Building an Online Resource for Candida Tropicalis

Final Report for CS39440 Major Project

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Abstract

Understanding data produced by next gen sequencing technologies is a difficult problem, not helped by a lack of well designed software and user experiences.

Researchers at Aberystwyth University are looking into the possibly of modifying a species of yeast, *Candida Tropicalis*, to metabolise xylose into xylitol.

If xylitol can be produced by *C. tropicalis* it would provide a cheaper supply of the sugar, which has antibacterial properties, as well as a very low glycemic index, making it suitable for diabetics.

To do this they have sequenced the DNA of three species of yeast and need an accessible and meaningful way to interact with the data produced.

Most genome database software is already over a decade old, and using very out of date practices, and technologies.

The aim for this project is to apply modern web development standards to this problem and produce a web site to display their data using Node JS, with the data stored in a NoSQL database, something that hasn't been done before.

The implementation of this database and website has shown that NoSQL is a viable approach to storing and viewing genomic information, especially for smaller genomes. However it does also act as a proof of concept and provide a starting step for the production of a larger open source project that aims to accommodate larger genomes.

Importantly it shows that modern technologies and approaches can have a meaningful benefit over the old monolithic solutions, especially in terms of producing high quality maintainable software.

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Chapter 1

Background & Objectives

1.1 Background

Researchers at Aberystwyth University have been studying three species of yeast, *Candida tropicalis*, *Candida boidinii*, and *Candida shehatae*. To study the genetics of these species, they have each had their genomes sequenced by Illumina sequencing machines. From that point the sequences of DNA that have been read are then assembled into contigs (contiguous DNA fragments). This process produces an accurate representation of the species DNA, which then can then be aligned to other better understood DNA and annotated as such.

Arabinose and Xylose are five carbon sugars ubiquitously found in plants such as grass, which can be used as a feedstock for industrial biotechnology. *Candida tropicalis* is able to convert arabinose & xylose into arabitol and xylitol respectively. Xylitol is a commercially valuable antibacterial food grade sugar use in the manufacture of chewing gum. However arabitol and xylitol are stereo isomers and cannot be easily separated. *Candida boidinii* cannot metabolise arabinose, for an unknown reason, but does metabolise Xylose but at a much slower rate which is commercially in-viable.

Understanding at a genomic level why *Candida boidinii* is unable to utilise arabinose and genetically modifying *Candida tropicalis* to have the same phenotype, may enable *Candida topicalis* to exclusively produce xylitol and not arabitol. This would mean a cheap and easy way to produce a source of Xylitol, which can be used for many applications. One exciting possibility is using it as a low glycaemic index table sugar that can be used by diabetics.

Currently the researchers have the genetic information for the three species of yeast in their raw data format, a fasta file. These files are large >16MB text files that are quite difficult to navigate and manipulate for less technically minded biologists. To make predictions about what genes perform what functions in the yeasts they will need to be able to search and browse this data in a much easier to interact with form.

This is why a website would be advantageous to them, as it would provide a simple and familiar user experience to other web based genome browsers such as the Candida Genome Database [2]. In addition to this functionality, one task that would be very useful for this is to be able to select a proteins coding sequence of nucleotides and then a user defined number of bases up and down stream of the gene, so that when they test out a theory in the wet lab experiments they can easily retrieve the bases around the gene that they intend to modify.

1.2   Existing Databases

As the core of the project focuses on having the data ingested into a database, the first action was to investigate the current database solutions that had been developed for genomic information.

The most well established was CHADO [3] which was initially developed back in 2005 for the FlyBase [4] project. It has since then grown to accommodate genomic information for eukaryotes, plants, and other complex multicell animals. It uses PostgreSQL [5] to store it's data and Perl [6] to setup and maintain the database. Due to it's monolithic approach of being a one size fits all solution, it has over 200 tables in a very complex schema. This makes working on it rather difficult, as there are many relations to consider when entering and manipulating data from the database.

When installing and populating CHADO, it was clear that the project wasn't very well maintained as during the installation several of the Perl modules that it depends on where failing their tests and not installing. This meant several modules had to be manually fixed just to get the application to install correctly. If it takes an experienced programmer and Linux admin several hours to follow the default installation instructions, debugging issues at several steps, it isn't going to be appropriate to impose such a system on potentially less technically minded Biologists. This combined with the fact that Perl has now been superseded by more modern scripting languages such as Python [7] and JavaScript [8], thanks to their superior package management, eco-systems and syntax, it was clear that a more modern approach would be more sustainable and maintainable for this project and for the future.

1.3   Alignment Tools

As the source of the data was uncertain, it was decided to investigate performing alignments in the event that more data, or different data was needed for the project than what was provided. The original alignment tool BLAST [?] was developed in the 90's and is very widely accepted as the de facto standard for performing alignments.

Alignment is when a DNA, or protein sequence is compared against a database of known protein sequences that have already been determined via previous research. This allows a researcher to match their sequences against known proteins and annotate them accordingly. These annotations aren't proof that the gene codes for that exact protein, but it is a strong indicator that it does. To prove it the researchers will test it in a lab experiment, however as there are many thousands of potential genes to test it is a lot more efficient to test genes that are predicted via an alignment.

An alignment is a computationally intensive task there have been many efforts to improve the efficiency of the algorithm and make the most of developments made in computing since the 90's. One area that has been pursued is the use of GPU's to perform alignments in parallel utilising hundreds or thousands of cores of the GPU, compared to the tens of cores on a modern CPU.

Initially Diamond [9] was tested and was able to align the data sets against the NCBI [10] non-redundant protein database [11], in about an hour and a quarter for each species, this was only using the CPU and may have been bottlenecked by the mechanical drives being used as storage.

The hardware being used for this was an Intel i7-6700k @ 4.6Ghz, 16GB of RAM, a 7200rpm Hard Disk Drive, and a Nvidia GTX 980.

