

VGN Completes First Leg of NECC Network

By James Vincent, Ph.D.

On October 31, a new fiber optic network connected the University of Vermont to Albany, NY, a regional hub for high capacity national networks. This is the first step in completion of a regional network being built by the North East Cyberinfrastructure Consortium (NECC). A second network leg from UVM to Hanover, New Hampshire is anticipated in early 2011. The NECC states include Vermont, New Hampshire, Maine, Rhode Island, and Delaware. Coordinated funding for this project comes from coordinated supplements to four INBRE and one COBRE grant and a collaborative NSF EPSCoR Track-2 grant.

When completed, the regional network will link institutions in the NECC states: Vermont, Maine, New Hampshire, Rhode Island and Delaware. The expanded capacity of the network will allow researchers to share large data sets among NECC members and to move these data sets to advanced resources such as the National Science Foundation Teragrid. The ability to move terabyte size data sets is becoming critical to research as generation of large data sets becomes the norm in many fields of science.

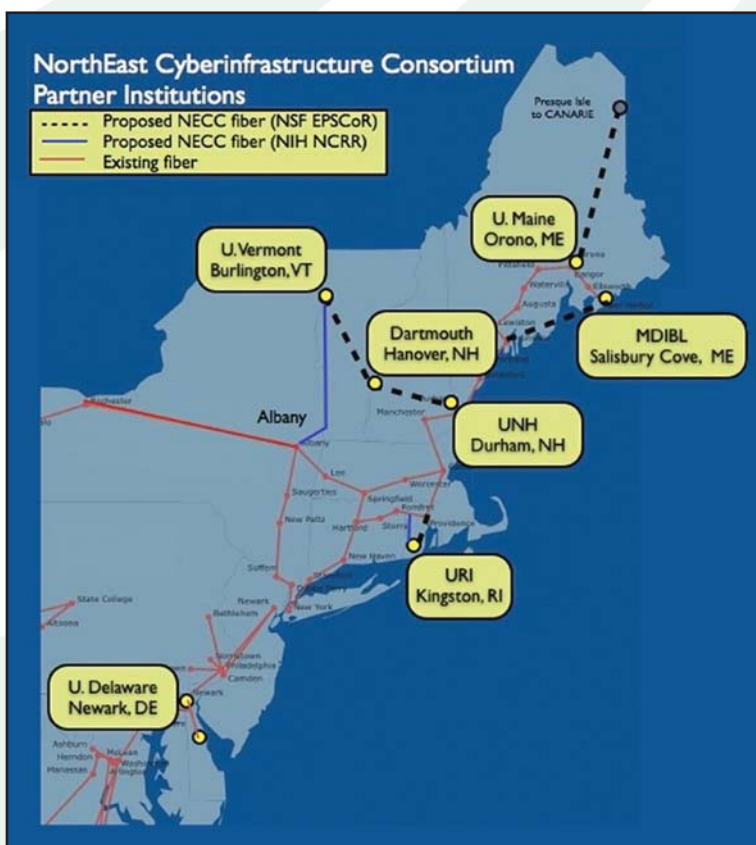
At an aggregate network capacity of 120 gigabits per second, the new network will provide UVM with capacity that rivals the largest research institutions. Through the connection point in Albany, NY the NECC

the northeast will be one of the first projects to take advantage of the new network. The project aims to develop insight into blooms and the reasons they become toxic through the use of massively parallel sequencing. The

new regional network, in conjunction with the NECC Shared Data Center, will allow multiple researchers from across the NECC easy access to the large data sets generated by the project.

Senator Leahy and UVM president Daniel Mark Fogel announced completion of the first part of the network on Thursday, October 28 at the ECHO Lake Aquarium and Science Center at the Leahy Center for Lake Champlain in Burlington. Senator Leahy stated the NECC network will complement the \$171 million in American Recovery and Reinvestment Act broadband funding awarded to Vermont providers and organizations earlier this year.

The National Science Foundation (NSF) and the National Institutes of Health (NIH) provided funding for the network under multi-year competitive awards. U.S. Senator Patrick J. Leahy provided key leadership in support of research funding at these agencies.



Network map of the North East Cyberinfrastructure Consortium states: Vermont, Maine, New Hampshire, Rhode Island and Delaware.

regional network may eventually connect directly to the United States Unified Community Anchor Network (U.S. UCAN), an advanced network backbone that will link regional networks across the nation.

A regional effort to determine the microbiomes of algal blooms in Lake Champlain and four other lakes in



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FROM THE DIRECTOR

Judith
Van Houten,
PhD



On October 28th Senator Patrick Leahy joined us at the ECHO Lake Aquarium and Science Center in announcing the completion of the first reach of fiber in the North East Cyber Infrastructure Consortium fiber ring for research and education. The University of Vermont's President Daniel Mark Fogel, Mr. Frank Cioffi of the Vermont Technology Council, and Mr. Greg Kelly, President of Teljet Longhaul spoke about the significance of this exciting project. See the headline article by Dr. James Vincent for a description of the fiber ring and metagenomics research that it will facilitate.

Our BPI Coordinators organized the 4th Annual VGN Faculty Professional Development Seminar that was held on Saturday, January 23rd at Saint Michael's College. The topic for the seminar was "Succeeding in a Faculty Position at a Predominantly Undergraduate Institution" and the guest speaker was Dr. Thomas Wenzel, Professor Chemistry from Bates College. Following, Dr. Wenzel's presentation, four of our BPI faculty members spoke about their own examples of how they balance all of their demands and then opened the discussion to an interactive question and answer period. We at VGN would like to thank Saint Michael's College BPI Coordinator Dr. Alayne Schroll for her efforts in hosting the event.

