UiO : Department of Informatics
University of Oslo

Esten Høyland Leonardsen

Master's Thesis Spring 2016

Esten Høyland Leonardsen

21st March 2016

Abstract

Contents

List of Figures

List of Tables

List of Theorems

Preface

Chapter 1

Introduction

Chapter 2

Background

2.1 Genetics

Deoxyribonucleic acid (DNA) is a molecule in which living organisms store genetic information. The information is encoded by *nucleotides* bound together by a sugar-phosphate backbone into strands. The nucleotides are smaller molecules which contain one of the nitrogenous bases *Adenine* (A), *Cytosine* (C), *Guanine* (G) or *Thymine* (T). Each of the bases are complementary to another, A with T and C with G. Due to the chemical structure of the nucleotides, a DNA strand can be said to have a direction: Upstream towards the 5' end or downstream towards the 3' end. DNA strands can be connected with a *reverse complementary* strand in a double helix. The two strands will have opposing directions, and every base in one of the strands will be connected to its complement. The paired nucleotides are called *base pairs*. Because either of the strands are easily deduced from the other, DNA is usually represented by only of them. DNA can be seen as a linear sequence of discrete units and can thus be represented by text strings, containing the four leading letters representing nucleotides. The text strings representations often also contain the letter N, referencing *aNy base*.

2.1.1 Genes

"What is a gene?" Helen Pearson. Coding/non-coding, synonymous/non-synonymous

2.1.2 Variation

Genetic information is prone to mutations, either as a result of environmental influence or as a consequence of imperfections in reproduction. The simplest mutations are *point mutations* which affect a single nucleotide base. Point mutations can either be *Single-nucleotide polymorphisms* (SNPs) where a single base is substituted for another, or *insertions* or *deletions* (indels) where a single nucleotide is removed or inserted into the genetic sequence. Mutations can also occur over larger areas of the genome, where longer subsequences can be deleted, inserted, moved or reversed. A

final type of mutations is *Copy number variations* where a longer sequence of DNA, typically at least 1 kb [6], is repeated a variable number of times. CNVs are also called *repeats*

2.1.3 A reference genome

contigs, scaffold, chromosomes. Haplotypes

2.1.4 The human genome

The human genome consists of roughly 3 billion base pairs (bp). These base pairs are spread over 22 paired chromosomes and is assumed to contain about 23 000 genes [14]. The current human reference genome is GRCh38, developed and maintained by the *Genome Reference Consortium* HOWTO: reference websites. GRCh38 contains 261 alternate loci, spread over 178 out of a total of 238 regions. An average human is estimated to deviate from the reference genome in 10.000-11.000 synonymous sites and 10.000-12.000 non-synonymous sites.

Major Histocompatibility Complex

The *Major Histocompatibility Complex* (MHC) is a genetic region spanning approximately 4 million base pairs (mb) [9]. In humans it is located on chromosome 6 and contains about 200 genes. MHC is a region known to contain genes which affect the functionality of the immune system [22]. Even more so MHC is known to be a highly variable region, containing variants that are directly associated with disease [7].

2.1.5 Sequencing

2.1.6 Alignment

Sequence alignment is the process of determining correspondence between text strings, in this case representing DNA, by mapping the elements from one to the elements of the other. The score of an alignment is determined by a *scoring schema*, which provides scores for mapping characters against characters through a *substitution matrix* and penalties for introducing *gaps*.

Definition 1 (Scoring schema)
A structure which provides functionality for mapping bases against bases with a function $mappingScore(c_1, c_2)$ *and for penalizing gaps with a function* $gapPenalty(distance)$*. The structure is defined through a substitution matrix, a gap opening penalty and a gap extension penalty*

Definition 2 (Consistent scoring schema)
A scoring schema is consistent if $mappingScore(c, c) = \max_{a \in \Sigma}(mappingScore(c, a))$ *for all* $c \in \Sigma$.

A gap refers to an element in one of the strings which has no counterpart in the other string when aligned (Fig. 2.1). The scoring schemas can be based around simple match/mismatch scores, which corresponds to the mathematical *Edit distance problem*, or more complex scores (Fig. 2.2). These complex models typically try to model the probabilities behind the physical processes responsible for change. The computational sequence alignment problem consists of finding the highest scoring alignment for any two strings. There exists two main variants of the problem, *global alignments* where to entire strings are aligned against each other and *local alignments* where a string is aligned against a substring of another. The two are traditionally solved respectively by the Needleman-Wunsch and Smith-Waterman algorithms which both are based on *dynamic programming* (Section 2.3.1).

```
ACGGGCCTA
|||| ||||
ACGGACCTA
```

(a) An alignment with no gaps, but one mismatch

```
ACGGGCCTA
||||  |||
ACGG--CTA
```

(b) An alignment with a single gap of length 2

Figure 2.1: Examples of aligned text strings

If more than two sequences are aligned the result is a *Multiple sequence alignment* (MSA). This is typically done on sequences which is expected to share a common ancestor to determine which traits of the individuals arised from the same origins and how the involved species have diverged over time. A final variant of the alignment problem is one involving large databases of sequences, where the algorithms does not only need to find the best alignment between two sequences, but also determine which sequence should be chosen in order to maximize the result. Both of the preceding techniques utilize heuristical methods to decrease the computational complexity.

	A	C	G	T
A	91	-114	-31	-123
C	-114	100	-125	-31
G	-31	-125	100	-114
T	-123	-31	-114	91

Figure 2.2: The HOXD70 substitution matrix

2.2 Graph-based genome representations

Representing genetic information as graphs instead of the traditional linear representations have some major advantages. Graphs are far more expressive structures compared to text strings, able to represent more complex relationships between the elements involved. Secondly, if biological questions can be rephrased to graph theoretical settings, the extensive mathematical field of graph theory can present more feasible approaches to previously hard problems. There is however a major problem: A more complex structure calls for more sophisticated variants of existing methods. Graph-based approaches have been used for some time in the assembly process, and more recently in relation to reference genomes. This section will present both of these approaches alongside some of the remaining unsolved problems. No graph theoretical foreknowledge is needed as all the involved elements will be defined before they are used, but for interested readers there exists good sources in the bibliography [21, Chapter 0] [24, Chapter 9] [1, Chapter 11]. Complexity in regards to the graphs and their operations is discussed using *big-O* notation [24, Chapter 2][1, Section 3.1]

Definition 3 (Graph-based reference genome (Graph))
A pair $G = \{V, E\}$ *where* V *is a set of vertices and* E *is a set of edges.* $|G|$ *denotes the number of vertices in* G.

