NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTMENT OF COMPUTER SCIENCE

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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

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Abstract

Since the dawn of mankind, our pursuit to understand the world has driven innovation and shaped the course of scientific discovery. Among the most profound achievements of this quest is the field of genomics, which has revolutionized our understanding of life itself. By decoding the genetic blueprint of organisms, genomics has unlocked insights into health, disease, evolution, and biodiversity, paving the way for transformative advancements in medicine, agriculture, and biotechnology.

Sanger sequencing remains a cornerstone of DNA analysis, yet its alignment results often necessitate labor-intensive manual verification to identify anomalies and inaccuracies. This process is not only time-consuming but also susceptible to human error, presenting significant challenges for laboratories handling high-throughput sequencing data. Addressing these limitations is critical for improving the efficiency and reliability of genomic workflows.

This master's thesis introduces an AI-driven tool designed to automate the verification of DNA sequence alignment results generated by the ABI3730xl sequencer. Leveraging machine learning models trained on real-world data from 96-well plates, the tool identifies anomalies, detects sequence homologies, and evaluates the acceptability of results with high precision. To ensure accessibility, the tool is deployed on 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, supporting advancements in precision genomics and biotechnology. Additionally, the tool's capability to detect inaccurately labeled wells further highlights its practical utility in high-throughput DNA analysis.

By addressing critical limitations in current practices, this work demonstrates the transformative potential of integrating artificial intelligence into genomic research. The tool contributes to the field by enabling more efficient and accurate DNA sequencing, paving the way for future innovations in genomics and biotechnology.

Keywords: Sanger Sequencing, Artificial Intelligence, DNA Verification, Machine Learning, Genomic Workflows, High-Throughput Sequencing, Automation, Precision Genomics

Resumo

Desde os primórdios da humanidade, a nossa busca para compreender o mundo tem impulsionado a inovação e moldado o curso da descoberta científica. Entre os feitos mais profundos desta busca está o campo da genómica, que revolucionou a nossa compreensão da vida em si. Ao decifrar o código genético dos organismos, a genómica revelou insights sobre saúde, doença, evolução e biodiversidade, abrindo caminho para avanços transformadores na medicina, agricultura e biotecnologia.

O sequenciamento Sanger é um método fundamental na análise de DNA, mas os seus resultados de alinhamento exigem frequentemente uma verificação manual, um processo moroso para identificar anomalias e imprecisões. Este processo não só consome tempo, como também está sujeito a erros humanos, representando um desafio significativo para laboratórios que lidam com dados de sequenciação de alto débito. Superar estas limitações é essencial para melhorar a eficiência e a fiabilidade dos fluxos de trabalho genómicos.

Esta tese de mestrado apresenta uma ferramenta baseada em inteligência artificial (IA) concebida para automatizar a verificação dos resultados de alinhamento de sequências de DNA gerados 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 avalia a aceitabilidade dos resultados com elevada precisão. Para garantir acessibilidade, a ferramenta foi implementada numa plataforma online, permitindo a sua utilização remota por laboratórios em todo o mundo.

A solução proposta melhora significativamente os fluxos de trabalho laboratoriais ao agilizar o processo de verificação e reduzir a dependência de supervisão manual. Isto não só minimiza erros, como também acelera o processamento de dados, contribuindo para avanços na genómica de precisão e na biotecnologia. Além disso, a capacidade da ferramenta para identificar poços incorretamente rotulados reforça a sua utilidade prática na análise de DNA de alto débito.

Ao abordar limitações críticas das práticas atuais, este trabalho demonstra o potencial transformador da integração da inteligência artificial na investigação genómica. A ferramenta contribui para o campo ao possibilitar uma sequenciação de DNA mais eficiente e precisa, abrindo caminho para futuras inovações na genómica e na biotecnologia.

Palavras-chave: Sequenciação Sanger, Inteligência Artificial, Verificação de DNA, Aprendizagem Automática, Fluxos de Trabalho Genómicos, Sequenciação de Alto Débito, Automação, Genómica de Precisão

Contents

List of Figures **vii**

1 Introduction **1**
1.1 Motivation . . . 1
1.2 Context . . . 2
1.2.1 Introduction to STAB VIDA . . . 2
1.2.2 Challenges in the Current Workflow . . . 3
1.2.3 The Need for Automation at STAB VIDA . . . 3
1.2.4 AI-Powered Solutions for Sequencing Workflows . . . 4
1.3 Objectives . . . 5
1.4 Structure . . . 5

2 State of the Art & Related Work **7**
2.1 Genomics . . . 7
2.1.1 Introduction to DNA . . . 8
2.1.2 DNA Sequencing and Sanger Sequencing . . . 9
2.2 Artificial Intelligence . . . 10
2.2.1 Data Science . . . 11
2.2.2 Machine Learning and Deep Learning . . . 12
2.2.3 Considerations for Model Selection & Development . . . 15
2.3 Related Work . . . 19
2.3.1 Isolation Forest: Unsupervised Anomaly Detection . . . 19
2.3.2 DBSCAN: Density-Based Clustering and Anomaly Detection . . . 20
2.3.3 Comparison with Thesis Approach . . . 21
2.3.4 Other Relevant Algorithms . . . 21
2.3.5 Conclusion . . . 22

3 Proposal **24**
3.1 Laboratory Processes . . . 24
3.1.1 Sample Preparation and Processing . . . 24

3.1.2 The ABI3730xl DNA Sequencer 26
3.2 Data Structure and Analysis 27

4 Planning **29**

Bibliography **30**

Appendices

A *NOVAthesis* covers showcase **36**
A.1 A section here 40

B Appendix 2 Lorem Ipsum **44**

Annexes

I Annex 1 Lorem Ipsum **49**

List of Figures

2.1 Objectives of Human Genome Project: The Human Genome Project was an international scientific endeavor with the aim of finding the base pairs that constitute human DNA as well as of identifying, mapping, and sequencing every gene in the human genome from both a physical and functional perspective [62]. 8

