NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTMENT OF COMPUTER SCIENCE

RICARDO DUARTE CARDOSO BERNARDINO

BSc in Computer Science and Engineering

DEVELOPMENT OF AN AUTOMATED TOOL FOR DNA SANGER ALIGNMENTS BASED ON AI

INNACURACY DETECTION ON DNA SANGER SEQUENCED DATA USING MACHINE AND DEEP LEARNING

MASTER IN COMPUTER SCIENCE AND ENGINEERING

NOVA University Lisbon

Draft: *January 15, 2025*

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RICARDO DUARTE CARDOSO BERNARDINO

BSc in Computer Science and Engineering

Adviser: Carmen Morgado
Assistant Professor, NOVA University Lisbon

Co-adviser: Rui Crespo
Biochemichal Engineer, STAB VIDA

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Abstract

Sanger sequencing, while a cornerstone of DNA analysis, frequently yields alignment results that require labor-intensive manual verification to identify anomalies and inaccuracies. This process is not only time-consuming but also susceptible to human error, posing significant challenges for laboratories managing high-throughput sequencing data. Addressing this issue is critical for improving efficiency and reliability in genomic workflows.

This thesis introduces an AI-driven tool designed to automate the verification of DNA sequence alignment results produced by the ABI3730xl sequencer. By utilizing machine learning models trained on real-world data from 96-well plates, the tool identifies anomalies, detects sequence homologies, and determines the acceptability of results with a high degree of precision. To facilitate broader adoption, the tool is accessible via an online platform, enabling remote usage by laboratories worldwide.

The proposed solution significantly enhances laboratory workflows by streamlining the verification process and reducing reliance on manual oversight. This not only minimizes errors but also accelerates data processing, paving the way for advancements in precision genomics and biotechnology. The tool's ability to identify inaccurately labeled wells further underscores its practical value in supporting high-throughput DNA analysis.

By addressing key limitations in current practices, this work demonstrates the transformative potential of integrating artificial intelligence into genomic research, contributing to a future of more efficient and accurate DNA sequencing.

Keywords: Sanger Sequencing, Artificial Intelligence, DNA verification, Machine Learning, Genomic Workflows

Resumo

A sequenciação Sanger, apesar de ser uma pedra angular na análise de DNA, frequentemente produz resultados de alinhamento que requerem verificação manual intensiva para identificar anomalias e imprecisões. Este processo não é apenas moroso, mas também suscetível a erros humanos, apresentando desafios significativos para laboratórios que gerem dados de sequenciação de alto desempenho. Abordar este problema é crucial para melhorar a eficiência e a confiabilidade nos fluxos de trabalho genómicos.

Esta tese apresenta uma ferramenta baseada em inteligência artificial (IA) concebida para automatizar a verificação dos resultados de alinhamento de sequências de DNA produzidos pelo sequenciador ABI3730xl. Utilizando modelos de aprendizagem automática treinados com dados reais de placas de 96 poços, a ferramenta identifica anomalias, deteta homologias de sequências e determina a aceitabilidade dos resultados com um elevado grau de precisão. Para facilitar a adoção ampla, a ferramenta é acessível através de uma plataforma online, permitindo a utilização remota por laboratórios em todo o mundo.

A solução proposta melhora significativamente os fluxos de trabalho laboratoriais ao simplificar o processo de verificação e reduzir a dependência da supervisão manual. Isto não apenas minimiza erros, mas também acelera o processamento de dados, abrindo caminho para avanços na genómica de precisão e na biotecnologia. A capacidade da ferramenta de identificar poços rotulados de forma incorreta destaca ainda mais o seu valor prático no suporte à análise de DNA de alto desempenho.

Ao abordar limitações-chave nas práticas atuais, este trabalho demonstra o potencial transformador de integrar inteligência artificial à investigação genómica, contribuindo para um futuro de sequenciação de DNA mais eficiente e preciso.

Palavras-chave: Sequenciação Sanger, Inteligência Artificial, Verificação de DNA, Aprendizagem Automática, Fluxos de trabalho genómicos

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1

Introduction

1.1 Motivation

The ever-growing demand for efficient and accurate DNA sequencing solutions has highlighted the limitations of traditional methods, particularly in high-throughput environments. Sanger sequencing remains a widely used technique due to its reliability and precision. However, the manual verification of alignment results introduces bottlenecks that hinder the scalability and efficiency of laboratories, especially those handling extensive genomic datasets.