2.2.1 Representation

Deciding upon the representation of the graph consists of defining the structure of the elements involved, namely the vertices and edges. As the graphs are built from genetic information the basic building blocks, the nucleotides, should obviously be represented. If the input data are more complex than single nucleotides, we must represent the relationships. Because the input data has variation, the structure needs to tolerate flexibility. There is however a risk of making the structures so flexible they present no consistency, and a flexibility/rigidness-tradeoff becomes apparent (fig. 2.3). How the structures are defined in detailed should be determined through the operations which are desirable to perform on them.

de Bruijn graphs

In the article "An Eulerian path approach to DNA fragment assembly"[20], Pevzner, Tang and Waterman proposes *de Bruijn* graphs as a solution to find the correct assembly of repeats during fragment assembly. A de Bruijn graph is a structure where vertices represent *k-mers* from an alphabet and edges represent relationships between the k-mers of two vertices (Fig. 2.4). Pevzner et al. lets the vertices contain strings of length $l-1$ and connects vertices with an edge wherever there exists a read of length l containing the two substrings. Formulating the problem in this fashion lets the problem be formulated as a *Eulerian path* problem, solvable in polynomial time,

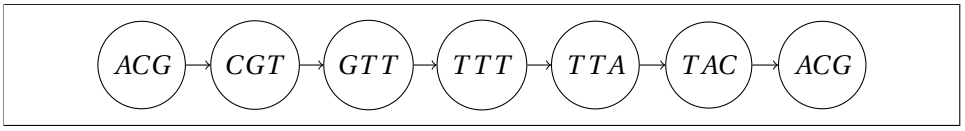
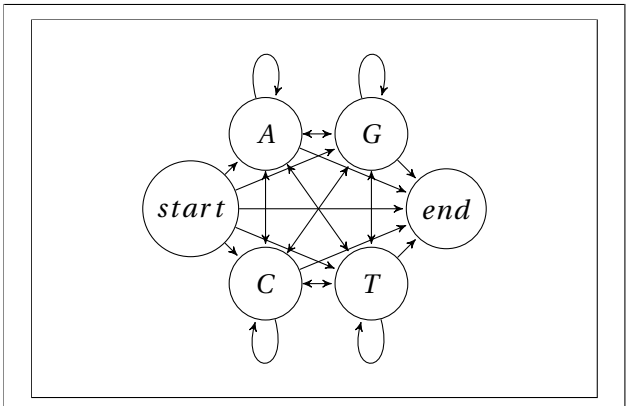
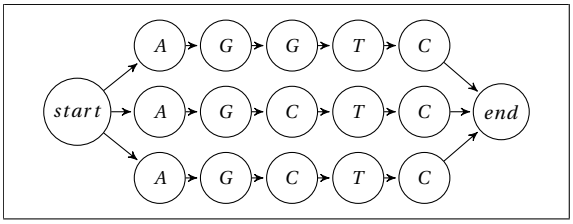


Figure 2.4: A de Bruijn graph with $k = 3$ corresponding to the sequence ACGTTTACG

rather than the traditional "overlap-layout-consensus" method which is equivalent to the NP-complete problem of finding a *Hamiltonian path*[1, Section 11.1]. A great benefit with de Bruijn graphs is that there is no disambiguity: Any legal k-mer has at no point more than one vertice representing it.



(a) A graph with paths corresponding to every possible DNA string



(b) A graph which is built through alignments without allowing variation

Figure 2.3: Two graphs with vertices representing nucleotides and edges representing sequences displaying too much flexibility (a) and (arguably) too much rigidity (b)

A more detailed type of de Bruijn graphs is the colored variant where the origins of edges and vertices are stored as colors. The entire sequence originating from a single individual sample can be seen by following a path with a given color. Similarities between samples can be seen as multicolored stretches, variation take the form of bubbles. Colored de Bruijn graphs can be used for de novo assembly as a more powerful method for detecting variation, compared to traditional assembly techniques[8].

Sequence graphs

The term *sequence graph* stems from the article "Mapping to a Reference Genome structure"[19] and describes a graph structure which is possibly more intuitively pleasing. Every vertice in the graph corresponds to a single nucleotide

from one or more genetic sequences used in building the graph. An edge represents two nucleotides which are consecutive in one of the original sequences. Paths symbolize subsequences of the originating input sequences. To handle the arising problem that the contents of nodes are no longer unique each vertice can be given an index which is exclusive for their associated graph. Whenever a graph is referenced without being specifically classified the following definitions are assumed:

Definition 4 (Graph genome vertice (Vertice))
A pair $v = \{b, i\}$ where $b \in \{A, C, T, G\}$ and i is a unique index. The vertice at index i is denoted v_i. Every graph G also has two special vertices $s_G = \{s, 0\}$ and $t_G = \{e, -1\}$ which represents unique start and end vertices. The notation $b(v_i)$ references the first element in the pair (the nucleotide).

Definition 5 (Graph genome edge (Edge))
An ordered pair $e = \{i_s, i_e\}$ where both elements are indexes for vertices.

Definition 6 (Path)
An ordered list P of indexes such that for all consecutive ordered pairs $\{i_x, i_{x+1}\} \in P$ there exists an edge $e = \{i_x, i_{x+1}\}$.

2.2.2 Mapping

Although the two terms are often used isomorphically we will in this thesis define mapping and alignment as two separate concepts. Mapping is the process of finding relationships between single characters of a string and single elements of a reference genome. Alignment is concerned with finding relationships between consecutive elements of an input string and substructures in the reference genome. A mapping score is defined as the score achieved by looking up two bases in a substitution matrix.

Definition 7 (Mapping score)
A score produced by mapping two characters $b_1, b_2 \in \{A, C, G, T\}$ against a substitution matrix contained in a scoring schema. Referenced by $mappingScore(b_1, b_2)$.

For linear strings mapping is easy. Every string has the same underlying coordinate system, represented by the positions of the characters, and two elements from two separate sequences are either in the same position or they are not. If they are not the difference in position can be derived from the difference between the indexes. Because the indexes of a graph has only one property, uniqueness, they do not hold the intrinsic value of describing relationships between vertices. Any mapping system which uses fixed coordinates would face problems when dealing with a fluent graph able to merge in new information, as the internal relations are bound to change. In de Bruijn graphs the problem is solved by moving the mappable quality away from positions and into the data: For any possible k-mer there either is a corresponding vertice or there is not. In sequence graphs, where nucleotides are the most basic information, there exists an equal number of

identically scoring positions for every base as there are vertices containing that vertice in the graph.

