Purchase of Applied Biosystems™ 3500 HID Genetic Analyzer to Establish DNA Sequencing Core Facility at Western Carolina University

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Abstract

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The expertise of the faculty and staff of the Forensic Science Program (FSP) at Western Carolina University (WCU) is in DNA sequence analysis. With over three decades of combined experience with DNA sequencing analysis, spanning nuclear and mitochondrial, human and non-human, we are prepared to provide the faculty of WCU as well as researchers from collaborating institutions, the ultimate service in DNA sequence analysis: a DNA sequencing core facility. Housed within the sequencing laboratory space of the FSP, a multitude of services will be available to assist researchers, including experimental design and methodology, sequencing instrumentation training, as well as data analysis and interpretation. Based on a successful previous collaboration with Dr. Brian Byrd from the School of Health Sciences, the FSP has established partnerships with other associated WCU investigators both on and off campus to assist with various aspects of the DNA sequencing needs of their research. These partnerships include members from the Biology Department, Environmental Health Sciences and Highlands Biological Station. These collaborations are the founding bases for a DNA sequencing core facility and will undoubtedly stimulate additional research related activities, providing a continuous exceptional environment for student growth and development. The esteemed primary investigators involved in this proposal are all exceptional within their respective fields of study.

The funding requested in this proposal will be used to purchase the Applied Biosystems™ HID 3500 Genetic Analyzer. This instrument is the state-of-the-art platform for capillary electrophoresis of DNA sequences for both nuclear and mitochondrial DNA. The Applied Biosystems™ 3500 HID Genetic Analyzer data collection software integrates seamlessly with several downstream software packages to provide comprehensive analysis of genetic data. Specifically, the combination of the instrument and software package can perform multiple aspects of DNA sequencing analysis including *de novo* sequencing, mutational profiling, single nucleotide polymorphism (SNP) detection, comparative sequencing, resequencing, sequence confirmation, DNA library construction and identification. On the fragment analysis side alone, a variety of platforms are available for microsatellite analysis including LOH, SNP, MLPA, AFLP and t-RFLP. Human identification analysis can be performed using the traditional STR typing analysis, but in addition microbial DNA typing can also be performed. The majority of these applications can be run in a single capillary array and polymer type, therefore allowing the ability to multitask various applications.

The investigators in this proposal have projects which utilize student efforts, and current funding exists to advance research in the areas of forensic science, public heath, plant systematics, population genetics, environmental biology, ecology, evolution and conservation. The specific advantages of the AB™ 3500 HID Genetic Analyzer

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varies among the different projects and needs of the researchers, but all have expressed a strong interest in learning how to apply this instrument to their ongoing and future projects. This instrument would be a fundamental component of the DNA sequencing core facility, fulfilling these demanding research requirements that span multiple areas of study. Hence this instrument would be a joint-use instrument, used in a variety of projects across several investigative disciplines.

In addition to research objectives, this instrument will meet the newly evolving teaching demands placed on the FSP and the Biology Department. An exceptional aspect of the laboratory training provided in the FSP and Biology Department at WCU is the ability to expose and train students on the current instrumentation available in the employment sector. With the purchase of the AB™ 3500 HID Genetic Analyzer, students at WCU would be educated in DNA sequencing and DNA typing processes, would gain valuable hands-on experience using the sequencer, and would master the data analysis and interpretation software, thus elevating student biotechnology education to a higher level of rigor and applicability. As numerous biotechnology laboratories worldwide have validated this instrument for criminal casework and diagnostic analysis, the purchase of this instrument is vital to the ongoing quality education provided by WCU.

Implementation of this instrument and the establishment of the DNA sequencing core facility at WCU will result in an evident educational advancement in biotechnology at WCU. The collaborations established among the researchers of these various disciplines will result in research projects for students that provide the fundamental training on state-of-the-art instrumentation for the next generation of researchers, technicians and educators for North Carolina. As a DNA sequencing core facility utilized by various individuals from the Biology Department, Environmental Health Sciences, FSP and Highlands Biological Station, the opportunity for advancement of biotechnology within multiple disciplines is evident. This is an outstanding opportunity for Western Carolina University to provide researchers in the western end of the state with an exceptional service otherwise not available.

Significance

The Forensic Science Program (FSP) is currently expanding in both size and scope. Student enrollment in the program nearly doubles every year. This includes increased student numbers in forensic science courses as well as student participation in forensic science research projects in the laboratory. The capstone course in the FSP is Forensic Biology, Bio 422/522. This course is an undergraduate and graduate course wherein students conduct mock criminal casework which includes mtDNA sequencing analysis and short tandem repeat (STR) analysis on questioned and known samples using the same protocols and instruments established in crime laboratories, culminating in a mock courtroom trial.

Research conducted within the FSP, under the direction of Dr. Mark Wilson, entails investigation of complex concepts such as mixture interpretation, heteroplasmy and single cell analysis, all of which utilize advanced instrumentation. The FSP has an Illumina® IIx Genome analyzer and a Roche®454 GS Junior DNA Sequencer. These two sequencers are state-of-the art, specializing in deep sequencing for mitochondrial DNA (mtDNA) analysis, with the ability to detect single nucleotide polymorphisms (SNPs) at the level of one percent or better. This is a tenfold increase in the level of sensitivity compared to traditional sequencing methods and is essential to resolving issues of heteroplasmy. In addition, the FSP also has an Applied Biosystems™ 3130xL capillary electrophoresis genetic analyzer, currently utilized for traditional fluorescent based sequencing and nuclear DNA short tandem repeat (STR) analysis. This research is not only pertinent to the field of forensic science but also to molecular genetics and molecular/cellular biology. In addition, the FSP is expanding its research objectives to include non-human DNA analysis such as microbial and domestic animal genetics, both of which are rapidly emerging fields in forensic science. Forensic microbial genetics is vital to investigations of terrorist acts, germ warfare and additional homeland security risks. Forensic animal genetics is a crucial component of humanitarian activities to resolve cases of animal cruelty, abuse, aggravated assault with canines and illegal dog fighting activities.

The expertise of the faculty of the FSP is in DNA sequence analysis. With over three decades of combined experience with DNA sequencing, spanning nuclear and mitochondrial, human and non-human, we are prepared to provide the faculty of Western Carolina University as well as researchers from collaborating institutions, the ultimate service in DNA sequence analysis: a DNA sequencing core facility. Housed within the sequencing laboratory space within the FSP, a multitude of services will be available to assist researchers, including development of experimental design, education and training on the various sequencing instruments, sequence generation methodology as well as sequence data analysis and interpretation. Based on a successful previous collaboration with Dr. Brian Byrd from the School of Health

Sciences, the FSP has established partnerships with other associated Western Carolina University investigators both on and off campus to assist with various aspects of the DNA sequencing needs of their research. These partnerships include members from the Biology Department, Environmental Health Sciences and Highlands Biological Station. These collaborations are the founding bases of the need for a DNA sequencing core facility.

Based on the activities outlined above the current demand on the existing capillary electrophoresis instrument is extensive. It is necessary to acquire a second capillary sequencing instrument which can perform both DNA fragment analysis and DNA sequencing analysis. The current top of the line instrument for this platform is the Applied Biosystems™ 3500 HID Genetic Analyzer (3500xL). This instrument would be a fundamental component of the DNA sequencing core facility, fulfilling the demanding research requirements that span multiple areas of study. Hence this instrument would be a joint-use instrument, used in a variety of projects across several investigative disciplines. In addition, this instrument will meet the teaching needs of the FSP capstone course Forensic Biology, Bio 422/522 and be implemented into the Biology Department's Biology 240, Introduction to Genetics course.

As a DNA sequencing core facility utilized by various individuals from the Biology Department, Environmental Health Sciences, FSP and Highlands Biological Station, the opportunity for advancement of biotechnology within multiple disciplines is evident. This is an outstanding opportunity for Western Carolina University to provide researchers in the western end of the state with an exceptional service currently not available. Furthermore, by providing students both classroom and research exposure to state of the art molecular biology equipment like the Applied Biosystems™ 3500 HID Genetic Analyzer, we are able to bring biotechnology into the classroom.

Objectives

This funding will be used for the purchase of the Applied Biosystems™ 3500 HID Genetic Analyzer, the computer and analysis software to interface with the instrument, reagents to conduct the initial performance check on the instrument and operational training provided by Applied Biosystems for four individuals. Training on the software is necessary. The data collection software provided with this instrument integrates seamlessly with several downstream software packages to provide comprehensive analysis of genetic data under a variety of applications. This instrument is essential to the development of the new DNA Sequencing Core Facility at Western Carolina University.

The Applied Biosystems™ 3500 HID Genetic Analyzer is a twenty-four capillary instrument which can perform a wide variety of applications, including but not limited to, *de novo* sequencing, microsatellite analysis, fragment length analysis and SNP analysis. A variety of applications can be run using a single type of capillary array and

polymer. This provides increased efficiency as the majority of applications will use the same reagents and consumable supplies.

