# A High-Quality Genome Assembly of SMRT Sequences Reveals Long-Range Haplotype Structure in the Diploid Mosquito *Aedes aegypti*

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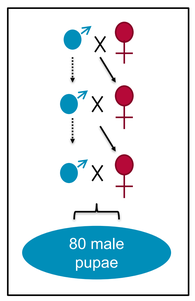
## Summary

*Aedes aegypti* is a tropical and subtropical mosquito vector for Zika, yellow fever, dengue fever, and chikungunya. We describe the first diploid assembly of an insect genome, using SMRT Sequencing and the open-source assembler FALCON-Unzip. This assembly has high contiguity (contig N50 1.3 Mb), is more complete than previous assemblies (Length 1.45 Gb with 87% BUSCO genes complete), and is high quality (mean base >QV30 after polishing). Long-range haplotype structure, in some cases encompassing more than 4 Mb of extremely divergent homologous sequence with dramatic differences in coding sequence content, is resolved using a combination of the FALCON-Unzip assembler, genome annotation, coverage depth, and pairwise nucleotide alignments.

## Strain Preparation

- 3 generations single-pair mating with same father each generation
- Founding strain: Liverpool
- Max ploidy = 4N
- Genomic DNA extracted with MagAttract Kit (Qiagen)




## Library Preparation and Sequencing

- Three SMRTbell libraries constructed with 20 and 30 kb size selection using BluePippin
- Libraries run on PacBio RS II using P6-C4 chemistry for 6 hour movies

| | |
|---|---|
| PacBio RS II SMRT Cells | 177 |
| Total Raw Sequence | 140 Gb |
| Genome Coverage | 110 fold |
| Subread N50 | 13,733 bp |

**Table 1**. Sequencing Results

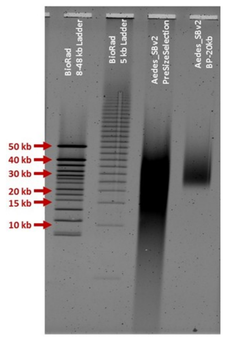



**Figure 1**. Size selection of a 20 kb library with BluePippin system

## FALCON-Unzip Phased, Diploid Genome Assembly

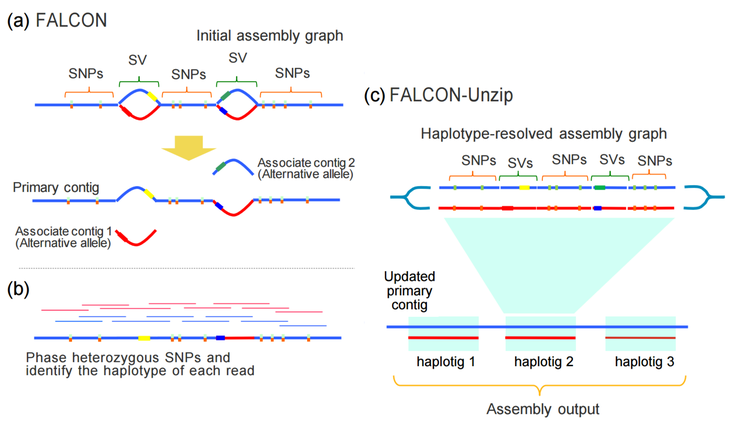



**Figure 2**. FALCON-Unzip assembler[2]

## Assembly Results

| Contig Set | Primary Contigs | Associated Haplotigs | Current Reference (L3.31)[1] |
|---|---|---|---|
| **Total Length** | 1.45 Gb | 0.59 Gb | 1.38 Gb |
| **Contig Number** | 3,462 | 4,328 | 36,204 |
| **Contig N50** | 1.43 Mb | 0.38 Mb | 0.083 Mb |

**Table 2**. **Assembly Statistics**. FALCON-Unzip[2] (v0.7.0) used for assembly followed by genome polishing with Arrow in SMRT Link.

## Identifying Divergent Haplotypes using Gene Annotations

- Genome was annotated with conserved, single-copy genes from BUSCO[3] arthropod dataset (N=2675)
- Shorter members of pairs of primary contigs with duplicated BUSCO genes and reduced raw read coverage were recategorized as haplotigs[7]

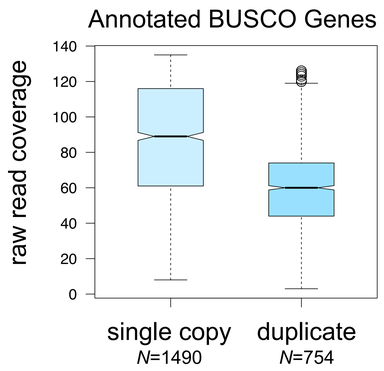



**Figure 3**. Reduced coverage in windows around duplicate BUSCO genes compared to single copy genes is consistent with haploid read coverage.