Paten et al.[19] introduce the concept of *context-based mapping* as a solution to the mapping problem when the reference is modeled as a graph. Context-based mapping is an approach where a vertice is identified by the surrounding environment in the graph. More technically a vertice has a set of *contexts* which are paths that pass through the vertice. Because these paths are linear and passes through vertices containing characters, the contexts can be treated as text strings. There are two concrete examples of approaches presented in the article: The *general left-right exact match mapping scheme* and the *central exact match mapping scheme*. The key words left-right and central refer to how a vertice defines it's contexts based on the surrondings. The former defines separate contexts for incoming and outcoming paths whereas the latter defines the vertice as a center of a path where the differences of the lengths of the two contexts are minimized. A *balanced central exact match mapping scheme* is a special case of the latter where both contexts are the same length, and the vertice thus is the center of a k-mer. This is a concept closely related to de Bruijn graphs.

Both of the examples use the word *exact* in their definitions. The term refers to the fact that every context is *unique* to a single vertice which means every possible context either maps unambiguously to a single vertice or does not map at all. Because the graphs have the possibility of branching a vertice can have several contexts contained in *context sets*. Because every context is unique a collection of such will also be unique, which means context-based mapping leads to a two-way unique mapping schema. This is an even strong notion of mappability than positions is strings, as a character of a string does not necessarily map uniquely back to its position. This strong notion has a drawback: There exists situations where a vertice does not have a unique context which yields it unmappable.

2.2.3 Alignment

Intro

Dynamic programming on graphs

PO-MSA

Context-based alignment

Canonical, Stable, General Mapping using Context Schemes

2.3 Techniques and tools

2.3.1 Dynamic programming

Dynamic programming (DP) is a problem-solving technique where a problem instance is solved by combining the results of smaller subproblems. DP is similar to recursion in that every instance is solved by a *recurrence relation* (Equation 2.1) which recurses on smaller and smaller problems until a *base case* is found. A base case represent the bottom of the recursion and is a value which can easily be computed without further lookups. The main difference between recursion and DP is that the latter usually stores its intermediate results to allow for fast lookups for reoccuring instances. DP is often used as an approach for optimization problems in order to minimize computational complexity while giving a guarantee for optimal results [1, Chapter 9]. A problem which is typically solved by dynamic programming is the previously mentioned edit distance problem which utilizes a 2-dimensional array to store the computed values (Fig. 2.5).For two strings S and P, every index $[i, j]$ in the edit distance table represents the problem instance of the strings $S[0 : i], P[0 : j]$. The base cases can be seen in the first row and column. There are often dropped from the table itself due to the simple nature of their computations. The remainder of the table is filled out with the following recurrence relation:

		a	l	g	o	r	i	t	h	m
	0	1	2	3	4	5	6	7	8	9
l	1	1	1	2	3	4	5	6	7	8
o	2	2	2	2	2	3	4	5	6	7
g	3	3	3	2	3	4	5	6	7	8
a	4	3	4	3	3	4	5	6	7	8
r	5	4	4	4	4	3	4	5	6	7
i	6	5	5	5	5	4	3	4	5	6
t	7	6	6	6	6	5	4	3	4	5
h	8	7	7	7	7	6	5	4	3	4
m	9	8	8	8	8	7	6	5	4	3

Figure 2.5: The 2-dimensional array used for solving the edit distance problem for the strings S="algorithm" and P="logarithm" (Note: This follows regular ED scoring where every operation is penalized +1)

$$D[i,j] = min \begin{cases} D[i-1,j]+1 \\ D[i,j-1]+1 \\ D[i-1,j-1]+score(S[i],P[j]) \end{cases} \tag{2.1}$$

where $score(x, y)$ is the identity function. The score for the entire problem

instance can be found in the cell with the highest indexes in the bottom right corner.

There are two separate ways of using Dynamic Programming. A *bottom-up* approach starts at the smallest cases and computes everything until it reaches the actual given problem instances. This corresponds to starting in the top left corner of the edit distance array and computing the cells iteratively moving downwards to the right. A *top-down* procedure starts at the given problem instance and recursively computes every subproblem that is needed. This means starting in the bottom right corner of the 2-dimensional array and recursing upwards to the right. For the edit distance problem the choice of approach bears no big significance as every cell has to be computed either way, but there are problems where using top-down can avoid some computations which are irrelevant to the final result. The latter can also be efficient for heuristical methods where an area of the search space can be overlooked.

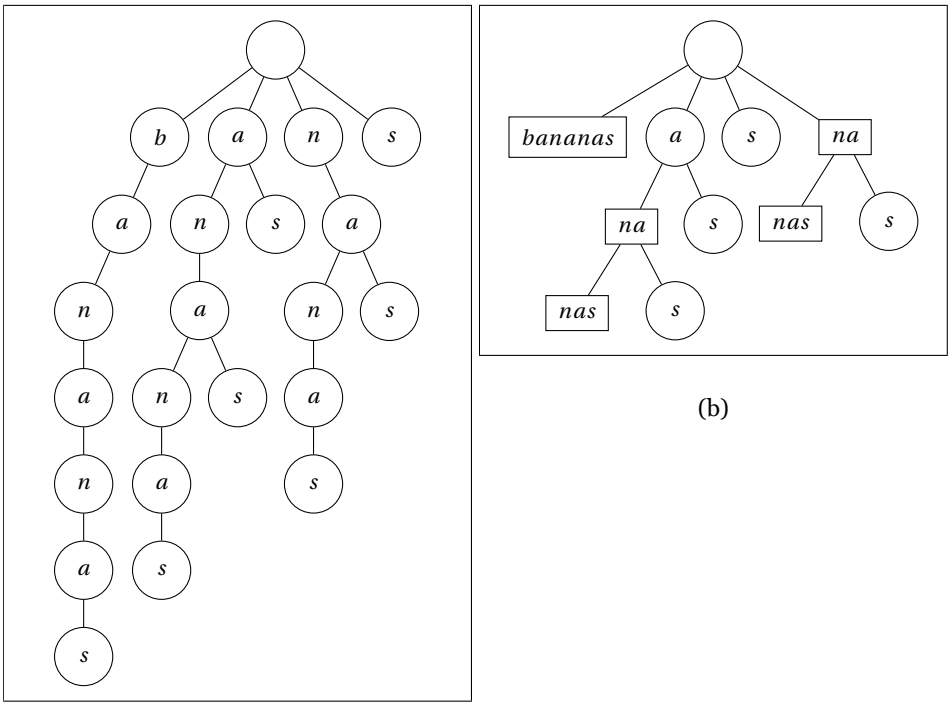
2.3.2 Implementing graphs

2.3.3 Suffix trees

A *suffix trie* is a special tree constructed for specifically for strings of text, containing vertices representing characters (Fig. 2.6a). Every suffix of a string has a corresponding leaf vertice, where the vertices along path from the root to the vertice contains the characters in the suffix. From this it follows that every substring has a matching path starting in the root node. A *suffix tree*, or *compressed suffix trie*, is a suffix trie in which every linear path is compressed into a single vertice (Fig. 2.6b). Suffix trees can easily be extended to hold collections of strings[1, Chapter 20]. An elementary solution has a space complexity of $O(s)$, where s is the length of the string (or the total length of all strings if the tree is built from a collection), and a string of length m can be looked up in $O(km)$ time for an alphabet of size k[1, Section 20.6.1]

