BiSCoT: Improving large eukaryotic genome assemblies with optical maps

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Motivation. Long read sequencing and Bionano Genomics optical maps are two techniques that, when used together, make it possible to reconstruct entire chromosome or chromosome arms structure. However, the existing tools are often too conservative and organization of contigs into scaffolds is not always optimal.

Results. We developed BiSCoT (Bionano SCaffolding COrrection Tool), a tool that post-processes files generated during a Bionano scaffolding in order to produce an assembly of greater contiguity and quality. BiSCoT was tested on a human genome and four publicly available plant genomes sequenced with Nanopore long reads and improved significantly the contiguity and quality of the assemblies. BiSCoT generates a fasta file of the assembly as well as an AGP file which describes the new organization of the input assembly.

[p]**Availability.** BiSCoT and improved assemblies are freely available on Github at http://www.genoscope.cns.fr/biscot and Pypi at https://pypi.org/project/biscot/.[p]

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2 # genome assemblies with optical maps

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9 ## ABSTRACT

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11 used together, make it possible to reconstruct entire chromosome or chromosome arms structure.
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14 **Results.** We developed BiSCoT (Bionano SCaffolding COrrection Tool), a tool that post-processes files
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22 ## INTRODUCTION

23 Assembling large and repetitive genomes, such as plant genomes, is a challenging field in bioinformatics.
24 The appearance of short reads technologies several years ago improved considerably the number of
25 genomes publicly available. However, a high proportion of them are still fragmented and few represent
26 the chromosome organization of the genome. Recently, long reads sequencing techniques, like Oxford
27 Nanopore Technologies and Pacific Biosciences, were introduced to improve the contiguity of assemblies,
28 by sequencing DNA molecules that can range from a few kilobases to more than a megabase in size
29 (Istace et al. (2017); Schmidt et al. (2017); Kim et al. (2019); Shafin et al. (2019)). Nevertheless and even
30 if the assemblies were greatly improved, the chromosome-level organization of the sequenced genome
31 cannot be deciphered in a majority of cases. In 2017, Bionano Genomics launched its Saphyr system
32 which was able to generate optical maps of a genome, by using the distribution of enzymatic labelling
33 sites. These maps were used to orient and order contigs into scaffolds but the real improvement came
34 in 2018, when Bionano Genomics introduced their Direct Label and Stain (DLS) technology that was
35 able to produce genome maps at the chromosome-level with a N50 several times higher than previously
36 (Belser et al. (2018); Formenti et al. (2018); Hu et al. (2019)).

37

38 However, scaffolds generated with the tool provided by Bionano Genomics do not reach optimal
39 contiguity. Indeed, when two contigs C_1 and C_2 are found to share labels, one could expect that the tool
40 would merge the two sequences at the shared site. Instead, the software chooses a conservative approach
41 and outputs the sequence of C_1 followed by a 13-Ns gap and then the C_2 sequence, thus duplicating the
42 region that is shared by the two contigs (Figure 1 case 1 and 2) and in numerous cases, these duplicated
43 regions could reach several kilobases. As an example, on the human genome we used to evaluate BiSCoT
44 (see Results), we could detect 515 of those regions, affecting 16 genes and corresponding to around
45 24.5Mb of duplicated sequences, the longest being 237kb in size. These duplicated regions affect the
46 contiguity and have to be corrected as they can be problematic for downstream analyses, like copy number
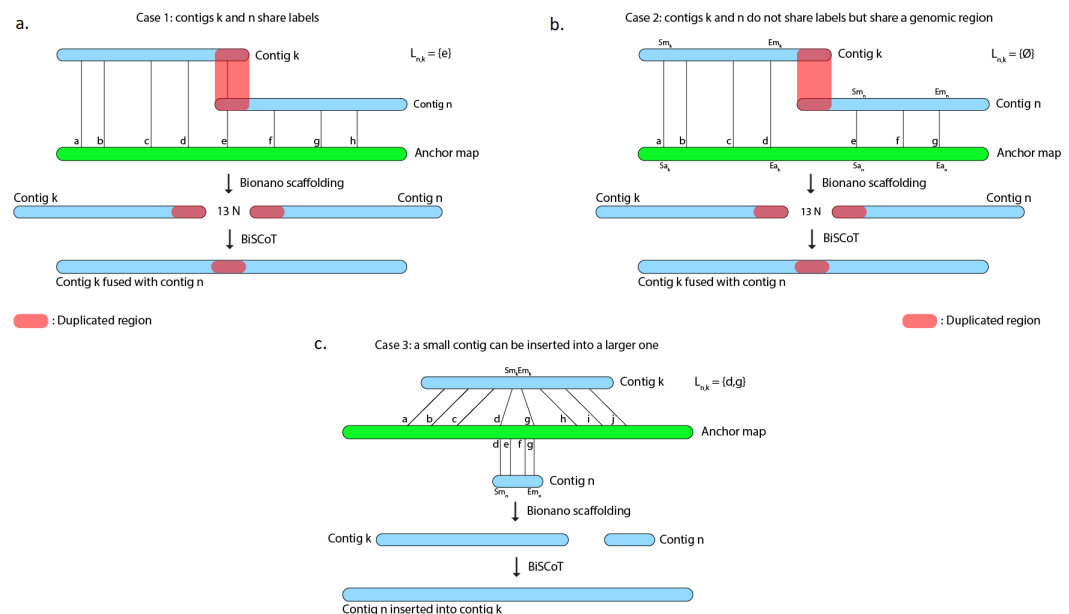
Figure 1. The Bionano scaffolding tool does not merge contigs even if they share labels. Instead, it inserts 13 N's gap between contigs, thus artificially duplicating the shared region. **a.** BiSCoT merges contigs that share enzymatic labelling sites. **b.** If contigs do not share labels but share a genomic region, BiSCoT attempts to merge them by aligning the borders of the contigs. **c.** The Bionano scaffolding tool does not handle cases where contigs can be inserted into others. BiSCoT attempts to merge the inserted map with the one containing it if they share labels.

