	Turkey	Ongoing Insect Genome Studies of the world and situation of Turkey
Yassel Ramos	Cuba	SDS-free PAGE: an alternative to OGE for peptide fractionation
Alexis Yero	Cuba	Interaction of human Inter-alpha Inibitor with dengue virus
Yassel Ramos	Cuba	Antitumor peptide CIGB-300 binds to B23/NPM and impairs in vivo Casein Kinase-2 (CK2)-mediated phosphorylation in myeloid leukemia cells
Arielis Rodriguez	Cuba	Proteomic profile modulated by the cardioprotective peptide CIGB500 in cardiomyoblast H9c2 cells.
Dianne Pupo	Cuba	Implementing an IgG purification methodology for analysis of the humoral response memory against domain III of Dengue virus.

Poster viewing and discussion with authors:

Posters can be viewed on:

Wednesday

- during the coffee break from 10:40 to 10:55
- at the end of the Oral Session

Thursday

- during the coffee break from 11:15 to 11:30
- at the end of the Oral Session

During this time viewers will have the opportunity to discuss posters with the authors on an individual basis. Accordingly, at least one of the poster authors should be present for the full duration of the viewing period.

Abstracts

Oral Presentations

Cuban Biotechnology and the Center for Genetic Engineering and Biotechnology

Author: **Gerardo Guillen Nieto**
Center for Genetic Engineering and Biotechnology. La Habana, Cuba.
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An extensive product pipeline has been built by the Cuban Biotechnology Institutions and by the Centre for Genetic Engineering and Biotechnology (CIGB). Growing under particular environment with limited resources, the biotechnology is tightly connected with the health system and is driven by a national collaboration instead of competition. The urgency for immediate application of scientific results reinforced the need for expanding collaboration across the sector. Research generated by the CIGB has developed a number of products with significant impact to society. An overview of the achievements including the R&D projects with a strong intellectual property position and the project pipeline comprising several hepatitis related projects will be introduced to the participants.

Prospects and Opportunities for Genomic Medicine in Latin America

Author: **King Jordan**
Director, Bioinformatics Graduate Program Georgia Institute of Technology
king.jordan@biology.gatech.edu

Technological developments in genomics and bioinformatics hold promise to revolutionize the practice of medicine and healthcare delivery. In this talk, I will provide an overview of several distinct areas of research and development in genomics and bioinformatics with respect to their relevance to human health. The common thread that unites these different areas of investigation is the interrogation of the relationship of genome sequence variation with human health and/or disease. This genome sequence variation can be inter-individual variation between human genome sequences or genome sequence variation of the microbial pathogens that cause infectious disease.
I will cover several specific examples of how genomic and bioinformatic techniques can be used to reveal the relationship of either human or microbial genome sequence variation to human health with an emphasis on their applicability to populations and countries in Latin America. These topics will be addressed in such a way as to highlight the unique challenges and opportunities facing the development of application of novel genomic medicine technologies in the region.

Ancestry, Admixture and Selection in Latino Genomes

Author: **King Jordan**
Director, Bioinformatics Graduate Program Georgia Institute of Technology.
king.jordan@biology.gatech.edu

Latin America has a highly admixed population with ancestry components from Africa, the Americas and Europe. We performed a large-scale comparative analysis of complete Latino genome sequences with ancestral source population sequences in an effort to characterize Latino admixture patterns and to explore the potential relationship between admixture, natural selection and determinants of human health. This analysis included a total of 685 complete genomes/genotypes: 61 Latino genome sequences from Medellin, Colombia, 212 additional Latin American genotypes and 412 sequences/genotypes from putative ancestral populations. We were able to define admixture patterns for the Colombian genomes at base pair resolution allowing for both sex-specific and regional-specific admixture analyses. As previously reported, Mestizo-Colombian genomes show sexually asymmetric patterns of admixture with predominantly European paternal contributions and Native American maternal contributions, whereas Afro-Colombian genome sequences show no such asymmetry. We developed and applied an admixture enrichment analysis technique to search for genomic regions that show anomalous levels of admixture from a single ancestral source population.

DNA methylation alterations in ovarian cancer: impact on cancer initiation, progression and response to therapy

Author: **Dimcho Bachvarov**[1,2], Mamadou Keita[1,3], Zhi-Qiang Wang[1,3], Jean-Francois Pelletier[1,3], Magdalena Bachvarova[3], Marie Plante[2,3] Jean Gregoire[2,3], Marie-Claude Renaud[2,3], Anne-Marie Mes-Masson[4,5], Éric R. Paquet[2]
[1]Dept. of Molecular Medicine and [2]Dept. of Obstetrics and Gynecology, Laval University, Quebec PQ, Canada; [3]Centre de recherche du Centre Hospitalier Universitaire de Quebec, Quebec PQ, Canada; [4]Dept. of Medicine, Université de Montréal, Montreal, PQ Canada; [5]CHUM - Institut du cancer de Montréal, Montréal (Québec), Canada

We used methylated DNA immunoprecipitation (MeDIP) in combination with CpG island tiling arrays to characterize at high resolution the DNA methylation changes that occur in the genome of serous epithelial ovarian cancer (EOC) during disease progression. The DNA methylation profiles of five serous borderline, five serous grade 1/stage III/IV, five serous grade 3/stage I and five serous grade 3/stage III/IV EOC tumors were compared with those of five normal human ovarian tissue samples.

