Rapid exploration, interpretation, and comparison of discrete basis vectors contributing to transcriptional signatures of single cells at the scale of the HCA with ProjectoR								
Personnel:		**Role**	**Base**	**Cal. Mos.**	**% Effort**	**Salary Req.**	**Fringe**	**YR1 Total**
Loyal Goff		PI	$ 132,651	1.80	15%	$19,898	$6,765	*$ 26,663*
Seth Blackshaw		Collaborator	$ -	0.00	0%	$0	$0	*$ -*
Brian Clark		Research Associate	$ -	0.00	0%	$0	$0	*$ -*
Genevieve Stein-O'Brien		Postdoctoral Fellow	$ 47,853	6.00	50%	$23,927	$4,618	*$ 28,544*
TBN		Graduate Student	$ 31,936	9.00	75%	$23,952	$4,400	*$ 28,352*
Briana Winer		Research Technologist	$ 34,000	6.00	50%	$17,000	$5,780	*$ 22,780*
Subtotal						*$84,776*	*$21,563*	**$ 106,339**
Expenses:								
Supplies & Materials:								
10x V2 library kit (16 samples per kit; 2X $21,000 each)								$ 42,000
Chips (6*8 samples/chip, 2x $1,440)								$ 2,880
Barcode Index kit (96 samples, 1x $805)								$ 805
Nextera XT Library Prep Kits								$ 2,000
Reagents for enzymatic dissociation of single cells and seq. library preparation								$ 3,000
Computational storage space and Rstudio server (Amazon web services)								*$ 6,000*
Laboratory Consumables (pipette tips, plasticware, etc)								*$ 4,867*
RNAScope probes for model validations								*$ 4,000*
Services								
Illumina 2500 Sequencing lanes (20*$2,000)								*$ 40,000*
Computers								
Laptop computer								*$ 3,000*
Travel to HCA meetings								
Travel expenses								*$ 2,500*
Subtotal								**$ 111,052**
Total Direct Costs, Year 1:								**$ 217,391**
						Facilities & Administration Costs	*15%*	**$ 32,608.67**
TOTAL COSTS, YEAR 1								**$ 250,000**

• Loyal A. Goff, Ph.D. (PI) will be responsible for overall project design and coordination, direction on ProjectoR development and collaborative efforts on single cell collection and biological interpretation (Goff, Blackshaw).

• Seth Blackshaw, Ph.D. (Collaborator) Dr. Blackshaw is a renowned expert in the biology of mammalian retinal development. Drs. Goff, Blackshaw, and collaborative network member Dr. Fertig have an existing collaboration built around a detailed characterization of retinal cell developmental biology that has contributed much of the preliminary data for this proposed project. Dr. Blackshaw will continue to provide biological interpretations to learned basis vectors and will provide necessary resources for validations.

• Brian Clark, Ph.D. (Research Associate) Dr. Clark is a research associate (K99) jointly mentored in the labs of the PI (Goff) and collaborator (Blackshaw). He has years of experience in studying mouse retina development and has extensive experience working with RNA. He will be responsible for tissue acquisition, processing, and will continue to generate the bulk and single cell libraries in mouse and human retina. Dr. Clark will also be primarily responsible for validation of learned basis vectors through in situ fluorescence hybridization analysis.

• Genevieve Stein-O'Brien, Ph.D. (Postdoctoral Fellow) is the chief developer and maintainer of the ProjectoR package and will be responsible for implementing the transfer learning statistics into the software package. Dr. Stein-O'Brien is a postdoctoral fellow co-supervised by Dr. Goff and collaborative network member Dr. Fertig. She is listed in both proposals. In this proposal, Dr. Stein-O'Brien is listed as responsible for the algorithm development and analyses proposed in this award. Her work will be completed in collaboration with all key personnel on this proposal and co-supervised by Dr. Goff and Dr. Fertig. If both awards are funded, a TBN postdoc will be hired to collaborate with Dr. Stein-O'Brien on these efforts.

• TBN (Graduate Student) in conjunction with the research technologist will perform the required single cell library preparations and metadata aggregation and will be primarily responsible for generating and processing the human retinal single cell benchmark data.