2.2 The structure of the DNA double helix. 8

2.3 Steps involved in Sanger sequencing [57]. 10

2.4 Understanding what Machine Learning and Deep Learning are, as subsets of Artificial Intelligence [51]. 12

2.5 Illustration of the main types of Machine Learning. 13

2.6 A deep neural network consists of an input layer, multiple hidden layers and an output layer, all consisting of nodes [55]. 14

2.7 A node in a hidden layer of a deep neural network takes an input, performs a calculation, and passes on an output to nodes in the next layer [55]. 14

2.8 Each neural node in a deep neural network performs a calculation by taking inputs from nodes in the previous layer, multiplying these by weights, and summing all outcomes. The resulting number gets processed by an activation function to allow for more complex calculations. Lastly, the output is passed on to the nodes in the next layer [55]. 14

2.9 The Optimum Regression compared to Underfitting and Overfitting the prediction. [2]. 17

2.10 (a) Graphical illustration of bias and variance. The aim is to obtain a model that has low bias and low variance. (b) Contribution of bias and variance to the total error of the machine learning model (reproduced from the reference [57]). With increasing model complexity, error function reduced significantly for training data set but perform poorly in validation dataset, i.e., higher variance. The task to define a model in such a way that the bias and variance can tackle in validation and blind test data set. [45]. 18

3.1 A 96-well PCR plate. 25

3.2 Structure and Organization of each PCR plate. 25
3.3 A closed ABI3730xl capillary electrophoresis DNA analyzer. 26
3.4 An opened ABI3730xl capillary electrophoresis DNA analyzer. 26

1

Introduction

In this initial chapter, the motivation for automating critical aspects of the Sanger sequencing pipeline is explored in detail. We discuss the operational challenges faced by laboratory personnel in manually verifying DNA sequencing results, the potential for artificial intelligence (AI) to alleviate these challenges, and the overall significance of introducing automated solutions in the context of genomic research.

The chapter also establishes the context in which this thesis was developed, explaining the collaboration between the company and the university to address specific problems in sequencing workflows. The importance of minimizing errors, optimizing throughput, and improving accuracy in genomic sequencing is emphasized, setting the stage for the problem addressed in this thesis.

Following the context, the defined objectives of the thesis are outlined, detailing the deliverables and results expected from this research. Finally, a brief overview of the thesis document's structure is provided to guide the reader through the subsequent chapters, ensuring a clear understanding of the overall thesis plan.

1.1 Motivation

DNA sequencing has become a cornerstone of modern science, driving advancements in fields such as medicine, agriculture, and evolutionary biology. The ability to decode genetic information has transformed our understanding of life, enabling breakthroughs in personalized medicine, disease diagnosis, and the development of targeted therapies [25]. In agriculture, sequencing has facilitated the engineering of crops with improved yield, resilience, and nutritional value, addressing critical challenges such as food security [70]. Similarly, in evolutionary biology, sequencing has provided unprecedented insights into the genetic diversity and history of species [63].

Despite its transformative potential, DNA sequencing faces significant challenges. The process of generating and interpreting sequencing data is complex, labor-intensive, and prone to human error. Sanger sequencing, a widely used method known for its accuracy, involves multiple manual steps that can introduce inefficiencies and inaccuracies into the

workflow. Laboratory personnel must meticulously verify sequencing results, a task that is both time-consuming and susceptible to oversight. These challenges are exacerbated by the growing demand for high-throughput sequencing, which places additional pressure on laboratory resources and increases the risk of errors [9].

The consequences of these inefficiencies are far-reaching. In medicine, for example, errors in sequencing can lead to misdiagnoses or inappropriate treatments, potentially jeopardizing patient outcomes. In agricultural biotechnology, inaccuracies in sequencing data can delay the development of improved crop varieties, hindering efforts to address global food security. Similarly, in evolutionary biology, errors in sequencing can distort our understanding of genetic relationships and evolutionary processes [40].

Addressing these challenges is critical to unlocking the full potential of DNA sequencing. By improving the accuracy, efficiency, and scalability of sequencing workflows, we can accelerate scientific discoveries and enhance the practical applications of genomics. Emerging technologies, such as artificial intelligence (AI), offer promising avenues for addressing these challenges. AI has demonstrated its potential to automate complex tasks, reduce human error, and enhance decision-making across various domains, including healthcare and biotechnology [18, 61]. Furthermore, artificial intelligence (AI) exhibits significant potential in domains characterized by large-scale datasets, particularly when such data contains discernible patterns and diverse variations. It's the ability of extracing meaningful insights from complex and heterogeneous data that enhances AI's value in addressing intricate challenges across various fields. In the context of DNA sequencing, AI-driven solutions could streamline the verification of sequencing results, enabling faster and more reliable analyses.

1.2 Context

Given that AI has non-stop increased relevance in the market, many companies seek to leverage it's capabilities for specific services, not only creating market share, but also providing competition, innovation, and bolstering the scienfitic advancement of the field. This thesis is situated within the context of a collaboration between STAB VIDA, a leading company in genomic sequencing, and the university. The partnership aims to address specific inefficiencies in Sanger sequencing workflows, with a focus on improving accuracy, scalability, and operational efficiency. By combining academic research with industry expertise, this collaboration seeks to explore and implement existing AI mechanisms that can transform the field of genomics.

1.2.1 Introduction to STAB VIDA

STAB VIDA is a leading company in the field of genomic sequencing, specializing in high-quality services for DNA and RNA analysis. With a strong focus on precision and reliability, STAB VIDA offers state-of-the-art sequencing technologies, including Sanger sequencing,

which remains one of the most trusted methods for accurate DNA analysis [59]. The company caters to a wide range of clients, from academic researchers to pharmaceutical and agricultural industries, providing critical data for projects related to personalized medicine, evolutionary biology, and biotechnological innovations [21].

