

Supplementary Materials 2. BioNano extended results

The entire optical mapping procedure from DNA isolation to hybrid assembly was performed at the UC Davis DNA Technologies Core.

BioNano molecule summary

A total of 1750X optical reads were collected from pooled Y_L male larvae and was downsampled to roughly 120X for the remainder of the pipeline. Table 1 summarizes the optical mapping data generated from the Saphyr Platform.

Table 1. Bionano molecule results for entire data (first two rows) and for downsampled data (following rows).

Total no. of molecules generated	334,498
Total length (Mb)	69911.101
Data collected (molecules > 150kb)	52.929 Gb
Molecule N50 (molecules > 150kb)	276.5 kb
Label density (labels per 100kb)	12.51
Map rate (% molecules aligned to reference)	52.2%
Effective coverage of reference	118.9X

Hybrid scaffold summary

For the hybrid scaffolding, we provided a genome consisting of the female *D. pseudoobscura* assembly before Hi-C stitching and our Y_L chromosome contigs. The hybridScaffold.pl was run using the following parameters: -f -B 2 -N 2.

Table 2 provides an overview of the assemblies from sequencing, optical mapping, and hybrid pipelines. In total, there were 32 hybrid scaffolds (“super-scaffolds”) from the pipeline. The autosomes and X were contained within 6 hybrid scaffolds (MullerA-AD, B, C, E, F) and the remaining 26 contained the Y.

Figure 1 shows the alignment of the hybrid assembly to the published *D. pseudoobscura* assembly using NUCmer (version 3.1) with visualization by mummerplot (version 3.5). The inversion in MullerA-AD (blue) likely indicates a mis-assembly in the published Hi-C stitched genome.

