**Understanding cuticular expansion in ticks for identification of new molecular targets**

1. **Background**

The black-legged tick, *Ixodes scapularis,* is an ectoparasite and an obligatory blood-feeder making it a vector of medical and veterinary importance. *Ixodes* ticks can transmit a wide variety of pathogens including bacteria, viruses, and protozoan parasites. *Ixodes scapularis* is the major vector of the spirochete *Borrelia burgdorferi* which causes over 400,000 cases of Lyme disease annually in the United States. The success rate of pathogens transmitted by ticks is influenced by the long-lasting blood-feeding (up to 10 days) unique to ticks. Strategies that target this vector rather than individual pathogens stand to protect against several diseases. Thus, there is an urgent need to understand unique aspects of the tick’s biochemistry and physiology so that new targets can be identified for species-specific management strategies. Female ticks increase in size by approximately 100-fold during their 7 to 10 day long feeding period before dropping off the host to lay eggs. To accommodate this huge bloodmeal, the cuticle must expand through remodeling. This phenomenon is barely understood at the biochemical or molecular level. A coordinated response modulating enzyme activity and structural protein production as well as trafficking accompanies and enables the rapid expansion of a feeding adult female. The goal of this study is to understand the process of blood-feeding in *I. scapularis* using a multi-omics approach. This would enable us to characterize or identify candidate genes and proteins that would serve as molecular targets for tick management.

We have currently sequenced the transcriptome of unfed, slow-feeding and rapid-feeding stage tick epidermis (cuticle). We would do transcriptomics analysis using tools and packages from anaconda (bioconda), and R while employing lessons we have had in linux, bash scripting, python, version control, and data visualization as described below.

1. **Methods**

**Sequence quality control**

The quality of raw fastq files would be characterized using FastQC and the results would be aggregated using MultiQC. Sequence pairs would be filtered for nucleotide base quality and trimmed using Trimmomatic. Sequence quality of the trimmed reads would be accessed with FastQC and summarized with MultiQC.

**Sequence Alignment and Feature Detection/ Read Count**

Trimmed read pairs would be aligned to the Ixodes scapularis genome using the HISAT2 splice-aware read alignment tool. The aligned reads would be compressed from the Sequence Alignment/Map (SAM) format to Binary Alignment/Map (BAM) format using SAMtools and raw counts of read and read pairs that aligned with genes would be summarized into a count matrix with featureCounts tool of the subread package.

**Differential Expression Analysis**

The R package, DESeq2 would be used to access genes that were differentially expressed in the slow-stage fed tick and the rapid-stage fed tick epidermis with reference to the unfed tick epidermis. Differentially expressed genes would be visualized in R using volcano plots and heatmaps.

**Gene Enrichment Analysis**

GO enrichment analysis on significantly overexpressed and under expressed genes would be done using the GOEnrichment python package in Bioconda. Enriched GO terms would be visualized using dot plots.