VGN students and their advisors made our 8th Annual Career Day a great success. We gathered on April 14th at the Doubletree Hotel. Dr. Karen Lounsbury, VGN Director of Networking & Professional Development organized a panel of four speakers who told us about their individual career paths. The panelists were especially diverse this year and included a dentist, the deputy chief medical examiner, a clinical psychologist and a forest and wildlife consulting services small business owner. The undergraduate students presented their VGN research in a poster presentation. Justin Bogart and Kristin Maletsky, both from Middlebury College received prizes for the best posters. Congratulations!

Our most important annual event, our 9th Annual Retreat, was held on August 10th at the Doubletree Hotel. We were delighted

to have Dr. Bryan Ballif Co-Director of the Proteomics Facility and Tim Hunter, Manager, Microarray Facility speak to the researchers about their facilities. Dr. Ballif's talk was titled "Using Mass Spectrometry to Quantify Site-Specific Protein Phosphorylation: A New Twist on an Old Oncogene" and Mr. Hunter's talk was titled, "Using Microarray in Your Research."

Four VGN funded researchers also gave presentations about their research while other researchers presented their research in a poster session. We would like to thank Dr. Elizabeth Dolci, Johnson State College, Dr. Kevin Fleming, Norwich University, Dr. Hans Haverkamp, Johnson State College, and Dr. Elizabeth Wuorinen, Norwich University for their informative updates of their VGN funded research. Julie Paris, VGN Program Manager hosted a Business & Grant Managers meeting for our BPI's with special guest William Harrison, Chief Internal Auditor, University of Vermont.

VGN would like to congratulate the following VGN Staff and Faculty for their promotions this year. Dr. Janet Murray has been promoted to Director of our Outreach Core. VGN has also welcomed some new faces this year. Marc Farnum Rendino and Panagiotis Lekkas are working on our ARRA Supplements through the end of August of 2011. We would also like to thank Dr. Chris Allen, Dr. Karen Lounsbury, Dr. Dwight Matthews and Mr. Pat Reed for their years of service with VGN.

Congratulations to our BPI faculty who submitted meritorious applications for nine project awards and four pilot awards. Please see the box (on page 12) for the PI names and project titles. Please help me to welcome and congratulate all of our VGN members. I wish you all another successful and productive year and thank you for making VGN a success.

Save the Dates!

Vermont Genetics Network
Professional Development Workshop
Saturday, January 22nd, 2011
Middlebury College

Vermont Genetics Network
Career Day
Wednesday, April 13th, 2011
Doubletree Hotel, South Burlington

*Details on both events to follow,
or check <http://vgn.uvm.edu/>*



Above left: Participants and instructors from the first Skate Genome Annotation Workshop held at the University of Delaware, Newark, Delaware
Above right: Leaders of the Skate Genome Annotation Workshop at MDIBL last week: Dr. James Vincent of the University of Vermont, Ben King of MDIBL, and Dr. Cecilia Arighi of the University of Delaware



Bioinformatics cores from the North East Bioinformatics Collaborative (NEBC) recently completed a four-day hands-on workshop for nearly two dozen students. Instructors from NEBC bioinformatics cores, including the VGN Bioinformatics Core, the Mount Desert Island Biological Laboratory (MDIBL), the University of Rhode Island and the University of Delaware led the Little Skate Annotation Workshop II, which was held at MDIBL.

The four-day workshop was the second in a series of three. The first five-day workshop, held at the University of Delaware in May, drew 27 participants and 10 instructors from universities and colleges in the Northeast Cyberinfrastructure Consortium (NECC) states. The third and final workshop will be held at the University of Delaware May 23-27, 2011. The workshops offer training and opportunities for faculty and students to work together.

"The skate genome project provides an ideal opportunity for students to participate in a scientific project that is literally charting new territory," says Ben King, a staff scientist at MDIBL and co-leader of the project. "Annotating the genome, or figuring out what parts play which role, requires an enormous effort that is best achieved through collaboration. It's exciting to have so many INBRE institutions working together."

The workshops train students in bioinformatics with the goal of annotating the genome of the little skate, *Leucoraja erinacea*. With 3.4 billion base pairs, the skate genome is slightly larger than the human genome. As the skate is believed to have evolved 450 million years ago, a genetic "road map" of this ancient creature will offer new insight into evolution and reveal which genes have been retained or "conserved" over those millions of years. The Little Skate is one of 11 organisms selected

LITTLE SKATE GENOME WORKSHOP

By James Vincent, Ph.D.

by a National Human Genome Research Institute panel with the greatest potential to fill gaps human biomedical knowledge.

The massive sequencing effort draws on expertise in all five of the NECC collaborating states. The original genetic material was obtained at MDIBL. Technicians and faculty at the University of Delaware have been determining the actual sequences, using pieces of skate DNA. The resulting data is analyzed at MDIBL and the University of Vermont. The annotation is taking place in Maine, Delaware, Rhode Island, Vermont, and New Hampshire, and will provide opportunities for follow-up research projects for years to come.

The North East Bioinformatics Collaborative (NEBC) is a subproject

of the Northeast Cyberinfrastructure Consortium (NECC). The NECC is developing cyberinfrastructure across New Hampshire, Maine, Rhode Island, Vermont, and Delaware for cyber-enabled research programs. The National Science Foundation and the National Institutes of Health fund the Consortium. Stimulus funds from the American Recovery and Reinvestment Act are also supporting the skate genome project through the INBRE program.

Excerpts for this article have been reprinted from http://www.mdibl.org/mdibl_press_releases/Skate_Genome_Workshop_Held_at_MDIBL/288/ with permission from Jerilyn Mitchell Bowers, Director of Development and Public Affairs, Mount Desert Island Biological Laboratory.



