Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Trustees of the University of Pennsylvania

2. **Reporting Period (start and end date of grant award period):** 1/1/2009-12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Gearline R. Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100047654

6. **Project Number and Title of Research Project:** 9 - Genome-based Bio-marker Discovery and Systems Biology Engineering

7. **Start and End Date of Research Project:** 1/1/2009-12/31/2012

8. **Name of Principal Investigator for the Research Project:** Junhyong Kim, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 1,655,102.45

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Carberry, N	Student Worker	Yr 1 11%	$4,223.30
Carey, J	Pre-Doctoral Trainee	Yr1 80%;Yr2 96%	$1,249.98
Caulin, A	Graduate Student Trainee	Yr 1 13%;Yr2 80%	$1,666.64
Chekholko, A	Systems Programmer	Yr 1 12%;Yr 2 15%; Yr 3 42%	$35,478.90
Chumo, B	Student Worker	Yr 1 80%; Yr 2 80%	$9,165.15
Ding D	Student Worker	Yr 1 80%; Yr 2 80%	$9,165.15
Ding, Y	Student Worker	Yr 1 60%; Yr 2 75%	$833.32
Feder, A	Temp Worker	Yr 1 82%	$4,223.30
Francis,C	Post-Doctoral Researcher	Yr 1 30%	$6,399.99
Hogenesch, J	Professor	Yr 1 83%;Yr 2 79%;Yr 3 71%	$178,711.69
Imran Alsous, J	Student Worker	Yr 1 11%	$4,223.30
Kim, J	Professor	Yr 1 5%	$54,820.63
Kwon, S	Student Worker	Yr 2 6%; Yr 3 60%	$4,220.00
Lee, H	Student Worker	Yr 1 80%; Yr 2 80%	$9,165.15
Lee, J	Student Worker	Yr 1 80%; Yr2 80%	$9,165.15
Lee, P	Student Worker	Yr 2 60%; Yr 3 60%	$1,987.20
Li, F	Graduate Student Trainee	Yr 1 13%;Yr 2 65%; Yr 3 40%	$2,554.30
Liu, Z	Student Worker	Yr 1 11%	$4,223.30
Madara, J	Graduate Student Trainee	Yr 1 13%; Yr 2 65%; Yr 3 40%	$2,554.30
Maganty, A	Student Worker	Yr 1 11%	$4,223.30
Murray, J	Asst. Professor	Yr 2 27%; Yr 3 15%	$97,067.75
Natarajan,S	Post-Doctoral Researcher	Yr 1 27%	$14,733.51
Peritz, A	Resource Technologist	Yr 1 46%; Yr 2 32%; Yr 3 17%	$120,141.62
Richards, J	Post-Doctoral Researcher	Yr 2 40%; Yr 3 40%	$36,554.64
Richardson, A	Student Worker	Yr 1 80%; Yr 2 80%	$9,165.15
Shah, A	Student Worker	Yr 1 10%; Yr 2 80%	$8,805.87
Singh,B	Student Worker	Yr 1 80%; Yr 2 80%	$9,165.15
Tsai, E	Graduate Student Trainee	Yr 1 100%; Yr 2 59%; Yr 3 5%	$1,720.98
Umfrey, L	Student Worker	Yr 1 12%; Yr 2 12%	$8,952.00
Walsh, A	Research Fellow	Yr 1 100%; Yr 2 68%; Yr 3 5%	$2,554.30
Walton, T	Research Specialist B	Yr 2 40%; Yr 3 13%	$52,283.40
Wang, C	Temp Lab Tech	Yr 1 11%;Yr 2 11%	$8,952.00
Zacharias, A	Post-Doctoral Researcher	Yr 2 40%; Yr 3 33%	$33,790.00

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if

percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Kim, J.	Professor and Co-Director PGFI	10%
Eberwine, J.	Professor and Co-Director PGFI	10%
Greenberg, R.	Professor	(5% Yr 1; 5% Yr 2)
Hogenesch, J.	Professor	(10% Yr2; 10% Yr 3; 10% Yr 4)
Anafi, R.	Postdoctoral Researcher	(25% Yr 2; 25% Yr 3; 25% Yr 4)
Ganguly, A.	Professor	(10% Yr 3; 10% Yr 4)
Dueck, H.	Graduate Student	(25% Yr 2; 25% Yr 3; 25% Yr 4)

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
High-Performance Computer Networked Storage System	This equipment enabled the storage and analysis of large-scale RNA sequencing data generated by this project. Each sample generates more than 50GB of data and the project would have been impossible without a high-performance computing facility.	$150,879.79

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Molecular, cellular, and physiological mechanisms of the mammalian circadian clock	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	September, 2011	$3,111,205	$3,111,205
Role of single cell mRNA variation in systems associated electrically excitable cells	X NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	January, 2012	$9,390,950	$9,390,950

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes__X_____ No_____

If yes, please describe your plans:

Two key results from this project include the retinoblastoma biomarker study and Chrono circadian rhythm gene identification. We will be submitting new NIH R01 grants to continue to characterize these genes and to develop these genes as biomarkers for dysfunction.

12. Future of Research Project. What are the future plans for this research project?

Three subprojects, the single cell phenotypic marker identification study, the circadian rhythm systems signature study, and the retinoblastoma genome-based marker study will be continued. The data from this project will be used to obtain additional external funding. The studies will concentrate on functional characterization of markers we have obtained as well as refining statistical methods for signal detection.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes__X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male			3	3
Female			2	1
Unknown	14			
Total	**14**	**0**	**5**	**4**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			5	4
Unknown	14			
Total	**14**	**0**	**5**	**4**

	Undergraduate	Masters	Pre-doc	Post-doc
White			2	3
Black				
Asian			3	1
Other				
Unknown	14			
Total	**14**		**5**	**4**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No___X____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This project enabled the Penn Genome Frontiers Institute to form interdisciplinary teams to work on biomarker discovery studies. It enabled collaboration across schools (Engineering,

Arts and Sciences, and Medical school) and helped pilot high-risk technology development that cannot be funded in other contexts. It also helped enhance an existing high-performance computational infrastructure to store and analyze next generation sequence data.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No__X____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending

publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

The overall goal of this project is to use genomic technologies to identify molecular states of cells and tissues, especially with respect to how measurement of key genes or functional states may serve as biological markers for cell/tissue states. Genome-based biomarker discovery often utilized large-scale sequence data analysis coupled with functional genomics data to suggest that certain gene sequences or gene expression patterns are indicative of organismal states. An important concept in biomarker identification is the concept of organismal states. Because an organism is a dynamical system with constantly changing states, it is difficult to distinguish distinct states except at a very coarse level. More recently, the definition and the utility of different tools as biomarkers have expanded to include novel detector systems. In addition, systems biology modeling and functional genomics data allow us to use statistical signatures of measurements to identify cell/tissue states and their phenotypic identities. The specific aims for this grant are:

Aim 1: Develop novel biomarkers for human normal and disease states using high-throughput sequencing, cell-based screening, bio-photonics, and single-cell genomics.

Aim 2: Develop systems models of identified biomarkers and develop single-platform diagnosis devices using identified biomarkers.

Results for Aim 1: Develop novel biomarkers for human normal and disease states using high-throughput sequencing, cell-based screening, bio-photonics, and single-cell genomics.

For this aim, we piloted a new measurement technology to assay double strand breaks of DNA in live organisms and we developed single cell transcriptome measurements to identify functional genomic states that are indicative of cell types.

Double strand breaks of DNA (DSB) initiate extensive alteration of genomic structure and they are indicative of somatic mutation states that can be markers for tumors. The breast cancer susceptibility genes BRCA1 and BRCA2 provide two prominent examples of this interplay between DSB repair and malignancy (Greenberg, 2008; Venkitaraman, 2002). BRCA mutations confer high penetrance breast and ovarian cancer phenotypes and hypersensitivity to anti-cancer DNA damaging agents. The strength of the DSB repair response thus appears to be a pivotal determinant of cancer susceptibility and chemotherapeutic response (Bartkova et al., 2005; Gorgoulis et al., 2005). We developed a novel reporter dependent on the ATM kinase and used the system to study an ATM-dependent transcriptional silencing program in cis to DSBs. We developed a single-cell assay by modifying a previously described transcriptional reporter system to allow simultaneous visualization of DNA damage responses and nascent transcription on a contiguous stretch of chromatin. Our method utilizes a FRET sensor that detects ATM kinase activity in vivo. The sensor consists of a multivalent fusion protein involving the following

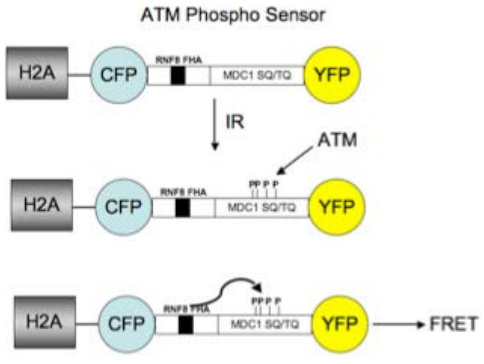

Fig 1. Schematic of FRET-based sensor for ATM-dependent double strand breaks.