Instrument setup is extensive and requires a performance check assay. In addition to company supplied calibration and performance-check reagents and protocols, the instrument will be evaluated by processing several samples through two different assays. One assay will be the traditional mitochondrial DNA Sanger sequencing methodology and the other will be a nuclear DNA short tandem repeat assay. The assays will use previously sequenced and typed samples to ensure correct results are obtained. This will be sufficient to verify the instrument's performance.

Due to the advances in capabilities, system design and software, which are extensive in comparison to the 3130xL model, advanced training on the instrument will also be required. This will ensure that the instrument is used to its full potential. Applied Biosystems offers two advanced courses which cover assay protocol development, troubleshooting, maintenance, and software training on this instrument. Four members of the FSP faculty and staff would attend these courses. These individuals would benefit the most from this training as they already have a strong foundation in DNA analysis methods and would be responsible for assisting other researchers with experimental design or sample processing. In addition, these individuals could then provide training to other researchers or graduate students as needed.

Project Plan

I.Equipment

General description

The Applied Biosystems™ (AB™) 3500 HID Genetic Analyzer is a 24-capillary DNA sequencer that can be utilized for both nuclear DNA (nucDNA) and mitochondrial DNA (mtDNA) analysis including DNA sequencing and fragment analysis. A superior feature of this instrument is substantially improved temperature control through an advanced thermal system design. Precise temperature control is essential for consistent sample migration, thereby reducing variation between DNA analysis runs as well as between the individual capillaries. This instrument also has the innovative “snap-in-and-go” consumable design. This feature provides consumables in premade cassettes that can be loaded onto the instrument directly and are tracked with radio frequency identification (RFID) technology. The software, in combination with the RFID, tracks and records the data from key consumables including lot numbers, amount consumed, amount remaining and the expiration dates for all reagents in support of quality assurance and quality control. This removes any potential ambiguities related to the stability of the reagents on the instrument as well as reducing potential reagent waste. Data analysis from the instrument has also been streamlined with a new, more powerful integrated data collection and quality control analysis software. The new features of this software provide real-time assessment of data quality which incorporates instrument control, data collection and autoanalysis of sample files. The software has also been designed to simplify run set up as well as software navigation. The 3500 data collection software integrates seamlessly with several downstream software packages to provide comprehensive analysis of genetic data. Specifically, the combination of the instrument and software package can perform multiple aspects of DNA sequencing analysis including *de novo* sequencing, mutational profiling, single nucleotide polymorphism (SNP) detection, comparative sequencing, resequencing, sequence confirmation, DNA library construction and identification. On the fragment analysis side alone, a variety of programs are available for microsatellite analysis including LOH, SNP, MLPA, AFLP and t-RFLP. Human identification analysis can be performed using the traditional STR typing analysis, but in addition microbial DNA typing can also be performed. The majority of these applications can be run in a single capillary array and polymer type, therefore allowing the ability to multitask various applications.

Applied Biosystems is the only manufacturer of the 3500 HID Genetic Analyzer and is renowned in the field as the signature provider of capillary electrophoresis instruments. The 3500 series genetic analyzers are accepted in the fields of molecular biology, genetics and forensic science as the state-of-the-art capillary electrophoresis platforms. Numerous crime laboratories as well as molecular genetics laboratories worldwide have validated Applied Biosystems instruments for criminal casework and diagnostic analysis. An exceptional aspect of the laboratory training provided here at

WCU, is the ability to expose and train students on the current biotechnology available in the employment sector. Hence purchase of this instrument is vital to the ongoing quality education provided by Western Carolina University in preparation for careers in biotechnology and related fields.

The Applied Biosystems quote included in the supporting documentation section of the application, includes the Applied Biosystems™ 3500 HID Genetic Analyzer with one year warranty, the data collection and data analysis software to process runs on the instrument and training for four individuals conducted on site, on the purchased instrument at Western Carolina University.

Justification of equipment selection

Applied Biosystems is the industry leader for capillary electrophoresis (CE) applications of DNA sequencing and DNA typing analysis. The 3500 HID Genetic Analyzer is recognized by the scientific community as providing the highest level of data quality and reliability in a capillary-based platform. This instrument has improved upon previous CE systems in both hardware and software, which have been designed to optimize data quality, reliability and performance. Since this platform supports a wide variety of DNA sequencing and typing applications, this instrument can be utilized across a variety of research fields.

This instrument is vital to the growth and development of the FSP at WCU. The forensic DNA typing community is implementing this instrument into criminal casework laboratories based on the familiarity and reliability of Applied Biosystems™ instruments in addition to the advantages provided with the new design. A critical improvement is the tracking, quality assurance and quality control of reagents, features that can be addressed in the new applications of the AB™ 3500 HID Genetic Analyzer. Since reagent dispensing is accomplished by the use of premeasured and preloaded cassettes, a significant reduction in wasted reagents is expected. In addition, the barcoded cassette provides easy tracking and troubleshooting of reagents.

The FSP at WCU is exceptional in scope, application and faculty. The program has been developed and staffed by former forensic science practitioners who hold doctoral degrees in the natural sciences. The mission of this program is to prepare students for technical positions in organizations that conduct physical analysis of evidentiary materials in both civil litigations and criminal procedures. Our program emphasizes hands-on experience with current state of the art instrumentation, representative of the equipment found in many biotechnology laboratories. Therefore, the purchase of the AB™ 3500 HID Genetic Analyzer is essential to the continued success and development of the FSP. Students in the program will gain crucial experience and knowledge of the applications of the AB™ 3500 HID Genetic Analyzer through analysis of mock casework samples in the program's capstone course, Forensic Biology 422/522.

The Biology Department would also benefit greatly by having student access to this instrument. The sophomore level Introduction to Genetics course, Biology 240, is a prerequisite course for more advanced molecular, cellular and biotechnology related courses. Among the fundamental techniques covered in this course is DNA sequencing. Using protocols which are widely established in the field of biotechnology, students extract their own mitochondrial DNA and amplify the HV1 and HV2 regions of the mtDNA genome. Currently, the amplified DNA product is sent away to another facility for sequencing, the data is returned and the students then review the data. This process leaves a lack in developmental knowledge of the sequencing procedure and analysis. Instead, with the purchase of the AB™ 3500 HID Genetic Analyzer, the students in this course would be educated in the sequencing process including protocol design, would gain invaluable hands-on experience on the sequencer, and would master the sequence interpretation software, thus elevating this course to a higher level of rigor and applicability.

This instrument is also essential to current research projects spanning multiple disciplines. Under the DNA sequencing core facility, research projects from such diverse disciplines as forensic science, plant systematics, bacterial biology, general biology and environmental health would be dependent on this instrument for various applications of sequencing analysis. These initial collaborations will undoubtedly stimulate additional research related activities, providing a continuous exceptional environment for student growth and development. The primary investigators involved in this proposal are all respected and exceptional within their respective fields of study.

Dr. Mark Wilson, Director of the FSP and a former Supervisory Special Agent of the Federal Bureau of Investigation, has an interest in using the AB™ 3500 HID Genetic Analyzer for traditional Sanger sequencing of human mtDNA. This is currently the standard in crime laboratories and is taught to students interested in forensic DNA analysis applications. In addition, Dr. Wilson is currently funded by the National Institute of Justice to evaluate the performance of two new next generation sequencers in comparison to each other, as well as to the current Sanger sequencing method. Within this study, mixture interpretation, sample read length, sample preparation, and cost analysis will be compared among all three sequencing platforms.

Dr. Patricia Foley of the FSP, formally with the Department of Defense Armed Forces DNA Identification Laboratory, is interested in establishing forensic short tandem repeat markers for domesticated cats and dogs. An emerging aspect of forensic science is the identification of hairs from domestic pets which are transferred from victim or crime scene to suspect. In addition, these markers could also be used in analysis of dog biting cases. The AB™ 3500 HID Genetic Analyzer is an excellent platform for typing DNA sequences to establish reliable markers with the appropriate amount of variability to use in a forensic application.

Dr. Sean O'Connell, Department Head of Biology and named as one of UNC's top teachers, is involved in two projects which will utilize the capabilities of the AB™ 3500 HID Genetic Analyzer. One project relies on DNA sequencing capabilities to characterize sequences of genes to evaluate the diversity of bacteria from the soil and water of the Great Smoky Mountain National Park. The other evaluates DNA sequences of prokaryotes associated with the Eastern Hemlock. Both of these projects generate nearly 500 sequences per year which can be processed efficiently on the AB™ 3500 HID genetic Analyzer. Additional protocols beyond DNA sequencing which are valuable to this research include the analysis of terminal restriction fragment length polymorphisms, single nucleotide polymorphisms and amplified fragment length polymorphisms, all of which are supported by the AB™ 3500 HID Genetic Analyzer.

