Path-set – A Newer Platform for Genome Assembly With Mate-Pairs

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Abstract

One of the key advances that has led to a significant improvement in contig lengths has been mate pairs, which facilitate the assembly of repeating regions. In most current assemblers, mate-pair information has been used in various post-processing steps to untangle the assembly graph or to link contigs into scaffolds. These methods, although having different names, share the same underlying mechanism with the mate-pair transformation procedure [6]: finding a unique path between mate-pairs in the assembly graph and transforming mate-pairs into single long reads by filling the gaps between them. When multiple paths matching the insert size's range exist between the left and right reads of a mate-pair, the traditional mate-pair transformation procedure fails and therefore the resulting assembly deteriorates as the variation of the insert size is high. Recently, [3–5] proposed similar platforms for genome assembly that incorporate the paired information in the graph structure rather than using it in the post-processing heuristics. However, these methods face difficulties when the variation of the insert sizes is high. To facilitate this problem, instead of using mate-pairs reads directly, we transform them into edge-pairs and further, combine the ideas of mate-pair transformation, paired de Bruijn graph and de Bruijn graph structure to create the so-called Path-set graph platform for genome assembly.

1 Introduction

The current generation sequencing platforms such as ABI Solid, Illumina, 454 Life Science, with short reads and high throughput allow genomes to be sequenced more quickly with lower cost and has enabled new experimental opportunities to a variety of biological applications, including DNA methylation study, cancer research, ChIP-seq and whole transcriptome sequencing. However, as the sequencing technologies improves, the available sequenced data does not make the assembly task easier, if not harder. In fact, current genome assemblers face the challenge of assembling from reads of much shorter length (hundreds of nucleotides) comparing to longer reads in the previous Sanger sequencing platform (thousands of nucleotides). In repeat-rich genomes, when the length of the repeat is longer than twice the read length, correctly matching up its upstream and downstream regions is difficult. Fortunately, all current sequencing platforms have been able to produce mate-pairs – pairs of short reads between which the genomic distance (called the insert size) is approximately known. Because insert sizes could be much longer than the read length, mate pairs were able to span long repeats and could potentially match up the regions surrounding a repeat.

Mate-pair information has played an important role in most genomes projects and many current assemblers have modules to incorporate mate-pair information to improve the contigs length in a post-processing steps (mate-pair transformation in EULERSR [2], Breadcrumb in Velvet [8], All-paths in ALLPATHS [1], etc.). Albeit having different names, most of these modules rely on the same basic observation: If there is a unique path in the assembly graph that connects the left and right reads of the mate-pair, the gap in the mate-pair can be filled in with the nucleotide sequence representing the found path. When multiple paths are found between the mate-pairs, it remains ambiguous which path should be used to filled in the gap. When the variation of insert size increases, the number of paths that match the possible values of the insert size will increase and therefore, the mate-pair transformation efficiency (percentage of unique paths) decreases. Unfortunately, current technologies have the variation in reads about 5% of the insert size. Could the distance between reads be estimated exactly, the mate-pair transformation efficiency would increase and therefore improved the assembly. In the case when all the mate-pair transformations were successful, it would clearly create an upper bound for any repeat resolving methods using mate-pair.

Only recently, [5] introduced the paired de Bruijn graph platform that incorporates mate-pair information in the graph structure rather than use them for the mate-pair transformation in the post-processing steps. [3, 4] also independently developed similar platforms for using mate-pairs. Unfortunately, the performance of these platforms deteriorates as the variation of the insert size increases and undoubtedly, plays as a major obstacle for most current assembly methods using mate-pairs.

The main contributions of this paper are as follow. In the method section, we address the variation of the insert size problem by transforming mate-pairs to edge-pairs (pair of edges in the condensed de Bruijn graph). Using a collection of edge-pairs, we further combine the ideas of mate-pair transformation and paired de Bruijn graph to a single platform for genome assembly. Namely, we present a basic data structure called path-set, which is a set of possible paths between edge-pairs, and path-set graph to further extend path-sets into contigs. In the result section, we compare the performance of the path-set graph platform to most of the widely used assemblers on multiple E.Coli datasets.