As there was a high performace graphics card available it would be advantageous to make use

a GPU accelerated alignment tool to make the most of the hardware available, and reduce the time that any future alignments may take.

The first attempt to use a GPU powered tool was BarraCUDA [12], which installed successfully and appeared to run fine, however it would attempt to read in the entire reference database into memory. This was an issue as the NCBI non-redundant database is around 66GB in size, and the system that was being used only had 16GB of RAM available.

The next tool tested was CLAST [13], which unfortunately had compilation errors on the system. Debugging this was out of scope for my project as in this initial stage it didn't seem necessary. Next tested was Cudasw++ [14] this did install, but ran into the same issues as Barracuda, being limited by the system memory available.

In an attempt to get GPU acceleration working, the NCBI nr database was split up into 10GB chunks for use with these tools, however even after being reduced in size, BarraCUDA and Cudasw++ both had errors and failed to complete an alignment.

It was decided that although the speed gains of a GPU accelerated alignment tool would be significant, the Diamond alignment was quick enough to not be a major bottleneck for the project, when compared with the extra time and effort that would have to be put into investigating how to get the GPU alignments to work.

1.4   Differences in Bioinformatics and Computer Science

From studying bioinformatics and researching the most common methods of manipulating and analysing the data gathered, a trend was in the solutions being used was observed.

In computer science, tools are highly developed and have evolved to a very stable state and the best tools are known to be industry standards for their specialised purposes.

However in bioinformatics a combination of factors including the relative youth of the field and the large number of competing projects that don't collaborate due to competing for research publications; there have been a wide array of tools created, often by biologists with no software engineering experience, none of which have been adopted and honed as the de facto standard.

This means that for every task in the bioinformatics space there is often many different solutions offered, to what can be a very simple problem. An example of this is the simple task of splitting a fasta file up into several chunks, this should be a very simple problemto solve, and once it's been solved once that tool should become the one tool to complete that task.

Unfortunately when trying to do this, several tools were discussed on biostars forum [15], none of which worked for the nr database that was trying to be split. Eventually a tool made by Gordon Gremme et al [16] was able to complete the task, yet it wasn't mentioned in the earlier discussion on the online forum.

As a computer scientist this is a very odd observation, as for most problems in the programming sphere will have had several tools developed to solve it. The community then quite quickly picks the best solutions and adopts them as the known standards. [?].

An example of this is data markup languages, there are really only three major competing standards, XML, JSON and YAML, and they each have their own niche. However in bioinformatics there seems to be many ways to represent data from alignments or sequences. Fasta is appearing

to be the most mature way however there are so many standards, tab separated, gff3, EMBL and SAM files to name a few.

It seems very misguided to have produced all these new file formats, instead of a standard mark up language to represent the data. Fasta could very easily be replace by YAML, which would save a huge amount of developer time as they wouldn't have to produce or implement a fasta parser in their language of choice, as YAML can be natively read by most modern languages.

1.5   Analysis

From researching the current solutions to the problem of annotating, storing and presenting genetic information, it was clear that a solution using modern web development technologies wasn't currently in the public or open source domain.

Of the open source projects that were currently in use the majority appeared to be very old and monolithic. This is often due to their integration with the most popular genome viewers GBrowse [17] and JBrowse [18] which are both written in Perl.

Perl is not suited to modern web development, mainly due to it's miniscule market share [19] and vastly smaller number of available third party modules.
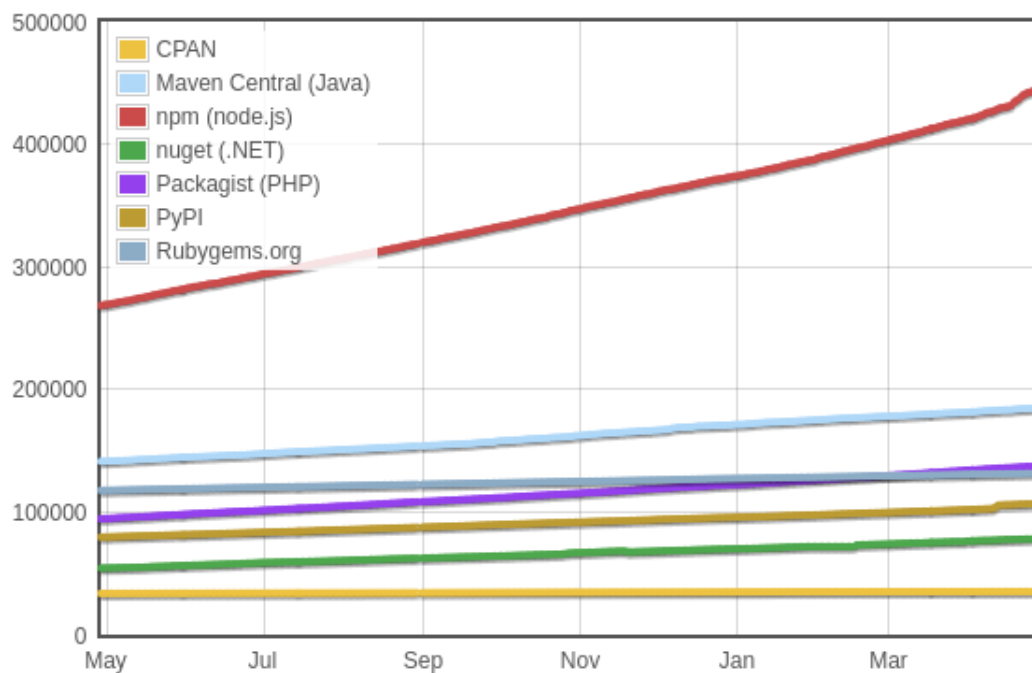

Figure 1.1: Number of modules available for server side languages, Perl (CPAN), Java (Maven), JavaScript (npm), .NET (nuget) PHP (Packagist), Python (PyPI), Ruby (Gems) [1]

Web development simply isn't done in Perl any more, and that means that the ecosystem

surrounding it just can't compete with newer technologies such as Ruby on Rails or Node JS.