modules: the histone H2B protein, CFP-RNF8 FHA domain, MDC1 SQ/TQ motifs, YFP. DSB activation of ATM will initiate ATM-dependent phosphorylation of MDC1 SQ/TQ motif and binding of the RNF8 FHA domain, creating proximity of CFP and YFP and FRET emission. Figure 1 above shows the schematic of the sensor. As previously reported, this sensor demonstrated FRET changes as indicated by a decrease in the YFP/CFP intensity ratio in response to DNA damaging agents. FRET changes were detected in response to 50 uM etoposide and to camptothecin, both of which create DSBs (see Fig 2). ATM kinase activity as reflected in FRET changes were detected only in the nucleus, despite the fact that the biosensor is expressed in both nuclear and cytoplasmic compartments. The first set of sensors did not have a chromatin targeting moiety (histone H2B) and freely diffused in nuclear and cytosolic compartments. We fused the sensor to histone H2B to target the sensor to the chromosome. Interestingly, the chromatin targeting sensor did not show FRET changes in response to etoposide treatment at concentrations similar to those shown in Fig 2. We hypothesized that ATM activity occurred locally at each DSB and that the sensor activity could be amplified when freely diffusible, allowing continuous exposure of the sensor to ATM activity at DNA damage sites. A tethered sensor would be only active at the DSB site, preventing sensor molecules at other nuclear sites from being phosphorylated. In support of this hypothesis, we observed that increasing the dose of etoposide in cells expressing the tethered sensor produced FRET changes (200 uM concentration). At this point in the project, we demonstrated the feasibility of a live-cell DSB reporter system using FRET signals. Additional development of this technology was inhibited by the observed loss of sensitivity; that is, we were able to detect large-scale damage as a function of high concentration of DSB inducing agents but the levels were too high to be useful as a native biosensor. In recent years, single molecule imaging has become feasible in live cells (e.g., Li, and Xie, 2011) but single molecule FRET imaging remains difficult due to a higher sensitivity to noise. However, the techniques learned from this feasibility study contributed greatly to our study of ATM-dependent double strand break chemistry (Shanbhag et al., 2010). [This paper acknowledges funding from PGFI that hosted HRFF, but due to author error that we did not catch in time, the paper failed to acknowledge Pennsylvania Commonwealth directly. We apologize for this mistake.]

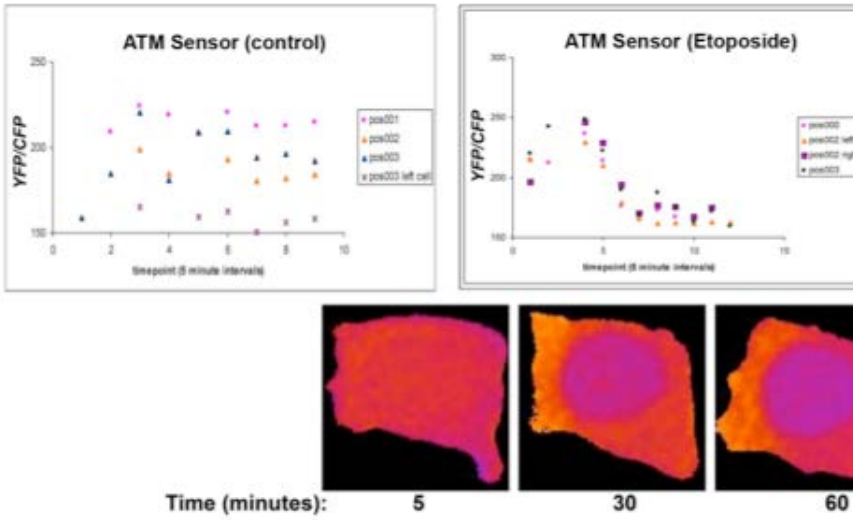

Fig 2: ATM sensor activity. In response to 50 uM etoposide (bottom image and right top panel) change in FRET signal is seen. FRET changes were not evident over time in the absence of etoposide (left top panel) in four different cells.

In our next set of research, we investigated the utility of using single cell whole transcriptome profiling to identify functional genomics signatures of cell identity and cell states. This work follows our original observation in Sul et al. (2009) that at the single cell level, a surprisingly large region of the transcriptome space supports the cell phenotype of traditionally identified cell types. The single cell transcriptome profiling suggests that biomarkers for cell types can be highly variable—however, we also hypothesized that single cell measurements will be more sensitive due to their ability to reduce averaging effects from large collections of cells. Therefore, we sought to identify statistical signatures of cell types. We isolated single cells from dispersed cell culture or live tissue slices using micro-pippettes. The RNA from individual cells were amplified using the Eberwine In Vitro Transcription (IVT) protocol (Morris, et al. 2011) and made into Illumina Hiseq RNA sequencing libraries. Each sample was multiplexed, three samples per lane, with the goal of obtaining 50 million to 80 million reads per sample. The resulting next-generation sequencing reads were processed by custom routines to filter for quality and then aligned using the Penn developed RUM alignment algorithm (Grant et al., 2012). The data were normalized using a combination of custom routines and the methods described in Srivastava and Chen (2010) and Robinson and Oshlack (2010).

We have described the broad characteristics of single cell transcriptomes and their utility as cell phenotype markers in Eberwine et al. (2012) and in particular we provided a model-based explanation of single cell variability in Kim and Eberwine (2010), where we hypothesized that the expression levels of any particular gene are constrained by the molecular physiology of the cell; but, nevertheless we expected molecular signatures of cell types. We assayed single cell transcriptomes from mouse cardiomyocytes (CRD), brown adipose cells (BAT), hippocampal neurons (HIP), ventral dorsal raphe cells (DRV), lateral dorsal raphe cells (DRL), and cortex neurons (CTX). Figure 3 shows the broad pattern of transcriptome correlation amongst single cells of the six cell types shown as a cluster diagram and correlation heat map (rank correlation). As shown in the figure, there are both coherent signatures as seen in the groupings of cardiomyocytes and CNS cells, and heterogeneity in the overall signature as seen in the scattering of the BAT cells and dorsal raphe cells. Figure 4 shows a subset of the genes that we have identified as potential markers for the cell types despite the variability. The figure shows a cluster diagram of the cell types, restricted to this subset, showing much better coherence, and the heat map of the expression of the genes. The heat map shows a strong block structure suggesting their utility as biomarkers for these cell types. We will continue this work to develop a multivariate signature that we expect to yield much more robust biomarker patterns.

In addition to single cell RNA levels, we also used the single cell RNA sequences to identify novel sequence features. In particular, we previously reported the presence of intronic sequences in cytoplasmic RNA (Buckley et al., 2010, Bell et al. 2010). In Bell et al. (2010) we showed evidence that these cytoplasmic intron-sequence retaining transcripts (CIRTs) can indicate a novel functional compartment of a neuron. Therefore, we hypothesized that the presence of particular CIRTs in cells might serve as biomarkers for physiological states. We used single cell RNA sequence data from mouse and rat neurons, cardiomyocytes, and BAT cells, along with micro-dissected dendrite RNA sequence data to survey the genomic prevalence of CIRTs to assess their utility as biomarkers. Surprisingly, we found ~59% of the mouse genes and ~44% rat genes in dissected dendritic samples showed evidence for the presence of CIRTs. The median read density in the intronic regions of these genes ranged 3-7 reads per 50 bp while the

maximum read density was in the range of 950-2350 reads per 50 bp. We also identified a set of transcripts in both dendritic as well as soma samples of rat and mouse wherein the sequence evidence comes only from within the intronic regions of a gene model (8-15%). For these transcripts the median read density was in the range of 1-4 reads per 50 bp and the maximum was in the range 290-950 reads per 50 bp. We also analyzed non-neuronal single cell datasets, namely four independently collected single cells from mouse brown adipose tissue (BAT) and seven single cells from mouse cardiomyocytes using the same analysis pipeline for CIRTs. For these two cell types we also saw a widespread prevalence of CIRTs. In BAT cells we observed ~68% of the genes giving rise to CIRTs whereas in cardiomyocytes there were 56% of genes that resulted in CIRTs. The pair-wise overlap of retained introns amongst the different BAT cells ranged between 8-22% with a small fraction of ~1.5% of the introns appearing in CIRTs in all the samples. Similarly, for the cardiomyocytes the pair-wise overlap of retained introns ranged between 10-24% with ~1.3% of the introns appearing in CIRTs in all the samples. Thus, the CIRT data showed evidence of utility as a discriminator of both cell types and cell states.

