This is provided as an example proposal.

It is important that you follow the current guidelines.

The mentor letter has been removed.

A PROPOSAL TO STUDENT ENHANCEMENT AWARD REVIEW COMMITTEE

TITLE OF PROJECT: Examining the interactions between virus and host with ChIP-seq

NAME OF APPLICANT: Mark Slayton

STATUS: _____ Undergraduate ✓ Graduate _____ Medical

CAMPUS/LOCAL ADDRESS: Life Sciences Building, 7 Depot Street
E-MAIL ADDRESS: ms915613@ohio.edu
DEPARTMENT: Bios/MCB

EXPECTED GRADUATION DATE (Month and Year): August 2018

RE-SUBMISSION: ___ YES (Original Submission Date _____) ✓ NO

PROPOSAL CATEGORY (select one):
✓ Life/Biomedical
_____ Social/Behavioral
_____ Arts/Humanities
_____ Physical Sciences/Engineering

BUDGET: Total Request $6,000
(May not exceed $6,000)

FACULTY MENTOR INFORMATION:
NAME: Bonita Biegalke
E-MAIL ADDRESS: biegalke@ohio.edu
CAMPUS ADDRESS: Life Sciences Building, 7 depot street
DEPARTMENT: Biological Sciences
DEPARTMENT ADMIN./EMAIL: Molly Morris / morrism@ohio.edu

IRB AND IACUC APPROVAL:
To ensure that the University is in compliance with all federal regulations, complete the checklist below. *Note: your proposal can be approved prior to IRB or IACUC approval (put "pending" or "to be submitted" instead of approval number), but funding will be withheld until notification of approval or exemption.*

Yes	***No***	***Office of Research Compliance***	***Policy #***
	X	Human Subjects in Research (including surveys, interviews, educational interventions): Institutional Review Board (IRB) Approval #: Expiration Date:	19.052
	X	Animal Species: Institutional Animal Care & Use Committee (IACUC) Approval #: Expiration Date:	19.049

SIGNATURES

Applicant's Signature		**Faculty Mentor's Signature**	
Signature	Mark Slayton	Signature	Bonita Biegalke
Name	Mark Slayton	Name	Bonita Biegalke
Dept/School	Bios/MCB	Unit	Heritage College
Date	1/21/16	Date	1/21/16

☒ **Optional:**
If selected for funding, I give permission to the Office of the Vice President for Research and Creative Activity to use my proposal as an example during training and workshop exercises.
(Sign below)
Signature: Mark Slayton Date: January 21st 2016

STUDENT ENHANCEMENT AWARD
APPLICATION CHECKLIST

Applicants **must** complete and sign the checklist. The checklist should be included as the second page of the application (following the cover page).

☑ Cover page	use SEA form
☑ Checklist	use SEA form
☑ Abstract*	1 double-spaced page
☐ ResubmissionSummary (***For Re-submissions Only***)*	1 double-spaced page
☑ Project Narrative	5 double-spaced pages
☑ Glossary/Definition of Terms* (***Not required***)	2 double-spaced pages
☑ Bibliography (***Not required***)	2 pages
☑ Presentation of Results	1 double-spaced page
☑ Mentor's Endorsement	1 page
☑ Biographical information (***Applicant(s) and key personnel***)	3 pages per person
☑ Budget and Justification	no limit specified (Including the OHIO-Affiliated Travel Form, if applicable)
☑ Appended Materials/Multimedia Files	5 pages; and no more than 10 minutes of footage
☑ Electronic copy of proposal	Single Acrobat file, containing entire proposal and required signatures

Sections marked with a bullet (*) identify text sections that should be written in language understandable by an informed layperson to assist the Committee in its review.