1.2.2 Challenges in the Current Workflow

Despite the advanced technology used by STAB VIDA, the manual processes associated with DNA sequencing present significant challenges. While the company's clients provide the DNA samples to be sequenced, the preparation of these samples often requires additional human intervention before the sequencing process can begin. Depending on the type of service requested, some samples must be mixed with specific primers, while others require adjustments to their concentration levels. These preparatory steps, though critical for ensuring successful sequencing, introduce opportunities for human error that can compromise the quality of the final results. For example, samples with high concentration levels can contaminate neighboring samples during the sequencing process. This contamination not only affects the reliability of the sequencing results but also necessitates repeat analyses, further delaying the workflow and increasing costs. These manual interventions exacerbate the challenges faced by laboratory personnel. In addition to these preparatory steps, the verification of DNA sequencing results in 96-well plates is another error-prone and time-consuming task. Laboratory personnel must manually inspect each well for abnormalities, such as abnormal signal scores, cross-well contamination, or unexpected DNA sequences that could indicate potential errors in the sequencing process [8]. These manual checks, while essential for ensuring accurate and high-quality sequencing results, introduce bottlenecks into the workflow. With the increasing demand for rapid sequencing services, the time and effort required for manual verification grow proportionally, raising the likelihood of human error [3]. Such errors can compromise the reliability of sequencing results delivered to clients, impacting both client satisfaction and the company's reputation [39].

The combination of manual sample preparation and post-sequencing verification creates a workflow that is not only labor-intensive but also vulnerable to inconsistencies. Addressing these challenges is critical to improving the accuracy, efficiency, and scalability of STAB VIDA's sequencing services.

1.2.3 The Need for Automation at STAB VIDA

Given the increasing complexity and volume of sequencing tasks, STAB VIDA recognizes the urgent need to automate certain aspects of the workflow. Specifically, the company seeks to leverage artificial intelligence to enhance the detection of anomalies in sequencing data [35]. AI has the potential to significantly improve the speed and accuracy of this process by automatically flagging wells that exhibit unusual patterns.

By automating the flagging process, STAB VIDA aims to:

- **Reduce manual labor**: Laboratory personnel will be freed from repetitive tasks and be able to focus on higher-order tasks that require human expertise, such as resolving flagged anomalies [76].
- **Increase accuracy and throughput**: AI-driven anomaly detection will reduce human error, improving the overall accuracy of sequencing results delivered to the client and enabling faster turnaround times [10].
- **Improve scalability**: Automation will allow STAB VIDA to handle larger volumes of sequencing data, facilitating growth and meeting the increasing demand for sequencing services [56].

The collaboration between STAB VIDA and the university has thus emerged as a critical step in addressing these challenges. This thesis, which focuses on the study and application of existing AI tools for anomaly detection in sequencing data, aligns directly with STAB VIDA's goals of operational efficiency and enhanced service quality [64].

1.2.4 AI-Powered Solutions for Sequencing Workflows

The proposed solution leverages existing AI mechanisms to integrate seamlessly into STAB VIDA's existing sequencing workflows. By analyzing sequencing data from the company's ABI3730xl sequencers, the AI tool will automatically flag wells that deviate from expected patterns based on specific criteria, such as signal quality or sequence consistency [23]. These flagged wells will then be quarantined for human review, where laboratory personnel can investigate and resolve any issues that might arise.

Key benefits of this AI-driven solution include:

- **Automated anomaly detection**: The AI tool will automatically identify and flag wells exhibiting abnormalities, such as contamination, poor signal quality, or unusual DNA sequences [75].
- **Detailed reporting**: For each flagged well, the system will provide detailed explanations and reasons for the anomaly, enabling more efficient review and resolution [77].
- **Integration with existing workflows**: The tool will be designed to work within STAB VIDA's current sequencing infrastructure, ensuring minimal disruption to ongoing operations and enabling smooth adoption by laboratory staff [11].

This thesis will contribute to STAB VIDA's mission to improve sequencing workflows, reduce operational inefficiencies, and enhance the quality and reliability of its sequencing services. By focusing on the study and implementation of existing AI mechanisms, the proposed solution has the potential to set a new standard for accuracy and efficiency in genomic sequencing.

1.3 Objectives

The primary objective of this thesis is to develop an AI-powered tool that automates the verification of alignment results in Sanger sequencing. This tool will address inefficiencies in the manual processes currently employed and enhance the overall reliability of sequencing workflows. Specific objectives include:

- **Data Exploration and Preprocessing**: Use data science techniques to explore, determine, and select the relevant data for model training, ensuring the data is preprocessed effectively.
- **Model Investigation**: Research an already existing robust AI algorithm fit and capable of detecting anomalies in the sequencing data, including signal inconsistencies, contamination, and unexpected DNA sequences.
- **Model Training and Validation**: Train and validate the AI algorithm using real-world datasets obtained from ABI3730xl sequencers, ensuring its applicability in practical laboratory settings.
- **Performance Evaluation**: Compare the AI tool's accuracy and efficiency against traditional manual verification methods, demonstrating its effectiveness in a real-world context.
- **Deployment Strategy**: Develop an accessible online platform for deploying the AI tool, ensuring seamless integration into existing laboratory workflows and maximizing user adoption.

By achieving these objectives, this research will not only improve the efficiency of STAB VIDA's sequencing workflows but also contribute to the broader adoption of AI in genomics, paving the way for future innovations in the field.

1.4 Structure

This document is organized into four main chapters, each focusing on a critical aspect of the thesis:

- **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.
- **Proposal**: 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.

Each chapter is designed to guide the reader through the research journey, from identifying the problem to proposing and validating a solution. This structured approach ensures clarity and coherence, making the thesis accessible to both technical and non-technical audiences. The following chapter will delve into the state of the art, reviewing existing literature and technologies relevant to this research.

