A Computational Platform for Assessing the Impact of Alternative Splicing in Cancer

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Abstract

Cancer is a disease that affects millions of individuals around the world every year. Although it is known by this single name it may have different causes. One such cause is based on an abnormal working of the genetic mechanisms. The emergence of high-throughput sequencing has brought substantial advances in cancer genomics research. The current pace of advance of this technology made it possible to assay with high depth of coverage the DNA and the transcriptomes (RNA-Seq) of tumour samples and cancer cell lines. Aberrant RNA processing of genes plays an important role in cancer.

The goal of this thesis is twofold: automate, as much as possible, the analysis process of genetic data for cancer studies; and develop a computational platform that makes the powerful computational resources required for such analysis easily manageable and in a scalable fashion. With these two goals in mind we have implemented part of a pipeline that performs the analysis from the RNA-Seq data (the set of reads) to the determination of the possible protein domains that may be in the origin of the disease. The platform also allows the enrichment of the analysis by searching for information in relevant databases available on the internet. We have also developed it in a way that hides the computational resources from the user making it easy to use by the life sciences experts. The platform is also very easy to manage enabling the use/updating/removing of resources on different operating systems and without impact to the analysis process. It also enables the execution of the complete set or individual steps of the analysis pipeline.

Although the platform is general purpose, the current version is tuned for to the application of methods that use high-throughput information from the transcriptome of cancer samples to identify events of aberrant splicing and their impact in the transcript structure. The developed platform was tested in case studies using different cell lines from databases available on the ENCODE project web site.
Resumo

O câncer é uma doença que afeta milhões de pessoas em todo o mundo todos os anos. Apesar de ser conhecida por este nome, pode ter diferentes causas. Uma dessas causas é baseada num mau funcionamento dos mecanismos genéticos. O surgimento do sequenciamento de alto rendimento trouxe avanços substanciais na área de investigação da genómica do câncer. Com o passo atual de desenvolvimento desta tecnológica, é possível determinar com grande cobertura o DNA e os transcriptomas (RNA-Seq) de exemplos células cancerígenas e de linhas celulares. O processamento aberrante de genes tem um papel importante no câncer.

O objetivo desta tese é duplo: automatizar ao quanto possível o processo de análise de dados genéticos para o estudo do câncer; e desenvolver uma plataforma computacional que permita o uso de poderosos recursos computacionais para estudos que envolvam análise de informação de alto rendimento do transcriptoma de amostras cancerígenas de forma fácil e escalável. Com estes dois objetivos em mente implementámos parte das sequências de tarefas que executam a análise a partir dos dados RNA-Seq (o conjunto de reads) até à determinação dos possíveis domínios de proteínas que possam estar na origem da doença. A plataforma permite o enriquecimento da análise ao procurar por informação em bases de dados relevantes disponíveis na internet. A plataforma foi desenvolvida de maneira a esconder os recursos computacionais do utilizador, fazendo com que esta seja simples de usar por parte dos especialistas das ciências vivas. A plataforma é também muito fácil de gerir ao permitir utilização/atualização/remoção de recursos em diferentes sistemas operativos e sem impacto no processo de análise. Também permite a execução individual de cada passo da sequência de tarefas.

Apesar de a plataforma ser de uso geral, a versão atual está otimizada para a aplicação de métodos que usam informação de alto rendimento de transcriptomas de amostras de câncer para identificar ocorrências de splicing alternativo e o seu impacto na estrutura transcrita. A plataforma desenvolvida foi testada em casos de estudo usando diferentes linhas celulares de bases de dados disponíveis na página web do projeto ENCODE.
Acknowledgements

I would like to thank my supervisor at FEUP, Professor Rui Carlos Camacho de Sousa Ferreira da Silva, for his suggestions and reviews on both the solution developed and this document. I would also like to thank my co-supervisor from the Swiss Institute of Bioinformatics, Doctor Pedro Gabriel Ferreira, for his help in understanding the pipelines related with alternative splicing analysis.

Vitor Moreira
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Abbreviations

API Application Programming Interface
ASP Active Server Pages
BAM Binary Sequence Alignment
BED Annotation Track Format
cDNA Complementary DNA
CRUD Create, Read, Update and Delete
CSS Cascading Style Sheets
DB Database
DNA Deoxyribonucleic Acid
FEUP Faculty of Engineering of the University of Porto (Faculdade de Engenharia da Universidade do Porto)
FTP File Transfer Protocol
GNU GNU's not UNIX!
GPL GNU General Public License
GTF Gene Transfer Format
HTML HyperText Markup Language
HTTP Hypertext Transfer Protocol
IIS Internet Information Services
JS JavaScript
JSON JavaScript Object Notation
mRNA Messenger RNA
MVC Model–View–Controller
NGS Next Generation Sequencing
NoSQL Not Only SQL
ORF Open Reading Frame
OS Operating System
OSI Open Source Initiative
REST Representational State Transfer
RGB RGB colour model (red, green, blue)
RNA Ribonucleic Acid
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA-Seq</td>
<td>RNA Sequencing</td>
</tr>
<tr>
<td>SAM</td>
<td>Sequence Alignment/Map</td>
</tr>
<tr>
<td>SHA</td>
<td>Secure Hash Algorithm</td>
</tr>
<tr>
<td>SQL</td>
<td>Structured Query Language</td>
</tr>
<tr>
<td>Stderr</td>
<td>Standard Error</td>
</tr>
<tr>
<td>Stdin</td>
<td>Standard Input</td>
</tr>
<tr>
<td>Stdio</td>
<td>Standard Input/Output</td>
</tr>
<tr>
<td>Stdout</td>
<td>Standard Output</td>
</tr>
<tr>
<td>SVM</td>
<td>Support Vector Machine</td>
</tr>
<tr>
<td>URL</td>
<td>Uniform Resource Locator</td>
</tr>
<tr>
<td>XML</td>
<td>Extensible Markup Language</td>
</tr>
</tbody>
</table>
Chapter 1

1. Introduction

This chapter gives an introduction to the problem being addressed by the thesis work and describes the solution adopted and implemented. We also provide a motivation for the work done and summarize the contributions.

1.1 Context

Cancer is a disease that affects millions of individuals around the world every year. Although it is known by this single name it may have different causes. One such cause is based on an abnormal working of the genetic mechanisms. Aberrant alternative splicing has been recognised as having a very important role in cancer development (Sette, Ladomery, and Ghigna 2013), (Venables 2004). The emergence of high-throughput sequencing, especially with the next generation sequencing (NGS) techniques, has brought substantial advancements in cancer genomics research. Newer NGS techniques are faster, cheaper and, with advancements in computing, allow us to analyse the process using cheap, off-the-shelf hardware. The current pace of advancements of this technology made it possible to assay with high depth of coverage the DNA and the transcriptomes (RNA-Seq) of tumour samples and cancer cell lines. Aberrant RNA processing of genes plays an important role in cancer.

Along with the advancements on sequencing technology, molecular biology has seen an incredible increase in the number and diversity of software developed to solve a wide range of biological problems and to speed up a large number a tedious task expert biologist have to perform. Data analysis in molecular biology has also seen considerable advancements with the crucial help of informatics. Despite the reported advancements in software applied to biological issues, there are important problems that have to be addressed. For complex studies the required information is scattered among a lot of different sources in the internet and encoded in different
Introduction

formats. Complex analysis (using genetic data for example) requires powerful computational resources. The available software used in complex analysis may encompass the use of a lot of tools (the user must know how to use them all). There are a lot of complex analyses that have to be done routinely by biologist. To all of these problems (bio)informatics may give a very useful contribution as we expect to show with our work.

1.2 Motivation and Goals

Advances in the study of the effect of aberrant alternative splicing in cancer may have an enormous social impact. Insights on the mechanism that originates cancer may suggest processes to prevent or reduce its occurrence. There is also an enormous amount of both data and software tools available on the web. The software available is usually collections of programs that solve a small part of biological problems. Our main motivation is to contribute to the availability of software that may speed-up and facilitate the work of biologist and physicians who are involved in the fight against cancer. We also take advantage of real data available on the web.

More specifically we are particularly motivated by the crucial help that informatics can provide to the studies requiring the analysis of massive amounts of (genetic) data.

1.3 Project

In this thesis we propose and have implemented a computational platform that may improve the analysis process by expert biologists when studying the effect of aberrant alternative splicing in the development of cancer. The platform is easy to use and allows the management of users, computational resources and experiments. It enables the execution of tasks to be distributed among several machines running different environments. Users have control over their experiments and can share information among them by making their resources public. There is also (implemented with the topic of aberrant alternative splicing in mind) the possibility to search for information on available databases on the internet in a user transparent manner. The platform provides a way to fetch, store and retrieve large amounts of data that are typical in human genomic based studies. General contributions of this thesis include:

- A general purpose architecture to run jobs in several machines running different operating systems;
- A web based interface that makes it easy for the user to automate processes requiring the execution of several tasks;
- A software tool to be used in a collaborative process among researchers sharing any information they think useful to others;
- A ubiquitous computational tool accessible from any place with access to the web.
Contributions specific for the study of aberrant alternative splicing include:

- A platform running "standard" tools used in aberrant alternative splicing studies;
- The possibility of chaining tools to build a pipeline that accepts the aligned reads as input and produces the aberrant alternative splicing results;
- An automatic process (using the API from the adequate web resource) to fetch the final information of the analysis concerning the domains of missing parts of the proteins encoded by the gene under analysis.

1.4 Structure of the Thesis

The rest of the thesis is structured as follows.

Chapter 2 describes the biological processes related to the domain of genetic-based cancer and molecular biology basic concepts. We also survey the main technologies that we have used in the development of the proposed computational platform.

Chapter 3 details the computational platform developed and how it works both internally and from an end user's perspective.

Chapter 4 describes the biological processes important for alternative splicing analysis and how they can be executed using our proposed computational platform.

Chapter 5 concludes this thesis and outlines future improvements.
Chapter 2

2. Basic Concepts and Survey on Technology

We first introduce basic biological concepts necessary to understand the scope of this thesis. We then describe the process of sequencing programs and the state-of-the-art in algorithms used to study the impact of alternative splicing.

The rest of the chapter addresses the technological tools and alternatives to implement the computational platform.

2.1 Basic Biological Concepts

The genome is composed by a set of genes, and it contains all of our hereditary information. The genes are found in chromosomes and made of deoxyribonucleic acid (DNA). Genes determine the various characteristics of all living organisms by telling our cells how to make proteins, as seen in Figure 2.1. Proteins do not give a human being big ears, per se, but their production is determined by your genes and that will be responsible for one's physical traits.
Genes are made of DNA, and the sum of an organism's DNA is the genome. DNA is made from different combinations of four base molecules -ACGT- repeated over and over again in different configurations. It is nowadays technically possible to determine the genome of an individual. To do that, pieces of RNA are collected (RNA sequencing) out of small sequences. These short RNA sequences are commonly referred to as reads.

Genome sequencing is the process of mapping out the order of the genome's bases that make up an organism's DNA. By knowing which genes do what, we can map an organism's characteristics to their equivalent genes and thus know which are responsible for what parts of that organism. When applied to diseases, this allows us to know which genes are responsible for their development. The human genome is made up of over 3 billion of these base molecules, and we are not one of the most complex species on earth as other species possess far more molecules.