Participants and instructors from the second Skate Genome Annotation Workshop held at Mount Desert Island Biological Laboratory, Salisbury Cove, Maine



VGN 2010 CAREER DAY:

STUDENTS PRESENT THEIR WORK AND EXPLORE CAREERS IN SCIENCE

Vermont Genetics Network hosted its 9th Annual Career Day on April 14th at the Doubletree Hotel in South Burlington, Vermont. Undergraduate students from UVM, CCV, Essex High School and our 7 Baccalaureate Partner Institutions (BPIs); Castleton State College, Green Mountain College, Johnson State College, Lyndon State College, Middlebury College, Norwich University and Saint Michael's College attended along with their faculty advisors. Other faculty and staff attended from these schools as well.

The evening began with student poster presentations. Undergraduate students who received funding by VGN for INBRE Project Year 5 presented posters describing their research projects. Also included this year was a high school outreach team from Essex High School. The two students, Anne Levy and Elizabeth Tobey and their teacher Adam Weiss worked in a UVM research laboratory during Summer 2009. A panel of judges chose two winning posters from the students who received 8GB flash drives. The winners were Justin Bogart and Kristin Maletsky, both of Middlebury College.

A buffet dinner followed the poster presentations. After dinner, Karen Lounsbury, VGN Director of Networking & Professional Development, introduced the panel of speakers. The presentations focused on the career paths panelists took to arrive at their current careers. The panelists included: Grace Dickinson Branon, DMD (Dentist with Dickinson and Branon Dental Care); Elizabeth Bundock, MD, PhD, Deputy Chief Medical Examiner (Vermont Department of Health); Elena Ramirez, PhD PLC, Clinical Psychologist and Allan Thompson Northern Stewards, Forestry and Wildlife Consulting Services.

Below: Panelist Allan Thompson, Forestry and Wildlife Consulting Services

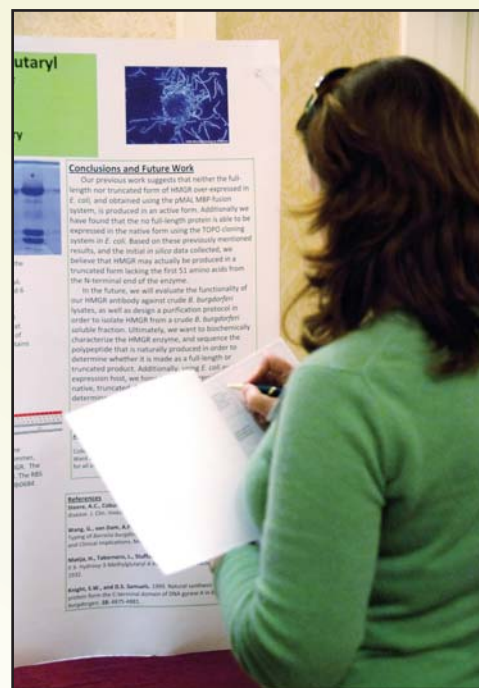


L to R: Panelists Elena Ramirez, PhD PLC, Clinical Psychologist and Grace Dickinson Branon, DMD, Dickinson and Branon Dental Care



Above: Panelist Elizabeth Bundock MD, PhD, Deputy Chief Medical Examiner

Below: Dr. Janet Murray judging student posters



WHAT'S NEW IN THE VGN MICROARRAY FACILITY?

By Tim Hunter and Scott Tighe



Microarray Staff (left to right):
Panos Lekkas, Tim Hunter, Scott Tighe

The VGN Microarray facility continually strives to improve the quality of services provided for our user base. This includes introducing new services as they become available on the market as well as explore new approaches for challenging sample types from our growing clientele. As the work load increases and the range of services become more diverse to accommodate these sample types, the facility has completed several studies to implement these needed techniques. Additionally, the facility has also added one new staff member to address the increasing work load.

Panos Lekkas joined the facility staff this summer to provide support on all aspects of the microarray facility, assist with outreach instructional activities, and provide support on the new metagenomic project studying blue-green algae blooms in Regional New England lakes. Panos comes with an extensive background in proteomics and has been a welcomed addition to our instructional team for the VGN Proteomics educational module, which has been developed and is currently being taught at both Norwich University and Green Mountain College this fall.

Laboratory studies investigating new methodologies to mitigate compromised RNA integrity has led to the addition of several new approaches for microarray target preparation methods for special samples such as those encountered with FACS (Fluorescence Activated Cell Sorting) and LCM (Laser Capture Microdissected) derived RNA sources. As reported in our previous newsletter, the

study used human brain reference RNA that was non-chemically degraded to final RIN (RNA Integrity Number) values of 10, 6, 4, 2, and results were presented at the Northeast Regional IDEa States meeting held in New Hampshire and the Advances in Microarray (AMT) conference in Stockholm, Sweden. This work culminated in a book chapter written by Scott Tighe and Matt Held on the subject of FACS-derived RNA and the downstream processing needed for microarrays

studies. Humana Press published the chapter in Microarray Methods for Drug Discovery, Methods in Molecular Biology, vol. 632, titled "Isolation of Total RNA from Transgenic Mouse Melanoma Subsets Using Fluorescence-Activated Cell Sorting". The chapter has been well received and widely requested by both the flow cytometry and microarray community alike.