1.5.1   Analysis of the problem

Looking at the data that I was provided with there was three clear stages to the development of my solution. The first would be to ensure that the data I had was valid and in the same format for each of the species that were being analysed. The next was to import that data into a database, and then from there develop a web front end to interact with the data in the database.

An alternative approach to this project would be to use one of the existing databases such as Intermine [20] or CHADO [3], and then use a web front end compatible with those databases such as GBrowse [17] or Jbrowse [18]. This would require slight changes to how the data was processed before going into these established systems, however the benefit of using this approach would be access toa ll of the bioinformatics tools that are compatible with these platforms.

As a computer scientist looking at this problem, there doesn't seem to be much need for a very complex solution. Once the data is generated, the hard bit, it just needs to be made into a database friendly format and then ingested to the database system. From there building a simple web front end to make the database accessible is a very simple task. There isn't any need to manipulate or modify the data, simply create and read it. Because of this it seemed that the current systems were greatly over complicating matters.

1.5.2   SQL vs NoSQL databases

Structured Query Language (SQL) databases such as MariaDB, PostrgeSQL and SQLite, store data in a relational manner, this means that data is stored across many linked tables, that are grouped by the data type. They are the interfaced with via SQL to select related data from multiple tables to return a meaningful result.

- SQL based systems have been used for a long time and have a large amount of support and compatibility with different frameworks.

- Efficient storage of data, not duplicated

- Complex queries can be ran with relative ease

- Requires a strict schema

- Development is more complex as you have to manage and maintain relations

NoSQL databases store data in collections of documents. Each collection is usually linked to a use case for the data, for example a website that has blog posts would have a "blog" collection, that stores all of the data for blog posts. The collection is made up of indivdual documents representing individual items, so the "blog" collection will store lots of individual blog posts. Each document is then made up of key value pairs, commonly represented as JSON.

- Collections are based on use cases so no complex selection logic

- Data is represented as JSON so it is very interoperable with JavaScript

- Dynamic schema, not every document has to have the same information as the other documents in the collection

- Newer technology so less legacy support

- Data can be stored twice which is less efficient

1.6   Research Method and Software Process

It was intended to use a blend of agile and extreme programming methodologies for this project. The idea was to ensure that as the understanding of the task at hand increased and new requirements or changes to the structure of the application became necessary, an agile workflow would allow adaptations to be made without setting the project's progress back.

For this project there were clear clients that could be accessed and used for feedback during development iterations. David Bryant and Abhishek Somani, the scientists conducting the research were available to meet and provide feed back. When suitable we would meet on a Friday at the end of an iteration to discuss the projects progress and any difficulties that may have been encountered. Towards the end of the project this time was used to get detailed feedback on how the website could be improved.

Unit testing was also used to develop the key algorithms, as well as continuous integration and deployment, this process is described in much more detail later on in the document. It was key to being able to deliver working software at regular intervals and demonstrate it to the clients.

Once requirements were gathered a Kanban board would be made with each individual task listed, based on the requirements of the application. Those tasks would then be weighted with an estimated difficulty, and then worked on. Once development had started on each task it would be moved to the "In progress" board, and then across to the "Done" board, once completed.
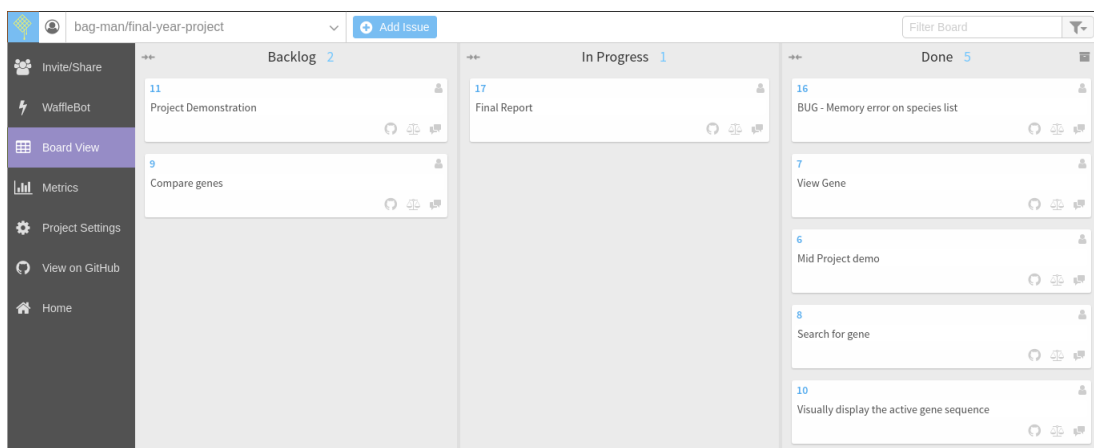

Figure 1.2: Kanban board in use. Waffle.io is the service providing the board

However as this project involved a lot of domain knowledge that wasn't familiar, there was a very significant amount of time spent learning what the data meant and how it should be used. During that time I was prototyping potential solutions to how the data could be interpreted, without

a specific set of requirements in mind. This lead to very vague requirements being created and development following a path closely linked to the data and my understanding of it.

An example of this process was the reverse compliment feature, that didn't appear to be necessary until a couple of weeks before the project deadline. This is exactly why a traditional structured development process like waterfall would not have worked for this project, as new requirements and features were appearing deep into the project, something that i wouldn't have been able to accommodated if I were using a strict waterfall model, where each phase of development is scheduled a head of time.

Chapter 2

Experiment Methods

2.1   Provided data

The data for this project was produced by a contracted researcher who had moved onto another project. Because of this there was limited opportunity to talk with them about the data, how it was produced and what needed doing to it. Unfortunately, the data did not include any form of documentation, or annotation as to how it was produced, the only metadata available was the filenames.

