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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

NOVA University Lisbon **Draft:** January 11, 2025



DEPARTMENT OF COMPUTER SCIENCE

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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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GLOSSARY

computer

An electronic device which is capable of receiving information (data) in a particular form and of performing a sequence of operations in accordance with a predetermined but variable set of procedural instructions (program) to produce a result in the form of information or signals. This is a test that adds a citation [2] to the glossary! (*p.* 13)

ACRONYMS

aaa acronym aaa (p. 13)aab acronym aab (p. 13)aba acronym aba (p. 13)

abbrev abbreviation of a longer text (p. 13)

bbb acronym bbb (*p.* 13)

novathesis NOVAthesis LAT_EX (pp. 1, 5–8)

xpto xpto xpto xpto xpto xpto xpto (p. 13)

Symbols

- μ Mu (p. 14)
- π the numerical value of pi (p. 13)
- r the radius of a circle (p. 13)

Introduction



This is the NOVAthesis LATEX (novothesis) LATEX template Version 7.2.5 from Template!date2024-11-03.

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1.1 Motivation

The ever-growing demand for efficient and accurate DNA sequencing solutions has high-lighted 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

Sanger sequencing, developed in the 1970s, revolutionized genetic research and remains a gold standard for DNA analysis. The ABI3730xl sequencer is one of the most prominent

instruments used for this purpose, providing high-quality sequence alignments. However, the process is not immune to errors, particularly in labeling and detecting sequence anomalies within 96-well plates.

In recent years, artificial intelligence has emerged as a powerful tool for tackling complex problems across various domains, including genomics. Machine learning models, trained on extensive datasets, can identify patterns and anomalies with a precision that surpasses human capabilities. Applying these advancements to the verification phase of Sanger sequencing presents an opportunity to overcome existing limitations and enhance the reliability of DNA sequencing results.

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 96well 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 96well 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.

STATE OF THE ART

2.1 State of the Art

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.

2.1.1 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 [dnastar, geneious].

2.1.2 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 [frontiers, pmc_ml].

2.1.3 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 [pmc_asap, bioinformaticsadvances].

2.1.4 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 [champuru2].

2.1.5 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 [base4].

2.2 Quick Start

ee

2.2.1 With a Local LATEX Installation

- 1. Download LATEX. There are two major LATEX distributions MikTEX and TEXLive that share lots of similarity, and LATEX documents are portable between them. This means that, for most users, both systems are equally usable.
 - **TeX-Live** is maintained by (La)TeX developers and is certainly the best distribution you may install in your computer: However, the default distribution will take more than 5 GB on your hard disk... so, if you are not short on disk space, install TeXLive!
 - **MikTeX** will, by default, install only a minimal set of packages. The extra/additional packages will be installed on the fly. Installing packages on the fly is useful if disk space is limited, but has its own caveats in the longer term. Definitely choose MikTeX if you're short on disk space.

Which one to download? There are pros and cons for both distributions so it is essentially a question of where does your heart fall first! Mine falls to TEXLive, but yours can fall elsewhere! :D

- 2. Install LATEX. Installation of LATEX is as hard as installing any other software. Just do your best and you will certainly succeed.
- 3. Update your LaTeX installation using the *TeXLiveUtility* program of the MikTeX console.
- 4. Download the NOVAthesis LATEX (novothesis) template by either:
 - Cloning the GitHub repository with

or

```
git clone --depth=1 https://github.com/joaomlourenco/novathesis.git
```

- Downloading the latest version from the GitHub repository as a Zip file.
- 5. Download additional School specific files if applicable:

```
Universidade do Minho (UMINHO) download the required NewsGotT font files
    from https://github.com/joaomlourenco/novathesis-extras/raw/main/Fonts/
    NewsGotT.zip
    then unzip the file and copy the 3 font files
        "n015002t.ttf", "n015003t.ttf", and "n015006t.ttf"
    to the folder
        "NOVAthesisFiles/FontStyles/Fonts".
```

Escola Superior de Enfermagem do Porto (ESEP) download the required Calibri font files from https://github.com/joaomlourenco/novathesis-extras/raw/main/Fonts/Calibri.zip then unzip the file and copy the 4 font files

"Calibri.ttf", "Calibrib.ttf", "Calibrii.ttf", and "Calibriz.ttf" to the folder

NOVAthesisFiles/FontStyles/Fonts.

