

Supplemental Table 1. Sequencing statistics before and after assembly with Canu.

	PacBio preassembled reads	Canu assembly
Total sequence length	34.84 Gb	622.30 Mb
Total contigs	3,255,924	3593
Contig N50 length	21,068 bp	541,786 bp
Max. contig length	87,617 bp	12,930,346 bp
Min. contig length	1000 bp	1028 bp
Mean contig length	10,701 bp	173,198 bp
Median contig length	5969 bp	56,654 bp

Supplemental Table 2. Nucleotide substitutions per site between three different populations and the reference genome assembly (Bearpaw Lake) and the reference Y chromosome assembly (Paxton Lake).

Population	Autosomes (Bearpaw)	X (Bearpaw)	Y (Paxton)
Puget Sound	0.0028	0.0022	0.00014
Lake Washington	0.0025	0.0022	0.00015
Paxton Lake benthic	0.0038	0.0031	0.000025

Supplemental Table 3. Log₂ fold change between testis tissue and three other tissues for genes that are duplicated on the Y chromosome and have an X-linked gametolog.

Comparison	Y duplicated	X homolog	P value
Testis versus Liver	-1.300	-0.186	0.025
Testis versus Brain	-3.379	-0.114	0.006
Testis versus Larvae	-5.395	-4.872	0.998

Supplemental Table 4. The effect of sequence identity threshold on the total number of X chromosome contigs identified.

Sequence identity threshold	Number of X chr. contigs	Total contig length	Difference from reference X chr.
94%	133	23,137,131 bp	2,518,665 bp
95%	124	21,909,126 bp	1,290,660 bp
96%	114	21,255,474 bp	637,008 bp
97%	90	18,109,484 bp	-2,508,982 bp
98%	73	15,892,396 bp	-4,726,070 bp

Supplemental Table 5. The effect of the 3D-DNA parameter --editor-repeat-coverage on the number of concordant BACs that align to the assembly.

Editor repeat coverage	Number of concordant BACs
8	62
9	48
10	89
11	92
12	92
13	92
14	89
15	89
16	89
17	88
18	88