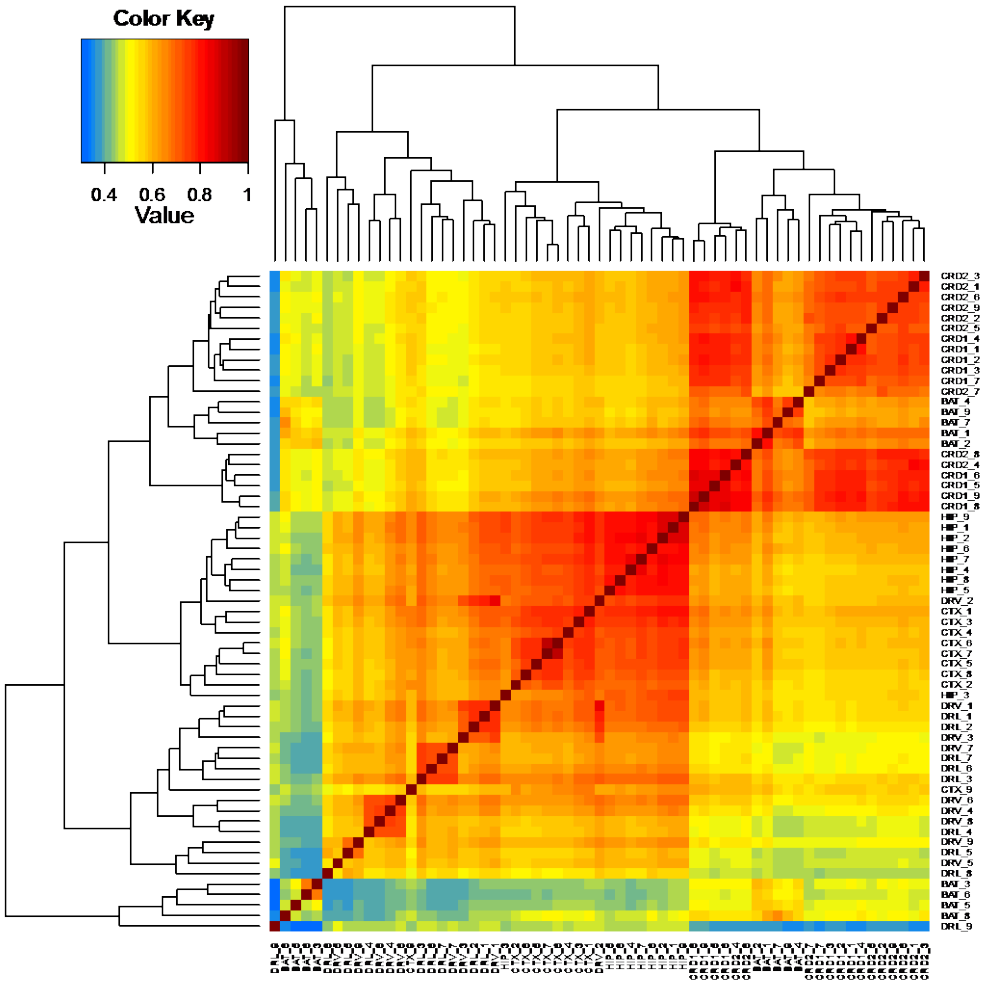

Fig 3: Clustering of six different single cell transcriptomes (with replicates) using rank correlation. The heatmap shows degree of correlation. The cell types are cardiomyoctye (CRD), brown adipose tissue (BAT), dorsal raphe ventral and lateral (DRV and DRL), hippocampal neurons (HIP) and cortex neurons (CRX).

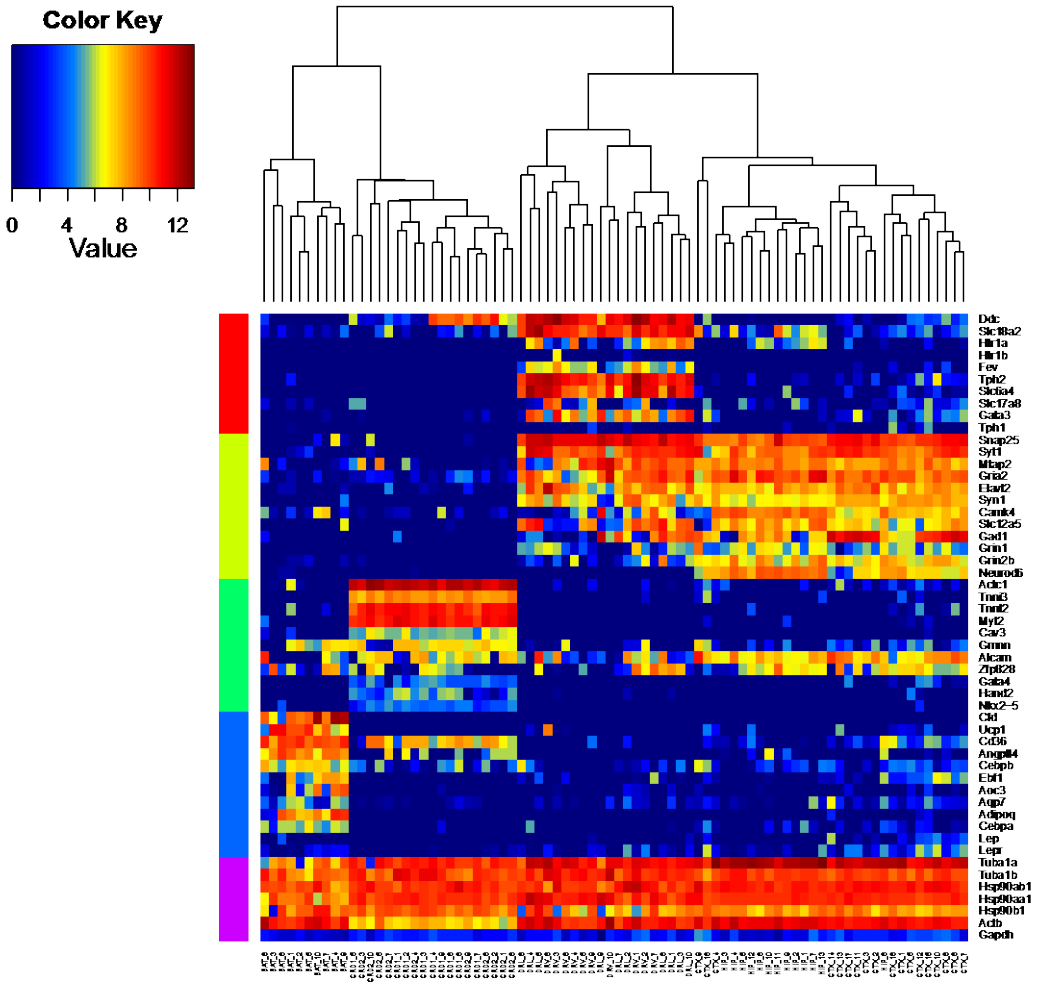

Fig 4: Marker genes in the single cell transcriptome that discriminates the six cell types described in Fig 3. The heat map shows expression levels of the genes indicated in row labels.

Results for Aim 2: Develop systems models of identified biomarkers and develop single-platform diagnosis devices using identified biomarkers.

To meet Aim 2, we worked on two subprojects: 1) to use systems level modeling and machine learning techniques to identify signatures of circadian rhythm core components, and 2) to develop molecular signatures of matched normal and retinoblastoma (RB) tumor pairs using next-generation sequencing.

Throughout the last decades, researchers have characterized a set of "clock genes" that drive daily rhythms in physiology and behavior. The importance of this work is highlighted by the involvement of these same genes in metabolic, neoplastic, and psychiatric disorders. To accelerate the discovery of core clock components, we integrated genome-wide data sets within a probabilistic framework and rank-ordered genes by the cumulative evidence that they have an essential circadian function. In order to find novel biomarkers for clock signal, we first enumerated features that embodied our definition of a core clock component: (1) Core clock transcripts cycle with a ~24-hour period; (2) Core clock gene mutation or knockdown affects circadian behavioral rhythms; (3) Core clock genes interact with other core clock genes; (4) Core clock genes are expressed in most tissues; and (5) Core clock genes are phylogenically conserved between vertebrates and invertebrates (e.g., *Drosophila melanogaster*). As none of these features are absolute requirements (e.g., known clock genes do not display all of these signatures), we sought to delineate a statistical signature using Bayesian integration. For each of these features, we developed a numerical measure (clock metrics) that quantified a scale for the feature and then computed an empirical distribution of the metrics as an estimate of the probability of that clock component. We then computed a Bayes' factor for each feature consisting of P(observed metric in interval I | gene in known clock component)/P(observed metric is in interval I | gene is non-clock). The interval, I, for each feature was determined from the empirical distribution of the known clock components (Fig 5). The Bayes' factors for all five features were multiplied assuming weak independence to yield an overall statistical signature of biomarkers for clock component. The top genes from this analysis are shown in Figure 5F.

This analysis yielded prediction of several novel putative clock components, including a gene previously annotated as Gm129. We carried out functional characterization of this gene assessing its interaction with known clock genes PER2 and BMAL1. Several assays suggested that Gm129, which we renamed Chrono, physically interacts with PER2 and BMAL and represses BMAL1 transcriptional activity. We obtained a knockout mouse from the Knockout Mouse Project (Skarnes et al., 2011) and carried out an activity assay. Under free-running conditions, homozygous Chrono knockouts exhibited a statistically significant ($p < 0.05$) ~25 minute increase in circadian period as compared to wild type controls (Fig 6). Heterozygous knockouts demonstrated an intermediate period. These data strongly suggest that Chrono plays an important regulatory role in the mammalian circadian clock. In sum, we developed a novel statistical signature of circadian rhythm components of the genome and validated its utility by identifying a novel clock component. This paper has been completed and is being submitted during the 1st quarter of 2013.