This lead to a lot of confusion as it wasn't immediately obvious what each bit of data was and what it meant. Examining the data and learning about the biology behind it took several weeks for me to ascertain what bits of data were actually relevant to the project, and what other data needed producing. Eventually it was determined that for each species there were three files that I would need to use.

- Raw assembled contigs of DNA, these would be used to get a genes position in the genome, and it's surrounding bases.

- Protein sequences annotated with Gene Ontology ID's, these had been extracted from an NCBI nr blast

- Coding sequences, these are the nucleotide sequences that coded for the proteins. Uncertain as to how they were produced.

Linking this data to the Candida Genome Database was a key bit of information that was missing. To do this the annotated proteins for *C. albicans* [21] were downloaded, then turned into a reference database with Diamond. With this the three species could then have their coding sequences aligned against *C. albicans* to produce accession ID's from the Candida Genome Database. The script that performed this step is in in $../../../data/albicans/run_{diamond}.sh.$

There was then several sets of data required to map these proteins to the Candida Genome Database, that would allow the researchers to easily compare the genes to well annotated species. First was a file of GO annotations for all the proteins stored in the Candida Genome Database. [22] This file conveniently also stores all the accession ID's for those proteins. This is what was used to map the alignment results to the Candida Genome Database, by selecting the accession ID from

the Diamond alignment results, and looking that up in this data set, the Candida Genome ID was able to be found.

In addition to this was a mapping file that mapped Candida Genome Database ID's to Uniprot ID's. With a small JavaScript script and some manual cleaning with a text editor, I was able to produce a complete mapping file in JSON, ready to be read in by the importation script. This allows a quick link to another database full of further information about the proteins that were being coded for.

Chapter 3

Software Design, Implementation and Testing

3.1 Requirements

There are two main components to the software being developed for this project. The first is the manipulation of the data into a database, and the second is a web front end for the data to be accessed via.

3.1.1 Database

The database has to store the following information for each hit from the alignment with *C. albicans*:

- ID of the hit that was found when the species coding sequences have been aligned with *C. albicans*.

- Species it came from.

- The gene name and ID from Candida Genome Database.

- UniProt ID.

- Contig of raw DNA.

- Coding sequence of nucleotides.

- Protein sequence that the coding sequence codes for.

- Annotations from NCBI nr database to provide a description of the proteins.

- A list of Gene Ontology [23] ID's for the protein.

- If it can be found, the positions of the start and end of the coding sequence in the raw contig.

From these requirements it is clear there are a few pieces of data that need to be extracted from the data that has been collated. For each alignment with *C. albicans* the protein sequence and GO annotations need to found, the coding sequence that was used in the alignment and the contig that it came from. In addition to this the metadata about the genes such as the name and ID's need to be read from the mapping file that was created earlier.

The final bit of data that needs to be added is the position of the coding sequence in the contig. An algorithm will have to be developed to detect this.

3.1.2   Website

The website will only have a few requirements:

- Display each record in the database in an easy to read manner.

- Highlight coding sequence in contig if possible.

- Copy coding sequence and +/- a user defined number of bases to the clipboard.

- Search the database for a gene, but name, description, ID, or even by nucleotide sequence.

3.2   Build Process

One of the first things that was setup for this project was the build process. This doesn't take long to setup but provides a huge benefit for the rest of the project. It helps to reduce errors and speed up the process of deployment and testing.

3.2.1   Development Environment

This project will be developed on an Arch Linux system, as it will only ever be ran on a Linux host this isn't an issue, as the production environment will match the development one.

All of the code and LaTeX will be written with the editor Vim, which has been configured to have many optimisations to this workflow. The full Vim configuration that was used can be found here [24].

3.2.2   Version Control

This project is being entirely tracked with Git version control. This allows the developer to have all of the work backed up, with an easily accessible history. It also allows for branching of the project to test out experimental features or bug fixes. The project is remotely hosted on Github [25] for remote backup and access via multiple hosts. Github also is widely supported for third party integrations, something that will be utilised quite extensively.

When pushing code to Github, a module called Husky [26] will run the test suite on the developers local machine. If the tests fail, the code will not be pushed.

3.2.3   Test suite

The test suite is set up to first clean the project of old builds and coverage reports, then run ESlint [27]. This will scan the entire codebase for files that do not adhere to the code style defined in the Clock configuration. This means that any badly formatted code will cause an error and prevent the code from being pushed until the developer fixes the issues.

After the linting check, the test suite will use Mocha [28] to run all of the JavaScript test files that are in the project. If any of the assertions made in the test files fail, the project will not be pushed.

Once the test's have been ran Istanbul [29] will provide a coverage report, that will show to the user the percentage of tested code that has been covered by the assertions. After this the code can finally be pushed to Github.

3.2.4   Continuous Integration

Once the code has been pushed to Github, a hook on Github will trigger Travis CI [30] to clone down the latest version of the code to it's servers and run the test suite again on it's own server. This is very useful as occasionally developers can bypass Husky to push code, or have something setup in their system that isn't defined in the application that causes test to pass on their system but not when the application is built from scratch.