*****Please Note: The committee has the right to return without review any proposals that do not conform to these format requirements*****

Applicant signature: Mark Dlugosz

Abstract

Human cytomegalovirus (HCMV) is a commonly occurring viral infection with a global infection rate of 90% (1). HCMV infection can cause severe developmental abnormalities in newborns, including blindness and deafness (2, 3). Typically, adults with a functional immune system do not experience symptoms of HCMV infection; however, HCMV has been implicated as a factor in hypertension and the development of cardiovascular disease (4). In order to replicate within human cells and cause disease, HCMV must successfully evade the immune system and replicate its DNA genome. Upon infection, the virus expresses several genes which are critical for the viral replication cycle. Expression of the viral gene UL34 is required for viral replication to occur (5, 6). Although UL34 is an essential viral gene, the functions of this gene remain primarily unknown. Previous work in our lab discovered that the UL34 proteins bind to the viral genome where they act to regulate the production of other viral genes (7–9); however, the potential effects of UL34 on human gene expression is unknown. The goal of the proposed research is to determine if the UL34 proteins bind to the human genome during viral infection. I hypothesize that the UL34 proteins bind not only to the viral genome, but also at sites within the human genome. The experiments proposed herein will provide a crucial step in examining the role of UL34 as a protein with gene regulating activity within both the HCMV and human genomes. UL34 is unique to cytomegalovirus and has the potential to serve as a target during drug development. My project will provide the foundation for investigating the control of viral and cellular gene expression by the viral UL34 gene. These results will be published in a peer-reviewed journal, such as the Journal of Virology, and will be of interest to virology and biomedical researchers around the world.

Project Narrative

Goals, scope, and context

Viral Replication: Human cytomegalovirus (HCMV) is a herpesvirus with a large double-stranded DNA genome. All known herpesviruses undergo two methods of viral replication: active replication, referred to as the lytic phase, or inactive replication which is termed latency. Lytic phase replication requires a temporally controlled cascade of viral gene expression, resulting in the production of infectious progeny. Several viral genes are essential to the lytic phase, with the removal of these genes from the cytomegalovirus genome resulting in a lack of viral replication (5). Genes which are essential to the lytic phase typically encode proteins that are involved in attachment, transcriptional regulation, replicating the viral DNA genome, virion assembly, or virion egress. Additionally, although the mechanisms are not well understood, HCMV modulates the expression of human genes to promote an environment suitable for lytic phase replication (10).

UL34: One of the genes essential to the lytic phase, called UL34, encodes two highly similar sequence-specific DNA-binding proteins (an early and a late protein) which recognize a ten base pair binding sequence (a UL34 binding site) (6, 9). The UL34 binding site occurs 14 or 15 times throughout the viral DNA genome, depending on the strain of the virus (8). The UL34 proteins bind within the promoter regions of the viral immunoevasion genes, US3 and US9, resulting in transcriptional down regulation (7). While several of the UL34 binding sites throughout the HCMV genome have been examined, the impact of UL34-DNA interactions within the human genome is unknown. Discovering if and where UL34 binds to human genomic DNA will implicate UL34 in the control of cellular gene expression.

Project Aim 1: Which UL34 binding sites within the HCMV genome are occupied during the viral replication cycle?

Previous work in our lab demonstrated that UL34 proteins bind to DNA containing the UL34 binding site *in vitro* (8). Additionally, UL34 acts to regulate the transcription of the viral immunoevasion genes, US3 and US9, during infection (7). Further work in our lab suggests that UL34 binds within the viral origin of lytic DNA replication and participates in viral DNA replication; taken together, these results suggest a multi-functional role for the UL34 gene as a transcriptional regulator and as a factor in viral DNA replication. The impact of UL34-DNA binding interactions at remaining sites throughout the viral genome have yet to be characterized. Chromatin immunoprecipitation with sequencing (ChIP-seq) will be used to examine UL34-DNA interactions throughout the viral genome during viral replication (at different time points post infection), providing an insight into the dynamics of this important viral protein.

Project Aim 2: What are the interactions of UL34 with the human genome?

2a. *Where are the UL34 binding sites located within the human genome as predicted via computational methods?* The complete sequence of the human genome has been publically available for more than a decade. However, the locations and frequency of the UL34 binding sequence (AAACACCGT[G/T]) have yet to be determined. I have performed a preliminary search for the UL34 binding sequence within experimentally validated human promoter regions through motif-identifying software (FindM, Swiss Institute of Bioinformatics). The UL34 binding site occurs within the promoter regions of several human genes; one such gene, called AIFM1, functions to regulate the apoptotic cascade (11). Dysregulation of this gene results in Cowchock syndrome, which presents as neuropathy, deafness, and mental retardation – intriguingly, all of these are potential indications of congenital CMV infection (2). To answer

this question further, an in-depth analysis of the human genome will be performed using the FindM software, allowing for UL34 binding sites to be grouped based on the regulatory regions in which they occur.

