

# Global Optimization for Scaffolding and Completing Genome Assemblies

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## Abstract

We develop a method for solving genome scaffolding as a problem of finding a long simple path in a graph defined by the contigs that satisfies additional constraints encoding the insert-size information. Then we solve the resulting mixed integer linear program to optimality using the Gurobi solver. We test our algorithm on several chloroplast genomes and show that it outperforms other widely-used assembly solvers by the accuracy of the results.

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\* Preliminary version of this paper has been presented at the Workshop on Constraint-Based Methods for Bioinformatics 2016.

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# 1 Introduction

Modern Next-Generation Sequencing (NGS) techniques are not able to output the whole genome sequence in one large sequence, but instead output billions of short DNA sequences, called *reads*. These reads are extremely redundant, erroneous, and, consequently, unusable practically. *Genome assembly* is the challenging computational task, consisting in reconstructing the full genome sequence from these fragmented raw data. This is a complex procedure, usually composed of three main steps: (1) generation of *contigs*, which are long contiguous DNA sequences issued from the overlapping of the reads; (2) orientation and ordering of these contigs, called the *scaffolding*; (3) gap-filling. A notable weakness of the contemporary approaches for *de-novo assembly* is to consider the above process as a set of independent tasks and not be able to propose a global optimal solution.

The first step generates a list of *contigs* (assembled ungapped sequences) that represent the "easily assembled regions" of the genome. Building contigs is currently supported by methods using a specific data structure called *de-Bruijn* graph [12].

Whereas the main challenge in the first step is in the sheer size of the raw data, in the second step, scaffolding, the data is of moderate size, but the problem remains largely open because of its NP-hard complexity [9]). The goal here is to provide a reliable order and orientation of the contigs in order to link them together into *scaffolds*—a sequence of contigs that overlap or are separated by gaps of a given length. The gap information is generated during sequencing based on *paired-end* or *mate-pair* reads [14], and has distance information associated with the gap. Specifically, each gap can be represented as a couple of fragments separated by a known distance (called *insert size*) and provides information about the distance between the corresponding contigs.

While the ultimate goal of the genome assembly is to generate a complete genome, the scaffolding phase usually produces a set of multiple scaffolds that, in addition, may contain inside them regions that have not been completely predicted. Further stages, such as *gap-filling* and a step that we call here *scaffold extension* (elongating and concatenating the contigs after the scaffolding step) are needed to complete the genome.

This paper focuses simultaneously on the above mentioned three steps (scaffolding, gap filling and scaffold extension) of *de novo* genome assembly. Given a set of contigs and their relationships—overlaps and/or remoteness in terms of distances between them (insert sizes)—we propose a global optimization-based approach for completing the genome assembly as

the longest sequence that is consistent with the given contigs and linkage information. A drawback of the typically used strategy of constructing a set of disjoint paths, rather than a single path, is that it would require additional steps of gap filling and scaffold extension, involving additional work. Moreover, it would make impossible to find a provably optimal final solution, since, even if each separate problem is implemented optimally, their combination may not be optimal.

Here we introduce a so called *contig graph*, that encodes information about contigs and distances between them, and reduce the scaffolding and gap-filling to finding a longest simple path in that graph such that as many as possible mate-pairs distances are satisfied (we call hereafter such path just a *longest path*). Since both conditions cannot generally be simultaneously satisfied, our objective function is a linear combination of them. We solve this problem by reformulating it as a mixed integer linear program (MILP) and develop a method that exactly solves the resulting program on genomes of up to 165 contigs and up to 6682 binary variables. We analyze the performance of the algorithm on several chloroplast genomes and compare it to other scaffolding algorithms.

An advantage of our approach is that the modeling of scaffolding as a longest path problem allows one to solve simultaneously all subtasks specific for completing the genome assembly. We are not aware of previous approaches on scaffolding based on the longest path problem reduction. There is no guarantee that the genome sequence corresponds to a longest path, but our experiments show that that is the case in many instances or, if not, there is a very small difference between the two. Unlike the shortest path problem with non-negative weights, for which efficient polynomial-time algorithms exist, the longest weighted path problem is NP-hard [5], which means that no polynomial time solution is likely to exist for it.

We tested this model on a set of chloroplast and bacteria genome data and showed that it allows to assemble the complete genome as a single scaffold. Compared to the publicly available scaffolding tools that we have tested, our solution produces assemblies of significantly higher quality.

