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 717-231-2825.

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

2. **Reporting Period (start and end date of grant award period):** 1/1/2011 – 12/31/2014

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

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

5. **Grant SAP Number:** 4100054874

6. **Project Number and Title of Research Project**: 4-Translational and Personalized Genomics of Inherited Retinal Degenerations

7. **Start and End Date of Research Project:** 1/1/2011 – 6/30/2014

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:

   $ 1,412,435

   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, First Name	Position Title	% of Effort on Project	Cost
Au, Elizabeth	Resource Technologist C	Yr. 1 – 29%	$2,364.17
Lozynska, Olga	Research Specialist D	Yr. 2 – 85%	$61,827.12
Black, Aaron	Graduate Student	Yr 2, 3 – 5%	$3,000
Drivas, Theodore	Combined Degree (MD-PhD) Student	Y. 2, 3 – 10%	$3,000
Gai, Xiaowu	Collaborator	Yrs. 2-3, 10%	$10,000
Pierce, Eric	Co-PI (until 2011)	Yr. 1 <1%	$1,000
Farkas, Michael	Graduate Student	Yr. <1%	$500
Grant, Gregory	Collaborator	Yrs 1-2: 8% Yr. 3 – 30%	Yrs 1-2: $363 Yr. 3: $20,000
Bennicelli, Jeannette	Sr. Res. Invest	Yr. 2 – <1%	Yr 2:$700

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, First Name	Position Title	% of Effort on Project
Kim, Junhyong	Principal Investigator	20%
Eberwine, James	Co-Principal Investigator	10%
Geskes, Jeanne	Sr. Technical Director	15%
Bennett, Jean	Co-PI	<1%

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
None		

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 awarded:
Gene therapy for choroideremia (NNRI) TA-GT-1211-0564-UPA-WG:	☐NIH ☐ Other federal (specify:____) ☒ Nonfederal source (specify: National Neurovision Research Institute	12/2011	$500,000	$500,000
The Pathogenesis of RNA Splicing Factor Retinitis Pigmentosa	☒NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	10/2011	$337,832	$337,832
Pediatric Center for Retinal Degenerations C-CL-0913-0626-UPA01)	☐NIH ☐ Other federal (specify:_____) ☒Nonfederal source (specify: Foundation Fighting Blindness	1/2013	$400,000	$400,000
Gene therapy for choroideremia	☐NIH ☐ Other federal (specify:_____) ☒Nonfederal source (specify: Spark	7/2014	$2,500,000	$2,500,000

	Therapeutics, Clinical Trials Agreement and Sponsored Research Agreement_)			
Gene therapy for Leber's congenital Amaurosis due to LCA5 mutations	☐NIH ☐ Other federal (specify:_) ☒Nonfederal source (specify: Foundation for Retinal Research and the Shapiro Foundation, Clinical Trials Agreement and Sponsored Research Agreement_)	5/14	$4,000,000	pending

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:

Data from the funded project was used to:

1. Identify the mutations that cause disease in many previously identified genes. For several of these orphan diseases (specifically, Leber's congenital Amaurosis (LCA) due to CEP290, RDH12 orLCA5 mutations; X-linked retinitis pigmentosa due to RPGR mutations; Choroideremia due to CHM mutations) we are planning to carry out a clinical trial to test for therapeutic effect of delivery of the normal copy of the defective gene (i.e. gene augmentation therapy). We have developed proof-of-concept of gene augmentation therapy for choroideremia and are far along in developing proof-of-concept for the other conditions. We are currently negotiating funding for a choroideremia gene therapy clinical trial and for an LCA5 clinical trial. There are additional parties interested in funding this research and so if the current negotiations fail, we will enter negotiations with other parties.

2. Identify new disease-causing genes. Analyses of next generation sequencing (NGS) data revealed the genes/mutations in several genes whose importance with respect to retinal health/function had not been previously known. We have generated induced pluripotent stem (iPS cell) models from additional blood samples from these patients and used them to generate personalized models. Using these models, we are delineating the cell biologic functions of proteins encoded by these genes. We will likely submit at least one R01 proposal to characterize function of the other genes/proteins.

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

As described in Question 11 above, we are seeking multiple additional funding sources to move the project to clinical trials. We are working with both private foundations and start-up companies for this purpose.

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	1		7	1
Female	3		5	2
Unknown				
Total	**4**		**12**	**3**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic			1	
Non-Hispanic	4		1	3
Unknown			10	
Total	**4**		**12**	**3**

	Undergraduate	Masters	Pre-doc	Post-doc
White			2	2
Black				
Asian	1		1	1
Other				
Unknown	3		9	
Total	**4**		**12**	**3**

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 funding allowed the operations of a high-throughput next generation sequencing facility and bioinformatics facility for data analysis. This capacity was critical for supporting this project and enhancing the research ability of this institution.

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___X_____ No_____

If yes, please describe the collaborations:

1) The research results led to collaborative interactions, which led the University of Pennsylvania Perelman School of Medicine to establish a new Center, the Center for Advanced Retinal and Ophthalmic Therapeutics (CAROT). This Center fosters collaborations between clinicians and scientists and aims to enhance translational studies involving blinding diseases.

2) The results of studies funded by the CURE program funds have led to plans for a multi-center clinical trial.
A clinical trials agreement, sponsored by Spark Therapeutics, Inc. is in process. The agreement covers collaborations between The Children's Hospital of Philadelphia (CHOP) and the Center for Advanced Retinal and Ophthalmic Therapeutics at the University of Pennsylvania Perelman School of Medicine. Two to three additional sites (outside of the university and entire hospital system) will likely be added in year 3.

Dr. Pierce, initially the key driving scientist of this program, has since become the Director of the Ocular Genomics Institute and Associate Director of the Berman-Gund Laboratory for the Study of Retinal Degenerations in the Department of Ophthalmology at the Massachusetts Eye and Ear Infirmary (MEEI) and Harvard Medical School. This extends the collaborative ventures of our program at University of Pennsylvania substantially.

The results of studies funded by the CURE program inspired us to organize a conference on the "Biology of Cilia and Relationship to Human Disease Research." This conference was held on April 25, 2012 on the University of Pennsylvania campus and attracted 3 dozen researchers from Pennsylvania.

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

Yes____X_____ No_____

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

A patent has been issued for the proviral plasmid generated to deliver the choroideremia gene in upcoming human clinical trials. Ultimately if the reagent proves to be both safe and effective, it could be commercially developed by Spark Therapeutics, Inc.

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

Yes___X_____ No_____

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

Over the course of the funding period, we have presented our research results in lectures at: Will's Eye Hospital, Drexel Medical Student Research Day, Scheie Eye Institute Alumni Day (University of Pennsylvania), the Philadelphia Chapter of Foundation Fighting Blindness, The Life Science Management Retreat for Wharton (University of Pennsylvania), Penn Science Student Research Day (University of Pennsylvania), The Howard Hughes Med-to-Grad Annual Symposium, the Philadelphia Veteran's Administration Low Vision Support Group, Radnor High School Science Club.

The Choroideremia Research Foundation has established a branch in the Philadelphia/Delaware area (C. Moen, MD, Director) and this group has been actively involved in design and promotion of an upcoming clinical trial for choroideremia. This group has also relayed information to patients in Pennsylvania and around the USA about ongoing research studies in which they may participate.