2b. *Do the UL34 proteins bind to the human genome in vivo?* Through bioinformatics, researchers can predict the likelihood that a DNA-binding protein will bind to its target. However, several factors regulate the accessibility of DNA in the human genome, such as methylation and the state of chromatin (open or closed). These naturally occurring variations in the human genome may influence the binding of UL34 at the sites predicted via computational methods. *I hypothesize that the UL34 proteins bind* in vivo *not only to the cytomegalovirus genome, but to the human genome as well.* The ChIP-seq experiment (see Project Aim 1) will be used in tandem to find the solution to this question.

Methods

Chromatin Immunoprecipitation (ChIP): To perform the ChIP experiment, an antibody against the protein of interest is required. However, the available antibody against UL34 is not qualified for use in ChIP experiments. A modified version of HCMV containing the UL34 protein fused to the myc epitope (myc-UL34 HCMV) was recently developed by Dr. Tanvir Hossain, a researcher in our laboratory. The myc epitope is small and does not interfere with protein function. This modified virus will be utilized along with a commercially available antibody against the myc epitope. Human diploid fibroblasts (HDFs) will be grown in plastic culture dishes before infection with myc-UL34 HCMV. At early and late time points post infection (12 hours and 72 hours, respectively), the cells will be treated with a formaldehyde solution to reversibly cross-link DNA binding proteins to their nucleotide targets (see Fig. 1). The cells will then be harvested, followed by sonication to fragment the protein-bound DNA into

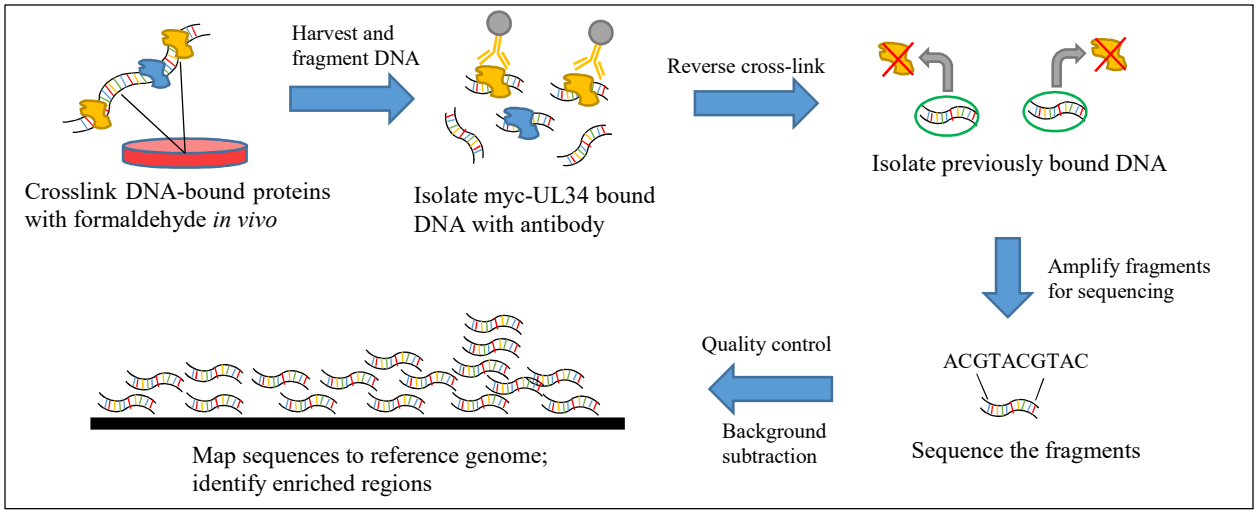

Figure 1. Overview of a ChIP-seq experiment. Typically, several million DNA fragments will require sequencing in a single sample, resulting in the need for next generation sequencers.

small pieces. The myc-UL34 bound DNA will be isolated by immunoprecipitation with an anti-Myc tag antibody. Then, the cross-linking reaction will be reversed, releasing the bound DNA for further purification.