Once those tests are successful, it will send the coverage report generated by Istanbul to another third party service called Codecov [31]. This tracks the test coverage over time and provides interactive charts highlighting areas that need more test coverage.
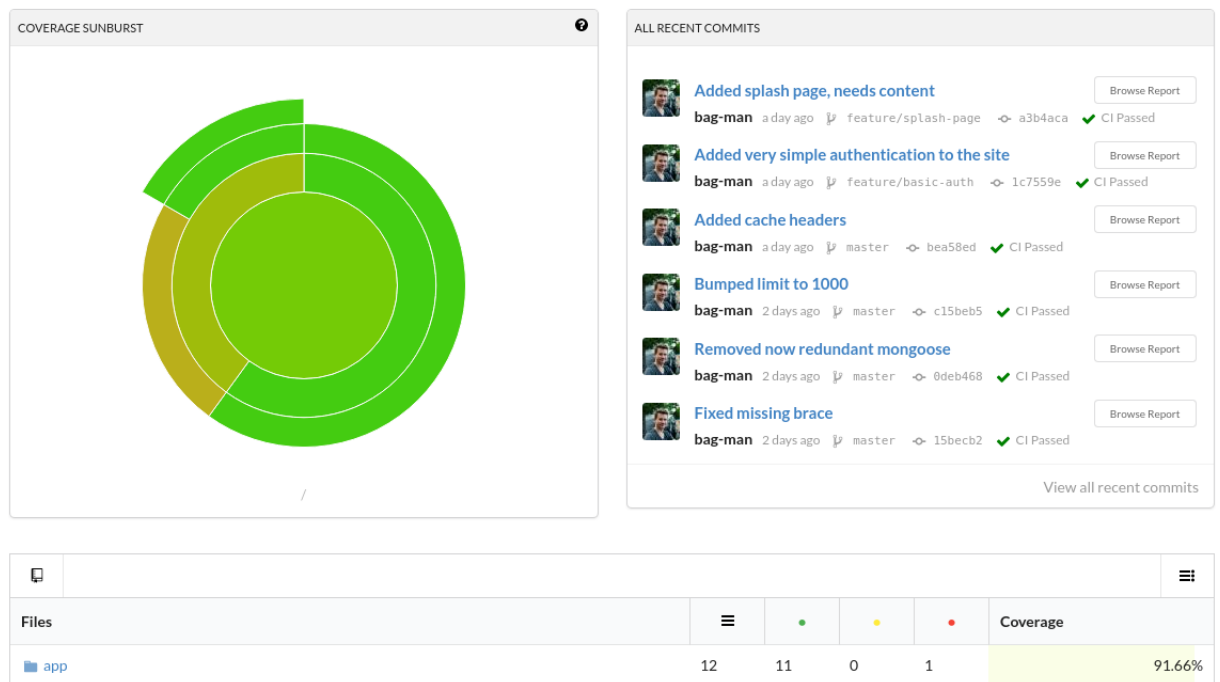

Figure 3.1: Codecov interactive coverage report

3.2.5 Deployment

If the tests pass on Travis CI, a Github hook will then activate the deployment. The project is setup to use Heroku [32], and to build on successful CI results. This means that when code is pushed to the master branch, it will automatically be deployed to their service. This is excellent for development as it allows changes made to the system to be almost immediately reflected on the live website. The only drawback of Heroku is that this project is using their free offerings which only allow 500MB's of database storage, which means that anything larger than that will be truncated. This isn't an issue for development, however it will mean that when the project is to go live, funding would need to be found to pay for more data storage, or alternatively the project can be hosted elsewhere, most likely within the University network.

The benefits of this build process are clear, firstly code is automatically deployed, which saves the developers a lot of time, as well as encouraging regular client feedback. However the main advantage is that before the deployment the code will have had the test suite ran on it twice, once on the developers local machine and then again on a brand new build on the CI service. This means that any mistakes are caught before they are put into production and the developer is notified of these issues so they can't be avoided.

3.3 Design

The first stage in designing this system was to decide upon what database technology would be used for the data store. As discussed in the analysis a NoSQL database called MongoDB has been chosen to store the data. This choice was also influenced by the choice of server side language, which for this project will be NodeJS.

NodeJS was chosen because:

- Client and server side languages are the same.

- Integrates well with MongoDB as it uses BSON which works with native JSON.

- Provides easy package management via npm.

- ES6 syntax is very nice.

- I have a lot of experience with it and learning a new language is outside of the scope of the project.

As outlined in Ioannis K. Chaniotis et al's paper, 'Our study concludes that Node.js offers client-server development integration, aiding code reusability in web applications, and is the perfect tool for developing fast, scalable network applications.' [33]

The website for this interface will be built off of nodestack [34], a set of boilerplate code that uses several technologies including:

- Node JS / ES6

  "Node.js is a JavaScript runtime built on Chrome's V8 JavaScript engine. Node.js uses an event-driven, non-blocking I/O model that makes it lightweight and efficient. Node.js'

package ecosystem, npm, is the largest ecosystem of open source libraries in the world." [35] Since Node ¿4.0 it has supported the ES6 standard, which provides a lot of syntactic sugar, and makes the formatting of the code a lot easier to read and write.

- Webpack

  Webpack is a module bundler that allows for easy packaging of client side javascript. It allows the client side javascript to be written in ES6, then when built it will transpile the ES6 code into more widely supported ES2015 code, minify it for efficient, and package it into one single file. This means that the code can be written in a nicely organised manner across multiple files in ES6, and then reduced to one small file for actual usage.

- Mongoose

  Mongoose is an Object Data Manager, it allows developers to define schema's and validation for their data objects as well as extensible models for those objects. This makes interactions with MongoDB a lot simpler, as well as easy enforcement of validation rules.

- Pug

  Pug is an HTML templating language that allows developers to write HTML with dynamic variables from the server side, and include some logic elements. The main benefit is that it has a much cleaner syntax than HTML so it is a lot easier to read and write.

- Stylus

  Stylus is an expressive CSS language that, like Pug and ES6, improves the syntax of CSS by minimising unnecessary elements. It has many other advantages over CSS, although for this project they aren't likely to be utilised as the front end design work of this project will be minimal.

- Bootstrap

  Bootstrap is a CSS and JS framework that is aimed at making responsive websites easier to write. With the addition of bootstrap to the project adapting the front end to work on mobile will be minimal. It also adds useful features for laying out the page and some attractive default CSS.

Using this boilerplate will save a huge amount of time in development as the basic framework of the web application is already laid out, so the development time can be focussed on developing software to meet the functional requirements rather than investing time in setting up a good web framework.