This bottleneck is not merely an operational issue but a scientific challenge. As genomic research expands its applications in fields like medicine, agriculture, and biotechnology, the need for automated tools capable of minimizing errors and optimizing workflows becomes increasingly evident. By integrating artificial intelligence (AI) into these processes, laboratories can achieve new levels of efficiency and accuracy, ultimately advancing the frontiers of genomic research.

1.2 Context

1.2.1 Introduction to DNA

DNA (deoxyribonucleic acid) is the molecular blueprint for all living organisms, carrying the genetic instructions necessary for growth, development, and reproduction. The structure of DNA was famously elucidated by Watson and Crick in 1953, a discovery that revolutionized molecular biology [33]. DNA consists of two complementary strands coiled into a double helix, with nucleotide bases (adenine, thymine, cytosine, and guanine) forming the rungs of the helix [2].

Figure 1.1: The structure of the DNA double helix.

1.2.2 DNA Sequencing and Sanger Sequencing

DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. Among the various sequencing methods, Sanger sequencing remains a widely utilized technique due to its accuracy and reliability. Developed by Frederick Sanger and colleagues in 1977, this method involves the selective incorporation of chain-terminating dideoxynucleotides (ddNTPs) by DNA polymerase during in vitro DNA replication [27].

Sequencing DNA is fundamental to understanding genetic information, enabling applications ranging from disease diagnosis and treatment to evolutionary biology [23]. By deciphering the nucleotide order, scientists can identify mutations, study gene expression, and uncover genetic diversity.

The steps of Sanger sequencing are enumerated as follows and illustrated in Figure 1.2:

1. **Primer annealing and chain extension**: DNA polymerase synthesizes complementary DNA strands using a primer and a mixture of deoxynucleotides (dNTPs).

2. **ddNTP binding and chain termination**: The incorporation of ddNTPs halts DNA synthesis at specific nucleotides, resulting in DNA fragments of varying lengths.

3. **Fluorescent labeling of DNA fragments**: The terminated DNA strands are fluorescently labeled for detection.

4. **Capillary gel electrophoresis and fluorescence detection**: DNA fragments are separated by capillary gel electrophoresis and identified based on fluorescence signals.

5. **Sequence analysis and reconstruction**: The nucleotide sequence is reconstructed from the fluorescence data and analyzed.

Figure 1.2: Steps involved in Sanger sequencing [26].

To better understand the components involved in this process:

- **DNA Template**: The single-stranded DNA to be sequenced.
- **Primer**: A short single-stranded oligonucleotide complementary to the target sequence, serving as the starting point for DNA synthesis.
- **DNA Polymerase**: An enzyme that synthesizes new DNA strands by adding nucleotides to the primer.
- **Deoxynucleotides (dNTPs)**: Normal nucleotides (A, T, C, G) used to elongate the DNA strand.
- **Dideoxynucleotides (ddNTPs)**: Modified nucleotides (ddATP, ddTTP, ddCTP, ddGTP) that terminate DNA strand synthesis upon incorporation due to the lack of a 3 -OH group.

1.2.3 The ABI3730xl DNA Sequencer

The ABI3730xl DNA sequencer is a high-throughput instrument widely used in genomics for Sanger sequencing. It automates the electrophoresis and fluorescence detection steps, corresponding to steps 3 and 4 of the Sanger sequencing process (Figure 1.2), allowing for the simultaneous processing of 96 samples in a single run [31, 10]. By leveraging this technology, laboratories can achieve precise and efficient DNA sequencing.

Figure 1.3: A closed ABI3730xl capillary electrophoresis DNA analyzer.

Figure 1.4: A opened ABI3730xl capillary electrophoresis DNA analyzer.

1.2.4 Role of Artificial Intelligence in DNA Analysis

Artificial intelligence (AI) revolutionizes genomic research by enhancing the accuracy and efficiency of data analysis. Machine learning (ML) models, trained on extensive genomic datasets, excel at recognizing patterns and anomalies. In Sanger sequencing, AI can:

- Detect mislabeled or erroneous sequences.
- Automate anomaly identification in 96-well plate outputs.
- Reduce human intervention while improving data reliability.

Ethical considerations must accompany AI integration to safeguard data privacy, address biases, and ensure equitable access to technology. The synergy between AI and DNA sequencing promises advancements in precision medicine and genomics.

1.2.5 Machine Learning and Deep Learning in Genomics

Machine Learning (ML) and Deep Learning (DL) are subfields of Artificial Intelligence (AI) that have revolutionized various scientific disciplines, including genomics, in which both of these fields have transformed the landscape of genomic research [21]. These computational approaches enable systems to learn patterns and make predictions based on large datasets, bypassing the need for explicitly programmed instructions. ML algorithms, such as support vector machines and random forests, excel in identifying patterns within large datasets, while DL models, like convolutional neural networks (CNNs), are adept at analyzing complex, high-dimensional data [22, 4].