Sequencing ChIP DNA: The ChIP isolated DNA fragments will be subjected to next generation sequencing via the Illumina MiSeq sequencer at the Ohio University Genomics Facility. I have communicated with the director of the Genomics Facility, Dr. Thomas Lisse, to negotiate a price which falls within the project budget (see Appended Materials). Additionally, Dr. Lisse invited me to help and observe during the sequencing process, thereby allowing me to learn more about next generation sequencing. After sequencing, data analysis will be performed using the CLC Genomics Workbench (CLC Bio) and BaseSpace (Illumina) software packages to identify enriched regions, thereby indicating binding by UL34 (see Fig. 2). Uninfected cells will serve as the negative control and the UL34-DNA binding interaction at the US3 promoter will serve as the positive control. All of the above experiments, including the controls, will be performed using biological duplicates. After data analysis and background subtraction, statistically significant peaks will be identified through the software packages listed above.

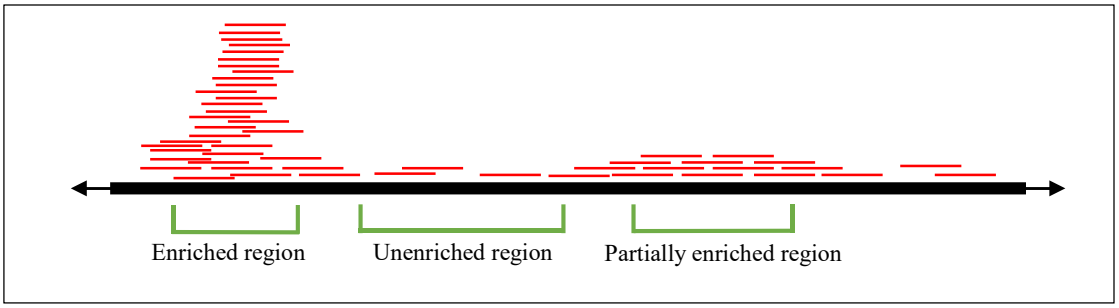

Figure 2. Hypothetical results of ChIP-seq data analysis. Red lines represent sequenced fragments while the thick black line represents the reference genome. Enriched, partially enriched, and unenriched regions are shown as example.

Significance

Human cytomegalovirus (HCMV), a ubiquitous herpesvirus, causes substantial disease in patients with underdeveloped or weakened immune systems (2, 12). Women with an active infection can pass HCMV to their developing child, resulting in possible developmental abnormalities and in rare cases, death (2, 3). Persons undergoing chemotherapy often experience the symptoms of a diminished immune response and must undergo prophylactic treatment against HCMV (13). Treatments for CMV infection primarily target the viral DNA polymerase but resistance to the drugs and toxicity to the patient are major concerns (14, 15). Additionally, a vaccine against HCMV does not yet exist. Through expanding our understanding of the viral UL34 gene, I hope to provide a contribution to the knowledgebase used to combat this disease.

Broader Impacts

This work will constitute the first use of ChIP-seq to analyze the binding of a human cytomegalovirus protein to the human genome. The proposed project will generate data that will be utilized to investigate the impact of UL34 on viral and cellular functions. Sequence-specific DNA-binding activity among HCMV proteins is uncommon and consequently of interest to both biomedical researchers as well as pharmaceutical companies. The results of this project are important not only for my dissertation research, but will also open up new fields for HCMV research through the investigation of virus-host interactions.

Glossary

Antibody: specialized protein which recognizes and binds to specific molecules called antigens.

Apoptosis: purposeful cell death which is controlled by a chain of events – viruses typically inhibit this to keep the cell alive while they replicate within.

ChIP-seq: Chromatin immunoprecipitation with sequencing - a technique which identifies the genomic positions at which DNA binding proteins (typically transcription factors) exhibit binding activity within a biological system.

Chromatin: tightly packed DNA, bound with histones.

Cross-linking: the use of treatment (often chemical or high energy) to create new, stronger bonds between two molecules.

Immunoevasion: methodologies used by infectious agents to evade the immune system of the host.

Immunoprecipitation: a technique in molecular biology which uses antibodies bound to solid substrates to selectively isolate protein via precipitation.

Methylation: the addition of a methyl group to a substrate, which is often a regulatory mechanism in biological entities.