2 Methods

2.1 Basic Notations

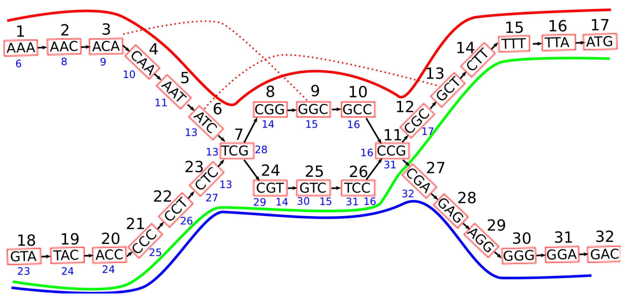
Define a k-mer as a string of length k. Given a k-mer $s = s_1 \dots s_k$, define $prefix(s) = s_1 \dots s_{k-1}$ and $suffix(s) = s_2 \dots s_k$. Given a set of k-mers A, the de Bruijn graph G is constructed with an edge $e = (u, v)$ for each k-mer where u and v are the prefix and suffix of the k-mer respectively. The condensed de Bruijn graph B is obtained by replacing every non-branching path in G by a single edge with length equal to the number of edges in the corresponding path. Each edge in the condensed graph can be represented by an edge-list — list of the normal de Bruijn graph edges. The length of a path in the condensed graph equals to sum of the length of all its edges.

Each k-mer corresponds (maps) to an edge in the de Bruijn graph G and therefore, uniquely identified by an edge and a position on that edge in the condensed graph B (the position of the edge in the edge list). For example, Fig. 1(b) is the condensed graph of the de Bruijn graph showed in Fig. 1(a).

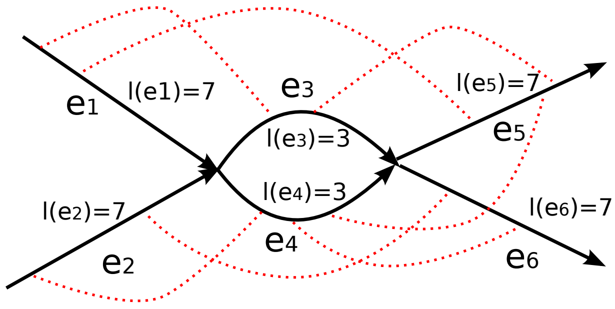
A pair of k-mers $(a|b)$ is called a (k, d)-mer if they are at a distance d in the original genome. We call a and b: the left and the right k-mers of $(a|b)$ respectively. Given parameters d_0 and Δ, a k-pair $(a|b)$ is called a (k, d_0, Δ)-mer of S it is a (k, d)-mer of S where $d \in [d_0 - \Delta, d_0 + \Delta]$. Given a set of (k, d_0, Δ)-mers, a pair of edges (e_1, e_2) in the corresponding condensed de Bruijn graph is called an edge-pair if there exists at least one (k, d_0, Δ)-mers that its left and right k-mers map to position p_1, p_2 on e_1 and e_2 correspondingly.

2.2 From (k, d, Δ)-mers to edge-pairs

From the mate-pair reads where the mean insert size and its variation is known, we can generate a set of all (k, d_0, Δ)-mers. This set can be easily



(a) De Bruijn Graph



(b) Condensed de Bruijn Graph

Figure 1: (a) De Bruijn graph and the corresponding mapping of mate-pairs. The number on top of each node is the node ID where the smaller number below each node shows the corresponding paired node. The bold red, blue and green sequences demonstrate how the genome traverses the graph. (b) The condensed de Bruijn Graph — non-branching paths of the graph in (a) correspond to condensed edges in this graph. The dotted red lines show the edge-pairs information