A Block-sorting Lossless Data Compression Algorithm

2.3.4 Visualization of graphs: The dot-format



(a)

(b)

Figure 2.6: The suffix tree and suffix trie of the string “bananas”

Chapter 3

Methodology

This chapter will present a formal definition of the problem of aligning DNA strings against a graph-based reference genome and the remaining elements involved. We will also present a the algorithm “Fuzzy context-based search” as solution to the problem through a conceptual overview.

3.1 The problem

3.2 An overview of the involved components

3.3 The solution

Chapter 4

Design

In this section we will present the algorithm "Fuzzy context-based search" as an approach for aligning text strings against graph-based reference genomes. First the remaining elements involved in precisely defining the problem will be described. Then the syntax of the reference graphs used are explained in more detail. Finally, the algorithm is presented both through a conceptual overview and in specific detail. The implementation details refers to the tool *Graph Genome Alignment* (See Supplementary XXX).

4.1 Definitions

Definition 8 (Incomplete path)
An ordered list L of indexes such that for all consecutive ordered pairs $\{i_x, i_{x+1}\} \in L$ there exists a path P which starts at $\{i_x\}$ and ends at $i_{x+1}\}$.

Definition 9 (Input sequence)
A string s over the alphabet $\{A, C, T, G\}$. The length of the string is given by $|s|$. An individual character on position x is referenced by s_x

Definition 10 (Path score)
A score produced by traversing an incomplete path P through a graph G to create a linear sequence, scoring gaps according to the gap penalties given by a scoring schema.

Definition 11 (Alignment)
Given a sequence s and a graph G, an ordered list A of indexes such that every $a_x \in A$ is either a valid index for a vertice in G or 0. 0 indicates an unmapped element of the input sequence

Definition 12 (Alignment score)
Given a sequence s, a graph G and an alignment A, the score produced by combining mapping scores for the pairs $\{a_x, s_x\} for 0 <= x < |s|$ with the path score for the incomplete path(s) provided by A aligned against both G and s.

Definition 13 (The optimal alignment score problem)
For any pair $\{G, s\}$, where G is a graph and s is a sequence, find the

alignment A which produces the highest possible alignment score. If multiple max scores: Provide all or chose one?

Definition 14 (The bounded optimal alignment score problem)
Given a triplet $\{G, s, T\}$ where G and s are as before and T is a numeric value, find the alignment A which produces the highest alignment score, iff alignment the score for A is higher than T. If no such alignment exists, s is unalignable.

4.2 The graph

As defined the graphs involved will be graphs where one vertice represents a single nucleotide. Every vertice also contains an identifying index, which maps uniquely to that vertice. A graph G is made by starting out with only the start and end vertices, and iteratively merging in new sequences. Every sequence merged into the graph has a corresponding path starting in s_G and ending in t_G called a *full path*.

Definition 15 (Full path)
A path p through a graph where the first element is s_G and the last element is t_G

There is no correspondence the other way, meaning there can be paths from s_G to t_G which does not originate from a single sequence (Fig. 4.1). There exists no information storing the origin of an edge and all paths are thus seen as equally probable when aligning a sequence. How the new sequences are merged is defined entirely through the alignment procedure which relies in part on the scoring threshold λ and the given scoring schema.

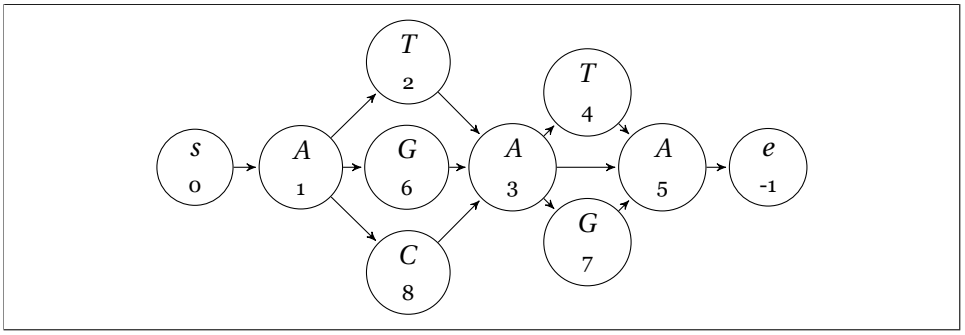


Figure 4.1: A graph G made from the three sequences “ATATA”, “AGAGA” and “ACAA” with 9 valid paths from s_G to t_G

4.3 Aligning sequences

4.3.1 Overview

“Fuzzy context-based search” is the algorithm we propose as a solution to the bounded optimal alignment score problem. The first step of the process is to build a searchable index based on the given graph G. This index is independent from the input sequences to be aligned, and can thus be reused for several searches. The alignment itself consists of two steps: Building a new graph G', from both G and an input string s, and search this newly formed graph for the optimal alignment. Searching for an alignment means combining nodes, representing bases, into a path which represents a linear sequence. This linear sequence can be aligned against the input sequence with regular string alignment tools and is therefore easily scorable. If the algorithm finds an alignment this is guaranteed to be one of the paths which produces the highest possible alignment score for any path in the graph (See supplementary XXX PROOF). There are some situations where the algorithm results in an empty alignment. These cases will occur when there are no paths in the graph which produces an alignment score higher than the defined threshold T, and the sequence s is identified as unalignable. When an empty alignment is provided as a basis for merging a new sequence into the graph, this results in a new full path which is separated from the original vertices (Fig. 4.2)

4.3.2 Building the index

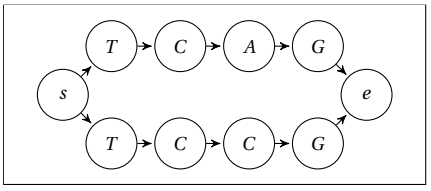
There are two data structures needed for aligning a string against the graph: a suffix tree for left contexts and a suffix tree for right contexts. Before either of the two are built the algorithm needs to decide a length for the contexts. Currently in the tool there are two ways of setting the context length: A user given parameter or an approximation based on the probability of sharing contexts Should probably ref somewhere. The length of the contexts does not impact the quality of the alignments found by the algorithm (See Supplementary XXX PROOF) but will have an impact on the

runtime (See Supplementary XXX COMPLEXITY ANALYSIS).