The Center for Retinal and Ocular Therapy that carried out the bulk of this research sponsored a Family Conference for patients and families affected with Leber's congenital Amaurosis in both July 2011 and July 2013.

We are sponsors and participants in the Foundation Fighting Blindness "Vision Walk" annually (2011-present). Members of our Center participated in fundraising efforts (a bicycle ride) to support research carried out by the University of Pennsylvania Orphan Disease Center (May 2014).

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 <u>for the period</u>

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.

General Summary/Introduction

Inherited retinal degenerations (IRDs) are important causes of blindness. The goals of the research are to use next-generation sequencing (NGS) approaches to identify new IRD disease genes, and to investigate the pathogenesis of RP caused by mutations in RNA splicing factors, with the long term goal of developing genetic therapies for these blinding disorders. We have made significant progress towards these goals thanks to support from the Commonwealth of Pennsylvania and the Penn Genomics Frontiers Institute (PGFI), as described below.

Summary of Research Progress

Specific Aim 1. The Genetics of Inherited Retinal Degenerations (IRDs)

When we started our program, we were able to identify the disease-causing gene in only a very small proportion of patients who came in for genetic testing for genes associated with blinding

disease. This is in part due to the fact that the disease genes for ~50% of patients with IRDs remain to be identified. It was also due to the fact that genetic testing for these conditions was extremely inefficient. The goal of this Aim was to test the hypothesis that NGS techniques will facilitate both genetic diagnosis and disease gene discovery in IRD patients. We used selective exon capture and whole exome capture – NGS approaches to identify disease genes in several cohorts of IRD patients.

Over the course of the project, we developed a genetic screening program for patients with IRDs that identifies more than 50-60% of the genes/variants that cause their disease. This program was built by progressively adding each of the genes/mutations that we found using both selective exon capture and whole genome sequencing as well as incorporating variants that are published in public data bases. The entire screening and variant identification process is now more efficient.

Specific Aim 2. The Pathogenesis of RNA Splicing Factor RP

The splicing factors affected in the RNA splicing factor forms of RP, pre-mRNA processing factor (PRPF) 3, PRPF8, PRPF31 and SNRNP200 are highly conserved components of the spliceosome, the complex which excises introns from nascent RNA transcripts to generate mature mRNAs. Since RNA splicing is required in all cells, it is not clear how mutations in these ubiquitous proteins lead to retina-specific disease. We used RNA-Seq transcriptome analyses to test the hypothesis that mutations in RNA splicing factors cause disease via the generation of aberrant transcripts in the retina and/or retinal pigment epithelium (RPE). We confirmed this hypothesis using both in vitro and in vivo studies. In the course of these studies, we developed an RNA-Seq Unified Mapper (RUM) analysis pipeline that is now routinely used to identify novel splicing events in normal human retinal transcriptome.

Details of Research Progress

Specific Aim 1. The Genetics of Inherited Retinal Degenerations (IRDs)

We used exon capture and Illumina sequencing to screen IRD patients for mutations in novel PSC genes.

In year 1 of the project, we performed these analyses for 25 patients with recessive IRD using a selective exon capture for 153 known IRD disease genes, and 596 novel PSC genes. These analyses led to the identification of the genetic diagnosis for 16 of the 25 patients. In several cases, pathogenic mutations were identified in IRD disease genes typically associated with phenotypes distinct from those displayed by the affected patients. For example, a 7 year old with chorioretinal atrophy similar in appearance to gyrate atrophy was found to have two null mutations in *NRL*, a gene usually associated with dominant retinitis pigmentosa.

In year 2 of the project, we performed these analyses for 14 patients with recessive IRD. These analyses led to the identification of the genetic diagnosis for 5 of the 14 patients. In one case, pathogenic mutations were identified in three different IRD disease genes (*CEP290, RPE65*, and

RPGRIP1). In this case, the disease is likely due to the two CEP290 mutations since only one pathogenic mutation was found in each of the *RPE65* and *RPGRIP1* genes.

In year 3 of the project, we performed genetic analyses for 147 individuals (probands and their close relatives) of families with IRD. In most of these individuals, we first carried out pre-screening of genes associated with clinical diagnosis for which there is a likely disease-causing gene. For example, for individuals with choroideremia or retinoschisis, there is a high likelihood that the disease-causing gene is *CHM* or *RS1*, respectively. For individuals with Leber's congenital amaurosis (LCA) and autosomal recessive Stargardt disease, many of the known disease-causing mutations can be identified by Arrayed Primer EXtension (APEX) screening. This screening step identified the disease-causing gene/mutations in approximately one quarter of the families (Table 1).

Table 1. Examples of Clinical and Genetic analyses from a subset of samples Note: multiple families were studied for many of these conditions.

	Genetic Diagnosis/Gene			
Clinical Diagnosis	**Previously known gene/ mutation**	**Previously known gene/new mutation**	**New gene identified**	**Unknown: Pending additional family members (likely new gene)**
Achromatopsia		X		
Choroideremia (CHM)	X	X		
Choroideremia				X
Cone-rod Dystrophy			X	
Cone-rod Dystrophy				X
Early onset Stargardt-like disease			X	
Heimler Syndrome			X	
LCA-AIPL1		X		
LCA-CEP290 (two different families)	X			
LCA-CEP290 (two different families)		X		
LCA-CRB1	X			
LCA-CRX	X			
LCA-Lebercilin	X	X		
LCA-RPE65		X		
LCA-new autosomal gene			X	
LCA-new autosomal gene			X	
LCA-new X-linked gene			X	
LCA-unknown (two different families)				X

Mental retardation and LCA-unknown			X	
Macular Degeneration				X
Retinoschisis (RS1); (two different families)	X			
RP-PRPH2 (two different families)		X		
RP (simplex)				X
RP-AR	X			
RP (X-linked) (RPGR)		X		
RP (X-linked)				X
Stargardt disease (AR) (ABCA4)	X			
Stargardt disease (simplex)				X
Usher's Syndrome (GPF98)		X		
Vitreoschisis and retinal detachment				X
TOTAL NUMBER CONDITIONS DIAGNOSES	**8**	**9**	**7**	**8**

DNA analyses led to the identification of novel variants in genes previously not associated with retinal disease in 7 families. DNA analyses also led to the identification of novel variants in 9 individuals in previously known retinal-disease causing genes (Table 1). These mutations were confirmed by testing in a clinically certified (CLIA) laboratory so that the information could be relayed to the patients and their doctors. The novel mutations in *RPE65* qualified 6 individuals (3 children and 3 adults) to be enrolled in gene therapy clinical trials. All three children were enrolled in a Phase III clinical trial at The Children's Hospital of Philadelphia (CHOP), where they received bilateral subretinal injections of a recombinant adeno-associated virus (AAV) carrying the wildtype *RPE65* cDNA. These individuals now have much improved retinal and visual function.

