PhD Proposal

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Introduction

The problem of *pattern matching* is one of the most studied topics in the field of algorithmics and bioinformatics. The interest in such problems is due to the need to align sequences or to search for specific patterns within the *DNA*. In this context, a large number of data structures and algorithms have been modeled. Among these, one of the most used is the **Burrows-Wheeler transform (*BWT*)**, thanks to the studies of Ferragina and Manzini, who proposed its use together with indexing via *FM-index*.
Furthermore, in recent years, there has been a change of interest in the field of bioinformatics. Until a few years ago the research was focused on the study of a **linear sequence of a genome** while now the researchers are beginning to deepen the topic of the **pangenome**, which term was introduced by Tettelin in 2005. In fact, the need to take into account the high variability in population genomes as well as the specificity of an individual genome in a personalized approach to medicine is rapidly pushing the abandonment of the traditional paradigm of using a single reference genome [1].
Thanks to the last developments in sequencing technologies, which had led both to reduce the costs of single sequencing and to produce sequences of ever higher quality in less and less time, the researchers were able to theorize the **pangenome graph**. Furthermore, the new amount of sequences has led to new algorithms regarding the problem of *pattern matching*. In 2021, Rossi et al. proposed *MONI* as a data structure to handle a **run-length encoded version of BWT (*RLBWT*)**, with the ultimate intention of indexing and using multiple genomes as a reference [2]. Together with this data structure, the authors proposed the concept of **matching statistics (*MS*)** in order to efficiently compute the matches between a pattern and a text. A recent improvement has been made through the implementation of *PHONI* [3], where the **longest-common-extension (*LCE*) queries** in order to further optimize the pattern search.
From a biological point of view, it is interesting to note that, as it has been pointed out by the study of *1000 Genome Project*, there are over 88 million variants between those human genomes. Among these variants 84.7 million are **Single Nucleotide Polymorphisms (*SNPs*)**, 3.6 million are **short insertions/deletions (*indel*)** and 60000 are **structural variants**, involving more than 50 nucleotides. All of them are now a limit to the traditional use of a *linear sequence of a genome*.
Against this background, various algorithms and data structures have been implemented in order to study *haplotypes* and *genotypes*, such as the **genotyping variants problem**. Briefly, we could define *haplotypes* as a combination of allelic variants, each one inherited from a parent. Instead, the *genotype* is the complete set of genes contained in the DNA. So, from 2005, publication of the **genome-wide association studies (*GWAS*)** has begun. The goal of this type of studies is to screen the *pangenome* looking for associations between genetic variants, in order, for example, to study also deseases outocomes. In this particular historical period it is also impossible not to mention the viruses. In fact, by nature, during infections, viruses replicate a lot but often in a not perfect way, thus producing many inexact clones, referred as **viral haplotypes** which, taken together, form the **viral pangenome**. The identification of all this haplotypes is crucial both for the effective study of the spread of the virus and for the production of effective drugs, in a content of high pharmacological resistance.
One of the most important data structure, developed in order to handle the study of haplotypes sequences, is the **positional Burrows-Wheeler transform (*PBWT*)**, proposed by Durbin in 2014 [4]. Using this particular data structure (which will be described below), it is possible to study efficiently a collection of haplotypes but only in the bi-allelic case. Furthermore variants of the *PBWT* have been studied for handling the multiallelic case. The use of the *PBWT* is found in many software for the study of haplotypes and in various genotype imputation methods, that are studies that infers unobserved genotypes in a sample of individuals [5].
During the development of my master thesis, I worked to create a run length encoded variant of the *PBWT*, the **RLPBWT**, using and adapting the various theories developed for the *RLBWT*, in collaboration with

the authors of *MONI* and *PHONI*. In this context, my PhD is going to be focused on the development of new algorithms in various topics related to open problems in the study of the *haplotyping/genotyping*, of the *genome variants* and of the imputation issues related. My intention is also to deepen experimental themes of *pattern matching* and of the *pangenome graph*, in detail the new developments on BWT and indexing structures, as well as *succinct data structures*, with particular attention to the use of *bitvectors*.

State of the art

Now I present a brief overview of the main algorithms, data structures, methods etc ... that will be the core of my studies during PhD.

Bitvectors **Bitvectors** are one of the most important data structure when mentioning *succinct data structures*.
A *bitvector* is an array on n bits which allows two particular operations, called **rank** and **select**, in addition to the classic operations on boolean arrays, such as *random access* in *constant time*. More in detail, the *rank function* allows to calculate how many occurrences of one are up to a certain index. Instead, the *select function* allows to obtain the index of every one present in the *bitvector*. Formally, given a bitvector B, such that $|B| = n$, and given an index i, such that $0 \leq i < n$, we can define $rank_B(i) = \sum_{k=0}^{k<i} B[k]$. Instead, about the select function, given an integer i, such that $0 < i \leq rank_B(n)$, where $n = |B|$, we can define $select(i) = \min\{j \mid rank_B(j+1) = i\}$.
From a theoretical point of view these two operations can be supported in *constant time*, with the additional cost of $\mathcal{O}(n)$ bits in memory. In more practical terms, there are several implementations of the same within **SDSL (*Succint Data Structures Library*)**, one of the most important C++ library used in bioinformatics. As the implementation changes (for example *plain bitvector, interleaved bitvector, sparse bivector* etc...) the computational time of the two operations varies (usually only one of the two is in constant time) as well as the amount of additional bits needed. An example of the use of bitvectors is tracking the runs in the run-length encoded implementations of BWT and $PBWT$, where we put one at each head of run, allowing fast operations along the runs themselves.