- 6. Compile the document with you favorite LaTeX processor (pdfLaTeX, XeLaTeX or LuaLaTeX).
 - The main file is named "template.tex", but you are free to rename it as you please.
 - Either load the main file in your favorite LaTeX text editor and press the appropriate (magic) button to generate a PDF file, or open a terminal and compile it with "latexmk -pdf template". If you use a LATeX text editor, please notice that the novothesis template uses "biber" and not "bibtex" to process the bibliography, which means that most probably you have to open the Editor Preferences and somewhere (depending on the Editor you are using) change "bibtex" to "biber".
 - Notice that, due to the external font sets used, pdfLATEX will not work for both UMINHO and ESEP, and you have to use either XALATEX ("latexmk -pdfxe template") or LuaLATEX ("latexmk -pdflua template").
- 7. Edit the files in the "Config" folder. See Table 2.2.
- 8. Recompile de document.
- 9. And you're done with a beautifully formatted thesis/dissertation! :D

2.2.2 With a Remote Cloud-based Service

Follow these steps to get started with a remote LATEX installation:

- Download the latest version from the GitHub repository as a Zip file.
- Login to your favorite LaTeX cloud service. I recommend Overleaf but there are alternatives. These instructions apply to Overleaf and you'll have to adapt for other providers.
- In the menu select | New project | Upload project |.
- Select "template.tex" as the main file.
- Follow from Step 5 above in Section 2.2.1 (With a Local LATEX Installation).

2.3 Folder and Files

The novothesis template is organized into many files and folders. At the main level it includes the following files and folders listed in Table 2.1.

Table 2.1: The folders and files (top level).

Name	Туре	Access	Contents
template.tex		DON'T TOUCH!	The main template file. You need to <i>compile</i> this file with one of pdfIATEX, XATATEX, or LuaIATEX to obtain the PDF file ("template.pdf"). I recommend the usage of the "latexmk" command or, if you use a UN*X-like OS, you may use "make" (and the ggiven "Makefile").
Config		docs.	Configuration files. Please customize your template by changing the files in this folder!
Chapters			Examples of document contents, including Chapters, Appendices, Annexes, Abstracts, Glossaries, Lists of Symbols, etc. Replace them with your own.
Bibliography			Where all your bibliography files should be located. You may have has many bibliography files as you want.
template.pdf			A possible result of applying pdfLATEX to the "template.tex" file. The look and feel of the document will depend on the parametrization/configuration (e.g., School) of this template.

novathesis.cls





The main class file.

NOVAthesisFiles



Additional files for the novothesis template. This is where all the juice is so, unless you are a TEXmagician, don't mess up with the files and folders inside this folder.

Table 2.2: The configuration files (Config folder).

Name	Туре	Access	Contents
O_memoir.tex		DON'T TOUCH!	Options specific for the memoir class. Don't touch this file unless you know what you are doing!
1_novathesis.tex			The main configuration file for the template, e.g., select the document type, the school, the used languages, etc.
2_biblatex.tex			Select how your citations and bibliographic references will be printed. The default is numbers inside square brackets, e.g. [11], but you can change it to other formats, such as author-year, e.g., Lourenço (2021).
3_cover.tex		(Control of the Control of the Contr	Configure cover contents (e.g., author's name, thesis/dissertation title, author, advisers, committee, etc)
4_files.tex		Sowe S	Select which files shall be included in the document as chapters, appendices, annexes, etc
5_packages.tex			User's customization, such as loading additional packages and declare user defined commands.
6_list_of.tex		DON'T TOUCH!	Configure the lists to be printed (table of contents, list of figures, list of tables, list of listings, etc). Don't touch this file unless you know what you are doing!
9_nova_fct.tex		Tools	Configurations specific to NOVA FCT. <i>Otherwise ignored</i> .
9_ulisboa_fmv.tex		Yours	Configurations specific to ULISBOA FMV. <i>Otherwise ignored</i> .
9_ulisboa_ist.tex		SOWE O	Configurations specific to ULISBOA IST. <i>Otherwise ignored</i> .