Motif: a "footprint" of sequence, either DNA, RNA, or protein, which has biological significance.

Origin of lytic replication: the region of DNA in a herpesvirus genome where the DNA replication machinery organizes to replicate DNA during the lytic phase.

Promoter: a region of DNA which contains regulatory elements important for the control of transcription.

Sonication: the use of ultrasonic frequencies to shear and fragment DNA or other biological molecules.

Transcription: the process by which DNA is converted into RNA which is used as a template for protein production.

Bibliography

1. **Cannon MJ**, **Schmid DS**, **Hyde TB**. 2010. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol **20**:202–213.

2. **Damato EG**, **Winnen CW**. 2002. Cytomegalovirus Infection: Perinatal Implications. J Obstet Gynecol Neonatal Nurs **31**:86–92.

3. **Nassetta L**, **Kimberlin D**, **Whitley R**. 2009. Treatment of congenital cytomegalovirus infection: implications for future therapeutic strategies. J Antimicrob Chemother **63**:862–867.

4. **Cheng J**, **Ke Q**, **Jin Z**, **Wang H**, **Kocher O**, **Morgan JP**, **Zhang J**, **Crumpacker CS**. 2009. Cytomegalovirus Infection Causes an Increase of Arterial Blood Pressure. PLoS Pathog **5**:427.

5. **Dunn W**, **Chou C**, **Li H**, **Hai R**, **Patterson D**, **Stolc V**, **Zhu H**, **Liu F**. 2003. Functional profiling of a human cytomegalovirus genome. Proc Natl Acad Sci **100**:14223–14228.

6. **Rana R**, **Biegalke BJ**. 2014. Human cytomegalovirus UL34 early and late proteins are essential for viral replication. Viruses **6**:476–488.

7. **LaPierre LA**, **Biegalke BJ**. 2001. Identification of a novel transcriptional repressor encoded by human cytomegalovirus. J Virol **75**:6062–6069.

8. **Liu Z**, **Biegalke BJ**. 2013. Human Cytomegalovirus UL34 Binds to Multiple Sites within the Viral Genome. J Virol **87**:3587–3591.

9. **Biegalke BJ**, **Lester E**, **Branda A**, **Rana R**. 2004. Characterization of the Human Cytomegalovirus UL34 Gene. J Virol **78**:9579–9583.

10. **Zhu H**, **Cong J-P**, **Mamtora G**, **Gingeras T**, **Shenk T**. 1998. Cellular gene expression altered by human cytomegalovirus: Global monitoring with oligonucleotide arrays. Proc Natl Acad Sci U S A **95**:14470–14475.

11. **Rinaldi C**, **Grunseich C**, **Sevrioukova IF**, **Schindler A**, **Horkayne-Szakaly I**, **Lamperti C**, **Landouré G**, **Kennerson ML**, **Burnett BG**, **Bönnemann C**, **Biesecker LG**, **Ghezzi D**, **Zeviani M**, **Fischbeck KH**. 2012. Cowchock Syndrome Is Associated with a Mutation in Apoptosis-Inducing Factor. Am J Hum Genet **91**:1095–1102.

12. **Emery V**. 2001. Investigation of CMV disease in immunocompromised patients. J Clin Pathol **54**:84–88.

13. **O'Brien SM**, **Kantarjian HM**, **Thomas DA**, **Cortes J**, **Giles FJ**, **Wierda WG**, **Koller CA**, **Ferrajoli A**, **Browning M**, **Lerner S**, **Albitar M**, **Keating MJ**. 2003. Alemtuzumab as treatment for residual disease after chemotherapy in patients with chronic lymphocytic leukemia. Cancer **98**:2657–2663.

14. **Andrei G**, **Clercq ED**, **Snoeck R**. 2009. Viral DNA Polymerase Inhibitors, p. 481–526. *In* Raney, KD, Gotte, M, Cameron, CE (eds.), Viral Genome Replication. Springer US.

15. **Tchesnokov EP**, **Gilbert C**, **Boivin G**, **Götte M**. 2006. Role of Helix P of the Human Cytomegalovirus DNA Polymerase in Resistance and Hypersusceptibility to the Antiviral Drug Foscarnet. J Virol **80**:1440–1450.