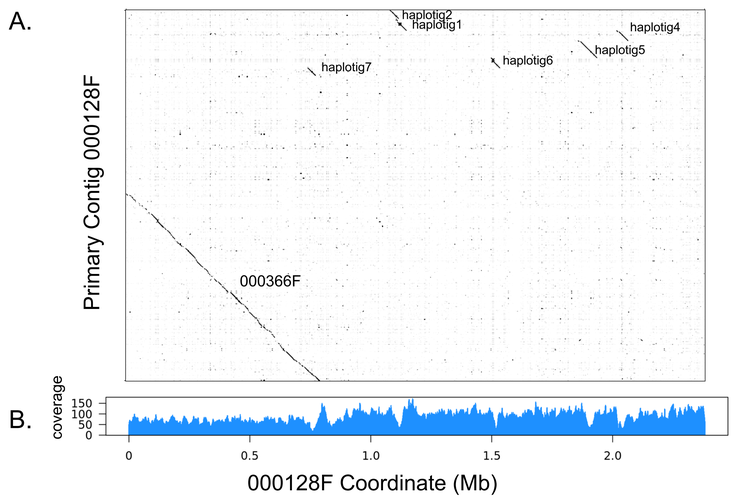



**Figure 4**. **Raw read coverage is reduced in regions of haploid assembly.** (**A**). Dot plot[5] of primary contig 000128F, its associated haplotigs, and an additional *homologous primary contig,* 000366F, which shares two BUSCO genes with 000128F. (**B**). Raw read coverage across primary contig 000128F is reduced by half in regions with multiple assembled haplotypes.

| Contig | Length (Mb) | Proportion Aligned | P-distance | Mean (s.d.) Read Coverage |
|---|---|---|---|---|
| **000128F** | 2.38 | 21.9% | NA | 80 (24) |
| **haplotig1** | 0.0336 | 100% | 0.32% | 21 (14) |
| **haplotig2** | 0.0492 | 81.2% | 0.42% | 50 (35) |
| **haplotig4** | 0.0420 | 100% | 0.50% | 59 (27) |
| **haplotig5** | 0.0703 | 100% | 0.40% | 51 (27) |
| **haplotig6** | 0.0413 | 99.1% | 0.36% | 48 (27) |
| **haplotig7** | 0.0316 | 100% | 0.92% | 10 (8) |
| **000366F** | 1.26 | 21.0% | 1.79% | 61 (13) |

**Table 3**. Contig 000366F is recategorized as haplotig of 000128F. Nucmer[4] alignments show it is more divergent than the other haplotigs identified by FALCON-Unzip.

## Genome Completeness and Quality

| Contig Set | Primary + Haplotig | Primary Contigs | Associated Haplotigs | Current Reference |
|---|---|---|---|---|
| **Complete** | 87% | 81% | 53% | 85% |
| **Duplicated** | 32% | 5.4% | 11% | 11% |
| **Fragmented** | 10% | 10% | 8.7% | 11% |
| **Missing** | 2.0% | 8.1% | 37% | 2.1% |

**Table 4**. BUSCOv1 analysis with arthropod dataset.

## Phased Assembly Identifies Dramatic Allelic Differences

- Long-range haplotype phasing spans 4Mb of contigs 000013F and 000043F, which align over <40% of their length but share 8 BUSCO genes
- Region contains heterozygous premature stop codon in AAEL005110, a DNA repair protein

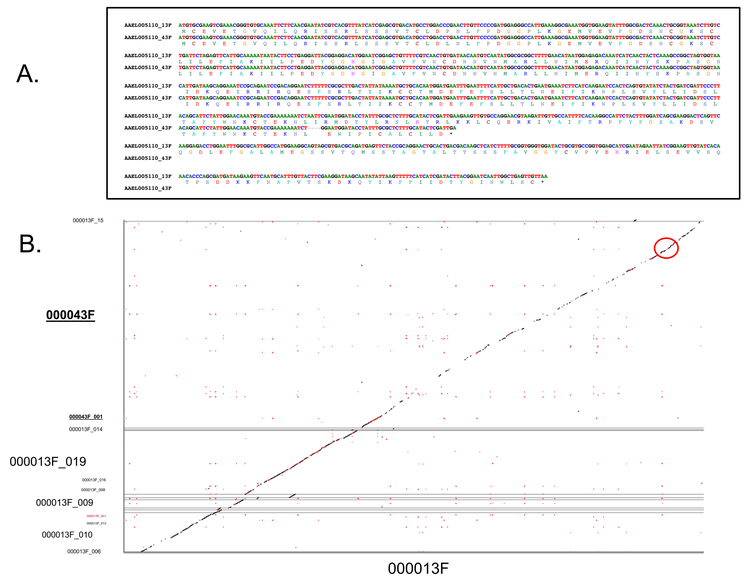



**Figure 5**. A. CDS and peptide alignment of AAEL005110 showing intact 302aa protein (above) and truncated 185aa allele (below). B. Location of heterozygous variant in dot plot between contigs.

## Conclusion

- FALCON-Unzip can efficiently assemble long-range phased haplotypes in heterozygous non-model organisms, elucidating allelic differences between parental chromosomes
- Annotation with BUSCO genes is a simple and powerful way to identify divergent homologous genomic regions, in conjunction with read depth data

## References

1. Nene et al. (2007) Genome sequence of *Aedes aegypti*, a major arbovirus vector. *Science*, 316(5832), 1718-1723.
2. Chin et al. (2016) Phased diploid genome assembly with single-molecule real-time sequencing. *Nature Methods*. 13(12),1050-105
3. Simão et al. (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*. 31(19), 3210-3212.
4. Kurtz et al. (2004) Versatile and open software for comparing large genomes. *Genome Biology* 5(2), R12.
5. Krumsiek et al. (2007) Gepard: a rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics*. 23(8),1026-1028.
6. Nattestad and Schatz (2016) Assemblytics: a web analytics tool for the detection of variants from an assembly. *Bioinformatics*. 32(19), 3021-3023.
7. Kingan (2016) https://github.com/skingan/HomolContigsByAnnotation

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