Two new recent additions to the facilities array repertoire are the miRNA and Phylogenetic GeneChips. The miRNA GeneChip is well suited for addressing epigenetic questions regarding the role of small RNA's, can be used for 71 different organisms, and contains sequences for 6,703 miRNA.. The workflow has been developed, refined, and implemented in the core, and the facility staff is currently working on two projects using these chips. The phylogenetic GeneChip (Phylochip), developed by Gary Anderson's lab at Lawrence Berkeley National Laboratory,

allows researchers to identify up to 32,000 taxa of bacterial species and organisms from complex microbial samples. The utility of this GeneChip extends beyond simple bacterial identification, but rather it is a means to studying metagenomics using a microarray approach.

Lastly, the VGN microarray facility will be hosting an upcoming seminar in the User Educational Core Seminar Series on GEO (Gene Expression Omnibus) on December 10th in 300 HSRF at noon. The seminar will be presented by Dr. Julie Dragon, VGN Bioinformatics, and is titled "Gene Expression Omnibus: Mining for Gold".

CONGRATULATIONS TO JAMES VINCENT, PH.D. DIRECTOR, BIOINFORMATICS CORE

Who was recently awarded the following patent:

Gene expressed in prostate cancer and methods of use

Abstract: A new polypeptide is disclosed that is specifically detected in the cells of the prostate, termed Novel Gene Expressed in Prostate (NGEP). Polynucleotides encoding NGEP are also disclosed, as are vectors including these polynucleotides. Host cells transformed with these polynucleotides are also disclosed. Antibodies are disclosed that specifically bind NGEP. Methods are disclosed for using an NGEP polypeptide, an antibody that specifically binds NGEP, or a polynucleotide encoding NGEP. Assays are disclosed for the detection prostate cancer. Pharmaceutical compositions including an NGEP polypeptide, an antibody that specifically binds NGEP, or a polynucleotide encoding NGEP are also disclosed. These pharmaceutical compositions are of use in the treatment of prostate cancer.





VGN FUNDED UNDERGRADUATE STUDENT POSTERS:

Justin Bogart, Middlebury College

"Transferrin Mediated Activation of Ruthenium (III) Anticancer Drugs"

Karina Lepeley, Johnson State College

"The Effects of the Alexander Technique on Aerobic Exercise Response"

Kristin Maletsky, Middlebury College

"Facilitating Ethanol Tolerance with Pregnenolone Sulfate and Intoxication Practice and Assessing the Impact of Tolerance on Ethanol Consumption in Swiss Webster Mice"

Ashley Panichelli, Middlebury College

"Expression and Purification of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase of *Borrelia burgdorferi*"

Mitchell Pesesky, Middlebury College

"Characterizing the SloR: DNA Binding Interface in *Streptococcus Mutans*"

DaWeon Ryu, Middlebury College

"Oxidation of Ruthenium Complexes"

ADDITIONAL POSTERS PRESENTED BY STUDENTS OF VGN FUNDED FACULTY:

Dustin Berry, Johnson State College

"Effects of Altered Pre-Exercise Airway Function on Exercise-Induced Bronchospasm in Asthmatic Humans"

Shannon Englemann & William Prince, Middlebury College

"Effects of Varying Levels of Prolonged Testosterone Exposure on Spatial Learning and Memory in Male Rats"

Susan Nader, Johnson State College

"Effects of Altered Pre-Exercise Airway Function on Exercise-Induced Airway Refractoriness in Asthmatic Humans"

Brittany Perdue, Johnson State College

"Infant Clowns: Humor Perception & Creation in 3- to 6-month olds"

VGN FUNDED HIGH SCHOOL STUDENTS:

Anne Levy & Elizabeth Tobey, Essex High School

"Determining Whether the DnaK Protein is Required for EmaA Secretion in *Aggregatibacter actinomycetemcomitans*"



Justin Bogart, Middlebury College
(poster award winner)



Karen Lounsbury, VGN Outreach Director
and Kristin Maletsky, Middlebury College
(poster award winner)

BIOINFORMATICS CORE HELPS BUILD INFRASTRUCTURE

By James Vincent, Ph.D.

The VGN Bioinformatics Core has been busy helping build shared research infrastructure both within the University of Vermont and through the North East Cyberinfrastructure Consortium (NECC).

NECC Shared Data Center

The NECC Shared Data Center (SDC) is fully operational. The SDC now supports five researchers with diverse projects and data storage needs. Researchers from multiple institutions are able to easily share large data sets through the SDC. This eases the burden on individual researchers in managing data. By using the SDC, researchers are able to control access to data sets through a simple web interface.

Sharing a private data set with researchers at another institution is as simple as sending

an SDC link. The SDC provides password protection and download limits as needed by the owner of the data. This allows maximum flexibility for researchers wishing to share data while relieving the researcher of storage and security details.

Researchers across all five states currently use the SDC. The VGN Bioinformatics Core plays a key role in development and management of the SDC.

Advanced Genome Technologies Core

The College of Medicine, the Vermont Cancer Center and the Vermont Genetics Network have begun working together to build support for massively parallel sequencing projects at the University of Vermont. Jeff Bond, Molecular Bioinformatics Shared Resource Director, Tim Hunter, VGN Microarray

Facility Manager and VCC DNA Analysis Facility Manager and James Vincent, VGN Bioinformatics Core Director have been working together to develop a massively parallel sequencing core facility named the Advanced Genome Technologies Core (AGTC). Our initial aim is to provide consultations for researchers undertaking massively parallel sequencing experiments.

The AGTC has reserved weekly time slots for meetings with researchers. Principal Investigators and lab members involved in projects are encouraged to meet with the AGTC early in the planning stage. Researchers can leverage the experience of the AGTC members in developing project plans.

Consultations are scheduled through the AGTC website at: <http://www.uvm.edu/agtc>



VGN Proteomics Mass spectrometers in MLS 335



Laser puller p-2000

After four years of serving numerous investigators inside and outside of Vermont, the Vermont Genetics Network (VGN) Proteomics Core has a new home in a newly renovated laboratory in Marsh Life Science Building Room 335 as of August 2010. Routine operations and services resumed after only a short one-week hiatus.