In order to obtain the whole human genome, one first splits it into pieces which are then sequenced and reassembled in the original order to get to the original genome. As you can imagine, this process is error prone because of incorrect reads from the machinery used and the repetition and mapping of the reads in their right order. The computer algorithms used to perform this have varying results depending on the species as some species have smaller strains with many repetitions where others have wider, more unique strains. Many techniques are used to perform genome sequencing. Some are faster than others at the cost of reliability. It is like solving a jigsaw puzzle, where many of the puzzle pieces are very similar, however, this process isn't necessary to understand for the success of this thesis as we will focus on the computational platform that will facilitate the execution of tasks related to this process. Our work starts after the genome assembly but uses information about the reads distribution.

![Diagram showing the translation of mRNA and the synthesis of proteins by a ribosome](http://en.wikipedia.org/wiki/DNA_Translation)
A gene (as seen in Figure 2.2) is composed of a sequence of smaller parts. These parts are of two types: exons and introns. Exons and introns alternate in sequence to make up the complete gene. Only the exons convey information to encode the proteins as introns are noncoding regions. It happens that at different times and circumstances a different set of exons may be *active* (may be translated). This means that the same gene may produce different products. This phenomenon is called alternative splicing. Some of the alternative products may cause diseases.

Figure 2.2: Principles of alternative splicing.

This process happens inside our cells and is responsible for our physical traits and some diseases such as certain types of cancer. To assess which genes are active in each organism, one must take the initial assembled genome, or parts of it, and count the number of times a certain sequence appears in it. The more times a sequence appears, the more active that particular gene is.

Once that count has been established, one can use it to compare to known traits and assay the probability of developing certain diseases. Software tools help us produce these counts from reads and others help us view and analyse the necessary information in order to produce a report about the functioning of those particular genes.

The output of the RNA sequence (one of the techniques for doing genome sequencing) is a histogram of the reads which tell us which genes (or parts of the genes) are more active or inactive. This is useful information to study genomic based diseases.

### 2.2 Software for the Biological Analysis

Software for the Biological analysis is divided by function. Most programs abide by the UNIX philosophy where each program is designed to do one thing only and do it well. Because of this, most programs are command-line programs that take the input of others and produce a new set of outputs for another program.

There are many available tools, each dealing with one or more steps of the process, notably:
Basic Concepts and Survey on Technology

- Read aligners align reads to a reference genome, such as the human genome;
- Read mappers work similarly to read aligners but try to identify splice junctions;
- Differential expression programs assemble reads and estimate their abundance for further analysis;
- Visualizers take input from differential expression analysis and present commonly used visualizations.

There are some publicly available genome browsers\(^2\) online that allow us to visualize and retrieve genomic information from a number of species. Some of the most well-known projects are:

- The Ensembl genome database project\(^3\);
- The UCSC (University of California Santa Cruz) genome browser\(^4\).

### 2.2.1 Standard File Formats for Bioinformatics Data

Many formats exist to represent the various stages of this process. One of the most common formats is the SAM (Sequence Alignment/Map) format and its binary equivalent, BAM.

The following format descriptions are taken from either Wikipedia\(^5\) or a web page describing the format from the format's web page or a public format description from a renowned institution.

**SAM/BAM**

The SAM\(^6\) (Sequence Alignment/Map) format\(^7\) and its binary equivalent, BAM, are the most common formats used for sequence data. It is used by many bioinformatics tools. Both formats store the same information.

The SAM format is a TAB-delimited text format consisting of a header section, which is optional, and an alignment section. If present, the header must be prior to the alignments. Header lines start with "@", while alignment lines do not. Each alignment line has 11 mandatory fields for essential alignment information such as mapping position, and a variable number of optional fields for flexible or aligner specific information.

Each alignment line has 11 mandatory fields:

---


\(^3\) Ensembl - [http://www.ensembl.org/](http://www.ensembl.org/)

\(^4\) UCSC Genome Browser - [http://genome.ucsc.edu/](http://genome.ucsc.edu/)

\(^5\) Wikipedia is a free encyclopaedia - [http://en.wikipedia.org](http://en.wikipedia.org)


\(^7\) SAM format - [http://samtools.sourceforge.net/SAMv1.pdf](http://samtools.sourceforge.net/SAMv1.pdf)
Basic Concepts and Survey on Technology

1. **QNAME** – Query template name. Reads/segments having identical QNAME are regarded as coming from the same template. A QNAME "*" indicates the information is unavailable;

2. **FLAG** – Reference sequence NAME of the alignment. See the full specification for a detailed description;

3. **RNAME** – Reference sequence NAME of the alignment. If @SQ header lines are present, RNAME (if not "*") must be present in one of the SQ-SN tag. An unmapped segment without coordinate has a "*" at this field. However, an unmapped segment may also have an ordinary coordinate such that it can be placed at a desired position after sorting. If RNAME is "*", no assumptions can be made about POS and CIGAR;

4. **POS** – 1-based leftmost mapping position of the first matching base. The first base in a reference sequence has coordinate 1. POS is set as 0 for an unmapped read without coordinate. If POS is 0, no assumptions can be made about RNAME and CIGAR;

5. **MAPQ** – Mapping quality. It equals $-10 \log_{10} Pr^8$, rounded to the nearest integer. A value 255 indicates that the mapping quality is not available;

6. **CIGAR** – CIGAR string. The CIGAR operations are given in the following table (set "*" if not available).

<table>
<thead>
<tr>
<th>Op</th>
<th>BAM</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>0</td>
<td>Alignment match (can be a sequence match or mismatch)</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>Insertion to the reference</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>Deletion from the reference</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>Skipped region from the reference</td>
</tr>
<tr>
<td>S</td>
<td>4</td>
<td>Soft clipping (clipped sequences present in SEQ)</td>
</tr>
<tr>
<td>H</td>
<td>5</td>
<td>Hard clipping (clipped sequences NOT present in SEQ)</td>
</tr>
<tr>
<td>P</td>
<td>6</td>
<td>Padding (silent deletion from padded reference)</td>
</tr>
<tr>
<td>=</td>
<td>7</td>
<td>Sequence match</td>
</tr>
<tr>
<td>X</td>
<td>8</td>
<td>Sequence mismatch</td>
</tr>
</tbody>
</table>

7. **RNEXT** – Reference sequence name of the primary alignment of the next read in the template. For the last read, the next read is the first read in the template. If @SQ header lines are present, RNEXT (if not "*" or "=") must be present in one of the SQ-SN tag. This field is set as "*" when the information is unavailable, and set as "=" if RNEXT is identical RNAME. If not "=" and the next read in the template has one primary mapping (see also bit 0x100 in FLAG), this field is identical to RNAME at the primary line of the next read. If RNEXT is "*", no assumptions can be made on PNEXT and bit 0x20;

8. **PNEXT** – Position of the primary alignment of the NEXT read in the template. Set as 0 when the information is unavailable. This field equals POS at the primary line of the next read. If PNEXT is 0, no assumptions can be made on RNEXT and bit 0x20;

---

8 Phred quality score - [http://en.wikipedia.org/wiki/Phred_quality_score](http://en.wikipedia.org/wiki/Phred_quality_score)
9. **TLEN** – signed observed Template length. If all segments are mapped to the same reference, the unsigned observed template length equals the number of bases from the leftmost mapped base to the rightmost mapped base. The leftmost segment has a plus sign and the rightmost has a minus sign. The sign of segments in the middle is undefined. It is set as 0 for single-segment template or when the information is unavailable;

10. **SEQ** – Segment sequence. This field can be a "*" when the sequence is not stored. If not a "*", the length of the sequence must equal the sum of lengths of M/I/S/=/X operations in CIGAR. An "=" denotes the base is identical to the reference base. No assumptions can be made on the letter cases;

11. **QUAL** – ASCII of base quality plus 33 (same as the quality string in the Sanger FASTQ format). A base quality is the phred-scaled base error probability which equals \(-10 \log_{10} P_r\). This field can be a "*" when quality is not stored. If not a "*", SEQ must not be a "*" and the length of the quality string ought to equal the length of SEQ.

![SAM file example with tabs replaced with spaces for readability.](image)

The SAM/BAM format (as seen in Figure 2.3) is a bit complex and is not fully explained in this document. For a more detailed description, see the format's specification.9

**GTF**

GTF10 (Gene Transfer Format) is a file format used to hold information about a gene and is the output format of differential expression software such as Cufflinks. It is an extension of the GFF11 (General Feature Format) which has 9 tab-separated required fields but with the last field different, as seen in Figure 2.5.

1. **SEQNAME** – The name of the sequence. Must be a chromosome or scaffold;
2. **SOURCE** – The program that generated this feature;
3. **FEATURE** – The name of this type of feature. Some examples of standard feature types are "CDS", "start_codon", "stop_codon", and "exon";

---

10 GTF format - [http://www.genome.ucsc.edu/FAQ/FAQformat.html#format4](http://www.genome.ucsc.edu/FAQ/FAQformat.html#format4)
11 GFF format - [http://www.genome.ucsc.edu/FAQ/FAQformat.html#format3](http://www.genome.ucsc.edu/FAQ/FAQformat.html#format3)
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4. **START** – The starting position of the feature in the sequence. The first base is numbered 1;

5. **END** – The ending position of the feature (inclusive);

6. **SCORE** – A score between 0 and 1000. If the track line USESCORE attribute is set to 1 for this annotation data set, the score value will determine the level of grey in which this feature is displayed (higher numbers = darker grey). If there is no score value, enter ".". Figure 2.4 shows the Genome Browser's translation of BED score values into shades of grey:

<table>
<thead>
<tr>
<th>Score Range</th>
<th>Grey Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 166</td>
<td>Light grey</td>
</tr>
<tr>
<td>167-277</td>
<td>Medium grey</td>
</tr>
<tr>
<td>278-388</td>
<td>Dark grey</td>
</tr>
<tr>
<td>389-499</td>
<td>Very dark grey</td>
</tr>
<tr>
<td>500-611</td>
<td>darkest grey</td>
</tr>
<tr>
<td>612-722</td>
<td>.....</td>
</tr>
<tr>
<td>723-833</td>
<td>.....</td>
</tr>
<tr>
<td>834-944</td>
<td>.....</td>
</tr>
<tr>
<td>≥ 945</td>
<td>.....</td>
</tr>
</tbody>
</table>

Figure 2.4: Range of score values and it corresponding grey colour.

7. **STRAND** – Valid entries include "+", ",", or "," (for don't know/don't care);

8. **FRAME** – If the feature is a coding exon, frame should be a number between 0-2 that represents the reading frame of the first base. If the feature is not a coding exon, the value should be ",";

9. **ATTRIBUTE** – A list of tag-value pairs, providing additional information about each feature. Attributes must end in a semi-colon, and be separated from any following attribute by exactly one space.

