DNA Analysis: Principles and Sequencing Algorithms

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Abstract: DNA discovery has put humans one step closer to deciphering their own structure stored as biological data. Such data could provide us with a huge amount of information, necessary for studying ourselves and learn all the variants that pre-determine one's characteristics. Although, these days, we are able to extract DNA from our cells and transform it into sequences, there is still a long road ahead since DNA has not been easy to process or even extract in one go. Over the past years, bioinformatics has been evolving more and more, constantly aiding biologists on the attempts to "break" the code. In this paper, we present some of the most relevant algorithms and principles applied on the analysis of our DNA. We attempt to provide basic genome overview but, moreover, the focus of our study is on assembly, one of the main phases of DNA analysis.

1 INTRODUCTION

Deoxyribonucleic acid, known as DNA, was firstly discovered and introduced by Watson and Crick (Watson and Crick, 1953). Authors stated that any existing organism's molecule stores all the necessary data for its living and grown by carrying instructions that are necessary for constant development and overall organism's functioning (Saenger and Wolfram, 1984), (Alberts et. al, 2002). For us, humans, DNA contains most of our characteristics as a person, such as, adaptation, character and so on but, more importantly, information about creation of proteins that form all of our parts. This protein is used by our organism to produce all the necessary substances, for example, keratin or our hair and nails, bio functioning proteins that transport oxygen inside our body and so on. DNA structure is presented as double helix, where on one helix are located Adenine (A) and Guanine (G) and on the other helix Thymine (T) and Cytosine (C). While creating the double helix, A connects with T and, respectively, C with G. Those are called base pairs.

Currently, there are several challenges related to the DNA study and manipulation. One of the limitations of the current technology is the capability of extracting the entire DNA chain, only parts of DNA may be read. That means that, these days, it is not possible to extract the entire genome. More than that, extracted parts can be easily corrupted, either by extraction errors or by transcription errors. Moreover, even if the part of DNA that is analyzed was correctly processed, there is a possibility of that sequence being just a part of necessary sequence or not meaning anything (trash). That means that even if the code was extracted correctly, the extracted sequence may not have expected importance. Another challenging task that follows is genome assembly.

In this paper we attempt describing some of the challenges that are related to the genome assembly as well as some of the current possibilities. Currently there are a lot of studies and papers, focusing on extracting ever more of the necessary and important data. However, we consider that it may be challenging for someone to begin study bioinformatics. Therefore, we describe the assembling algorithms and the origin of those as well as their base, so readers can understand some of the associated challenges with genome sequencing.

The remaining of the paper is structured as follows: Section 2 describes related work and some of the studies that have been performed over the past years. Section 3 describes some of the available assemblers that are used for extracting and sequencing DNA. Section 4 provides an overview over algorithms that are used for DNA sequencing (genome assembly). Finally, section 5 presents our conclusions and future work.