• Briana Winer (Research Technologist). In conjunction with the TBN graduate student, Briana will be responsible for performing the additional 10x genomics and sci-RNA-Seq datasets under the direct supervision of the PI, as well as aggregation and organization of all associated metadata.

Other Support

GOFF, L.A.

ACTIVE:

IOS-1665692 (Brown/Goff) 03/01/2017 – 02/28/2021 1.20 Calendar
National Science Foundation $225,000
Cell type specific gene expression differences induced by experience-dependent plasticity

2016-MSCRFI-2805 (Goff/Bjornsson) 06/01/2016 – 05/31/2019 2.40 Calendar
Maryland Stem Cell Research Commission $200,000
Single cell analysis of hippocampal neurogenesis defects in Kabuki Syndrome 1

SLI (Goff/Brown) 06/01/2016 – 05/30/2018 1.20 Calendar
Johns Hopkins Science of Learning Institute $100,000
Cell-type specific heterogeneity in experience-induced gene expression

Target ALS (Goff – Co-PI) 05/01/2017 – 04/30/2018 1.80 Calendar
TargetALS Foundation $120,000
Cellular Mechanisms of Cortical Hyperexcitability
This project will explore the cell-type-specific effects of familial ALS mutations on hyperexcitability of cortical neurons, and the common and distinct gene expression changes that evoke this phenotype in ALS mouse models.

Synergy Award (Goff) 07/01/2017 – 06/31/2018 1.20 Calendar
Johns Hopkins School of Medicine Discovery Fund $100,000
Systematic characterization of transcriptional variation in retinal development at single cell resolution

PENDING:

SPARC (Chakravarti/Goff) 1/1/2018 - 12/31/2020 2.40 Calendar
NIH Common Fund (SPARC OT2) $310,000
Comprehensive mapping and characterization of the intrinsic and extrinsic connection matrix of the enteric Nervous system.

NSF (Fertig) 07/01/2018-06/30/2022 1.20 Calendar
National Science Foundation $941,508
Scalable Methods for Smooth-spare non-negative Matrix Factorization in Genomics
This project is to develop efficient algorithms for pattern detection in genomics with smooth-sparse matrix factorization.

1R21AI139358-01 (Potter) 07/01/2018-06/30/2020 .6 Calendar
NIH/NIAID $275,000
Identification and characterization of mosquito sensory neurons detecting human-related cues
Mosquitoes utilize a diverse array of senses to navigate and identify human hosts for biting, and a better understanding of these sensory systems could lead to new effective methods to control mosquito populations.

OVERLAP: None

Statement on Sharing / Code and Data Dissemination / Collaboration

We commit to making all scripts, code, and software available via github under the permissive MIT license consistent with the standard operating procedures of the Goff Lab. Each software package and analysis will have an independent, open-source repository for coordinating development with collaborators. Proposed work in benchmark data annotation, model identification and characterization, and analysis will also be conducted on a publicly available github repository as they are generated. Additional communication/coordination will be through Slack, Github Issues, and Pull Requests as required. The proposal has been developed in the open in conjunction with the proposed collaborative network and is currently publicly shared at https://github.com/gofflab/czi-rfa-2017/. Additional collaboration in the development of this joint proposal was conducted on Slack. We acknowledge that the final proposal will be made public and submitted to a specific Github repository / wiki established by CZI. All primary and benchmark data will be deposited with the HCA data coordination platform, as well as freely accessible online, and archived on the NCBI short read archive along with all available metadata. Learned basis vectors, workflows, and analyses will be publicly available on a custom web site, archived in conjunction with published manuscripts, and posted to Biorxiv. Stable software builds will be released in the ProjectoR package through the Bioconductor project. Any experimental protocols developed will be depositied with Protocols.io. Preprints derived from this work will be made available through BioRxiv at the time of submission for publication and any published works will cite the CZI grant number for this proposed project as requested.