transformed to a set of edge-pair using the above definition. Each edge-pair (e_1, e_2) is represented as a triple $(e_1, e_2, \mathbf{h})$. In this triple, $\mathbf{h}$ is a histogram where $h(x)$ equals to the number of (k, d_0, Δ)-mers that supports the *genomic* distance x between e_1 and e_2. Namely, $h(x) = \Sigma I(d_0 + p_{a_i} - p_{b_i} = x)$ where p_{a_i}, p_{b_i} are the positions that a_i and b_i map to e_1, e_2 respectively; I is the indicator function; the summation is taken for all (k, d, Δ)-mers $(a_i|b_i)$ that map to the edge-pair. Since the insert size follows a Gaussian-like distribution, with enough coverage the problem of estimating the distance less demanding when the histogram contain a single distribution.. In this case we can just use the peak of the distribution as the estimated distance. The complication raises when the histogram is a mixtures of multiple distribution. In this case, we choose a threshold and disregards the regions of the histogram where the values are below the threshold [1]. The histogram results in one or more components, each component represents a tighter estimation of the distance between these edges. The original edge-pair can be separated into multiple edge-pairs, each with a histogram supporting the distances for each component. Figure 2(a) shows an pair of edges (e_1, e_2) that is traversed there times, each time passing P_1, P_1, and P_3. The first two of these repeats $(e_1P_1e_2,\ e_1P_2e_2)$ have similar length while the last one $(e_1P_3e_2)$ is well separated in length. Setting the threshold to 650 we can separate them into 2 components, one that has length varied from 130 to 147 and the other range from 156 to 165. As a result, the original edge pair $(e_1, e_2, \mathbf{h})$ is transformed into two edge pairs: $(e_1, e_2, \mathbf{h_1})$ and $(e_1, e_2, \mathbf{h_2})$ where

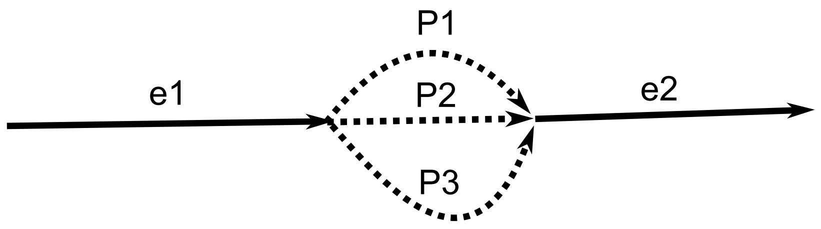
$$h_1(x) = \begin{cases} h(x) & \text{if } x \in [130, 147] \\ 0 & \text{otherwise} \end{cases}$$

and

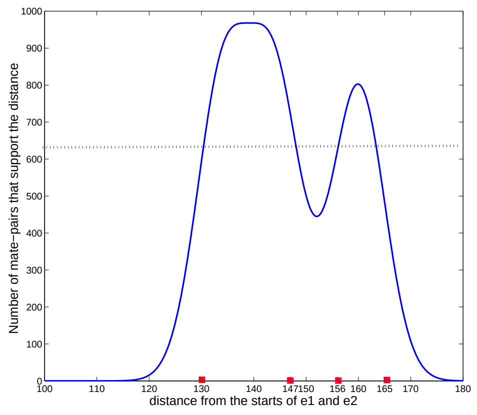
$$h_2(x) = \begin{cases} h(x) & \text{if } x \in [156, 165] \\ 0 & \text{otherwise} \end{cases}$$

While edge-pair is similar to mate-pair in that both of them have a gap sequence between both ends, edge-pair possesses multiple advantages. In edge-pair, the distance between both ends is better estimated and therefore more suitable for the previous methods for resolving repeats: mate-pair transformation [7], paired de Bruijn graph [5], paired graph [4], etc. Moreover, since the edge-pairs set is a compact representation of the original mate-pairs data, many redundant operations could be omitted directly, for instances, in mate-pair transformation, multiple function calls for mate-pairs that map the same pair of condensed edges can be replaced by a single mate-pair transformation on the corresponding edge-pair.

[1]An analytical formula for choosing the threshold would be beneficial



(a)



(b)

Figure 2: (a). The sequence traverses from e_1 to e_2 by 3 paths: P_1, P_2, and P_3. The length of e_1, P_1, P_2, and P_3 is 100, 34, 40 and 60 correspondingly. (b) The histogram of mate-pairs that support 3 possible distances. Setting the threshold to 600, we obtained two separated components, one from 130 to 147 that supports the traversal via paths P_1 and P_2 while the other component (from 156 to 165) that supports the traversal through path P_3.