RLBWT The **Burrows-Wheeler Transform (*BWT*)** was introduced in 1994 in order to compress texts but it has been used widely in bioinformatics, above all thanks to the already cited *FM-index*.
Speaking of *pangenome*, linear indexing via FM-index is no longer the best solution as it does not handle the large repetitions there are in this new type of sequences. In 2005 Mäniken and Navarro defined the **Run-Length Burrows–Wheeler Transform (*RLBWT*)**. Given a text T, $RLBWT_T$ is a rappresentation of BWT_T with a compact storage of consecutive equal characters, the so-called *runs*. With this new perspective, the algorithms have changed from being linear over the length of the text, n, to being linear over the number of runs, r, so sub-linear over the length of the text.
The new indexing method, introduced by Gagie et al., was called **r-index** and it corresponds to the $RLBWT$ plus the *suffix array sampling* at the beginning and at the end of every run. The algorithm for querying through the $RLBWT$ takes advantage of other methods, such as the use of **thresholds** (minimum LCP value between two consecutive runs of the same character) in *MONI*, and the use of **longest common extension (*LCE*) query** (to compute the right equal common extension between two position in the text) in *PHONI*. Both solutions use **straight-line programs (*SLP*)**, for *random access* in *MONI* and for *Longest Common extensions (LCE) queries* in *PHONI*. The purpose of the two projects is computation of the **matching statistics (*MS*)**. Given a pattern P and a text T, the MS of P in respect to T is an array M of pairs position/length (pos/len), $|M| = |P|$, such that $T[M[i].pos : M[i].pos + M[i].len - 1] = P[i : i + M[i].len] - 1]$ and $P[i : i + M[i].len]$ does not occur in T. Given MS, we can compute every **Maximal Exact Match (*MEM*)** of a pattern in a text. Given a text T and a pattern P, a substring of the pattern $P[i : i + l - 1]$, of length l, is a MEM of P in T if $P[i : i + l - 1]$ is a substring of T but neither $P[i - 1 : i + l - 1]$ nor $P[i : i + l]$ are, so if the substring cannot be extended either right or left. Furthermore, using a particular function called φ (and and its inverse φ^{-1}), based on the use of the **inverse suffix array (*ISA*)**, it has been possible to find all starting positions of all copies of P in T from starting position of the match extracted by MS, quickly calculating, given a position p in SA, the previous and next position in the suffix array.
Thanks to these and other methods, it was possible to perform pattern matching efficiently even on long sequences of nucleotides, such as those studied in a pangenomic context. In fact, most studies in the field

of bioinformatics start with the resolution of pattern matching problems and, as a direct consequence, of alignment problems.

PBWT Based on the theories of the *BWT*, in 2014, Durbin devised the **positional Burrows–Wheeler transform (*PBWT*)**, in order to solve the problem of pattern matching on panels (matrices) of haplotypes. In detail, he analyzed a panel X with M haplotypes and N biallelic sites. This data structure is based on a *reversed-prefix ordering* at each column k that produces two different multidimensional arrays. The first one is the set of the **prefix arrays**, denoted by a, which contains the index of the haplotype m in the original panel, for each column k and for each position i of a_k. More formally, we can say that $a_k[i] = m$ iff X_m is the i-th haplotype in the reversed-prefix ordering at column k. We can note x_m, such that $a_k[i] = m$, could be denoted by y_i^k. The second bidimensional array is the set of the **divergence arrays**, denoted by d, which indicates the index of the starting column of the longest common suffix, ending in column k, between a row and its previous one, at reversed-prefix ordering at column k. More formally, we can define $d_k[i] = h$ iff h is the smallest column index such that $y_i^k[h, k) = y_{i-1}^k[h, k)$.
Thanks to these two bidimensional arrays, it is possible to compute all matches within X longer than a minimum length L, all set-maximal matches within X in linear time and all set-maximal matches (which we could also call "MEMs") from a new sequence z to X in $\mathcal{O}(M^2N)$. Despite the fact that *PBWT* has been poorly regarded in the scientific community in the early years of its development, there has been a growth in research based on it, both in terms of variant design, such as the already mentioned multiallelic version or the **dynamic PBWT (*d-PBWT*)**, and in terms of its use to study haplotype panels.
A first example could be found in the paper of Alanko et al., published in 2021, where the authors used the *PBWT* to look for *maximal perfect haplotype block*, within a haplotypes panel. These types of algorithms are essential for the identification of genomic regions that show signatures of natural selection.
Another interesting paper is the one by Wlliams and Mumey, published in 2020, who also studied *maximal perfect haplotype blocks*, but with the addition of *missing data*, handled with the help of wildcards, using the *PBWT*.
A very intriguing tool, talking about *GWAS*, to be cited is **IMPUTE5**, proposed by Rubinacci et al. in 2020. The main purpose of this software is to make genotype imputation in order to predict unobserved genotypes from a panel with milions of haplotypes. Due to the size of the panel, the use of *PBWT* proved inevitable, further demonstrating the importance of this data structure.
Durbin himself, the author of the *PBWT*, with Shchur et al. in 2019, proposed a use of his data structure in *GWAS* context. Infact they studied the associations between genetic variants in order to identify signals of natural selection to build the so-called **ancestral recombination graph**, that contains complete informations about samples history.
For the sake of completeness, an example where *PBWT* is not used is **Ranbow**, proposed by Moeinzadeh et al. in 2020. The aim of this project was the haplotype reconstruction of polyploid genome from short read sequencing data, studying also the multi-allelic case.