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2 RELATED WORK

DNA study has been a hardly invested area over the past years and, therefore, there is a lot of data about genetic study and evolution. Munib et al. (2015) present and describe the entire process of shotgun sequence assembly in the realm of high-performance computing. They describe main phases of genome assemble and some of the algorithms that may be used among those phases. Also, Mount (2004) provides an examination of the computational methods needed for analyzing DNA, RNA – structure that DNA is transformed after has been extracted from the nucleus of the cell, and protein data, as well as genomes. Differently, we focus our paper on one specific area, providing more detail, instead of entire process overview. Polyanovsky et al. (2011) present a comparison approach between alignment algorithms. Authors based they work mainly on Polyanovsky et al. (2008), Sunyaev et al. (2004), Domingues et al. (2000) and compared results produced by local and global alignment and concluded that both local and the global alignment algorithms rely on positions and relative lengths of the parts of the sequences that will be aligned and depending of the sequence positioning, global or local algorithms provide better results. Global algorithm proved to that when the core part of one sequence was positioned above the core of the other sequence but when the cores were positioned asymmetrically, the local algorithm was more stable. Posada (2009) describes methods to analyze DNA sequences as well as some available tools that are free to use, describing recognition of similar sequences using BLAST, sequence alignment, DNA compositions and molecular evolution and other. This book is more oriented for biologists that do not have much knowledge about computer science and, moreover, have a bigger biological background that most of computer engineers do not have. Jones and Pavel (2004) provide a large overview over existing algorithms that are used in bioinformatics by presenting the foundations of algorithms, describing principles that drove their design, and their most important results. DNA sequencing challenges and processing are described in El-Metwally et al. (2013), Niedringhaus et al. (2011), Voelkerding et al. (2009), Zhou et al. (2010), Wheeler et al. (2008). Authors describe some of the past and current technologies, solutions for DNA sequencing and available next-generation sequencing (NGS) platforms and their future (Hert et al., 2008). NGS platforms perform sequencing of millions of small fragments of DNA in parallel which means that millions of small fragments of DNA can be sequenced at the same time, creating a massive pool of data. All the sequence fragments are analysed several times in order to reduce error possibility.

3 GENOME ASSEMBLERS

After years of genome study and research there is a variety of assemblers used to extract and transcript genome. Constant interest and technological evolution have been contributing for different generations of assemblers, attempting to provide better results and surpass each other. The first next-generation DNA sequencing machine, the GS20, was introduced to the market by 454 Life Sciences in 2005 (Gilles et al., 2011) That technology was based on a large-scale parallel pyrosequencing system, which relies on fixing DNA fragments to small DNA-capture beads in a water-in-oil emulsion.

While trying to understand what is genome assembling and what its stages are and elements that are take part, one of the first things we hear of is de-novo assembly. So what is de-novo how it is important? De-novo is a method of new generation sequencing that attempts to sequence genome without any reference. That means that short reads are not mapped against original sequence (when it is available). On the other hand, reference assembling considers available parts of the sequence (reads) and only considers those that can be mapped into an original sequence. It may be used to assemble genomes of already extinct organisms (Era7, 2016).

One of the first challenges consisted of the extractions of the DNA from the cells. Biological advance and evolution had contributed to this task and currently it is possible to extract DNA. That possibility would provide all of the answers to one's "construction" and allow us to manipulate our own constituent and fight many diseases. DNA structure is presented as double helix, where on one helix are located Adenine (A) and Guanine (G) and on the other helix Thymine (T) and Cytosine (C). While creating the double helix, A connects with T and, respectively, C with G. Those are called base pairs.

After extracting a DNA sequence (input), it is processed by overlapping the extracted sequences. This overlap process is called sequence alignment and it allows creating a larger sequence that contains parts of small pieces. The output of an assembler is generally decomposed into contigs, or contiguous regions of the genome which are nearly completely resolved, and scaffolds, or sets of contigs which are approximately placed and oriented with respect to each other (Gregory, 2005). Currently DNA sequencing technology allows short reads of 500 and up to 700 nucleotides. This sequences are parts from one complete DNA genome.

Genome assembling from shorter sequences is like reassembling the magazine from the millions of small pieces of each page. In both cases will exist errors. In case of magazine it would be ragged edges that difficult putting pieces together. In case of genome assembly those would be reading errors. More than that, pieces may be lost in both cases and also, in case of DNA, nucleotides may be transcripted incorrectly. Nevertheless, efforts to determine the DNA sequence of organisms have been remarkably successful, even in the face of these difficulties. Salzberg et al. (2011) describe some of the most popular Assemblers.