Most previous work on scaffolding is heuristics based, e.g., SSPACE [1], GRASS [6], and BESST [13]. Such algorithms may find in some cases good solutions, but their accuracies cannot be guaranteed or predicted. Exact algorithms for the scaffolding problem are presented in [15], but the focus of that work is on finding structural properties of the contig graph that will make the optimization problem of polynomial complexity. In [11], integer linear programming is used to model the scaffolding problem, with an objective to

maximize the number of links that are satisfied. In order to avoid sub-cycles in the solution, the authors use an incremental process, where cycles that may have been produced by the solver are forbidden in the next iteration. While our focus is on accuracy, [11] focuses on efficiency, and indeed their algorithm, being a kind of heuristics, is faster than ours. However, integrating the distances between contigs and accounting for possible multiplicities of the contigs (repeats) is indicated as future improvement in [11], while it has been realized in our approach.

The contributions of this study are as follows:

- Our modeling of the scaffolding problem as a longest path problem allows to solve *simultaneously* the set of subtasks specific for completing the genome assembly like: contigs orientation and ordering, repeats, gap filling and scaffold extension, which in other approaches are separate phases.
- The scaffolding problem is reduced to finding a longest path in a particular graph. In addition, these paths need to satisfy a set of distances between couples of vertices along these paths. We are not aware of previous approaches on scaffolding based on the longest path problem.
- We formulate the above problem as a mixed integer linear program (MILP) with several interesting properties like: cycles elimination constraints and using binary variables for the edges of the graph only. Vertices are modeled with real variables, but we prove that the integrality of these variables follows from other constraints. Moreover, the commonly used approach for solving the longest weighted simple path when the initial (source) and final (target) vertices are unknown consists in artificially adding these two vertices and  $2|V|$  edges in the graph  $G = (V, E)$  [2]. This increases by  $2|V|$  the number of binary variables, which is a drawback when the density of the graph is small (as is the case of scaffolding graph). In contrast, our modeling does not require such a graph transformation and requires fewer binary variables.
- We tested this model on a set of chloroplast and bacteria genome data and showed that it allows to assemble the complete genome as a single scaffold. None of the publicly available scaffolding tools that we have tested targets single scaffolds (this is corroborated by the obtained numerical results).
- Our numerical experiments indicate that the relaxation of the mixed integer model is tight and produces upper bounds of excellent quality. This suggests a promising direction of research towards the scalability of our approach.

## 2 Modeling the scaffolding problem

### 2.1 Graph Modeling

We model the problem of scaffolding as path finding in a directed graph  $G = (V, E)$  that we call a contig graph, where both vertices  $V$  and edges  $E$  are weighted. The set of vertices  $V$  is generated based on the set  $C$  of the contigs according the following rules: the contig  $i$  is represented by at least two vertices  $v_i$  and  $v'_i$  (forward/inverse orientation respectively). If the contig  $i$  is repeated  $k_i$  times, it generates  $2k_i$  vertices. Denote  $N = \sum_{i \in C} k_i$ , therefore  $|V| = 2N$ .

The edges are generated following given patterns—a set of known overlaps/distances between the contigs. Any edge is given in the graph  $G$  in its forward/inverse orientation. We denote by  $e_{ij}$  the edge joining vertices  $v_i$  and  $v_j$  and the inverse of edge  $e_{ij}$  is  $e_{j'i'}$ . For any  $i$ , the weight  $w_i$  on a vertex  $v_i$  corresponds to the length of the contig  $i$ , while the weight  $l_{ij}$  on the edge  $e_{ij}$  corresponds to the value of the overlap/distance between contigs  $i$  and  $j$ . The problem then is to find a path in the graph  $G$  such that the total length (the sum over the traversed vertices and edges) is maximized, while a set of additional constraints are also satisfied:

- For any  $i$ , either vertex  $v_i$  or  $v'_i$  is visited (participates in the path).
- The orientations of the nodes does not contradict the constraints imposed by mate-pairs. This is at least partially enforced by the construction of  $G$ .

To any edge  $e \in E$  we associate a variable  $x_e$ . Its value is set to 1, if the corresponding edge participates in the assembled genome sequence (the associated path in our case), otherwise its value is set to 0. There are two kinds of edges: edges corresponding to overlaps between contigs, denote them by  $O$  (from overlaps), and edges associated with mate-pairs relationships, denote them by  $L$  (from links). We therefore have  $E = L \cup O$ . Let  $l_e$  be the length of the edge  $e = (u, v)$ . We have  $l_e < 0$  and  $|l_e| < \min \{w(u), w(v)\}$ ,  $\forall e \in O$ , and  $l_e > 0 \forall e \in L$ . Let  $w_v$  be the length of the contig corresponding to vertex  $v$  and denote  $W = \sum_{v \in V} w_v$ .