Presentation of Results

My results will be presented at the American Society for Virology 36th Annual Meeting, which will take place from June 24th to 28th, 2017. The location has been determined and will be at the University of Wisconsin-Madison within the Monona Terrace Community and Convention Center at downtown Madison. I had a meeting with my mentor and we both agreed that this is the best conference for us to attend together where my results will be presented. This conference brings together virologists from all of the different sub-disciplines of virology, including viruses than infect humans, animals, plants, insects, fungi, and bacteria. Presenting my results to this diverse group will grant me feedback from researchers with similar, yet different, strategies to solving research questions. Several opportunities for development are available at the conference including symposia by top virologists, workshops about specific types of viruses, a poster session, and a unique opportunity for students to meet with pharmaceutical company representatives and discuss strategies for finding work within the research industry. The presentation of my results will be of interest not only to other herpesvirus researchers, but to biomedical researchers in general whom are in attendance as the ChIP-seq technique is currently a hot topic within these fields. While at the conference, I will represent Ohio University as a courteous and respectful student learner with much knowledge to gain from fellow researchers.

Biographical Sketch of the Applicant

Qualifications & Skillset

- Experienced in techniques for genetic manipulation of bacteria and viruses. Technical skills include, but are not limited to, DNA, RNA, and protein manipulation and purification, q/RT-PCR, cell culture work with several mammalian lines, Southern, northern, and western blotting, film development, bacterial transformation and competency, Red recombination, agarose and polyacrylamide electrophoresis, *in vitro* transcription/translation, and light/fluorescent microscopy.

- Undergoing Ph.D. training to gain expertise in experimental design using logic and critical thinking to provide timely and cost effective solutions to questions of interest.

- Background in working on research projects independently, supervising and training undergraduate researchers, and presenting scientific results.

Educational Training

2013 – Present Ohio University, Athens, OH – Molecular and Cellular Biology Ph.D. Program
Dissertation advisor: Dr. Bonita Biegalke
Cumulative GPA: 3.52
Anticipated date of graduation: August 2018

2009 – 2013 Wayne State University, Detroit, MI
Bachelor in Science, Biological Sciences

Research Experience

2013 – Present: Ohio University
Doctoral Thesis Research with Dr. Bonita Biegalke
Project title: Determining the role of UL34 in HCMV genome replication

2012– 2013 Wayne State University
Undergraduate Directed Study with Dr. Aleksander Popadić
Project title: Genetic Mechanisms of Insect Appendage Development

Exhibitions

2015 Ohio University Student Expo, Ohio University

International Herpesvirus Workshop, Boise, Idaho

Appalachian Regional Cell Conference, Marshall University

Relevant Coursework

BIOS 5260 – Molecular Genetics
BIOS 5240 – Virology
BME 5170 – Data Mining in the Life Sciences
CHEM 5900 – Special Topics in Chemistry: Proteomics
CHEM 7150 – Advanced Special Topics in Biochemistry: Chemical Biology
CS 6150 – Computational Genomics
MCB 7200 – Molecular Biology
MCB 7300 – Molecular and Cellular Biology Laboratory
MCB 7600 – Advanced Cell Biology
PBIO 5150 – Statistical Methods in Plant Biology
PBIO 5170 – Biological Research Ethics

Funding and Awards

2015 **Ohio University Graduate Student Senate Original Work Grant**

Choose Ohio First Bioinformatics Scholarship

Appalachian Regional Cell Conference, Marshall University

- 1st place poster presentation award –
  "Involvement of UL34 in HCMV Lytic Phase DNA Replication"

Ohio University Student Expo, Ohio University

- 1st place poster presentation award –
  "Human Cytomegalovirus DNA Replication is Regulated by UL34"