We found widespread DNA hypermethylation at CpG islands in serous tumors that occurs even in less invasive/early stages of ovarian tumorigenesis. This hypermethylation preferentially included key developmental/homeobox genes and is possibly associated with repressive chromatin marks such as Polycomb group proteins-mediated gene silencing. Contrary to DNA hypermethylation, significant DNA hypomethylation was observed only in high-grade (G3) serous tumors. The later observation was further confirmed when comparing the DNA methylation profiles of primary cell cultures derived from matched tumor samples obtained prior to, and following chemotherapy treatment from two serous EOC patients with advanced disease. To our knowledge this is the first report that has shown the presence of massive DNA hypomethylation in advanced serous EOC, associated with the possible induction of a number of oncogenes, implicated in cancer progression, invasion/metastasis and probably chemoresistance. Our data raise the concern that demethylating drugs that are currently being used in advanced EOC disease (representing the majority of serous EOC

cases) might have adverse effects due to activation of oncogenes and prometastatic genes. Understanding the relative roles of hypomethylation and hypermethylation in cancer could have clear implications on the therapeutic use of agents targeting the DNA methylation machinery. Our epigenomic approach has also led to the identification of novel aberrantly methylated gene targets in serous EOC, including hypermethylated genes with potential tumor-suppressor gene function, and hypomethylated genes, involved in disease progression. These genes could represent new therapeutic targets and/or novel biomarkers indicative for EOC etiology.

Using a combination of chemical genomics, proteomics, lipidomics and metabolomics for discovery of natural anti-aging and anti-cancer compounds and defining mechanisms of their action.

Authors: Vincent R. Richard, Adam Beach, Anna Leonov, Amanda Piano, Rachel Feldman, Michelle Burstein, Anthony Arlia-Ciommo, Veronika Svistkova and **Vladimir I. Titorenko**
Department of Biology, Concordia University, Montreal, Quebec, Canada

Caloric restriction and dietary restriction extend lifespan across species and improve health by delaying the onset of age-related diseases. All currently known anti-aging drugs: (1) mimic life-extending and health-improving effects of CR and DR without restricting caloric and nutrient intake; and (2) target signaling pathways that are under the stringent control of calorie and/or nutrient availability. It was believed therefore that all longevity pathways are “adaptable” by nature because they modulate longevity only in response to certain changes in the extracellular and intracellular nutrient and energy status of an organism. However, it is possible that that some longevity pathways could be “constitutive” or “housekeeping” because they control longevity irrespective of calorie and/or nutrient availability. We designed a high-throughput chemical genetic screen for compounds that increase the lifespan of yeast under CR by modulating such housekeeping pathways. Our screen identified lithocholic acid (LCA) as one of them. Using proteomics, lipidomics, metabolomics and cell biological approaches, we found that LCA delays aging by: (1) remodeling lipid metabolism in the endoplasmic reticulum, lipid droplets and peroxisomes thereby preventing liponecrotic programmed cell death caused by the age-related accumulation of fatty acids; (2) remodeling the repertoire of mitochondrial membrane lipids thereby reducing the number of mitochondria, increasing their size, expanding their cristae, elevating the abundance of respiratory supercomplexes in the inner mitochondrial membrane, and altering the age-related dynamics of changes in mitochondrial respiration, membrane potential and reactive oxygen species; (3) attenuating age-related mitochondrial fragmentation thereby suppressing mitochondria-controlled apoptosis; and (4) promoting “mitohormesis” through the activation of several stress response-related processes in mitochondria. Our findings also imply that, in addition to its robust anti-aging effect, LCA exhibits a potent and selective anti-tumor effect in cultured human neuroblastoma, glioma, breast cancer and prostate cancer cells by activating both the intrinsic and extrinsic pathways of apoptotic death.

Highly sensitive feature detection for LC-MS-based metabolomics

Author: **Oliver Kohlbacher**
Center for Bioinformatics, Quantitative Biology Center, and Dept. of Computer Science, University of Tübingen, Germany

LC-ESI-MS permits a quick and cost-effective screening for metabolites and is increasingly becoming popular in clinical applications.
Sensitive and reliable detection as well as accurate quantification of metabolites is required in particular for large-scale studies.
We present a novel algorithm for quantifying small molecule features [Kenar et al., MCP, 2014, 13(1):348-59]. It combines a sensitive mass trace detection with an efficient feature assembly algorithm based on a machine-learning model for recognize metabolite isotope cluster.
Compared to other algorithms, we can demonstrate a higher sensitivity and excellent linearity of the quantification. The algorithm is available as part of the OpenMS software package.

Transcriptome analysis of tropical plants facilitated by next generation sequencing

Author: **Leonardo Mariño Ramirez**
Computational Biology Branch, Bioinformatics of Chromatin Structure Group
NCBI
Next generation sequencing technologies and bioinformatics tools for the analysis of massive parallel sequencing allows the exploration of transcriptomes in non-model organisms. We are investigating the transcriptomes of Cape gooseberry and Banana under a variety of conditions to understand their unique metabolic pathways and contribute to crop improvement efforts. Here I will present recent advances in computational strategies used to assemble and annotate the Cape gooseberry and Banana transcriptomes.

COMMD1 is a target for a cytotoxic peptide CIGB-552

Authors: **Fernández Massó JR[1]**, Oliva Argüelles B[3], Palenzuela Gardon D[1], Guillén Perez I[1], Vázquez Blomquist D[1], Delgado-Roche L[2], Novoa Perez L[1], Guerra Vallespi M[3].[1]
Department of Genomics, Center for Genetic Engineering and Biotechnology Havana, Cuba; [2] Center of Studies for Research and Biological Evaluations, Pharmacy and Food Sciences College, University of Havana,[3] Pharmaceutical Department, Laboratory of Cancer Biology, Center for Genetic Engineering and Biotechnology, Cuba Julio.fernandez@cigb.edu.cu

CIGB-552 is a second-generation peptide with increased cytotoxic activity on murine and human tumor cells. Its cell-penetrating capacity is an associated useful property as drug targeting molecule. Although, the antitumor effects of the peptide involve a negative regulation of cell-cycle progression and apoptosis, little is known regarding the mechanism of action. By combining proteomics and gene expression analysis, we now propose COMMD1 as the most likely target of the CIGB-552. shRNAs against COMMD1 reduce the cytotoxic activity of NCI-H460 cells. In addition, COMMD1 accumulation is necessary for apoptosis. As we have shown in this study, the combined use of these approaches is valuable in identifying new therapeutic targets and biological mechanisms.