Dr. Katherine Mathews of the Biology Department and previous Director of the WCU Herbarium is currently funded to establish a superiorly modified cultivar of Black Cohash, which can be genetically identified, patented and grown commercially. This research is directly related to biotechnology and drug development as Black Cohash is popular as an herbal remedy for menopausal symptoms. The data generated is immense as the study is heavy in sample volume, requiring analysis of twenty plant specimens from twenty different plant populations, each screened by hundreds of different genetic markers. Although the DNA analysis work in this project is currently conducted by collaborators, augmenting the DNA analysis capabilities at WCU would serve to further this area of research beyond the current scope of the effort. By providing access to a higher capacity instrument such as the AB™ 3500 HID Genetic Analyzer, which has a more efficient run time, data can be generated more expeditiously, a vital component given the competitive nature of drug development.

Dr. Brian Byrd of the College of Health and Human Sciences, a recipient of the 2011 College of Health and Human Sciences Student Engagement Faculty Award, collaborated with the FSP on a project that resolved a sixty year old scientific question involved in mosquito taxonomy, namely differentiating between *Aedes* and *Ochlerotatus* mosquitoes by analyzing their DNA sequences. Both of these mosquitoes are vectors for a multitude of diseases and are detrimental to public health. Proper identification of mosquitoes is essential to the mosquito control industry, yet currently identification is conducted based on morphological characteristics, leading to some inconsistencies and confusion. Dr. Byrd's molecular biology approach to this problem has the potential to lead to the development of reliable, fast nucleic acid assays for mosquito identification. By further collaborating with the FSP under the DNA core sequencing facility, continued work on this project can be conducted in a highly efficient and cost effective manner.

Dr. James Costa, renowned entomologist, Darwinian expert and the Director of Highlands Biological Center, UNC's mountain biological research station, is completely supportive of this initiative and proposal. The mission of Highlands Biological Station is to promote research and education in the ample biodiversity present in Western North

Carolina. Scientists from all over the world come to this unique area to conduct research in the areas of ecology, systematics, evolution and conservation. By providing the scientists at Highlands Biological Station with access to equipment in the DNA sequencing core facility, research projects can be designed to examine the diversity unique to this region with a molecular biology or molecular genetics focus. The expansion in scope for the research and educational courses conducted at the HBS is endless. The WCU DNA sequencing core facility would bring a level of biotechnology to Highlands Biological Station otherwise unavailable to these scientists and students and will expand the collaborations between Highlands Biological Station, Western Carolina University and the North Carolina Arboretum.

Current capabilities and justification of need

Currently, no DNA Sequencing core facility exists at Western Carolina University. However, the location is convenient to serve the needs of a wide variety of researchers in the western part of the state. Although the FSP and the Biology Department each have an older Applied Biosystems™ capillary electrophoresis instrument, a 3130 and a 3130xL, these two instruments are not readily available to faculty either due to current demand of usage, limited ability of scope, or maintenance issues. Both of these instruments, being older, do not have some of the applications that are currently in demand for the research projects discussed in this proposal. In addition, Applied Biosystems is phasing out the 3130 series of genetic analyzers and will soon no longer provide technical and maintenance support for these instruments.

By establishing a DNA sequencing core facility at WCU, managed by members of the FSP, biology and biotechnology researchers at WCU can expect assistance with training on the instrument, experimental design, sample preparation and analysis, and/or the interpretation of data. Members of the Biology Department, Environmental Health Program and Highlands Biological Station who may be interested in expanding their research projects into DNA analysis will have one location to provide them with quality DNA typing and/or DNA sequencing and data analysis. The expertise of the faculty of the FSP is with DNA analysis and sequencing, and hence these needs will be met. By integrating this strength with a facility that provides all of the equipment required for various DNA sequencing applications, a successful DNA sequencing core facility will be established at WCU. This facility would be the only location in western North Carolina to serve the academic and biotechnology researchers located here.

II. Research Projects

Overview

The AB™ 3500 HID Genetic Analyzer is an essential piece of equipment for the continued educational success of students at WCU. The investigative interest that is sparked from student research projects, combined with the ability to gain hands-on

experience with state of the art equipment, compels students to take biotechnology from the classroom to the community. Several primary investigators have projects which utilize student efforts, and current funding exists to advance research in the areas of forensic science, public heath, plant systematics, population genetics, environmental biology, ecology, evolution and conservation. The specific advantages of the AB™ 3500 HID Genetic Analyzer varies among the different projects and needs of the researchers, but all have expressed a strong interest in learning how to apply this instrument to their ongoing and future projects.

In addition to supporting specific research projects, this instrument will also serve an educational purpose. The instrument will be used in the capstone course of the FSP, Biology 422/522. This course is both a graduate and undergraduate course which provides hands-on training to students on the current DNA analysis equipment and protocols used in crime laboratories. Crime laboratories across the nation have implemented the AB™ 3500 Genetic Analyzer. By providing training to our students on this instrument we will facilitate job placement of our graduates into technical and analytical positions at crime laboratories. This instrument will also be used to instruct students in mtDNA sequencing in the Biology Department's genetics course, Biology 240. This course is a fundamental prerequisite course for upper level biotechnology related courses in biology, premedical, and pre-professional heath and science majors.

Implementation of this instrument and the establishment of the DNA sequencing core facility at WCU will result in an evident educational advancement in biotechnology at WCU. The collaborations established among the researchers of these various disciplines will result in research projects for students that provide the fundamental training for the next generation of researchers, technicians and educators for North Carolina.

Projected usage table

Name	Affiliation	Annual Percent Usage
Mark Wilson	Director, Forensic Science Program Department of Chemistry and Physics	15 percent
Patricia Foley	Associate Professor Forensic Science Program Department of Chemistry and Physics	15 percent
Sean O'Connel	Head of the Department of Biology	15 percent
Katherine Mathews	Associate Professor Department of Biology	20 percent
James Costa	Director Highlands Biological Station Professor, Department of Biology	15 percent
Brian Byrd	Assistant Professor Environmental Health Program College of Health and Human Sciences	20 percent

Major users

Research Summary: Mark Wilson, PhD

Research Project Title: Evaluation of newly emerging DNA sequencing technologies as applied to Forensic DNA Analysis.

The addition of an Applied Biosystems™ 3500 HID Genetic Analyzer to ongoing, as well as projected, research and teaching activities within the FSP at Western Carolina University is considerable. Students in the program are provided with a hands-on, experiential curriculum that emphasizes technical acumen on instrumentation that is currently employed in crime laboratories throughout the world. The AB™ 3500xl fits the needs of the program from both a teaching and research perspective.

The Applied Biosystems™ 3500 HID Genetic Analyzer instrument is part of the newest generation of DNA analysis instrumentation employed in forensic laboratories in the U.S. In order to serve our students in the most effective manner and prepare them for jobs in a DNA section of a crime laboratory, it is important that the FSP acquire and employ the latest instrumentation, software, and reagents. This instrument, with its innovative design and use of RFID technology, promises to be present in crime laboratories for many years. The 3500xl instrument would be used to generate DNA fragment size data as well as DNA sequence data from both evidentiary and exemplar samples which will then be compared with each other and the results interpreted according to established protocols. The comparisons would be of both short tandem repeat (STR) profiles as well as human mitochondrial DNA templates.

Ongoing research projects within the FSP include DNA sequencing of amplified human mitochondrial DNA templates. The Program currently is funded by the National Institute of Justice (NIJ) to evaluate newly emerging DNA sequencing technologies and compare them to the existing instrumentation used in crime laboratories. Accordingly, the AB™ 3500xl would be employed to generate traditional Sanger sequence information from templates that can then be compared to data arising from the newly emerging instruments.

In short, it has become important that the FSP continue to provide high quality training to students as well as generate high quality DNA sequence data to support ongoing and future research projects. The acquisition of the AB™ 3500 HID Genetic Analyzer would be invaluable to these important endeavors.

Research Summary: Patricia Foley, PhD SAMPLE DO NOT COPY

Research Project Title: Development of Short Tandem Repeat markers for identification and association of domestic cats (Felis catus) and domestic dogs (Canis familiaris).

The FSP at Western Carolina University is exceptional by providing real-world experience and education on state of the art equipment and protocols which are utilized in crime laboratories across the nation and worldwide taught by former practitioners in the field of forensic science. In order to provide students with the most current and pertinent training, the program stays abreast of newly emerging technologies in the field. One such topic is the development of nuclear DNA markers for individualization of non-human biological samples used as an alternative biological target of forensic investigation. Current use of non-human biological material in forensic investigation has been limited by the absence of well-defined DNA hypervariable markers, the lack of a population databases for statistical application and the unavailability of standardized, validated protocols. There is no commercially available kit for which crime laboratories may use to establish the identity of animal hairs collected as trace evidence. Given that for a vast majority of criminal cases the only biological evidence which can link the suspect to the victim is trace amounts of hair transferred from domestic pets, the inability to pursue this evidence as an investigative lead is unfortunate to say the least.