The VGN Proteomics Facility has improved its ability to provide its users with cutting edge technologies for analyzing proteins and peptides through the combination of current equipment (three mass spectrometers—two Thermo-Finnigan LTQ Linear Quadrupole Ion Traps and one Thermo-Finnigan LTQ-Orbitrap) as well as the addition of new equipment. The facility has added a new SpeedVac, which is used for sample preparation and a Laser Puller P-2000 for use in making packed separation columns.

Since last year over 2000 samples have been analyzed, eight peer-reviewed papers have been published and three new grants have been funded due to expertise from the VGN Proteomics Facility. The user base is growing and includes over 59 active faculty, post docs and staff; 35 graduate students; and 40 undergraduates. At least 25 seminars and poster presentations have been presented by users/staff that included data from the VGN Proteomics Facility. Since its beginnings in 2006 the VGN Proteomics Facility has experienced a rapid growth in its user base and in total samples analyzed. However, we are expecting even more users and collaborative projects in the facility given the critical nature of proteomic data to various lines of biological inquiry.

Please visit the following link to sign up for a consultation or to fill out an online sample submission form: <http://vgn.uvm.edu/proteomics/>

VERMONT GENETICS NETWORK PROTEOMICS FACILITY HAS NEW HOME

By Bin Deng, Ph.D.



SpeedVac and Lyophilizer



Sample preparation bench in MLS 335



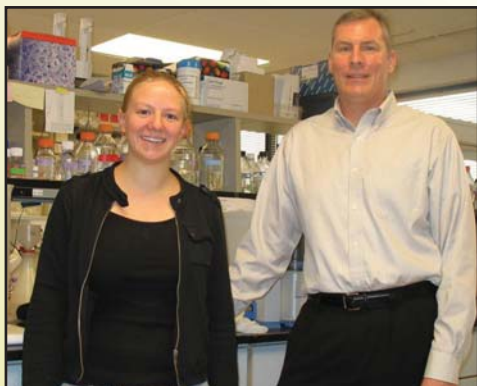
Right: Proteomics Outreach Team in consultation room in MLS 337

VGN Proteomics Spotlights Successful Investigators

In this issue we spotlight two teams of investigators whose projects benefited by mass spectrometry analysis at the VGN Proteomics Facility. The two projects also highlight the emerging capabilities of the facility in two important research avenues: whole protein mass spectrometry and the analysis of glycoproteins.

TRANSCRIPTION FACTOR-ssDNA INTERACTION STUDY PUBLISHED

The purine-rich element binding proteins are a small, evolutionarily-conserved family of nucleic acid-binding proteins whose signature biochemical feature is specific and high affinity interaction with guanosine-rich single-stranded DNA (ssDNA) or RNA sequences. The founding member of this family, Pura, has been linked to aspects of nucleic acid homeostasis required for regulated cell growth and genome stability. On the other hand, we



Pictured are the first author Ph.D. student, Amy Rumora, and her advisor, Dr. Robert Kelm Jr. who was the senior author of the study. Both are members of the UVM Biochemistry Department.

and others have reported that a related but less studied member of this family, Purb, functions as a potent repressor of genes required for the differentiation of cell types found in connective tissue and muscle. In a recent paper¹, we identify the core domain of Purb that mediates the sequence-specific binding of the protein to the purine-rich strand of an enhancer from the smooth muscle α -actin gene. Based on the modular arrangement of putative secondary structural elements in Purb, we hypothesized that the protein might fold in such a way as to protect the central region from proteolysis while leaving the putatively more flexible N- and C-terminal segments exposed. As expected, limited tryptic digestion of recombinant Purb generated a 30 kDa ssDNA-binding fragment devoid of N- and

C-terminal epitopes. Efforts to sequence the N-terminus of the core fragment via Edman chemistry were not successful so we turned to Dr. Ballif in the VGN Proteomics Facility to help determine the peptide composition and mass of the Purb core tryptic fragment via a combination of peptide mapping and intact protein mass spectrometry. Dr. Ballif's results confirmed predictions about the sites of tryptic cleavage by establishing that the core fragment corresponds to residues 29-305/306. This information enabled us to move forward in characterizing the foldedness, quaternary state, and ssDNA-binding properties of the isolated core domain of Purb in comparison to the full-length protein. Future studies will focus on evaluating the functional roles of certain conserved modules within the Purb core domain in facilitating protein-protein interaction, nucleoprotein complex assembly, and target gene repression.

¹Amy E. Rumora, Ashley N. Steere, Jon E. Ramsey, Anna M. Knapp, Bryan A. Ballif, and Robert J. Kelm Jr. (2010) Isolation and characterization of the core single-stranded DNA-binding domain of purine-rich element binding protein B (Purb). *Biochem. Biophys. Res. Commun.* 400, 340-345. PMID: 20728429

CHARACTERIZATION OF CARBOHYDRATES FOUND ON COAGULATION PROTEINS

Hemostasis is the process in which bleeding is stopped by the formation of a clot, and numerous plasma and integral membrane proteins are essential components. Studies of these proteins at the molecular level are defining how their structure dictates their function, and how those relationships may be altered by post-translational modifications (PTMs) such as glycosylation. In collaboration with the VGN, two hemostasis research groups at UVM are employing some exciting new methodologies to define how differences in glycosylation alter protein function. A recent study¹ from Dr. Saulius Butenas' laboratory (UVM Department of Biochemistry) has focused on tissue factor



