

Optimizing Genomic Sequencing Workflows for Pathogen Detection in Northeastern Tick Populations

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Introduction: Tick-borne diseases represent a growing public health concern in the Northeastern United States, underscoring the need for rapid, high-resolution surveillance methods. Traditional molecular screening approaches may be limited in their ability to detect unexpected organisms or characterize complex coinfections. This study aimed to optimize an integrated genomic workflow combining *in silico* assay design, dual RNA and DNA extractions, and complementary short- and long-read sequencing to improve pathogen detection in regional tick vectors.

Methods: Field-collected ticks underwent optimized dual RNA and DNA extractions and were processed using short-read Illumina (Illumina Microbial Amplicon Prep) and long-read Oxford Nanopore Technologies (ONT GridION) workflows. Long-read sequence datasets were assembled *de novo* with Flye, followed by taxonomic assignment using NCBI BLAST and computational filtering to distinguish microbial sequences from host genomic background.

Results: The analytical workflow successfully distinguished tick host sequences, including sequences assigned to *Haemaphysalis longicornis*, from microbial signatures. Filtering of long-read assemblies enriched bacterial and endosymbiotic contigs, supporting targeted microbial identification while reducing host-sequence interference.

Conclusions: Integration of Illumina short-read sequencing with ONT long-read sequencing provided complementary resolution for tick-associated metagenomic analysis. This workflow offers a scalable approach to reducing host-sequence interference and improving microbial characterization in ticks, with potential applications in pathogen surveillance and vector-borne disease monitoring.