Although some polymorphic sites have been determined for many members of domestic dogs, the literature is confusing lacking in consistent nomenclature or standardization of the DNA marker sites. In addition, these regions have not been thoroughly examined for the ease of amplification within a multiplex system, which would be essential to implementation in a crime laboratory. Furthermore, very little genetic information is known about polymorphic sites for domestic cats, leaving this potential forensic lead completely unutilized.

The goal of this research project is to establish several short tandem repeat markers which provide enough genetic information to resolve the identity of a particular domestic pet, yet can be multiplexed together in a single amplification system for ease of implementation into the crime laboratory setting. Purchase of the Applied Biosystems™ 3500 HID Genetic Analyzer, which has high throughput capability, is essential to the success of this project.

Research Summary: Sean O'Connell, PhD SAMPLE DO NOT COPY
Research Project Title: Bacterial diversity in Great Smoky Mountains National Park

The research we are conducting is part of two ongoing projects including describing the culturable diversity of bacteria from soils and waters of Great Smoky Mountains National Park (GSMNP) and the molecular diversity of prokaryotes associated with Eastern Hemlock (*Tsuga canadensis*). In the first project, approximately 30 students from class each fall isolate and characterize bacterial isolates and use a DNA sequence of the 16S rRNA gene to identify these cultures. The methods they learn include microbial cultivation, microscopy, and biochemical techniques that are well suited for applications in biotechnology. Generation and analysis of their DNA sequences give them experience in handling and interpreting nucleic acids. The hemlock work involves students in independent and thesis research projects and is more intensive than in class. These students generally extract DNA from the rhizosphere and construct clone libraries from PCR-generated 16S rDNA products. Sequencing involves separating and analyzing dozens to hundreds of clones. Exposure to bioinformatics and other software to handle large DNA datasets as well as the field and lab methods gives students a great experience in biotechnology.

All of our research projects critically rely on DNA sequencing. Over the last ten years, we have generated thousands of sequences on slab gel and capillary machines. Our output is not large enough to require a high throughput or next generation system. The 3130 and 3130xL have served us well, in part because they can be left idle for a week or two at a time with little care required. However, the systems we have are aging and will eventually not be serviced by Applied Biosystems. We will require a system such as the 3500xL sooner rather than later and would like to be proactive in updating our research infrastructure and exposing students to the newest systems available.

Our needs are fairly basic, usually requiring a DNA sequence of 500-600 bases per sample. We generate approximately 75-500 sequences per year depending upon how many and what sort of independent research projects are active. The 16S rDNA sequences are sufficient to identify bacterial cultures to genera and sometimes to species while clone libraries contain many sequences related to uncultured bacteria. These latter sequences can often describe diversity at the genus level, but in many cases, a family, order, or class description can be very valuable. The ABI 3500 or 3500xL are representative of the lower throughput, "workhorse" sort of DNA sequencer we need. These machines also can perform other analyses that we use including terminal restriction fragment length polymorphism (T-RFLP) analyses as well as SNP and amplified fragment length polymorphism (AFLP) methods. Other sequencers either require constant care (and expense) such as high throughput models or are unable to do analyses other than sequencing.

Research Summary: Katherine Mathews, PhD SAMPLE DO NOT COPY
Research Project Title: Population Genetics, DNA Fingerprinting and Marker-Assisted Selection for the Medicinal Herb, Black Cohosh

Black cohosh (*Actaea racemosa*), a flowering plant native to the Appalachian range, is popular as an herbal remedy for menopausal symptoms (Blumenthal 2008). Due to extensive wild harvesting of rhizomes, the sustainability of this species in the wild is of great concern. The goal of this multidisciplinary project is to select and breed a regional cultivar of black cohosh with superior levels of biologically active compounds and best growth characteristics for western North Carolina that may be identified with genetic markers, patented and grown commercially. New, faster DNA sequencing equipment will advance our research objectives much more quickly than is currently possible. Increasing the speed of our data generation is important in the competitive field of drug development and to allow us to rapidly move on to the selection and breeding phase of the project.

Preliminary work for this project has been funded by a two-year grant from the North Carolina Biotechnology Center to Western Carolina University (PIs Jason Clement, Katherine Mathews and Joe-Ann McCoy). Providing a biotechnologically enhanced product that can guarantee specific levels of triterpenoid glycosides (the market standard, Upton 2002) from well-characterized plant material correlated to identifiable molecular markers may give a commercial advantage to growers in our region. Molecular markers may also be used to rule out the presence of adulterants (other species of *Actaea*) in commercial preparations of black cohosh.

For the genetics component of this project, we have already outsourced the development of microsatellite (short tandem repeat, STR) markers for black cohosh and have been screening five individuals from 20 wild-collected populations grown in a common garden at the North Carolina Arboretum for amplification and variability. So far we have discovered a large amount of genetic variation across the range of this species. To generate significant results, we will need to include more samples from each population in our data set. A typical population genetics study includes at least 20 individuals per population to accurately assess the range of within and between population variability in molecular markers. We plan to revisit populations from which the original accessions were made to collect additional samples.

Also, we have screened only 48 primer pairs from over 100 that were developed, and only 19 of these amplify consistently. To do marker-assisted selection, a larger number of markers should be generated. Thus, we would like to increase both the number of plants sampled and the number of genetic markers screened. We are currently sharing a 4-capillary sequencer among several lab groups and biology classes. With this capacity, data generation is relatively slow. Having access to a high-throughput sequencer would make this data generation much more expedient.

Research Summary: Brian Byrd, PhD SAMPLE DO NOT COPY
Research Project Title: Molecular Characterization and Identification of *Aedes/Ochlerotatus* Mosquitoes

Many *Aedes* and *Ochlerotatus* mosquitoes are important primary or potential secondary vectors for diseases such as dengue fever, arboviral encephalitis (e.g., West Nile, St. Louis, La Crosse, and Eastern Equine), canine heartworm, and others. Recent taxonomic reclassifications and nomenclature changes for *Aedes/Ochlerotus* mosquitoes have created confusion and inconsistencies in the published literature resulting in significant burdens for the public health community at large. The lack of molecular evidence and phylogenetic analyses hinders the stabilization of these taxa. Our work seeks to address this timely problem within mosquito systematics with sequence and phylogenetic analyses of nuclear and mitochondrial loci which will allow for the development of molecular assays to rapidly and reliably identify these important public health pests.

Our work relies on molecular techniques (e.g., DNA extraction, PCR amplification, cloning, and sequencing) that are commonly used in the biotechnology sector. Because this research is conducted with undergraduate and graduate students, we provide significant training and practical “hands-on” experiences for students that decide to pursue biotechnology careers or graduate biomedical training. The identification of mosquito species and the pathogens they vector are critical elements of the mosquito control industry. Currently most mosquito control districts or state agencies rely on morphological identification of the mosquitoes and nucleic acid testing (i.e., rt-PCR) for arbovirus pathogens (e.g., West Nile, St. Louis encephalitis, Eastern Equine encephalitis, and La Crosse viruses). We expect that our work will lead to the development of nucleic acid assays which identify the principle mosquito vectors responsible for disease transmission.

The acquisition of the AB 3500 by the WCU Forensic Science Program will increase our capacity to generate novel sequences “in-house” and allow us to conduct this work in a collaborative and cost-effective manner. Students will benefit greatly by being provided the opportunity to conduct experiments on state of the art equipment which previously was not available at Western Carolina University.

Our work depends on sequencing reads of 300-650 base pairs in length and currently requires different polymer and experimental design than what is commonly used on the current instrument available in the Forensic Science Program. Switching polymers is expensive, time consuming, and causes unnecessary downtime. The AB 3500 Genetic Analyzer has more versatility in experimental design as well as a higher throughput of sample volume, hence the acquisition of this sequencer will increase productivity and decrease the overall cost of operation.

Research Summary: James Costa, PhD SAMPLE DO NOT COPY

Research Project Title: Analysis of diversity of the Appalachian natural environment

The value of the proposed Applied Biosystems™ 3500 Genomic Sequencer to research conducted at the Highlands Biological Station (HBS) is considerable, and extends far beyond any single research project. As a University of North Carolina center closely affiliated with WCU, HBS hosts researchers from a diversity of UNC universities as well as other institutions nationwide conducting field- and laboratory-based investigations in ecology, organismal biology, systematics, population genetics, conservation biology, and evolution. All of these disciplines now include important molecular genetic applications; accordingly, it is critical for traditionally field-oriented stations such as HBS to provide molecular research facilities and related educational opportunities.