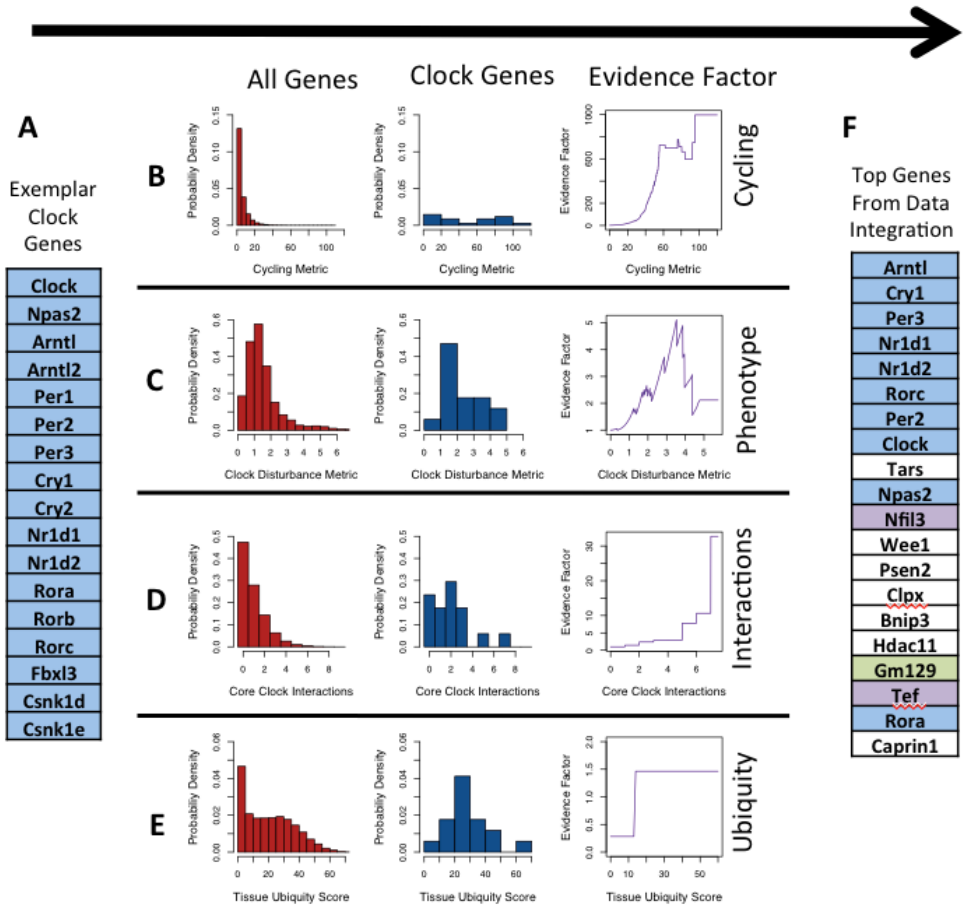

Fig 5: System signature of clock components and empirical distribution of exemplar clock genes (panel A and blue histogram) versus background (red histogram) for four metrics of clock score (panels B-E). Panel F shows the top 20 scoring genes.

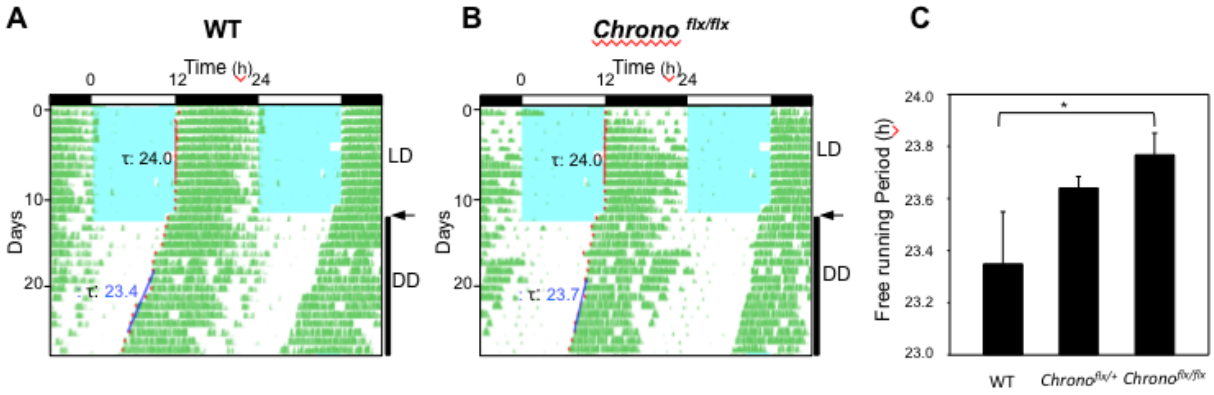

Fig 6: Phenotype activity assay of Wild Type (A) versus Chrono knockout mouse (B and C).

As previously reported, we also carried out a pilot study using next-generation sequencing to identify markers for Retinoblastoma. Retinoblastoma (RB) is the most common intraocular cancer in children. Sporadic RB is caused by de novo germline mutations in RB1 gene in ~80% of newly diagnosed bilateral cases and ~15% of unilateral cases. De novo germline mutation may be the result of either a pre-conception mutation in a parental germ cell, or an early post-zygotic mutation giving rise to somatic and/or germline mosaicism. Detection of a mosaic mutation, which is challenging, alters the future risk of second cancer for the affected individual. Deep sequencing methods are significantly more efficient in detecting low-level mosaic mutations missed by traditional sequencing. We performed deep sequencing of RB1 gene on DNA isolated from blood of 69 RB probands with unilateral disease, with 93 somatic mutations identified in tumors, but without any germline mutation. The individual exons of RB1 gene with known somatic mutations were amplified using DNA of the respective individual. The amplicons were pooled in equimolar concentrations, barcoded libraries prepared and sequenced on the Ion PGM using 316 chip and 100b sequencing kit. Sequencing was performed in duplicate. To estimate the level of background sequencing error, all the tested exons were amplified using DNA from three healthy individuals, pooled, and sequenced following the same procedure. Sequence data were analyzed by Torrent Suite (Life Technologies) and NextGENe software (Softgenetics). About 250 Mb data were generated on a single 316 chip run and the average coverage for each exon was above 10,000. Five low-level mosaic mutations were identified. Sanger sequencing missed three of these mutations and two were detectable upon re-examination of the chromatograms. These variants were called with high statistical significance ($p< 0.0001$). Thus, the incidence of mosaic germline mutation is estimated to be 7.2% in unilateral RB probands. Use of deep sequencing platform, Ion PGM, yielded highly sensitive detection of low-level mosaic mutations in RB1 gene. While previous methods using allele-specific PCR had predicted presence of ~3% mosaic mutations in unilateral RB, the current platform indicates that the rate can be as high as 7%. Considering, 15% as the standard germline mutation frequency in sporadic unilateral RB, this is a significant increase. This finding changes the genetic counseling for risk of second cancer in the proband and for future affected offsprings.

References Cited

Anders, S. and W. Huber. 2010. Differential expression analysis for sequence count data. Genome biology **11**(10): p. R106.

Bartkova, J., Horejsi, Z., Koed, K., Kramer, A., Tort, F., Zieger, K., Guldberg, P., Sehested, M., Nesland, J.M., Lukas, C., et al. (2005). DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 434, 864-870.

Bell, T.J, Miyashiro, K.Y., Sul, J.-Y., Buckley, P.T., Lee, M.T., McCullough, R., Jochems, J., Kim, J. Cantor, C.R., Parsons, T.D. and J.H. Eberwine. 2010. Intron retention facilitates splice variant diversity in calcium-activated big potassium channel populations. PNAS 107(49):21152-21157.

Buckley, P.T., Lee, M.T., Sul, J.-Y., Miyashiro, K.Y., Bell, T.J., Fisher, S.A., Kim, J. and J. Eberwine. 2010. Broad pattern of intron retention promotes multiple mechanisms for localization of dendritic RNA. Neuron 69:877.-884

Eberwine, J., Lovatt D., Buckley, P., Dueck, H., Francis, C., Kim, T.K., Lee, J., Lee, M., Miyashiro K., Morris, J., Peritz, T., Schochet, T., Spaethling, J., Sul, J.-Y., and J. Kim 2012. Quantitative biology of Single Cells. J. Roy. Soc. Interface doi:10.1098/rsif.2012.0417.

Gorgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., Venere, M., Ditullio, R.A., Jr., Kastrinakis, N.G., Levy, B., et al. (2005). Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 434, 907-913.

Grant, G.R., et al., 2011. Comparative analysis of RNA-Seq alignment algorithms and the RNA-Seq unified mapper (RUM). Bioinformatics **27**(18): p. 2518-28.

Greenberg, R.A. (2008). Recognition of DNA double strand breaks by the BRCA1 tumor suppressor network. Chromosoma 117, 305-317.

J. Kim and J. Eberwine. 2010. RNA as the state memory of cellular phenotype. Trends in Cell Biology, DOI: 10.1016/j.tcb.2010.03.003

Li, G.-W. and X.S. Xie. 2011. Central dogma at the single-molecule level in living cells. Nature 475:308-315.

Morris, J., Singh, J.M., and J. H. Eberwine. 2011. Transcriptome analysis of single cells, J. Vis. Exp. 50:2634.

Robinson, M.D. and A. Oshlack. 2010 A scaling normalization method for differential expression analysis of RNA-seq data. Genome biology **11**(3): p. R25.

Skarnes, W.C., Rosen, B., West, A.P., Koutsourakis, M., Bushell, W., Iyer, V., Mujica, A.O., Thomas, M., Harrow, J., Cox, T., et al. 2011. A conditional knockout resource for the genome-wide study of mouse gene function. Nature *474*, 337–342.

Shanbhag, H.M., Rafalska-Metcalf, I.Y., Balane-Bolivar, C., Janicki, S. M., and R. A. Greenberg. 2010. ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. Cell 141:970-981.

Srivastava, S. and L. Chen, A two-parameter generalized Poisson model to improve the analysis of RNA-seq data. Nucleic acids research, 2010. **38**(17): p. e170.

Sul, J.-Y., Wu, C.K., Zeng, F., Jochems, J., Lee, M.T., Kim, T.K, Peritz T., Buckley, P., Cappelleri, D.J., Maronski, M., Kim, M., Kumar, V., Meaney, D., Kim, J., and Eberwine, J. 2009. Transcripome transfer produces a predictable cellular phenotype. PNAS USA doi: 10.1073/pnas.0902161106.

Venkitaraman, A.R. (2002). Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 108, 171-182.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
X____ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two

publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes___X_____ No_____

If yes, please describe your plans:

Three papers have completed manuscripts and are in the process of being submitted or being revised. These are:

Chen, Z., Moran, K., Yutz, J.R., Gehardt, D., Ganguly, T., Shields, C.L., and A. Ganguly, 2013. Enhanced sensitivity of germline mosaic RB1 mutation detection on IOPGM Next-generation sequencing platform. To be submitted to Cancer Biology.

Anafi, R.C., Lee, Y., Sato, T.K., Venkataraman, A., Ramanathan, C., Hughes, M.E., Baggs, J.E., Liu, A.C., Kim, J. and J.B. Hogenesch. 2013. A machine learning approach identifies Chrono as a novel circadian clock repressor. To be submitted to Cell.