Let  $A^+(v) \subset E$  (resp.  $A^-(v) \subset E$ ) denote the subset of arcs in  $E$  leaving (resp. entering) node  $v$ .

### 2.2 Mixed Integer Linear Programming Formulation

We associate a binary variable for any edge of the graph, i.e.

$$(1) \quad \forall e \in O : x_e \in \{0, 1\} \text{ and } \forall e \in L : g_e \in \{0, 1\}.$$

Furthermore, to any vertex  $v \in V$  we associate three variables,  $i_v$ ,  $s_v$ , and

$t_v$ , which stand respectively for intermediate, source, and target for some path, and satisfy

$$(2) \quad 0 \leq i_v \leq 1, \quad 0 \leq s_v \leq 1, \quad 0 \leq t_v \leq 1.$$

All three variables are set to zero when the associated vertex  $v$  participates in none of the paths. Otherwise,  $v$  can be either a source/initial (noted by  $s_v = 1, t_v = 0, i_v = 0$ ), or a target/final ( $t_v = 1, s_v = 0, i_v = 0$ ), or an intermediate vertex, in which case the equalities  $i_v = 1, t_v = 0$  and  $s_v = 0$  hold. Moreover, each vertex (or its inverse) can be visited at most once, i.e.

$$(3) \quad \forall (v, v') : i_v + i_{v'} + s_v + s_{v'} + t_v + t_{v'} \leq 1.$$

The four possibles states for a vertex  $v$  (to belong to none of the paths, or otherwise, to be a source, a target, or an intermediate vertex in some path) are provided by the following two constraints

$$(4) \quad s_v + i_v = \sum_{e \in A^+(v)} x_e, \quad t_v + i_v = \sum_{e \in A^-(v)} x_e.$$

Finally, only one sequence (a single path) is searched for

$$(5) \quad \sum_{v \in V} s_v = 1 \text{ and } \sum_{v \in V} t_v = 1.$$

**Theorem 2.1** *The real variables  $i_v, s_v, t_v, \forall v \in V$  take binary values.*

**Proof.** Given in [4]. □

We introduce a continuous variable  $f_e \in R^+$  to express the quantity of the flow circulating along the arc  $e \in E$

$$(6) \quad \forall e \in E : 0 \leq f_e \leq Wx_e.$$

For  $e \in O$ , the value of  $x_e$  is set to 1, if the arc  $e$  carries some flow and 0, otherwise. In other words, no flow can use the arc  $e$  when  $x_e = 0$ .

We use the flows  $f_e$  in the following constraints,  $\forall v \in V$ ,

$$(7) \quad \sum_{e \in A^-(v)} f_e - \sum_{e \in A^+(v)} f_e \geq (i_v + t_v)(w_v + \sum_{e \in A^-(v)} l_e x_e) - Ws_v, \quad Ws_v \leq \sum_{e \in A^+(v)} f_e.$$

The purpose of the last two constraints is manifold. When a vertex  $v$  is a source ( $s_v = 1$ ), (7) generates and outputs from it an initial flow of sufficiently big value ( $W$  is enough in our case). When  $v$  is an intermediate vertex ( $i_v = 1$ ), constraint (7) forces the flow to decrease by at least  $l_{(u,v)} + w_v$  units when it moves from vertex  $u$  to its adjacent vertex  $v$ . The value of the flow thus is decreasing and this feature forbids cycles in the context of (4). When  $v$  is a final vertex, (7) is simply a valid inequality for the input flow.

We furthermore observe that because of (4), the constraint (7) can be written as follows

$$(8) \quad \forall v \in V : \sum_{e \in A^-(v)} f_e - \sum_{e \in A^+(v)} f_e \geq (i_v + t_v)w_v + \sum_{e \in A^-(v)} l_e x_e - W s_v.$$

The constraint (8) is linear and we keep it in our model instead of (7).

Furthermore, binary variables  $g_e$  are associated with links. For  $(s, t) \in L$ , the value of  $g_{(s,t)}$  is set to 1 only if both vertices  $s$  and  $t$  belong to the selected path and the length of the considered path between them is in the given interval  $[\underline{L}_{(s,t)}, \bar{L}_{(s,t)}]$ . Constraints related to links are :

$$(9) \quad g_{(s,t)} \leq s_s + i_s + t_s \text{ and } g_{(s,t)} \leq s_t + i_t + t_t$$

$$(10) \quad \forall (s, t) \in L : \sum_{e \in A^+(s)} f_e - \sum_{e \in A^-(t)} f_e \geq \underline{L}_{(s,t)} g_{(s,t)} - M(1 - g_{(s,t)})$$

$$(11) \quad \forall (s, t) \in L : \sum_{e \in A^+(s)} f_e - \sum_{e \in A^-(t)} f_e \leq \bar{L}_{(s,t)} g_{(s,t)} + M(1 - g_{(s,t)}),$$

where  $M$  is some big constant.