2

State of the Art & Related Work

This chapter provides a comprehensive review of the current state of the art in genomics, emphasizing the transformative role of artificial intelligence (AI) in modern DNA sequencing and analysis. It begins with an introduction to genomics as a scientific discipline, exploring the structure, function, and evolution of genomes, along with the foundational advancements enabled by projects such as the Human Genome Project.

The chapter delves into DNA sequencing techniques, with a primary focus on Sanger sequencing, highlighting its historical significance, methodology, and key components.

Core concepts of machine learning and deep learning are introduced in latter sections, emphasizing their application in automating and enhancing genomic analysis tasks. Important topics such as overfitting, bias-variance tradeoff, and model optimization are thoroughly discussed, reflecting the relevance of these challenges in the context of AI-driven genomics research. This chapter aims to establish a clear understanding of the foundational and emerging methodologies that define the field today.

2.1 Genomics

Genomics is the study of the structure, function, and evolution of genomes—the complete set of DNA within an organism. It encompasses various aspects, including gene identification, genome sequencing, and the analysis of genetic interactions. The field has advanced rapidly since the completion of the Human Genome Project, which mapped the entire human genome, enabling breakthroughs in personalized medicine, evolutionary biology, and disease research [41, 72, 32].

Modern genomics leverages high-throughput sequencing technologies, such as next-generation sequencing (NGS), which generate vast amounts of data at unprecedented speeds. These technologies, combined with computational tools, have transformed our ability to explore genetic information on a massive scale. Genomics not only provides insights into fundamental biological processes but also underpins critical applications in biotechnology, agriculture, and healthcare [49, 46].

Figure 2.1: Objectives of Human Genome Project: The Human Genome Project was an international scientific endeavor with the aim of finding the base pairs that constitute human DNA as well as of identifying, mapping, and sequencing every gene in the human genome from both a physical and functional perspective [62].

2.1.1 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 [74]. 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 [1].

Figure 2.2: The structure of the DNA double helix.

2.1.2 DNA Sequencing and Sanger Sequencing

DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. Among the sequencing methods, Sanger sequencing, developed by Frederick Sanger in 1977, has long been the gold standard due to its high accuracy and reliability [60]. This method, also known as the chain termination method, involves the selective incorporation of chain-terminating dideoxynucleotides (ddNTPs) during DNA replication, resulting in DNA fragments of varying lengths that can be analyzed to determine the DNA sequence [48].

While Sanger sequencing remains indispensable, next-generation sequencing (NGS) technologies have emerged in recent years. These technologies offer higher throughput and faster sequencing capabilities, enabling the sequencing of entire genomes quickly and cost-effectively [49]. However, Sanger sequencing retains its significance for specific applications such as sequencing small, targeted regions of the genome, validating NGS results, and scenarios where longer read lengths are advantageous [46].

Despite advancements in NGS, Sanger sequencing continues to be favored for its simplicity, cost-effectiveness in low-throughput projects, and unparalleled accuracy. Its enduring role in genomic research and clinical diagnostics highlights its importance, particularly for applications requiring meticulous validation of genetic data [63].

This section explores the principles and methodologies of Sanger sequencing, emphasizing its relevance and applications in modern genomic studies. Sequencing DNA is fundamental to understanding genetic information, enabling applications ranging from disease diagnosis and treatment to evolutionary biology [47]. By deciphering the nucleotide order, scientists can identify mutations, study gene expression, and uncover genetic diversity.

To better understand the components involved in the Sanger Sequencing 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.

The steps of Sanger sequencing are enumerated as follows and illustrated in Figure 2.3:

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 2.3: Steps involved in Sanger sequencing [57].

2.2 Artificial Intelligence

Artificial intelligence (AI) represents a paradigm shift in how data is analyzed and interpreted across multiple fields, including genomics. AI encompasses a broad range of computational techniques aimed at simulating human intelligence, including learning, reasoning, and self-correction [58]. These capabilities enable AI systems to process and analyze vast amounts of data, uncover hidden patterns, and make informed predictions. The journey of AI began with early algorithms that could perform basic logical reasoning.

Over time, advancements in computational power and data availability led to the development of sophisticated methods like **Machine Learning (ML)** and **Deep Learning (DL)**, which are now at the forefront of AI innovation. The development of AI has been greatly accelerated by advancements in computational power, data availability, and algorithmic improvements [7].

2.2.1 Data Science

Data is the cornerstone of achieving optimal model performance in Artificial Intelligence (AI). The effectiveness of AI models hinges on the quality and relevance of the data used for training. These models learn from the data provided, and their ability to generate accurate responses is directly tied to the insights they extract during this process. In the context of Data Science, this underscores the critical importance of data preparation, including cleaning, transformation, and feature engineering, as these steps lay the foundation for robust and reliable AI systems [24, 54]. Data Science is an interdisciplinary field that combines domain expertise, programming skills, and knowledge of mathematics and statistics to extract meaningful insights from structured and unstructured data [52]. It serves as the foundation for advanced technologies like Machine Learning (ML) and Deep Learning (DL), offering essential tools and methodologies for data processing and analysis [16]. The importance of Data Science lies in its ability to bridge the gap between raw data and actionable insights. By leveraging techniques such as data cleaning, transformation, and visualization, it enables the identification of patterns, trends, and correlations that would otherwise remain hidden [33]. This process lays the groundwork for training ML and DL models by ensuring that the data is accurate, complete, and relevant.

The key components of Data Science include:

- **Data Collection:** Gathering data from diverse sources, such as experiments, databases, or web scraping [27].
- **Data Cleaning:** Addressing issues like missing values, duplicates, and inconsistencies to ensure data quality [38].
- **Exploratory Data Analysis (EDA):** Using statistical techniques and visualization tools to understand the underlying structure of the data [69].
- **Feature Engineering:** Transforming raw data into meaningful features that enhance the performance of ML models [26].
- **Model Evaluation:** Assessing the performance of predictive models using metrics like accuracy, precision, and recall [19].