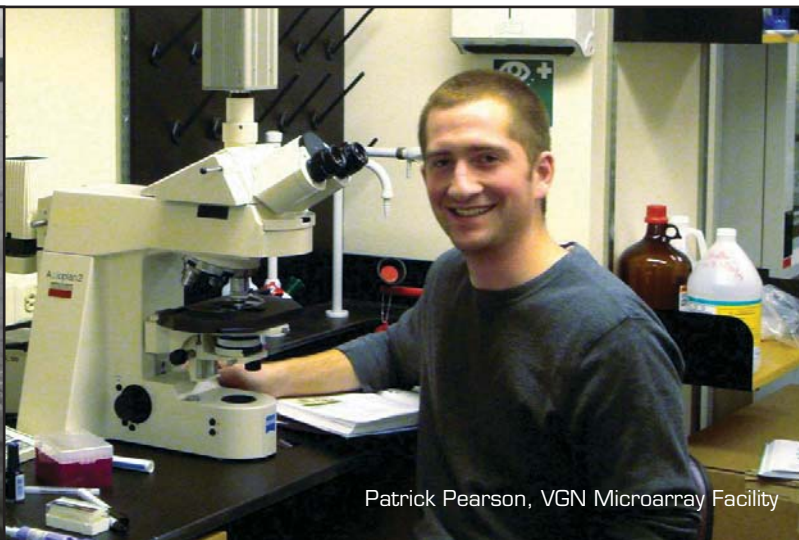
(L to R) Jeremy Wood, Dr. Jay Silveira, Jolanta Krudysz-Amblo (UVM Department of Biochemistry)

development of a simplified method for site-specific profiling of the glycans on proteins.² These analyses revealed the presence and composition of the N-linked glycans at asparagines 11, 124, and 137 in TF, and indicated that there are substantial differences in the composition of carbohydrates at each site between the recombinant and natural forms. Combining the data from these detailed glycan characterizations with enzymatic deglycosylation and functional studies of the different TF forms demonstrated that glycosylation had significant impact on the biological activity of TF. A second UVM study³ in Dr. Paula Tracy's laboratory has been applying the same technique to characterize the glycans of factor Va (FVa), an essential cofactor for clot formation. Two different forms of the FVa protein exist, with differences in PTMs that are thought to be responsible for their dramatic functional differences. To address this hypothesis, Drs. Jeremy Wood and Jay Silveira in collaboration with the VGN, are characterizing glycosylation differences in the proteins. Analyses of plasma-derived FVa have identified and characterized the N-linked glycans at asparagines 269, 432, 439, 1675, and 218. Detailed compositional analyses have identified high mannose and complex glycan chains with relatively little compositional heterogeneity, as well as potential N-linked glycosylation sites that appear to lack glycan addition. Similar analyses are currently being

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Vermont Genetics Network Leads Multi-State Metagenomics Study

By Patrick Pearson



Patrick Pearson, VGN Microarray Facility

This summer, a metagenomic study of algae existing in Northeastern lakes and ponds was initiated by North East Cyberinfrastructure Consortium and the Vermont Genetics Network through ARRA funding. Metagenomics is the culture-independent analysis of a microbial community. Direct sequencing of an environmental sample, such as a water sample from a pond or lake, bypasses the need for isolation and cultivation of species in a laboratory.

During the summer warm temperatures and plentiful sunlight provide favorable conditions for algae growth. The potentially dangerous and toxic algal blooms can have adverse effects on human and animal health, and the local economy. Therefore, this project was undertaken to gain a better understanding of the factors at play that contribute to this problem; such as the diversity of the algae before, during, and after a bloom.

Overall, samples from seven lakes and ponds from four states were included in the study: Sebasticook Lake in Maine, Lake Winnepesaukee in New Hampshire, four ponds from Rhode Island, and Lake Champlain in Vermont. At each site, DNA-free bottles were supplied to sites for collection of three water samples weekly. Water samples were collected from mid-July to mid-October and shipped to the VGN Microarray Facility at the University of Vermont. Upon arrival the samples were immediately subjected to a spectrum of laboratory assays. Arguably,

the most important of these assays was to obtain a DNA sample for further downstream sequencing. This was accomplished by filtering the water through a very fine membrane, thereby separating the water from its algal constituents. DNA from the samples will be extracted and subjected to Massively Parallel Sequencing for identification of microbes present and the genetic diversity within the cyanobacteria (blue-green algae) providing an unbiased census of all the organisms in the environmental sample. Further filtering was completed also to collect a sample for a microcystin analysis. Microcystin is a toxin produced by certain cyanobacteria that has been studied extensively. In addition to filtering, several assays were completed to visually count, identify and determine the abundance of algae species using microscopy techniques and flow cytometry. Also, a designated volume of each sample was archived for potential further studies in the future.

Currently, the counting and identification of the algae species present in each water sample has begun, and samples are being selected for sequencing and microcystin analysis. Additionally, during our sampling time blooms have been detected and recorded in Lake Champlain. Therefore, the results from further downstream work will hopefully shed light on the intricacies of bloom production and aid in the development of novel remediation techniques.

CHARACTERIZATION OF CARBOHYDRATES FOUND ON COAGULATION PROTEINS

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extended to the platelet-derived FVa molecule for comparison, as are analyses of other PTMs including phosphorylation, sulfation, and O-linked glycosylation using the tools and techniques available through the VGN.

¹Jolanta Krudysz-Amblo, Mark E. Jennings II, Kenneth G. Mann, and Saulius Butenas. Carbohydrates and Activity of Natural and Recombinant Tissue Factor. *Journal of Biological Chemistry*. 2010 Jan 29; 285(5): 3371-3382.