The two LCA patients with novel mutations in *AIPL1* were referred to a Phase I clinical trial that is pending initiation in London, England. The two retinoschisis patients with novel mutations in the *RS1* gene were referred to NEI/NIH, which is planning a gene therapy clinical trial for that condition; The two LCA patients with *GUCY2D* mutations were referred to University of Florida, Gainesville, which is planning a gene therapy clinical trial for that condition; the patient with *RPGRIP1* mutations was referred to Harvard/Mass Eye and Ear, which is planning a gene therapy clinical trial for that condition; and four patients with X-Linked retinitis pigmentosa (XLRP) due to *RPGR* mutations were referred to the NIH/NEI, which is planning a clinical trial for that condition. An achromatopsia patient with *CNGB3* mutations was referred to a group planning a gene therapy clinical trial for this condition. Ten different individuals with choroideremia were referred to groups that are carrying out (University of Oxford, England) or planning (Children's Hospital of Philadelphia/University of Pennsylvania, Philadelphia, PA) gene therapy clinical trials for choroideremia. A number of the individuals with these and other

conditions (Choroideremia (due to *CHM* mutations), RP due to *PRPH2* (a splicing factor gene) mutations, Stargardt disease due to *ABCA4* mutations and LCA due to *CEP290, CRX, CRB1, AIPL1*, and *Lebercilin* mutations) have also enrolled in additional research studies aiming to gain a better understanding of the pathogenesis of their diseases through study of induced pluripotent stem cell (iPSC) models. These individuals have also enrolled in clinical studies aimed at developing improved outcome measures for future human clinical trials.

We note that these clinical trials were not part of this research project.

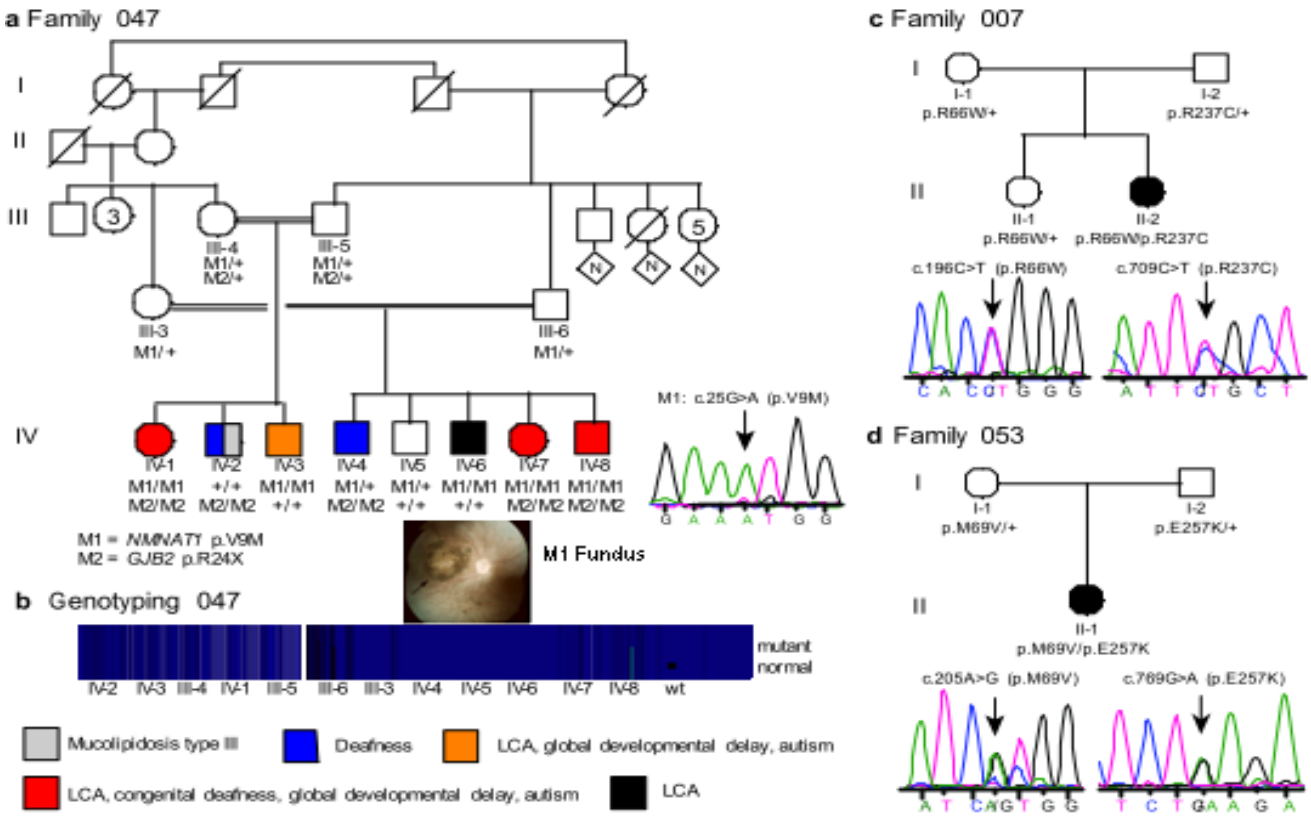
Exome sequencing for disease gene discovery

We used exome sequencing of families that do not have mutations in known IRD disease genes or the 596 novel PSC genes in our selective exon capture experiment to identify novel IRD disease genes.

In year 1 of the project, we sequenced exomes from 8 families with RP and LCA. Results suggested the identification of a novel LCA gene in one of these families. Functional analysis of the variant detected in this family revealed a loss of function of the enzyme encoded by the putative novel disease gene. In collaboration with Dr. Marni Falk, we also sequenced the exomes of two families with retinal degeneration associated with mitochondrial disease, and identified a novel disease gene in one of these families.

In year 2 of the project, we sequenced exomes from 43 different individuals, including 38 probands in 38 different families with retinitis pigmentosa (RP) and Leber's congenital Amaurosis (LCA). Analyses of the results this year led to the identification of a novel LCA gene in two of these families. The gene, *NMNAT1*, encodes the nuclear isoform of nicotinamide mononucleotide adenylyltransferase, a rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD+) biosynthesis. There are three functionally non-redudndant mammalian NMNAT isoforms encoded by different genes. The NMNAT1 isoform localizes to the nucleus where it is involved in nuclear NAD+ homeostasis necessary for both DNA metabolism and cell signaling.(Lau et al., 2009) Studies in patient-derived cells of one of the NMNAT1 mutant proteins, p.Val9Met, shows that the mutation decreases NMNAT1 enzyme activity. The results raise several interesting questions, including why a deficit is observed only in retinal cells given that the *NMNAT1* gene is expressed in many different cells types. Clues to the answer to this question may come from studies showing that NmnatI is the principle component of the mouse Wallerian degeneration (Wld8) fusion protein and this protein has neuroprotective activity (Coleman and Freeman, 2010). Additional DNA studies involving 285 unrelated LCA probands provided by institutions in France and India showed that *NMNAT1* mutations are found in 14 of these families, or 4.9% of unrelated cases. Thus mutations in *NMNAT1* are a relatively common cause of LCA. Figure 1 shows pedigrees of three LCA kindreds in whom NMNAT1 mutations were identified.

Figure 1. Pedigrees of three LCA kindreds (a-c) in whom mutations were identified (by whole exome sequencing with Sanger sequencing validation) in *NMNAT1*. Fundus photo of proband in family 047 is shown along with RFLP results of PCR amplification of exon 2 of *NMNAT1*.