Human Tissue / Vertebrate Animals:

Use of human tissue:

All human tissue used will be obtained from sources that have broadly consented donors for use in this proposed study and were collected for uses other than this specific study. All proposed activities meet the criteria for human subjects exemption, as defined by the National Institutes of Health, as tissue will be de-identified prior to acquisition and all investigators will be blinded to the donors and will not be able to ascertain the identity of the donors. Investigators will not have access to personally identifiable information and will not generate additional identifiable genomic data.

Detailed description of the proposed use of animals:

Both male and female C57BL/6J or CD-1 IGS wildtype mice, ages E11 to P30, will be used for the proposed experiments to provide retinal tissue for the single cell analysis. These studies will require approximately 20 pregnant females and 30 male and female mice (newborn to adult). Experiments will be carried out on excised, and enzymatically dissociated retinal tissue. The tissue will be isolated and immediately processed as required for each specific assay. Prior to sacrifice, animals will be housed in the Johns Hopkins American Association of Laboratory Animal Care (AALAC) accredited animal facility.

Justification for the use of animals, choice of species and numbers of animals to be used: The major goal of this research is to describe the developmental organization of the mammalian retina across time and space using single cell measurements. Because of fundamental gaps in our knowledge of retina development, no effective computational models have been developed that could replace the experimentation with animals proposed here. Nor can the precise and complex interplay of activity in intact tissue organization be mimicked in cell culture or other *in vitro* systems. Because of the increasing sophistication of the genetic tools available in the mouse, mice have become a standard model organism for studying the function of neural circuits. Therefore, all the proposed experiments will be performed in wild type mice. The use of experimental animals has been carefully considered in the design of this proposal. We are restricting our investigations *in vivo* to questions where an *in vivo* investigation is critical. The number of animals assigned to each experiments represents the minimum that we expect to make possible statistical analysis and thus interpretation of results. This number takes into account that some animals may be needed for unanticipated validation experiments. Based on experience, we computed approximately 10% more mice than those necessary for statistical analysis. Every effort will be made to minimize the number of animals used for the described experiments.

Veterinary care: Mice will be housed in a barrier facility at Johns Hopkins (BRB), an AALAC accredited facility. All procedures will be performed in the BRB according to governmental and NIH requirements. Veterinary care is provided by the Research Animal Resources (RAR) at Johns Hopkins. JHU maintains the expectation that all veterinary care for JHU research animals will be provided by RAR veterinarians or with RAR veterinary guidance to ensure quality and contemporary standards of care. Veterinary care is available 24/7 via routine rounds and a rotating on-call schedule. Veterinary care is provided by 4 faculty veterinarians, and 6 veterinary fellows. Surgical and technical support is provided by RAR rodent technical specialists. Daily husbandry procedures are provided by trained animal technicians. The veterinarians work directly with the animal care staff on programs designed to reduce the prevalence of infectious disease, the monitoring of animal health, and the diagnosis and treatment of illness and disease. Veterinary staff reserves the right to intervene in all cases in which animals are experiencing unalleviated pain or distress that has not been justified in the protocol as necessary to accomplish scientific objectives and for which provisions for palliative care have not been provided. An animal protocol, which is reviewed annually, has been approved for these studies by the Institutional Animal Care and Use Committee at Johns Hopkins

Procedure to minimize pain and discomfort: Every effort will be made to minimize any discomfort or distress to the animals being used. The surgical procedures are relatively straightforward and will be performed after extensive training and under supervision by experienced laboratory personnel and RAR veterinary staff. Mice will be carefully monitored for signs of distress as they recover from anesthesia and in the days following the surgery. If there is any sign of distress, animals will be euthanized immediately.

Euthanasia: Animals are inspected daily by the veterinary care support staff. Any moribund animals are noted and treated as appropriate (hydrogel addition, saline injection) and monitored closely over the next 24 hours. Those animals not responding to treatment will be euthanized. Euthanasia in all instances will be terminal inhalation of carbon dioxide or isoflurane vapor, followed by secondary euthanasia through cervical dislocation or decapitation. Methods of euthanasia are consistent with the American Veterinary Medical Association (AVMA) Guidelines on Euthanasia, and in accordance with protocols approved by the Animal Care and Use Committee at Johns Hopkins University.