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Transcript ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB000381 Twinscan CDS</td>
<td>380 401 . + 0 gene_id &quot;001&quot;; transcript_id &quot;001.1&quot;;</td>
</tr>
<tr>
<td>AB000381 Twinscan CDS</td>
<td>501 650 . + 2 gene_id &quot;001&quot;; transcript_id &quot;001.1&quot;;</td>
</tr>
<tr>
<td>AB000381 Twinscan CDS</td>
<td>700 707 . + 2 gene_id &quot;001&quot;; transcript_id &quot;001.1&quot;;</td>
</tr>
<tr>
<td>AB000381 Twinscan start_codon</td>
<td>380 382 . + 0 gene_id &quot;001&quot;; transcript_id &quot;001.1&quot;;</td>
</tr>
<tr>
<td>AB000381 Twinscan stop_codon</td>
<td>708 710 . + 0 gene_id &quot;001&quot;; transcript_id &quot;001.1&quot;;</td>
</tr>
</tbody>
</table>

Figure 2.5: GTF file example with tabs replaced with spaces for readability.

**BED**

BED12 is a file format used to hold sequence annotations. It has 3 mandatory fields and 9 optional ones, as seen in Figure 2.7. The order of the optional fields must be preserved where previous fields are not left empty. This format is sometimes called BED6 or BED12 depending on the number of fields it has.

The first three required BED fields are:

1. **CHROM** – The name of the chromosome (e.g. chr3, chrY, chr2_random) or scaffold (e.g. scaffold10671);

2. **CHROMSTART** – The starting position of the feature in the chromosome or scaffold. The first base in a chromosome is numbered 0;

---

12 BED format - [http://www.genome.ucsc.edu/FAQ/FAQformat.html#format1](http://www.genome.ucsc.edu/FAQ/FAQformat.html#format1)
3. **CHROMEND** – The ending position of the feature in the chromosome or scaffold. The CHROMEND base is not included in the display of the feature. For example, the first 100 bases of a chromosome are defined as CHROMSTART=0, CHROMEND=100, and span the bases numbered 0-99.

The 9 additional optional BED fields are:

4. **NAME** – Defines the name of the BED line. This label is displayed to the left of the BED line in the Genome Browser window when the track is open to full display mode or directly to the left of the item in pack mode;
5. **SCORE** – A score between 0 and 1000. If the track line useScore attribute is set to 1 for this annotation data set, the score value will determine the level of grey in which this feature is displayed (higher numbers = darker grey). Figure 2.6 shows the Genome Browser's translation of BED score values into shades of grey:

```
<table>
<thead>
<tr>
<th>Score Range</th>
<th>Grey Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 166</td>
<td>Light Grey</td>
</tr>
<tr>
<td>167-277</td>
<td>Medium Grey</td>
</tr>
<tr>
<td>278-388</td>
<td>Medium Dark</td>
</tr>
<tr>
<td>389-499</td>
<td>Dark Grey</td>
</tr>
<tr>
<td>500-611</td>
<td>Very Dark</td>
</tr>
<tr>
<td>612-722</td>
<td>Very Dark</td>
</tr>
<tr>
<td>723-833</td>
<td>Very Dark</td>
</tr>
<tr>
<td>834-944</td>
<td>Very Dark</td>
</tr>
<tr>
<td>≥ 945</td>
<td>Black</td>
</tr>
</tbody>
</table>
```

Figure 2.6: Range of score values and it corresponding grey colour.

6. **STRAND** – Defines the strand - either "+" or "-";
7. **THICKSTART** – The starting position at which the feature is drawn thickly (for example, the start codon in gene displays);
8. **THICKEND** – The ending position at which the feature is drawn thickly (for example, the stop codon in gene displays);
9. **ITEMRGB** – An RGB value of the form RGB (e.g. 255,0,0). If the track line ITEMRGB attribute is set to "On", this RBG value will determine the display colour of the data contained in this BED line. NOTE: It is recommended that a simple colour scheme (eight colours or less) be used with this attribute to avoid overwhelming the colour resources of the Genome Browser and your Internet browser;
10. **BLOCKCOUNT** – The number of blocks (exons) in the BED line;
11. **BLOCKSIZES** – A comma-separated list of the block sizes. The number of items in this list should correspond to BLOCKCOUNT;
12. **BLOCKSTARTS** – A comma-separated list of block starts. All of the BLOCKSTART positions should be calculated relative to CHROMSTART. The number of items in this list should correspond to BLOCKCOUNT.

Track definition lines can be used to configure the display further, e.g. by grouping features into separate tracks. Track lines should be placed at the beginning of the list of features they are to affect.

The track line consists of the word "track" followed by space-separated key=value pairs - see the example below. Valid parameters used by Ensembl are:
1. **NAME** – unique name to identify this track when parsing the file;
2. **DESCRIPTION** – Label to be displayed under the track in Region in Detail;
3. **PRIORITY** – integer defining the order in which to display tracks, if multiple tracks are defined;
4. **USESCORE** – a value from 1 to 4, which determines how scored data will be displayed. Additional parameters may be needed, as described below:
   - Tiling array;
   - Colour gradient – defaults to Yellow-Green-Blue, with 20 colour grades. Optionally you can specify the colours for the gradient (cgColour1, cgColour2, cgColour3) as either RGB, hex or X11 colour names, and the number of colour grades (cgGrades);
   - Histogram;
   - Wiggle plot.
5. **ITEMRGB** – if set to "on" (case-insensitive), the individual RGB values defined in tracks will be used.

```
track name="pairedReads" description="Clone Paired Reads" useScore=1
chr22 1000 5000 cloneA 960 + 1000 5000 0 2 567,488, 0,3512
chr22 2000 6000 cloneB 900 - 2000 6000 0 2 433,399, 0,3601
```

Figure 2.7: BED file example with tabs replaced with spaces for readability.

**FASTA**

FASTA\(^\text{13}\) is a text based file format used to hold nucleotide or amino acid sequences which are represented using single-letter codes.

A sequence in FASTA format begins with a single-line description, followed by lines of sequence data, as seen in Figure 2.8. The definition line (defline) is distinguished from the sequence data by a greater-than (>) symbol at the beginning. The word following the ">" symbol is the identifier of the sequence, and the rest of the line is the description (optional). Normally, identifiers are simply protein accession, name or Entrez gi's (e.g., Q5I7T1, AG10B_HUMAN, 129295), but a bar-separated NCBI sequence identifier (e.g., gi|129295) will also be accepted. Any arbitrary user-specified sequence identifier can also be used (e.g., CLONE00073452) but you are advised to use sufficiently long unique words in such case. There should be no space between the ">" and the first letter of the identifier. It is recommended that all lines of text be shorter than 80 characters in length.

---

\(^{13}\) FASTA format - [http://genetics.bwh.harvard.edu/pph/FASTA.html](http://genetics.bwh.harvard.edu/pph/FASTA.html)
2.2.2 Genomic Assemblage Software

Here we present the tools that are being considered for each phase. Descriptions are taken from their respective web pages.

Cufflinks

Cufflinks\(^{14}\) (Trapnell et al. 2010), (Roberts, Trapnell, et al. 2011), (Roberts, Pimentel, et al. 2011), (Trapnell et al. 2013) assembles transcripts, estimates their abundances, and tests for differential expression and regulation in RNA-Seq samples. It accepts aligned RNA-Seq reads and assembles the alignments into a parsimonious set of transcripts. Cufflinks then estimates the relative abundances of these transcripts based on how many reads support each one, taking into account biases in library preparation protocols.

TopHat

TopHat\(^{15}\) (Trapnell, Pachter, and Salzberg 2009), (Langmead, Trapnell, et al. 2009), (Kim and Salzberg 2011), (Kim et al. 2013) is a fast splice junction mapper for RNA-Seq reads. It aligns RNA-Seq reads to mammalian-sized genomes using the ultra-high-throughput short read aligner Bowtie, and then analyses the mapping results to identify splice junctions between exons.

Bowtie


\(^{14}\) Cufflinks - http://cufflinks.cbcb.umd.edu/
\(^{15}\) TopHat - http://tophat.cbcb.umd.edu/
\(^{16}\) Bowtie - http://bowtie-bio.sourceforge.net
\(^{17}\) Bowtie 2 - http://bowtie-bio.sourceforge.net/bowtie2

Figure 2.8: FASTA file example.
Other

There are many other software packages for the various stages of the process that won't be enumerated here because of their large number, including variations of these made to scale in computer clusters.

On the SEQAanswers wiki\(^{18}\), an on-line community for next generation sequencing, there are more than 600 software packages listed for solving one or more stages of the processes involved in next generation sequencing.

2.2.3 Databases

The ENCODE project\(^{19}\) aims to identify all functional elements in the human genome sequence. There are several types of data referenced, including RNA-Seq data. We can obtain data aligned to a reference genome from there.

There are various cell lines referenced in the ENCODE project that are taken from human tissue samples and later transformed in a laboratory so that they can be replicated and researched throughout various laboratories around the world. Some of these cell lines are derived from cancer tissues. You can find more information about the different cell lines on the project’s page\(^{20}\).

This is the description from the two samples we will use taken from the project’s page: K\textsuperscript{562} and GM\textsuperscript{12878}.

“\textit{K562 is an immortalized cell line produced from a female patient with chronic myelogenous leukemia (CML). It is a widely used model for cell biology, biochemistry, and erythropoiesis.”}

“\textit{GM12878 is a lymphoblastoid cell line produced from the blood of a female donor with northern and western European ancestry by EBV transformation.”}

The mapped data can be obtained from here: http://genome.crg.es/encode\_RNA\_dashboard/hg19/\n
\(^{18}\) SEQAanswers wiki - \url{http://seqanswers.com/wiki/}\n
\(^{19}\) The ENCODE project - \url{http://www.genome.gov/10005107}\n
\(^{20}\) The ENCODE project common cell types - \url{http://www.genome.gov/26524238}
2.3 Data Mining

Here we present the algorithms that are being considered for the data analysis phase and software packages that can be used to apply them. Descriptions are taken from Wikipedia\textsuperscript{21} and/or their web pages for the tools.

2.3.1 Data Analysis Algorithms

Decision Trees

A decision tree is a flowchart-like structure in which internal node represents test on an attribute, each branch represents outcome of test and each leaf node represents class label (decision taken after computing all attributes). A path from root to leaf represents classification rules.

Rule Induction Algorithms

Rule induction algorithms are an area of machine learning in which formal rules are extracted from a set of observations. The rules extracted may represent a full scientific model of the data, or merely represent local patterns in the data.

Many branches of machine learning apply this technique, namely inductive logic programming.

Support Vector Machines

Support vector machines (SVMs), also known as support vector networks, are supervised learning models with associated learning algorithms that analyse data and recognize patterns, used for classification and regression analysis. Given a set of training examples, each marked as belonging to one of two categories in the case of classification, an SVM training algorithm builds a model that assigns new examples into one category or the other, making it a non-probabilistic binary linear classifier. An SVM model is a representation of the examples as points in space, mapped so that the examples of the separate categories are divided by a clear gap that is as wide as possible. New examples are then mapped into that same space and predicted to belong to a category based on which side of the gap they fall on.

In addition to performing linear classification, SVMs can efficiently perform a non-linear classification using what is called the kernel trick, implicitly mapping their inputs into high-dimensional feature spaces.