variation studies. They originate from overlaps that are not fused in the input assembly and usually correspond to allelic duplications. In addition, contigs can sometimes be inserted into other contigs, these cases are not handled by the Bionano scaffolding tool that discards the inserted contigs (Figure 1 case 3).

We developed BiSCoT, a python script that examines data generated during a previous Bionano scaffolding and merges contigs separated by a 13-Ns gap if needed. BiSCoT also re-evaluates gap sizes and searches for an alignment between two contigs if the gap size is inferior to 1,000 nucleotides. BiSCoT is therefore not a traditional scaffolder since it can only be used to improve an existing scaffolding, based on an optical map.

METHODS

Mandatory files loading

During the scaffolding, the Bionano scaffolder generates a visual representation of the hybrid scaffolds that is called an 'anchor'. It also generates one '.key' file, which describes the mapping between map identifiers and contig names, several CMAP files, which contain the position of enzymatic labelling sites on contig maps and on the anchor, and a XMAP file, that describes the alignment between a contig map and an anchor.
BiSCoT first loads the contigs into memory based on the key file. Then, the anchor CMAP file and contig CMAP files are loaded into memory. Finally, the XMAP file is parsed and loaded.

Scaffolding

Alignments of contigs onto anchors contained in the XMAP file are first sorted by their starting position on the anchor. Then, alignments on one anchor are parsed by pairs of adjacent contigs, i.e alignment of contig C_k is examined at the same time as contig C_n, with C_k aligned before C_n on the anchor. Aligned anchor labels are extracted from these alignments and a list of shared labels $L_{n,k}$ is built. For the following cases, we suppose C_k and C_n to be aligned on the forward strand (Figure 1).

Case 1: contig maps share at least one anchor label

The last label l from $L_{n,k}$ is extracted and the position P_l of l on both contigs C_k and C_n is recovered from the CMAP files. In the resulting scaffold, the sequence of C_k will be included up to the P_l position and the sequence of C_n will be included from the P_l position. In this case, the gap is removed, both contigs C_k and C_n are fused and BiSCoT generates a single contig instead of two contigs initially separated by a gap in the input assembly.

Case 2: contig maps do not share anchor labels

Let $Size_k$ be the size of the contig C_k, Sm_k and Em_k the start and end of an alignment on a contig map and Sa_k and Ea_k the corresponding coordinates on the anchor. The number n of bases between the last aligned label of C_k and the first aligned label of C_n is then:

$$n = Sa_n - Ea_k \tag{1}$$

We then have to subtract the part d_k of C_k after the last aligned label of C_k and the part d_n of C_n before the first aligned label of C_n:

$$d_k = Size_k - Em_k \tag{2}$$
$$d_n = Sm_n \tag{3}$$

Finally, we can compute the gap size g with:

$$g = n - d_k - d_n \tag{4}$$

If $g \leq 1000$, a BLAT(Kent (2002)) alignment of the last 30kb of C_k is launched against the first 30kb of C_n. If an alignment is found and if its score is higher than 5,000, C_k and C_n are merged at the starting position of the alignment and, as in case1, BiSCoT generates a single contig instead of two contigs initially separated by a gap in the input assembly. Otherwise, a number g of Ns is inserted between C_k and C_n.