Rs12979860 and rs8099917 polymorphisms of IL28b: simultaneous genotyping in Cuban health donors and HCV genotype 1 patients treated with CIGB230 in combination with INF-α plus ribavirin.

Author: **Daniel Palenzuela** daniel.palenzuela@cigb.edu.cu

Several host and viral factors have been found to be associated with differences in HCV clearance or persistence. In 2009 three independent studies strongly correlated the presence of IL28B single nucleotide polymorphism (SNP) with sustained virogical response (SVR) and spontaneous clearance (SC) in HCV chronic patients of genotype 1, treated with pegylated interferon alpha plus ribavirin PEG-INF/RBN [i,ii,iii]. Recent evidence shows that determination of one single IL28B SNP, rs12979860, is sufficient for predicting treatment outcome. We evaluate two IL28B SNP, rs12979860 and rs8099917 in a cohort of genotype 1b chronic HCV patients and in a reference sample of Cuban health donors. The allele frequencies found in the health donor sample was rs12979860 C (55%), rs12979860 T (45%) and for rs8099917 T and G were 80% and 20%, respectively. In the health donor cohort of 400 individuals, the overall genotype distribution of IL28B rs1297860 CC, CT, and TT was 30%, 49%, and 21% and the distribution of rs8099917 TT, TG, and GG was 65%, 32%, and 3%, respectively. SVR rates in HCV cohort were 55%, 42%, and 35% for rs1297860 CC, CT and TT and 52%, 30%, and 25% for rs8099917 TT, GT, and GG, respectively. Because rs8099917 T allele was the most frequent in Cuban population, with high percent of SVR for homozygous responder rs8099917 TT and its high PPV for treatment success and failure; we recommend combined genotyping rs12979860 and rs8099917, to predict SVR in Cuban chronic HCV type 1 infected patients.

Precision HLA typing based on combinatorial optimization with OptiType

Author: **Oliver Kohlbacher**
Center for Bioinformatics, Quantitative Biology Center, and Dept. of Computer Science, University of Tübingen, Germany

The human leukocyte antigen (HLA) gene cluster plays a crucial role in adaptive immunity and is thus relevant in many biomedical applications. While next-generation sequencing data are often available for a patient, deducing the HLA genotype is difficult because of substantial sequence similarity within the cluster and exceptionally high variability of the loci. Established approaches, therefore, rely on specific HLA enrichment and sequencing techniques, coming at an additional cost and extra turnaround time.
We present OptiType, a novel HLA genotyping algorithm based on integer linear programming, capable of producing accurate predictions from NGS data not specifically enriched for the HLA cluster. We also present a comprehensive benchmark dataset consisting of RNA, exome and whole-genome sequencing data. OptiType significantly outperformed previously published in silico approaches with an overall accuracy of 97% enabling its use in a broad range of applications.

The National Center for Biotechnology Information (NCBI) services and databases for human variation and disease analysis

Author: **Leonardo Mariño Ramirez**

Computational Biology Branch, Bioinformatics of Chromatin Structure Group
NCBI

The NCBI integrates large and complex biomedical information that comprises literature, health, genomic, proteomic and chemical related resources. The genomic data explosion catalyzed by the widespread adoption of next generation sequencing technologies opens new research avenues but also generate significant challenges for data storage, analysis and interpretation to build biological knowledge. In this talk, I will be presenting recent resources for the analysis of human variation in relation to disease and how these resources facilitate population genomic studies that could be translated into better human health in the near future.

BisoGenet 3.0: Building Biological Networks in the Big-Data Era

Authors: Alexander Martin Tornet, Maria Elena Ochagavia Roque, Jamilet Miranda Navarro, Carelys Suarez Arencibia, Jorge Fernandez de Cossio, **Ricardo Bringas Perez**
Center for Genetic Engineering and Biotechnology. ricardo.bringas@cigb.edu.cu

Network Biology represents components of complex system as nodes and relations between them as edges. The main aim of such representation is to decipher the changes that take places in such networks when the system is perturbed by one or more factors such as the development of a disease, a viral or bacterial infection, the effects of a drug or some other environmental factors. Several bioinformatics application has been developed for biological network analysis. One such applications is BisoGenet a multi-tier application for building, visualization and analysis of biological networks developed at CIGB. From public data sources an in-house database (data tier) integrates information on genes, proteins, protein-protein interactions, transcription factor-DNA regulatory interactions an microRNA-gene silencing interactions. In the middle tier (the server) a network is created representing the bioentities and their relationships. The client is a CytoScape plugin with a friendly user interface. Recently BisoGenet was redesigned to comply with CytoScape 3.x new requirements. Another recent addition was the incorporation of ChIP-Seq experiment results from the Encyclopedia of DNA Elements at UCSC (Encode Project). In the last few years, as we enter the so called Big-Data era, an increasingly amount of information is generated from ChIP-Seq experiments. The Encode project collects data from such experiments for a high variety of transcription factors and cell lines. BisoGenet 3.0 now incorporates data from Encode project as “transcription factors”-gene regulatory interactions. Data on 161 transcription factors in 91 cell lines generated more than 400 000 regulatory interactions now incorporated into BisoGenet data tier.

