De novo assembly of a long-read *Amblyomma americanum* tick genome

We generated a whole-genome assembly for the lone star tick to serve as a reference for downstream efforts where whole-genome maps are required. We created our assembly using pooled DNA from salivary glands of 50 adult female ticks that we sequenced using PacBio HiFi reads.

Purpose

This pub introduces our first public draft assembly of an *Amblyomma americanum* genome. We've continued to refine and explore this assembly in a variety of projects, including for genome annotation, gene expression, and comparative genomics [1]. We're sharing the work that led to our initial assembly in the hope that it will serve as a valuable reference dataset for the tick research community. We think there's more work to be done with this genome. For example, we found sequences from the endosymbiont *Coxiella* in the salivary gland, consistent with previous reports [2], but haven't followed up on this at all.

- This pub is part of the **project**, "Ticks as treasure troves: Molecular discovery in new organisms." Visit the project narrative for more background and context.

- We followed up on this work in a **subsequent pub**, "[Predicted genes from the _Amblyomma americanum_ draft genome assembly](#)." We predicted proteins from our _A. americanum_ assembly, which are now available on GenBank ([GCA_030143305.2](#)) and UniProt ([UP001321473](#)).

- **Raw genomic data** is available via NCBI at [BioProject PRJNA932813](#).

- Our **pseudo-haploid genome** is in GenBank under accession [GCA_030143305](#). We cover assembly version [GCA_030143305.1](#) in this pub.

- Our **full assembly** is on [Zenodo](#).

- Our **code** is available in [this GitHub repository](#).

Background and goals

We want to [understand how ticks manipulate humans](#), so we've been studying the lone star tick, _Amblyomma americanum_. Given that there are over 900 species of ticks identified so far, it's easy to overlook _A. americanum_ in particular. However, to many in the eastern and southern United States, _A. americanum_ is a pest with a rapidly growing presence and predilection for humans as hosts [3][4].

One of our long-time pain points with respect to the study of _A. americanum_ has been operating without a reference genome. Despite their relevance to humans, we suspect the deficit in publicly available genomic references for _A. americanum_ might stem from their surprising genetic complexity. Based on flow cytometry estimates, the tick's haploid genome is expected to be approximately 3 gigabases (Gb) [5]. For comparison, _Escherichia coli_ (bacterium) have 5 megabase (Mb) genomes, _Drosophila melanogaster_ (fruit fly) have 140 Mb genomes, _Mus musculus_ (mouse) have 2.7 Gb genomes, and _Homo sapiens_ (human) have 2.9 Gb genomes [6][7]. Based on these figures, one might not expect that tiny ticks could hold so much genetic information, but believe it or not, the tree of life is peppered

with examples more extreme than this. Our ability to accurately and comprehensively map these edge cases with reasonably ordinary resources is a recent phenomenon.

Previously, we and others generated transcriptome assemblies using tissue extracted from *A. americanum* ticks [8][9]. These datasets provide snapshots of the genes being transcribed in the cells we collected. This information was instrumental for generating the protein databases we needed to do mass spectrometry-based proteomics on the same tick species. While these transcriptome datasets have proven useful, they can fall short when mass spectrometry detects peptide features that don't correspond to reference transcripts. In these cases, the mass spectrometry features will likely go unidentified in analyses until more transcriptomic data becomes available.

Rather than iteratively sequence tick transcriptomes under various conditions (to capture broader transcript landscapes), we decided that a whole-genome assembly could give us more bang for our buck by capturing all genes encoded by *A. americanum*.

The approach

We felt that the time might be perfect to generate an *A. americanum* genome because long-read DNA sequencing technologies have become more accessible in recent years, meeting the challenge of mapping large and complex genomes. These long reads should, in theory, make the assembly of large genomes less computationally expensive (compared to short reads) while reducing assembly errors. Many groups have rallied around long reads as a key technology for tick genome assembly in particular, with a notably high-quality *Ixodes scapularis* (deer tick) example unveiled very recently [10][11]. To the best of our knowledge, no such assembly exists for *A. americanum*.

Using long-read HiFi DNA sequencing from Pacific Biosciences (PacBio), we assembled an unphased diploid whole-genome map for female *A. americanum* ticks using the pooled DNA of approximately 50 individuals. We hope that our efforts will provide the research community with a useful resource for advancing work in this important tick species.

Detailed methods

Tick salivary gland extraction

We dissected 50 female ticks for DNA extraction. In an effort to reduce potential contamination from microbes that might inhabit the tick gut, we chose to isolate salivary glands, which incidentally comprise a major mass fraction of the internal tick organ system. We pooled these salivary glands in distilled water, chilled on ice, and extracted DNA immediately after dissection.