Two ongoing research projects at HBS are molecular characterization of a developing pheromone gland in plethodontid salamanders, and a population genetic study of goldenseal [*Hydrastis canadensis*]. However, HBS lacks sequencing capabilities. Presently, researchers must send their material off-site to another institution for sequencing. A DNA sequencing core facility at WCU would be greatly beneficial to researchers at HBS who need to generate and analyze sequence data while in residence at the station. Similarly, HBS-based classes in molecular methods could easily travel to the core facility at WCU for sequencing.

A fully equipped DNA sequencing core facility at WCU would also prove invaluable in furthering the collaborative research efforts of HBS, WCU, and the NC Arboretum. One joint research partnership of these three regional institutions will focus on the level and distribution of genetic diversity in selected native plant species which are of potential economic importance. Currently no population genetic data exist for these species. Characterization of mitochondrial and nuclear DNA markers such as RAPDs and microsatellites, as well as subsequent population genetic analyses of these species, will be greatly augmented by the availability of a DNA sequencing core facility at WCU. Moreover, access to a DNA sequencing core facility at WCU will considerably enhance the competitiveness of local researchers as they seek further extramural funding.

In short, it has become critically important for institutions like HBS to provide researchers with the equipment to efficiently process, manipulate and analyze genetic material in support of a diversity of research endeavors. The addition of the proposed Applied Biosystems™ 3500 Genomic Analyzer and the development of the proposed DNA sequencing core facility at WCU will be of considerable benefit to HBS researchers and students, and will further augment the growing collaborative relationship between HBS, WCU and the NC Arboretum.

III. Administration and Operation

Administrative responsibility

Ultimate authority for administration of the facility, long-term maintenance, user fees, and equipment operations will be held by Dr. Mark Wilson. Dr. Wilson is the director of the FSP and a retired Supervisory Special Agent of the F.B.I. where he served a total of 23 years, the majority of which was spent in the Laboratory Division. Dr. Wilson is a forerunner in the field of mitochondrial DNA analysis and was instrumental in the development of microbial DNA analysis. His work established the protocols for isolation, amplification, sequencing analysis and interpretation of mtDNA as a forensic tool. These protocols have become the standard operating procedure currently used for mtDNA analysis throughout the world. Dr. Wilson was the first mtDNA examiner in the United States and has years of experience processing forensic samples as well as providing expert witness testimony as a DNA examiner. He has published a combined total of approximately 40 articles and book chapters dealing with the topic of forensic DNA analysis. Dr. Wilson's extensive expertise in mtDNA analysis provides unparalleled instruction and experience. He remains instrumental in the continuing development of forensic mtDNA analysis.

Technical responsibility

Ms. Brittania Bintz will have the primary responsibility of day-to-day technical operations including routine maintenance, user scheduling and training. Ms. Bintz is a Forensic Science Research Scientist with a M.S. degree in chemistry. Ms. Bintz has extensive experience and training on all of the instrumentation, protocols, and analytical procedures conducted for both nuclear and mtDNA forensic analysis. Her all-embracing background in instrumentation and methodology is essential to the successful operation of investigative procedures conducted in the laboratory. Ms. Bintz has also established herself as an excellent independent scientist in mtDNA analysis, having devised a method for amplification of the entire human mtDNA genome utilizing one primer pair in a single PCR reaction.

Supporting Ms. Bintz with the technical responsibilities will be Ms. Erin Burnside. Ms. Burnside is also a Forensic Science Research Scientist with a M.S. degree in biology. Ms. Burnside has extensive validation and molecular biology experience from both an industrial and academic setting. In addition, Ms. Burnside has three years of experience as a Research Operations Manager, providing unsurpassed knowledge of various forms of equipment and standard operating procedures. Her impressive background in experimental design and implementation stemming from her validation work provides unparalleled insight to our program.

In addition, Dr. Patricia Foley will evaluate the performance of the instrument and assist with any troubleshooting aspects. Dr. Foley is the Forensic Scientist in Residence and Associate Professor at WCU. Dr. Foley has a combined eight years of forensic

nuclear DNA analysis experience from the Department of Defense Armed Forces DNA Identification Laboratory (AFDIL) and the Baltimore County Police Department Forensic Services Section. Dr. Foley has processed over 400 cases, representing over 2,000 tissue and bone specimens for the identification and re-association of human remains from military casualties as a result of acts of terrorism or war. Her caseload includes many high profile and time sensitive cases such as the Space Shuttle Columbia Mishap, Operation Enduring Freedom, Operation Iraqi Freedom and the Tri-State (Georgia) Crematorium case. Dr. Foley has presented many of the cases at scientific meetings around the country. In addition, Dr. Foley has processed over 150 criminal cases and established herself as an accomplished expert witness for testimony in nuclear DNA analysis. She has first rate knowledge of validation and implementation of new equipment and standard operating procedures. Dr. Wilson and Dr. Foley both serve as ASCLD/LAB assessors and are members of the American Academy of Forensic Sciences.

Location

The DNA sequencing core facility will be located at WCU in the Stillwell Building, room 134. This is the sequencing laboratory of the Forensic Science Program. The room currently houses the Illumina® IIx Genome analyzer, the Roche®454 GS Junior DNA, and the Applied Biosystems™ 3130xL Genetic Analyzer. This room has been modified to provide the essential temperature control required by DNA sequencers. Room temperature fluctuations can alter the rate of migration of DNA fragments and cause complications in data interpretation. The FSP has installed an independent temperature control system that maintains a constant temperature in that room. In addition, the instruments are installed on an uninterrupted power supply. This verifies that if there is a fluctuation or disrupt in power, the instruments will not be affected. This option is essential for assurance of quality data and no data loss or waste of reagents.

User access

Initially user access will be limited to the participants involved in this grant application as these are projects in which a collaborative relationship has been established. However, it is the goal of the FSP to extend this service of a core facility to all researchers at WCU. We expect that as we continue to engage ourselves in the research activities of others, more collaborative projects will become available. It is expected that the growth of the sequencing facility will be rapid. Scheduling of activity on the instrument will be conducted by Ms. Bintz and Ms. Burnside so that usage of reagents and supplies is optimized for efficiency. There will be no prioritization of availability to the instrument based on any one particular project or individual, but rather sample types will be grouped together to minimize turnaround time for results and maximize quality data. Sample preparation for loading onto the AB™ 3500 Genetic

Analyzer will be conducted by trained individuals only. All user activity on the instrument will be monitored by Ms. Bintz and Ms. Burnside. Certainly, researchers are welcome to obtain training on the instrument, but we expect that as the demand for sample processing increases, graduate students from the various collaborators as well as from the Forensic Science Program will be trained on the instrument in order to maintain effectiveness. Until the facility is established with additional permanent technical positions held by trained individuals, the facility will not be accessible to individuals outside WCU.

User training

Dr. Wilson, Dr. Foley, Ms. Bintz and Ms. Burnside will be trained to use the instrument by Applied Biosystems in a two class series. These individuals can in turn train other researchers and students who will have access to the instrument.

IV. Long-term support

The College of Arts and Science and the Office of the Provost at WCU, recognize that the preliminary collaborations presented in this grant focused around the utilization of the requested Applied Biosystems™ 3500 HID Genetic Analyzer, are but the foundation to a larger vision. The establishment of the DNA sequencing core facility would provide an environment where both students and researchers from multiple disciplines will thrive by having access to the latest technologies as well as to provide the opportunity for informal discussions which often times spark imagination and new research avenues. It is through these types of interactions that future scientific relationships both inside and outside WCU may be established.

As indicated by the letter of support provided by interim Dean Knotts of the College of Arts and Sciences, Western Carolina University endorses this project fully and has made the DNA sequencing core facility a priority. Therefore, the 25% matching fund requirement for the cost of the instrument will be provided for by a combination of funds from the College of Arts and Sciences, the Office of the Provost and the Forensic Science Program. The College of Arts and Sciences and the Office of the Provost will each contribute $9,000, for a total of $18,000 toward the matching funds requirement. The remainder ($25,250) will be provided for by the Forensic Science Program.

In order for Western Carolina University to support science, it must support the equipment used to facilitate scientific research. Therefore, funds will be provided by the University for Service Contracts on this instrument after the initial first year contract has expired. Users of the instrument will not be charged any fees, however, each independent researcher will be expected to purchase their own disposables and reagents used on the instrument.

Budget Justification

Equipment:

We are requesting funds to purchase the Applied Biosystems 3500 HID 24 capillary Genetic Analyzer which is a state of the art capillary electrophoresis DNA sequencer. This instrument is recognized as the top capillary electrophoresis instrument by forensic scientists and is currently being implemented into crime laboratories across the country and aboard. This instrument has additional features including but not limited to, *de novo* sequencing, microsatellite analysis, fragment length analysis and SNP analysis that service a wide variety of scientific disciplines. Hence the purchase of this instrument is fundamental to the establishment of a DNA sequencing core facility and the collaborative projects presented in this application.