Applications of Pyrosequencing Technology in Personalized Medicine

Author: **Enrique Sánchez**

(PENDIENTE)

Marine Conus Toxins, A Strategy from Nature to Engineering Proteins from Big to Small Size

Author: **Alexei Fedórovish Licea Navarro**
Molecular Immunology and Biotoxin Laboratory, Biomedical Development Unit/ Marine Biotechnology Department, CICESE alicea@cicese.mx

We have been working in the isolation of new drugs for the last 20 years. The Conus toxins are one of the most fascinating molecules for this porpoise.

These toxins interact with different membrane proteins from our cells. These toxins can regulate several cell activities, they can increase the secretion level of proteins, they can kill the different type bacteria or parasites, they can block chronic pain in degenerative disease and they can stop the cancer cell division.

However, these toxins have been engineering by nature with a huge number of posttranslational modifications in order to reduce at maximum level the size of the proteins without losing the activity and specificity.

Bioinformatics analysis of a sweet potato RNASeq experiment: from sequences to pathways.

Authors: **Maria Elena Ochagavia**[1], Cathie Martin[2], Chris Watkins[3], Aylin Nordelo Valdivia[4] and Rolando Morán[4*] [1]*Bioinformatics Department, CIGB, Havana, Cuba* [2]*John Innes Centre, Norwich, UK* [3]*The Genome Analysis Centre (TGAC), Norwich, UK* [4]*CIGB, Camagüey, Cuba*
**Corresponding author, email: rolando.moran@cigb.edu.cu*

The sweet potato (*Ipomoea batatas L*) is widely grown around the world due to its strong adaptability, rich nutrient content and multiple usages. Currently, sweet potato is the fifth most important food crop in developing countries. Nevertheless, no complete and well annotated genome is still available and only a few transcriptome sequencing studies have been published so far. As a result, sweet potato breeding and their applications has been progressing slowly.
In this study, we report a high-throughput RNA sequencing study for analyzing the transcriptome of the sweet potato in two plant tissues and two developing stages (young and mature leaves as well as young and mature roots). After de novo transcriptome assembly, a total of 135087 unigenes were obtained and ranged from 201 to 13100 nt with an average length of 879 nt. The average expression of one unigene was 9.1 reads per kb per million reads (RPKM) with a maximum expression of 61440.9 RPKM. Unigenes annotation was obtained by scanning major protein databases and PFAM protein families. Gene Ontology annotations and KEGG metabolic pathways were also inferred. Among other findings, most enzymes involved in the terpenoid synthesis pathways, which have been associated with the synthesis of secondary metabolites that attract oviposition of sweet potato weevil pest (Cylas formicarius elegantulus) and transcription factors involved in flavonoid synthesis were identified. Our results could contribute to identify gene targets to combat sweet potato weevil pest and to conceive strategies for the development of expression systems of flavonoids in plant cells.

Qiagen Solution for Personalized Medicine by Real time PCR

Author: **Enrique Sánchez**

(PENDIENTE)

Advances in Quantitative Proteomics

Author : **Bruno Domon**
Luxembourg Clinical Proteomics Center, CRP-Sante, Luxembourg

During the past decade hybrid high-resolution accurate mass spectrometers have undergone tremendous advances. High-frequency data acquisition has enabled large-scale qualitative analyses, and the identification of a major cross-section of a proteome has become achievable in one single LC-MS experiment. This type of instrumentation, and more specifically quadrupole-orbitrap mass spectrometers, can also be applied to quantitative measurements. While quantification is routinely performed on triple quadrupole instruments using a targeted acquisition strategy, the orbitrap based platform presents unique characteristics that have opened new avenues for such analyses. First, the high-resolving power of the mass analyzer effectively separates the analytes from the interferences of the matrix, resulting in an increased analytical precision. Second, the accurate mass measurements allow unambiguous assignment of the precursor and the fragments ions, and thus a higher level of confidence in the results. Third, the trapping capabilities of the instrument enable the detection and quantification of low concentration analytes, as ions can be collected over a longer period of time.
A new quantification workflow was developed and applied to the analysis of clinical samples, in which the peptide is first identified using the full MS/MS spectrum, and then subsequently quantified using stable isotope dilution. The internal standards are used as landmarks during the data acquisition to trigger the actual measurements of the analytes of interest. The technique was applied to measure lung cancer biomarkers in plasma samples, and an extension of the technique allows to the analysis of protein mutations in tissue samples.

Structural characterization of sulfated N-glycans in a serine protease from the neotropical moth Hylesia metabus (Cramer [1775])(Lepidoptera, Saturniidae)

Authors: ***Gleysin Cabrera****, Raquel Montesino, Yanet Támbara, Teresa Núñez-Villavicencio, Ada Triguero, Annia González-Hernández, Madelón Portela, Rosario Durán, Ulf Lundberg, Eva Vonasek, Luis Javier González* gleysin.cabrera@cigb.edu.cu

Contact with the urticating setae from the abdomen of the adult females and the egg-nests of the neo-tropical moth Hylesia metabus gives rise to a condition known as lepidopterism, characterized by an urticating dermatitis, ocular lesions and generalized malice. The urticating setae contains a toxic glycoprotein with proteolytic and proinflammatory properties. This glycoprotein has a significant sequence homology with other serine proteases of insects belonging to S1A subfamily. N-glycans obtained by digestion with PNGase-A and PNGase-F were analyzed by Normal Phase HPLC, Weak Anion-Exchange HPLC and MALDI-MS. Five main different complex paucimannosidic N-glycans were identified, half of which were exclusively α1-6- fucosylated at the proximal N-acetylglucosamine. No antennal α1-3-fucosylated Lewis-like structures were found in the assigned glycoforms therefore they could not be associated to the lepidopterism. Weak Anion-Exchange chromatography separated the N-glycans into two pools of neutral and negatively-charged species. A methanolysis specific to release the sulfate groups, revealed that the structures of a negatively-charged species are the same reported for the neutral species but bearing a single sulfate group. MALDI-TOFTOF analysis and tandem exoglycosidase digestion complemented with a methanolysis at the last step suggested that the sulfate group is located in one the mannose residues linked a(1-6) or a(1-3) to the innermost mannose of the core. This is the first report of sulfate groups in N-glycans of insects. The possible role of the sulfatation in the N-glycans of this protease is discussed.