2014 **John J. Kopchick Molecular and Cellular Biology Research Fellowship**

Budget and Justification

Consumable Research Supplies			
Item	**Supplier**	**Cost per item**	**Total cost**
Growth media (10 liters)	Gibco	$35 / liter	$350
60mm cell culture dishes, 100 count	Corning	$93 / set	$93
Trypsin	Gibco	$38 / 500 ml	$38
20% formaldehye, 100ml	Ladd Research	$37 / bottle	$37
Anti-Myc tag antibody, ChIP grade	Abcam	$419 / 100ug	$419
Dynabeads® M-280 Sheep Anti-Mouse IgG	ThermoFisher Scientific	$391 / 2 ml	$391
QIAquick PCR purification kit, 50 reactions	QIAGEN	$112 / kit	$112
NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina® (2 kits)	New England Biolabs	$285 / kit	$570
NEBNext® Multiplex Oligos for Illumina®, Index Primers Set 1	New England Biolabs	$105 / set	$105
NEBNext® Multiplex Oligos for Illumina®, Index Primers Set 2	New England Biolabs	$105 / set	$105
Bioanalyzer QC procedure (10 samples)	Ohio University Genomics Facility	$50 / sample	$500
MiSeq® Reagent Kit v3 - 600 cycle	Illumina	$1,742 / kit	$1,742
		Total	$4,462

Travel	**Cost**
• 2017 American Society of Virology Annual Meeting, Madison, Wisconsin	
➢ Registration fee – includes meals	$450
➢ Airfare (roundtrip)	$450
➢ Hotel – 6 days at $110/day	$660
Total	$1,560

Budget Summary	
Consumable Research Supplies	**Total: $4,462**
Travel to Present Results	**Total: $1,560**
Project Total Cost:	**$6,022**
Final amount requested:	**$6,000** Excess cost covered by laboratory funds

- Funding for Travel: The requested amount reflects the current rate for registration of the 2016 American Society of Virology Annual Meeting, which is expected to remain the same for the 2017 meeting. Airfare will be for a round trip flight from Port Columbus, Columbus, Ohio to Dane County Regional Airport, Madison, Wisconsin. The rate for the hotel was determined based on the current rate for an average hotel stay during the time of the conference.

- Consumable Research Supplies: This portion of the budget reflects the cost of performing the proposed research project, including costs of human cell line maintenance and the preparation of samples for ChIP-seq.

    - Human diploid fibroblasts are cultured in growth media (typically DMEM). The estimated cost includes the additional serum and antibiotics required for cell maintenance.

    - 60mm cell culture dishes will be used for maintaining our current human diploid fibroblast cell line on a surface that they readily attach to, while additionally providing a sterile environment.

    - Trypsin is a protease which, at low concentrations, assists in releasing adherent cells from cell culture plastic ware.

    - 20% formaldehyde will be used during the cross-linking reaction which temporarily binds proteins and DNA together.

    - Anti-Myc tag antibody (ChIP grade) is required for the immunoprecipitation reaction which allows for the isolation of myc-UL34 bound DNA.

    - Dynabeads® M-280 Sheep Anti-Mouse IgG are tiny beads coated with an antibody that will recognize the anti-Myc tag antibody, allowing for the separation of myc-UL34 proteins from the mixed sample.

    - The QIAQuick PCR purification kit is used to purify the amplified DNA library before sequencing.

- NEBNext® ChIP-Seq Library Prep Master Mix Set for Illumina® is a kit which contains the reagents necessary to prepare the amplified DNA library for sequencing on any Illumina sequencer (such as MiSeq).

- NEBNext® Multiplex Oligos for Illumina® are used to tag the fragments with barcodes allowing for multiple samples to be sequenced together and still be distinguished from one another.

- The Bioanalyzer QC procedure will be performed at the OU Genomics Facility. This procedure checks the ChIP library to make sure that the quality is good enough for sequencing.

- MiSeq® Reagent Kit v3 (600 cycle) is a kit containing all of the necessary reagents to perform next generation sequencing on the MiSeq platform.

Appended Materials

RE: Request for extension

Male'-Brune, Roxanne

To:
Slayton, Mark;
Fri 1/15/2016 12:27 PM
Inbox

Your request is approved.
Please append this email to you SEA proposal.
Roxanne

From: Slayton, Mark
Sent: Friday, January 15, 2016 11:33 AM
To: Male'-Brune, Roxanne <male-bru@ohio.edu>
Subject: Request for extension

Hi Dr. Male'-Brune,

I am writing for the Student Enhancement Award and would like to request a 6 month extension on the award timeline (should I be selected) in order to attend the conference with my advisor where I will present my results. The conference will take place in June of 2017. Please let me know if this is possible.