4 ALGORITHMS

In the 1950s, Seymour Benzer applied graph theory to show that genes are linear (Jones and Pevzner, 2004). Therefore, in this section we will describe some of the most popular algorithms for genome assembly, while most of those being Graph algorithms. Short read assemblers generally use one of two basic algorithms: overlap graphs and de Bruijn graphs. The overlaps between each pair of reads is computed and compiled into a graph, in which each node represents a single sequence read. This algorithm is more computationally intensive than de Bruijn graphs, and most effective in assembling fewer reads with a high degree of overlap (Illumina¸ 2010). De Brujin Graph started from Bridges of Königsberg problem (Compeau et al., 2011). The bridges problem is presented on the Figure 1.

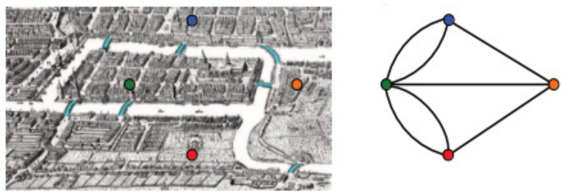


Figure 1: Bridges of Königsberg (Compeau et al.,2011).

The problem statement consisted on passing each bridge only once considering that the islands could only be reached by the bridges and every bridge once accessed must be crossed to its other end. The starting and ending points of the route may or may not be the same. This problem was firstly solved by Leonard Euler, a Swiss mathematician, physicist, astronomer and logician. At first Euler analysed the problem and decided that it could not be solved (Paoletti, 2011). However, Euler could not just accept that. He decided to create a logical formula for this type of problems. Euler observed that in order to be able to solve this type of problems it would be necessary zero or two nodes with odd degree. This theory was posteriorly proved by Carl Hierholzer. This author stated that in order to be able to solve a graph using Euler's path, each connected node has to have an even degree, except for the starting and terminal nodes (Barnett, 2009). On August 26, 1735, Euler presented a paper containing the solution to the Konigsberg bridge problem. He addressed Bridges of Königsberg, problem as well as provided a general solution where any number of bridges could be used (Hopkins and Wilson, 2004).

In this section we describe some of the basic DNA assembly algorithms and problems that those try to solve.

4.1 Shortest Superstring Problem

Since all the DNA reads are performed as parts, it is necessary to be able to overlap those and create one superstring that would better represent and include considered pieces of sequences. This is important since different sequences may be part of the same superstring. However, this superstring creation may be challenging. As more simplistic approach, superstring could result from complete concatenation of available reads. But this approach would not be very correct. Best superstring created should be the shortest one possible. That would represent the sequence that somehow concatenates available reads but, also, does not contain unnecessary information.

INPUTS: Set of sequences.

OUTPUT: One sequence that contains all the input sequences while being as short as possible.

In order to be able to create one superstring it is necessary to align input sequences and be able to identify which ones may be same part of different reads. We should be able to define any overlapping regions and after that create one complete sequence. Below we present an example of Shortest Superstring Solution. Given:

INPUTS: {00, 01, 10, 11}

FULL SEQUENCE: 00011011

SHORTEST SUPERSTRING:

00

01

10

11

001011

As it is possible to understand, instead of just concatenating all the available stings, in order to solve Shortest Superstring Problem, it is necessary to evaluate all the sequences and find overlaps. After those overlaps are considered, final sequence is shorter than just full sequence concatenation.

As computational approach, in order to solve these problems may be used greedy algorithms (Vazirani¸ 2001). Those algorithms continuously build up the final solution, through interactive cycles. Each cycle the algorithm attempts to find optimal solution. It is important to notice that greedy algorithms are not exhaustive and when the problem has considerable difficulty it may not be possible to obtain the best optimal solution, instead just local maximum. Also, depending on the problem difficulty, those algorithms require a considerable amount of cycles in order to be able to produce optimal solution that may, never less, be local. While applied to the Shortest Superstring Problem, greedy algorithms iterate input sequences and integrate (assemble) those one by one. In the approach presented below the algorithm considers two of the most similar sequences and overlap them, creating just one sequence. After, this sequence will be aligned with the third one and so on, for example:

INPUT: {ACTG, CTGA, GACC, TGAC, ACCC}

STAGE 1 – consider most similar string ACTG and CTGA

ACTG

CTGA -> ACTGA

STAGE 2 – consider most similar ACTGA and TGAC

ACTGA

TGAC -> ACTGAC

STAGE 3 – consider most similar ACTGAC and GACC

ACTGAC

GACC -> ACTGACC

STAGE 4 – overlap the rest – ACTGACC and ACCC

ACTGACC

ACCC -> ACTGACCC

OUTPUT: ACTGACCC

As we can see, in the previous example, greedy algorithm was able to create a shortest superstring with 4 steps. However, the problem presented is very simplistic. In real case scenario, inputs string would have been larger and number of inputs would be bigger. Therefore, it may be difficult for the algorithm to find the best superstring. Also, the algorithm may consider different possibilities of sequences to match, especially at start. This also highly affects the final sequence.

4.2 Hamiltonian Path Problem

Hamiltonian Path in an undirected graph is a path that visits each vertex exactly once. A cycle that uses every vertex in a graph exactly once is called a Hamilton cycle. It is important to notice that in order for Hamiltonian Path to exist, graph edges must be biconnected. That means that two or more vertices of the graph when removed could not disconnect the graph. Problem statement is, as follows:

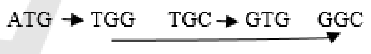
INPUT: Read sequences

OUTPUT: Created graph as well as Spectrum S

Available sequences must be connected if there is an overlap. For example,

INPUT: {ATG, TGG, TGC, GTG, GGC}

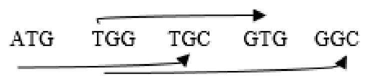
OUTPUT:



ATGGCGTGC

In this example there is a possibility of the second path that could be obtained by overlapping sequences in different order (way):

OUTPUT:



ATGCGTGGC

This simple example allows us to create different path by overlapping existing sequences. Due to the nature of the sequences (available nucleotides) there were two possible path to choose from. However, in the more complex problem the solution is not a trivial one.

As first approach could be used brute-force (exhaustive) search. But, a better approach is presented by Frank Rubin Author proposed an algorithm that divides a complete problem into pieces, each being solved easier than them all together (Rubin, 1974). All the available graphs are divided into three categories: used in path, not used in path and undecided. The functioning of this algorithm (identification and study of small problems) resembles dynamic programming principles, where each stage of the problem (part) is independent from previous stages (Vazirani¸2001).

Also, Michael Held and Richard M. Karp presented their solution using dynamic programming and based on the traveling-salesman problem (Held and Karp, 1962). Traveling-salesman problem is a well knows problem, similarly to the Bridges of Königsberg problem. Salesman must visit each city just once and using shortest route. Therefore, authors provided a solution as cyclic iteration of vertices and verify if there is a possibility of covering a path in set of vertices S. For further vertices the path is evaluated only if it existed in the previous stage.

4.3 Eulerian Path Problem

Similarly, to the previously stated problem, the purpose of the Eulerian Path is to find a route to visit each node of the graph just once. Euler Circuit is a circuit that uses each vertex of the graph just once. The main difference between Eulerian path and Circuit is that path ends on the different vertex, while circuit represents the complete cycle where it starts and end with the same vertex. Differently from the Hamiltonian Path problem, while working with Eulerian Path, we shall consider more linear traveling approach. That means that sequences are connected linearly (v1 -> v2 -> …) which not happens in Hamiltonian Path. Moreover, while working with Eulerian path it is necessary to consider the following theorem: “A connected graph is Eulerian if and only if each of its vertices is balanced.” (Jones and Pevzner, 2004). Consider that vertex is balanced when indegree = outdegree for presented vertex. In order to create a Eulerian cycle, it is necessary to start drawing path from the arbitrary vertex v and traversing edges that have not already been used. We stop the path when we encounter a vertex with no way out, that is, a vertex whose outgoing edges has already been used in the path.