\textsuperscript{21} Wikipedia is a free encyclopaedia - http://en.wikipedia.org
Ensembles

In statistics and machine learning, ensemble methods use multiple models to obtain better predictive performance than could be obtained from any of the constituent models. Unlike a statistical ensemble in statistical mechanics, which is usually infinite, a machine learning ensemble refers only to a concrete finite set of alternative models, but typically allows for much more flexible structure to exist between those alternatives.

Random Forrest

Random forests are an ensemble learning method for classification (and regression) that operate by constructing a multitude of decision trees at training time and outputting the class that is the mode of the classes output by individual trees.

K-NN

K-Nearest neighbours’ algorithm (or k-NN for short) is a non-parametric method used for classification and regression. In both cases, the input consists of the k closest training examples in the feature space. The output depends on whether k-NN is used for classification or regression.

Inductive Logic Programming

Inductive logic programming (ILP) is a sub field of machine learning which uses logic programming as a uniform representation for examples, background knowledge and hypotheses. Given an encoding of the known background knowledge and a set of examples represented as a logical database of facts, an ILP system will derive a hypothesised logic program which entails all the positive and none of the negative examples.

This is a good candidate to start with as it has been used in these types of problems, mainly because of its ease of handling multi-relational data. ILP can build complex but comprehensible models that make it easy to explain the phenomena that produced them.

2.3.2 Data Analysis Tools

Here we will describe packages used for the data analysis part. Some of these tools are used by other tools.

CummeRbund
CummeRbund\textsuperscript{22} is an R package that is designed to aid and simplify the task of analysing Cufflinks RNA-Seq output. R is program and programming language used in statistical analysis.

**RapidMiner**

RapidMiner\textsuperscript{23} is a software platform developed by the company of the same name that provides an integrated environment for machine learning, data mining, text mining, predictive analytics and business analytics. It is used for business and industrial applications as well as for research, education, training, rapid prototyping, and application development and supports all steps of the data mining process including results visualization, validation and optimization.

RapidMiner is developed on a business source model which means the core and earlier versions of the software are available under an OSI-certified open source license. A Starter Edition is available for free download; Personal, Professional and Enterprise Editions are available.

**KNIME**

KNIME\textsuperscript{24}, the Konstanz Information Miner, is an open source data analytics, reporting and integration platform. KNIME integrates various components for machine learning and data mining through its modular data pipelining concept. A graphical user interface allows assembly of nodes for data pre-processing (ETL: Extraction, Transformation, Loading), for modelling and data analysis and visualization.

Since 2006, KNIME has been used in pharmaceutical research, but is also used in other areas like customer data analysis, business intelligence and financial data analysis.

**WEKA**

Weka\textsuperscript{25} (Waikato Environment for Knowledge Analysis) is a popular suite of machine learning software written in Java, developed at the University of Waikato, New Zealand. Weka is free software available under the GNU General Public License.

**Orange**

Orange\textsuperscript{26} is a component-based data mining and machine learning software suite, featuring a visual programming front-end for explorative data analysis and visualization, and Python

\textsuperscript{22}CummeRbund - http://compbio.mit.edu/cummeRbund/
\textsuperscript{23}RapidMiner - http://rapidminer.com/
\textsuperscript{24}KNIME - http://www.knime.org/
\textsuperscript{25}Weka - http://www.cs.waikato.ac.nz/~ml/weka/
\textsuperscript{26}Orange - http://orange.biolab.si/
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bindings and libraries for scripting. It includes a set of components for data preprocessing, feature scoring and filtering, modelling, model evaluation, and exploration techniques. It is implemented in C++ and Python. Its graphical user interface builds upon the cross-platform Qt framework.

Orange is freely distributed under the GPL. It is maintained and developed at the Bioinformatics Laboratory of the Faculty of Computer and Information Science, University of Ljubljana, Slovenia.

R

R\textsuperscript{27} is a free software programming language and software environment for statistical computing and graphics. The R language is widely used among statisticians and data miners for developing statistical software and data analysis.

The Bioconductor project provides R packages for the analysis of genomic data, such as Affymetrix and cDNA microarray object-oriented data-handling and analysis tools, and has started to provide tools for analysis of data from next-generation high-throughput sequencing methods.

2.4 Algorithm Evaluation Methods and Measures

In order to assess the quality of the algorithms, some techniques commonly used in algorithm evaluation will be applied to avoid overfitting\textsuperscript{28}, where the testing data and the actual data vary enough to hinder the algorithms used.

One of these techniques consists on having two sets of data to test against; one for training and another for verifying (train/test and cross validation\textsuperscript{29}). Hold-out is one of such techniques, where a part of the data is used for training and the remaining part for testing.

Another technique which can help is resampling\textsuperscript{30}, where many permutations of data are used to train the algorithms in order to add randomness to the training set by omitting data from training using a series of techniques and using the omitted data to validate the algorithm, and then re-training with the same data but switching the testing data with a part of the training data until all data is used in both training and testing.

An associated error rate will be used to assure the result, in order to cope with the inherent errors that such an enormous amount of data can have. One of these metrics is named F-

\begin{thebibliography}{9}
\bibitem{r} R - \url{http://www.r-project.org/}
\bibitem{overfitting} Overfitting - \url{http://en.wikipedia.org/wiki/Overfitting}
\bibitem{crossvalidation} Cross Validation - \url{http://en.wikipedia.org/wiki/Cross-validation_(statistics)}
\bibitem{resampling} Resampling - \url{http://en.wikipedia.org/wiki/Resampling_(statistics)}
\end{thebibliography}
Basic Concepts and Survey on Technology

measure\textsuperscript{31}, which takes into account both the precision and the recall of the test to compute the final score.

2.5 Web Services

Web services are a technology that works over computer networks by providing a means of communication between machines using a communication interface that is understood by all machines. This is usually implemented on top of HTTP\textsuperscript{32} (Hypertext transfer protocol) protocol used to access the internet from a web browser in conjunction with other web standards and formats.

The usefulness of web services is that they sit on top of well-established and standard protocols that are widely available on nearly all computer systems and thus can have a broader reach. The HTTP protocol works by requesting an operation pointed at a hyperlink that will in turn reply with an appropriate response. For example, when we navigate on a web browser to Google's search engine hyperlink (http://www.google.com), we are performing the GET request method on that hyperlink and will get Google's initial web page as a response. There are other request methods we can specify that allow us to perform more operations. These standards are referred to as web APIs (application programming interfaces).

Many standards exist that sit on top of these technologies. They vary in the way that the web service's API is specified and used and in which format the messages should be transferred. The two most widely used formats to transmit structured documents or data objects over the internet are JSON\textsuperscript{33} and XML\textsuperscript{34}.

Another big advantage of having web services sit on top of HTTP is that the same API can be used by both web browsers and programs that consume that API. If the web service detects that a web browser is making the request, it can return an HTML document to display in the browser. If the web service detects that a consumer application is making the request, it can return a simpler response with the data in one of the named document formats to be processed by the application in its own way.

Detecting which client is accessing the API can be done either by analysing the HTTP user agent (a header in the protocol that identifies the browser or HTTP library being used), or by having the programs that consumes that API specify that they do not want a visual response in HTML but a JSON or XML response to process in their own way. This can be done in a number of ways and varies depending on the standard used.

\textsuperscript{31} F-measure - http://en.wikipedia.org/wiki/F-measure
\textsuperscript{32} HTTP protocol - http://en.wikipedia.org/wiki/HTTP
\textsuperscript{33} JSON - http://en.wikipedia.org/wiki/JSON
\textsuperscript{34} XML - http://en.wikipedia.org/wiki/XML
2.5.1 Representational State Transfer

One of such web service (or web API) standards is representational state transfer\(^{35}\) (REST). It differs from other web service standards by being simpler and relying on a small subset of the HTTP protocol. It is also not a protocol definition but an architectural style of achieving common operations. This means we are not forced to implement the web service in a specified way but have the liberty to deviate from the reference style to accommodate our solution. The REST protocol works based on these simple aspects:

- A base URL, such as http://www.example.com/resource/;
- Standard HTTP methods (usually GET, PUT, POST and DELETE);

Here are two examples (Tables 2.1 and 2.2) of how the management of users can look like using REST:

<table>
<thead>
<tr>
<th>URL</th>
<th><a href="http://www.example.com/users/">http://www.example.com/users/</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>GET</td>
<td>Gets a list with all the users.</td>
</tr>
<tr>
<td>PUT</td>
<td>Not usually used.</td>
</tr>
<tr>
<td>POST</td>
<td>Adds a new user to the collection.</td>
</tr>
<tr>
<td>DELETE</td>
<td>Deletes all users.</td>
</tr>
</tbody>
</table>

Table 2.1: REST operations for user collection.

<table>
<thead>
<tr>
<th>URL</th>
<th><a href="http://www.example.com/user/Item1">http://www.example.com/user/Item1</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>GET</td>
<td>Gets the user that corresponds to Item1.</td>
</tr>
<tr>
<td>PUT</td>
<td>Updates the user's attributes.</td>
</tr>
<tr>
<td>POST</td>
<td>Not usually used.</td>
</tr>
<tr>
<td>DELETE</td>
<td>Deletes this user.</td>
</tr>
</tbody>
</table>

Table 2.2: REST operations for user item.

Because REST is an architectural style, we are not forced to implement these specific operations using these HTTP methods. One common modification is to use POST for both adding elements and editing them. Distinction between both operations is then done by appending /edit to the end of the URL. This is not a specification but a common usage style. These modifications have many reasons to be, namely firewall restrictions or web browser incompatibilities.

2.5.2 Web Frameworks

Many web frameworks support writing RESTful web APIs. Because many of the described steps are common and repetitive, they abstract away the burden of implementing REST from scratch using only an HTTP framework and provide much functionality that eases the development of these APIs. Some of these common functionalities are:

- **Routing** – Allows the specification of the URL and which method should be executed for each request. It takes care of figuring out which route will handle which request;
- **Model binding** – Allows sending of data in an easy way as a response to a request;
- **View engines** – Allows the usage of view engines that make it easier to create dynamic web pages;
- **Localization** – Makes it easy to have web pages translated into multiple languages without having to modify a lot of files in the process;
- **Testing** – Provides mechanisms to test REST routes to make sure they are working properly;
- **Content negotiation** – Allows the client to negotiate what type of content it wants in an easy way. Whether it is an HTML page or a JSON/XML response.

Many web frameworks provide mechanisms to deal with these issues and other common ones. For the solution we are developing, Routing, model binding, content negotiation and view engine support are the most important ones.

2.6 Job Scheduling

Since most bioinformatics applications are command-line based and abide by the Unix philosophy of having programs chained together to produce a final result from a set of inputs, the web service that will manage the data analysis will be a job scheduling application where jobs are created by the user to perform a specific task and ran in a worker bot that will be idle until a task is given to it.

Because job scheduling is a problem that has been addressed many times before, there are already many solutions available. There are also some solutions made specifically for bioinformatics.

Sequence Manipulation Suite

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Sequence Manipulation Suite is a collection of JavaScript programs for generating, formatting, and analysing short DNA and protein sequences. It is commonly used by molecular biologists, for teaching, and for program and algorithm testing.

This suite does not offer user management or a workspace for each user.