Design of beta-hairpin peptides as entry inhibitors of Dengue virus

Authors: **Glay Chinea**, Vivian Huerta, Noralvis Fleitas, Patricia Toledo, Alejandro Martín, Viviana Falcón, Dianne Pupo, Yamilé Vidal, Osmany Guirola, Mónica Sarría, Osvaldo Reyes, Hilda Garay, and Luis Javier González, Centre for Genetic Engineering and Biotechnology, Havana, Cuba, glay.chinea@cigb.edu.cu

Beta-hairpins are common structural motifs of proteins often involved in protein-protein interactions. Here we show the design of structurally constrained synthetic peptides based on the beta-hairpin FG from domain III (DIII) of the envelope protein of Dengue virus (DV), which lead to the ultimate development of peptides showing potent antiviral activity. DIII is involved in key virus/host-cell interactions and it is target of potent anti-DV neutralizing antibodies (Abs). Proteomics analysis indicates that DIII interact with serum proteins and putative membrane receptors.

First we demonstrate that designed peptides mimic efficiently a functional surface patch of DIII; peptides bind neutralizing antibodies and other DIII binding host-proteins. Moreover, peptides inhibit infection but the dominant epitope recognized by type specific neutralizing mAbs and the structural determinants for antiviral activity seems to be topologically distinct. We further explore the impact of several structural properties of peptides on its biological activity, including sequence space, secondary structure, loop length, turn type, cyclization, supramolecular structure, etc. We also show that antiviral activity of peptides correlates with putative receptor binding properties.

CIGB-300: A novel peptide-based CK2 inhibitor for cancer therapy. Proteomic tools for mechanism elucidation.

Authors: **Yasser Perera[1]***, Arielis Rodríguez-Ulloa [2], Teresa Nuñez[2], Yassel Ramos[3], Jeovanis Gil[3], Lila Castellanos-Serra[3], Yairet García[3], Lázaro Betancourt[3], Vladimir Besada[3], Jorge Fernández-de-Cossio[2], Aniel Sanchez [3], Hernán Farina[4], Luis J. González[3], Gabriel Padrón[3], Silvio E. Perea[1]

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CIGB-300 is a novel competitive substrate inhibitor that impairs the CK2-mediated phosphorylation by binding to the conserved phosphoaceptor domain on its substrates. Compelling data shown this peptide exerts antineoplastic effects both *in vitro* and *in vivo* in suitable preclinical cancer models. Whereas setting up the anticancer properties of a new compound is usually straightforward, dissecting the molecular basis of the antineoplastic effects is a daunting task, even in the current molecular target therapies age. Such notion is particularly true for CIGB-300, a peptide inhibitor that could potentially inhibits hundreds of CK2 substrates in a tumor cell. Here, by using different proteomics (*i.e.* interactomics, comparative proteomics, and phosphoproteomics) and bioinformatics tools we identified major targets and biological processes modulated by CIGB-300 in lung cancer cells. Such findings were corroborated at the molecular and cellular level by using suitable markers and experimental models. Importantly, the proteomic profile modulated by CIGB-300 uncovered for the first time the molecular basis to explain its observed pro-apoptotic, anti-angiogenic, anti-metastatic and chemo sensitization effects in cancer cells. Moreover, the proteomic analysis also provided potential biomarkers that are currently evaluating in cancer clinical trials with this novel anti-CK2 inhibitor.

Charge state selective separation of tryptic peptides for enhanced proteomic studies.

Authors: Lázaro H. Betancourt[a], Pieter-Jan De Bock [b,c], An Staes [b,c], Evy Timmerman[b,c], Yasset Perez-Riverol[a], Aniel Sánchez[a], **Vladimir Besada**[a], Luis Javier González[a], Jorge Fernández-Cossío[d], Joël Vandekerckhove[b,c], Kris Gevaert[b,c]

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Multidimensional peptide fractionation is widely used in proteomics to reduce the complexity of peptide mixtures prior to mass spectrometric analysis. Here, we describe the sequential use of strong cation exchange and reversed phase liquid chromatography in both basic and acidic pH buffers for separating tryptic peptides from complex mixtures of proteins. Strong cation exchange exclusively separates peptide by their charge state into neutral, singly and multi-charged species. To further reduce complexity, each peptide group was separated by reversed phase liquid chromatography at basic pH and the resultant fractions were analyzed by LC–MS/MS. The high selectivity displayed during the SCX step (93% to 100%) and the overlaps between proteins identified from the SCX-separated peptide groups, are interesting assets of the procedure. Application of this workflow to several biological samples allowed quantification of four experimental conditions and improved identification outputs.

Abstracts

Poster Presentations

Comprehensive Characterization of the Pangenome of *Acidithiobacillus caldus*

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Acidithiobacillus caldus is a sulfur oxidizing extreme acidophile and the only known thermo-tolerant member of the order Acidithiobacillales. It is an important, and sometimes the numerically-dominant, microorganism in mineral bioprocessing carried out at temperatures of ca. 35-50 ^{o}C. Multi-Locus Sequence Typing based population structure analyses have shown that only certain *At. caldus* strains prevail in heap leaching industrial settings, while others occur in reactors and oxidation tanks. To gain further insight into these preferences, the genomic diversity and lineage specific adaptations of several strains of the species were explored. Genomes were sequenced using Next Generation Sequencing (Illumina) and paired-end libraries with ~500 bp inserts. Quality-filtered reads were assembled against the type strain genome. Draft genomes were annotated and cross-compared using previously reported tools and mobile genetic elements (MGE) were identified using an in-house designed workflow. Comparative sequence analysis revealed that, despite sharing a conserved and highly syntenic genomic core, each strain has a unique gene complement encompassing more than 20% of their respective genomes. A predominantly pool of actively excising MGEs occurs in the *At. caldus* population and points to a greater frequency of gene exchange in this econiche than previously recognized. Here we predict the potential function of some of the accessory gene products carried by these MGEs and provide inferences on the ecological significance of these functions in strain linages adaptation to different industrial settings. This work further contributes to the elucidation of the species pangenome and the adaptative features of the flexible genome of the *Acidithiobacilli*.