9_uminho.tex





Configurations specific to UMINHO (all schools). *Otherwise ignored*.

2.4 Customizing the novathesis template

The novathesis template can be customized by editing the files in the Config folder.

2.4.1 Options in 1_novathesis.tex

2.4.1.1 Most Relevant Options (1_novathesis.tex)

doctype=OPT phd, phdprop, phdplan, msc, mscplan, bsc, plain	The type of the document. phd → PhD thesis (← default). phdprop → PhD thesis proposal (for FCT-NOVA). phdplan → PhD thesis plan. msc → MSc thesis. mscplan → MSc thesis plan. bsc → BSc report. plain → Other report.
--	--

school=OPT

nova/fct, nova/fcsh, nova/ims,
nova/ims/mcsig, nova/ims/mgt,
nova/ensp, nova/itqb/green,
nova/itqb/gray, ulisboa/ist, ulisboa/fc,
ulisboa/fmv, uminho/eaad, uminho/ec,
uminho/ed, uminho/eeg, uminho/eeng,
uminho/elach, uminho/emed,
uminho/epsi, uminho/ese, uminho/i3bs,
uminho/ics, uminho/ie, iscteiul/eta,
ips/ests, ipl/isel, ipl/isel/meb, ulht/deisi,
ulht/mge, other/esep

Selection of the university and of the school (and degree variant).

⇒ Default: school=nova/fct

This option changes the typesetting of the de document to some specific School formating and layout, like covers, margins, fonts, paragraph spacing and indentation, etc.

docstatus=OPT

draft, provisional, final

The current status of the document.

working \rightarrow Working version (\Leftarrow *default*). provisional \rightarrow Version for submission. final \rightarrow Final version.

lang=OPT en, pt, de, es, fr, gr, it	The main language for the document. en \rightarrow Enlgish (\Leftarrow default). pt \rightarrow Portuguese. de \rightarrow German. es \rightarrow Spanish. fr \rightarrow French. gr \rightarrow Greek. it \rightarrow Italian.
media=OPT screen, paper	The target media for the PDF. screen \rightarrow No empty/white pages (\Leftarrow default). paper \rightarrow Empty/white pages are added when necessary.
print/webography=OPT User defined title	Generate a separate bibliography for @online references. ⇒ Default: print/webography=undefined If undefined, the @online references are list in the main bibliography. If defined, the @online references will be printed in a separate bibliography titled as given in the option.
color/links=OPT Color name	The color for the hyperlinks (URLs, cross references, citations). ⇒ Default: color/links=DarkBlue The valid color names as listed in "xcolor" manual, the "svgname" color set.
color/gls=OPT Color name	The color for the glossary managed hyperlinks (glossary, symbols, etc). ⇒ Default: color/gls=Black The valid color names as listed in "xcolor" manual, the "svgname" color set.
$print/index=OPT$ $true$, $false (\Leftarrow default)$	Print the (words) index at the end of the document. Print the index (in Portuguese <i>Índice Remissivo</i>).