Machine Learning: ML refers to algorithms that improve their performance on a task with experience. In the context of DNA sequencing:

- Supervised learning algorithms are trained using labeled datasets, such as annotated DNA sequences, to classify or predict sequence anomalies.
- Unsupervised learning algorithms, like clustering methods, identify patterns in unlabeled data, such as grouping similar DNA sequences.
- Reinforcement learning methods optimize decision-making processes, like adjusting sequencing parameters for improved accuracy.

Deep Learning: DL, a subset of ML, uses artificial neural networks inspired by the human brain. These networks, especially when designed as deep architectures, are particularly effective in handling large and complex datasets such as genomic data. In Sanger sequencing:

- Convolutional Neural Networks (CNNs) can process visual data from electropherograms to identify signal anomalies.
- Recurrent Neural Networks (RNNs) or their advanced variants (e.g., Long Short-Term Memory networks, LSTMs) are ideal for analyzing sequential data, such as nucleotide chains.
- Autoencoders and Generative Adversarial Networks (GANs) are employed for noise reduction and synthetic data generation, enhancing training datasets.

In genomics, these techniques are employed for tasks such as sequence alignment, variant calling, and the identification of genetic mutations [34]. The integration of AI into Sanger sequencing workflows, as in this thesis, enables the automation of error detection

and data validation, enhancing the accuracy and efficiency of sequencing processes [28, 15].

Applications in Sanger Sequencing: Integrating ML and DL into Sanger sequencing workflows enables:

- Automated classification of sequencing errors, such as those caused by fluorescent signal overlap or ddNTP misincorporation.
- Enhanced post-sequencing analysis to flag inaccurate well outputs in 96-well plates.
- Streamlined data analysis pipelines, reducing the time and labor required for manual verification.

The combination of these AI-driven methodologies with established sequencing technologies, such as the ABI3730xl, provides a robust framework for addressing critical challenges in genomic research. It bridges the gap between raw sequencing data and actionable biological insights, reinforcing the importance of interdisciplinary approaches in modern science.

1.2.6 Broader Scientific Implications

Studying DNA and developing advanced sequencing tools contribute to:

- **Precision Medicine**: Personalizing treatments based on genetic profiles.
- **Evolutionary Biology**: Understanding species evolution and genetic diversity.
- **Agriculture**: Enhancing crop yields and pest resistance through genetic insights.
- **Biotechnology**: Innovating in synthetic biology and gene editing applications.

Through the combination of traditional techniques, cutting-edge sequencing technologies, and AI, this thesis addresses the critical challenge of post-sequencing analysis, pushing the boundaries of what is possible in modern genomics.

1.3 Objectives

The primary goal of this thesis is to develop a tool that automates the verification of alignment results obtained from Sanger sequencing. The specific objectives include:

- Designing an AI algorithm capable of detecting anomalies and homologous sequences in alignment data.
- Training the algorithm using real-world datasets from ABI3730xl sequencers 96-well plates.

- Designing an AI algorithm capable of detecting anomalies and homologous sequences in alignment data.
- Training the algorithm using real-world datasets from ABI3730xl sequencers 96-well plates.
- Validating the tools performance against traditional manual verification methods.
- Deploying the tool via an online platform to ensure accessibility for laboratories worldwide.

1.4 Structure

This document is organized as follows:

- Introduction: Provides the motivation, context, objectives, and structure of the thesis.
- State of the Art & Related Work: Reviews existing literature and technologies in Sanger sequencing and AI applications in genomics.
- Methodology: Details the design and implementation of the proposed AI driven tool, including data collection, model training, and deployment.
- Planning: Presents the planning of the proposed work, composed by desired checkpoints and finish dates.

2

State of the Art & Related Work

Sanger sequencing remains a cornerstone of genomic studies due to its high accuracy and reliability. However, the manual analysis of chromatograms and sequence data has traditionally been labor-intensive and prone to human error. Recent advancements have introduced automated tools and methodologies to streamline this process, with artificial intelligence (AI) playing a transformative role in enhancing efficiency and accuracy.

2.1 Automated Sequence Alignment

Tools like DNASTAR's SeqMan Ultra and Geneious have become benchmarks in sequence alignment, offering robust algorithms capable of assembling sequences into contigs and generating consensus sequences. These tools integrate features such as ClustalO, MUSCLE, MAFFT, and LASTZ algorithms to support precise alignment and visualization [13, 18].