Data Science is a crucial step for ML and DL because the quality and relevance of data directly impact model performance. Poor data quality can lead to inaccurate predictions, while well-prepared data ensures that models can learn effectively and generate reliable results [5].

2.2.2 Machine Learning and Deep Learning

One of the key approaches within AI is Machine Learning (ML), which has become central to modern AI development. A more specialized area within ML is Deep Learning, which utilizes neural networks with multiple layers to model and solve highly complex problems. Deep Learning is considered a subset of ML, focused on using deep neural networks to automatically learn hierarchical representations of data.

The overall structure of AI can be thought of as a hierarchy, where AI is the overarching field that encompasses various approaches, with ML being a significant subset. Within ML, Deep Learning stands as a specialized technique designed to handle large, unstructured data by leveraging multi-layered networks. This structure allows each level to address different aspects of problem-solving, with Deep Learning excelling in tasks that require high levels of abstraction and complex pattern recognition.

Figure 2.4: Understanding what Machine Learning and Deep Learning are, as subsets of Artificial Intelligence [51].

2.2.2.1 Machine Learning

Machine Learning: Machine Learning (ML) is a subset of AI focused on developing algorithms that enable systems to learn from data and make decisions without explicit programming. In ML, algorithms are trained to recognize patterns and infer insights from large datasets [5]. ML techniques are typically divided into three main categories based on the nature of the training data:

- **Supervised Learning**: The algorithm is trained on labeled data, where the input data is paired with the correct output. The goal is to learn a mapping from inputs to outputs, as seen in classification or regression tasks [50].

- **Unsupervised Learning**: This method involves training on data that has no labels. The algorithm tries to identify hidden patterns or structures, such as grouping similar data points into clusters [33].

- **Reinforcement Learning**: The algorithm learns by interacting with its environment and receiving feedback through rewards or penalties. It is particularly useful in dynamic environments where decisions have long-term consequences [68].

Figure 2.5: Illustration of the main types of Machine Learning.

2.2.2.2 Deep Learning

Deep Learning: Deep Learning, a specialized area of machine learning, leverages artificial neural networks with multiple layers (hence "deep") to model and solve highly complex problems. By simulating the way humans learn from data, deep learning excels in identifying patterns, handling large datasets, and making predictions with high accuracy. Key architectures in deep learning include:

- **Convolutional Neural Networks (CNNs):** CNNs are particularly effective for analyzing data with a spatial structure, such as images or signals. They have found applications in areas like object detection, image classification, and medical imaging [43, 42].

- **Recurrent Neural Networks (RNNs):** RNNs are designed to handle sequential data, making them well-suited for tasks like natural language processing and time series analysis. Advanced variants, such as Long Short-Term Memory networks (LSTMs) and Gated Recurrent Units (GRUs), address challenges like vanishing gradients in longer sequences [31, 12].

- **Transformers:** Transformers revolutionized sequential data processing by introducing self-attention mechanisms, allowing the model to consider relationships between all elements in a sequence simultaneously. Transformers underpin models like BERT and GPT, which have demonstrated state-of-the-art performance in natural language understanding and generation [71, 14].

- **Autoencoders:** Autoencoders are unsupervised learning models that focus on efficient data encoding. They are widely used for dimensionality reduction, anomaly detection, and data denoising [30, 73].

Figure 2.6: A deep neural network consists of an input layer, multiple hidden layers and an output layer, all consisting of nodes [55].

Figure 2.7: A node in a hidden layer of a deep neural network takes an input, performs a calculation, and passes on an output to nodes in the next layer [55].

Figure 2.8: Each neural node in a deep neural network performs a calculation by taking inputs from nodes in the previous layer, multiplying these by weights, and summing all outcomes. The resulting number gets processed by an activation function to allow for more complex calculations. Lastly, the output is passed on to the nodes in the next layer [55].

2.2.3 Considerations for Model Selection & Development

Despite the vast array of deep learning techniques available, the choice of model should always align with the problem's context. Not all tasks require complex, deep networks; in fact, for simpler problems, simpler models may be more effective.

2.2.3.1 Overfitting

Overfitting is a common issue in machine learning, occurring when a model learns not only the underlying patterns in the training data but also the noise. This results in poor generalization to new, unseen data. Overfitting is particularly problematic when using overly complex models for relatively simple tasks.

For example, handwritten digit recognition tasks (such as the MNIST dataset) can be effectively solved using shallow neural networks or simpler models like Support Vector Machines (SVMs). Using a deep architecture, such as a Convolutional Neural Network (CNN), which is more suitable for complex datasets like ImageNet, would add unnecessary complexity and increase the risk of overfitting [28, 29].

To understand overfitting, it is essential to differentiate between training error and test error.

Training Error refers to the error a model makes on the data it was trained on. A model that memorizes the training set perfectly will have a very low training error, but this does not necessarily mean it will perform well on new data.

Test Error , on the other hand, measures the model's performance on unseen data. A well-generalized model maintains a balance between training error and test error. However, an overfitted model will exhibit a large gap between the two: it performs exceptionally well on training data but poorly on test data [22, 5].

Causes of Overfitting Overfitting is not solely caused by model complexity. Several factors contribute to this issue:

- **Model Selection:** Choosing a model that is too complex for the given dataset can lead to overfitting. For instance, using a deep neural network for a simple classification problem may result in unnecessary memorization rather than pattern recognition [16].

- **Insufficient Training Data:** When the dataset is too small, the model may not capture the true distribution of the data and instead learn noise as patterns. Expanding the dataset through data augmentation, synthetic data generation, or collecting more samples can mitigate this issue [65].