**EMBOSS**

EMBOSS\(^{38}\) is "The European Molecular Biology Open Software Suite". EMBOSS is a free Open Source software analysis package specially developed for the needs of the molecular biology (e.g. EMBnet) user community. The software automatically copes with data in a variety of formats and even allows transparent retrieval of sequence data from the web. Also, as extensive libraries are provided with the package, it is a platform to allow other scientists to develop and release software in true open source spirit. EMBOSS also integrates a range of currently available packages and tools for sequence analysis into a seamless whole. EMBOSS breaks the historical trend towards commercial software packages.

Because EMBOSS is a collection of tools for the most common bioinformatics operations, there are a few web interfaces that use it\(^ {39} \) and provide user account management and a workspace per user.

There are also some workflows\(^ {40} \) or pipelines that use EMBOSS and work similar to job schedulers as they allow job management and distributed computing.

**Other**

There are many other job scheduling solutions\(^ {41} \) that offer many features common to these types of problems like:

- Script storage where missing scripts are automatically transferred to the target system;
- Event driven where jobs are ran when a worker bot is available;
- Agents where host systems install a program that connects to the central job server;
- Multi-platform;
- Stdout/Stderr transfer;
- File events where a job is started when a file is created;
- File transfer to transfer files from/to the server;
- Authentication and role-based security;
- User interfaces.

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39 EMBOSS interfaces - [http://emboss.sourceforge.net/interfaces/](http://emboss.sourceforge.net/interfaces/)
40 EMBOSS workflows - [http://emboss.sourceforge.net/interfaces/#workflows](http://emboss.sourceforge.net/interfaces/#workflows)
2.7 Chapter Conclusions

In this chapter we outlined some of the tools and algorithms we will use to perform our work. Some of the algorithms are best suited for large amounts of data while others are not.

The biological analysis tools are not thoroughly mentioned since they will not all be used and the ones mentioned are most likely to be chosen as they are usually paired together. Because the focus will be on the computational platform, the tools are not that important.

One advantage of using the tools mentioned is that they were made to work well with each other and were partially developed by the same people.

Regarding the algorithms tools, they themselves pose challenges related to data integration. The algorithms themselves can have different behaviour depending on the size of the training sets and the amount of data to analyse.
Chapter 3

3. A Website for Alternative Splicing Analysis

Most of the applications involving the analysis of genomic data require powerful computational resources. In this chapter, an architecture and implementation of a computational platform that we think is adequate for genomic-scale data analysis is presented. We first describe the proposed architecture then we present the functionalities and lastly the implementation technologies and choices made.

3.1 Web Service

In bioinformatics, the tasks described in the previous chapter are not user friendly and are error prone if the person executing the task is not familiarized with the tools involved.

In order to streamline the process of executing these tasks and other tasks commonly used in bioinformatics, a web service was developed to tackle the most common usage scenarios and user’s needs.

3.1.1 Main Use Cases

Before starting the development of the web service, a set of requirements (both functional and non-functional) and use cases (Figure 3.1) were made regarding the web service, namely:

- The solution must be cross platform – In order to run from any computer, the proposed solution was to build a web service, since it will be accessible from any system with a web browser.
A Website for Alternative Splicing Analysis

- The solution must be user friendly – Since the target audience is not tech-savvy, the solution should be simple to use and hide the inherent complexity in the underlying process.

- The solution must work well with tablets – In today’s world, many of the interactions with the internet are made from touch screens, whether phones or tablets. Therefore, it is necessary to have the solution work well with touch centric devices by avoiding certain user interface choices such as small buttons and relying on the mouse to perform specific tasks (such as mouse hover tooltips\(^{42}\)).

- The solution must have the notion of users and authentication – It should not be possible to access any of the web service’s functionality without authenticating first with the system. New user accounts should also be validated by an administrator account before being allowed to access the system.

- The solution must allow the creation of projects – A project is a mere description of an experiment where a user can try new things and have its own personal areas to work in.

- The solution must allow the creation of jobs – A job is a background task that is to be executed by the solution and have its output saved for later use.

- The solution must be able to run jobs in a different computer – In order to have a job run in a more powerful computer or have more than one computer available to run jobs, the solution should be able to send jobs to another computer and have the results sent back when done.

- The solution must be able to download files – A user should be able to download files produced by the solution.

- The solution must allow file descriptions – A user should be able to add a description to a file describing it in more detail.

- The solution must enforce edit restrictions – A user should not be able to alter or delete another user’s artefacts unless he is an administrator.

- The solution must use open source technologies – The solution should not use technologies with restrictive licenses that prevent usage in commercial applications or require the purchase of a license to use.

\(^{42}\) Mouse hover - [http://en.wikipedia.org/wiki/Mouseover](http://en.wikipedia.org/wiki/Mouseover)
3.2 Architecture

The web service’s high level architecture consists of a server that manages the logic and a number of worker bots (Clusters) that are idle and ping the web service for pending jobs, as seen in Figure 3.2. Worker bots download all necessary files from the server before running a job and then upload back the results. This allows the service to have many worker bots executing tasks asynchronously.
A Website for Alternative Splicing Analysis

Figure 3.2: Web service architecture.

Internally, the web service is split into the following projects (Figure 3.3):

- **WebService** – contains the web service web logic including views and JavaScript. Uses the logic layer to access the database;
- **WebService.Common** – Contains common code between all projects such as settings code;
- **WebService.Common.Logic** – contains the code to interface with the database;
- **WebService.Common.WS** – contains the code to interface with the web service via the REST API;
- **WebService.Self** – program that creates a self-hosting web service instance;
- **WebService.Aspnet** - program that creates a web service instance hosted under Microsoft's Internet Information Services\(^{43}\) (IIS);
- **WebService.WorkerBot** – program that uses the WS layer to access the web service.

Figure 3.3: Web service project layers.

---

\(^{43}\) Internet Information Services
A Website for Alternative Splicing Analysis

There is a common set of functionalities that are used by both web service and worker bot projects. These include utility functions for common operations such as handling settings files.

The logic layer interfaces with the database and provides an abstraction to manage the business logic.

The logic layer is used only by the web service layer, which implements the user interface and a REST API to access it remotely. The web service layer is used by both the Self and Aspnet projects which are stub projects with minimum functionality that create an instance of the web service layer in different contexts (a self-hosting application or an IIS\(^{44}\) hosted one).

The common WS layer (short for web service) consumes the web service using the provided REST API.

Finally, the worker bot uses the common WS layer to access the web service without having to know the underlying implementation.

The web service implements the following concepts:

- **Clusters** – Accounts used by the worker bots to login into the service;
- **Files** – Files stored in the web service’s server;
- **Jobs** – Job management for creating different types of jobs to be run by a worker bot;
- **Projects** – Project describing an experiment. Jobs can be associated to a project for easier management;
- **Users** – User accounts for authentication into the system and authorization of operations.

### 3.2.1 Database Architecture

The database that supports the web service is a MongoDB\(^{45}\) database, a document oriented NoSQL database that does not require a schema definition before usage.

All the artefacts are stored in the database, except for files, which are stored and managed by the underlying file system.

Below is the schema of the database used by the web service (Figure 3.4):

---

\(^{44}\) IIS – Internet Information Services, a set of Internet-based services for servers using Microsoft Windows.

\(^{45}\) MongoDB - [http://www.mongodb.org/](http://www.mongodb.org/)
A Website for Alternative Splicing Analysis

Figure 3.4: Database schema of the web service.

All tables have a generated id named _id which is a 24 char unique identifier composed of the current time, machine id, process id and random data. Here is a description of each field:

**Files**

- **Name** – File’s path that acts as a unique identifier for a file;
- **Description** – A string with the file’s description.

**Jobs**

- **Name** – Job’s name;
- **Status** – Current status of the job. New jobs can be pending or ready;
- **CommandName** – Name of the command to execute;
- **Args** – List of arguments to pass to the command;
- **CreateDate** – Timestamp with the creation date;
- **OwnerId** – Id of the user who created the job;
- **ProjectId** – Optional id of a project to associate the job with.

**Projects**

- **Name** – Project’s name;
- **Description** – A string with the project’s description;
- **CreateDate** – Timestamp with the creation date;
- **OwnerId** – Id of the user who created the project.

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46 MongoDB ObjectId - [http://docs.mongodb.org/manual/reference/object-id/](http://docs.mongodb.org/manual/reference/object-id/)
A Website for Alternative Splicing Analysis

Users

- **Login** – string used to uniquely identify a user and sign in into the web service;
- **Name** – User’s name;
- **PassHash** – User’s password in hashed form;
- **CreateDate** – Timestamp with the creation date;
- **IsAdmin** – Boolean value indicating whether the user is an administrator or not;
- **IsCluster** – Boolean value indicating whether the user is a cluster account or not;
- **Pending** – Optional boolean value indicating whether the user account is pending validation by an administrator account.

MongoDB allows the creation of arrays as values of a field. This is used in the job’s args field to store a variable length array of strings that are used as arguments for the job’s command.

3.2.2 Web Service Architecture

The web service requires an account to be able to create new jobs. Most operations require authentication before being used. A menu bar is shown at the top of the page with access to the various functionalities, as seen in Figure 3.5.

![Welcome to the web service!](web_service_initial_menu.png)

Figure 3.5: Web service initial menu.
A Website for Alternative Splicing Analysis

A user can create an account from the web service. An admin account must approve new user accounts before these can be used, for security reasons. Administrator accounts must be created manually for security reasons by a person with direct access to the database.

Cluster accounts are a special kind of account used by worker bots to ping the service for pending jobs. We can have more than one worker bot account configured to execute more jobs asynchronously.

Jobs are defined by a name and a command to execute with a list of parameters whose meaning varies depending on the job. For a download job, the parameters are the download URL and an optional new file name.

Currently there are these types of job:

- **Bam2Sam** – Converts a BAM file to the SAM format, as seen in Figure 3.6;
- **Download** – Downloads a file to a folder from an URL;
- **InterProScan** – Uses the InterProScan\(^{47}\) sequence search web service to scan FASTA files for known proteins;
- **Orf Find** – Runs an ORF finder job using a FASTA file as input and outputs an ORF file;
- **Sam2Bam** – Converts a SAM file to the BAM format;
- **Sam2Fasta** – Converts a SAM file to the FASTA format.

![Jobs](image)

**Figure 3.6:** BAM to SAM conversion job example.

---

\(^{47}\) InterProScan web service - [http://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap](http://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap)
The worker bot checks the command name and constructs the command to execute and takes care of downloading the necessary files from the server and uploading the newly created files to the server. A job can have an associated project, in which case the projects associated files can be used as input and the output is saved in the project’s folder.

Because of the generic nature of the job not having specific information such as the command-line to execute, but the action to perform and the list of parameters, a worker bot can execute the job using whatever tools there are on the platform and correct and improve the command pipeline to execute for each action without needing to alter the job. For instance, to execute a conversion job, we can use one program and later on change that program to a different version as long as the job's parameters are used in the same way. This is useful when upgrading the pipeline with a different program to test performance or accuracy, as a worker bot can use a newer version than another bot.

Once a job is completed, the program’s stdout and stderr output are saved and synchronized with the service so the user can see their output (if any).

The worker bots poll the web service every 10 seconds for a pending job. We can monitor the status of the worker bots by viewing the clusters page and see the last polling time.