In addition to the novel LCA gene, we used exome sequencing to identify a novel mutation in the Pre-mRNA Processing Factor 31 Homologue (*PRPF31*) gene a large (55 individuals) family with autosomal dominant retinitis pigmentosa (AD-RP). The PRPF31 protein is part of a spliceosome complex and is localized to the nucleus. The mutation segregates with the disease. We are currently carrying out studies of how this mutation affects the structure, density, and function of cone photoreceptors in individuals in this family (Morgan et al, manuscript in preparation).

Exome sequencing results have also led so far to identification of retinal disease candidate genes in 9 additional different families. These have been confirmed by Sanger sequencing and studies in DNA samples from additional family members have verified appropriate segregation of a disease-causing gene in 8 of these families.

In year 3 of the project, we sequenced exomes from 118 different individuals (probands and close relatives) from families with retinitis pigmentosa (RP), choroideremia and Leber's congenital Amaurosis (LCA), Stargardt disease, cone-rod dystrophy, macular degeneration, inherited retinal detachment and several different syndromes, including Heimler Syndrome (macular degeneration, hearing disorder and dental anomalies), a syndrome involving mental retardation and LCA, and Usher's Syndrome (retinal and cochlear degeneration).

We are close to confirming the identity of four additional LCA-causing genes (one of which will be the first X-linked gene correlated with this condition), one new early onset Stargardt disease gene, a form of LCA with mental retardation, and Heimler Syndrome. Conclusive demonstration that these genes/mutations cause the various conditions is currently being established by testing for segregation of the phenotype along with the mutation in additional family members. Where possible, we are also verifying malfunction of the mutant gene in cell culture studies. In addition, we believe that we will be able to identify the genetic basis of another form of LCA as well as a condition involving vitreoschisis and retinal detachment once we gather samples from additional family members. Once we confirm the genetic diagnoses and establish the biology of the defects, we will publish the results, acknowledging the support of the Pennsylvania Commonwealth.

Examples of the identification of these novel retinal disease-causing /mutations are as follows:

a) Early onset and severe Stargardt disease.

A 6yo was evaluated in the clinic for visual symptoms and received the diagnosis of very early onset and severe Stargardt disease (inherited macular degeneration). The pedigree of this family shows autosomal recessive inheritance (Figure 2A). A screen for mutations in the *ABCA4* gene, the most common cause of Stargardt disease, was negative. DNA from the probands and his unaffected parents was evaluated by Next Gen sequencing. Rare compound heterozygous mutations were found in a gene that is highly expressed in the retina and associated with cilia formation. Both of these mutations were predicted to be damaging by both polyphen and sift analyses. There were relatively rare compound heterozygous changes in *HEATR5A* and rare variants in *MT-ATP6, MT-CYB*, and *HUWE1*. The cilia gene that is highly expressed in the retina became our first choice for analyses. Our second choice is *HEATR5A*, which appears to be involved in ubiquitination and is likely expressed in multiple cell types. *MT-ATP6* and *MT-CYB* are mitochondrially-expressed genes and were excluded since the disease in this family shows AR inheritance. Since *HUWE* is on the X chromosome and the pedigree indicates autosomal recessive inheritance, this gene was eliminated as a potential candidate. We are currently confirming that the compound heterozygous mutations found in the retina/cilia-associated gene segregate with the phenotype.

b) Heimler Syndrome

Heimler syndrome is a rare syndrome of tissue derived ectodermally. Primary features are macular dystrophy, enamel hypoplasia, sensorineural hearing loss, and anomalies in nails (transverse ridges and leukonychia = white spots). So far, no genetic cause of this syndrome has been identified. We obtained blood samples from a 13yo boy with Heimler Syndrome and his mother (Figure 2B). There were no other affected family members. DNA analyses showed non-synonymous rare variants in two different genes. One of the genes is an intracellular trafficking protein. The other is a homeobox gene thought to be associated with early embryonic development. We recently obtained a blood sample from the proband's father and will determine whether the variants in either of these two genes segregate with the disease. This study has expanded to be an international collaborative effort (including Dr. Alex Levin at Will's Eye Hospital, Dr. Stephen Tsang at Columbia University and Dr. Chris Inglehearn at University of Leeds (UK)).

c) Leber's Congenital Amaurosis – a Potential new X-Linked Gene

Leber's congenital amaurosis (LCA) is one of the most severe inherited forms of blindness as it is symptomatic in infancy. There are currently 17 genes implicated in LCA, all of which result in autosomal recessive disease.

We studied a 21yo male, JB-17, diagnosed with LCA in infancy (Fig. 2C). A screen for mutations in known LCA-causing genes was negative. We obtained blood samples from his unaffected mother, grandmother and sister. His father was not reachable. Next Gen sequencing identified a single variant in a gene located on the X chromosome. The mutation results in amino acid change arginine to threonine, predicted to be harmful with SIFT and PolyPhen-2 analyses. This protein encoded by this gene is normally expressed in photoreceptors at synaptic termini. Mutations in this gene may thus alter transmission of signal from photoreceptors to retinal bipolar cells, thereby accounting for the retinal phenotype. This gene has been implicated previously in X-linked congenital stationary night blindness (CSNB), a condition that is much more mild that LCA. Typically in CSNB there is a lack of progression of disease. The disease is relatively mild, with reduced visual acuity ranging from 20/30 to 20/200 (the Snellen eye chart), defective dark adaptation, refractive error (low myopia), nystagmus, strabismus, normal color vision and normal fundus examination. The symptoms of JB-17 are much more severe. His visual acuity is approximately 20/1280 and he could not navigate independently even under bright light. We are studying the effects of this new mutation in cell lines using patch-clamping techniques. The mutation appears to substantially reduce electrical signaling of these cells and to substantially increase inactivation time. Once we understand the biology of this condition, it may be possible to assess efficacy of FDA-approved channel activators/blockers for this condition.

d) Leber's Congenital Amaurosis – a Potential new Gene

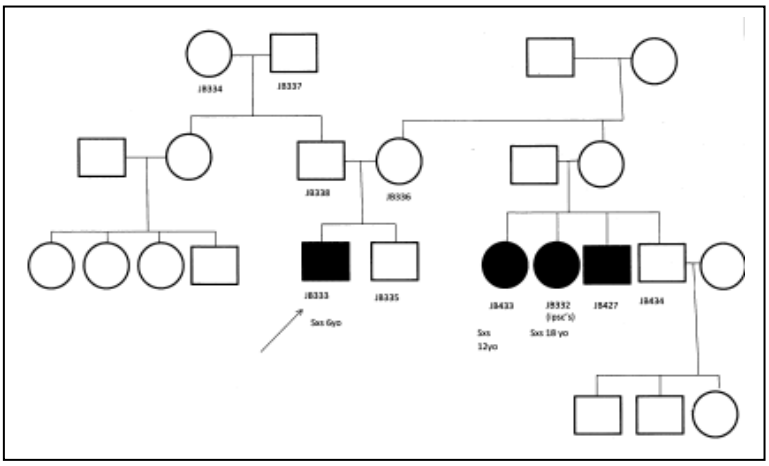
We studied a 23yo female diagnosed with LCA in infancy, JB-148 (Fig. 2D). A screen for mutations in known LCA-causing genes was negative. We obtained a blood sample from her unaffected mother. Genetic analyses of the probands revealed homozygous variants in a retina-specific gene. We are awaiting a sample from her unaffected father to determine whether the gene change segregates with the disease.