- **Lack of Regularization:** Regularization techniques such as L1/L2 regularization (ridge and lasso regression), dropout in neural networks, and early stopping help prevent overfitting by constraining the model's complexity [67].

- **Training for Too Many Epochs:** Over-training a model without early stopping can cause it to learn noise from the training data, reducing generalization to new examples [53].

- **High Feature Dimensionality:** If a model has too many features relative to the number of training samples, it may fit noise instead of meaningful relationships. Feature selection and dimensionality reduction techniques, such as Principal Component Analysis (PCA), can help mitigate this risk [36].

Mitigating Overfitting To reduce overfitting and improve generalization, various strategies can be employed:

- **Cross-validation:** Splitting data into multiple folds for training and validation helps estimate a model's performance on unseen data [37].

- **Early stopping:** Monitoring validation loss and stopping training before overfitting occurs prevents unnecessary memorization of noise [53].

- **Regularization:** Techniques like dropout, weight decay, and batch normalization help prevent overfitting by introducing constraints [67].

- **Data augmentation:** Applying transformations such as rotation, scaling, and flipping to training samples increases dataset diversity and improves model robustness [65].

- **Reducing model complexity:** Choosing a simpler model that still captures essential patterns prevents unnecessary parameterization [16].

By understanding and addressing the causes of overfitting, models can be optimized to balance training and test performance, ensuring their reliability in real-world applications.

Figure 2.9: The Optimum Regression compared to Underfitting and Overfitting the prediction. [2].

2.2.3.2 Balancing Bias and Variance

Bias and variance are two fundamental sources of error in machine learning models, and understanding their trade-off is crucial for building models that generalize well to new data [22].

Bias: refers to the error introduced by approximating a real-world problem with a simplified model. A model with high bias makes strong assumptions about the data and may fail to capture the underlying patterns, leading to underfitting [20]. This means that the model performs poorly on both training and test data. For example, using a linear regression model to fit a dataset with a highly nonlinear relationship would result in high bias. The model would fail to capture the complexity of the data, leading to systematic errors in its predictions [20].

Variance: refers to the model's sensitivity to small fluctuations in the training data. A model with high variance captures noise in the training set instead of just the underlying patterns, leading to overfitting [5]. This results in good performance on training data but poor generalization to unseen data. For instance, a deep neural network with a large number of parameters trained on a small dataset may memorize the training data but fail to generalize. The model's high variance means it is too dependent on the specifics of the training data, leading to poor performance on unseen samples [5].

The Trade-Offs: The trade-off between bias and variance is fundamental when selecting a model. Simpler models, such as linear regression or decision trees, generally exhibit higher bias but lower variance. These models are often preferable for tasks with limited data or when interpretability is critical. On the other hand, more complex models like Gradient Boosted Machines (GBMs) or neural networks tend to have lower bias but higher variance. Regularization techniques are often employed to control overfitting in these

cases [5, 16]. The goal of machine learning is to find a balance between bias and variance to minimize total error. A model with high bias and low variance tends to underfit, while a model with low bias and high variance tends to overfit. The optimal trade-off ensures that the model has just the right complexity to generalize well [16]. Regularization techniques, cross-validation, and careful model selection help manage this trade-off effectively.

Figure 2.10: (a) Graphical illustration of bias and variance. The aim is to obtain a model that has low bias and low variance. (b) Contribution of bias and variance to the total error of the machine learning model (reproduced from the reference [57]). With increasing model complexity, error function reduced significantly for training data set but perform poorly in validation dataset, i.e., higher variance. The task to define a model in such a way that the bias and variance can tackle in validation and blind test data set. [45].

2.2.3.3 Occam's Razor for Model Selection & Simplicity

Occam's razor [6] serves as a guiding principle: among competing models, the simplest one that adequately explains the data should be preferred. For instance, Pedro Domingos highlights the importance of understanding when complexity adds value and when it merely adds noise [16]. By adhering to this principle, one can avoid unnecessary complexity and focus on building models that are both effective and efficient.

2.2.3.4 Conclusion

In the context of machine learning and deep learning, choosing the right model depends on the specific problem, available data, and computational resources. While complex models like deep neural networks are powerful, they should be used judiciously. For many tasks, simpler models may offer equal or better performance with fewer risks of overfitting, lower computational costs, and greater interpretability. A thoughtful approach to model selection ensures that the chosen algorithm is both efficient and appropriate for the task at hand.

2.3 Related Work

The integration of Artificial Intelligence (AI) in genomics has revolutionized the field, enabling breakthroughs in DNA sequencing, variant calling, and anomaly detection. For this thesis, which aims to automate the detection and quarantining of anomalous DNA wells in a 96-well plate format, AI offers powerful tools to address the challenges of manual verification and error-prone workflows. Specifically, unsupervised and semi-supervised learning techniques are highly relevant, as they can identify patterns and anomalies in unlabeled sequencing data.

This section explores two state-of-the-art AI algorithms that are best suited for the task: Isolation Forest and DBSCAN. Both algorithms are unsupervised and excel at identifying anomalies in complex datasets. A comparative analysis is presented to evaluate their suitability for automating the quarantining process of DNA wells.

2.3.1 Isolation Forest: Unsupervised Anomaly Detection

Isolation Forest is an unsupervised anomaly detection algorithm that isolates anomalies instead of profiling normal data points. It works by randomly selecting a feature and then randomly selecting a split value between the maximum and minimum values of the selected feature. Anomalies are identified as data points that require fewer splits to be isolated [44].

Methodology Isolation Forest operates by constructing an ensemble of isolation trees. Each tree is built by recursively partitioning the data space until all data points are isolated. Anomalies, which are typically few and different, are isolated closer to the root of the tree, while normal points require more splits to be isolated. The anomaly score for each data point is calculated based on the average path length from the root to the leaf across all trees.