²Mark E. Jennings II, Jolanta Krudysz-Amblo, Kenneth G. Mann, Saulius Butenas, and Dwight E. Matthews. Site-Specific Glycoproteomics Simplified Using an Ion Trap Mass Spectrometer. 58th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, Salt Lake City, Utah, May 23-27, 2010.

³Jeremy P. Wood, Adam J. Blanchard, Mark E. Jennings II, Jay R. Silveira, and Paula B. Tracy. Characterizing the N-linked Glycosylation of Factor Va Using Mass Spectrometry. Gordon Research Conference on Hemostasis, Waterville Valley Resort, Waterville Valley, NH, July 25-30, 2010.

HIGHLIGHTS FOR VGN OUTREACH 2009/2010

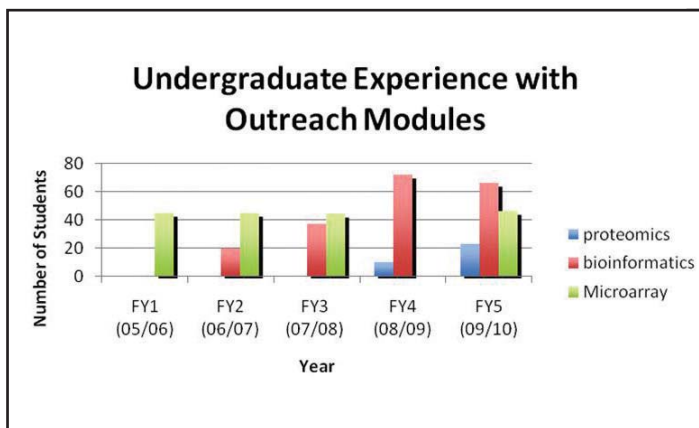
By Janet Murray, Ph.D.

The 2009/2010 academic year was a very busy year for the VGN outreach team with twelve visits to eight different undergraduate colleges where we shared three different outreach modules. We are seeing more investment from our partner colleges in terms of curriculum integration and increased budgetary investments. We in the outreach core are very happy with the success of our outreach programs and working with faculty at colleges throughout the state.

Microarray Outreach

In the 2009/2010 academic year we brought the microarray module to five of our outreach partners. To date, we have worked with eight current outreach partner colleges to bring Microarray into the classroom. Five of these colleges have or are planning to integrate this module into their curricula. Faculty at Norwich University and Green Mountain College were able to obtain department funds to support a large portion of the module's cost during the 2009/2010 academic year. Other colleges are beginning to cover the costs of disposable items as a way to invest more in the program.

A class in Bates College in Maine participated in microarray outreach. Maine is an IDEA state with an active INBRE program. This was our first attempt at collaborating with another INBRE state to share our INBRE program. This very successful initial work, with a small class of four students will hopefully grow to an ongoing collaboration in



We are seeing an increase in the number of students being introduced to cutting edge science modules provided by the VGN Outreach Program.

a larger class setting. We hope to encourage exchange of outreach ideas with our other INBRE neighbors in the future.

All our outreach modules are available for review on the VGN website: <http://vgn.uvm.edu/>

Bioinformatics Outreach

We worked with two new colleges, Castleton State College and Lyndon State College, during the 2009/2010 academic year to bring the Bioinformatics Core into the classroom. Dr. Alan Giese at Lyndon State College is planning to integrate this module into a course offered every two years. The bioinformatics module is continually being upgraded as links change and databases mature. Our most recent upgrade was the incorporation of video guides to the tutorial.

The initial feedback from the students enrolled in bioinformatics tutorial was very

positive. The module will be going through a major update over the winter break due to the massive changes introduced to the NCBI website.

This course has been integrated into the curricula at several colleges readily. Our role in curriculum integration is to maintain the site with updates and to provide any necessary supporting in regards to the management system or faculty questions.

Proteomics Outreach

The Proteomics Outreach Module is our newest outreach module. It was developed during 2008 and beta tested at UVM in the spring of 2009. Classes at Saint Michael's College (16 students) and Johnson State College (7 students) in the fall of 2009 and spring of 2010 respectively. Dr. Alayne Schroll at Saint Michael's college is planning to integrate the module into a yearly course starting in the spring of 2011.

This course begins with protein purification, followed by 2D gel electrophoresis and Mass Spectrometry. We have seen a growing interest in this course and are currently bringing the proteomics module to Norwich University with Dr. Dick Milius and to Green Mountain College with Dr. Natalie Coe. Dr. Coe's research focused on beech bark disease which is wide spread in the Northeast. Dr. Coe has worked with the outreach team to modify the proteomics module to look at protein expression in beech tree buds from trees that are susceptible and resistant to beech bark disease. This is the first known proteomics investigation in beech trees. We are excited to be working with Dr. Coe and the students at Green Mountain College in this endeavor.



Fall foliage frames Coram Library, home to the Bates Imaging Center.

VERMONT GENETICS NETWORK 9TH ANNUAL RETREAT

FEATURES CURRENT VGN RESEARCH AND RESOURCES

The Vermont Genetics Network (VGN) hosted its 9th Annual Retreat on Tuesday, August 10, 2010 at the DoubleTree Hotel in South Burlington. VGN Director Dr. Judith Van Houten opened the program by welcoming all of the guests. Dr. Van Houten also gave a special thanks to all VGN staff, faculty, researchers and representatives from the BPI Institutions for their hard work toward the VGN renewal that began on August 1, 2010. The day began with four presentations by VGN funded faculty:

Elizabeth Dolci, PhD, Johnson State College
"Microbial Communities of the Vermont Asbestos Group Mine"

Kevin Fleming, PhD, Norwich University
"Decisions to Shoot in a Weapon Identification Task"

Hans Haverkamp, PhD, Johnson State College
"Lung Function During Exercise in the Asthmatic Human"

Elizabeth Wuorinen, PhD, Norwich University
"The Effect of Varied Exercise Intensity Over a 20-Week Training Period in Middle-Aged Women"

Dr. Bryan Ballif, Director, VGN Proteomics Core and Tim Hunter, Manager, VGN Microarray Core gave the keynote talks. Dr. Ballif's talk was titled, "Using Mass Spectrometry to Quantify Site-Specific Protein Phosphorylation: A New Twist on an Old Oncogene." Mr. Hunter gave a talk titled "Using Microarray in Your Research."