Research goals

Therefore, for my master's thesis, I had to rethink the concept of *Matching Statistics* for *PBWT* (tracking a *row* of the panel instead of the *pos*), how to compute the *SLP* for the panel, how to use *thresholds/LCE queries* and how to get the same behaviour of the φ function, in order to obtain the **RLPBWT**, combining the ideas related to the *RLBWT* with those of the *PBWT*, eventually, as for example for the φ function, developing a simple new data structure.
Obviously, there are some limitations, as in Durbin's *PBWT*, such as studying only biallelic panels and ignoring the management of missing data, which are very frequent in real cases.
Especially during my master's degree I have deepened both the most theoretical issues related to algorithms, especially in the field of bioinformatics, that the most modeling and inference issues, related to the field of computational systems biology, so it is my interest to continue my studies with the PhD in order to be able to deepen the computer science potential in the biological context. Summarizing, some of the research goals of my *Phd* are:

1. **Multiallelic data**. Thanks to the increasing growth in the production of genotype data the number of multiallelic sites is expected to grow, as the number of samples increases, even in the human genome, although now, for example, there is only a 2% presence of triallelic sites.

Moreover, not only could such sites be more than expected but they are usually not considered by most tools. A first step in this direction was made by Naseri with the already cited *m-PBWT*. From the point of view of spatial complexity, especially about stored arrays for the *FM-index*, a run-length encoded version of the same could allow the management of increasingly large data for the imputation phases. In this context the aim is to implement a new version of the *RLPBWT* that can handle multiallelic data, adapting the current use of bitvectors to handle efficiently the *LF-mapping* also with more than two alleles.

2. **Missing data**. A second extension, that would be more complicated to handle, is a *RLPBWT* version that admits the presence of missing data. Most algorithms and data structures mentioned above assume that they work on exact data. In reality, real data can contain errors or even gaps, both mainly due to sequencing machines that are not yet perfect, although they now have very high correctness rates. Unfortunately, although most of them are corrected as a preliminary step (mostly by heuristic methods), this is still an open problem. The development of a data structure based on the *PBWT*, and as a next step on the *RLPBWT*, that can manage missing data would allow the creation of more and more complete tools for the study of haplotypes and for the various *GWAS*. Handling missing data is known to be *hard* so I should probably deal with parametric algorithms or approximate algorithms, based on researches already developed in *BIAS*.
3. **Genotype imputation**. As introduced, having developed a wide-range interest in computer science potential in biological studies, I do not intend to forget the actual use of the data structures that I will study and develop during my PhD. Including the management of missing data and multiallelic data can further improve the state of the art of genotype imputation, allowing even more precise inference of unobserved genotypes, and, in more detail, of *GWAS*. All this must be read in the perspective of a continuous increase of the available data, moreover not not only regarding the human sequencing.

From a more technical point of view I mainly focused on the use of the **C++ programming language**, during both my bachelor and master thesis projects. I managed to do this because of the availability of efficient libraries, as the already cited *SDSL*, that has become a standard in bioinformatics. However, in addition to *C++*, I had the opportunity to deepen **Python**, with libraries such as *biopython*, and **Rust**, with recently developed libraries such as *bio-rust* (even if still not complete from an algorithmic point of view). Another point of interest is the analysis and the development of efficient algorithms based on parallel computing (also on GPU), that are increasingly in use in both *bioinformatics* and *computational systems biology*.
To conclude this proposal, I also would point out the intention to remain in contact with various researchers, with whom I have already collaborated during my master thesis project, including Christina Boucher (University of Florida) and Travis Gagie (Dalhousie University), in order to improve the quality of my PhD program.

References

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