Thank you!

Mark D. Slayton
Doctoral Student, Molecular and Cellular Biology
Ohio University
Biegalke Lab, Life Sciences 230
ms915613@ohio.edu | (313) 204-7776

RE: Question about pricing for Illumina sequencer

Lisse, Thomas

To: Slayton, Mark;
Thu 1/14/2016 8:45 AM
Inbox.

Yes you can do that, the p5/7 adapters are for the miseq flowcell, so it will work.

So the only cost would be for QC (dna bioanalyzer) and the sequencing kit.

You are welcomed to see how we do the sequencing and post analysis.

From: Slayton, Mark
Sent: Wednesday, January 13, 2016 10:25 PM
To: Lisse, Thomas <lisse@ohio.edu>
Subject: Re: Question about pricing for Illumina sequencer

Hi Dr. Lisse,

If I prepared my fragmented DNA library for ChIP-seq myself, would this reduce the total cost? This grant I am applying for is a student grant and I am worried that the funds will not cover the whole cost of ChIP-seq.

I looked around and found a kit from New England Biolabs that would prepare my ChIP samples for the Illumina sequencer (see https://www.neb.com/products/e6240-nebnext-chip-seq-library-prep-master-mix-set-for-illumina)

Then, I could use the kit from New England Biolabs which adapts the amplified library for multiplex sequencing on Illumina sequencers (see https://www.neb.com/products/e7335-nebnext-multiplex-oligos-for-illumina-index-primers-set-1 and https://www.neb.com/products/e7500-neb-next-multiplex-oligos-for-illumina-index-primers-set-2)

There are a few additional things I would need to buy to perform these protocols, but I think it would end up costing less than $243 per sample. Additionally, as a student, I would benefit from performing the protocols myself and learning how to do it. This way I would end up purchasing the 600 cycles kit from you guys to perform the sequencing and offset some of the library prep cost.

Let me know if this is possible!

Thank you,

Mark D. Slayton
Doctoral Student, Molecular and Cellular Biology
Ohio University
Biegalke Lab, Life Sciences 230
ms915613@ohio.edu | (313) 204-7776

From: Lisse, Thomas
Sent: Wednesday, January 13, 2016 9:57 AM
To: Yoho, Rachel
Cc: Slayton, Mark
Subject: RE: Question about pricing for Illumina sequencer

Hi Mark,

Which Chip-seq kit to use will depend on the approximate DNA fragment size from your post-chip preparation. If you are getting in the 200-800bp range, then I would recommend the 150cyclePaired END kit (75bp single reads). If your fragment size is larger (>300) then I would recommend the 600 cycle PE (300bp single read) kit.

There is a price difference obviously for increased output. Also, there is a cost for library/barcoding generation per sample. You can multiplex samples onto the miSeq sequencing kits. The number of samples to multiplex depends on species, binding factor (general versus specific), and level of coverage (ultra-low detection versus major binding targets). If this is for a grant, I would suggest running 6-10 samples max on one kit. Could be 3 treated, 3 untreated, something like that. If you have other conditions, you will need to add another kit.

Quote:

Library (per sample) = $243

miSEQ sequencing kit:

600 cycle kit: $1742
150 cycle kit: $1124

High sensitivity bio analyzer chip (QC of library):

$50 dollars per chip (11 samples per chip).

If you are opting for the 600 cycle kit, the cost per sample would be $408 (based on multiplexing 10 samples)
“” 150 cycle = $346 per sample.
Estimate

Good luck

Thomas

From: Slayton, Mark
Sent: Tuesday, January 12, 2016 12:49 PM
To: Yoho, Rachel
Subject: Question about pricing for Illumina sequencer

Hi Rachel,

I am planning to perform a ChIP-seq experiment and was wondering the cost of using the new Illumina Mi-seq machine. For this experiment I would prepare DNA fragments post-ChIP and amplify them before handing them to you guys.

Could you give me a cost per sample or per runs for the machine? I need to include this information in my grant. Thank you!

Take care,

Mark D. Slayton
Doctoral Student, Molecular and Cellular Biology
Ohio University
Biegalke Lab, Life Sciences 230
ms915613@ohio.edu | (313) 204-7776