Strengths

- **Anomaly Detection**: Isolation Forest is specifically designed for anomaly detection, making it highly effective for identifying outliers in sequencing data.
- **Handles Mixed Data Types**: While traditionally used for numerical data, Isolation Forest can be adapted to handle categorical data (e.g., primer types) and sequence data by encoding them appropriately.
- **Explainability**: The algorithm provides anomaly scores, which can be used to explain why a well was flagged (e.g., "High concentration level detected" or "Unexpected primer used").
- **Scalability**: Isolation Forest is computationally efficient and scales well to large datasets, making it suitable for high-throughput sequencing workflows.

- **Robustness to Noise**: The algorithm is robust to noisy data, which is important given the potential for errors in sequencing data.

Limitations

- **Feature Engineering**: The algorithm requires careful feature engineering to handle mixed data types effectively.
- **Parameter Tuning**: The performance of Isolation Forest depends on the choice of parameters, such as the number of trees and the contamination rate, which may require tuning.

Relevance to Thesis Isolation Forest is highly relevant to the unsupervised nature of the task in this thesis. Its ability to detect anomalies in mixed data types (e.g., numerical and categorical) aligns well with the goal of automating the quarantining of DNA wells. Additionally, its explainability and scalability make it a strong candidate for high-throughput sequencing workflows.

2.3.2 DBSCAN: Density-Based Clustering and Anomaly Detection

Density-Based Spatial Clustering of Applications with Noise (DBSCAN) is an unsupervised clustering algorithm that groups data points based on their density. It can identify clusters of arbitrary shapes and mark outliers as noise [17]. DBSCAN is particularly effective for detecting anomalies in datasets with irregular structures.

Methodology DBSCAN works by defining clusters as dense regions of data points separated by sparse regions. The algorithm requires two parameters: the minimum number of points (minPts) and the maximum distance (epsilon) between points to be considered part of the same cluster. Points that do not belong to any cluster are marked as noise (anomalies).

Strengths

- **Anomaly Detection**: DBSCAN explicitly identifies outliers as noise, making it suitable for anomaly detection tasks.
- **Handles Irregular Clusters**: Unlike K-Means, DBSCAN can detect clusters of arbitrary shapes, which is useful for complex genomic data.
- **No Need for Predefined Clusters**: DBSCAN does not require the number of clusters to be specified in advance, making it more flexible than K-Means.
- **Robustness to Noise**: DBSCAN is robust to noisy data, which is important for sequencing workflows.

Limitations

- **Parameter Sensitivity**: The performance of DBSCAN depends on the choice of parameters (epsilon and minPts), which may require tuning.
- **Scalability**: While DBSCAN is efficient for moderate-sized datasets, it can struggle with extremely large datasets due to its computational complexity.
- **Handling Mixed Data Types**: DBSCAN is traditionally designed for numerical data and may require adaptations to handle categorical or sequence data.

Relevance to Thesis DBSCAN is highly relevant to the unsupervised nature of the task in this thesis. Its ability to detect anomalies and handle irregular cluster shapes aligns well with the goal of automating the quarantining of DNA wells. However, its parameter sensitivity and scalability limitations may require careful consideration.

2.3.3 Comparison with Thesis Approach

Table 2.1: Comparison of Isolation Forest, DBSCAN, and Thesis Approach

Aspect	**Isolation Forest**	**DBSCAN**	**Thesis Approach**
Algorithm	Unsupervised Anomaly Detection	Density-Based Clustering	Anomaly Detection & Similarity-Based Quarantining
Data Type	Mixed (Numerical & Categorical)	Numerical (with adaptations for mixed data)	Mixed (Numerical & Sequence Data)
Supervision	Unsupervised	Unsupervised	Unsupervised
Output	Anomaly Scores	Cluster Assignments & Noise (Anomalies)	Cluster and Quarantine Visualization
Scalability	High	Moderate	High
Computational Cost	Moderate	Moderate	Moderate

2.3.4 Other Relevant Algorithms

In addition to Isolation Forest and DBSCAN, several other algorithms and techniques have been developed for genomic data analysis. These methods represent a mix of supervised, unsupervised, and transformer-based approaches, providing a broader context for the field.

DNABERT: Transformer-Based Genomic Sequence Analysis DNABERT is a transformer-based model designed for genomic sequence analysis. It leverages the self-attention mechanism of transformers to capture long-range dependencies in DNA sequences, enabling tasks such as sequence classification, motif discovery, and variant calling [34]. DNABERT is pre-trained on large-scale genomic datasets and fine-tuned for specific tasks, achieving state-of-the-art performance in several benchmarks.

Nucleotide Transformer: Pre-trained Models for Genomics The Nucleotide Transformer is another transformer-based model that has been pre-trained on diverse genomic datasets. It is designed to handle a wide range of genomic tasks, including sequence alignment, variant calling, and gene expression prediction [13]. Like DNABERT, the Nucleotide Transformer demonstrates the potential of pre-trained models for genomics, particularly in supervised learning tasks.

Autoencoders for Anomaly Detection Autoencoders are a type of neural network used for unsupervised learning, particularly for dimensionality reduction and anomaly detection. They work by compressing the input data into a lower-dimensional representation (encoding) and then reconstructing the original data from this representation (decoding). Wells with high reconstruction errors can be flagged as anomalies [**kingma2013autoencoding**].

Graph Neural Networks (GNNs) for Relationship Modeling Graph Neural Networks (GNNs) can model relationships between entities in a graph, making them suitable for tasks like gene clustering or pathway analysis. In your case, GNNs could be used to model relationships between wells based on shared features like primer usage, DNA ID, or client information [**kipf2016semi**].

2.3.5 Conclusion

The review of related work highlights the strengths and limitations of two state-of-the-art AI algorithms in genomics: Isolation Forest and DBSCAN. Isolation Forest is specifically designed for anomaly detection and excels at identifying outliers in mixed data types, making it highly suitable for the task of quarantining anomalous DNA wells. DBSCAN, on the other hand, offers robust clustering and anomaly detection capabilities, particularly for datasets with irregular structures, but may require adaptations to handle mixed data types.