e) Retinitis pigmentosa (RP)

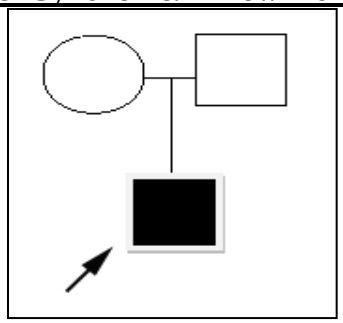
We studied a 94yo male with advanced retinitis pigmentosa. A pedigree was not available upon receipt of his sample. No variants were observed upon screening for known RP-associated genes. DNA analyses revealed a novel hemizygous mutation in the Retinitis Pigmentosa GTPase Regulator (*RPGR*) gene. The *RPGR* gene is on the X chromosome, and thus this diagnosis established the inheritance pattern for this individual's family. Follow-up with his daughter, an obligate carrier, revealed the fact that there were other affected individuals in this family. A pedigree was taken (Figure 2E) and testing for the *RPGR* mutation was carried out. The affected boy who was identified after this testing was referred to the NEI/NIH, which is planning a gene therapy clinical trial for this condition.

Figure 2. Pedigrees of families described in text.

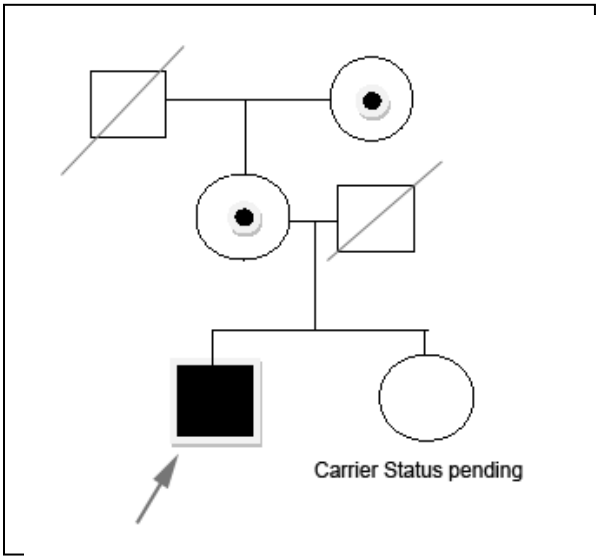
A) Pedigree of family with early onset, severe Stargardt disease. Arrow indicates the proband. The pedigree suggests autosomal recessive inheritance.



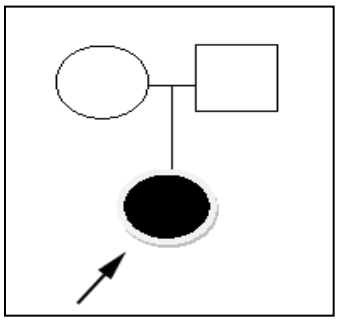
B) Pedigree of family with Heimler Syndrome. Arrow indicates the proband.



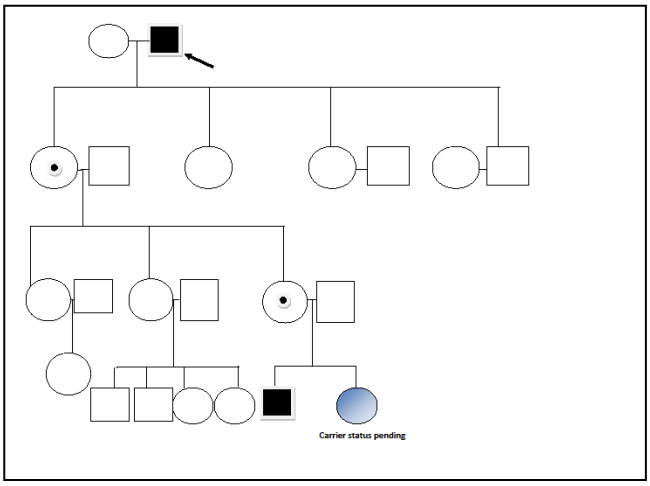
C) Pedigree of Family with XL-LCA



D) Pedigree of family with LCA



E) Pedigree of family with XLRP



Bioinformatics

A bioinformatic pipeline was developed in collaboration with Dr. Xiaowu Gai and Dr. Greg Grant to analyze the exon and exome capture data. Independent sequencing of detected variants has demonstrated a >95% validation rate. Further development and enhancement of the pipeline to add additional analysis tools and a web interface has occurred as the project evolved. Progress in this area extended to analyses of RNA-Seq data (Aim 2) as it allowed filtering of exome data for genes expressed in the retina. Over the project, the team has developed and extensively utilized a whole exome data analysis package that is highly modular with three major components: variant calling pipeline, variant databases, and annotation databases.

The variant calling pipeline is built with a combination of open-source software and custom programs. Briefly, alignments of Illumina sequence data are performed using BWA. Samtools is used to remove potential duplicates and make initial SNP and indel calls, which we then refine with our custom program that uses a false discovery rate approach to adjust raw base counts at a candidate position after performing a Benjamini and Hochberg correction based on quality

values of all base. High accuracy is achieved, as supported by both computational analysis and Sanger validations.

Resulting variant calls are first stored in the variant database, which are then annotated by querying against the annotation databases with the results stored in the variant annotation database.

Annotation databases consists of our custom human codon database, which maps each base position in the human reference genome based on Ensembl Release 65 gene annotations (http://uswest.ensembl.org/index.html) to its corresponding, if any, transcripts, genes, codons, and translation frames. Other types of chromsomal position-based annotations are each stored in their own database. These include known SNPs from dbSNP, variant calls from the 1000 Genomes Project (http://www.1000genomes.org/), variant calls from the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/), SIFT and PolyPhen-2 predictions, phastCons conservation scores, know retina disease genes, and gene expression patterns in retina. The later utilizes the RNA-Seq data generated by this project.