Projects exist to allow the user to describe the project and the files associated with it. Projects have a description which uses markdown\(^{48}\) (Figure 3.7) as a text description to allow for richer descriptions.

---

### 1.1 Introduction

Paragraphs are separated by a blank line.

Text attributes *italic*, **bold**, `monospace`.

A [link](http://example.com).

Testing files:

- genome.reference.gtf
- testing.set.bam

---

\(^{48}\) Markdown - [http://en.wikipedia.org/wiki/Markdown](http://en.wikipedia.org/wiki/Markdown)
File management is done using the files browser where a user can browse public files and project’s specific files, as seen in Figure 3.8. He can also delete files and add descriptions to them to better know what they are and where they came from.

The web service and the worker bot can be configured using App.config files located in the program’s folders. These can be used to configure server name, port, and the location of the data folders.

Before running the web service and the worker bot, these must be configured with valid paths to a folder to store the data files and a folder for the stdio files. Default folder names are used in case these are not set.

The web service is written in C# using the Nancy\textsuperscript{49} web framework, a lightweight web framework designed to be simple, modular and easy to extend where a user can choose which functionalities to use. Authentication is provided by Nancy’s forms authentication module. The view engine used to process the HTML pages is the Razor view engine\textsuperscript{50}.

On the client side, Twitter Bootstrap\textsuperscript{51} is used as a front-end framework for creating common interface components such as forms, buttons and lists. This allows us to focus on the business logic. Bootstrap takes advantage of the various screen resolutions to rearrange content based on screen size. Bootstrap allows us to change the appearance simply by modifying the default cascading style sheet (CSS) file. The default template is a modified version of the cyborg\textsuperscript{52} theme.

jQuery is used for common user interface tasks on the client side.

The worker bot uses the RestSharp\textsuperscript{53} REST API to communicate with the web service.

All dependencies are managed using NuGet\textsuperscript{54}, the package manager used by .NET.

The web service was tested in both Windows and Linux machines (using Mono\textsuperscript{55} - an open source implementation of .NET).

\textsuperscript{49} Nancy web framework - http://nancyfx.org/
\textsuperscript{50} Razor view engine - http://razorengine.codeplex.com/
\textsuperscript{51} Twitter Bootstrap - http://getbootstrap.com/
\textsuperscript{52} Cyborg theme for bootstrap by Thomas Park - http://bootswatch.com/cyborg/
\textsuperscript{53} RestSharp - http://restsharp.org/
\textsuperscript{54} NuGet package manager - http://www.nuget.org/
3.3 Chapter Conclusions

Due to the nature of bioinformatics tools being mostly command-line based, the web service is designed to be a generic job scheduling application, where new jobs can be added to the web service and processed by background workers. Some of the solutions named in Chapter 2 were studied before starting the implementation but they were dismissed because they were not quite what was required.

The .NET framework was chosen because it has many of its components available as open source with a big user community and good development tools. The Nancy framework was chosen because of its simplicity and ease of use and for allowing for a bit more freedom in comparison to standard model-view-controller (MVC) frameworks.

Other technologies and frameworks were considered, namely node.js and other free frameworks but were dismissed either because of their poorer development tools or because of difficulties achieving true cross platform targeting because of OS specific modules in these frameworks.

The same binary file is used to run on both Windows and Linux machines without modifications.

MongoDB is used as a database because the application does not require the full power of a SQL database and because it is simpler to model the database as we do not need to explicitly define a data schema. It also allows us to store variable arrays more easily in comparison to a relational database, as it is the case with a job’s parameter list.

File management uses the file system for storing files. This allows us to use the underlying operating system’s file API to manage files.

The worker bot transfers files from and to the web service’s server. This adds overhead to the process but allows for more flexibility as web can have the server running on a standard computer but the worker bot running on a faster machine.

Because worker bots have their own accounts, we can have multiple worker bots executing pending jobs in parallel by having several worker bots configured and thus have a more scalable solution. Note however that due to the transfer of files from and to the central server, this one might become a performance bottleneck due to the network traffic involved in the file transfers and the internal sharing of the same hard disk drive. It is therefore recommended not to use a high number of worker bots on the same web server.

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55 Mono - [http://www.mono-project.com/](http://www.mono-project.com/)
58 Node.js software platform - [http://nodejs.org/](http://nodejs.org/)
Chapter 4

4. Alternative Splicing Analysis

Process

This chapter describes the application of the developed computational platform to some of the steps taken to analyse data for the problem of aberrant alternative splicing in cancer. The data, as mentioned in Chapter 2, was downloaded from the ENCODE project\(^\text{59}\) that aims at the identification of all functional elements in the human genome. The project hosts various cell lines taken from human tissue samples and later transformed in a laboratory to be replicated and researched throughout various laboratories around the world. The samples we use are cancer cell lines: K562 and GM12878.

The very first step in the whole process is to download the data. When using the platform to downloading the K562 cell line CSHL1 took 1 hour 50 minutes and 13 GB were downloaded. For GM12878 cell line CSHL2, 1 hour and 54 minutes was required to download 15 GB of data (the BAM file with the aligned reads).

4.1 Junctions Identification and Open Reading Frame Detection

Alternative splicing is a process where during gene expression a particular exon may be included or excluded from the final processed messenger RNA (mRNA) from that gene. The proteins translated from the alternative spliced mRNA will have differences in their structure, as explained in Chapter 2. The platform was used to identify the following five basic modes of alternative splicing:

\(^{59}\) The ENCODE project - [http://www.genome.gov/10005107](http://www.genome.gov/10005107)
Alternative Splicing Analysis Process

**Exon skipping** – also known as cassette exon, where the exon can be spliced out of the transcript or retained with its flanking introns. This is the most common mode of alternative splicing in mammals (Figure 4.1).

![Exon skipping alternative splicing](image)

Figure 4.1: Exon skipping alternative splicing.

**Mutually exclusive exons** – One of two exons is retained in mRNAs after splicing, but not both in a mutually exclusive manner (Figure 4.2).

![Mutually exclusive exons alternative splicing](image)

Figure 4.2: Mutually exclusive exons alternative splicing.

**Alternative 3’ acceptor site** – An alternative 3’ splice junction (acceptor site) is used, changing the 5’ boundary of the downstream exon (Figure 4.3).

![Alternative 3’ alternative splicing](image)

Figure 4.3: Alternative 3’ alternative splicing.

**Alternative 5’ donor site** – An alternative 5’ splice junction (donor site) is used, changing the 3’ boundary of the upstream exon (Figure 4.4).

![Alternative 5’ alternative splicing](image)
Intron retention – An intron can remain in the mature mRNA molecule or be spliced out. This differs from exon skipping because the retained sequence is not flanked by introns. If the retained intron is in the coding region, the intron must encode amino acids in frame with the neighbouring exons, or a stop codon or a shift in the reading frame will cause the protein to become non-functional (Figure 4.5).

There are two more modes of alternative splicing, although they are less frequent (alternative first exon and alternative last exon).

One useful measure to extract is the count of each type of junction. Many programs can achieve this. They usually require an annotation file (GTF) and a file with mapped reads (SAM/BAM).

As explained in Chapter 2, if a genome has an error and misses it's ending frame, it will end when it finds the next ending frame and this will generate a potentially harmful protein. As such, we can use open reading frame (ORF) analysis to correlate which open reading frames are more present in tissue samples with certain diseases and try to determine if those ORFs have a greater impact in causing that disease.

4.1.1 Obtaining the Junctions Type Count

In order to get the number of gene types from a SAM/BAM file, one needs an annotation file of the whole human genome (GTF). An up-to-date version of the human genome sequence can be obtained from the GENCODE project web page.\(^{60}\)

The GTF format has a field named `gene_status` that indicates if the gene is known or new.\(^{61}\) These fields are present in both the annotation file (GTF) and the mapped read file to analyse (BAM):

\(^{60}\) The GENCODE project - [http://www.gencodegenes.org/](http://www.gencodegenes.org/)
Alternative Splicing Analysis Process

- The name of the chromosome;
- The starting position of the feature in the chromosome;
- The ending position of the feature in the chromosome.

In order to count the number of each type of function, we must count the `gene_status` field which can be one of these values:

- KNOWN – the gene is known;
- NOVEL – the gene is new;
- PUTATIVE – the gene is not known but it is believed to be a gene by its open reading frame.

To achieve this, we run the following command to convert a BAM file to a BED with 12 fields:

```
samtools view -h infile.bed | awk '{if(($6 ~ /N/)|| $1 == "@SQ") print}' | samtools view -bS | bamToBed -bed12 -i stdin > outfile.bed
```

This command will rearrange the data in the BAM file to the BED format. It takes an `infile.bam` as input and outputs an `outfile.bed`. After this step, a command is ran to cross reference this file with a GTF file and count the gene status column. This will give us a count of the known and unknown genes:

<table>
<thead>
<tr>
<th>Gene Status</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNOWN</td>
<td>548118</td>
</tr>
<tr>
<td>NOVEL</td>
<td>1626827</td>
</tr>
<tr>
<td>PUTATIVE</td>
<td>1532709</td>
</tr>
</tbody>
</table>

Another very important step in the analysis process is the identification of open reading frames. ORFs are the regions of the nucleotide sequence from the start codon to the stop codon. Gene finding is usually started by searching for open reading frames.

An ORF is a sequence of DNA that starts with start codon “ATG” (not always) and ends with any of the three termination codons (TAA, TAG, TGA). There is a task in the platform to execute this as well.

---

61 GENCODE GTF format - [http://www.gencodegenes.org/gencodeformat.html](http://www.gencodegenes.org/gencodeformat.html)
Alternative Splicing Analysis Process

4.2 Search for Known Proteins Domains

A useful analysis that can be performed is searching for known proteins domains. The interest of the last step relies on the possibility of this information to be quite relevant to explain diseases. When there is an aberrant splicing, the translation of DNA into RNA might be stopped too early which may have the consequence that the coded protein will be too short and some very important domains of such protein will be missing. This is done using InterProScan\(^{62}\) (Zdobnov and Apweiler 2001), (Quevillon et al. 2005), a web service for automated sequence analysis of proteins that can identify regions of interest, based on the InterPro consortium member databases. This allows us to quickly detect a novel sequence with considerable confidence.

There are two versions available: a standalone version and a web service. We use the web service with the following command (C# version of the web service client):

```
IPRScan5CliClient -email email@example.com -sequence infile.fasta -outfile outfilename
```

The web service version requires a valid email address and a FASTA file as input with the nucleotide sequences to scan. This tool can be used from a BAM file by converting it to a FASTA file using our web service.

4.3 Other

Other steps important for data analysis involve conversion between standard formats. The following commands are used to convert between formats:

BAM to SAM (using SAMtools):

```
samtools view -h -o outfile.sam infile.bam
```

SAM to BAM (using SAMtools):

```
samtools view -bS infile.sam > outfile.bam
```

SAM to FASTA (using EMBOSS):

```
seqret infile.sam outfile.bam
```

\(^{62}\) InterProScan web service - [http://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap](http://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap)
Chapter 5

5. Conclusions

This chapter describes the solution developed regarding goals achieved and the contributions that were made. Some suggestions for improvements are also made.