Edge-pair data can be adapted into most previous methods that are susceptible to the variation of the insert size of mate-pairs. While the use of edge-pair in mate-pair transformation methods is straightforwards, the use of edge-pair in the paired de Bruijn graph requires some adjustments in the way the graph is constructed as the length of both ends of edge-pair are usually not equal. In the next section, rather than focusing on the use of edge-pairs and adapt it to each of the previous repeat resolving methods individually, we combine mate-pair transformation with an ad hoc version of paired de Bruijn graph on edge-pairs to build a more advanced platform: *path-set* graph.

2.3 Adapting edge-pairs to mate-pair transformation and paired de Bruijn graph — Path-set and Path-set Graph

Path-set. Similar to the classic mate-pair transformation procedure, for each mate-edge $(e_1, e_2, \mathbf{h})$ we also look for paths between e_1 and e_2 that have length in the valid range of $\mathbf{h}$. Essentially, this amounted to transforming an edge-pair into long found path and thus equivalent to having a much longer reads. In different from the previous approach, where the procedure fails when there are multiple paths that match the distance's range , we store all paths that correspond to possible traversals from edge e_1 to e_2 that match the distance constrain into a structure called *path-set* (a set of paths). For example, in Fig. 1(b), edge pair (e_1, e_5) can be transformed to a path-set containing two paths $PS = \{e_1e_3e_5, e_1e_4e_5\}$

Path-sets have two important properties that make it useful: (a) path-sets are not intersected, (b) each path-set contains at least one path that is present in the genome. Theoretically, it may exist an exponential number of such paths in the graph and an naive search for all paths between two nodes in the graph that has a certain length is computationally expensive. In the appendix, we describe an algorithm with polynomial time and space for finding and storing these paths.

Path-set Processing. Path-set plays the role as a single long read when it contains a single element (path). When the path-set contains more than a single path, it remains ambiguous which path should be followed and this is a result of the following reasons: (1) There are multiple repeats with similar length that shares both ends of the edge-pairs and vary in between, (2) There is only one valid connection between the two edges of the edge-pair but because of the intervention of other repeats, multiple paths that match the distance can be found. Were the read length that could span all the gap between the edge-pairs available, all path-set would simply be singletons and provides an upper bound for any repeats resolution approaches using only mate-pairs. Given a collection of path-sets obtained from edge-pairs, we now use the paired de Bruijn graph and the underlying de Bruijn graph structure to increase the number of path-set and reduce the number of paths in each path-set while keeping the two basic properties of the path-sets.

(a) Using the de Bruijn graph for path-set splitting. Initially, using the graph structure, we want to separate the path-sets into multiple non-disjoint path-sets while remaining their two basic properties. The goal is to distinguish repeats that share the both end edges but have variations in between. A path P is called a mandatory path if it corresponds to a sequence

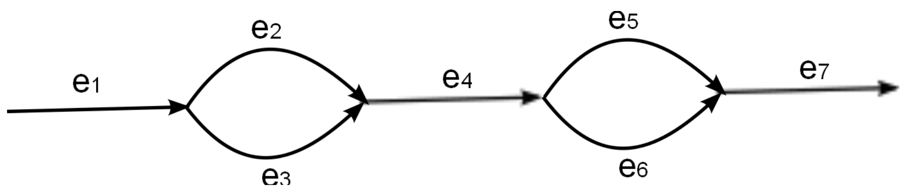


Figure 3: The path-set corresponding to the edge pair (e_1, e_7) contains 4 paths $PS = \{e_1e_2e_4e_5e_7, e_1e_3e_4e_5e_7, e_1e_2e_4e_6e_7, e_1e_3e_4e_6e_7\}$. — all possible connection from edge e_1 to e_7. Edges e_2 and e_3 are independent bridges and therefore, PS can be split into 2 path-sets: $PS_1 = \{e_1e_2e_4e_5e_7, e_1e_2e_4e_6e_7\}, PS_2 = \{e_1e_3e_4e_5e_7, e_1e_3e_4e_6e_7\}$

that is present in the genomes. Intuitively, if all members of a path-set PS are mandatory paths, we can split this path-set into multiple path-sets, each contains a single mandatory path. Even when we can not separate each path-sets into all singletons, it is still helpful to divide them into multiple non-disjoin path-sets to reduce the ambiguity of traversal in each path-set.