Case 3: insertion of small contigs

Let Sm_k and Em_k the start and end of an alignment on a contig map. If $[Sm_n, Em_n] \subset [Sm_k, Em_k]$, then the left-most shared label identifier l_l and right-most shared label identifier l_r are extracted. If C_n has more of its labels mapped in this region than C_k, the sequence of C_n will be inserted between l_l and l_r in the scaffolds. Otherwise, the sequence of C_k remains unchanged and C_n will be included as a singleton sequence in the scaffolds file.

Finally, if an Illumina polishing step was done before or after Bionano scaffolding, we recommend doing one additional round of polishing using Illumina reads after BiSCoT has been applied. Indeed, short reads tend to be aligned only against one copy of the duplicated regions, leaving the other copy unpolished.

RESULTS AND DISCUSSIONS

Validation on simulated data

In order to simulate a genome assembly, we downloaded the chromosome 1 of the GRCh38.p12 human reference genome and fragmented it to create contigs. We generated 120 contigs with an N50 size of 2.4Mb and a cumulative size of 231Mb. Contigs were generated with either overlaps or gaps between them. We introduced 50 gaps with a mean length of 50kb, the smallest being 3.4kbp long and the largest 99.6kb long, and 50 overlaps with a mean size of 44kb, the smallest being 278b long and the largest 98.6kb long. We also generated five contigs, with an N50 of 254kb, that were subsequences of larger contigs, to simulate contained contigs.

Then, we used these contigs and Bionano DLE and BspQI optical maps available on the Bionano Genomics website as input to the Bionano scaffolder. We gave the results of this scaffolding to BiS-CoT and aligned all assemblies to the chromosome 1 reference using Quast (Gurevich et al. (2013), v5.0.2).

BiSCoT was able to resolve 39 overlaps out of the 50 we introduced (Supplementary Table 1), 31 using shared labels and 8 using a Blat alignment. The 11 remaining overlaps could not be resolved

	Nanopore contigs	Bionano		BiSCoT	
		Contigs	Scaffolds	Contigs	Scaffolds
Cumulative size	2,818,937,673	2,818,997,568	2,878,230,106	2,810,480,725	2,868,077,379
N50	11,821,944	10,566,783	86,858,024	**12,894,141**	86,833,728
L50	67	71	14	**64**	14
N90	2,143,851	1,863,173	26,054,782	**2,321,940**	26,037,000
L90	280	301	36	**254**	36
auN*	15,164,719	14,547,428	82,760,251	**15,977,835**	82,474,548
# Ns	0	0	59,232,538	0	57,596,654
NGA50	5,794,944	5,729,014	10,816,842	**6,360,576**	11,713,900
NGA75	1,511,206	1,495,174	2,701,541	**1,596,102**	2,938,187
# misassemblies	1,356	1,299	1,602	**1,278**	1,515
Complete BUSCOs	**235 (92.2%)**	234 (91.8%)	231 (90.6%)	**235 (92.2%)**	231 (90.6%)
Duplicated BUSCOs	5 (2.0%)	**4 (1.6%)**	4 (1.6%)	**4 (1.6%)**	4 (1.6%)
Missing BUSCOs	11 (4.3%)	**10 (3.9%)**	13 (5.1%)	**10 (3.9%)**	13 (5.1%)

(*) auN is a new metric to measure assembly contiguity (Li (2020))

Table 1. Metrics of the NA12878 scaffolds and contigs before or after BiSCoT treatment. Bold formatting indicates the best scoring assembly among contigs.

114 due to contigs not sharing enough labels or the overlap being too small to produce an alignment of
115 sufficient confidence. BiSCoT was also able to integrate all contained contigs back to their original place
116 in the assembly. Furthermore, BiSCoT did not close any of the real gaps introduced during the assembly
117 generation.
118   Regarding assembly metrics (Supplementary Table 2), The N50 decreased by 1.4% in scaffolds and
119 increased by 22% in contigs. The number of Ns in scaffolds decreased from 20.7Mb to 20.4Mb. Moreover,
120 the number of misassemblies decreased by 68% after applying BiSCoT and the duplication ratio estimated
121 by Quast decreased from 1.026 in Bionano scaffolds to 1.021 in BiSCoT scaffolds.
122   In order to estimate the accuracy of gap sizes, we compared the gap sizes we introduced in the input
123 assembly to the ones that were estimated using optical maps (Supplementary Figure 1). We found that
124 estimated gap sizes were very close to the reality, with a mean scaled absolute error of 0.8%.