5.1 Achievement of Objectives

During this thesis, the development of the web service to automatize the execution of the bioinformatics jobs was completed. It is multi-platform, having been tested in both Windows and Linux. The web framework is divided into 2 main parts: the web service itself and the worker bot that uses the web service to run tasks from another computer. The web service meets all the requirements set in terms of functionality, namely being multi-platform, having a user interface that works well with small screens and touch screens, user accounts and authentication, management of files, management of projects describing experiences, management of jobs to be run on worker bots, being able to run jobs from another computer, taking care of file transfers between both computers automatically.

Jobs in the web service are generic enough to allow the creation of new types of jobs without having to rewrite the underlying logic by abstracting away command-line programs and storing only the name of the command or action to perform and a list or arguments to pass to the command. This allows us to maintain backwards compatibility by ignoring newer arguments and change the programs used in a job without having to alter existing jobs, as long as the new command interprets the arguments in the same order as before.

In summary, the thesis work provides: a general purpose architecture to run jobs in several machines running different operating systems; makes available to the community a web based interface that makes it easy for any user to automate processes requiring the execution of several tasks; a software tool capable of collaborative work among researchers sharing any information they think useful to others; a ubiquitous computational tool accessible from any place with access to the internet. The work was tested in a very specific domain, namely with procedures
Conclusions

mainly used to test the impact of aberrant alternative splicing in cancer. The proposed and
developed platform enables: running "standard" tools used in aberrant alternative splicing
studies; the possibility of chaining tools to build a pipeline that accepts the aligned reads as
input and produces the aberrant alternative splicing results; an automatic process (using the API
from the adequate web resource) to fetch the final information of the analysis concerning the
domains of missing parts of the proteins encoded by the gene under analysis, using the
InterProScan web service.

5.2 Future Work

Few issues were left unresolved in the web service. Most features were implemented. There are,
however, some aspects that can be improved, namely the web service could return different
views depending on the command executed and show the data in a more obvious way for the
user to analyse. Although this was not implemented, it is fairly easy to do and merely requires
the job view route in the web service to return a different view depending on the command
name and create the custom views using HTML and JavaScript.

Another issue that exists is the download command that is executed remotely only to have
the downloaded file sent back to the server. This is because of the distributed nature of the web
service and the fact that the download command is a job. An option to run downloads locally
could be added in the future.

When transferring files from the web service, worker bots will overwrite existing files with
the same name in their workspace, even if these are the same as the ones in the server. An
improvement could be done by checking the file sizes and modification dates or by using an
error detection code or a cryptographic hashing algorithm to verify that the files are the same.

Regarding the existing types of jobs, more jobs useful for alternative splicing analysis
could be added to add more functionality to the solution.

Adding unit testing to the web service’s public API and to helper functions is a future
improvement to ensure it works correctly with future changes. The web framework used
provides a way to test the web service\textsuperscript{63} and one of the many unit testing libraries can be used to
test other code.

Although the complete set of modules of the pipeline, starting at the raw RNA-Seq set of
(unaligned) reads and ending at the identification of the missing domains of proteins, is very
easily accommodated in the proposed framework, we have concentrated only on the pipeline
stages concerning the analysis of the gene expression (generation and analysis of ORFs). To
make the whole process complete the steps that compute the gene expression from raw reads
need to be incorporated.

\textsuperscript{63} Testing Nancy applications - \url{http://github.com/NancyFx/Nancy/wiki/Testing-your-application}
Conclusions

It is quite common in molecular biology to have tasks that are frequently done and are composed by a set of steps (a pipeline). A nice extension of the proposed web interface is an interface where a computer scientist could “assemble” easily such type of pipelines. He/she would upload or indicate the software to be used (if already available), scripts to convert between file formats if necessary, and the pipeline would be stored as a single software tool. Some steps in the pipeline could even get information from web data bases to be used in the pipeline analysis, relieving the user from collecting some of the useful information from several web sites (for example reference genomes). As users, biologists would only need to provide the input file(s) and get the results without the need to call several programs and with the need to know the inner working of the process. This work is partially done, however it can be improved further by making it easier to build job pipelines.

We would also be very interested in a near future to use Data Mining tools to help in the analysis process. Tasks like the use of clustering as a tool for outliers' detection could be useful for identifying and explaining junctions with an origin in aberrant alternative splicing. Classification methods that discriminate between "normal" and aberrant alternative splicing could also help in the explanation of the phenomena. However for a proper and complete use of such approaches, we need more time to enrich the information available in the ENCODE project's database that we used.
References


References


Appendix A

A. Web Service User Manual

A.1 Introduction

This document describes the necessary steps to have the web service up and running.

A.2 Installation

A.2.1 Prerequisites

The solution is written in C# and targets the .NET framework 4.0. In order to run the solution, you need one of the following frameworks installed on the operating system:

- .NET framework 4.0 or newer\(^{64}\);
- Mono 3.2.8 or newer\(^{65}\).

The solution has two main projects. Their prerequisites are:

Web service

- MongoDB\(^{66}\) – The web service requires a valid connection to a MongoDB database. The MongoDB database doesn't need to be on the same computer.

Worker bot

\(^{64}\) Microsoft .NET framework - http://www.microsoft.com/net
\(^{65}\) Mono - http://mono-project.com/Main_Page
\(^{66}\) MongoDB - http://www.mongodb.org/
Web Service User Manual

- **A command-line shell** – The worker bot uses a shell to run jobs locally. On Windows, cmd.exe is used and is installed with the system. On Linux, Bourne shell\(^67\) (sh) is used and either that or a compatible shell must be installed;

- **A list of packages** – The worker bot uses command-line programs to run the jobs and these programs must be installed and be available to be run from the default shell. Currently, these are the required programs:
  - Wget\(^68\) – Wget is a program to download content from HTTP and FTP servers. It is usually installed on Linux and can be installed on Windows using Gnu on Windows\(^69\);
  - EMBOSS\(^70\) – EMBOSS is a collection of command-line programs for bioinformatics. Some of those programs are used by some jobs;
  - SAMtools\(^71\) – SAMtools is used to work with SAM/BAM files.
  - InterProScan – InterProScan\(^72\) in a web service that uses EMBOSS tools in the background to search for proteins in their database from FASTA sequences. The web service uses the C# client (IPRScan5CliClient) which can be found on the web service’s web page.

On Linux based systems, most of these programs can be installed easily using the default package manager. On Debian\(^73\) based operating systems, you can use the following commands to install all the necessary dependencies (requires root access):

```
apt-get install mongodb
apt-get install mono-runtime
apt-get install emboss
apt-get install samtools
apt-get install wget
```

To install the InterProScan client, you need to download the C# client (IPRScan5CliClient.exe) to a folder that will be available from any command-line prompt. On a Debian based system, this folder can be /usr/bin. After that, make sure the program can be run without the .exe extension. Simply rename the file to remove the extension. Mono must be installed to run the program. On Windows, you do not need to rename the program, but simply make it available from the command-line prompt.

### A.2.2 Compiling

The root of the solution should look like this:

---


\(^69\) Gnu on Windows - [http://github.com/bmatzelle/gow/wiki](http://github.com/bmatzelle/gow/wiki)

\(^70\) EMBOSS - [http://emboss.sourceforge.net/](http://emboss.sourceforge.net/)


\(^72\) InterProScan web service - [http://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap](http://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap)

\(^73\) Debian operating system - [http://www.debian.org/](http://www.debian.org/)
Web Service User Manual

- /WebService/*
- /WebService.Aspnet/*
- /WebService.Common/*
- /WebService.Common.Logic/*
- /WebService.Common.WS/*
- /WebService.Self/*
- /WebService.WorkerBot/*
- WebService.sln

Each project folder contains a .csproj project file for compilation. The root of the folder has a .sln file that compiles the whole application (WebService.sln).

To compile the code, you need to have a version of Visual Studio\textsuperscript{74} or MonoDevelop\textsuperscript{75} that supports C# 4.0 projects and NuGet\textsuperscript{76} packages (these should be automatically restored when building). Visual Studio 2010 or newer and the latest version of MonoDevelop (Mono > 3.2.8) should be able to compile the project.

The solution uses MSBuild\textsuperscript{77} for compilation. It is a build automation tool from Microsoft that uses an XML based project file format. Besides being able to compile from Visual Studio or MonoDevelop, you can also compile using the command-line using MSBuild in Windows or XBuild\textsuperscript{78} in any platform supported by Mono.

To compile the project, simply run:

msbuild.exe WebService.sln
xbuild.exe WebService.sln

This command compiles the solution in its default configuration, which is Debug. To compile in Release mode, use:

msbuild.exe WebService.sln /property:Configuration=Release
xbuild.exe WebService.sln /property:Configuration=Release

Note: Compiled binaries with either MSBuild or XBuild should work without modifications on all platforms. It is not recommended to mix and match binaries compiled with both versions.

Each project is compiled to each of the project’s bin folder (bin/Debug or bin/Release). After building the project, all the necessary dependencies for the web service project should be in the WebService.Self bin folder:

- /Content/*
- /Views/*

\textsuperscript{74} Visual Studio express editions - \url{http://www.microsoft.com/express/download/}
\textsuperscript{75} MonoDevelop - \url{http://monodevelop.com/}
\textsuperscript{76} NuGet package manager - \url{http://www.nuget.org/}
\textsuperscript{77} MSBuild - \url{http://msdn.microsoft.com/en-us/library/dd393574.aspx}
\textsuperscript{78} XBuild - \url{http://www.mono-project.com/Microsoft.Build}
Web Service User Manual

- MongoDB.Bson.dll
- MongoDB.Driver.dll
- Nancy.Authentication.Forms.dll
- Nancy.dll
- Nancy.Hosting.Self.dll
- Nancy.ViewEngines.Razor.dll
- WebService.Common.dll
- WebService.Common.Logic.dll
- WebService.dll
- WebService.Self.exe
- WebService.Self.exe.config

The Content folder contains static web content files, namely CSS, JavaScript and static HTML pages.

The Views folder contains the Razor view engine’s views files (.cshtml) that render the HTML views at runtime.

The other project in the solution is the worker bot. All the necessary dependencies should be in the WebService.WorkerBot bin folder:
- Newtonsoft.Json.dll
- RestSharp.dll
- WebService.Common.dll
- WebService.Common.WS.dll
- WebService.WorkerBot.exe
- WebService.WorkerBot.exe.config

A.2.3 Installing

In order to install either the web service or the worker bot, all it is needed is to copy the necessary files into their destination and to run the application (the .exe file).

To run the .exe on Windows simply run it as administrator or call it from an administrator command-line, since a user mode command-line will fail to open the necessary port. On Mono, you must first append mono to the command-line:

```
mono WebService.Self.exe
```
Both projects require prior configuration to work properly. Configuration is achieved by using .NET framework’s built in application configuration files\(^79\). These files contain settings specific to the application and usually have the same name of the application with .config appended to the end. These files should have special write permission from the operating system, even if they are running in an account without writing permissions in the application’s folder. This behaviour is guaranteed to happen on Windows, but might not be on other operating systems.