Khaladkar, M., Buckley, P.T., Lee, M., **Eberwine, J**. and **J. Kim** 2012. A genome-wide survey of cytoplasmically retained intron sequence transcripts through single cell sequencing. In revision In revision for Genome Research.

One paper is in manuscript preparation stage:

Dueck, H., Kim, T.K., Spaethling, J., Khaladkar, M., Eberwine, J. and J. Kim 2013. Single

cell transcriptome variation and cell type determinants in mouse genome. To be submitted to Science.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

We have identified a novel component of the circadian rhythm called Chrono. We have developed a list of single cell transcriptome signatures to identify mammalian cell types at high resolution.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No___
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No___

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes__ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow			
NAME Junhyong Kim	POSITION TITLE Professor		
eRA COMMONS USER NAME junhyong			
EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral*			
INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Seoul National University, Seoul, Korea	B.S.	1984	Microbiology
SUNY at Stony Brook, Stony Brook , NY	MS/PhD	1986/1992	Ecology and Evolution
University of Arizona, Tucson, AZ	Postdoc	1992-1994	Drosophila Genetics

A. Personal Statement:

My laboratory works at the intersection of genomics, evolutionary biology, and quantitative biology. I have 25 years of experience in computational and theoretical biology. In my lab, we typically ask evolutionary questions applied to genomic models systems using computational, statistical, and mathematical tools. Recently we have been working on the evolution of temporal regulation using yeast transcriptome analysis, cell differentiation and transformation using rat neurons, single-cell transcriptome variability, and evolution of dendritic function in mammalian neurons. In particular, our key goal is to understand the regulation and evolution of single cell function in vertebrates. We have published in the fields of evolution, ecology, systematics, human genetics, anthropology, computational biology, genomics, neuroscience, and biophysics. In training, I have personally mentored 40 undergrads, graduate students, and postdocs. I have created new diversity training programs within Penn Genome Frontiers Institute and have develop a public high school genomics curriculum.

B. Positions and Honors

Edmund J. and Louise W. Kahn Professor, July 2002—present, Department of Biology, University of Pennsylvania, joint appointment in Department of Computer and Information Science, Penn Center for Bioinformatics.
Co-Director, Penn Genomics Institute, Oct 1, 2006--present
Jan 1994 – June 2002: Assistant and Associate Professor (with tenure), Department of Biology
(currently Ecology and Evolutionary Biology), Yale University. Joint appointment in Department of Statistics (since 1998), Department of Molecular, Cellular, and Developmental Biology, and Biomedical Engineering program (since 1997).
Sept. 2001- June 2002: Director, BBS graduate track in Bioinformatics and Computational Biology,
Yale Univ.
Sept. 1992 - Dec. 1993: Research associate in Department of Ecology and Evolutionary Biology, University of Arizona.
Steering Committee for Life Sciences, National Academies Koshland Museum.
Associate Editor, Systematic Biology, IEEE Transactions in Computational Biology and Bioinformatics

Member, NIH Genetics of Variations Study Section
Ellison Medical Foundation Senior Scholar in Aging, 2010
Guggenheim Fellow, 2010
Yale Senior Faculty Award

Yale Seessel Anonymous Award for studies in Biochemistry
Alfred Sloan Foundation Young Investigator Award in Molecular Evolution

C. SELECTED RECENT PUBLICATIONS

*Simola, D., and **J. Kim**. 2011. Using multi-locus Bayesian model and redundantly mapped sequence reads to enhance SNP discovery with next-generation sequencing. Genome Biology 12:R55.

*Kim, T., Sul, J.Y., Peternko, N., Lee, M., Patel, V., Kim, J. and J. Eberwine. 2011 Transcriptome transfer provides a model for understanding the phenotype of cardimyocytes. PNAS doi:10.1073/pnas.1101223108.

*Buckley, P.T., Lee, M.T., Sul, J.-Y., Miyashiro, K.Y., Bell, T.J., Fisher, S.A., **Kim, J. and J. Eberwine**. 2010. Broad pattern of intron retention promotes multiple mechanisms for localization of dendritic RNA. Neuron 69:877.-884

*Bell, T.J, Miyashiro, K.Y., Sul, J.-Y., Buckley, P.T., Lee, M.T., McCullough, R., Jochems, J., Kim, J. Cantor, C.R., Parsons, T.D. and J.H. Eberwine. 2010. Intron retention facilitates splice variant diversity in calcium-activated big potassium channel populations. PNAS 107(49):21152-21157.

Simola, D.F., Francis, C., Sniegowski, P.D., and **J. Kim**. 2010. Heterochronic evolution reveals modular timing changes in budding yeast transcriptomes. Genome Biology 11:R105.

***J. Kim and J. Eberwine.** 2010. RNA as the state memory of cellular phenotype. Trends in Cell Biology, doi:10.1016/j.tcb.2010.03.003.

*Guo, S., and **J. Kim**. 2009. Dissecting the molecular mechanism of Drosophila odorant receptors through statistical modeling and comparative sequence analysis. Proteins: Structure, Function, and Bioinformatics. DOI: 10.1002/prot.22556.

*Sul, J.-Y., Wu, C.K., Zeng, F., Jochems, J., Lee, M.T., Kim, T.K, Peritz T., Buckley, P., Cappelleri, D.J., Maronski, M., Kim, M., Kumar, V., Meaney, D., **Kim, J., and Eberwine**, **J.** 2009. Transcripome transfer produces a predictable cellular phenotype. PNAS USA doi: 10.1073/pnas.0902161106.

*Kreher, S.A., J. Kim and J.R. Carlson. 2008. Translation of sensory input into behavioral output via an olfactory system. Neuron 59:110-124.

*Lee, M.T. and **J. Kim**. 2008. Self-containment, a property of modular RNA structures, distinguishes MicroRNAs. PLoS Comp. Biol. 4(8):e1000150.

***Kim, J. and M.J. Sanderson**. 2008. Penalized likelihood phylogenetic inference: bridging the parsimony-likelihood gap. Syst. Biol. 57(5):665.

*Hartling, J. and **Kim, J.** 2007 Mutational robustness and geometrical form in protein structures. J. Exp. Zool. Part B: Mol. Dev. Evol. 10.1002/jez.b.21203.

*Guo, S. and **J. Kim.** 2007. Molecular evolution of Drosophila odorant receptor genes. Mol. Biol. Evol. doi:10.1093/molbev/msm038

*Hadley, D., Murphy, T., Valladares, O., Hannenhalli, S. Ungar, L., Kim, J. and Bucan, M. 2006. Patterns of sequence conservation in presynaptic neural genes. Genome Biol. 7:R105.

*Ge, F., Wang, L.S., **Kim, J**. 2005. Cobweb of life revealed by genome-scale estimates of horizontal gene transfer. PLOS Biology, 3(10):e316.

*Rifkin, S. A., Houle, D**., Kim, J. and White, K.P.** 2005. A mutation accumulation assay reveals extensive capacity for rapid gene expression evolution. Nature, 438: 220-223.

*Magwene, P. M. and **J. Kim**. 2004. Estimating genomic co-expression networks using first-order

conditional independence. Genome Biology,5(12):R100.
*Rifkin, S. A., J. Kim, and K. P. White. 2003. Evolution of gene-expression during metamorphosis in the *Drosophila melanogaster* subgroup. Nature Genetics 33(2):138-144.
Kim, J. Computers are from Mars, Organisms are from Venus: An interrelationship guide to Biology and Computer Science. IEEE Computer, July 2002.
*Clyne, P. J., C. G. Warr, M. R. Freeman, D. Lessing, J. Kim, and J. Carlson. 1999. A novel family of seven transmembrane proteins: candidate odorant receptors in Drosophila. Neuron. 22(2):327-338.

BIOGRAPHICAL SKETCH

James H. Eberwine

eRA COMMONS USER NAME eberwine

EDUCATION/TRAINING

POSITION TITLE

Professor of Pharmacology and Psychiatry

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Yale University, New Haven, CT	B.S.	1978	Biochem and Philosophy
Columbia University, New York, NY	Ph.D.	1984	Biochemistry
Stanford University, Stanford, CA	Postdoc	1984-1989	Molecular Neurobiology

A. Personal Statement.

Over the last 20 years I have pioneered the molecular analysis of pharmacological and disease-associated mRNA from small amounts of tissue including single cells by developing approaches to enable this level of analysis. For example, using RNAs isolated from single dendrites followed by aRNA amplification (developed in my lab), we found that dendrites have a greater RNA complexity than was suspected (~1000 mRNAs localized in dendrites). In another study, we demonstrated protein synthesis in dendrites by transfecting an epitope-tag encoding mRNA into isolated live dendrites and showing that the tag was translated. Such analyses of dendrites provide insight into the functioning of this important substrate of cognition. With regard to molecular analysis of CNS disorders the aRNA procedure has been used to generate probes for >70% of all published mRNA microarray studies thereby dramatically impacting biomarker discovery and therapeutic development. To make this scientific progress, I have had to develop many techniques including those involved in RNA binding protein and mRNA cargo identification (PAIR and APRA procedures), phototransfection for introducing mRNA into individual dendrites and cell somas and single cell PCR and the aRNA procedures for the analysis of mRNA in single cells. Among these discoveries is the surprising fact that many dendritically localized mRNAs have retained introns that can be spliced in the cytoplasm of the cell to give rise to the functionally relevant mRNAs. This is a developing story in my lab. Recently our TIPeR (transcriptome induced phenotype remodeling) paper pioneered an approach for converting one cellular phenotype to a distinct phenotype through the transfer of RNA populations.