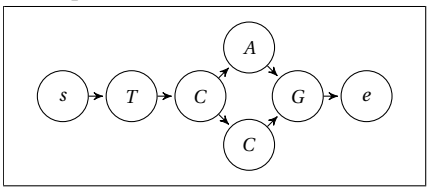
When a context length $|c|$ is set, the algorithm can start building the index. Two sets of strings, a left context set and a right context set, is generated for every vertice in the graph G. The generation of the two sets happen by the same procedure by swapping around the starting point and the direction of the iteration. When creating left contexts the algorithm starts in the start-node of G and traverses following the direction of the edges, for right contexts the opposite is done. Apart from this the two are equal. To generate the context set $c(n_x)$ for a given node n_x the algorithm looks at every string $c \in c(n_y)$ for every incoming neighbouring node n_y. Every c is modified into a new context string c' by trimming away the last character and prefixing the context with the character $b(n_y)$. All the generated strings c' is added to $c(n_x)$. As sets per definition does not allow duplicates the impact of a branching occuring in the graph will fade away after exactly $|c|$ steps as the difference is trimmed away (see Fig. 4.3), and thus avoid explosive exponentiality in the context set sizes. Unlike Paten et al. [19] there are no requirements for contexts to be uniquely mappable to exactly one vertice. Because the last step of the algorithm does a search for an incomplete path through all the found vertices this presents no difficulties when finding the alignment. Furthermore, dropping this precondition assures every node has two valid contexts and are thus present in both suffix trees.

	T	C	A	G
T	0	-1	-2	-3
C	-1	0	-1	-2
C	-2	-1	-1	-2
G	-3	-2	-2	-1

(a) The dynamic programming table for aligning the two strings "TCAG" and "TCCG" using edit distance as a scoring schema



(b) The result of aligning and merging the sequences with $T = 0$



(c) The result of aligning and merging the sequences with $T = -1$

Figure 4.2: Different scoring thresholds T yields different reference graphs

The iteration starts in the node defined as the starting point which has the empty string ϵ as its only context. Whenever a node has finished producing its contexts it enqueues everyone of its outgoing neighbours in a regular FIFO queue. If a vertice has more actual incoming neighbours than incoming neighbours which are finished generating contexts, the node puts itself back in the queue. Thus happens to ensure that when a vertice

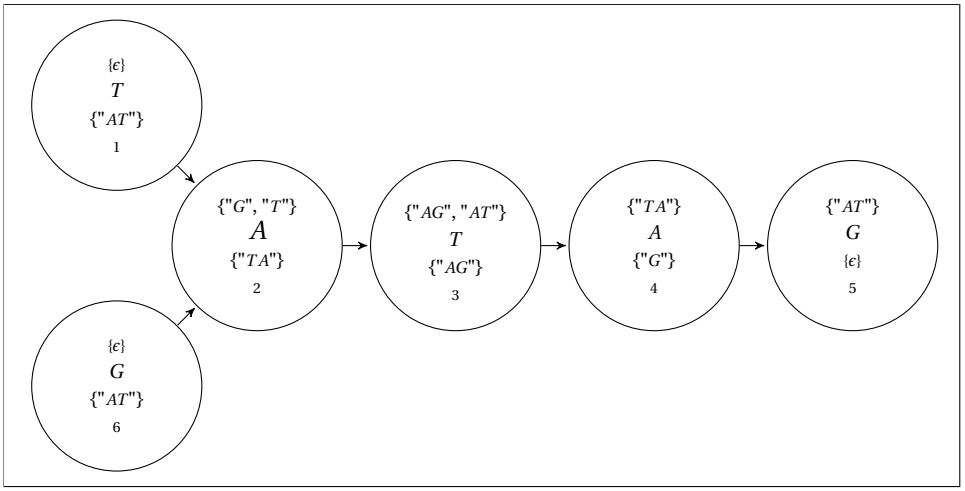


Figure 4.3: A small reference graph with left contexts (top) and right contexts (bottom) of length 2 shown

is finished generating context, both the vertice itself and all preceding vertices are finished, and the remaining nodes can safely fetch contexts from its neighbours. The algorithm halts when the queue is empty. Every node has to be visited exactly once to generate its context, looking up its approximately b neighbours, and as the procedure runs twice to generate both sets the total runtime for the operation is $O(2|G|b)$.

After generating the two context sets for every node, the elements of each one is inserted into their corresponding suffix tree. Every suffix is stored as a key with the index of it's originating node as a value (fig. 4.4). In theory every node can have $4^{|c|}$ contexts in each set, in practice a more fair approximation is $b^{|c|}$ where b is the observed branching factor for the graph. Should contain something about probable values of B. Find an article on it. The current implementation uses a naive suffix tree implementation where insertion is $O(|c|)$ (Discuss more efficient suffix possibilities somewhere?), giving a total time complexity of $O(b^{|c|}|c|)$ per node per context set and $O(2|G|b^{|c|}|c|)$ for the entire graph. Building the entire index can thus be done in $O(2|G| + 2|G|b^{|c|}|c|)$.

4.3.3 Generating the modified graph

The motivation behind building an entirely new graph is the realization that whenever reads are mapped against a reference genome the read is typically vastly shorter than the reference. We can therefore do a *linear pruning* where we determine which parts along the direction are interesting for the alignment. The same argument can be made for extremely complex graphs, where only a small number of the branches are relevant, in a process which can be seen as a *vertical pruning*. The result of both kinds of pruning is a graph consisting of *candidate vertices* which is far smaller in size compared

to the original graph.

Creating G' is the process of determining which vertices qualifies as candidate vertices for a given input string s and how they should be connected. In order to determine actual candidates for the given string, the algorithm needs to know how much *fuzzyness* to allow. This is a measure which decides how different a read can be from its optimal counterpart in the graph before it is categorized as not mappable. The algorithm takes in a fuzzyness parameter λ which can be used to set a threshold $T = maxScore(x) - \lambda$. The maximal score is found by mapping the string x, be it the entire input string or a context string, against itself with the scoring function provided by the scoring schema. Both λ and T is used throughout the entire process as cutoff variables. Whether T is a threshold for the entire string, for a path or for a substring is either explicitly defined or unambiguous in the given context frame.