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Expression and identification of intrinsically disordered proteins of *in vitro* sugar beet cell line during abiotic stress

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Drought and heavy metal stress represent major abiotic stresses that adversely affect growth and yield of plants, changing expression of thousands of proteins. Suitable experiments on the proteomic level mostly analyze expression of globular proteins. Intrinsically disordered proteins (IDP) represent a paradigm shift in our understanding how protein structure affects functioning under physiological conditions. Due to their partially disordered structure, the proteins are involved in cell regulation, signalization and control pathways. In our study we sought to unravel the expression of IDPs during water and heavy metal stress, using model organism of an industrially important plant, sugar beet (*Beta vulgaris* L.) cell lines. Recently published sugar beet genome allowed us to analyze it using bioinformatics tools for a number and percentage of IDPs. DisEMBL predictor and a *hotloops* criterion showed that sugarbeet genome contains 41,75% of IDPs that contain at least one long disordered region (over 30 aminoacids). Sugar beet cells were grown for two weeks on solid PG0 nutrient medium containing 300 mM mannitol (water stress) or 10, 40 or 100 μM $CdCl_2$ (heavy metal stress). Cells were collected at 8 and 14

days. Mannitol treatment caused necrotic changes in phenotype while and a slower growth than the control cells. Only the highest used Cd concentration slowed growth of the cells and provoked necrosis, while lower concentrations improved the growth of the cells. Activities of guaiacol peroxidases, an abiotic stress biomarker, were increased after 8 and 14 days of growth of cells on mannitol and on the highest Cd concentration. Total proteins were extracted and IDPs were separated from globular proteins using native polyacrylamide gel electrophoresis (PAGE) and 8M PAGE in second dimension. Seventeen protein spots were observed after silver staining and mannitol upregulated expression of 15 proteins. Regulation of expression by Cd was concentration dependent, and the highest concentration upregulated 11 proteins. Mass spectrometry of excised protein spots identified a few confirmed IDPs but also several new ones that seem to be involved in drought and heavy metal stress.

Comparative transcriptional and translational analysis of *Bordetella pertussis* Tohama I reference strain and clinical isolates growing in biofilm

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Bordetella pertussis is a strict human pathogen and the primary etiological agent of whooping cough or pertussis, a respiratory disease that is highly prevalent among infants. After widespread use of vaccines from the 1950´s the incidence of the disease decreased noticeably. However, in the last decades pertussis has become re-emergent with significant mortality in infants. Clearly, the efficacy of current vaccines, formulated from bacteria grown in stirred bioreactors is limited, showing the need to develop a new generation of vaccines. Physiological and pathogenic features of *B. pertussis* have been extensively studied focusing on the planktonic mode of growth, while only a few works so far have considered the biofilm lifestyle of this bacterial pathogen. With the hypothesis that *B. pertussis* colonizes and persists in their host through biofilm formation, in this work we examined biofilm forming capacity of eight clinical Argentineans isolates recovered from 2001 to 2007, against the reference strain Tohama I (a strain sub-cultured *in vitro* since 1950s). Clinical isolates showed higher ability to grow as biofilm compared to the Tohama I strain. Transcriptional and translational analysis of the isolate *B. pertussis* 2723, an isolate with high biofilm biomass production capacity, showed elevated expression of several virulence factors regulated by BvgAS system, including adhesins involved in biofilm development. In addition, a high expression of energy metabolism enzymes in the clinical isolate was observed. These results were associated with the consumption of the carbon and nitrogen source (glutamate) in the culture medium. In conclusion, the biofilm development has a distinctive impact on *B. pertussis* physiology indicating that it might be considered for the formulation of novel vaccines.

Integrating Bioinformatics Tools to Handle Glycosylation

Authors: **Yuliet Mazola**, Glay Chinea, and Alexis Musacchio

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Glycosylation is a co- and post-translational modification that involves the selective attachment of carbohydrates to proteins. The enhancement of glycosylation by applying glycoengineering strategies has become widely used to improve properties for protein therapeutics. Bioinformatics applications at the early stages of glycoengineering can aid the rational design and insertion of N-glycan sites in proteins. In this work, a workflow integrating available bioinformatics resources to assist protein glycosylation is exposed. In particular, the rational manipulation of the native N-glycosylation pattern, including in silico tools, is illustrated.

"Protium" a Platform for Analysis of Mass Spectrometric Data

Author: **Carlos Manuel Aleaga**
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Protium is a platform created by the Bioinformatics group of the Center for Genetic Engineering and Biotechnology, with the objective of processing and storing proteomics data projects. The platform runs on Windows Server 2012, .Net Framework 4.5, managed with a SQL Server 2012, and published through Internet information Services 8. Raw HPLC MS/MS data is selected with a client desktop application and their references annotated in a queue. Modules for peptide and protein identification are integrated by external scripts, and the references of the identification results are registered in the queue. The queue is attended in the background by a service which parse, process index and store the input data in the database, matching identification results with their respective raw MS data. The business logic is wrapped in a Web Service, for data access, information processing, quantification and analysis. Data retrieval and visualization of results is in charge of a client application.