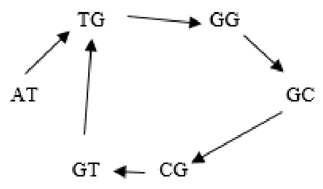
Below is presented an example of the Eulerian Path problem:

INPUT: {ATG, TGG, TGC, GTG, GGC}

STAGE 1: Create vertices of lengh k-1

{AT, TG, GG, GC, CG, GT}

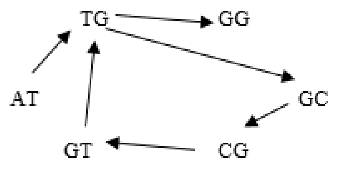
OUTPUT:



ATGGCGTGC

As we have seen in the previous sub-section, there is another path:

OUTPUT:



ATGCGTGGC

In order to solve Eulerian path problems there are two main algorithms: Fleury's algorithm and Cycle finding algorithm. Fleury's algorithm was originally created in 1883 to find Eulerian paths (Fleury, 1883). It proceeds by repeatedly removing edges from the graph in such way, that the graph remains Eulerian. The algorithm starts at a vertex of odd degree, or, if the graph has none, it starts with an arbitrarily chosen vertex. At each step it chooses the next edge in the path to be removed considering that that edge would not disconnect the graph itself (is not a bridge). If there is no such edge algorithm cannot proceed. It then moves to the other endpoint of that vertex and deletes the chosen edge. At the end of the algorithm there are no edges left, and the sequence from which the edges were chosen (removed) forms a Eulerian cycle if the graph has no vertices of odd degree, or a Eulerian trail if there are exactly two vertices of odd degree.

5 CONCLUSIONS

This work provides a basic overview over genome assembly processes and their challenges. We started by describing the overall genome sequencing problem and discussing its relevance. Posteriorly, we portrayed some of the most popular assemblers and their inner workings. Most of the assemblers, including some not mentioned in this work, use de Bruijn graphs for sequence assembling. But, sequence alignment, used for creating common substrings (overlaps) also has an high impact and weight in DNA sequencing performance. Finally, to provide a clear insight for computer scientists into genome assembly algorithms, we draw some parallelism between the Selling-travelers problem, as well as with the Bridges of Königsberg problem, with the genome sequencing problems, showing that both have highly contributed to the evolution and the development of graph algorithms and their use in bioinformatics.

After many years of research and work, genome assembly is still a hard and cumbersome task. As future work, we plan to implement and optimize some of the described approaches using state of the art parallel and distributed computing strategies.

REFERENCES

Alberts, B., Johnson, A., Lewis,J., Raff, M., Roberts, K., Walters, P. 2002. Molecular Biology of the Cell; Fourth Edition. New York and London: Garland Science. ISBN 0-8153-3218-1.

Barnett, J. H. 2009. Early Writings on Graph Theory: Euler Circuits and The Königsberg Bridge Problem.

Compeau, P. E. C., Pevzner, P. A. and Tesler, G. 2011. How to apply de Bruijn graphs to genome assembly.

Domingues, F.S., Lackner, P., Andreeva, A., Sippl, M.J. 2000. Structure-based evaluation of squence coparison and fold recognition alignment accuracy. J Mol Biol. 2000;297:1003–1013.

El-Metwally, S., Taher, H., Magdi, Z. and Helmy, M. 2013. Next-Generation Sequence Assembly: Four Stages of Data Processing and Computational Challenges. PLoS Comput Biol. 2013 Dec; 9(12): e1003345.

Era7. 2016. https://era7bioinformatics.com/en/page.cfm?id=1500 retrieved on 20.01.2016.

Fleury, M. 1883. Deux problèmes de Géométrie de situation. Journal de mathématiques élémentaires, 2nd ser. (in French) 2: 257–261.