A pair of edge (e_i, e_j) is called a constraint of e^* if e^* lies between e_i and e_j for any covering tour of the graph, and e^* is called a bridge[2] of the constraint (e_i, e_j). In other words, all paths that can traverse e^*, e_i, e_j have the following forms $e_i \dots e^* \dots e_j$ and we call them the constraint paths of edge e^*. Two bridge e_1^* and e_2^* (if exists) of the same constraint (e_i, e_j) are called independent if none of the constraint paths of e_1^* contains e_2^* and vise versa. For example, in Fig. 2.3, (e_1, e_7) is a constraint of e_2, e_3, e_5, e_6; these four edges are called bridges. Among them, e_2 and e_3, e_5 and e_6 are independent.

If a path-set contains all paths that connects the start edges to the ends edges, we can find a maximum set of edges in these paths, such that they are pairwise independent and transform that path-set to multiple non-disjoint path-sets, each contains the constraint paths of an edge in the chosen set.

(b) Using edge-pairs to remove invalid paths in path-sets — An ad hoc Paired de Bruijn Graph Approach. Since not all paths in each path-set are required to appear in the genome, we can remove the paths that are not supported by mate-pair information (edge-pairs). Edge e_i is called extendible by e_j if the following conditions hold: (1) if e_j follows e_i in the graph. (2) There exist mate-pairs $(e_i, e_i^*, \mathbf{d_i})$[3] and $(e_j, e_j^*, \mathbf{d_j})$ such that either

[2]Note that this terminology is not related to the common meaning of *bridge* in graph theory

[3]Note that after the mate-pair transformation procedure, the histogram in the edge-pair can be replaced by a set of possible distances $(\mathbf{d_i})$

$e_i^* = e_j^*$ and $d_i + l(e_i) = d_j$ or e_j^* follows e_i^* and $d_i + l(e_i) = d_j - l(e_j^*)$, where $d_i \in \mathbf{d_i}, d_j \in \mathbf{d_j}$. (3) There exist mate-pairs $(e_i^*, e_i, \mathbf{d_i})$ and $(e_j^*, e_j, \mathbf{d_j})$ such that either $e_i^* = e_j^*$ and $d_i + l(e_i^*) = d_j$ or e^*j follows e_i^* and $d_i + l(e_i) = d_j - l(e_j^*)$, where $d_i \in \mathbf{d_i}, d_j \in \mathbf{d_j}$. A path $P = e_i e_{i+1} \dots e_{i+t}$ is called a supported path if edge e_j is extendible by $e_{j+1}, \forall j = 1..t-1$ and if the distance between any pair of edges e_m, e_n in the path falls in the insert-size range, there exists an edge-pair of (e_m, e_n) that supports the distance (in the path) from the starts e_m to e_n.

Path-set Graph

Given a collection of path-sets generated from mate-pairs where each path-set contains at least one path that is present in the genome and two different path-sets do not have any path in common, the problem of assembling the genome from this collection is similar to the traditional genome assembly from single reads. Below, we define the path-set graph, namely the connection between path-sets to get longer contigs.

Path p_1 is an absolute prefix of path p_2 if p_2 can be obtained from p_1 by concatenating some non-zero-length path p' to the end/start of p_1. A path p'_2 follows a path p'_1 if they have the following form: $p'_1 = e_1 e_2 \dots e_k$ and $p'_2 = e_2 \dots e_k \dots e_{k+t}$ where $t \geq 0$. A path-set C_1 is called an absolute prefix of path-set C_2 if each path of C_1 is an absolute prefix of at least one path in C_2. The path-set graph is constructed from the collection of path-sets as follows: (1) Remove all the absolute prefix path-sets. (2) Represent each remaining path-set as a node and form an edge from node $C_i \to C_j$ if there exists paths $p_i \in C_i$ and $p_j \in C_j$ such that p_j follows p_i.