Understanding the structural basis of permeation and selectivity in Hv1 proton channel

Authors: **Amaury Pupo**1,4, Ester Otarola1, David E. Baez-Nieto1, Gustavo Contreras1, Wendy González2, Karen Castillo1, Peter Larsson3, Ramon Latorre1 and Carlos Gonzalez1
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Voltage-gated proton channel (Hv1) is an integral membrane protein with the capacity to permeate elementary particles in a voltage and pH dependent manner. This protein has been found in several species and is involved in various physiological processes. Hv1 channel lacks the pore domain present in other voltage-gated channels, so permeation must occur through its voltage sensing domain. In this work we demonstrate that residue N264, in *Ciona intestinalis*

Hv1, is crucial for permeation and channel selectivity to protons. This residue is completely conserved between all species with known Hv1 sequences. Using non-stationary fluctuation analysis we found that single channel conductance is affected by mutations in position N264. When N264 is replaced by positive residues (R or K) there is no conductance, when replaced by negative or small residues there is an increase in the single channel conductance. To verify N264 mutants specificity we determined their reversion potential with a ramp protocol, and we found that N264 is part of the Hv1 selectivity filter, in addition to the previously reported D160 residue. As there is not a structure of Hv1 in its open conformation we analyzed all the existing Hv1 homology models, their assumptions, predictions and the experimental facts that support them. From this analysis we were able to create a better model of the open conformation of Hv1. This model was used to realize 200 ns molecular dynamics simulations of WT and N264 mutants, which suggest an electrostatic effect of mutations on channel permeation and selectivity.

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SDS-free PAGE: an alternative to OGE for peptide fractionation.

Authors: **Yairet García**, Yassel Ramos, Annia González, Yasset Perez-Riverol, Lila Castellanos-Serra, Jeovanis Gil, Aniel Sánchez, Luis J. González and Vladimir Besada.

Currently shotgun proteomics based on peptide fractionation via liquid chromatography has become the common procedure for proteomic studies, although in the very beginning of the field, protein separation via electrophoresis was the main tool. During the last decade, the applications of electrophoretic techniques for peptide mixtures fractionation have emerged as an alternative to liquid chromatography systems. We recently proposed the combination of SDS-PAGE for protein fractionation and SDS-free PAGE for peptide separation as a novel procedure for proteomic studies. Here, SDS-free PAGE technique is compared to Off-gel electrophoresis; a system commonly used for peptides fractionation previous to MS analysis. The results demonstrated the highest resolution of SDS-free PAGE and its advantage to fractionate at a unique pH along the lane. Also, a new device for SDS-free PAGE is presented to get the fractionated peptides in solution and increase the number of identified peptides.

Antitumor peptide CIGB-300 binds to B23/NPM and impairs *in vivo* Casein Kinase-2 (CK2)-mediated phosphorylation in myeloid leukemia cells

Authors: **Yiliam Cruz**, Yasser Perera, Yassel Ramos, Teresa Nuñez, Luis D Cruz, Luis J González, Silvio Perea

Despite sustained advances in the treatment of AML (acute myeloid leukemia), mortality rates are still unacceptably high, stressing the necessity to develop new therapeutic drug candidates. Recent findings suggest that CK2 alpha, the catalytic subunit of the CK2 holoenzyme, is an unfavorable prognostic marker and an attractive therapeutic target in AML. Here, we explored for the first time the impact of the anticancer peptide CIGB-300, designed to block CK2-mediated phosphorylation, on AML cells. CIGB-300 had a strong anti-proliferative effect on AML cell lines, displaying at least twofold more potency in leukemic cells than in cell lines derived from solid tumors. Moreover, confocal microscopy assays revealed differences in the subcellular localization of the peptide among the cell lines used as experimental model (HL-60 and OCI-

AML3). Interestingly, the peptide clearly accumulated in the nucleolar region of HL-60 cells while accumulation seemed more diffuse in OCI-AML3 cells. The results of *in vivo* pull-down experiments combined with mass spectrometry allowed the identification of nine previously validated CK2 substrates and nine that were predicted using bioinformatic tools. The analysis of the molecular complexes to which these substrates are associated suggests that 16 of the 18 potential targets of CIGB-300 selected are identified through their interaction with B23/NPM. Importantly, we unambiguously identified that the oncogenic CK2 substrate B23/NPM interacts with CIGB-300, as confirmed by Western Blot experiments following the in vivo pull-down assays. Inhibition assays evaluating CK2-mediated phosphorylation impairment suggested that NPM/B23 may be an important target for CIGB-300 in AML. Considering the role of B23/NPM on cell proliferation and apoptosis, our data suggests that this protein could be one of the key targets mediating the antineoplastic effect of peptide CIGB-300. Moreover, our findings provide the first evidences of the suitability of pharmacological intervention on CK2-mediated signaling as an anti-AML therapy.

Interaction of human Inter-alpha Inhibitor with dengue virus.

Authors: **Alexis Yero Díaz**, Vivian Huerta Galindo, Dayron Martí Prieto, Alejandro Miguel Martín Dunn, Dianne Pupo Gómez, Yassel Ramos Gómez y Glay Chinea Santiago

The outcome of Dengue virus (DENV) infections, which produce a wide range of symptoms going from mild febrile disease to hypovolemic shock, depends on interactions between viral and host factors whose interplay, despite ongoing research, remains poorly understood. During previous efforts using affinity chromatography with domain III (DIII) of E protein from DENV2 as a ligand, addressed at dissecting the interaction network of human plasma proteins and DENV virions, we identified Inter- alpha Inhibitor (IaI) as a potential interactor. IaI is an abundant serum multisubunit complex consisting of two heavy chains (HC1 and HC2) and one light chain called bikunin. In the present work we have purified the human IaI complex from human plasma through a combination of ion exchange and heparin affinity chromatography, characterizing the resulting preparation by SDS-PAGE, mass spectrometry and the inhibition of trypsin activity. Using the purified IaI and recombinant DIII preparations from all four DENV serotypes, we demonstrated, using ELISA, that IaI interacts directly with DENV virions. The influence of IaI on DENV2 infection of the human hepatoma cell line Huh-7.5 was studied as well, examining the effect of incubating IaI with DENV virions pre-, during and post-infection and also determining the effect of pre-incubation only with the target cells or during virion adhesion (at 4°C). These experiments showed that IaI inhibits infection *in vitro* both during adhesion and in the intracellular stages of viral replication.