B. Positions and Honors.

Positions and Employment

1990-1994 Assistant Professor, Depts. of Pharmacology and Psychiatry, Univ. of Penn School of Medicine
1994-1996 Associate Professor, Depts. of Pharmacology and Psychiatry, Univ. of Penn School of Medicine
1996-present Professor, Depts. of Pharmacology and Psychiatry, Univ. of Pennsylvania School of Medicine
2006-present Co-Director, PENN Genomics Institute
2007-present Elmer Holmes Bobst Chair of Pharmacology, University of Pennsylvania School of Medicine

Other Experience and Professional Memberships (selected)

1987-1993 Founding Course Director for "Cloning of Neural Genes" for first 7 years, Cold Spring Harbor
1992-1997 Established Investigator of the American Heart Association
1997 Presidential Lecturer for Society for Neuroscience Meeting
1998 Matthew Moore Distinguished Lecture, AANP
1998 Director's Lecture, NIH
1998 Dresel Lectureship - Dalhousie University
1998 Distinguished Visiting Scientist, Albany Medical College
1999 Plenary Lecturer at 1st Korea/USA Joint Workshop on Neuroscience
2000 Dean's Lecture at University of Kentucky
2001 Presidential Symposium Association for Research in Otolaryngology
2002 Presidential Symposium – Society for the Study of Reproduction
2003 Andre McInvale Memorial Lecturer, University of Colorado
2005-09 Course Co-Director, Advanced Technologies in Molecular Neuroscience, Cold Spring Harbor

2010 Keynote Lecturer at Royal Society International Symposium on "Making light deliver: Optical nanosurgery for targeted drug delivery"
2012 Founding Course Director, Single Cell Analysis, Cold Spring Harbor
2012 Organizer – Society for Neuroscience Short Course entitled "Transcriptomics"
2012-13 Course Director, Single Cell Analysis, Cold Spring Harbor (1st Course of this type)
2013-18 External Advisory Board – Institute for Genomic Biology – Univ. of Illinois

Honors (Selected)

2001-2011 MERIT Award
2005 Stephen and Constance Lieber Distinguished Investigator for the (NARSAD)
2008 NIH Director's Pioneer Award Recipient
2009 EUREKA grant (co-recipient with Junhyong Kim)
2011 Ellison Medical Foundation Senior Scholar in Aging
2012 McKnight Technological Innovations in Neuroscience Award

C. Selected Peer-Reviewed Publications (in chronological order).

Eberwine J, Yeh H, Miyashiro K, Cao Y, Nair S, Finnell R, Zettel, M, Coleman P. (1992) Analysis of gene expression in single live neurons. PNAS 89: 3010-3014. [PMC48793]

Miyashiro K, Dichter M, Eberwine J. (1994) On the nature and distribution of mRNAs in hippocampal neurites: implications for neuronal functioning. PNAS. 91: 10800-10804. [PMC45113]

Crino P and Eberwine J. Molecular characterization of the dendritic growth cone: Regulated mRNA Transport and Local Protein Synthesis. Neuron 17:1173-1187, 1996.

Miyashiro, K., Beckel-Mitchener, A., Purk, T. P., Belt, B., Kelly, A., Becker, K., Barret, T., Weiler, I.J., Greenough, W. and Eberwine, J.: Deficits in Cellular Functioning Associated with the Loss of FMRP in the CNS of Knockout Mice as Revealed by Antibody Positioned RNA Amplification. Neuron. 37:417-431, 2003.

Glanzer, J., Belt, B., Sul, J-Y., Barrett, L., Haydon, P. and Eberwine, J.: RNA Splicing Capability of Live Neuronal Dendrites, Proc. Natl. Acad. Sci. 102:16859-64, 2005. [PMC1277967]

Job C and Eberwine J. (2001) Localization and translation of mRNA in dendrites and axons. Nature Reviews Neuroscience 2: 889-898. [PMID: 11733796]

Barrett L, Sul J, Takano H, Van Bockstaele E, Haydon P, Eberwine J. (2006) Region-directed phototransafection reveals the significance of a dendritically synthesized transcription factor. Nature Methods 3: 455-460. [PMID: 16721379]

Bell T, Miyashiro K, Sul J-Y, McCullough R, Buckley P, Jochems J, Meaney D, Haydon P, Cantor C, Parsons T, Eberwine J. (2009) Cytoplasmic BK Ca channel intron-containing mRNAs contribute to the intrinsic excitability of hippocampal neurons. PNAS 105(6): 1901-1906. [PMC2538856]

Sul JY, Wu CK, Zeng F, Jochems J, Lee MT, Kim TK, Peritz T, Buckley P, Cappelleri D, Maronski M, Kim, M, Kumar V, Meaney D, Kim J, Eberwine J. (2009) Transcriptome transfer produces a predictable cellular phenotype. PNAS 106(18): 7624-7629. [PMC2670883]

Bell, T., Miyashiro, K., Sul, J-Y, Buckley, P., Lee, M., Jochems, J., Kim, J., Parsons, T. and Eberwine, J. (2010) Cytoplasmic intron retention facilitates exon-choice and structural diversity within BKCa channel splice variants, Proc. Natl. Acad. Sci. 107:21152-21157. [PMID 21078998]

Kim J and Eberwine J. (2010) RNA: state memory and mediator of cellular phenotype. Trends in Cell Biology, (2010) 20:311-318. [PMC 2892202]

Eberwine, J. and Bartfai, J.(2011): Single cell transcriptomics of hypothalamic warm sensitive neurons that control core body temperature and fever response: Signalling asymmetry and extension of chemical neuroanatomy. Pharmacology and Therapeutics. 129:241-259. PMID: 20970451]

Buckley, P., Lee, M., Sul, J-Y, Miyashiro, K., Bell, T., Fisher, S., Kim, J. and Eberwine, J.(2011): Cytoplasmic intron sequence-retaining transcripts (CIRTs) can be dendritically targeted via ID element retrotransposons. Neuron, 69:877-884. [PMID 21382548]

Kim, T-K., Sul, J-Y., Peternko, N., Lee, M., Patel, V., Kim, J. and Eberwine, J. (2011): tCardiomyocyte: a model for generating and understanding the phenotype of cardiomyocytes. Proc. Natl. Acad. Sci., 108(29):11918-23. [PMID:21730152].

Sul, J-Y., Kim, T. K., Lee, J. and Eberwine, J.: Perspectives on cell reprogramming with RNA. Trends in Biotechnology*, doi:10.1016/j.tibtech.2012.02.004, 2012.*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Hogenesch, John B.

eRA COMMONS USER NAME hogenesch

POSITION TITLE Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Southern California	B.A.	1989	History
University of Southern California	B.S.	1991	Biology
Northwestern University	Ph.D.	1999	Neuroscience
University of Wisconsin-Madison	Postdoc	1999	Neuroscience
Genomics Institute of the Novartis Research Foundation	Postdoc	2000	Neuroscience

A. Personal Statement

I've spent the last 18 years acquiring expertise in genomics and informatics in both Pharma and academia. This includes large-scale data generation and analysis efforts such as the Gene Atlas, where we defined the expression patterns for human and mouse genes in a comprehensive set of tissues. I also serve as Associate Director of the Penn Genome Frontiers Institute, which houses sequencing and HPC facilities. In addition, I currently serve as Interim Director of the Penn Center for Bioinformatics, which is home of 10 academic bioinformatics laboratories and the Genomics and Computational Biology Graduate Program. My basic research experiences leverage functional genomics approaches including cell based screening to find those genes and networks that play important roles in mediating biological pathways (see refs 5-13). These methods leverage small molecules and RNAi in dose response to define quantitative edges in biological networks (see refs 13-14).

B. Positions and Employment

2000-2004 Program Manager of Genomics, the Genomics Institute of the Novartis Research Foundation, La Jolla, CA

2003-2004 Assistant Professor, Department of Neuropharmacology, the Scripps Research Institute, La Jolla, CA

2004-2006 Professor of Biochemistry and Director of Genome Technology, the Scripps Research Institute, Jupiter, FL

2006-2011 Associate Professor, Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA

2006-present Associate Director of the Penn Genome Frontiers Institute, University of Pennsylvania, Philadelphia, PA

2009-present Interim Director of the Penn Center for Bioinformatics, University of Pennsylvania School of Medicine, Philadelphia, PA

2012-present Professor, Department of Pharmacology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

Other Experience and Professional Memberships:

Molecular Biology Training Program, Northwestern University, 1994-1995; Society of Neuroscience; Society for Research in Biological Rhythms; Federation of American Societies for Experimental Biology (FASEB);

Scientific External Planning Committee, The National Center for Biotechnology Information (NCBI), 2003-2004; Scientific Advisory Panel, National Institute of Neurological Disorders and Stroke (NINDS), The Gensat Project, 2003-2005; Scientific Advisory Board (SAB), 2004, Scientific Advisory Committee (SAC), 2006

Chemical Industry Institute of Toxicology; SAB, The Ryan Licht Sang Bipolar Foundation, 2005-present; SAB, Qiagen N.V., 2006-present; Member, Defense Science Study Group, Institute for Defense Analyses, 2006-2007; Ad Hoc Reviewer, 2006-2008, Member, 2008-2012, Genomics, Computational Biology and Technology Study Section (GCAT); Ad Hoc Reviewer, 2007, Tumor Microenvironment (TME) Study Section; External Reviewer, 2005,2007, Genome Canada, 2005, 2007; Ad Hoc Reviewer, 2005, 2008, Neurogenesis and Cell Fate Study Section; Advisory Board, 2008-present, Journal of Biological Rhythms; Member, Research Review Board, 2009-, Genomics Institute of the Novartis Research Foundation, Member, Scientific Advisory Board, Bio-RAD, 2012-; Penn Fellow, 2012-2014

