Sequencing Sex Chromosome Telomeres to Quantify Degradation in Wild Lizard Population

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Telomeres are protective sequences of DNA located at the end of a chromosome that are pertinent to the maintenance of cell survival. Knowledge of the sequences of telomeres on sex chromosomes is especially limited due to their highly repetitive nature and high GC content. We optimized a method to sequence chromosome specific telomeres in the lizard, *Anolis sagrei*. Nanopore sequencing allows a single, long read through the telomere providing chromosome level information that is not acquired through other quantification methods such as qPCR. The aims of this project are to answer these questions: (1) What effect does sex have on telomere length across the lifespan? (2) Do the telomeres on the X and Y chromosomes degrade at different rates? I hypothesize that because women tend to live longer than men, and female lizards live longer than male lizards [1,3], the telomere lengths on sex chromosomes will be longer and degrade more slowly over time in females (i.e., the X chromosome), relative to the Y chromosome in the males.

To address these questions two sequencing trials were planned. The first trial took an adaptive sequencing approach to only enrich for telomere sequences in a sample size of three hatchlings and three adults, consisting of both males and females. Once the sequencing data was collected, it was moved onto Auburn University's high-performance computer, Easley. High Accuracy Basecalling was performed using Guppy (v.6.4.6). In order to assess the quality of the sequence data, FastQC was run on each of the barcoded samples. Each sample met the Nanopore requirement for good quality reads. A summary of the basecalled data was collected using Nanoplot. The total number of reads sequenced was 19,353 and the average read length of those reads was 6,785 bp (Fig 1).

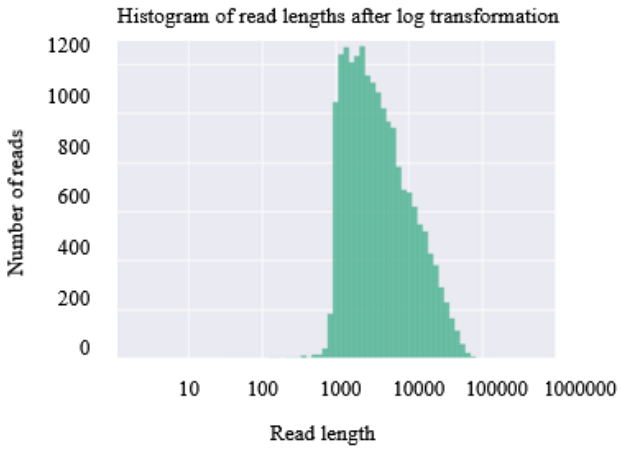

Fig. 1 Nanoplot graph of the distribution of sequenced read lengths

PoreChop (v.3.8.6) was applied to cut off adapters and barcodes, and the data quality for each sample was assessed again with FastQC. The PoreChop data was then mapped to the AnoSag2.1 genome assembly as a reference with Minimap2 (v.2.26). [4]

Findings from the first trial suggest that although the adaptive sequencing collected tandem repeats, many did not map to the ends of the reference genome. This is possibly due to unsequenced regions between the ends of the assembly and where the telomeres begin. We are currently testing other bioinformatic approaches for cataloging and quantifying the telomere repeats sequenced. [2] Due to this delay in the bioinformatic stage of analysis, telomere quantification from the first sequencing trial is still underway.

Moving forward, in the second trial, I will perform a revised Nanopore sequencing method, with the addition of TeloTags to target the telomere lengths in longitudinal blood samples, from the "Field Aging Anoles

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Dataset," to look at sex specific differences at the telomere level of the chromosome. [5] The Field Aging Anoles Dataset is from a mark-recapture study on a wild island population in Florida, in which the individuals were tracked throughout their lifespan. The sample size for this sequencing trial is four males and four females each with three blood samples from across their lifespans.

This research will provide insight on the relationship between sex and telomere length at the level of each chromosome, and how these change with senescence. In addition to filling a gap in knowledge, this research will improve the quality of the *Anolis sagrei* draft genome assembly.

Statement of Research Advisor

The field of aging biology has limited vertebrate models to study sex-specific aging, and we have limited understanding of telomere dynamics in reptiles. This is the first study to sequence sex-specific chromosomes and quantify changes in chromosome-specific telomeres for any reptile. Upon completion of this project, Ms. Payne will have made a major contribution in our ability to use reptiles as a model for sex-specific aging in the field of comparative aging biology as a whole.
- Dr. Tonia Schwartz, Department of Biological Sciences. College of Science and Mathematics

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Authors Biography

Brynleigh E. Payne is a senior-year student pursuing a B.S. degree in Genetics at Auburn University. She has been a member of The Schwartz Lab of Functional and Ecological Genomics since the Spring 2021 semester and is a Summer 2023 Undergraduate Research Fellow. Her research area of interest is in the biomedical field, specifically in diseases related to the aging process.

Dr. Tonia Schwartz is an Associate Professor in the Department of Biological Sciences. Her Lab of Functional Genomics addresses questions about how animals respond to environmental stressors. She served as a mentor to Ms. Brynleigh Payne as she conducted her research on the sex specific telomeres.