Proteomic profile modulated by the cardioprotective peptide CIGB500 in cardiomyoblast H9c2 cells.

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The synthetic peptide CIGB500, formally known as GHRP6 (growth hormone-releasing peptide 6), functions as a growth hormone secretagogue. CIGB500 also exerts potent cardioprotective actions. Previously has been demonstrated that CIGB500 treatment reduces myocardial infarct

size following an acute and sudden ischemic event (Berlanga J et al Clin Sci (Lond) 2007, 112(4):241-50). In this work, we studied the CIGB500 cardioprotective effect using a proteomic approach. The rat cardiomyoblast cell line H9c2 was used to identify the proteomic profile modulated in four experimental conditions: 1-) control, 2-) CIGB500 treatment [400µmol, 1h], 3-) Doxorubicin treatment [1µmol, 1h] and 4-) CIGB500 [400µmol] + Doxorubicin [1µmol, 1h] concomitant treatment. Proteins derived from whole H9c2 cell lysates were identified using liquid chromatography and tandem mass spectrometry (LC-MS/MS). To quantify differentially modulated proteins, proteolytic peptides were isotopically labeled with C_{13}- and D_0 – acetic anhydride. As a result, 111 proteins were significantly modulated in at least one condition respect to control. Proteins related to inhibition of apoptosis, cell survival and antioxidant defenses were found modulated in CIGB500 treated cells. Dilated cardiomyopathy induced by doxorubicin down-regulates ATP levels and increases intracellular calcium levels (Octavia Y et al. J Mol Cell Cardiol. 2012, 52(6):1213-25). Importantly, CIGB500 treatment up-regulates proteins that counteract such biological processes. This work gives an approach to the molecular basis by which CIGB500 elicits its cardioprotective effect.

Implementing an IgG purification methodology for analysis of the humoral response memory against domain III of dengue virus.

Authors: **Dianne Pupo Gómez**, Marian Mirabent Casals, Vivian Huerta Galindo, Alexis Yero Díaz, Alejandro M. Martín Dunn, Mónica Sarría Núñez, Glay Chinea Santiago.

Dengue, a mosquito-borne disease caused by any of the four serotypes of Dengue Virus (DENV1-4) for which no antiviral drug of vaccine is yet available, is rapidly becoming a serious public health problem worldwide. It is known that domain III (DIIIE) of the DENV envelope protein plays a fundamental role in binding to viral receptors in the host, and therefore considerable effort has been directed at characterizing the antibody response to this domain. In this presentation we describe the development of a methodology for the purification of IgG from serum samples, which was implemented as part of preliminary work for a serological survey of DENV-infected patients. This methodology, which consists of a single chromatography step combining gel filtration and ion exchange principles, requires processing times very similar to those of simpler alternatives while providing a more effective elimination of IgM and other contaminants as well as acceptable IgG yields. Surprisingly, when applied to the analysis of 52 serum samples from DENV-infected patients from Santiago de Cuba and La Habana corresponding to the 1997-2004 period, we found much higher anti-DIIIE titers than those commonly reported in the literature for non-fractionated serum samples. This preliminary finding has important implications for vaccine and antiviral drug development, as it implies that anti-DIIIE responses might play a bigger role in the control of viral replication than previously realized, and that there may be serum factors that interact with DENV virions via DIIIE, protecting them from antibody-mediated inactivation and thus influencing the outcome of DENV infections.

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OMICs Varadero 2014

The CIGB Biomedical Research Direction is pleased to announce as part of the series of events on OMICs and Bioinformatics the International Meeting **OMICs Varadero 2015** which will be held on October 27th-30th in Varadero, Cuba.

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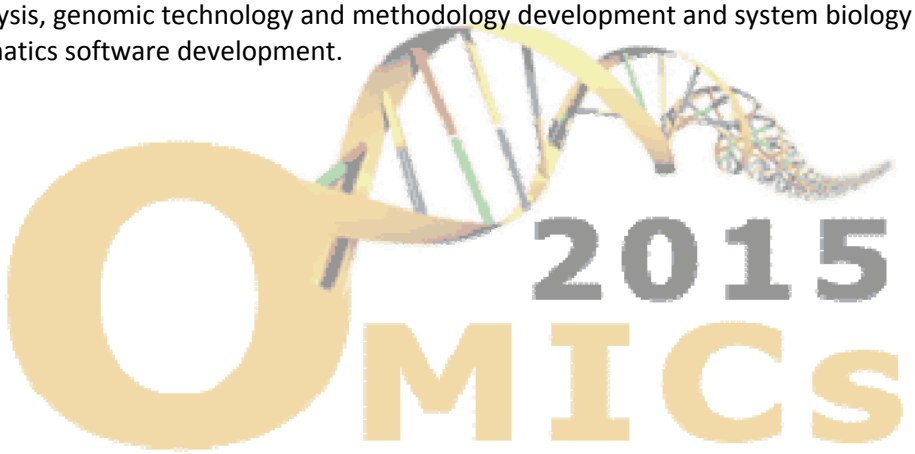

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