2.2 AI in DNA Sequence Analysis

Machine learning (ML) and AI have emerged as powerful technologies in genomic data analysis. ML algorithms are utilized for alignment, classification, clustering, and pattern mining, significantly improving the efficiency of DNA sequence analysis. AI techniques also enhance sequencing accuracy, particularly in detecting genetic variations and anomalies [9, 24].

2.3 Automated Pipelines for Sanger Sequencing

Automated pipelines such as the Automated Sanger Analysis Pipeline (ASAP) and SangeR have revolutionized the processing of Sanger sequencing data. These tools facilitate rapid data analysis with minimal user intervention, allowing for high-throughput and reproducible results [25, 3].

2.4 Online Accessibility of Sequencing Tools

The proliferation of online tools has democratized access to sequencing analysis. Tools like Champuru 2 provide web-based platforms for analyzing mixed Sanger chromatograms, making advanced functionalities accessible to a broader audience [1].

2.5 Automation in DNA Sequencing Laboratories

Automation in modern DNA sequencing laboratories has increased sample throughput, enhanced reproducibility, and reduced human error. These advancements enable precise sample tracking and high-throughput sequencing, meeting the demands of large-scale genomic studies [7].

3

Methodology

This Chapter aims at detailing the design and implementation of the proposed AI driven tool, including data collection, model training, and deployment.

3.1 Document Structure

3.2 Dealing with Bibliography

Citing something online [11, 16, 19].

3.3 Inserting Tables

3.4 Importing Images

3.5 Floats, Figures and Captions

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(a) One sub-figure (b) Another sub-figure

Figure 3.1: A figure with two sub-figures!

And this is a small text that references the Figure 3.1 and its Subfigures 3.1(a) and 3.1(b).

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3.6 Text Formatting

3.7 Generating PDFs from LaTeX

3.7.1 Generating PDFs with pdflatex

You may create PDF files either by using `latex` to generate a DVI file, and then use one of the many DVI-2-PDF converters, such as `dvipdfm`.

Alternatively, you may use `pdflatex`, which will immediately generate a PDF with no intermediate DVI or PS files. In some systems, such as Apple, PDF is already the default format for LaTeX. I strongly recommend you to use this approach, unless you have a very good argument to go for `latex` + `dvipdfm`.

A typical pass for a document with figures, cross-references and a bibliography would be:

```
$ pdflatex template
$ bibtex template
$ pdflatex template
$ pdflatex template
```

You will notice that there is a new PDF file in the working directory called `template.pdf`. Simple :)

Please note that, to be sure all table of contents, cross-references and bibliographic citations are up-to-date, you must run `latex` once, then `bibtex`, and then `latex` twice.

3.7.2 Dealing with Images

You may process the same source files with both `latex` or `pdflatex`. But, if your text include images, you must be careful. `latex` and `pdflatex` accept images in different (exclusive) formats. For `latex` you may use EPS ou PS figures. For `pdflatex` you may use

JPG, PNG or PDF figures. I strongly recommend you to use PDF figures in vectorial format (do not use bitmap images unless you have no other choice).

3.7.3 Dealing with Citations

Para fazer citações, deverá usar-se a chave da referência no ficheiro BibTeX. Se for uma única referência [6], usar um "~" para ligar o `\cite{...}` à palavra que o precede (...`referência~\cite{Art`
Caso queira fazer múltiplas citações [29, 30, 14], deverá agrupá-las dentro de um úinico `\cite{...}`.

Note que o ficheiro de bibliografia pode ter tantas entradas quantas quiser. Apenas aquelas cuja chave seja referenciada no texto é que serão incluidas na listagem de bibliografia.

3.7.4 Footnotes

Footnotes[1] will be numbered and shown in the bottom of the page.

3.7.5 Tables

The Table 3.1 illustrates some important concepts associated with table construction:

i) Do not use vertical lines;

ii) The caption should be above the table;

iii) Use the macros `\toprule`, `\midrule` and `\bottomrule` to make the top, inner and bottom horizontal lines, respectively.

Table 3.1: Test results summary.