C. Selected peer-reviewed publications (in chronological order of 84). *h-index* **48**

1. **Hogenesch JB**, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray-Grant M, Perdew GH, Bradfield CA. Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. ***J Biol Chem*** 272: 8581-8593, 1997.
2. **Hogenesch JB**, Gu YZ, Jain S, Bradfield CA. The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. ***Proc Natl Acad Sci USA*** 95: 5474-5479, 1998.
3. **Hogenesch JB**, Ching KA, Batalov S, Su AI, Walker JR, Zhou Y, Kay SA, Schultz PG, Cooke MP. A comparison of the Celera and Ensembl predicted gene sets reveals little overlap in novel genes. ***Cell*** 106: 413-415, 2001.
4. Panda S, Antoch, MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, **Hogenesch JB**. Coordinated transcription of key pathways in the mouse by the circadian clock. ***Cell*** 109: 307-320, 2002.
5. Conkright MD, Canettieri G, Screaton R, Guzman E, Miraglia L, **Hogenesch JB**, Montminy M. TORCs: novel transducers of regulated CREB activity. ***Molecular Cell*** 12: 413-423, 2003.
6. Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, **Hogenesch JB**. A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. ***Neuron*** 43: 527-537, 2004.
7. Willingham AT, Orth AP, Batalov S, Peters EC, Wen BG, Aza-Blanc P, **Hogenesch JB***, Schultz PG*. A strategy for probing the function of noncoding RNAs finds a repressor of NFAT. ***Science*** 309: 1570-1573, 2005. *Co-Corresponding Authors
8. Sato TK, Yamada RG, Ukai H, Baggs JE, Miraglia LJ, Kobayashi TJ, Welsh DK, Kay SA, Ueda HR, **Hogenesch JB**. Feedback repression is required for mammalian circadian clock function. ***Nat Genet*** 38: 312-319, 2006.
9. Amelio AL, Miraglia LJ, Conkright JJ, Mercer BA, Batalov S, Cavett V, Orth AP, Busby J, **Hogenesch JB***, Conkright MD*. A coactivator trap identifies NONO (p54nrb) as a component of the cAMP-signaling pathway. ***Proc Natl Acad Sci USA*** 104: 20314-20319, 2007. *Co-Corresponding Authors
10. Halsey TA, Yang L, Walker JR, **Hogenesch JB**, Thomas RS. A functional map of NFκB signaling identifies novel modulators and multiple system controls. ***Genome Biol*** 8: R104, 2007.
11. Warzecha CC, Sato TK, Nabet B, **Hogenesch JB**, Carstens RP. ESRP1 and ESRP2 are epithelial cell-type-specific regulators of FGFR2 splicing. ***Mol Cell.*** 2009 Mar 13;33(5):591-601.
12. McCarthy EV, Baggs JE, Geskes JM, **Hogenesch JB**, Green CB. Generation of a Novel Allelic Series of Cryptochrome Mutants via Mutagenesis Reveals Residues Involved in Protein: Protein Interaction and CRY2-Specific Repression. ***Molecular and cellular biology*** Aug 2009. [Epub ahead of print]
13. Zhang EE, Liu AC, Hirota T, Miraglia LJ, Welch G, Pongsawakul PY, Liu X, Atwood A, Huss JW, Janes J, Su AI, **Hogenesch JB***, Kay SA*. A Genome Wide RNAi Screen for Modifiers of the Circadian Clock in Human Cells. ***Cell***. Oct 2009 [Epub ahead of print]
14. Baggs JE, Price TS, DiTacchio L, Panda S, Fitzgerald GA, **Hogenesch JB**. Network features of the mammalian circadian clock. ***PLoS Biol***. 2009 Mar 10;7(3):e52.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Ron C. Anafi

eRA COMMONS USER NAME Ranafi

POSITION TITLE Instructor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Tulane University, New Orleans, LA	BSE	1991-1995	Biomedical Engineering
University of Minnesota, Minneapolis, MN	PhD	1998-2002	Mechanics
University of Minnesota, Minneapolis, MN	MD	1996-2004	Medicine
University of Vermont, Burlington, VT	Resident	2004-2007	Internal Medicine
University of Pennsylvania, Philadelphia, PA	Clinical Fellow	2008-2009	Sleep Medicine
University of Pennsylvania, Philadelphia, PA	Postdoctoral	2009-2011	Circadian Bioinformatics

A. Personal Statement.

My training and research have focused on the application of mathematical and engineering techniques to biomedical problems. During graduate school I developed a differential equation based model of pulmonary bronchioles to explain the spatial heterogeneity of asthmatic bronchoconstriction. Over the last years my research and clinical interests have focused on sleep and circadian rhythms. As a research fellow under the direction of John Hogenesch and Junhyong Kim, I worked to develop informatics approaches that couple machine learning algorithms with an understanding of the relevant molecular biology. We have applied these strategies to identify new core circadian genes and interpret circadian signatures in human blood samples. I have also collaborated closely with Dr. Allan Pack to in interpreting microarray studies describing the effect of sleep deprivation on peripheral tissues. My training in both engineering and sleep medicine is uniquely suited for the interpretation of the large data sets resulting from proposed work.

B. Positions and Honors

Clinical Appointments

2007-2008 Academic Hospitalist, University of Vermont/Fletcher Allen Hospital
2009-2011 Clinical Associate, Division of Sleep Medicine, University of Pennsylvania
2011- Instructor, Division of Sleep Medicine, University of Pennsylvania

Awards and Honors

2012 American Sleep Medicine Foundation: Circadian Rhythms Section Award
2010 American Sleep Medicine Foundation Best Science Award
1996-2004 N.I.H./University of Minnesota Medical Scientist Training Program Fellowship
1998-2002 Whitaker Foundation Pre-doctoral Fellowship in Biomedical Engineering
1995 Summa Cum Laude with Tulane Departmental Honors
1991-1995 Tulane University Dean's Honor Scholarship (Full Tuition)
1991 National Merit Scholar

Editorial Positions

2010- Reviewer - American Journal of Physiology – Lung Cellular and Molecular Physiology
2008- Reviewer - Journal of Applied Physiology

Teaching Responsibilities

2009-2011 Medical Student Small Group Leader – Sleep Related Breathing Disorders
2009-2011 Medical Student Small Group Leader – Disorders of Sleep Wake Regulation
2010 Lecturer – Graduate Course – Neurobiology of Sleep and Arousal – Modeling Approaches
2012 Course Co-Director - Neurobiology of Sleep and Arousal

C. Peer-Reviewed Publications

1. Wilson TA, **Anafi RC**, and Hubmayer RD. Mechanics of edematous lungs. J Appl Physiol 90: 2088-93, 2001.
2. **Anafi RC** and Wilson TA. Airway stability and heterogeneity in the constricted lung. J Appl Physiol 91:1185-1192, 2001.
3. **Anafi RC** and Wilson TA. Empirical model for the dynamic force-length behavior of airway smooth muscle. J Appl Physiol 92:455-460, 2001.
4. **Anafi RC**, Beck KC, and Wilson TA. Impedance, gas mixing, and bi-modal ventilation in constricted lungs. J Appl Physiol 94: 1003-1011, 2003.
5. Bates JHT, Bullimore SR, Politi AZ, Sneyd J, **Anafi RC,** and Lauzon AM. Transient oscillatory force-length behavior of activated airway smooth muscle. Am J Phys Lung Cell Mol Phys 297(2):362-72 2009.
6. **Anafi RC** and Bates JHT. Balancing Robustness Against the Dangers of Multiple Attractors in A Hopfield-Type Model of Biological Networks. PLOS One 5(12): e14413, 2010.
7. Schwartz BL, **Anafi RC**, Aliyeva M, Figueroa JT, Allen GB, Lundblad, LK, and Bates JHT. Effects of central airway shunting on the mechanical impedance of the Mouse Lung. Ann Biomed Eng 39: 497-07, 2011

C. Research Support

Ongoing Research Projects

R01-NS054794-0 Hogenesch (PI) 05/101/2012-05/011/2017
The goal of this project is to computationally identify candidate circadian genes and then test their function in cellular and animal models
Role: Co-Investigator

American Sleep Medicine Foundation Anafi (PI) 07/01/2010 – 07/01/2012
The goal of this project is to explore how the circadian oscillator coordinates the transcription of downstream output genes.
Role: P.I.

BIOGRAPHICAL SKETCH

NAME
Arupa Ganguly, Ph.D
eRA COMMONS USER NAME

POSITION TITLE
Professor C-E

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Calcutta University	B.S.	1974	Physics
Calcutta University	M.S.	1977	Physics
University of Calcutta	Ph.D.	1984	Biophysics
American Board of Medical Genetics	FACMG	1999	Clinical Molecular Genetics
American Board of Medical Genetics	FACMG	2009	Clinical Molecular Genetics

A. Personal Statement.

I am a molecular biologist with interest in the molecular basis of human genetic diseases with special emphasis on ocular cancer. I have been working with Dr. Jerry Shields and Dr. Carol Shields, Wills Eye Hospital for the last 7 years developing a clinical assay for genetic testing of Uveal melanoma. In 2006 we started to offer prognostic testing as a clinical test. During this time I have developed different assays looking for prognostic markers of uveal melanoma. My special interest stems from the use of next generation sequencing on small amounts of DNA isolated from formalin fixed paraffin embedded archived tissue, fine needle aspirate biopsy samples and interrogate the mutation profiles and copy number variations in the tumor genome.
On the clinical side, I have been directing the Genetic Diagnostic laboratory, Department of Genetics for the last 17 years. This laboratory is a reference laboratory for testing retinoblastoma, Hereditary Hemorhagic Telangiectasis, Uveal melanoma and Hemophilia A in the US. In addition, this laboratory is also an ABMG accredited laboratory for training clinical molecular genetics fellows.
Thus I have been involved with translation of current genomic technologies to better classification of ocular tumors, and help clinical management of the diseases.