We search for a long path in the graph and such that as much as possible mate-paired distances are satisfied. The objective hence is :

$$(12) \quad \max \left( \sum_{e \in O} x_e l_e + \sum_{v \in V} w_v (i_v + s_v + t_v) + p \sum_{e \in L} g_e \right)$$

where  $p$  is a parameter to be chosen as appropriate (currently  $p = 1$ ).

### 3 Computational results

Here we present the results obtained on a small set of chloroplast and bacteria genomes given in Table 1. Synthetic sequencing reads have been generated for these instances applying ART simulator [8]. For the read assembly step required to produce contigs we applied the well-known Minia [3] with parameter **unitig** instead of **contig** (a unitig is a special kind of a high-confidence contig). Minia generates the set of unitigs, their repetition factor (the value of  $k_i$ ), the overlaps between them, as well as the mate-pair edges and distances. Based on this data, we generate a graph as explained in Section 2.1.

Our results were obtained on an Intel(R) Xeon(R) CPU E5-2670 v2 @ 2.50GHz with 20 cores, 64 GB of RAM, and using Gurobi 6.5.1 solver for solving the MILP models. We compared our results with the results obtained by three of the most recent scaffolding tools– SSPACE [1], BESST [13], and Scaffmatch [10]. In order to evaluate the quality of the produced scaffolds,

Datasets	Total	#unitigs	#nodes	#edges	#mate-pairs
Acinetobacter	3 598 621	165	676	8344	4430
Wolbachia	1 080 084	100	452	7552	2972
Aethionema Cordifolium	154 167	83	166	898	600
Atropa belladonna	156 687	18	36	114	46
Angiopteris Evecta	153 901	16	32	144	74
Acorus Calamus	153 821	15	30	134	26

Table 1  
Scaffolding datasets.

we applied the QUAST tool [7]. The results are shown on Table 2. We observe that our tool GST (from Genscale Scaffolding Tool) is the only one that consistently assembles the complete genome (an unique scaffold in #scaffolds column) with more than 98% (and in four cases at least 99.9%) correctly predicted genome fraction and zero misassemblies.

Our next computational experiments focussed on comparing various relaxations and other related formulations for the above described models. Due to lack of space these results are not presented here, but the interested reader can find them in [4].

## 4 Conclusion

We developed and tested algorithms for scaffolding and gap filling phases based on a version of the longest path problem and MILP representation. Our algorithms significantly outperform three of the best known scaffolding algorithms with respect to the quality of the scaffolds. Regardless of that, we consider the current results as a work in progress. The biggest challenge is to extend the method to much bigger genomes. We plan to use some additional ideas and careful implementation to increase the scalability without sacrificing the accuracy of the results.

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Datasets	Scaffolder	Genome fraction	#scaffolds	# misassemblies	N's per 100 kbp
Acinetobacter	GST	98.536%	1	0	0
	SSPACE	98.563%	20	0	155.01
	BESST	98.539%	37	0	266.65
	Scaffmatch	98.675%	9	5	1579.12
Wolbachia	GST	98.943%	1	0	0
	SSPACE	97.700%	9	0	2036.75
	BESST	97.699%	49	0	642.90
	Scaffmatch	97.994%	2	2	3162.81
Aethionema Cordifolium	GST	100%	1	0	0
	SSPACE	95.550%	20	0	13603.00
	BESST	81.318%	30	0	1553.22
	Scaffmatch	82.608%	7	7	36892
Atropa belladonna	GST	99.987%	1	0	0
	SSPACE	83.389%	2	0	155.01
	BESST	83.353%	1	0	14.52
	Scaffmatch	83.516%	1	0	318.93
Angiopteris Evecta	GST	99.968%	1	0	0
	SSPACE	85.100%	4	0	0
	BESST	85.164%	2	0	1438.54
	Scaffmatch	85.684%	1	0	454.23
Acorus Calamus	GST	100%	1	0	0
	SSPACE	83.091%	4	0	126.39
	BESST	83.091%	4	0	127.95
	Scaffmatch	83.271%	1	1	3757.13

Table 2

Performance of different solvers on the datasets from Table 1. GST is our tool.

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