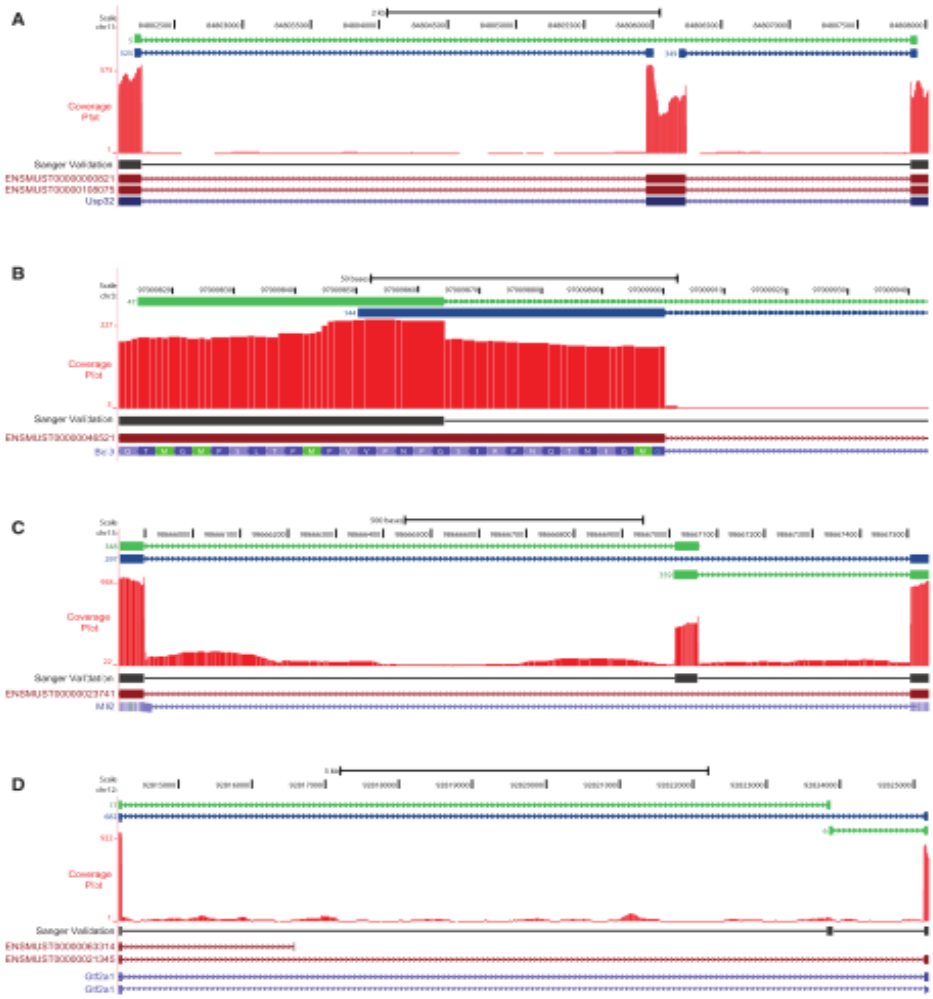
The package was designed to be primarily database driven and highly modular, so that search, analysis, and update can be done in a speedy manner and recurrently as needed. This is to accommodate frequent addition, update, and change of not only new data, but also new releases of new annotations and new types of annotations.

Specific Aim 2. The Pathogenesis of RNA Splicing Factor RP

The splicing factors affected in the RNA splicing factor forms of RP, pre-mRNA processing factor (PRPF) 3, PRPF8, PRPF31 and SNRNP200 are highly conserved components of the spliceosome, the complex which excises introns from nascent RNA transcripts to generate mature mRNAs. Since RNA splicing is required in all cells, it was not clear how mutations in these ubiquitous proteins lead to retina-specific disease. We used RNA-Seq transcriptome analyses to test the hypothesis that mutations in RNA splicing factors cause disease via the generation of aberrant transcripts in the retina and/or retinal pigment epithelium (RPE). We used three knock-in mouse models of the RNA splicing factor forms of RP, and human iPS-derived RPE cells from patients with these disorders for these studies.

We performed RNA-Seq analyses on multiple tissue samples from *Prpf3*, *Prpf8* and *Prpf31* mutant mice. We also performed RNA-Seq analyses on three human retina samples, and iPS-derived RPE cells from a patient with RNA splicing factor RP. RNA-Seq analyses of biologic replicate samples were used to identify aberrant transcripts in the mutant tissues. Figure 3 shows validation of novel splice junctions detected by RUM.

Figure 3. Validation of novel splice junctions detected by RUM. Exon junctions detected by RUM are displayed as a track using the UCSC Genome Browser. The reads with annotated junctions are displayed in blue; reads with novel junctions are shown in green. The depth of uniquely mapped sequence reads is shown in the Coverage Plot in red. The BLAT aligned Sanger sequenced reads from RT-PCR products are shown in black under the coverage plot. Annotated Ensemble and UCSC genes are indicated at the bottom of the images. (A) RUM aligned five RNA-seq reads cleanly across a putative novel junction between exons 29 and 31 of the Usp32 gene, compared with 525 and 349 reads that detected the 5' and 3' ends of annotated exon 30, respectively. RT-PCR and Sanger sequencing in independent biological samples confirmed the presence of the mRNA lacking exon 30. (B) The 47 reads aligned to a putative novel alternate splice junction at the 5' end of the 7th and final exon of Bcl9, while 144 reads aligned to the known junction. The novel junction removes 36 bases, and 12 amino acids in frame from the coding sequence. RT-PCR and Sanger sequencing in independent biological samples confirmed the presence of the mRNA with this novel junction. (C) An abundant putative novel exon was detected between exons 50 and 51 of Mll2 gene. In addition to detection by the RUM junction track, this exon is also evident in the coverage plot. The 48 bp novel exon is predicted to add 16 amino acids in frame to the Mll2 protein. RT-PCR and Sanger sequencing in independent biological samples confirmed the presence of this novel transcript. (D) A low abundance putative novel exon was detected between exons 2 and 3 of the Gtf2a1 gene. RT-PCR and Sanger sequencing in independent biological samples validated the expression of the novel Gtf2a1 transcript containing this 81 bp exon.



Bioinformatics

We developed the RNA-Seq Unified Mapper (RUM) analysis pipeline in collaboration with Dr. Greg Grant. The RNA-Seq Unified Mapper (RUM) analysis simulator addresses challenges to RNA alignment, including alternative splicing, insertions, deletions, substitutions, sequencing errors and intro signal. The RUM pipeline performance was compared with the performance of established tools for this problem and performed favorably with respect to the best current aligners and with accuracy, speed and ease of use. The RUM pipeline identified 10,000s of novel splicing events in normal human retinal transcriptome, including dozens of novel coding exons in known IRD disease genes (Figure 3).

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, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

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):
Adeno-associated virus 8-mediated gene therapy for choroideremia: preclinical studies in in vitro and in vivo models	Aaron Black, Vidyullatha Vasireddy, Daniel C. Chung, Albert M. Maguire, Rajashekhar Gaddameedi, Tania Tolmachova, Miguel Seabra, Jean Bennett	Journal of Gene Medicine	Dec, 2013	☐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:

We will soon be submitting the following manuscripts:

Theodore Drivas, Adam Wojno, Budd Tucker, Edwin Stone, Jean Bennett, "Basal exon skipping, a novel model of disease pathogenesis". This paper describes a mechanism by which mutations in a single gene can result in multiple phenotypes.

Thu Duong, Theodore Drivas, Olga Lozynska, Jean Bennett, "A ciliopathy resulting from mutations in an evolutionarily conserved cilia-associated structural gene."

Aaron Black, Sergei Nikonov, Olga Lozynska, Jean Bennett, "Mutation-dependent effects on disease pathogenesis of a gene encoding a calcium channel"

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.

This study has had the following impacts on outcome, impact, and effectiveness:

1) Several novel genes have been identified as causes of inherited blinding disease. So far, we have published on one of these genes, the NMNAT1 gene, which had a heretofore unknown role in any disease, but is now known to result in 4.9% of cases of early onset blindness/Leber's congenital Amaurosis. Plans are underway for developing proof-of-concept for gene therapy for NMNAT1 so that clinical trials using gene augmentation therapy can be undertaken. Manuscripts describing additional novel disease-causing genes/mutations are in process.
2) In our studies, we have identified genes/mutations causing a wide variety of blinding diseases, including retinitis pigmentosa, choroideremia, Leber's congenital Amaurosis, congenital stationary night blindness, Best's disease, macular dystrophy, Stargardt disease, cone-rod dystrophy, and achromatopsia. We have identified two potential candidate genes for Heimler Syndrome, a disorder that results in abnormalities of the macula, the nails and the teeth and for which a causative gene has not yet been identified.
3) Our genotype-phenotype studies, in combination with RNA seq experiments, have revealed a mechanism which can explain how different mutations in a single gene can lead to vastly different phenotypes. This finding will have widespread application to a diverse set of inherited diseases and may also lead to new therapeutic strategies.

