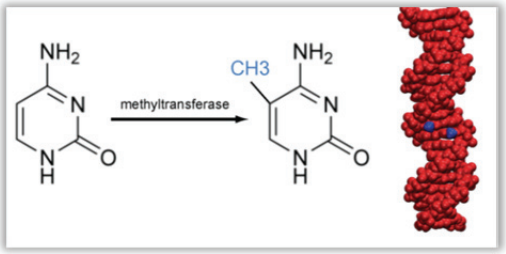
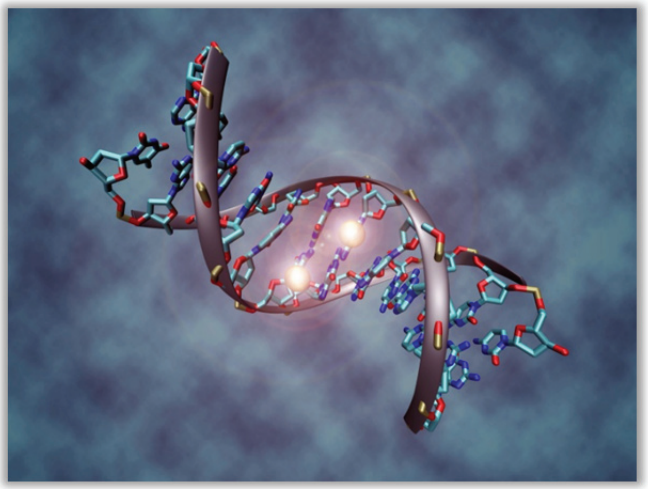
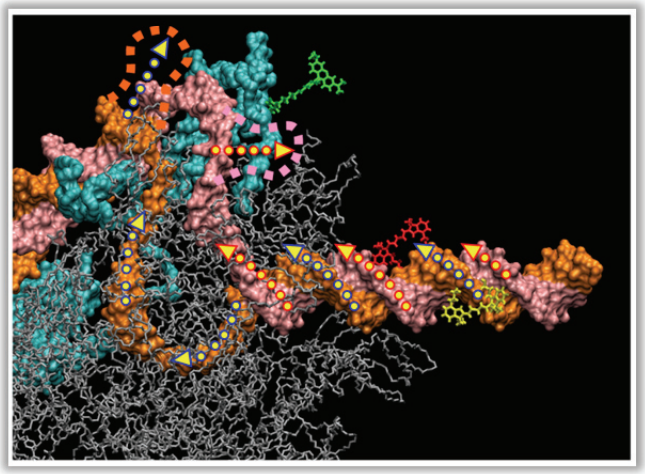
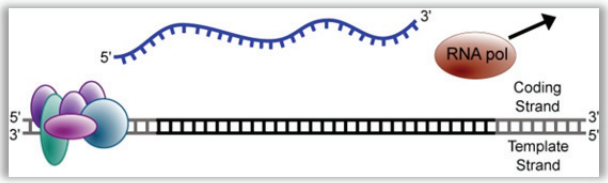
Satellite Education Workshop (SW4):

Epigenomics: Design, Implementation and Analysis for RNA-seq and Methyl-seq Experiments









Saturday March 17, 2012
Orlando, Florida

Workshop Description:

This full day Educational Workshop will provide an overview of current platforms in the rapidly changing world of the next-generation sequencing (NGS) market, with emphasis on epigenomics applications. The workshop is aimed at the beginner/intermediate level and will be useful for core laboratory personnel, graduate students, postdoctoral fellows and principle investigators interested in utilizing or understanding these powerful technologies and methods. The workshop will focus on RNA-seq and Methyl-seq applications; particularly regarding practical considerations, from sample preparation to data analysis, related to performing these workflows in the laboratory. Real-life experiments will be presented as examples, enabling the trainees to appreciate the power of these approaches and understand the efforts involved in executing them.

Organizer:

Anoja Perera, Senior Laboratory Manager at the Stowers Institute for Medical Research

Education Committee Liaison:

Michael R. Zianni, Senior Research Associate at the Ohio State University

Instructors:

J.A. Jeddeloh, PhD

Jeff is currently the Director of Business Development for Roche NimbleGen, Inc. He has been studying DNA methylation for over 12 years since he received his PhD from Washington University. After a postdoctoral position he became the Director of Science and Technology at Orion Genomics LLC where he was instrumental in developing a novel restriction enzyme for locating methylation sites in the genome in combination with arrays. For the past 4 years at Nimblegen he continued his efforts in the development of arrays while also studying DNA methylation. As a result of this work he has a patent for array technology he helped to develop.

David Smith PhD

David is a Professor in the Department of Laboratory Medicine and Pathology at the Mayo Clinic in Rochester, MN. He is also the Chairman of the Technology Assessment Group for the Center for Individualized Medicine and has been responsible for helping to set up the necessary infrastructure for Next Generation sequencing at the Mayo Clinic. David's research focuses on two main areas. The first is the role of long non-coding RNAs on cancer development. The second is to utilize the power of Next

Generation sequencing to better understand the precise alterations that underlie the development of cancers of the head and neck. He is also utilizing Next Generation sequencing to search for molecular markers in head and neck cancers that can be utilized to direct better clinical treatments for individual patients with head and neck cancer.

Ting Wang, PhD.

Ting is an assistant professor in the Department of Genetics and Center for Genome Sciences at Washington University in St. Louis. He has a PhD in Computational Biology. His research focuses on understanding genetic and epigenetic impact of transposable element on human regulatory networks and their role in human diseases. Ting develops algorithms for identifying regulatory motifs, and analytical and visualization methodologies to integrate genomic and epigenomic data, including a visualization platform for NIH's Roadmap Epigenomics project (http://VizHub.wustl.edu/). Dr. Wang is a co-inventor of the UCSC Cancer Genomics Browser, co-inventor of the Human Epigenome Browser and a co-investigator of the Epigenome Roadmap Mapping Centers.