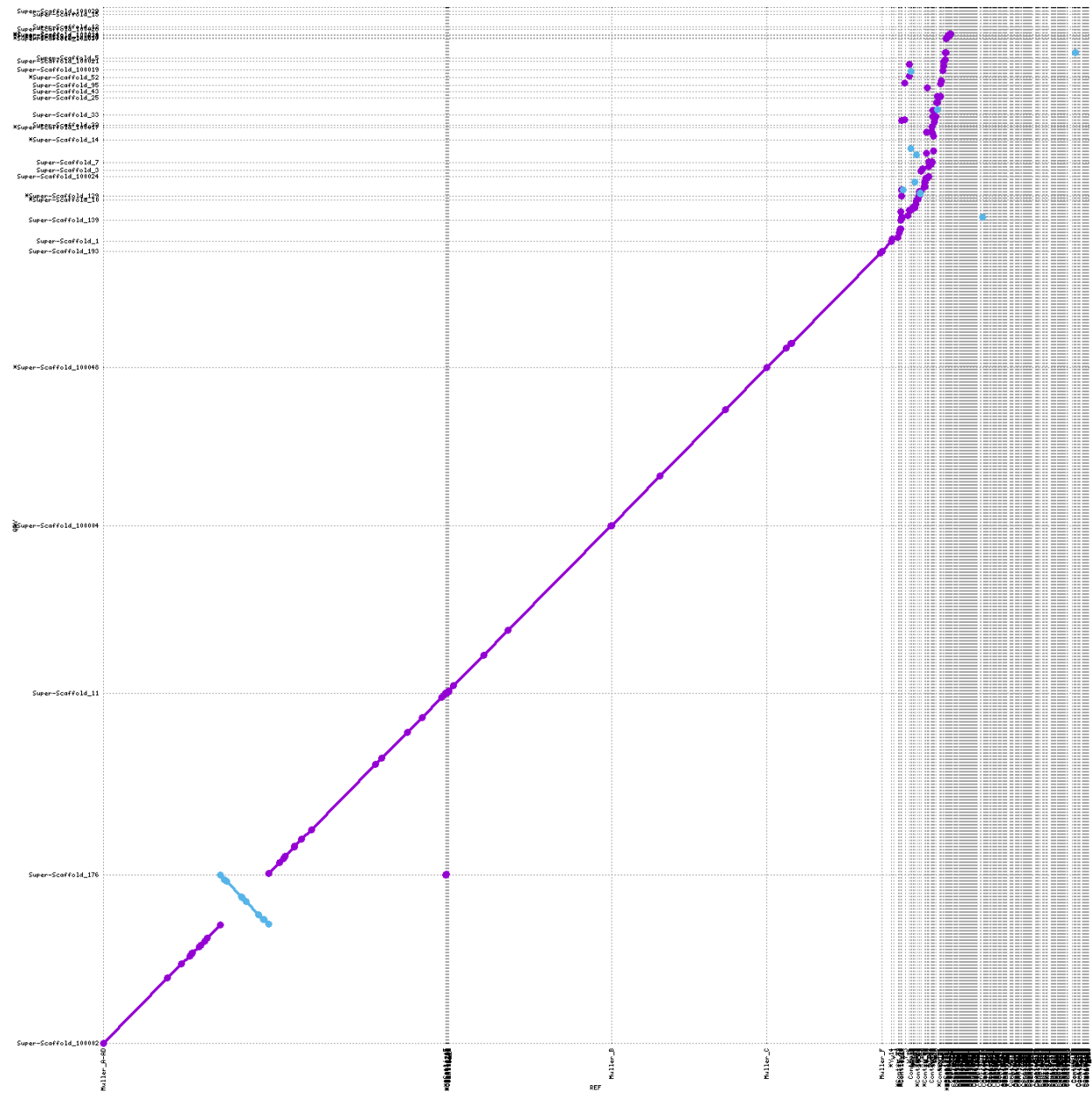


Figure 1. Bionano hybrid assembly aligned to *D. pseudoobscura* genome from Bracewell et al 2019. X-axis is the published genome, Y-axis is the hybrid assembly.

Table 2. Overview of assemblies in Bionano pipeline

	Original NGS	Bionano Map	Hybrid	Hybrid+unscaffolded
Contigs	386	54	32	328
Min length	0.004	0.068	0.133	0
Median	0.566	0.981	1.582	0.057
Mean	4.743	3.899	6.27	0.687
N50	20.319	30.926	30.573	30.573
Max	20.319	35.121	35.25	35.25
Total	218.638	210.562	200.631	225.367

Y chromosome scaffold summary

Table 3 shows the overview of the subset of hybrid scaffolds that belong to the assembled Y chromosome. We then verified that the restriction cut sites of the molecules and the mappings corroborated each other. Many hybrid scaffolds were of 1:1 BioNano map to NGS contig as in Figure 2 but there were some scaffolds that contained multiple NGS contigs as in Figure 3. Table 4 shows the breakdown of contigs and their overall placement, or lack thereof, into the hybrid assembly. Roughly 78% of the NGS contigs have support from the BioNano molecules.

Table 3. Overview of Y assemblies in the Bionano pipeline. The number of Hybrid Y contigs and unscaffolded contigs (column 2) is higher than expected given the number of fused contigs because many contigs are split and retained.

	Original Y NGS	Hybrid Y contigs	Hybrid Y contigs + unscaffolded contigs
Contigs	139	26	121
N50	0.816342	3.272	1.600714
Max	3.351422	4.459	4.459917
Number of Ns	0	6.202195	6.202195
Total	55.037118	45.271005	61.075317

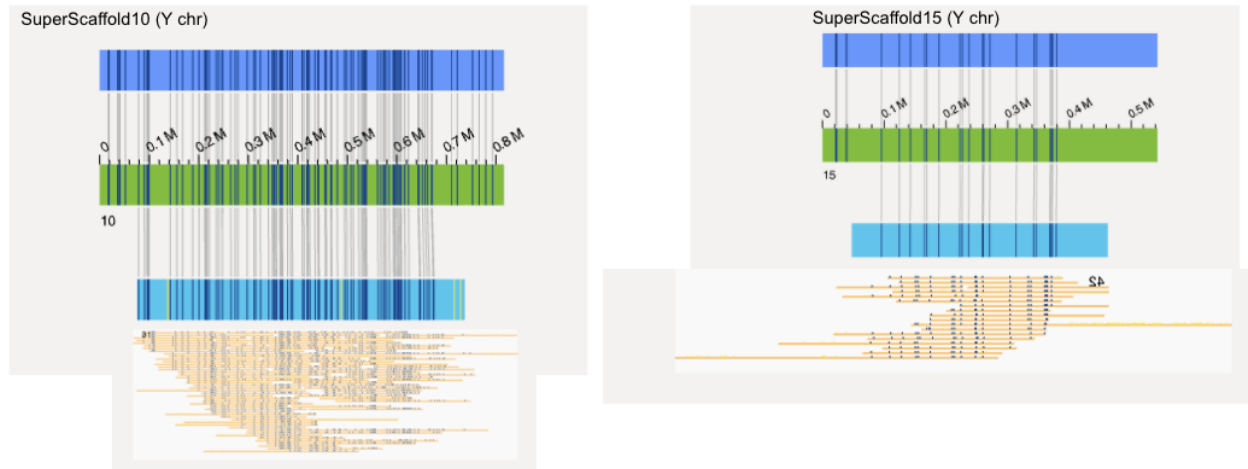


Figure 2. One to one verification of Y-linked contigs by BioNano maps and molecules. Shown are two examples of hybrid scaffolds (#10 and #15) that provide verification of two NGS contigs. Blue track is NGS, green is hybrid scaffold, lightblue is BioNano map, yellow pileup are BioNano molecules.