For more detail on how the boiler plate works, you can read the guide here: `http://blog.owen.cymru/nodejs-es6-boiler-plate/`

3.3.1 Database Import

To import the genenomic data into the database from the fasta file format, a script will be written that will use a npm module called fasta2json [36] to read the fasta files into JSON format.

From there it will use the blast results generated by the diamond script to look up the cotnig, coding sequence, and amino acid sequence (protein) for each of the blast results.

It will also compare the hit ID with the mappings file that was produced to get the ID's for the equivalent Uniprot and Candida Genome Database hits, if they are available.

That data will then be inserted into MongoDB, and an index built on the searchable fields. This index will allow for a very fast and efficient search for the key fields that will be searched for in the database, description, gene name and ID's.

```
1   { id: Schema.ObjectId
2   , hitid: String
3   , species: String
4   , name: String
5   , cgdid: String
6   , uniprot: String
7   , contig:
8     { head: String
9     , seq: String
10    }
11  , codingseq:
12    { head: String
13    , seq: String
14    }
15  , protein:
16    { head: String
17    , seq: String
18    , goids: [String]
19    , desc: String
20    }
21  , codingRange:
22    { start: Number
23    , end: Number
24    , fail: Boolean
25    }
26  }
```

Listing 3.1: The database schema that will represent the hit object

3.3.2   Overall Architecture

As the application only has one data object to model, the architecture isn't that complex. However it is still beneficial to follow good design principles which is why a traditional Model View Controller (MVC) design pattern was followed.

The MVC design pattern allows for each data object that is being modelled to have three components, a model which defines the data's structure and it's functionality, a controller that is how the application interacts with that model, and finally a view, which is what is displayed to the user and how they interact with the model. For example a user may click on a gene, which sends a request to the controller, the controller then requests that gene from the model, which in turn updates the view, that the user can then see the gene that they had selected.

Abstracting the functionality of the project out into these three components allows for the development to be a lot easier to manage and work with, than having one monolithic class to handle the entire functionality of a data object.

There will only be two routes in the application, one to the home page, which will simply list the first 100 results in the database, mainly to show the user what search results look like, before

they have searched.

The other route handles searching and viewing genes, this will be done by have a URL parameter for the gene ID in the database. If this is present then the view to display a gene in full will be displayed to the user. If it isn't present then a search must have been performed and the query string in the URL will provide the options for the search. A search will return just a few key fields for each search results and then display the results in a list.

When viewing a gene, the coding sequence will have to be highlighted inside of the contig, to do this the coding range that was determined when the data was imported will be used to select the substring.

As the coding sequences are stored in the database in only one compliment, there will be a reverse compliment button to reverse the compliment of the coding sequence. This is useful if the coding sequence is highlighted in the contig as the reverse compliment, it will allow the users to check that the data is correct.

3.3.3   User Interface

Before building the user interface for this project, I examined several other similar sites to see what information they were displaying and how it was laid out. The first was the Candida Genome Database [2] and it's "Locus" page. It lists column headings on the left, and the the infromation on the right. This design is easy to read and doesn't clutter up the page too much.
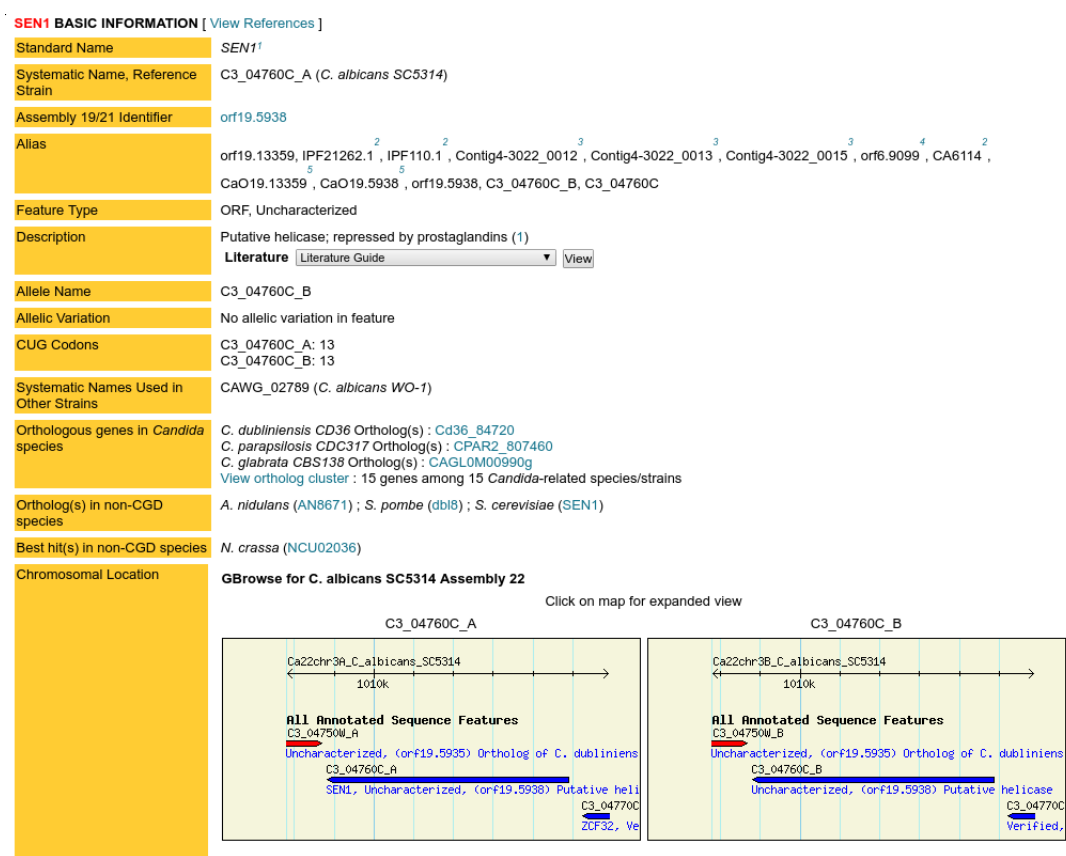
Figure 3.2: Candida Genome Databases's locus page

Next the Yeast Genome [37] project's locus page was inspected:

Figure 3.3: Yeast Genome Project's locus page

This page was very nicely formatted and laid out, it clearly had a lot of development, as it had integrations with two genome browsers as well as a large amount of extra information and graphical representations of the genes locations. Replicating this would be ideal, however it is too far out of the scope of this project, as many extra systems would have to be setup to get this data and make it as interactive.