2.4.1.2 Less Relevant Options (1_novathesis.tex)

abstractorder=OPT	Forces the abstracts languages and order for				
$L_0 = \{L_1, L_2, , L_n\}$	documents in language L_0 .				
	\Rightarrow Default: abstractorder={en={en,pt}} for english				
	\Rightarrow Default: abstractorder={L={L,en}} for lang L				
	L_i is a two-letters language code from the set of valid				
	language codes, following ISO 3166-1 (alfa-2).				
lang/extra=OPT	List of additional languages are used in the doc-				
$\{L_1,L_2,,L_n\}$	ument besides the main laguage and those used				
	in the abstracts (above).				
	\Rightarrow Default: lang/extra={}				
	L_i is a two-letters language code from the set of valid				
	language codes, following ISO 3166-1 (alfa-2).				
gnumberlist=OPT	Shall the glossary entries list the page numbers				
$true$, (\Leftarrow default)	where those entries are used? (Like a reverse in-				
false	dex!)				
numberallpages=OPT	Shall all the pages (except cover) be numbered?				
true,					
false (← default)					
tocintoc=OPT	Shall table of contents be listed in the table of				
true,	contents?				
$false \ (\Leftarrow default)$					
tocintoc=OPT	Shall a second cover page be forced?				
true,	If the contents for the second page are not defined, the				
$false (\Leftarrow default)$	second cover will be a replica of the first cover.				
print/committee=OPT	Shall the evaluation committee be printed?				
true,	\Rightarrow Default: print/committee=true if docstatus=fina				
false	\Rightarrow Default: print/committee=false otherwise				
print/frontmatter=OPT	Shall the front matter be printed?				
true, (← default)	Set to false to generate a PDF with only the cover and				
false	the book chapters.				
print/statement=OPT	Shall the honor/originality statement be printed?				
$true$, $false (\Leftarrow default)$					

print/copyright=OPT

Shall the copyright message be printed?

true, (\Leftarrow default)

false

false

print/timestamp=OPT

Shall a timestamp (with the PDF generation date/time) be printed in the cover?

true, date/time) be printed in the cover?

 \Rightarrow Default: print/timestamp=true if docstatus=working

 \Rightarrow Default: print/timestamp=false otherwise

style/url=OPT

default, (\Leftarrow default)

Use the same (main) font in URLs?

same

style/font=OPT W

arial, bookman, calibri, erewhon, kieranhealy, kpfonts, libertine, newpx, newsgott, scholax

Which font set to use in the document?

arial → Use 'arial' font. Requires XelaTeX or LualaTeX.

bookman \rightarrow Use 'bookman' font.

calibri → Use 'calibri' font. Requires XeI₄TEX or LuaI₄TEX.

erewhon \rightarrow Use 'erewhon' font. kieranhealy \rightarrow Use 'kieranhealy' font. kpfonts \rightarrow Use 'palatino' font. libertine \rightarrow Use 'libertine' font.

newpx \rightarrow Use 'palatino' font. (\Leftarrow *default*)

newsgott → Use 'newsgott' font. Requires XelaTeX or LualaTeX.

scholax \rightarrow Use 'scholax' font.

style/chapter=OPT

See list on the side!

Which chapter style to use in the document?

the standard memoir chapter (default, section, article, reparticle, hangnum, companion, demo, bianchi, bringhurst, brotherton, chappell, culver, dash. demodemoell, lyhne, madsen, pedersen, southall, thatcher, veelo, verville, crosshead, dowding, komalike, ntglike, tandh, wilsondob), the customized list of chapter styles below is also available. \rightarrow Use 'bar' chapter style. (\Leftarrow *default*) bar

 $\begin{array}{lll} bar\text{-}compact & \rightarrow Use \ 'bar\text{-}compact' \ chapter \ style. \\ bluebox & \rightarrow Use \ 'bluebox' \ chapter \ style. \\ compact & \rightarrow Use \ 'compact' \ chapter \ style. \\ elegant & \rightarrow Use \ 'elegant' \ chapter \ style. \\ fmv & \rightarrow Use \ 'fmv' \ chapter \ style. \\ hansen & \rightarrow Use \ 'hansen' \ chapter \ style. \\ ist & \rightarrow Use \ 'ist' \ chapter \ style. \\ ist & \rightarrow Use \ 'ist2' \ chapter \ style. \\ \end{array}$