Agenda:

7:00am – 6:00pm	**REGISTRATION OPEN**
7:00am - 8:00am	**CONTINENTAL BREAKFAST**
8:00am – 8:30am	**Introductions** Anoja Perera, Stowers Institute
8:30am – 9:30am	**Sequencing-based DNA Methylomics** Ting Wang, Washington University
9:30am – 10:30am	**Using Next Generation Sequencing to Analyze the Transcriptional Output of Cells.** David I Smith, Mayo Clinic
10:00am - 10:30am	**AM BREAK**
10:30am – 11:30am	**cDNA Capture and Sequencing Reveals Unappreciated Diversity of the Transcriptome** J.A. Jeddeloh, Roche NimbleGen
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11:30am – 12:00pm	**Discussion**
12:00 - 1:00 pm	**LUNCH**
1:00pm – 1:30pm	Presentation by **Life Technologies**
1:30pm – 2:30pm	**Analysis of Methyl-Seq data** Ting Wang, Washington University
2:30pm - 3:00pm	**PM BREAK**
3:00pm - 4:00pm	**Using Next Generation Sequencing to Characterize the Transcriptome, Exome and Methylome of Cancers of the Head and Neck** David I Smith, Mayo Clinic
4:00pm – 4:30pm	**Discussion**

Abstracts:

Sequencing-based DNA Methylomics
Ting Wang, Washington University

DNA methylation plays a vital role in regulation of cellular processes including host defense of endogenous parasitic sequences, embryonic development, transcription, X chromosome inactivation, and genomic imprinting. Recent advancement in genomic technology, in particular in sequencing-based DNA methylation profiling methods provides an unprecedented opportunity to map complete DNA methylome and comprehensively compare DNA methylomes of different cell types, developmental stages, and healthy and diseased tissues. In this presentation, widely used as well as the latest status of modern genomic technologies will be reviewed.

Using Next Generation Sequencing to Analyze the Transcriptional Output of Cells
David I Smith, Mayo Clinic

Next generation sequencing is a powerful technology that utilizes massively parallel sequencing to analyze billions of DNA fragments simultaneously. Just one powerful uses of this technology is its' ability to characterize the transcriptional output of cells. This technology is considerably more powerful than gene expression microarrays for many reasons. The first is that RNAseq can be used to get a digital output of transcription even for transcripts of very low abundance. In addition, since this technology is actually sequencing the transcripts it also provides information on transcript isoforms produced, allele-specific expression and for the more abundantly expressed transcripts can also be used to characterize mutations in different transcripts. The state of Next Generation sequencing (which is increasing its' output 5-10 fold each year) and how this powerful technology can be utilized to study transcription will be reviewed

cDNA Capture and Sequencing Reveals Unappreciated Diversity of the Transcriptome
J. A. Jeddeloh, Roche NimbleGen

Transcriptomic analyses have revealed an unexpected complexity to the human transcriptome, whose breadth and depth likely exceeds current RNA sequencing capabilities. Using microarray arrays to capture and sequence portions of the transcriptome, unannotated transcripts, whose rare or transient expression is below the detection limits of conventional sequencing approaches, were identified and characterized. The consequence of focusing the unprecedented depth of coverage afforded by this technique was revelation of widespread, regulated and remarkably complex noncoding transcription in intergenic regions. Remarkably, the focusing power of the approach maintained the ability to quantify gene expression across 5 logs of base representation. Validation, using both long and short read sequencing technologies and

RT-PCR, demonstrated that intermittent sequenced reads observed in conventional RNA sequencing data sets, previously dismissed as noise, are in fact indicative of unassembled rare transcripts. Mixed read ab-initio transcript assembly revealed the diversity of alternative spicing patterns in both genes and intergenic regions, Lastly, unannotated exons were discovered in even intensively studied protein-coding loci such as p53 and HOX. Collectively, these results reveal the range, depth and complexity of a human transcriptome that is far from fully characterized.

Using Next Generation Sequencing to Characterize the Transcriptome, Exome and Methylome of Cancers of the Head and Neck

David I Smith, Mayo Clinic

Next generation sequencing can be utilized for many things in addition to full genome sequencing. This technology can be used to characterize the transcriptional output of cells, and it also can be used to sequence just the coding portion of the genome, the exome. Finally this technology can also be used to study changes in genome wide methylation. We have been studying a sub-set of head and neck cancers, namely oropharyngeal cancers (which are cancers of the base of the tongue, the tonsils and the larynx). This is an interesting cancer because it can be caused by a history of smoking and drinking, but can also be caused by the presence of the human papillomavirus (HPV). We have been studying different oropharyngeal cancers using Next Generation sequencing in an attempt to compare and contrast the alterations that occur depending upon whether the person who develops this cancer has a history of smoking and drinking or not, but also comparing those patients that are HPV positive to those that have no HPV present. We will present our results of using Next Generation sequencing to characterize transcriptomes, exomes and methylomes. The real challenge is developing tools to integrate these three distinct datasets together to better understand the molecular alterations that occur depending upon the underlying etiology. This work gives powerful insights into the molecular changes that occur in different oropharyngeal cancers.

Acknowledgements:

We would like to thank our sponsors, Life Technologies and Illumina, for their generous support of the workshop. The workshop was also supported through a grant from the National Science Foundation, DBI-1157708**.** We would like to extend our gratitude to the Education Committee and the ABRF for providing critical and necessary support for this workshop.

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