$173,000 one Applied Biosystems™ 3500 HID Genetic Analyzer 24 capillary capacity with one year extended warranty which includes one annual planned maintenance service.

The College of Arts and Sciences, the Office of the Provost and the Forensic Science Program combined will provide the 25% matching funds requirement of $43,250.00.

Other Direct Expenses:

Software:

We are also requesting funds for the interfacing DNA software, the DNA sequencing analysis software and the short tandem repeat genetic analysis software. The interfacing software conducts the analysis of the raw data from the instrument, converts the data files so that these files may be imported into various analysis software applications and also runs the programming for storage of the data files after a run is completed. The sequencing software is used for DNA sequencing which includes both human and non-human sequence analysis under a variety of assay applications. The premier short tandem repeat data analysis software utilized in crime laboratories across the country is GeneMapper ID-X. One full copy of this software will be installed on the interfacing computer of the instrument. The five GeneMapper ID-X client licenses will be installed on five laptop computers which the Forensic Science Program currently owns. This will permit GeneMapper ID-X data analysis to be conducted in a different location than the instrument facilitating teaching and research interactions.

$5,000 one full copy of interfacing and sequence analysis software

$15,000 one full copy of GeneMapper ID-X software

Training:

We are requesting training by Applied Biosystems for four individuals, Dr. Wilson, Dr. Foley, Ms. Bintz and Ms. Burnside. The training consists of two sessions. Session one (2 days) covers the general applications and design of the instrument, sample preparation, running the instrument and data files. Session two (3 days) covers the maintenance and troubleshooting on the instrument, data analysis using all software applications and experimental design for obtaining best results. The training will be conducted on site at WCU on the purchased instrument and will be tailored to meet the specific research objectives that will be conducted on the instrument. These are objectives which are not part of a routine training course offered by Applied Biosystems.

$12,360 training for four individuals, session 1 (2 days)
$12,480 training for four individuals, session 2 (3 days)

Project Timeline

The instrument will be ordered within a month of when funds become available. The instrument should be delivered approximately eight weeks after ordering. The computer will be ordered at the same time as the instrument. Software and instrument installation will be completed once the computer and AB 3500 are both in house. Training should occur within a month after installation. Once installation and training are complete the AB 3500 will be brought online for use immediately. Total time from purchase to implementation is approximately twelve weeks.

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Mark Wilson

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Wilson, Mark R., et. al. (2002) "Recommendations For Consistent Treatment of Length Variants in the Human mtDNA Control Region" Forensic Sci. Int. 129 (1): 35-42.

Wilson, Mark R. and Allard, Marc W. (2004) "Phylogenetics and Mitochondrial DNA", Forensic Sci. Rev: I (16): 38-62.

Allard MW, Polanskey D, Miller K, Wilson MR, Monson KL, Budowle B. (2005) "Characterization of human control region sequences of the African American SWGDAM forensic mtDNA data set" Forensic Sci Int. Mar 10;148 (2-3):169-79.

Patricia Foley

Mary A. Kosir, Patricia A. Foley-Loudon, Raphaela Finkenauer and Steven Tennenberg. 2002. Multiple Heparanases Are Expressed in Polymorphonuclear Cells Journal of Surgical Research 103:100-108.

Patricia A. Foley and Leo S. Luckinbill. 2001.The Relationship between Stress Response, Metabolism and Aging; the use of D. melanogaster as a Model in Nutrient Restriction. Evolution 55:108-117.

Leo S. Luckinbill and Patricia A. Foley. 2000. The Role of Metabolism in Aging Age 23:85-93.

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Biogerontolgy 1:3-13.

Sean O'Connell

O'Connell, SP and JL Garland. 2002. Differential response of microbial communities in Biolog GN and GN2 microplates. Soil Biology and Biochemistry 34:413-416.

O'Connell, SP, RM Lehman, O Snoeyenbos-West, VD Winston, DE Cummings, ME Watwood, and FS Colwell. 2003. Detection of *Euryarchaeota* and *Crenarchaeota* in an oxic basalt aquifer. FEMS Microbiology Ecology 44:165-173.

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Lehman, RM, SP O'Connell, A Banta, JK Fredrickson, A-L Reysenbach, TL Kieft, and FS Colwell. 2004. Microbiological comparison of core and groundwater samples collected from a fractured basalt aquifer with that of dialysis chambers incubated in situ. Geomicrobiology Journal 21:169-182.

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Katherine Mathews

Gould, K. R. and L. Struwe. 2004. Phylogeny and evolution of *Symbolanthus*. (Gentianaceae-Helieae) in the Guayana Highlands and Andes, based on ribosomal 5S-NTS sequences. *Annals of the Missouri Botanical Garden* 91: 438-446.

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Mathews, K., N. Dunne, E. York, and L. Struwe. 2009. A phylogenetic analysis and taxonomic revision of *Bartonia* (Gentianaceae-Gentianeae), based on molecular and morphological evidence. *Systematic Botany* 34: 162-172.

James Costa

Shoemaker, D. D., J. T. Costa, and K. G. Ross. 1992. Comparisons of heterozygosity in two social insects using a large number of electrophoretic markers. *Heredity* 69: 573-582.

Costa, J. T. and K. G. Ross. 1993. Seasonal decline in the intracolony genetic relatedness of eastern tent caterpillars: Implications for social evolution. *Behavioral Ecology and Sociobiology* 32: 47-54.

Costa, J. T. and K. G. Ross. 1994. Hierarchical genetic structure and gene flow patterns in macrogeographic populations of the eastern tent caterpillar (*Malacosoma americanum*). *Evolution* 48: 1158-1167.

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Costa, J. T. and K. G. Ross. 2003. Fitness effects of group merging in a social insect. *Proceedings of the Royal Society of London, B* 70: 1697-1702.

Brian Byrd

Kang S, Sim C, **Byrd BD**, Collins FH, and Hong Y. *Ex vivo* promoter analysis of antiviral heat shock cognate 70B gene in *Anopheles gambiae*. *Virology Journal*. 2008 5: 136

Colborn JM, **Byrd BD**, Koita OA, and Krogstad DJ. Estimation of Copy Number using SYBR Green: Confounding by AT-rich DNA and by Variation in Amplicon Length. *American Journal of Tropical Medicine and Hygiene*, 79(6), 2008, pp. 887-892

Byrd BD, Wesson DM, and Harrison BA. Regional Problems Identifying the Fourth Instar Larvae of *Orthopodomyia kummi* (Coquillett) and *Orthopodomyia signifera* Edwards (Diptera: Culicidae). *Proceedings of the Entomologic Society of Washington*. 111(3), 2009, 751-753

Byrd BD, Gymburch EE, O'Meara GF, Wesson DM. Molecular Identification of *Aedes bahamensis* (Diptera: Culicidae). *Florida Entomologist.* In Press: December 2011.

Byrd BD, Harrison BA, Zavortink TJ, and Wesson DM. Sequence, secondary structure, and phylogenetic analyses of the ribosomal internal transcribed spacer 2 (ITS2) in the *Orthopodomyia signifera* group (Diptera: Culicidae). Manuscript in revision: *Journal of Medical Entomology*.

Current and Pending Grants

Mark Wilson, PhD –Director Forensic Science Program
Awarded
National Institute of Justice
Assessing Deep DNA Sequencing Technologies for Human Forensic mtDNA Analysis
$397,098.46
2 years

Pending
National Institute of Justice
Forensic DNA Analysis of Microdissected Individual Human Cells
$461,145.28
2 years
National Institute of Justice
Forensic DNA Analysis Training Course
$1,035,352.90
3 years

Patricia Foley, PhD –Forensic Scientist in Residence
Awarded
None

Pending
National Institute of Justice
Forensic DNA Analysis Training Course (Co-Author with Mark Wilson)

Katherine Mathews, PhD -Associate Professor Biology Department
Awarded SAMPLE DO NOT COPY
North Carolina Biotechnology Center
Use of Genetic Markers and Chemical Quantification to Identify Populations of *Actaea racemosa* (Black Cohosh) with Desirable Properties for Breeding a Regional Cultivar. (Co-Author with Jason Clement)
$280,551
2 years

Pending
None

James Costa PhD –Director Highlands Biological Station, Professor Biology Department
Awarded
National Oceanic and Atmospheric Administration
A Prototype Phenological Observatory in the Southern Blue Ridge for Research and Education
$24,420
1 year

Pending
None

Sean O'Connell, PhD -Head of the Department of Biology
Awarded
None

Pending
None

Brian Byrd PhD –Assistant Professor, School of Health Sciences
Awarded
None

Pending
None

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel. **DO NOT EXCEED ONE PAGE.**

NAME	POSITION TITLE
Mark R. WIlson	Director of the Forensic Science Program, Western Carolina University
INSTITUTION Western Carolina University	