The proposed approach in this thesis builds on the strengths of these algorithms while addressing their limitations through careful feature engineering and parameter tuning. By leveraging anomaly detection and explainability, the proposed solution aims to improve the efficiency and accuracy of DNA well quarantining in high-throughput sequencing workflows. The model quarantines wells it deems contaminated or anomalous, which are

then reviewed by laboratory personnel for further action (e.g., re-trying or approving). This workflow ensures that only high-quality sequencing results are delivered to clients, enhancing the reliability and reputation of the sequencing service.

3

Proposal

This chapter details the choice and implementation of the proposed AI-driven tool, outlining all the important steps for it's development. From the laboratory processes, the data collection, to the AI model proposal itself that will be used to automate well-quarantining, the ultimate goal of this proposal is to develop a system that enhances the efficiency and accuracy of delivered sequencing results while reducing manual effort. By leveraging machine learning techniques, particularly unsupervised learning, the proposed tool will provide an automated approach to categorizing DNA samples based on their similarity according to multiple fields. This will address the current limitations of manual analysis, improving both speed and reliability in laboratory workflows.

The chapter begins with an overview of the laboratory processes involved in sequencing, including the preparation of samples in a 96-well PCR plate, and it's sequencing using the ABI3730xl DNA sequencer. Next, it discusses the data collection and storage procedures, emphasizing the structured format of sequencing outputs and metadata handling. Finally, it introduces the proposed AI model for use, detailing the methodology for clustering the DNA sequences and automating quality assessment.

3.1 Laboratory Processes

The laboratory processes described here are central to the generation of the data used in this work. These processes involve the handling of DNA samples, their preparation for sequencing, and the subsequent analysis of the results.

3.1.1 Sample Preparation and Processing

The laboratory workflow begins with the reception of DNA samples from various clients. These samples are organized into 96-well PCR plates, where each well is identified by a unique coordinate system: letters A to H for the rows and numbers 1 to 12 for the columns (Figure 3.1). This organization ensures traceability and efficient handling of multiple samples simultaneously. Samples from the same client are grouped together in

neighboring wells, although not all wells need to be occupied for sequencing to proceed. Each plate must contain at least one control well.

Figure 3.1: A 96-well PCR plate.

Figure 3.2: Structure and Organization of each PCR plate.

Upon receipt, some samples require human intervention to prepare them for sequencing. For instance, samples with uneven DNA concentrations must be normalized to ensure consistent sequencing quality. Additionally, samples that do not include a primer must have the appropriate primer added by laboratory staff. These steps are critical to ensure the accuracy and reliability of the sequencing results.

Once prepared, these plates are then loaded into the ABI3730xl DNA sequencer, which includes DNA denaturation, primer annealing, extension with fluorescently labeled dideoxynucleotides, and capillary electrophoresis. The ABI3730xl sequencer then

generates raw data files containing fluorescence intensity readings, which are subsequently processed to produce DNA sequence alignments.

3.1.2 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 2.3), allowing for the simultaneous processing of 96-well plates in a single run [66, 4]. By leveraging this technology, laboratories can achieve precise and efficient DNA sequencing.

Figure 3.3: A closed ABI3730xl capillary electrophoresis DNA analyzer.

Figure 3.4: An opened ABI3730xl capillary electrophoresis DNA analyzer.

3.2 Data Structure and Analysis

The efficiency of any artificial intelligence model is heavily dependent on the quality and structure of the data used for training. In this work, the data originates from customer samples sequenced using the ABI3730xl sequencer. The sequencer outputs a variety of files per plate well, including raw fluorescence data, the processed sequence itself, and additional quality metrics. Each PCR plate generates a significant volume of data, with millions of files produced over time, as multiple plates are processed each day. Since each PCR plate contains 96 wells, and each well generates 7 files, this results in 672 files per plate. In this work, the files taken into account are the following:

- **Ab1 File**: Contains intensity readings for each of the four fluorescent dyes (A, T, C, G) used in Sanger sequencing, resulting in fluorescent traces. This file is optimized for long sequences (more than 500 bases).
- **Phd1 File**: Contains the DNA sequence derived from the fluorescence traces, along with quality scores and scan numbers.
- **Scf File**: Similar to .ab1 files, .scf files include quality information about the base calls, the chromatogram (also called the electropherogram), and the DNA sequence.
- **Seq File**: A plain text file containing the DNA sequence.
- **Qual File**: Includes the quality scores of each base in the DNA sequence.
- **Qualtrace File**: Contains additional information about the sequencing process, such as PeakStart, DyeSignal, Noise, and Spacing.

These files adhere to a strict naming convention: **ReactionID_Pos_DNAID+PrimerID**. For example, the file name `2338715_A1_AA8117+Clon_2+ORB501` can be broken down as follows:

- `2338715`: The unique reaction ID.
- `A1`: The position of the well in the plate (row A, column 1).
- `AA8117+Clon_2`: The DNA ID, identifying the specific DNA sample.
- `ORB501`: The primer ID, which is optional as some customers provide samples with primers already included.

Thus, 6 files are generated for this specific well:

- `2338715_A1_AA8117+Clon_2+ORB501.ab1`
- `2338715_A1_AA8117+Clon_2+ORB501.phd.1`
- `2338715_A1_AA8117+Clon_2+ORB501.scf`

- `2338715_A1_AA8117+Clon_2+ORB501.seq`
- `2338715_A1_AA8117+Clon_2+ORB501.qual`
- `2338715_A1_AA8117+Clon_2+ORB501.qualtrace`

The database thus contains Dailybooks for each day, with reactions processed during that specific day

4

Planning

TODO

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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 [15] in the appendix.

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I

Annex 1 Lorem Ipsum

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