DNA extraction

We attempted high-molecular-weight DNA extraction using several different commercially available kits and found that in our hands, the Circulomics high-molecular-weight DNA tissue kit was most consistent for isolating well-distributed, ≥ 30 kb genomic DNA fragments (as judged by femto-pulse analysis).

Sequencing

We submitted raw genomic DNA to UC Berkeley's QB3 genomics core for fragment analysis, shearing, and 12–17 kb size selection. Subsequently, we prepared HiFi libraries using PacBio's SMRTbell prep kit 3.0. We sequenced these libraries using two SMRT Cells (8M) and a Sequel II instrument. UC Berkeley's Vincent J. Coates genomics sequencing lab processed raw sequencing data into circular consensus (CCS) HiFi reads and sent us data in HiFi FASTQ format.

Data analysis

Our long-read assembly and assessment workflow is summarized in Figure 1. We tried assembling the genome with Shasta, Flye, and

Hifiasm with default settings on a 10-core 3.7 GHz Xeon workstation containing 224 GB of RAM, and ultimately moved forward with Flye.

The following code blocks depict the command line scripts we used for the assembly and assessment processes:

Concatenate FASTQ files (optional):

```
cat *.fastq > concatenated_file_name.fastq
```

Run Flye assembler (v2.8.3; default settings):

```
flye –pacbio-hifi concatenated_file_name.fastq -g 3g -o output_directory -t 19 –min-ovlp 5000
```

BUSCO assessment (v5.4.4):

```
busco -i assembly.fasta -l arachnida_odb10 -o output_directory -m genome
```

Purge_dups (v1.2.6):

Instructions for execution are available <u>here</u>.

Data deposition

We deposited raw HiFi reads (FASTQ files) into NCBI (<u>bioproject PRJNA932813</u>) and our pseudo-haploid genome assembly (FASTA file) into <u>NCBI/GenBank</u> (accession GCA_030143305.1). We also uploaded our full, unphased assembly (FASTA file) to <u>Zenodo</u> (DOI: <u>10.5281/zenodo.7747102</u>).
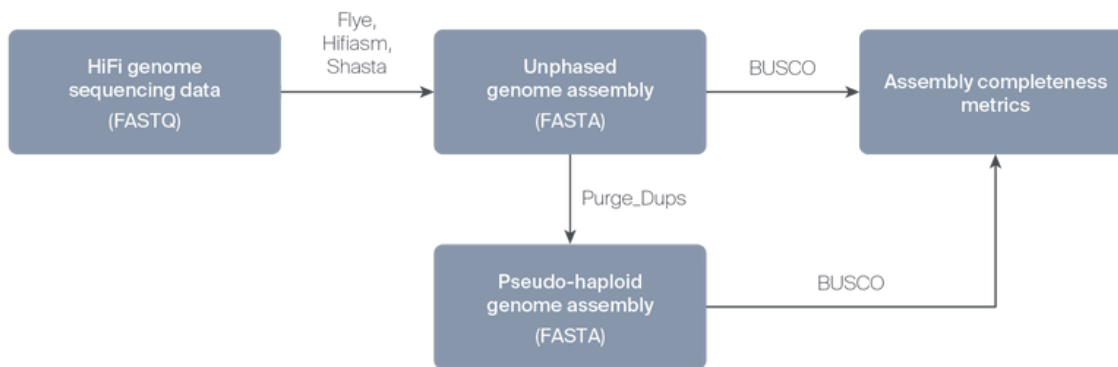
The data

Figure 1. **Bioinformatics workflow**.