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.

1) We have identified half a dozen new genes in which mutations lead to blindness. This paves the way for developing rational treatments for these particular conditions.
2) Studies of the structure and function of the newly identified blindness-causing genes and the genes/proteins with which they interact will lead to the identification of new disease candidate genes.
3) The identification of the genetic causes of blindness has led to clinical laboratory tests which provide patients with genetic diagnosis. Clinical laboratories which provide certified testing have recently included these genes in their screens. The information from the clinically certified tests can also be used for genetic counseling and will be required for any individual who ultimately enrolls for a clinical trial for gene therapy.
4) The identification of specific gene mutations in individuals who were screened has paved the way for them to enroll in clinical trials. Three of the individuals identified in our studies have been enrolled in Phase 3 gene therapy clinical trials for Leber's congenital Amaurosis due to RPE65 mutations. More than 80 individuals are now candidates for upcoming choroideremia gene therapy clinical trials. Two individuals are now candidates for a gene therapy clinical trial for achromatopsia, two are candidates for a trial for Best disease, a dozen are candidates for a trial for X-linked retinitis pigmentosa due to RPGR mutations, and more than 4 dozen are candidates for gene therapy clinical trials for ABCA4-mediated disease.
5) Delineation of biologic mechanism to explain genotype-phenotype relationships.

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__X_____ No_____

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: Gene therapy for disorders related to Cep290

b. Name of Inventor(s): Theodore Drivas, Jean Bennett

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

Defects in primary cilium formation and function are responsible for a variety of human diseases and developmental disorders, collectively termed ciliopathies. While the ciliopathies are diverse in both phenotype and etiology, specific genes, including CEP290 have been implicated as having causative roles in multiple cilium-associated disorders. These disorders range in severity from isolated retinal degeneration to renal dysfunction, central nervous system malformations, hepatic development defects, and

embryonic lethality. This invention comprises delivery of a CEP290 mini-gene to the diseased cells in order to restore structure and function.

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 __X No__

If yes, indicate date patent was filed: July, 2013

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 X

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___X

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___X

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_____X___ No_____

If yes, please describe your plans:

Our goal is to help develop treatments for people who are blind or who are going blind. The licensing will be intended to aid in the development of a therapy.

1. We may license intellectual property pertaining to gene therapy for choroideremia
2. We may license intellectual property pertaining to gene therapy for Leber's congenital Amaurosis due to CEP290 mutations.
3. We may license intellectual property relating to the delivery of other genes other than those listed above (but that have been identified as causes of blindness).

The licensing for the above items may be carried out in Pennsylvania, the rest of the USA, Europe, and other continents/countries.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators.

Co-Investigator, Jean Bennett, MD. PhD

Education:

Yale University: B.S. (Honors Biology) 1976.
University of California, Berkeley: Ph.D. (Cell and Developmental Biology/Zoology) 1980.
Harvard Medical School: M.D. 1986.

Awards, Honors and Membership in Honorary Societies (since 2006):

2006	Elected to Association of American Physicians
2008	Elected to Institute of Medicine
2009	Board of Director's Award, Foundation Fighting Blindness, Orlando, FL
2010	The Association for Retinopathy of Prematurity and Related Diseases (ROPARD) award for development of a treatment for vision loss in children, Detroit, MI
2011	The Retina Research Foundation Pyron Award for Outstanding Achievement in Retina Research, American Society for Retina Surgeons (ASRS) meeting, Boston, MA
2012	The Bressler Award for Vision Research, The Jewish Guild for the Blind, NY, NY
2013	The Clinical Research Forum Distinguished Clinical Research Achievement Award, Washington, DC.

Research Interests:

- Molecular genetics of retinal degenerations
- Gene therapy-mediated treatment of ocular disease

Description of Research:

Jean Bennett studies the molecular genetics of inherited retinal degenerations with the idea of using this knowledge to develop rational approaches for treatment of these diseases. Target diseases include retinitis pigmentosa and age-related macular degeneration. Studies in her laboratory range for identifying the molecular bases of retinal degenerations, generating animal models for these diseases and rescuing vision in animal models through gene based treatments. Dr. Bennett was one of the first investigators to use viral vectors to deliver transgenes to specific cells in the retina and also led the first team to demonstrate proof-of-principle of ocular gene therapy. She has developed a number of strategies for gene therapy-mediated treatments for retinal disease. Dr. Bennettís work leads naturally to translational research. For example, a recent study conducted in her lab and with collaborators at UPenn, Cornell and University of Florida led to a remarkable reversal of blindness in a canine model of a blinding disease affecting infants. This treatment will be tested in a human clinical trial.

She is interested in the identification and characterization of genes that are defective in blinding and currently untreatable hereditary retinal degenerations such as retinitis pigmentosa, macular degeneration and choroideremia. In order to gain an understanding of the pathogenetic mechanisms which lead to these diseases, we generate and study animal models for the diseases.

She is also interested in intervening with the progression of retinal degeneration by introducing corrective genes or molecules which delay programmed cell death into the retina. For this purpose, the laboratory has developed virus-based methods for delivering functional genes into differentiated retinal photoreceptors, retinal pigment epithelial, trabecular meshwork, iris and corneal endothelial cells. The viruses are being used to test the possibility of genetic therapy in spontaneous and genetically engineered animal models with retinal degeneration. Additional studies focus on the immune system's response to adenovirus-mediated gene delivery to the retina.

Recent Publications:

Testa, F, Maguire, AM, Rossi, S, Pierce, EA, Melillo, P, Marshall, K, Banfi, S, Surace, EM, Sun, J, Acerra, C, Wright, JF, Wellman, J, High, KA, Auricchio, A, Bennett, J and Simonelli, F, Three year follow-up after unilateral subretinal delivery of AAV in Italian patients with LCA2, Ophthalmology, S0161-6420(12)01168-2 (2013); PMID: 23474247

Mackay, DS, Borman, AD, Sui, R, van den Born, LI, Berson k,EL, Ocaka, LA, Davidson, AE, Heckenlively, JR, Branham, K, Ren, H, Lopez, I, Maria, M, Azam, M, Henkes, A, Blokland, E, LCA5 Study Group, Andreasson, S, de Baere, E, Bennett, J, Chader, GJ, Berger, W, Golovleva, I, Greenberg, J, den Hollander, AI, Klaver, CC, Klevering, BJ, Lorenz, B, Preising, MN, Ramsear, R, Roberts, L, Roepman, R, Rohrshchneider, K, Wissinger, B, Qamar, R, Webster, AR, Cremers, FP, Moore, AT, Koenekoop, RK, Screening of a large cohort of leber congenital amaurosis and retinitis pigmentosa patients identifies novel LCA5 mutations and new genotype-phenotype correlations; Hum Mutat 34(11):1537-46 (2013); PMID: 23946133.