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
Azusa-Pacific College, Azusa, CA	B.S.	1979	Biology/Chemistry
California State University, Fullerton, CA	M.A.	1983	Biology
George Mason University, Fairfax, VA	Ph.D.	2004	Biosciences

A. Positions

1990 - 1997: Supervisory Special Agent Examiner, Hairs and Fibers Unit, FBI Laboratory
1997 - 2000: Supervisory Special Agent Examiner, DNA Unit II, Program Manager FBI Laboratory
2000 - 2002: Supervisory Special Agent Examiner, DNA Unit II, FBI Academy, Quantico, VA.
2002: Research Scientist, Counterterrorism and Forensic Science Research Unit, FBI Laboratory, Quantico, VA
2002 –2006: Biology Program Manager, Supervisory Special Agent, Chemical-Biological Sciences Unit, Operational Response Section, FBI Laboratory, Quantico, VA.
2006 –2007: Acting Unit Chief, Supervisory Special Agent, Chemical –Biological Sciences Unit, Operational Response Section, FBI Laboratory, Quantico, VA.
2008 – present; Director, Forensic Science Program, WCU, Cullowhee, NC 28723

Honors

June, 1996, Director's Award, Scientific and Technical Achievement, Federal Bureau of Investigation, Implementation of Mitochondrial DNA Testing in the FBI Laboratory

B. List the 5 most relevant peer-reviewed publications (in chronological order).

Wilson, M.R., DiZinno, J.A., Polanskey, D., Replogle, J., and Budowle, B. (1995) "Validation of Mitochondrial DNA Sequencing in Forensic Casework Analysis" Int. J. Leg. Med: 10 (2): 68-74.

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NAME	POSITION TITLE AND DEPARTMENT
Patricia A Foley	Forensic Scientist in Residence, FS Program Associate Professor Dept of Chemistry and Physics
INSTITUTION Western Carolina University	

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 Michigan	BS	1992	Biology
Wayne State University	PhD	2000	Biology
Veteran Affairs Medical Center	Postdoc	2002	immunology

A. Positions and Honors.

August 2010-Present Forensic Scientist in Residence, Forensic Science Program, WCU
August 2010-Present Associate Professor, Dept of Chemistry and Physics, WCU
July 2006-August 2010 Forensic DNA Biologist III Assistant Technical Leader Baltimore County Police Department, Maryland
July 2006 –May 2010 Adjunct Professor Department of Biology Stevenson University, Maryland
March 2005-June 2006 Supervisory DNA Analyst Department of Defense Armed Forces DNA Identification Laboratory, Maryland
April 2002-March 2005 DNA Analyst Department of Defense Armed Forces DNA Identification Laboratory, Maryland
June 2000-March 2002 Research Scientist II Veteran Affairs Medical Center, Detroit, MI

Guest Presentations

The Use of Reference DNA Samples to Establish DNA Identification, Confirmation and Re-association of Combat Casualties during Operation Iraqi Freedom and Beyond
2004 Promega –International Symposium on Human Identification
Identification of Human Remains at the Tri-State Crematorium in Noble, Georgia
2004 International Congress on Military Medicine

B. List the 5 most relevant peer-reviewed publications (in chronological order).

Leo S. Luckinbill and Patricia A. Foley. 2000. The Role of Metabolism in Aging Age 23:85-93

Leo S. Luckinbill and Patricia A. Foley. 2000.Experimental and Empirical Approaches in the Study of Aging
Biogerontolgy 1:3-13

Patricia A. Foley and Leo S. Luckinbill. 2001.The Relationship between Stress Response, Metabolism and Aging; the use of D. melanogaster as a Model in Nutrient Restriction. Evolution 55:108-117

Mary A. Kosir, Patricia A. Foley-Loudon, Raphaela Finkenauer and Steven Tennenberg. 2002. Multiple Heparanases Are Expressed in Polymorphonuclear Cells Journal of Surgical Research 103:100-108

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel. **DO NOT EXCEED ONE PAGE.**

NAME	POSITION TITLE AND DEPARTMENT
Seán O'Connell	Associate Professor and Department Head Biology
INSTITUTION Western Carolina University	

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
Sullivan County Comm. College, Loch Sheldrake, NY	AS	1990	Environmental Studies
Johnson State College, Johnson, VT	BS	1994	Biology; Secondary Ed.
Idaho State University	PhD	2002	Microbiology

A. Positions and Honors.

2001 – 2007, Assistant Professor, Microbial Ecology, Biology Department, WCU

2007 – 2010, H.F. "Cotton" and Katherine P. Robinson Professor of Biology

2007 – Present, Associate Professor, Microbial Ecology, Biology Department, WCU

2009 – Present, Department Head, Biology Department, WCU

2010-2011 – President-Elect, North Carolina Branch of the American Society for Microbiology

Honors

Rising Star/Young Alumni Award from Johnson State College, 2002

Graduate School Teaching-Research Faculty Award, WCU, 2003

Outstanding Scientist of the Year, Biodiversity Research and Education, Discover Life in America, 2004

Arts & Sciences Teaching Award, WCU, 2006

Board of Governors' Award for Excellence in Teaching (2008-2009)

Chancellor's Meritorious Award for Engaged Teaching (2008-2009)

Western Carolina University Graduate School Commencement Speaker (2009)

B. List the 5 most relevant peer-reviewed publications (in chronological order).

O'Connell, SP and JL Garland. 2002. Differential response of microbial communities in Biolog GN and GN2 microplates. Soil Biology and Biochemistry 34:413-416.

O'Connell, SP, RM Lehman, O Snoeyenbos-West, VD Winston, DE Cummings, ME Watwood, and FS Colwell. 2003. Detection of *Euryarchaeota* and *Crenarchaeota* in an oxic basalt aquifer. FEMS Microbiology Ecology 44:165-173.

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Lehman, RM, SP O'Connell, A Banta, JK Fredrickson, A-L Reysenbach, TL Kieft, and FS Colwell. 2004. Microbiological comparison of core and groundwater samples collected from a fractured basalt aquifer with that of dialysis chambers incubated in situ. Geomicrobiology Journal 21:169-182.

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Provide the following information for the key personnel. **DO NOT EXCEED ONE PAGE.**

NAME	POSITION TITLE AND DEPARTMENT
Katherine Gould Mathews	Associate Professor
INSTITUTION Western Carolina University	Biology Department

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
Harvard University	BA	1992	Fine Arts
University of Texas at Austin	PhD	1997	Botany
Harvard University	postdoc	1998	Systematic Botany

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions:

1998-2001 Research Taxonomist, Brooklyn Botanic Garden, New York, NY
2001-2002 Assistant Professor, Austin Peay State University, Clarksville, TN
2002-2003 Grant Writer, University of Tennessee at Chattanooga
2003-2009 Assistant Professor, Western Carolina University, Cullowhee, NC
2009-present Associate Professor, Western Carolina University, Cullowhee, NC

Honors:

2010-2013 H.F. Cotton & Katherine P. Robinson Professorship in Biology

B. List the 5 most relevant peer-reviewed publications (in chronological order).

Gould, K. R. and L. Struwe. 2004. Phylogeny and evolution of *Symbolanthus*. (Gentianaceae-Helieae) in the Guayana Highlands and Andes, based on ribosomal 5S-NTS sequences. *Annals of the Missouri Botanical Garden* 91: 438-446.

Struwe, L. and **K. R. Gould**. 2004. Redefinition of *Symbolanthus* to include *Wurdackanthus*(Gentianaceae--Helieae). *Novon* 14: 354-359.

Struwe, L., V. A. Albert, M. F. Calió, C. Frasier, K. B. Lepis, **K. G. Mathews**, & J. R. Grant. 2009. Evolutionary patterns in neotropical Helieae (Gentianaceae): evidence from morphology, chloroplast and nuclear DNA sequences. *Taxon* 58(2): 479-499.

Mathews, K. G., J. Huguelet, M. Lanning, T. Wilson, and R. S. Young. 2009. Clonal diversity of fruiting culms within
stands of *Arundinaria gigantea* (Poaceae; Bambusoideae) in western North Carolina assessed using AFLP fingerprints. *Castanea* 74(3): 213-223.

Mathews, K., N. Dunne, E. York, and L. Struwe. 2009. A phylogenetic analysis and taxonomic revision of *Bartonia* (Gentianaceae-Gentianeae), based on molecular and morphological evidence. *Systematic Botany* 34: 162-172.