### A.2.3.1 Web Service

WebService.Self.exe

WebService.Self.exe.config

The web service supports the –h or --help parameters that print the application’s supported configuration parameters and their default values:

- **URL** – URL to use to start the web service. The HTTP protocol string must be present otherwise an exception will be thrown. Default is http://localhost:8080;
- **MongoDBHost** – Name of the MongoDB server host. Default is localhost;
- **MongoDBPort** – Name of the MongoDB server port. Default is 27017;
- **MongoDatabase** – Name of the MongoDB database name. Default is WebServiceDB;
- **PageSize** – This applies to all views that list items and defines the number of items to display per page. Default is 10;
- **DataFolder** – Name of the folder to store the data files. Default is ./data;
- **StdioFolder** – Name of the folder to store the command's stdio files. Default is ./stdio.

These settings can be configured in the WebService.Self.exe.config file (Figure A.1):

```
<?xml version="1.0" encoding="utf-8"?>
<configuration>
  <appSettings>
    <add key="DataFolder" value="./data" />
    <add key="StdioFolder" value="./stdio" />
  </appSettings>
</configuration>
```

Figure A.1: Web service example configuration file.

The data and stdio folders must exist on the disk and have read and write permission from the operating system. It is recommended that these folders be symbolic links\(^80\) if the


folders are on a different disk or a network drive to avoid problems that might arise from parsing unusual paths.

For security reasons, the web service doesn't allow the creation of administrator accounts. Users cannot create new user accounts without an administrator first allowing such an account. Administrator accounts must be created manually by directly importing users to the database. This can be done using `mongoimport`, a program that comes with MongoDB:

```
mongoimport --host localhost:27017 -d WebServiceDB --collection users --file users.json -upsert
```

This command will import users to the default database (`WebServiceDB`) on the default host (`localhost`) into the `users` collection from a JSON file (`users.json`). The `users.json` file looks like this:

```json
{
    "_id": {
        "$oid": "000000000000000000000001"
    },
    "login": "admin",
    "name": "Administrator",
    "passhash": "753068535f964205070a59af8a0c64aacc9883d03febd7ab8d2b92ed29c3dd93",
    "createdate": {
        "$date": "2014-01-01T00:00:00.000+0100"
    },
    "isadmin": true
}
```

Please note that the `oid` must not be 0, otherwise the system will stop working correctly. Notice the `isadmin` boolean set to true, indicating an administrator account. The `passhash` provided is for the password `demodemo`. You can change the password using the web service. The password hash stored in the database is composed of a SHA256\(^1\) hash of the SHA1\(^2\) password hash with a salt appended to it. The password salt value is defined in `WebService.Common/Settings.cs` as `PassSalt`.

```
PassHash = SHA256(SHA1(password) + PassSalt)
```

The web service uses Twitter Bootstrap\(^3\) theming (version 3) for its user interface. The default theme is a custom theme based on the ones found in `bootswatch.com` by Thomas Park. You can alter the theme by replacing the files `bootstrap.css` and `bootstrap.min.css` located in the `Content` folder with new ones.

Note however that the fonts file path should start with `fonts/` and not `../fonts/`. Most themes haven't got this path correctly and must be manually changed.


\(^3\) Twitter Bootstrap - [http://getbootstrap.com/](http://getbootstrap.com/)
The worker bot supports the `-h` or `--help` parameters that print the application’s supported configuration parameters and their default values:

- **URL** – URL pointing to the web service. The HTTP protocol string must be present otherwise an exception will be thrown. Default is `http://localhost:8080`;
- **Login** – Name of the cluster account to use to authenticate. This account must exist on the web service this worker bot is connecting to;
- **PassHash** – Hash of the password used to authenticate using the SHA1 hashing algorithm;
- **LocalBot** – Boolean value indicating if bot is sharing the data and the stdio folders with the web service in the same computer. This prevents the worker bot from trying to download remote files and upload the results back. Default is `false`;
- **DataFolder** – Name of the folder to store the data files. Default is `./data`;
- **StdioFolder** – Name of the folder to store the command's stdio files. Default is `./stdio`.

These settings can be configured in the `WebService.WorkerBot.exe.config` file (Figure A.2):

```xml
<?xml version="1.0" encoding="utf-8" ?>
<configuration>
  <appSettings>
    <add key="URL" value="http://localhost:8080/" />
    <add key="Login" value="Cluster1" />
    <add key="PassHash" value="5863d9e4cbdf522eaa62e0747fceb1c5b249ba13" />
    <add key="DataFolder" value="./data" />
    <add key="StdioFolder" value="./stdio" />
    <add key="LocalBot" value="true" />
  </appSettings>
</configuration>
```

Figure A.2: Worker bot example configuration file.

If the worker bot is running in the same computer, it is recommended that both the web service and worker bot share the data and stdio folders. To do this, make sure that both configuration files point to the same folders on the disk or create symbolic links in one of the program’s root folder that link to the other program’s folders. You also need to set the LocalBot parameter to true. Both folders should have read and write permission from the operating system.
A.3 User Guide

You can use the web service by first opening the web service's URL using a web browser. In order to access the web service, you must first login into the web service or create a new user account.

A.3.1 Sign In

You can sign in using the sign in link in the top right corner of the home page. You need to enter the following fields to sign in (Figure A.3):

- **Login** – Unique account login. Must be at least 3 characters long;
- **Name** – User name. Must be at least 3 characters long;
- **Password** – Password used to authenticate into the web service. Must be at least 4 characters long.

![Sign in form](Image)

Figure A.3: Sign in form.
A.3.2 Login

In order to access the web service, you must first login into the web service. You can do this by using the top menu login and entering your login and password (Figure A.4). If you try to access a restricted page, you will be redirected to a sign in page.

![User account menu](image)

Figure A.4: User account menu.

One logged in, you will see a welcome message with access to your user profile, where you can change your password, your jobs and projects and a logout form (Figure A.5).

![Login menu](image)

Figure A.5: Login menu.
A.3.3 Web Service Menu

After logging into the web service, you have access to a menu with all the functionality provided by the web service (Figure A.6).

![Web Service Menu](image.png)

Figure A.6: Web service menu.

The menu gives access to the following items:

- **Clusters** – Manages cluster accounts used by worker bots;
- **Files** – Simple file viewer to view and download files stored in the server;
- **Jobs** – Manages jobs to be run by worker bots;
- **Projects** – Manages projects describing experiments;
- **Users** – User account management.
A.3.4 Clusters

Cluster accounts are used by worker bots to access the web service. You can list cluster accounts and check their status by viewing the last activity. Cluster accounts, when in use, poll the web service every 10 seconds, which means that if a cluster account is idle, it should have a last activity below 10 seconds (Figure A.7).

![Cluster Accounts](image)

Figure A.7: Cluster accounts listing.

A.3.4.1 Create Cluster

You can create a cluster account from the clusters menu. You need to enter the following fields to create an account (Figure A.8):

- **Login** – Unique account login. Must be at least 3 characters long;
- **Password** – Password used to authenticate into the web service. Must be at least 4 characters long.

![Clusters](image)

Figure A.8: Add cluster account.
A.3.5 Files

You can use the files menu to browse files in the server (Figure A.9).

![File browser](image)

Figure A.9: File browser.

You can perform the following actions:

- Add a description to a file (Figure A.11);
- Edit that description (Figure A.10);
- Rename a file (Figure A.12);
- Delete a file (Figure A.13).

File descriptions use Markdown and come with an editor for easier editing.

![Edit file description](image)

Figure A.10: Edit file description.
Figure A.11: File description.

Figure A.12: Rename file dialog.

Figure A.13: Delete file confirmation dialog.
Folders are used by projects for their own files and identify themselves with the project's name for easy identification (Figure A.14). You can also navigate between folders using the folder bar at the top or by using the links present in sub folders:

- (.) – Root folder;
- (..) – Up one folder.

Figure A.14: Project's file browser.
A.3.6 Jobs

Jobs are actions that are to be performed by an available worker bot. There currently are these types of jobs:

- **Bam2Sam** – Converts a BAM file to the SAM format;
- **Download** – Downloads a file to a folder from an HTTP or FTP server;
- **InterProScan** – Uses the InterProScan\(^\text{84}\) sequence search web service to scan FASTA files for known proteins;
- **Orf Find** – Runs an ORF finder job using a FASTA file as input and outputs an ORF file;
- **Sam2Bam** – Converts a SAM file to the BAM format;
- **Sam2Fasta** – Converts a SAM file to the FASTA format.

A.3.6.1 Add Job

You can add a job from the menu. You need to enter the following fields to create a job (Figure A.15):

- **Name** – Job name;
- **Status** – Job status from the following:
  - **Pending** – awaiting execution. Use this to create a job but not have it executed right away;
  - **Ready** – ready for execution by a worker bot. Use this to signal the job is to be executed when a worker bot is available.
- **Project** – Optional field associating a job to one of the user's projects. This will run the job in that project's work area and create new files there instead of in the root folder. You also have access to input files located inside that project's work area.
- **List of parameters** – Each job takes a list of parameters that vary from job type. Usually it is an input file or more and a name of an output file.

\(^\text{84}\) InterProScan web service - https://www.ebi.ac.uk/Tools/webservices/services/pfa/iprscan5_soap
Figure A.15: Add job example.
A.3.6.2 Job Details

Once the job is executed, it will change its state depending on whether it is executing or it has already finished with success or because of an error (Figure A.16):

- **Executing** – Job is being executed by a worker bot;
- **Completed** – Job execution has completed without errors;
- **Error** – Job execution was halted because of an error. This usually means the command-line ran in the worker bot encountered a problem which can be due to an incorrect parameter or another unforeseen error. If the job ran with errors, but successfully, the state should be completed.

![Job Details](image)

Figure A.16: Job details.

The standard output and standard error streams are saved so you can check a job's success. Once you stop needing these files, you can delete the using the small delete button next to the output label.

You can also edit a job. Usually you do this to change its state. Manually editing job parameters is not recommended.

Deleting jobs can be done after the job ran and you don't need it anymore. Stdio files related to that job are also deleted. All files produced by that job are not, so you can use them in other jobs.
A.3.7 Projects

Projects are descriptions of experiments a user wants to have for reference. Each project has its own working folder, so users can have a separate work area for each project. Jobs that are associated to projects will output their files on that job's work area.

A.3.7.1 Create Project

You can create projects from the clusters menu. You need to enter the following fields to create a project (Figure A.17):

- **Name** – Project name;
- **Description** – Project description. Descriptions use Markdown and come with an editor for easier editing.

![Figure A.17: Add project.](image-url)
A.3.7.2  Project Details

Once the project is created, you can add jobs associated with it and view the project's files. The description is useful to describe the experiment and to add links to the files and special notes regarding the experiment (Figure A.18).

Figure A.18: Project details.
A.3.8 Users

User accounts are used for authentication. They are created using the singed in menu link as described in the beginning of this guide.

A.3.8.1 Pending User

When a new user is created by a guest, it is created in a pending state and you can't login into the system using that account until an administrator clears the pending state. Administrators are notified in the menu bar when pending users exist (Figure A.19).

![Pending users menu](image1)

Figure A.19: Pending users menu.

You can clear the pending state by clicking the clear pending button in the users listing (Figure A.20).

![Users listing](image2)

Figure A.20: Users listing.