 $pedersen \longrightarrow Use \ 'pedersen' \ chapter \ style.$

lang/cover=OPT en, pt, de, es, fr, gr, it	The main language for the cover. ⇒ Default: The same as the main language.
lang/copyright=OPT en, pt, de, es, fr, gr, it	The main language for the copyright message. ⇒ Default: The same as the main language.
spine/layout=OPT no, full, trim	Print the "book spine" at the end of the document? ⇒ Default: `trim' if docstatus=final ⇒ Default: `no' otherwise no → do not print the book spine. full → print the book spine in a full page. trim → print and trim the page to the width of the book spine.
spine/width=OPT LATEX dimension	Force the width of the "book spine". \Rightarrow <i>Default: The "natural width"</i> . The defailt width for the book spine will be the width of the number of pages of the document if printed in standard paper $(80g/m^2)$.
debug=OPT cover, spine	Activate debug mode for cover and/or book spine. ⇒ Default: debug={}

2.5 How to Write Using LATEX

Please have a look at Chapter 3, where you may find many examples of LaTeX constructs, such as Sectioning, inserting Figures and Tables, writing Equations, Theorems and algorithms, exhibit code listings, etc.

2.6 Example glossary, acronyms, and symbols

 please see the definition of μ .

A SHORT LATEX TUTORIAL WITH EXAMPLES

This Chapter aims at exemplifying how to do common stuff with LATEX. We also show some stuff which is not that common! ;)

Please, use these examples as a starting point, but you should always consider using the *Big Oracle* (aka, Google, your best friend) to search for additional information or alternative ways for achieving similar results.

3.1 Document Structure

3.2 Dealing with Bibliography

Citing something online [4, 7, 9].

3.3 Inserting Tables

3.4 Importing Images

3.5 Floats, Figures and Captions

Lorem ipsum dolor sit amet, consectetuer adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetuer id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

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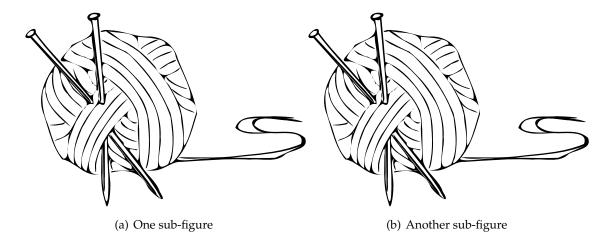


Figure 3.1: A figure with two sub-figures!

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 [2], usar um "~" para ligar o \cite{...} à palavra que o precede (...referência~\cite{Art Caso queira fazer múltiplas citações [12, 13, 6], 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

Footnotes¹ 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.

St Anomalies Warnings Correct

Test	Anomalies	Warnings	Correct	Categories	Missed
Connection [3]	2	2	1	С	1
Coordinates'03 [1]	1	4	1	2B, 1C	0
Local Variable [1]	1	2	1	A	0
NASA [1]	1	1	1		0
Coordinates'04 [2]	1	4	1	3C	0
Buffer [2]	0	7	0	2A, 1B, 2C, 2D	0
Double-Check [2]	0	2	0	1A, 1B	0
StringBuffer [8]	1	0	0		1
Account [14]	1	1	1		0

¹This is a simple footnote.