We received a sufficient amount of data from each SMRT Cell 8M. Our combined yield totaled 3.3 million HiFi CCS reads, composed of approximately 44.5 billion HiFi CCS bases, for an average HiFi insert length of 13.6 kb. We expected this amount of data to provide approximately 15-fold coverage of the *A. americanum* genome. We subjected the data to a long-read assembly and assessment workflow (Figure 1) starting with some cursory test assemblies using Shasta, Flye, and Hifiasm with default settings on a 10-core 3.7 GHz Xeon workstation containing 224 GB of RAM [12][13][14]. We found that Flye and Hifiasm provided the most BUSCO complete assemblies using data from just one SMRT Cell 8M (Figure 2).
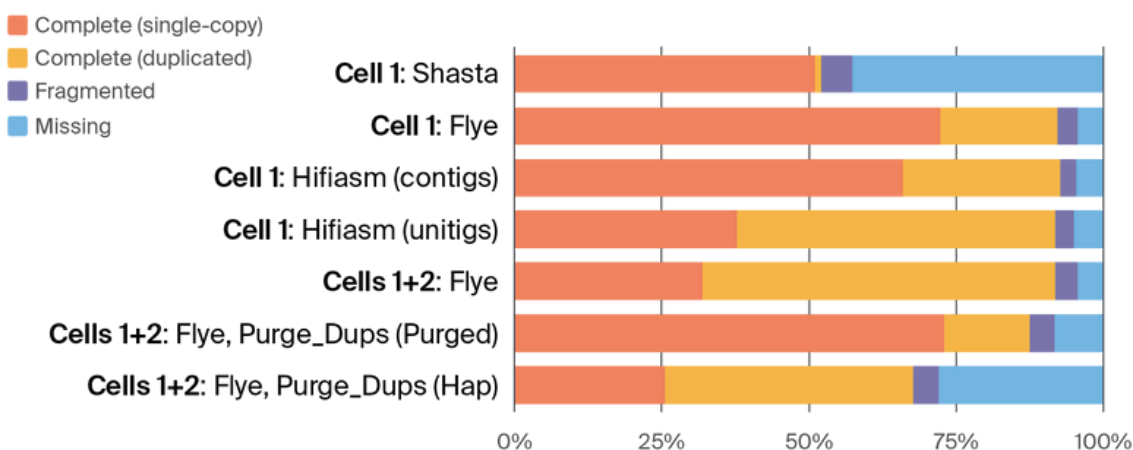

Figure 2. **BUSCO results for each assembly**.

Note that we deposited the entry labeled "Cell 1+2:Flye, Purge_Dups (Hap)" in Zenodo and deposited the entry labeled "Cell 1+2:Flye, Purge_Dups (Purged)" at NCBI/GenBank.

In our experience, Flye was the fastest assembler that produced reasonably (> 75%) complete assemblies. However, Hifiasm produced several assemblies and the largest (unitig) assembly contained the most duplicated BUSCO genes. Flye consumed approximately 1–2 days of processing time for one SMRT Cell worth of data and 3–4 days of processing time using the combined data from both SMRT Cells. Hifiasm consumed 1–2 days for one SMRT Cell and didn't complete processing after three weeks for two SMRT Cells. We suspect that Hifiasm might have had trouble with our dataset because the genomic DNA we sequenced came from 50 ticks rather than one individual, which would have been the ideal scenario.

For our initial draft assembly, we chose to move forward with Flye due to its speed, convenience, and simplicity of output. However, the unitig assembly that Hifiasm produced is a bit larger and potentially more information-rich than the contig Hifiasm assembly and the default Flye assembly. This could lead to higher read-mapping for RNA-seq mapping and more complete protein database assembly for proteomics.

During genome deposition at NCBI/GenBank, the raw assembly that Flye produced triggered a few automated error messages, indicating that our assembly needed some light cleanup. Specifically, we had several contigs of less than 200 nucleotides and several duplicate contigs that we needed to remove. We also had a contig containing an adaptor sequence requiring adaptor excision. We generated a Python-based Jupyter notebook to take care of these issues.

The final issue, which a simple Python script could not resolve, was the fact that our assembly was too large compared to NCBI/GenBank estimates. To solve this issue, we used Purge_Dups to split our unphased assembly [15]. This generated a pseudo-haploid assembly

which we then cleaned up using our aforementioned Python-based Jupyter notebook. We deposited the resultant assembly at NCBI/GenBank, available under accession GCA_030143305.1. We also deposited our unphased diploid genome into Zenodo for anyone interested in accessing our full dataset.

Key takeaways

We've assembled an 88% BUSCO-complete long-read pseudo-haploid *Amblyomma americanum* genome of approximately three gigabases from 50 individual female ticks. It's available for download and use in GenBank. The unphased diploid genome is also available in a Zenodo repository.

Next steps

We've continued to make refinements to the *Amblyomma americanum* assembly. We identified sequences from a *Coxiella*-like endosymbiont in the salivary glands (as seen previously [2]), but haven't done any work to analyze them beyond that. In a follow-up pub, we identified and removed those and other contaminant contigs [1] from our deposited assembly. We also performed gene-finding operations for this assembly, which are now available on GenBank (GCA_030143305.2) and UniProt (UP001321473). In the future, we plan to use this genome in research projects that involve expression profiling and comparative genomics.

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Contributors (A–Z)

- **Seemay Chou**: Conceptualization, Supervision

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