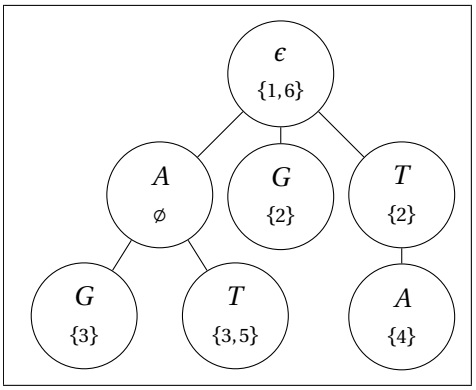


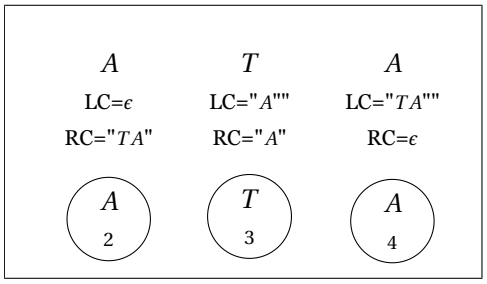
Figure 4.4: The left suffix tree corresponding to the graph in 4.3

After generating the two context sets for every node, the elements of each one is inserted into their corresponding suffix tree. In theory every node can have $4^{|c|}$ contexts in each set. When the graph is more or less linear with few branches a more fair approximation is $B * |c|$ where B is the observed branching factor. The current implementation in the tool uses a naive suffix tree where insertion is $O(|c|)$. This is done for every node in the graph, yielding a total time complexity of $O(|G|B|c|^2)$. A discussion on more efficient suffix structures can be found in SOMEWHERE IN DISCUSSION. Every suffix is stored as a key with the index of it's originating node as a value. The total runtime for building a searchable index for a graph is $O(3|G||c|^2B)$

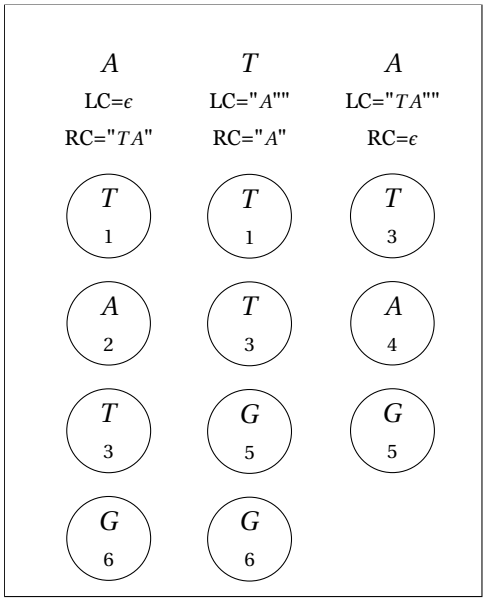
For every character $s_x \in s$ a left-context string and a right-context string is generated by looking at the $|c| + maxPossibleGapGivenFuzzyness(\lambda)$ surrounding characters. The two strings are treated as contexts, one left and one right, and used as a basis for a fuzzy search in it's corresponding suffix tree. The search is a recursive function based on PO-MSA. The root node is supplied with a one-dimensional scoring array corresponding to the context string c, which is initialized with all zeroes. Then, for every child, a new scoring array is computed by regular edit distance rules: For each index i take the maximal score for either a gap in the graph, a gap in the string or matching the character c_i with the character contained in the child node (Reference actual code in supplementary?), (more explanation needed?). This newly created array is supplemented to the same recursive function in

the child. When a leaf node is reached the last index of the supplied scoring array corresponds to mapping the entire string c against the entire context achieved by concatenating the characters contained in the path through the tree traversed by the recursion. If the score is higher than the threshold T for the given context string, every index contained in the node is stored as a pair on the form $\{index, score\}$ in the candidate set. If an index is stored several times, only the pair containing the highest score is saved.

In theory every leaf node has to be visited in order to check the score for every represented context in the tree. In practice the tree can be pruned by cutting off the search whenever the *maximal potential score* falls below the threshold T for the provided context. The maximal potential score for a node is found by adding together the currently highest score in the scoring array with the maximal matching score for the remainder of the string. This reduces the number of nodes to be searched from $O(4^c)$ to (something alot smaller. Needs calculations).



(a) $T = 0$



(b) $T = 1$

Figure 4.5: The resulting candidate sets for mapping the string "ATA" against the reference genome from fig. 4.3 with varying T values

After the fuzzy search is concluded there are two sets of candidates for every index, one containing the nodes matching the left context and an equivalent for nodes matching the right context. These two sets are intersected to produce a final candidate set for the index i, where the score is created by adding together the scores from the two original sets. When the intersection happens the final set can again be pruned by removing all vertices which has a combined score that is lower than the combined threshold T for both contexts. When the vertices are found the edges need to be generated in order to finish the graph. Intuitively there should be an edge whereever there is a gap which is traversable without having the gap penalty exceeding λ. In practice this is a step which is done during the next step of the algorithm.

The newly formed graph G' can be defined formally:

$$G'(G, s, T) = \{V', E'\}$$

where V' is an ordered set of sets of length $|s|$ where each set V'_i is a set of nodes such that

$$V'_i = \{v_x | v_x \in G \wedge \exists [c \in c(v_x)](alignmentScore(c, c(s_i)) >= T)\}$$

and E' is a list of weighted edges such that

$$E' = \{e' = \{i_s, i_e, w\} | v_{i_s} \in V'_x \wedge v_{i_e} \in V'_y \wedge gapPenalty(y - x) <= \lambda \wedge w = distance(v_{i_s}, v_{i_e}) \wedge gapPenalty(w) <= \lambda\}$$

where $alignmentScore(x, y)$ and $gapPenalty(x, y)$ are scoring functions provided by the scoring schema and $distance(x, y)$ is the distance of the shortest path from node x to node y in the graph. (Mixing up nodes and indexes in the definitions)

4.3.4 Searching G' with a modified PO-MSA search

When the candidate nodes for each position has been chosen the next step is to find out how they can be combined into a single linear path. This is equivalent to finding the path through G' which traversal gives the best score within the given scoring schema. Conceptually this is in many ways similar to a regular PO-MSA search. The difference is that the roles are switched: Instead of searching through the reference graph with an input string we are searching through the indices of the string with the candidate nodes from the reference graph as our input. Instead of giving every node a score for every index in the string we give every index of the string a score for every candidate node. These scores are found through dynamic programming by filling out an array *scores* which has the same dimensions as the structure storing the candidate node sets. Because sets are not indexable, the indexes of the candidate nodes are also stored in an integer array $indexes$ such that $indexes[i][j]$ references the index for the j-th candidate node in the set V'_i and $scores[i][j]$ references the score for mapping the substring s' of s spanning the indexes 0 to i to the a path ending in the node with index $indexes[i][j]$. In order to store the actual path yielding the score a third array of pairs, $backpointers$, of the same dimensions, is also needed.