Much like the rest of this project the UI was prototyped along with the understanding of the data at the time. This means that the UI was evolving with the data, and wasn't truly decided upon and polished until the data that was being displayed was known to be correct, and wouldn't change. Unfortunately this didn't happen until quite late in the project.
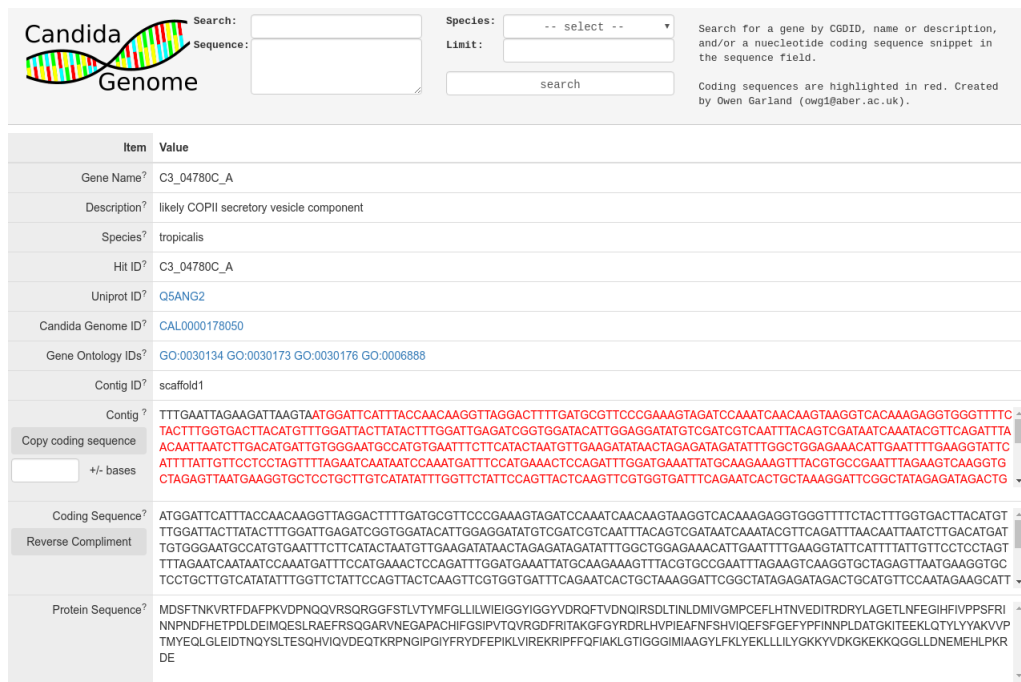
3.3.3.1   Final design

The focus for this design was to make it as simple to implement and use as possible, as this isn't a public facing service it doesn't need to be a cutting edge design aimed at grabbing users attention. Instead it just needs to be functional and represent the data clearly.

There are only two pages on the website, a search view, and a locus page for specific genes. These share the same template, and adapt based on the data provided to them. The tasks for these pages are simply to display the data in the database, and provide a form to query it.

Tooltips were used to display information about each row. As the site is only going to be used by a couple of researchers there wasn't much benefit to creating a help section or a how to guide on how to use the site, as firstly it has been changing a lot during development and secondly it is quicker to just have a direct conversation with them about areas that they might not understand. However if in the future this were to become a public service, I would create an extra information page that lists more background information about the project, the data and how the website is to be used.

The page was constructed with bootstrap's [38] grid system, which allows the content to be dynamically repositioned to fit different size displays.

Figure 3.4: The final locus page for the project

Figure 3.5: The final search page for the project

Figure 3.6: A locus page viewed on a mobile device

Figure 3.7: Tooltip on mouse hover describing what row means

3.4   Implementation

During the investigation into the data, many issues were encountered. Mainly due to a lack of understanding of what the data meant, and how it was produced. Initially the plan was to take the raw contigs for each species and use diamond to align them against the NCBI nr database. Then from those results link the data back to the Candida Genome Database via the RefSeq ID's that the NCBI nr database uses. However it was later discovered that there was an annotated set of blast data in the provided data, meaning that this step was no longer necessary.

A prototype was built around this blast data, however it was then realised that the data for *C. boidinii* was not aligned against the NCBI nr database, but rather another unknown data set. This meant that to pursue this line of prototyping I would need to either reproduce the *C. Boidinii* dataset, or reproduce all three with another pipeline, as the results from the alignments weren't consistent.

The core problem was that it was unknown what tools had been used to produce this data, it appeared that a proprietary tool blast2go [39] had been used to align and annotate the data. However after using a trial copy to try and replicate the results it was evident that this tool had not actually been used to create the alignments with the NCBI nr database.

Eventually it became clear that the alignment had just been performed with the BLAST tool, on the universities high performance cluster, with an XML format that wasn't known to have existed at the time. This meant that the data could actually be reproduced quite easily with Diamond, using the newly discovered XML output that BLAST offers.

After these revelations, and another meeting with the researchers, we discovered in the original datasets that there were in fact already annotated protein sequence files and coding sequence files.

This meant that the data was already processed and largely annotated already. This meant that it wasn't needed to generate our own data set. There was however one missing piece which was the link back to the Candida Genome Database, something that would be invaluable to the researchers who were already familiar with CGD.