125 **Validation on real data**
126 We downloaded genome assemblies for which a DLE optical map was available: the NA12878 human
127 genome (Jain et al. (2018)), *Brassica oleracea* HDEM (PRJEB26621, Belser et al. (2018)), *Brassica rapa*
128 Z1 (PRJEB26620, Belser et al. (2018)) and *Musa schizocarpa* (PRJEB26661, Belser et al. (2018)) and
129 *Sorghum bicolor* Tx430 (PRJNA472170, Deschamps et al. (2018)).
130 The QUAST and BUSCO (Simão et al. (2015), v4.0.5) tools were used respectively to evaluate the number
131 of misassemblies to the GRCh38.p12 human reference genome and the number of conserved genes among
132 eukaryotes.
133 In all cases, we first used the Bionano workflow to scaffold the draft assembly and launched BiSCoT
134 using the files generated by the Bionano tools (Table 1, Supplementary Table 3, 4, 5 and 6). The output of
135 the Bionano workflow and BiSCoT are scaffolds, but we generated a contig file for each assembly by
136 splitting each scaffold at every position with at least one N.
137
138   Concerning the NA12878 genome, we could detect 515 overlapping regions with a mean size of 47kb
139 and representing in total 24.5Mb of duplicated sequences. Among these 515 regions, 499 were corrected
140 by BiSCoT using either shared labels (113 regions) or a BLAT alignment (386 regions) when no shared
141 labels were found.
142   Globally, the contig NX and NGAX metrics increased drastically: the contigs NGA50 of NA12878
143 increased by around 10%, going from 5.8Mb to 6.3Mb. The scaffolds NGAX metrics also increased: the
144 scaffolds NGA50 increased from 10.8Mb in Bionano scaffolds to 11.7Mb in BiSCoT scaffolds. Moreover,
145 the number of Ns decreased marginally and the number of complete eukaryotic genes stayed the same in
146 scaffolds. More importantly, when aligning the assemblies against the reference genome, we could detect
147 a decrease in the number of mis-assemblies going from 1,602 in Bionano scaffolds to 1,515 in BiSCoT
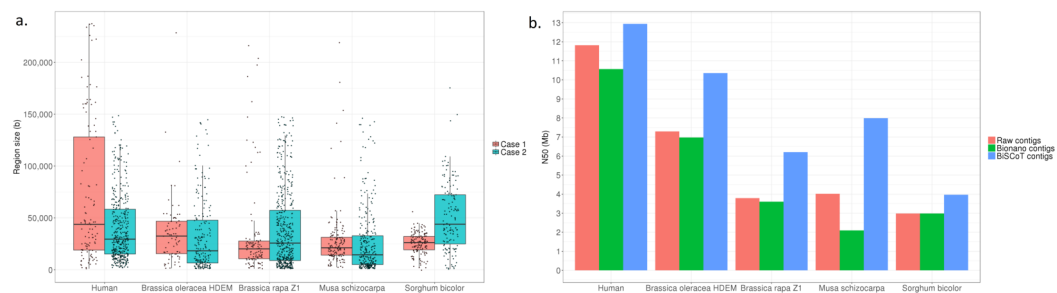
Figure 2. a. Distribution of the sizes of overlapping regions in the raw assemblies. Detection was done using either Bionano labels (Case 1) or a BLAT alignment (Case 2). **b.** N50 contigs of raw assemblies and assemblies before or after BiSCoT treatment.

scaffolds. The same kind of results were observed in the four plant genomes with a slight decrease in scaffolds NX metrics and number of Ns but an increase in contigs NX metrics(Figure 2 and Supplementary Tables 2, 3, 4 and 5).

SUMMARY

Thanks to the advent of long reads and optical maps technologies, it is now possible to obtain high-quality chromosome-scale assemblies. However, the official Bionano scaffolding tool does not always perform optimally when joining two contigs. Indeed, it does not merge two sequences when they share a genomic region, creating artificial gaps in the assembly. We developed BiSCoT, a tool that corrects these problematic regions in a prior Bionano scaffolding and showed that it increased significantly contiguity metrics of the resulting assembly, while preserving its quality.

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REFERENCES

Belser, C., Istace, B., Denis, E., Dubarry, M., Baurens, F.-C., Falentin, C., Genete, M., Berrabah, W., Chèvre, A.-M., Delourme, R., Deniot, G., Denoeud, F., Duffé, P., Engelen, S., Lemainque, A., Manzanares-Dauleux, M., Martin, G., Morice, J., Noel, B., Vekemans, X., D'Hont, A., Rousseau-Gueutin, M., Barbe, V., Cruaud, C., Wincker, P., and Aury, J.-M. (2018). Chromosome-scale assemblies of plant genomes using nanopore long reads and optical maps. *Nature Plants*, 4(11):879–887.