A	T	A
1	1	3
2	3	4
3	5	5
6	6	

(a) Indexes

A	T	A
-1	-2	-2
0	0	0
-1	-3	-2
-1	-3	

(b) Scores

A	T	A
-1:-1	0:0	1:1
-1:-1	0:1	1:1
-1:-1	0:2	1:1
-1:-1	0:3	

(c) Backpointers

Figure 4.6: The 4 arrays used by the searching algorithm when using the candidate sets from Fig 4.5 and $T = -1$

The search is initialized by looping over every node $n_x \in V'_0$ with a counter j, setting

$$indexes[0][j] = x$$
$$scores[0][j] = mappingScore(b(n_x), s_0)$$
$$backPointers[0][j] = -1 : -1$$

Then the nodes $n_x \in V'_i$ for the remaining candidate sets at the indexes $1 <= i <= |s|$ are looped over with j as a counter, and $indexes[i][j]$ is set to x. For every such entry a list of pairs is made with other indexes (i', j') such that i' is a preceding index $i' < i$ and j' is variable looping over $indexes[i']$. Every entry-pair $((i, j), (i', j'))$ can be scored by a scoring function $\theta((i, j), (i', j'))$. The scoring function works by combining the score contained in the preceding entry, $scores[i'][j']$, a gap penalty, and a mapping score for the current index $mappingScore(n_{indexes[i][j]}, s_i)$. The gap penalty is found by combining a gap penalty for a gap of length $i - i'$ and for a gap of length $distance(n_{indexes[i'][j']}, n_{indexes[i][j]})$. The final score stored in $scores[i][j]$

is the maximal achievable score θ produced by one of these pairs. $backpointers[i][j]$ is set to the to the index-pair (i', j') responsible for producing this score. The recursive formulas for the three arrays are defined by:

$$indexes[i][j] = x \quad \text{for } n_x \in V'_i$$
$$scores[i][j] = \max_{i',j'} \theta((i,j),(i',j')) \quad \text{for } 0<=i'<=i, 0<=j'<length(scores[i'])$$
$$backPointers[i][j] = \underset{i',j'}{argmax}\, \theta((i,j),(i',j')) \quad -||-$$

where θ is a scoring function defined as:

$$\theta((x_1,y_1),(x_2,y_2)) = scores[x_2][y_2] + gapPenalty(x_1 - x_2) + gapPenalty(distance(n_{indexes[x_2][y_2]}, n_{indexes[x_1][y_1]})) + mappingScore(b(n_{indexes[x_1][y_1]}), s_{x_1}))$$

There are no restrictions in the recursive formulas to avoid alignments with aligned gaps in both the sequence and the graph. Because both gap penalties are counted the algorithm will however choose a path where only one gap is chosen in all scoring schemas where gaps are penalized with negative values, if such a path exists. By deciding the prioritized order of operations the algorithm can also handle scoring schemas with a gap penalty of 0. Within these types of schemas, the only scenario where an alignment with aligned gaps can be produced are scenarios where there are no valid candidate nodes with context scores larger than T for a subset of indexes i, which means there are no possible traversal of these nodes as a path which would give a score higher than T. This again means the path is not interesting because aligning it against the sequence would result in not mappable.

There are two components of the dynamic programming algorithm where a search is performed to find possible paths: The search backwards in the indexes and the search for distances between nodes in the graph. Both of these searches can be halted whenever the resulting gap penalty exceeds the parameter λ. This means the final time complexity is much closer related to the tunable fuzzyness parameter than the size or complexity of the graph. Because of this the time complexity for the entire dynamic programming step is $O(SOMETHINGIDIDNTPUSHTOGIT)$ (See supplementary XXX complexity analysis).

4.3.5 Handling invalid threshold values

An invalid threshold value is in this context seen as the cases where a string s is aligned against a graph G with a threshold T, but there exists no alignments for s and G which produces a score higher than T. By the definition of the problem this should result in s being unalignable which is equivalent to every element $s_x \in s$ mapping to no vertice in G. There are two cases in which this can happen: Either the algorithm finds an optimal path where the score is too low or there are no valid paths through G'. The fact that these two are the only cases can be proven by looking at their inverse: If none of the above are true there exists atleast one valid path through G and

the algorithm is able to find a path with a satisfactory score.

The first of the cases provides no problems programmatically as the search is able to find alignments, score them and determine which is the best. Why can this alignment not be output as optimal? Firstly one can argue this is done in order to provide consistency regarding the definition of the problem. Another, and more convincing, argument is that the search only find the best alignment present in G'. Already at the point of choosing candidate nodes actual elements of the real optimal alignment can have been pruned away as a result of not having a good enough context scores. Although these alignment do not fall within the strict definition they can be utilized for heuristical applications REF SOMEWHERE. DISCUSSION MAYBE.

Intuitively, the second case can be seen as instances where there are no paths from a set of startnodes indexes to a set of endnodes. Programmatically this is harder, as the edges are computed in real time. The algorithm recognices these instances when it finds a consecutive set of indexes which cannot be traversed without receiving a penalty larger than λ, and every vertice in every one of these candidate sets has no possible backpointers. Because of the possibilities of gaps at the beginning and the end of the alignment, the sets of start and endnodes can't be the candidate sets for the first and last index, but has to include all candidates for all indexes which are reachable without being penalized more than λ.

