**Research question and rationale**

Meiotic recombination occurs in nearly all sexual eukaryotes. **In spite of its ubiquity, there is still much unknown about the basic biology of recombination.** For example, recombination rates vary considerably within and among species, but evolutionary mechanisms maintaining this variation remain unclear. One compelling idea is that recombination rates are, like many organismal traits, optimized by natural selection. For example, theoretical models predict that recombination should evolve to higher rates in populations that experience spatially or temporally variable regimes of natural selection. This is because genotypes that more frequently “remix” their genomes will, on average, adapt more quickly to the shifting optima that characterize these regimes.

However, these predictions have remained untested, largely because studying recombination as a quantitative phenotype is difficult. This has prevented us from applying the tools of quantitative evolutionary genetics to the study of recombination rate – that is, studying recombination rate as we might study a quantitative morphological character. Studying recombination in this manner requires estimates of recombination rate from **multiple individuals within several populations – that is, multiple standardized genetic maps.**

As part of a test of the role of natural selection in shaping recombination rates, we are currently working towards quantifying recombination rate in sixteen naturally-derived lines of *Drosophila pseudoobscura*. However, estimating rates of recombination across the genome requires genotyping many thousands of individuals at a moderate number of informative markers. This need makes typical high-throughput methods ineffective, as they focus on genotyping a moderate of individuals at a large number of markers. A new method, GT-Seq, inverts the typical individuals-to-loci ratio by using multiplexed PCR to enrich samples for a small number of target regions (say, 1000 SNPs), which are then barcoded and sequenced as a pool. This provides the ideal individual-to-marker ratio for the construction of high-resolution genetic maps. However, this method requires *a priori* knowledge of informative markers (e.g. SNPs that are homozygous for different alleles in the grandparent cross) throughout the genome. **Thus, we are requesting funding to perform whole-genome sequencing on our mapping lines to identify mapping-informative markers throughout the genome.** These data will also provide us with the tools to begin tying natural variation in recombination rate to its underlying molecular mechanisms.

**Proposed plan and justification for amount requested**

We seek to obtain whole-genome sequence for our 19 naturally-derived inbred lines. 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. The library preparation and sequencing will be carried out at the Duke Center for Genomic and Computational Biology, at a total estimated cost of $5500. After obtaining the sequencing data, we will align the data to a 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 a genetic map for each of our 19 lines.

The amount requested will allow us to sequence our experimental lines to sufficient depth for confident calling of mapping-informative SNPs. Sequencing at a lower depth would result in dropout of sites across the genome – note that our 80x estimate is for the *average* depth of sequencing, and many sites will have much lower depth. A lower depth would impair our ability to identify informative markers for all 19 lines.

Recombination is a fundamental process, but we still know very little about how it itself evolves in actual populations. **The funding we are requesting will allow us to develop the genomic resources we need to apply the full force of modern evolutionary genetics to the study of recombination rate.** These data will also greatly increase the likelihood of extramural funding for our research by allowing us to leverage these approaches to generate pilot data for applications in the next funding cycle.