Deschamps, S., Zhang, Y., Llaca, V., Ye, L., Sanyal, A., King, M., May, G., and Lin, H. (2018). A chromosome-scale assembly of the sorghum genome using nanopore sequencing and optical mapping. *Nature Communications*, 9(1):4844.

Formenti, G., Chiara, M., Poveda, L., Francoijs, K.-J., Bonisoli-Alquati, A., Canova, L., Gianfranceschi, L., Horner, D. S., and Saino, N. (2018). SMRT long reads and Direct Label and Stain optical maps allow the generation of a high-quality genome assembly for the European barn swallow (Hirundo rustica rustica). *GigaScience*, 8(1). giy142.

Gurevich, A., Saveliev, V., Vyahhi, N., and Tesler, G. (2013). QUAST: quality assessment tool for genome assemblies. *Bioinformatics*, 29(8):1072–1075.

Hu, L., Xu, Z., Wang, M., Fan, R., Yuan, D., Wu, B., Wu, H., Qin, X., Yan, L., Tan, L., Sim, S., Li, W., Saski, C. A., Daniell, H., Wendel, J. F., Lindsey, K., Zhang, X., Hao, C., and Jin, S. (2019). The chromosome-scale reference genome of black pepper provides insight into piperine biosynthesis. *Nature Communications*, 10(1):4702.

Istace, B., Friedrich, A., d'Agata, L., Faye, S., Payen, E., Beluche, O., Caradec, C., Davidas, S., Cruaud, C., Liti, G., Lemainque, A., Engelen, S., Wincker, P., Schacherer, J., and Aury, J.-M. (2017). De novo

183 assembly and population genomic survey of natural yeast isolates with the Oxford Nanopore MinION
184 sequencer. *GigaScience*, 6(2). giw018.

185 Jain, M., Koren, S., Miga, K. H., Quick, J., Rand, A. C., Sasani, T. A., Tyson, J. R., Beggs, A. D., Dilthey,
186 A. T., Fiddes, I. T., Malla, S., Marriott, H., Nieto, T., O'Grady, J., Olsen, H. E., Pedersen, B. S., Rhie,
187 A., Richardson, H., Quinlan, A. R., Snutch, T. P., Tee, L., Paten, B., Phillippy, A. M., Simpson, J. T.,
188 Loman, N. J., and Loose, M. (2018). Nanopore sequencing and assembly of a human genome with
189 ultra-long reads. *Nature Biotechnology*, 36:338 EP –.

190 Kent, W. (2002). Blat–the blast-like alignment tool. *Genome Research*, 12:656–664.

191 Kim, H.-S., Jeon, S., Kim, C., Kim, Y. K., Cho, Y. S., Kim, J., Blazyte, A., Manica, A., Lee, S., and Bhak,
192 J. (2019). Chromosome-scale assembly comparison of the Korean Reference Genome KOREF from
193 PromethION and PacBio with Hi-C mapping information. *GigaScience*, 8(12). giz125.

194 Li, H. (2020). aun: a new metric to measure assembly contiguity.

195 Schmidt, M. H.-W., Vogel, A., Denton, A. K., Istace, B., Wormit, A., van de Geest, H., Bolger, M. E.,
196 Alseekh, S., Maß, J., Pfaff, C., Schurr, U., Chetelat, R., Maumus, F., Aury, J.-M., Koren, S., Fernie,
197 A. R., Zamir, D., Bolger, A. M., and Usadel, B. (2017). De novo assembly of a new solanum pennellii
198 accession using nanopore sequencing. *The Plant Cell*, 29(10):2336–2348.

199 Shafin, K., Pesout, T., Lorig-Roach, R., Haukness, M., Olsen, H. E., Bosworth, C., Armstrong, J., Tigyi,
200 K., Maurer, N., Koren, S., Sedlazeck, F. J., Marschall, T., Mayes, S., Costa, V., Zook, J. M., Liu, K. J.,
201 Kilburn, D., Sorensen, M., Munson, K. M., Vollger, M. R., Eichler, E. E., Salama, S., Haussler, D.,
202 Green, R. E., Akeson, M., Phillippy, A., Miga, K. H., Carnevali, P., Jain, M., and Paten, B. (2019).
203 Efficient de novo assembly of eleven human genomes using promethion sequencing and a novel
204 nanopore toolkit. *bioRxiv*.

205 Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. (2015). BUSCO:
206 assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*,
207 31(19):3210–3212.