4.4 Merging aligned sequences

After aligning a sequence s against a graph G there exists an alignment A where every element $s_x \in s$ either has a mapping to an element $v_y \in G$ or does not map to anything. If we model s as a linear graph G_s with a single path, where the vertices have indexes corresponding to their position in s and edges corresponding to consecutive vertices, we can use the alignment as an equality relation such that $s_x \in G_s = v_y \in G \iff a_x = v_y$ to determine overlap between the vertices in G_s and G. The merged graph $G*$ will then contain a vertice set V_{G*} which is equal $V_G \cup V_{G_s}$, using the alignment as our equality determinator. Conceptually this means that every character c which maps to a vertice v is merged into that vertice, whereas every c which does not map to anything creates a new vertice. The edges of $G*$ is created the same way, however here there are 4 possibilites: Mapped vertice to mapped vertice, unmapped vertice to mapped vertice (and its inverse) and unmapped vertice to unmapped vertice. The 3 latter cases can all be generalized to an edge which is not already existing in G, because one of the vertices does not exist in G, and the operation is thus the same. The usage of the union-operator is critical because it ensures both that all new information from the sequence is stored aswell as the fact that the graph does not lose previously seen information, which would lead to deteriation over time.

Chapter 5

Experiments

The following chapter describes the details of the experiments conducted to produce the results in the succeeding chapter. The experiments are divided along a natural border, decided by the size of the input data, into two classes. Each class has its own section describing the motivation behind the experiments and the details specific to that class. Elements which are common to both classes are described once in the section preceding the class-specific sections.

5.1 Proof of concept

Whenever a graph is built from a set of sequences one can get an intuition concering what the final result should look like. These experiments are attempts to formalize the notion of intuition into stable, testable results. Due to readability and shortcomings of printed media only a small set of the experiments are presented here. A more exhaustive set of tests can be found as unit tests in the tool ref tool.

5.1.1 Test data

Because the motivation behind these tests are to determine the behaviour of the algorithm, the input data consists of small, handcrafted sequences which for each experiment contains exactly one easily identifiable trait. These traits are crafted in a way which reflects the nature of variation in genetic sequences. Because the negated edit distance scoring schema is a flat scoring schema which penalizes all errors the same it is prone to display order of operations characteristics of the underlying algorithm. Because the order of operations of the implementation is well known to the authors this is taken into account when creating the data.

5.1.2 Validation

There are two main concepts which the validation of this experiment class wish to capture: The intuition and the formalization. The intuition is captured through visualizable results. Every experiment will be provided

with a visualization of both the inputs and the outputs. The output visualizations will be directly produced by the tool using the -print parameter, by porting the resulting dot-file to the tikz syntax used in this thesis. The formalization is carried out through a set of statements from first order logic concerning the state of the visual output. The previously mentioned unit tests are created be representing these statements through Java syntax.

5.2 Efficiency

In order to determine the usefulness of an approach it should be compared to other approaches. The goal of these experiments is to run the implementation in the tool against similar applications to determine the grade of correctness and the computational feasibility of the approach. Because of its stableness PO-MSA2.2.3 is chosen as a baseline, through an own implementation

5.2.1 Test data

5.2.2 Validation

5.3 Common elements

5.3.1 Scoring schema

The scoring schema used in all the experiments will be regular edit distance where mismatches and gaps yield a penalty of -1. The reason for the negation compared to "regular" edit distance is that the implemented algorithm seeks to maximalize a score, not minimalize a loss. Edit distance is used because of the intuitive nature of the scores involved. A final result of $-x$ means there is something wrong exactly x places. This provides a good foundation for validating the qualitative tests visually.

Chapter 6

Results

6.1 Proof of concept

6.1.1 Equal sequences

6.1.2 SNPs

6.1.3 Indels

6.1.4 Structural variations

6.2 Efficiency

6.2.1 Building the index

6.2.2 Runtime as a function of |G|

6.2.3 Runtime as a function of |s|

6.2.4 Runtime as a function of λ

Chapter 7

Discussion

Chapter 8

Conclusion

Chapter 9

Further work

9.1 Improvements to the algorithm

9.2 Heuristical approaches

Bibliography

[1] Kenneth A. Berman and Jerome L. Paul. *Algoritms: Sequential, Parallell and distributed*. Thomson/Course Technology, 2005.

[2] M. Burrows and D.J. Wheeler. 'A Block-sorting Lossless Data Compression Algorithm'. In: (1994).

[3] Deanna M. Church et al. 'Extending reference assembly models'. In: (2015).

[4] The 1000 Genomes Project Consortium. 'A map of human genome variation from population-scale sequencing'. In: (2010).

[5] Alexander Dilthey et al. 'Improved genome inference in the MHC using a population reference graph'. In: (2015).

[6] Jennifer L. Freeman et al. 'Copy number variation: New insights in genome diversity'. In: (2006).

[7] Roger Horton et al. 'Variation analysis and gene annotation of eight MHC haplotypes: The MHC Haplotype Project'. In: (2008).

[8] Zamin Iqbal et al. 'De novo assembly and genotyping of variants using colored de Bruijn graphs'. In: (2012).

[9] Charles A. Jr. Janeway et al. *Immunobiology: The Immune System in Health and Disease. 5th edition*. 2001.

[10] Schneeberger K. et al. 'Simultaneous alignment of short reads against multiple genomes'. In: (2009).

[11] Birte Kehr et al. 'Genome alignment with graph data structures: a comparison'. In: (2014).

[12] Christopher Lee, Cathrine Grasso and Mark F. Sharlow. 'Multiple sequence alignment using partial order graphs'. In: (2001).

[13] Arthur M. Lesk. *Introduction to Bioinformatics*. Oxford University Press, 2014.

[14] Artur M. Lesk. *Introduction to genomics*. Oxford University Press, 2012.

[15] Shoshana Marcus, Hayan Lee and Michael Schatz. 'SplitMEM: Graphical pan-genome analysis with suffix skips'. In: (2014).

[16] Joong Chae Nal et al. 'Suffix Array of Alignment: A Practical Index for Similar Data'. In: (2013).

[17] Ngan Nguyen et al. ‘Building a Pan-Genome Reference for a Population’. In: (2015).

[18] Adam Novak et al. ‘Canonical, Stable, General Mapping using Context Schemes’. In: (2015).

[19] Benedict Paten, Adam Novak and David Haussler. ‘Mapping to a Reference Genome Structure’. In: (2014).

[20] PA. Pevzner, H. Tang and MS. Waterman. ‘An eulerian path approach to DNA fragment assembly’. In: (2001).

[21] Michael Sipser. *Introduction to the Theory of Computation*. CENGAGE Learning, 2013.

[22] Simone Sommer. ‘The importance of immune gene variability (MHC) in evolutionary ecology and conservation’. In: (2005).

[23] Esko Ukkonen. ‘On-line construction of suffix trees’. In: (1995).

[24] Mark Allen Weiss. *Data Structures and Algorithm Analysis in Java*. Pearson Education, 2007.