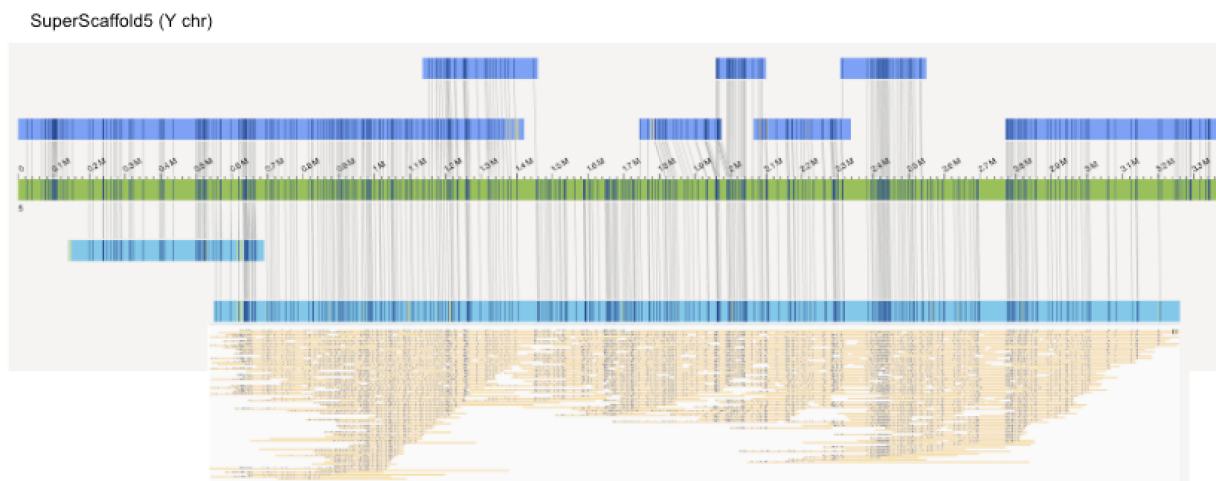


Figure 3. Many to one NGS contigs to hybrid scaffolds. Shown is one example of hybrid scaffold #5 that places 7 NGS contigs into one scaffold. Tracks are the same as described in Figure 2. Note that some NGS contigs overlap which provides one explanation to the decrease in Y chromosome length in the hybrid assembly compared to NGS assembly.

Table 4. Overview of NGS contigs placed/unplaced into hybrid scaffolds. NGS contigs with multiple hybrid scaffold placements are cut during the BioNano pipeline.

NGS Contig	Hybrid Scaffold Placement	NGS Length
tig00000625_pilon	Super-Scaffold_95	1480853
000015F_pilon	Super-Scaffold_7	1982054
tig00000771_pilon	Super-Scaffold_7	1021014
000058F_pilon	Super-Scaffold_7	900917
000079F_pilon	Super-Scaffold_7	327961
tig00000383_pilon	Super-Scaffold_7	220108
000198F_pilon	Super-Scaffold_52	1515852
tig00000676_pilon	Super-Scaffold_516, Super-Scaffold_5	918914
000068F_pilon	Super-Scaffold_5, Super-Scaffold_33	1331247
000012F_pilon	Super-Scaffold_5	1420331
000063F_pilon	Super-Scaffold_5	609973
000074F_pilon	Super-Scaffold_5	377702
000101F_pilon	Super-Scaffold_5	273022
000144F_pilon	Super-Scaffold_43	326142
tig00000746_pilon	Super-Scaffold_43	192962
tig00000843_pilon	Super-Scaffold_33	366968
000118F_pilon	Super-Scaffold_33	263537
000163F_pilon	Super-Scaffold_33	180489
000216F_pilon	Super-Scaffold_33	114741
tig00000142_pilon	Super-Scaffold_33	78017
000114F_pilon	Super-Scaffold_32	514009
tig00000750_pilon	Super-Scaffold_32	402975
000123F_pilon	Super-Scaffold_32	227200
000143F_pilon	Super-Scaffold_32	218895
000131F_pilon	Super-Scaffold_32	203890
000297F_pilon	Super-Scaffold_32	161440
tig00000184_pilon	Super-Scaffold_32	133204
000010F_pilon	Super-Scaffold_3	1563104
000083F_pilon	Super-Scaffold_25	1303374
000060F_pilon	Super-Scaffold_15	542665
000042F_pilon	Super-Scaffold_14	503240
tig00000386_pilon	Super-Scaffold_14	423437