Gilles, A., Meglécz, E., Pech, N., Ferreira, S., Malausa, T., Martin, J.F. Accuracy and quality assessment of 454 GS-FLX Titanium pyrosequencing, 2011, 12:245.

Gregory, S. 2005. Contig Assembly. Encyclopedia of Life Sciences.

Held, M. and Karp, R. M. 1962. A dynamic programming approach to sequencing problems. J. Siam 10 (1): 196–210.

Hert, D.G., Fredlake, C.P., Barron, A.E. 2008. Advantages and limitations of next-generation sequencing technologies: a comparison of electrophoresis and non-electrophoresis methods. Electrophoresis. 29(23): 4618-26.

Hopkins, B, and Wilson, R. The Truth about Königsberg. College Mathematics Journal (2004), 35, 198-207.

Illumina, Inc. 2010. De Novo Assembly Using Illumina Reads. Nature 171:737–738.

Mount, DM. 2004. Bioinformatics: Sequence and Genome Analysis (2nd ed.). Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY. ISBN 0-87969-608-7.

Munib, A., Ishfaq, A., Mohammad S. A. 2015. A survey of genome sequence assembly techniques and algorithms using high-performance computing. The Journal of Supercomputing. Vol. 71 (1), pp 293-339.

Jones, N.C. and Pevzner, P.A. 2004. An Introduction to Bioinformatics Algorithms. © 2004 Massachusetts Institute of Technology.

Niedringhaus, T.P., Milanova, D., Kerby, M.B., Snyder, M.P., Barron, A.E. 2011 Landscape of next-generation sequencing technologies. Anal Chem 83: 4327– 4341.

Paoletti, T. 2011. Leonard Euler's Solution to the Konigsberg Bridge Problem. Convergence 2011.

Polyanovsky, V. O., Roytberg, M. A., Tumanyan, V. G. 2011. Comparative analysis of the quality of a global algorithm and a local algorithm for alignment of two sequences. Algorithms for Molecular Biology 6.

Polyanovsky, V., Roytberg, M.A., Tumanyan, V.G. 2008. Reconstruction of genuine pair-wise sequence alignment. J Comp Biol. 2008;15:379–391.

Posada, D. 2009. Bioinformatics for DNA Sequence Analysis. (Ed.). ISBN 978-1-59745-251-9.

Rubin, F. 1974. A Search Procedure for Hamilton Paths and Circuits. Journal of the ACM 21 (4): 576–80.

Saenger, W. 1984. Principles of Nucleic Acid Structure. New York: Springer-Verlag. ISBN 0-387-90762-9.

Salzberg, S. L., Phillippy, A.M., Zimin, A., Puiu1, D., Magoc, T., Koren, S., Treangen, TJ., Schatz, M.C., Delcher, A.L., Roberts, M., Marçais, G., Pop, M. and Yorke, J.A. 2011. GAGE: A critical evaluation of genome assemblies and assembly algorithms.

Sunyaev, S.R., Bogopolsky, G.A., Oleynikova, N.V., Vlasov, P.K., Finkelstein, A.V., Roytberg, M.A. 2004. From analysis of protein structural alignments toward a novel approach to align protein sequences. Proteins: Structure, Function and Bioinforrmatics. 2004; 54:569–582.

Vazirani, U. V. 2001. Algorithms.

Voelkerding, K.V., Dames, S.A., Durtschi, J.D. 2009. Next-generation sequencing: from basic research to diagnostics. Clin Chem 55: 641–658.

Watson J, Crick. 1953. Molecular structure of nucleic acids: a structure for deoxyribose nucleic acid.

Wheeler, DA, Srinivasan, M., Egholm, M., Shen, Y., Chen, L., McGuire, A. 2008. The complete genome of an individual by massively parallel DNA sequencing. Nature 2008; 452:872-876.

Zhou, X., Ren, L., Meng, Q., Li, Y., Yu, Y. 2010. The next-generation sequencing technology and application. Protein Cell 1: 520–536.