Complete applications will include a one-page proposal describing the research question to be investigated, rationale, proposed plan, and justification for amount requested (11 pt font, 0.5” margins), and a brief letter from the core facility director attesting to the availability of the proposed technical service. Applications are limited to one per investigator, and only new applications will be considered.

Research question

* Meiotic recombination is a fundamental aspect of reproduction in sexual eukaryotes.
* Recombination shapes how evolution occurs, and can both promote and constrain many evolutionary processes.
* In spite of its fundamental importance, many aspects of our understanding of recombination remain unclear
* For example – rates of genome-wide recombination vary greatly among species and potentially among populations and individuals.
* Theory predicts that natural selection optimize rates of recombination – is this indeed the case?
* The first step in testing this question is to understand the quantitative genetics of recombination rate. Compared to other traits, the basic genetics of recombination rate variation are vastly understudied.
* The primary reason for this is that quantifying recombination rate is difficult. Standardized genetic maps must be made from multiple individuals from multiple populations.
* We are currently undertaking a project to quantify the recombination rate in sixteen inbred lines of Drosophila pseudoobscura. These lines are unique in that they were recently inbred from wild populations, and thus contain a great deal of natural variation, including potential for recombination rate variation.
* Constructing genetic maps requires crossing each line to a standardized “tester line”, intercrossing the resultant F1 offspring, and then mapping marker distances in the resulting F2 offspring.
* This presents a unique challenge, as each individual only will have (on average) a single cross over per chromosome arm. Thus, estimating rates of recombination across the genome requires genotyping many hundreds of individuals at a large number of informative markers.
* This makes typically high-throughput methods ineffective, as they focus on genotyping low numbers of individuals at large numbers of markers.
* A new method, GT-Seq, overcomes this method. The method turns e
* While it has potential to massively transform our ability to measure recombination in the laboratory, it has so far been used for population genetic analyses in wild populations.
* Using this new method requires a prior knowledge of SNPs throughout the genome that can be used as informative markers for the construction of genetics maps.
* Thus, we seek to obtain whole-genome sequence for our 16 inbred lines and three tester lines (19 lines in total).
* This can be achieved via multiplex whole genome sequencing in two lanes on an Illumina HiSeq 4000. Given a genome size of 130Mb, this will net approximately 80x coverage for each individual (Illumina estimates 715x total coverage of a single drosophila per lane, 19 genomes in two lanes, (715x)\*2 / 19 = ~80x).
* The library preps and sequencing will be carried out at the duke sequencing center. With a total estimated cost of $5500.
* After obtaining the sequencing data, we will align the data to the reference genome, call SNPS using GATK and identify mapping-informative markers.
* We will then use these markers to design a 196 GT-seq amplicon panel for high-throughput construction of 16 genetic maps for each line.
* The amount requested will allow us to sequence our experimental lines to sufficient depth for confident calling of mapping-informative SNPs. Lower depth sequencing would greatly reduce our ability to build an informative panel of SNPs for genetic map construction.

Rationale

Proposed Plan

Justification for Amount Requested