Jigsaw [14]	1	2	1	С	0
Over-reporting [14]	0	2	0	1A, 1C	0
Under-reporting [14]	1	1	1	_	0
Allocate Vector [10]	1	2	1	C	0
Knight Moves [3]	1	3	1	2 <i>B</i>	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

$$e = mc^2 (3.1)$$

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

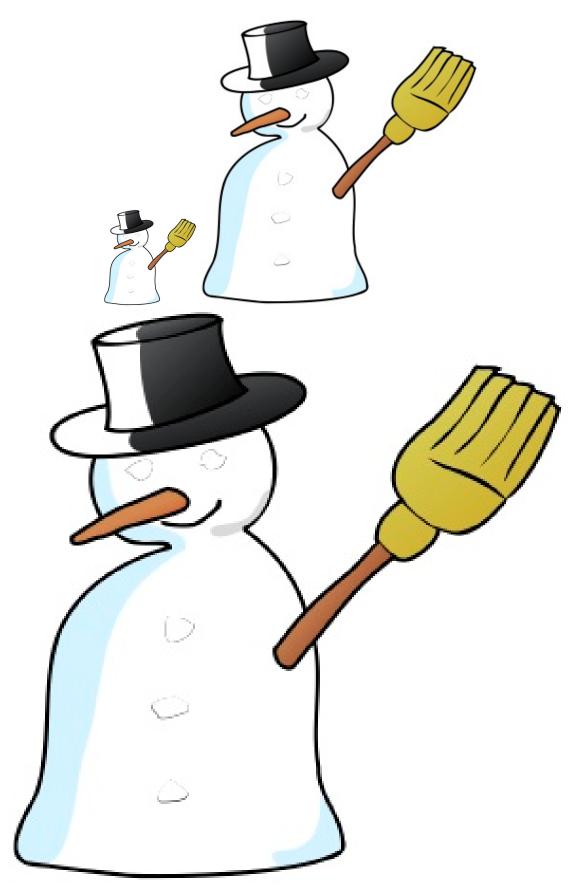


Figure 3.2: Bitmap image (JPG/PNG)

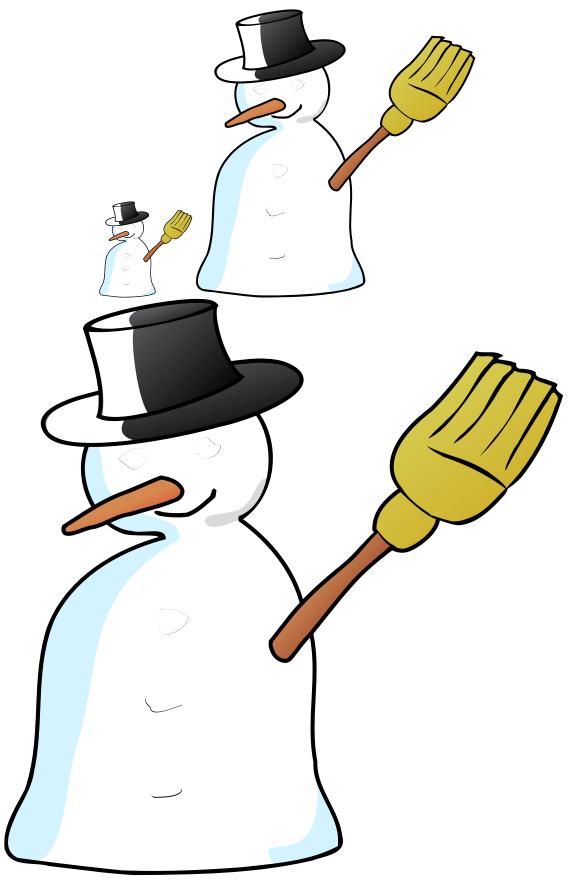


Figure 3.3: Vectorial image (PDF)

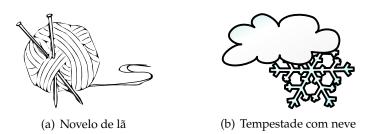


Figure 3.4: Exemplo de utilização de subbottom

\ntaddlistof{listofalgorithms}

in file "8_list_og.tex".

Adding Support to a New School (work in progress)

My advice to customize the novothesis 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 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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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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В

APPENDIX 2 LOREM IPSUM

This is a test with citing something [5] in the appendix.

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Annex 1 Lorem Ipsum

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