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel. **DO NOT EXCEED ONE PAGE.**

NAME	POSITION TITLE AND DEPARTMENT
James T. Costa	Executive Director, Highlands Biological Station & Professor, Department of Biology
INSTITUTION Highlands Biological Station and Western Carolina University	

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
SUNY Cortland, Cortland, New York	B.S.	1981-1985	Biology and Philosophy
University of Georgia, Athens, Georgia	M.S.	1985-1988	Insect Community Ecology
University of Georgia, Athens, Georgia	Ph.D.	1988-1992	Insect Population Genetics
Harvard University, Cambridge, Massachusetts	Postdoctoral	1992-1996	Pop. Gen., Social Evolution

A. Positions and Honors.

Assistant Professor, Western Carolina University, 1996-2002
Hunter Scholar Award, Western Carolina University, 1999
University Scholar Award, Western Carolina University, 2002
Associate Professor, Western Carolina University, 2002-2005
Jeanne Rousselet Fellow, Radcliffe Institute for Advanced Study, Harvard University, 2004-2005
H. F. and Katherine P. Robinson Professor of Biology, Western Carolina University, 2004-2007
Professor, Western Carolina University, 2005-Present
Interim Director, Highlands Biological Station, Sept. 2005-Jan. 2006
Executive Director, Highlands Biological Station, Feb. 2006-Present

B. List the 5 most relevant peer-reviewed publications (in chronological order).

Shoemaker, D. D., J. T. Costa, and K. G. Ross. 1992. Comparisons of heterozygosity in two social insects using a large number of electrophoretic markers. *Heredity* 69: 573-582.

Costa, J. T. and K. G. Ross. 1993. Seasonal decline in the intracolony genetic relatedness of eastern tent caterpillars: Implications for social evolution. *Behavioral Ecology and Sociobiology* 32: 47-54.

Costa, J. T. and K. G. Ross. 1994. Hierarchical genetic structure and gene flow patterns in macrogeographic populations of the eastern tent caterpillar (*Malacosoma americanum*). *Evolution* 48: 1158-1167.

Hanfstingl, U., A. Berry, E. A. Kellogg, J. T. Costa, W. Rüdiger, and F. Ausubel. 1994. Haplotypic divergence coupled with lack of diversity at the *Arabidopsis thaliana* alcohol dehydrogenase locus: Roles for both balancing and directional selection? *Genetics* 138: 1-18.
Costa, J. T. and K. G. Ross. 2003. Fitness effects of group merging in a social insect. *Proceedings of the Royal Society of London, B* 70: 1697-1702.

Other publications: http://www.wcu.edu/7426.asp

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BIOGRAPHICAL SKETCH

NAME	POSITION TITLE AND DEPARTMENT
Brian David Byrd	**Assistant Professor**
INSTITUTION **Western Carolina University**	**Environmental Health Sciences**

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
UNC-Asheville, Asheville NC	B.A.	2000	Biology
Tulane School of Public Health and Tropical Medicine New Orleans, LA	M.S.P.H.	2002	Public Health Parasitology
Tulane School of Public Health and Tropical Medicine New Orleans, LA	Ph.D.	2009	Medical Entomology

A. Positions and Honors.

Pre-Doctoral Fellow, Tulane University/Centers for Disease Control and Prevention Vector-Borne Infectious Disease Training Program (2003-2008), Tulane University School of Public Health and Tropical Medicine, New Orleans, LA 70112

Assistant Professor (2008-Present), Environmental Health Sciences Program, College of Health and Human Sciences, Western Carolina University, Cullowhee, NC 28723

Honors.

Student Engagement Award, College of Health and Humans Sciences, WCU
Tulane University/Centers for Disease Control Graduate Research Training Fellowship Award
Louisiana Board of Reagents Doctoral Incentive Award
UNC-Asheville “Order of Pisgah” Alumni Award
Louisiana Mosquito Control Association Student Research Grant Award

B. Peer-reviewed Publications (in chronological order).

Kang S, Sim C, **Byrd BD**, Collins FH, and Hong Y. *Ex vivo* promoter analysis of antiviral heat shock cognate 70B gene in *Anopheles gambiae*. *Virology Journal*. 2008 5: 136

Colborn JM, **Byrd BD**, Koita OA, and Krogstad DJ. Estimation of Copy Number using SYBR Green: Confounding by AT-rich DNA and by Variation in Amplicon Length. *American Journal of Tropical Medicine and Hygiene*, 79(6), 2008, pp. 887-892

Byrd BD, Wesson DM, and Harrison BA. Regional Problems Identifying the Fourth Instar Larvae of *Orthopodomyia kummi* (Coquillett) and *Orthopodomyia signifera* Edwards (Diptera: Culicidae). *Proceedings of the Entomologic Society of Washington*. 111(3), 2009, 751-753

Byrd BD, Gymburch EE, O’Meara GF, Wesson DM. Molecular Identification of *Aedes bahamensis* (Diptera: Culicidae). *Florida Entomologist.* In Press: December 2011.

Byrd BD, Harrison BA, Zavortink TJ, and Wesson DM. Sequence, secondary structure, and phylogenetic analyses of the ribosomal internal transcribed spacer 2 (ITS2) in the *Orthopodomyia signifera* group (Diptera: Culicidae). Manuscript in revision: *Journal of Medical Entomology*.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel. **DO NOT EXCEED ONE PAGE.**

NAME	POSITION TITLE AND DEPARTMENT
Brittania J. Bintz	Forensic Research Scientist
INSTITUTION Western Carolina University	Chemistry and Physics

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 South Florida	B.S.	1998-2004	Microbiology
Western Carolina University	M.S.	2004-2006	Chemistry

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Western Carolina University, 2008 – Present, Cullowhee
Forensic Research Scientist

Western Carolina University, 2006 – 2008, Cullowhee, NC
Visiting Assistant Professor, Chemistry

AB Technical Community College, 2007, Asheville, NC
Adjunct Instructor

Western Carolina University, 2006, Cullowhee, NC
Advanced General Chemistry Laboratory Coordinator

Western Carolina University, 2005, Cullowhee, NC
Stockroom Technician

B. List the 5 most relevant peer-reviewed publications (in chronological order).

No Publications

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BIOGRAPHICAL SKETCH

Provide the following information for the key personnel. **DO NOT EXCEED ONE PAGE.**

NAME	POSITION TITLE AND DEPARTMENT
Erin Burnside	Forensic Research Scientist
INSTITUTION Western Carolina University	Department of Chemistry and Physics

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
East Tennessee State University	BS	2003	Health Sciences
East Tennessee State University	MS	2005	Biology

A. Positions and Honors. List in chronological order previous positions, concluding with your present position. List any honors. Include present membership on any Federal Government public advisory committee.

Positions:

Research Assistant II, East Tennessee State University, Johnson City, TN (May 2005 – July 2006)

Validation Specialist, Vi-Jon, Inc., Smyrna, TN (August 2006 – March 2008)

Research Operations Manager, Western Carolina University, Cullowhee, NC (September 2008 – May 2011)

Forensic Research Scientist, Western Carolina University, Cullowhee, NC (May 2011 – present)

Honors:

Corporate Activities Program Student Travel Grant Award, American Society for Microbiology, 2004

Dean's Travel Grant Award, College of Public and Allied Health, East Tennessee State University, 2004

B. List the 5 most relevant peer-reviewed publications (in chronological order).

(Published under Erin P. Storey)

Chakraborty, R., E. Storey, and D. van der Helm. 2007. "Molecular Mechanism of Ferricsiderophore Passage through the Outer Membrane Receptor Proteins of *Escherichia coli*." Biometals. 20(3-4):263-74.

Storey, E. P., R. Boghozian, J. Little, D. Lowman, and R. Chakraborty. 2006. "Chemical Characterization of 'Schizokinen;' a Dihydroxamate-type Siderophore Produced by *Rhizobium leguminosarum* IARI 917." Biometals. 19:637-649.

North Carolina Biotechnology Center

Institutional Development Grant Budget

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Project Title:
Purchase of Applied Biosystems 3500 Genetic Analyzer to establish DNA Sequencing Core Facilty at WCU

EQUIPMENT • Itemize and briefly describe each item • 25% matching requirement

Equipment name/description	Amount available from other sources	Amount requested from Center	Total Amount
4442013 AB 3500HID 24 Genetic Analyzer w/1 year ext warrenty	$43,250	$129,750	$173,000
TOTAL EQUIPMENT COSTS:	$43,250	$129,750	$173,000

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OTHER DIRECT EXPENSES • IDG grants do not support salaries, service contracts, or indirect costs.

Description of other direct cost	Amount available from other sources	Amount requested from Center	Total Amount
4443261 GenMapper ID-X full software package		$15,000	$15,000
4360966 Software for sequencing analysis and fragment analysis		$5,000	$5,000
TRN00194 Training 2 day 4 people		$12,360	$12,360
TRN00080 Training 3 day 4 people		$12,855	$12,855
TOTAL OTHER DIRECT EXPENSES:		$45,215	$45,215

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TOTAL PROJECT COSTS	**$43,250**	**$174,965**	**$218,215**
MATCHING	**25%**		

• • • All funds requested in this budget must be justified in the grant proposal. • • •