**Senior Personnel.**

**Alan O. Bergland, Ph.D.** (51% effort). Principal Investigator will be responsible for the overall administration and direction of the project. Summer support is being requested for 1 month during the first two years of the proposed project (startup funds provided by the University of Virginia have been allocated to provide 2 months of summer support for 2016 and 2017). In years 3-5 of the proposed project PI Bergland is requesting 3 months of summer support

**Personnel at University of Virginia**

**TBD, Post-doctoral Research Associate,** (100% effort). Will be responsible for generating and overseeing data-analysis of allele specific expression and cis-eQTL experiments in Drosophila.

**TBD, Graduate Research Assistant,** (100% effort for 3 summer months). Will be responsible for generating and analyzing whole-genome resequencing of *Daphnia*.

**TBD, Graduate Research Assistant,** (100% effort for 3 summer months). Will be responsible for assessing cis-eQTL in *Daphnia.*

**TBD, Lab Technician,** B.Sc.Will assist in Drosophila and Daphnia field and laboratory experimental work and for DNA/RNA extraction and library construction in this project.

**Benefits.**

Benefits are calculated at 6% for the PI, 27% for the Post-Doc, 38.9% for the Lab Technician, and 6% for the Graduate Research Assistants.

**Equipment**

We will purchase one near infra-red (NIR) spectrophotometer in year one of this grant (~$50,000). NIR spectrophotometry is a useful technique for identifying species identity, age and Wolbachia status of individual *D. melanogaster*. Being able to measure these parameters for individual flies enables us to infer demographic parameters of natural populations and to properly interpret mRNA-seq profiles from wild caught individuals after accounting for physiological covariates.

**Materials and Supplies**

High-throughput sequencing services. High-throughput sequencing will be performed on the NextSeq 500 platform (services provided by NGX-bio, San Francisco, CA). Each NextSeq 500 run produces ~400 million 2x150 bp reads, equivalent to ~800-1000X coverage of the fly or Daphnia genome for ~$5000/run. We will perform highly-multiplexed resequencing of fly and Daphnia samples and will leverage existing resequenced strains/clones in order to reduce our sequencing effort as much as possible (see Proposal for further information). We anticipate preparing samples requiring 5-10 runs of NextSeq data per year, each year for a total cost of $25,000 to 50,000 per year.

Reagents for DNA extraction. We will utilize our BioMek liquid handling robot for DNA and RNA extractions. Using this technology, each extraction costs ~$1.50. We anticipate generating DNA and RNA from ~5000 individuals per year for a total cost of $15,000/year.

Reagents for library preparation. We will utilize a modified version of Illumina’s Nextera library preparation method that enables us to efficiently generate individual sequencing libraries for roughly 1/20th the cost of the standard Nextera prep method. Accordingly, we will require roughly two (standard) “96 reaction” kits per year (~$10,000). We will generate custom synthesized dual-indexed primers for multiplexing. These primers will allow us to multiplex >5000 individuals per NextSeq run. This primer set will cost ~$2000 and will be a fixed cost, being purchased in year one and lasting the duration of the project.

Reagents for SureSelect. We will use custom designed SureSelect oligos from Aglient to perform targeted resequencing of genomic regions of interest. Because we will be using barcodes, we will actually require very little SureSelect reagent (~10 reactions worth). This amount of oligo will cost ~$10,000-20,000 and will be purchased during year 2.

**Travel**

We request $1500 a year for travel. Travel funds will be required to access field sites and to attend meetings. International travel to England to collect Daphnia will be paid out of PI Bergland’s startup-funds.

**Publication costs.**

We request $2000 to cover publication costs in open access journals.