Fig. 1(b) illustrates the construction of the path-set graph for a toy example. In this example, there are 8 path-sets. After removing all the prefixes path-sets, 6 path-sets remains. Among them, there are 3 path-sets that contains 2 paths, and 3 path-sets are singletons. After using mate-pair information to remove invalid paths, we obtains all 6 singletons path-sets. The graph is now simple with 3 non-branching paths corresponding to 3 contigs.

Note that the path-set graph shares similarities with both the de Bruijn graph and the overlap graph. It is similar to the de Bruijn graph that the edge $PS_i \to PS_j$ represents a $t-1$ overlapping edges where t is the number of a path in PS_i. It is similar to the overlap graph that the genome corresponds to a covering walk that covers all nodes in the graph. With the input as a set of all mate-pair, the path-set graph platform can be summarized as follows:

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Input: Set of mate-pairs
1: Construct the de Bruijn graph from reads
2: Construct the condensed de Bruijin graph
3: Transform mate-pair into a collection of edge-pairs with better estimated
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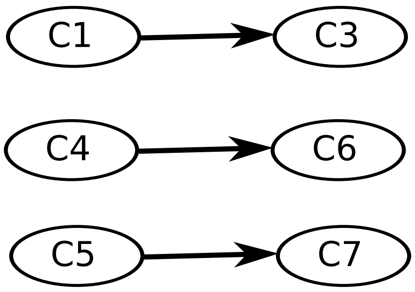

Figure 4: The path-set graph constructed from the graph and mate-pair information in figure 1. Initially there are 8 path-sets: $C_1 = \{e_1e_3e_5, e_1e_4e_5\}$, $C_2 = \{e_1e_3\}$,$C_3 = \{e_3e_5\}$,$C_4 = \{e_2e_3e_5, e_2e_4e_5\}$,$C_5 = \{e_2e_3e_6, e_2e_4e_6\}$,$C_6 = \{e_4e_5\}$,$C_7 = \{e_4e_6\}$, $C_8 = \{e_2e_4\}$. Among them, C_1, C_4 and C_5 each contains two paths. Using the edge-pair information, we remove one invalid path in each of these path-sets. The path-set graph is then constructed by firstly remove all prefixes path-sets (C_2, C_8). The path-set graph has 6 nodes and spell out 3 different contigs.

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   distance.
4: Transform each edge-pair to path-set
5: **Foreach** path-set
6:     Split the path-set by using the graph structure
7:     Remove invalid paths by using mate-pairs
8: Construct path-set graph and output contigs as non-branching paths.
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3 Results

4 Discussion

In this paper, we have presented the path-set graph — a framework for assembling genomes using mate-pairs data. In stead of using mate-pair transformation on a set of mate-pair directly, which is computationally expensive and susceptible to failure when the insert size is high, we first transform them to edge-pairs. The distance between two edges in the edge-pairs can be estimated by using the distribution of all mate-pairs that maps to these condensed edges. Furthermore, all mate-pair transformation operations for mate-pairs that correspond to the same edge-pair can be replaced by a single edge-pair transformation. In different from the traditional mate-pair transformation approach, where a unique path between paired reads in the de Bruijn graph is required, this new approach stores all the paths in a structured called path-set, and latter using the paired information to remove invalid paths and utilizing the underlying graph structure to further distinguish the repeats in each path-set. We latter provide a way to construct the path-set graph to further extend path-set into contig.

Multiple libraries with different insert size can also be utilized in the path-set graph approach. The paired information in different libraries can be used to remove invalid paths in each path-set. A further direction is to extend the path-set to a more stable platform that can work even in the case no path can be found between edge-pair — which is caused by the gap in coverage in most current single cell dataset.

5 Appendix

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