000059F_pilon	Super-Scaffold_14	404781
tig00000642_pilon	Super-Scaffold_14	341715
000011F_pilon	Super-Scaffold_139	3351422
tig00000875_pilon	Super-Scaffold_139	449123
tig00000674_pilon	Super-Scaffold_129, Super-Scaffold_43	1445882
000006F_pilon	Super-Scaffold_129	1648969
000081F_pilon	Super-Scaffold_129	336668
000154F_pilon	Super-Scaffold_129	231781
tig00000580_pilon	Super-Scaffold_129	168617
000053F_pilon	Super-Scaffold_12	911661
000073F_pilon	Super-Scaffold_12	482370
000048F_pilon	Super-Scaffold_11	451959
000138F_pilon	Super-Scaffold_100029	215000
000093F_pilon	Super-Scaffold_100028	268752
tig00000310_pilon	Super-Scaffold_100028	177291
000132F_pilon	Super-Scaffold_100024, Super-Scaffold_100029	579595
000037F_pilon	Super-Scaffold_100024	548194
tig00000683_pilon	Super-Scaffold_100024	402603
tig00000682_pilon	Super-Scaffold_100021, Super-Scaffold_129	1055918
000014F_pilon	Super-Scaffold_100019	1200401
000064F_pilon	Super-Scaffold_100019	379135
000125F_pilon	Super-Scaffold_100017	335447
000180F_pilon	Super-Scaffold_100017	161059
000235F_pilon	Super-Scaffold_100017	152107
000077F_pilon	Super-Scaffold_100014	335848
000085F_pilon	Super-Scaffold_100014	299838
000045F_pilon	Super-Scaffold_10	816342
tig00000690_pilon	Super-Scaffold_1, Super-Scaffold_5	1351039
000005F_pilon	Super-Scaffold_1	2592044
000098F_pilon	Super-Scaffold_1	278028
000097F_pilon	Super-Scaffold_1	261656
000112F_pilon	NA	812288
tig00000811_pilon	NA	666670
tig00000616_pilon	NA	542448
tig00000686_pilon	NA	527170

tig00000647_pilon	NA	523306
tig00000020_pilon	NA	407740
tig00000809_pilon	NA	393744
tig00000253_pilon	NA	391689
tig00000608_pilon	NA	356790
000078F_pilon	NA	354427
tig00000619_pilon	NA	337116
tig00000640_pilon	NA	316104
000142F_pilon	NA	267487
tig00000688_pilon	NA	253541
tig00000376_pilon	NA	242141
000126F_pilon	NA	215873
tig00000201_pilon	NA	215439
tig00000836_pilon	NA	214123
tig00000194_pilon	NA	212548
tig00000898_pilon	NA	198019
tig00000787_pilon	NA	196327
000136F_pilon	NA	194175
000161F_pilon	NA	189776
000160F_pilon	NA	189547
000200F_pilon	NA	188034
tig00000030_pilon	NA	186167
000139F_pilon	NA	183953
000244F_pilon	NA	164230
tig00000889_pilon	NA	157986
tig00000054_pilon	NA	157959
000155F_pilon	NA	157092
000166F_pilon	NA	152958
tig00000064_pilon	NA	146934
tig00000813_pilon	NA	146438
tig00000646_pilon	NA	145617
000167F_pilon	NA	142078
000220F_pilon	NA	141954
tig00000017_pilon	NA	139541
tig00000783_pilon	NA	127322

000254F_pilon	NA	127051
tig00000768_pilon	NA	120729
tig00000380_pilon	NA	116698
tig00000025_pilon	NA	115717
tig00000105_pilon	NA	104564
tig00000353_pilon	NA	104427
tig00000260_pilon	NA	96514
tig00000023_pilon	NA	94099
tig00000318_pilon	NA	88063
tig00000232_pilon	NA	87880
tig00000749_pilon	NA	87477
tig00000155_pilon	NA	85704
tig00000242_pilon	NA	83270
000310F_pilon	NA	82502
tig00000145_pilon	NA	78184
tig00000766_pilon	NA	78108
tig00000371_pilon	NA	76659
tig00000362_pilon	NA	73597
tig00000406_pilon	NA	71855
tig00000154_pilon	NA	70523
tig00000720_pilon	NA	68759
000438F_pilon	NA	67866
tig00000258_pilon	NA	62038
tig00000245_pilon	NA	60794
tig00000405_pilon	NA	59768
tig00000079_pilon	NA	58165
tig00000354_pilon	NA	57221
tig00000639_pilon	NA	56858
tig00000801_pilon	NA	52457
tig00000080_pilon	NA	52410
tig00000264_pilon	NA	51826
tig00000261_pilon	NA	50974
tig000008195_pilon	NA	45953
tig00000808_pilon	NA	44001
tig00000571_pilon	NA	40516

tig00000753_pilon	NA	39371
tig00000645_pilon	NA	32287