Test	Anomalies	Warnings	Correct	Categories	Missed
Connection [8]	2	2	1	*C*	1
Coordinates'03 [5]	1	4	1	*2B, 1C*	0
Local Variable [5]	1	2	1	*A*	0
NASA [5]	1	1	1	—	0
Coordinates'04 [6]	1	4	1	*3C*	0
Buffer [6]	0	7	0	*2A, 1B, 2C, 2D*	0
Double-Check [6]	0	2	0	*1A, 1B*	0
StringBuffer [17]	1	0	0	—	1
Account [32]	1	1	1	—	0
Jigsaw [32]	1	2	1	*C*	0
Over-reporting [32]	0	2	0	*1A, 1C*	0
Under-reporting [32]	1	1	1	—	0
Allocate Vector [20]	1	2	1	*C*	0

[1] This is a simple footnote.

Knight Moves [8]	1	3	1	*2B*	0
Total	12	33	10	5A, 6B, 10C, 2D	2

3.7.6 Figures

The images inserted in the document must be of good quality, preferably in vector format (vector PDF) and not in *bitmap* (PNG, JPG, etc.). *bitmap* images (Figure 3.2) do not scale well and have negative effects on the quality of your document. On the other hand, *vector* images Figure 3.3 scale as much as necessary without degrading the quality of the image.

You should only use *screenshots* for your plots, charts, etc, if you absolutely have no other alternative. Instead of generating a *screenshot*, try using a virtual PDF printer and printing to a PDF file. As a general rule, you will get a vector PDF. Even if your PDF contains images, they will always be of higher or equal quality than what you would get with a *screenshot*.

To combine several figures into a single one… You can then reference the set as Figure 3.4 or the sub-figures separately as 3.7.6 and 3.7.6.

3.8 Equations

LaTeX is a powerful tool for writing in a mathematical style. It allows you to insert formulas into the text, such as this: $ax^2 + bx + c = 0$. It also allows formulas to be highlighted on a separate line and centered on the page.

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

or numbererd

$$e = mc^2 \tag{3.1}$$

which can latter be referenced as equation 3.1

3.9 Test for algorithms

Uncomment the algorithms source below and add the following to file "`5_packages.tex`"

```
\usepackage{algorithm2e}
\RestyleAlgo{ruled}
```

and uncomment

```
\ntaddlistof{listofalgorithms}
```

in file "`8_list_og.tex`".

Figure 3.2: Bitmap image (JPG/PNG)

Figure 3.3: Vectorial image (PDF)

(a) Novelo de lã

(b) Tempestade com neve

Figure 3.4: Exemplo de utilização de *subbottom*

4

Planning

My advice to customize the novathesis template to another School/University/Department/Degree is to browse the existing supported degrees to find one that is *close enough*, and depart from there!

The multitude of layouts supported by the novathesis template is based in a three-tier naming scheme, separated by slashes: University / School / Department-or-Degree. This three-tier naming scheme is also reflected in a three-tier directory (folder) structure in: `<project_root>a/NOVAthesiFiles/Schools/`…. For example:

```
…
|
+-- nova
|   +-- Images
|   +-- fct
|   |   \-- Images
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|   |   \-- Images
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\-- uminho
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    +-- ea
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    +-- ec
    |   \-- Images
    …
```

The directory `uminho` contains the customization for all Schools of Universidade do Minho. This university is an example of the case where the regulations are defined at University level and all the schools apply the same thesis layout and organization. So,

the all the customization is done in the file `uminho/uminho-defaults.ldf`, except the definition of the name and logo of each individual school.

As another example, the directory `nova` contains the customization for all Schools from NOVA University Lisbon. This university grants a lot of freedom in the definition of the thesis layouts. In some cases, they are defined at the School level (e.g., NOVA FCT), while is some other cases they are defined separately for each degree (e.g., NOVA IMS).

1. Try all the already supported schools and check which one is closer to your needs;

   a) Edit `Config/1_novathesis.tex` and near line 28 uncomment the line with key `\ntsetup{school=<SOMETHING>}`;

   b) For each school supported (see the comment), replace `<SOMETHING>` with the school name, e.g., make it `\ntsetup{school=ulisboa/fmv}`

   c) Recompile and check the document. Particularly, check the cover layout, the front-page (second cover) layout, the front-matter contents, the bibliography style;

   d) Repeat for the next school, until you find one close enough.

2.

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A

NOVAthesis covers showcase

This Appendix shows examples of covers for some of the supported Schools. When the Schools have very similar covers (e.g., all the schools from Universidade do Minho), just one cover is shown. If the covers for MSc dissertations and PhD thesis are considerable different (e.g., for FCT-NOVA and UMinho), then both are shown.

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B

Appendix 2 Lorem Ipsum

This is a test with citing something [12] in the appendix.

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Annex 1 Lorem Ipsum

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