Julie Paris, VGN Program Manager, held a business and grant managers meeting for representatives from each of the BPI Institutions. William Harrison, Chief Internal Auditor, University of Vermont gave an informal talk about Audit Services and Sponsored Programs.

The Baccalaureate Partner Institutions (BPI) Coordinators met to discuss their roles with VGN for the next year and to discuss updates as the new round of INBRE funding has begun. After presentations and meetings concluded, attendees networked and visited the posters that were presented by the BPI faculty and UVM graduate students who received VGN funding last year. There were 14 posters presented under many different disciplines.



Elizabeth Dolci, PhD
Johnson State College



Kevin Fleming, PhD
Norwich University



Hans Haverkamp, PhD
Johnson State College



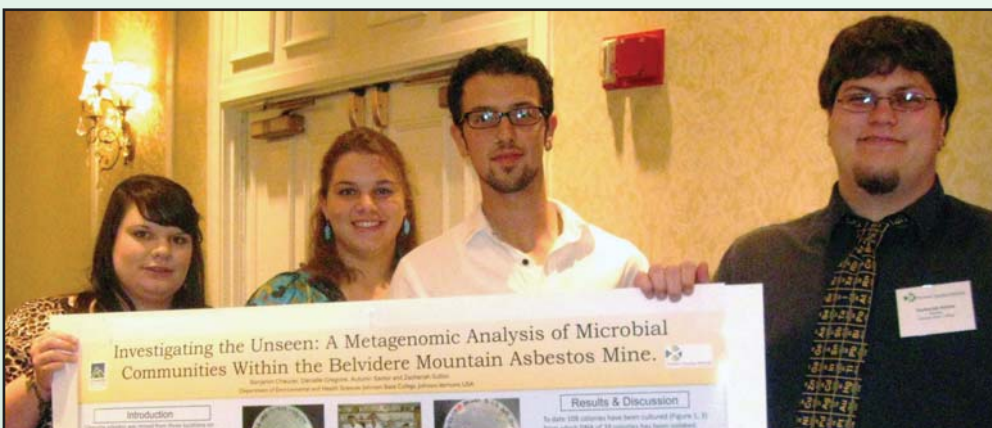
Mr. Tim Hunter



Elizabeth Wuorinen, PhD
Norwich University



Dr. Bryan Ballif





VGN PROJECT AWARD WINNERS

Natalia Blank PhD, Norwich University
"1,2-diamines via Asymmetric Addition of RLi to 1,2-diimines"

Kevin Fleming PhD, Norwich University
"Vigilance and Threat Perception in Military Veterans and Cadets"

Hans Haverkamp PhD, Johnson State College
"Baseline Airway Mechanical Function and Airway Function During Exercise in Asthma"

Shane Lamos PhD, St. Michael's College
"New Chemical Tools for the Quantitative Assessment of Biological Metabolomes"

Gina Mireault PhD, Johnson State College
"Social Referencing to Parent "Clowns": Infant Humor Perception and Attachment"

Mark Spritzer PhD, Middlebury College
"Effects of Testosterone and Social Interactions on Memory and Adult Neurogenesis"

Mark Stefani PhD, Middlebury College
"The role of GABAergic Signaling in Schizophrenia-related Cognitive Disorders"

Elizabeth Wuorinen PhD, Norwich University
"Exercise Intensity Effects on Weight and Fat Loss in Middle Aged Adults"

VGN PILOT AWARD WINNERS

Deborah Alongi PhD, Castleton State College
"Physiological Plasticity in Carbohydrate Metabolism and Transport in Arabidopsis"

Elizabeth Dolci PhD, Johnson State College
"Metagenomic Analysis of an Aquatic Environment at the Lowell/Eden Asbestos Mines"

Karen Hinkle PhD, Norwich University
"Investigation of Mitochondrial DNA Damage in TFM-treated *S.cerevisiae*"

Jennifer Sellers PhD, Green Mountain College
"The Roles of Testosterone & Estradiol on Dominant & Affiliative Behavior"

VGN STUDENT AWARD WINNERS

Elizabeth Dolci PhD/Benjamin Chaucer, Johnson State College
"Asbestos Mine Microbial Communities"

Elizabeth Dolci PhD/Danielle Gregoire, Johnson State College
"Asbestos Mine Microbial Communities"

Mark Stefani PhD/Evan Masseau, Middlebury College
"The Role of GABAergic Signaling in Schizophrenia-related Cognitive Disorders"

Mark Spritzer PhD/William Prince, Middlebury College
"The Effects of Prolactin on Hippocampal Neurogenesis in Male Rats"

Natalia Blank PhD/Anthony Sassi, Norwich University
"Mechanistic Studies of PhLi Addition to N,N'-bis (4methoxyphenyl)-ethylenediimine"

Mark Spritzer PhD/Leanne Shulman, Middlebury College
"Effect of Sexual Experience on Spatial Learning and Memory in Male Rats"



RETURN SERVICE REQUESTED

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Burlington, Vermont 05405-0086

Vermont Genetics Network



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