B. Positions and Honors.

Positions and Employment

1985	Lecturer, Department of Biochemistry and Biophysics, Kalyani University, Kalyani, West Bengal, India
1985-1990	Post Doctoral Fellow, Thomas Jefferson University
1992-1993	Instructor, Department of Biochemistry, Jefferson Institute of Molecular Medicine
1993-1995	Research Assistant Professor, Department of Biochemistry, Jefferson Institute of Molecula
1995-1996	Adjunct Assistant Professor of Genetics, University of Pennsylvania School of Medicine
1995-present	Director, R&D Genetic Diagnostic Laboratory, University of Pennsylvania
1996-1998	Fellow in CHOP-UPENN, Genetics Program

1996-2003 Assistant Professor of Genetics at the Hospital of the University of Pennsylvania

1996-2003 Adjunct Assistant Professor, University of Pennsylvania, School of Nursing

2003-2010 Associate Professor of Genetics at the Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine
2003-Present Adjunct Associate Professor, University of Pennsylvania, School of Nursing
2009-present Member, Advisory Committee, Molecular Genetic Technology ProgramU.T. M.D. Anderson Cancer Center, Houston, Texas
2010-present Professor of Genetics at the Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine

Other Experience and Professional Memberships

Testing Working Group, HHT Foundation International, USA

1990-present American Society of Human Genetics
2003-2004 The Wellcome Trust, UK , External grant reviewer
2005-present Hereditary Hemorrhagic Telangiectasia Foundation International, USA , Chair, Genetic

2006-present Association for Research in Vision and Ophthalmology
2006-present Children's Oncology Group, member, Retinoblastoma working committee
2007-2012 Cancer Biomarker Study Section, NIH, Permanent member since July, 2008

C. Selected peer-reviewed publications (selected from 39 peer-reviewed publications)

10. Bunin Greta R, Felice Marc A, Davidson William, Friedman Debra L, Shields Carol L, Maidment Andrew, O'Shea Michael, Nichols Kim E, Leahey Ann, Dunkel Ira J, Jubran Rima, Rodriguez- Galindo Carlos, Schmidt Mary Lou, Weinstein Joanna L, Goldman Stewart, Abramson David H, Wilson Matthew W, Gallie Brenda L, Chan Helen S L, Shapiro Michael, Cnaan Avital, Ganguly Arupa, Meadows Anna T (2011). Medical radiation exposure and risk of retinoblastoma resulting from new germline RB1 mutation. International journal of cancer. Journal international du cancer 128(10): 2393-404 PMCID: PMC3124307

11. Shields Carol L, Ramasubramanian Aparna, Ganguly Arupa, Mohan Diwakar, Shields Jerry A (2011). Cytogenetic testing of iris melanoma using fine needle aspiration biopsy in 17 patients. Retina (Philadelphia, Pa.) 31(3): 574-80

12. Macmullen Courtney M, Zhou Qing, Snider Kara E, Tewson Paul H, Becker Susan A, Aziz Ali Rahim, Ganguly Arupa, Shyng Show-Ling, Stanley Charles A (2011). Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the β-cell sulfonylurea receptor SUR1. Diabetes 60(6): 1797-804 PMCID: PMC3114386

13. Shields Carol L, Ganguly Arupa, Bianciotto Carlos G, Turaka Kiran, Tavallali Ali, Shields Jerry A (2011). Prognosis of uveal melanoma in 500 cases using genetic testing of fine-needle aspiration biopsy specimens. Ophthalmology 118(2): 396-401

14. Bunin Greta R, Tseng Marilyn, Li Yimei, Meadows Anna T, Ganguly Arupa (2012). Parental diet and risk of retinoblastoma resulting from new germline RB1 mutation. Environmental and molecular mutagenesis 53(6): 451-61

15. Shields Carol L, Ganguly Arupa, O'Brien Joan, Sato Takami, Shields Jerry A (2012). Uveal melanoma trapped in the temple of doom. American journal of ophthalmology 154(2): 219-21

BIOGRAPHICAL SKETCH

NAME: Roger A. Greenberg

POSITION TITLE
Associate Professor, Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing include postdoctoral training and residency training if applicable)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Haverford College, Haverford, PA	BA	1987-1991	Chemistry
Albert Einstein College of Medicine, Bronx, NY	MD, PhD	1993-2000	Biology, Medicine
Brigham and Women's Hospital, Boston, MA		2000-2003	Pathology
Dana-Farber Cancer Institute, Boston, MA		2002-2006	Cancer Biology
Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, PA		2007-present	Cancer Biology

A. Positions and Honors

Positions and Employment

2012-present Director of Basic Science, Basser Center for BRCA Research, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2012-present Associate Investigator, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2012-present Associate Professor with tenure, Department of Cancer Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2007-2012 Assistant Professor, Department of Cancer Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2007-2012 Assistant Investigator, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
2006-2007 Instructor in Pathology, Harvard Medical School, Boston, MA
2002-2006 Post-doctoral Fellow, Dana Farber Cancer Institute, Boston, MA – Mentored by David M. Livingston, M.D.
2000-2003 Resident in Clinical Pathology, Brigham and Women's Hospital, Boston, MA
1991-1993 Associate Scientist, Medicinal Chemistry, DuPont-Merck Pharmaceuticals, Wilmington, DE

Honors

2013 Elected to American Society of Clinical Investigation
2013 Harrington Institute Scholar-Innovator Award
2012 Promoted to Associate Professor with Tenure, Perelman School of Medicine, University of Pennsylvania
2010 Michael S. Brown New Investigator Award for Basic Research, Perelman School of Medicine, University of Pennsylvania
2008 Kimmel Scholar Award in Translational Science
2008 Charles E. Culpeper Scholarship in Medical Sciences

2008 Mary Kay Award Foundation Grant in Innovative/Translational
2003 Leukemia Research Foundation Physician-Scientist Award

B. Selected Peer-reviewed Publications

1. Tang J, Cho NW, Cui G, Manion EM, Shanbhag NM, Botuyan MV, Mer G, **Greenberg RA**: TIP60 limits 53BP1 accumulation at DNA double-strand breaks to promote BRCA1-dependent homologous recombination. *Nat Struct Mol Biol Feb 2013*.
2. Domchek SM*, Tang J, Jill Stopfer, Lilli DR, Tischkowitz M, Foulkes WD, Monteiro ANA, Messick TE, Powers J, Yonker A, Couch FJ, Goldgar D, Nathanson KL, **Greenberg RA***:Biallelic deleterious *BRCA1* mutations in a woman with early-onset ovarian cancer. *Cancer Discovery* Dec 2012 epub ahead of print. Notes: *co-corresponding authors.
3. Solyom S, Aressy B, Pylkäs K, Patterson-Fortin J, Hartikainen JM, Kallioniemi A, Kauppila S, Nikkilä J, Kosma VM, Mannermaa A, **Greenberg RA***, Winqvist R* Recurrent breast cancer predisposition- associated *Abraxas* mutation disrupts nuclear localization and DNA damage response functions of BRCA1. *Science Trans Med* 22;4(122):122ra23, 2012.* co-corresponding authorship.
4. **Greenberg RA**: BRCA1, Everything but the RING? *Science* 334 (6055): 459-460, 2011.
5. Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, Janicki SM, and **Greenberg RA**. ATM dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141(6): 970-81. 2010
6. Coleman KA, **Greenberg RA**. The BRCA1-RAP80 Complex Regulates DNA Repair Mechanism Utilization by Restricting End Resection. *J Biol Chem* 286(15): 13669-80. 2011
7. Patterson-Fortin J, Shao G, Bretscher H, Messick TE, **Greenberg RA**. Differential regulation of JAMM domain deubiquitinating enzyme activity within the RAP80 complex. *J Biol Chem* 285(40): 30971-81, 2010
8. Shao G, Lilli DR, Patterson-Fortin J, Messick TE, Feng D, Shanbhag N, Wang Y, and **Greenberg RA**. The Rap80-BRCC36 Deubiquitinating Enzyme Complex Antagonizes RNF8-UBC13 Dependent Ubiquitination Events at DNA Double Strand Breaks. *PNAS* 106(9): 3166-71. 2009
9. Nikkilä J, Coleman K, Morrissey D, Pylkäs K, Erkko H, Messick TE, Karppinen SM, Amelina A, Winqvist R*, and **Greenberg RA***. Familial breast cancer screening reveals an alteration in the *RAP80* UIM domain that impairs DNA damage response function. *Oncogene*. 28(16): 1843-52. 2009. * co-corresponding authorship
10. Messick TE, **Greenberg RA**. The ubiquitin landscape at DNA double-strand breaks. *J Cell Biol* 187(3): 319-26, 2009.
11. Sobhian B, Shao G, Lilli DR, Culhane AC, Moreau L, Xia B, Livingston DM* and **Greenberg RA***. RAP80 targets BRCA1 to specific ubiquitin structures at DNA damage sites. *Science* 316(5828): 1198-202, 2007 * co-corresponding authorship
12. Schaetzlein S.,Kodandaramiredd KR, Ju Z, Lechel A, Stepzynska A, Lilli DR, Clark AB, Rudolph C, Wei K, Schlegelberger B, Schirmacher P, Kunkel TA, **Greenberg RA**, Edelmann W, Rudolph KL. Exo1 deletion impairs DNA damage signal induction and prolongs lifespan of telomere dysfunctional mice. *Cell* 130(5): 863-877*,* 2007