Hiebler, S, Masuda, T, Hacia, JG, Moser, AB, Faust, PL, Liu, A, Chowdhury, N, Huang, N, Lauer, A, Bennett, J, Watkins, PA, Zack, DJ, Braverman, NE, Raymond, GV, Steinberg, SJ, The Pex1-G844D mouse: a model for mild human Zellweger spectrum disorder, Mol Genet Metab 111(4):522-32 (2014); PMID: 24503136.

Minella, AL, Mowat, FM, Willett, KL, Sledge, D, Bartoe, JT, Bennett, J, Petersen-Jones, SM, Differential targeting of feline photoreceptors by recombinant adeno-associated viral vectors: implications for preclinical gene therapy trials; Gene Ther doi: 10.1038/gt2014.65 (2014); PMID: 25056608

Black A, Vasireddy V, Chung DC, Maguire AM, Gaddameedi R, Tolmachova T, Seabra M, Bennett J. AAV8-mediated gene therapy for Choroideremia: preclinical studies in in vitro and in vivo models, J Gene Med. 16(5-6):122-30 (2014 Jun 24). doi: 10.1002/jgm.2768.(2014) PMID: 24962736

Cronin, T, Vandenberghe, LH, Hantz, P Juttner,' J, Reimann, A, Kacsó, A-E, Huckfeldt, R, Busskamp, V, Kohler, H, Lagali, PS, Roska, B, Bennett, J, Efficient transduction and optogenetic stimulation of retinal bipolar cells by a synthetic adeno-associated virus capsid and promoter, EMBO Molecular Medicine, e201404077 (2014). PMID 25092770.

NAME	POSITION TITLE
Gregory Robert Grant	Research Assistant Professor
eRA COMMONS USER NAME	
GRANT_G	

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 Maryland, College Park MD	Bachelor	1988	Mathematics
University of Maryland, College Park MD	Ph.D.	1995	Mathematics
University of Pennsylvania, Philadelphia PA	Masters	1999	Computer Science
University of Pennsylvania, Philadelphia PA	Postdoctoral Researcher	1998-2003	Computational Biology/Bioinformatics

A. PERSONAL STATEMENT

My primary focus is on transcriptomics and statistical modelling. I started working with microarray analysis from 1998 and expanded into sequencing analysis since 2008. I work on methods development, methods benchmarking and performing primary statistical and computational analyses for collaborations with bench investigators. I can program and perform statistical analysis at every level. I supervise a group of five people in the bioinformatics group within the ITMAT institute.

B. POSITIONS AND HONORS

Positions and Employment

- 1996-1997 Visiting Assistant Professor, Johns Hopkins University, Baltimore MD
- 1998-2003 Postdoctoral, Researcher, Center for Bioinformatics, Univ. of Pennsylvania, Philadelphia, PA
- 1/1/2000-12/31/2005 NHGRI Mentored Career Transition grantee. K25 HG00052-01A1.
- 2003-2007 Research Manager, Center for Bioinformatics, Univ. of Pennsylvania, Philadelphia, PA
- 2007 to present. Research Assistant Professor, Dept. Genetics, Univ. of Pennsylvania, Philadelphia, PA
- 2013 to present. Director of Bioinformatics. Institute for Translational Medicine and Therapeutics (ITMAT).

Research Interests

- Statistical and computational methods for genomic data analysis and significance testing.
- Next Generation Sequencing Analysis, RNA-Seq, ChIP-Seq, etc.
- Development of Integrated Web Resources for Biological Data Access and Analysis

C. PUBLICATIONS (selected from 52 peer reviewed publications)

- Ruth Heller, Elisabetta Manduchi, Gregory R. Grant, Warren J. Ewens. *A Flexible Two Stage Procedure for Identifying Gene Sets that are Differentially Expressed Bioinformatics* (2009) *25(8): 1019-1025.*
- Arupa Ganguly, Kim Nichols, Gregory R. Grant, Eric Rappaport, Carol Shields. *Molecular Karyotype of Sporadic Unilateral Retinoblastoma Tumors. RETINA* (2009) *29(7): 1002-1012.*

- Ruth Heller, Elisabetta Manduchi, Gregory R. Grant, Warren J. Ewens. *A Flexible Two Stage Procedure for Identifying Gene Sets that are Differentially Expressed Bioinformatics* (2009) *25(8): 1019-1025.*
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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 training.)*			
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 as well as 15 years of experience in molecular biology and genomics. In my lab, we typically explore molecular mechanisms of cellular control and their evolution using genomic technologies and quantitative approaches. The major focus in my lab is Single Cell RNA Biology. We are one of three NIH Roadmap U01 centers for Single Cell Analysis Program-Transcriptomics and I also coordinate the informatics for all three centers. We study single cell transcriptomes from a broad range of organisms: humans, mouse, rats, zebrafish, and C. elegans. We are also investigating spatial localization of RNA using single molecule in situ techniques. My lab carries out both computational and experimental work and we have developed both new genomic technologies and new algorithmic methods. 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 over 40 undergrads, graduate students, and postdocs. I have created new diversity training programs within Penn Genome Frontiers Institute and have developed a public high school genomics curriculum.

B. Positions and Honors

Co-Director, Penn Program in Single Cell Biology, School of Medicine, University of Pennsylvania.
Patricia M. Williams Professor, Sept. 2014-present, Department of Biology, joint appointment in Department of Computer and Informatics Science, University of Pennsylvania.
Edmund J. and Louise W. Kahn Professor, July 2002—2014, Department of Biology, University of Pennsylvania.
Co-Director, Penn Genome Frontiers Institute, Oct 1, 2006--2014
Assistant and Associate Professor (with tenure), 1994-2001. 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.
Ellison Medical Foundation Senior Scholar in Aging, 2010
Guggenheim Fellow, 2010
Yale Senior Faculty Award

Alfred Sloan Foundation Young Investigator Award in Molecular Evolution

C. Ten Selected Citations

1. Middleton, S. and **J. Kim** 2014 NoFold: RNA structure characterization without folding or alignment. *RNA* doi: 10.1261/rna.041913.113. PMID:25234928.

2. Francis, C., Natarajan, S., Lee, M.T., Khaladkar, M., Buckley, P.T., Sul, J.-Y., **Eberwine, J. and J. Kim**. 2014. Divergence of RNA localization between rat and mouse neurons reveals the potential for rapid brain evolution. *BMC Genomics 15:883.*

3. **Eberwine, J.**, Sul, J.-Y., Bartfai, T., and **J. Kim** 2013. The promise of single cell sequencing. Nature Methods 11:25-27.

4. Khaladkar, M., Buckley, P.T., Lee, M., **Eberwine, J**. and **J. Kim** 2013. A genome-wide survey of cytoplasmically retained intron sequence transcripts through single cell sequencing. *PLoS One 8(10):e76194*.

5. Daugharty, E., Goodman, A., and **J. Kim** 2012. Pervasive antisense transcription is conserved in budding yeast. *Mol. Biol. Evol.*, doi:10.1093/molbev/mss240. PMID:23079418

6. 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.

7. 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. PMID:21382548

8. 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. PMID:20969771

9. **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. PMID:20382532

10. 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. PMID:19380745