Because of this, thankfully the weeks of effort spent learning about alignments and annotations weren't wasted, as I was able to produce a new alignment using Diamond. Comparing the coding sequence files, against the proteins found in *C. albicans*, the latter being provided by CGD. This data generated was then able to be used to link the coding sequences found in our data sets to the

well documented genes in *C. albicans*. In addition to this the other annotations provided by the original dataset, such as Gene Ontology ID's, are able to be enhanced by using a mapping from the Candida Genome Database to get UniprotKB ID's which offer even more information on the known genes.

Now each species had, the raw assembled contigs, the coding sequences, the amino acid sequences, the annotated protein information, a link to the Candida Genome Database, and in some cases a link to the UniprotKB database.

The next key piece of information to recover that would be of great use to the researchers was the position of the gene (coding sequence) inside the raw assembled contig. With this information they would be able to easily find the sequence of bases that surround the gene, making wet lab tests a lot easier.

To do this mock data was produced manually, that contained the correct results, and unit tests made to check if a dummy function was returning the correct results as defined in the mock data. Then an algorithm was developed to find the position of a coding sequence inside a contig.

The initial algorithm was finding around fifty percent of the genes in the dataset, which was a worry. Thankfully after some discussions with my tutor, it became apparent that the reason for this is that the coding sequences were stored "in one direction", but the alignment results were finding genes that were the reverse compliment of that. This explains why around fifty percent of the genes weren't found.

Modifying the algorithm to search for the reverse compliment of the coding sequence if it wasn't found "the first way", resulted in every gene being detected. The algorithm, simply found the index of the first twelve bases of the coding sequence in the contig, and the last twelve bases of the coding sequence in the contig. If the start or end couldn't be found it was assumed that the gene was spread across two contigs and the start or end respectively were marked to indicate that the gene was split across two contigs.

```
module.exports = function findCodingRange (codingSeq, contig, reverse) {
  let codingLength = codingSeq.length
    , start = 0
    , end = 0
    , selector = 12
    , fail = false

  start = contig.indexOf(codingSeq.substring(0, selector))
  end = contig.indexOf(codingSeq.substring(codingLength - selector, codingLength)) + selector

  if (start < 0 && end <= selector) fail = true
  if (start === -1) start = 0
  if (end <= selector) end = contig.length

  if (fail && !reverse) {
    return findCodingRange(reverseCompliment(codingSeq), contig, true)
  } else {
    return { start, end, fail }
  }

  function reverseCompliment (sequence) {
    let reverse = sequence.split('').reverse().join('')

    return reverse.replace(/[ACTG]/g, (base) => {
      return 'ACTG'.charAt('TGAC'.indexOf(base))
    })
  }
}
```

Figure 3.8: Algorithm to find coding sequence inside a contig.

3.5   Testing

Initially it was planned to develop this application in a test driven manner, as it leads to high quality code and stable solutions.

The difficulty with this approach was that when developing prototypes rapid development is key, as it allows you to try out many different solutions. If a strict TDD pattern was followed during this phase the prototyping would have been drastically slowed as the amount of development work would have doubled. Unfortunately this wasn't a worthy endeavour as time was limited and developing tests for prototype code that may never end up in the final codebase was not going to be feasible.

Another difficulty in testing this project was that the majority of the projects logic is in the seed script that imports the data into the database. Testing this would be difficult because the only way of really checking the validity of what it was producing was to look at what was in the database and to see if that made sense in a biological context.

Unfortunately it would be beyond the scope of this project to test the biological accuracy of the data that was imported. However I was able to manually verify that the data was correct at several stages, by aligning sequences from this project against other genomic databases such as the Candida Genome Database [2] and the Yeast Genome [37] project.

3.5.1   Automated Testing

For the area's of logic that were testable, a TDD design was followed, mainly for the selection of the coding sequence in the contig, as this was the only real area of logic in the project that had it's own custom algorithm to be tested. In addition to this the test suite that was being used for the project also checked the project for code formatting issues with the eslint [27] tool. This will cause the tests to fail if potentially dangerous assertions are made in the code. For example if a '==' was used instead of a '===' it will throw and error highlighting the issue to the developer.

3.5.1.1   Unit Tests

Where possible test driven development was used to produce unit tests, these were mainly written for the logic that detects the coding sequence inside the contig. This is a crucial algorithm as the sites functionality depends on this data being accurate, so it was important that the algorithm be thoroughly tested. To do this several mock database records were created that had all the different possibilities that a gene could be found in, a normal hit, a reverse compliment hit, a missing hit and a hit that was split above and below the contig.

Tests were then written to compare the result of the finding function against the correct values stored in the mock data. You can see this in the file 'find-coding-range.test.js' where the tests are located. The function was then able to be developed ensuring that the data it was returning was correct.

3.5.2 User Interface Testing

The user interface hasn't had automated tests written for it unfortunately, as there was only one HTML page and only two sets of data that were being returned it didn't feel particularly necessary to write tests to check that the data was coming through correctly.

That being said the UI has been manually tested on Chrome, Firefox and Internet Explorer to check for any functional issues. None were found, however it was noted that in Internet Explorer there was some font rendering issues, but this isn't a concern as it doesn't impact the functionality of the site. I will be recommending that the site is used on Chrome though, as that it was it was developed on, so is the most thoroughly tested.

Once the site was at a stage where it could be demonstrated the UI was shown to the researchers and they were asked to provide any feedback that might make the site easier to use for them. This feedback was.... yaydadada .... because of this feedback xyz was done to make it abc for them to use.

3.5.3 Stress Testing

As this site is hosting commercially sensitive data, it won't be publicly accessible, this means that only the researchers who are working on this data will be accessing it. Because of this there isn't a need to perform any stress testing on the service, as the stack is more than capable of handling ¡ 10 users at a time, and isn't vulnerable to public attacks.

3.5.4 Integration Testing

As listed in the build process section 3.2.4, continuous integration services were used throughout this project, meaning that every time a new build was pushed to github, it had to pass tests on the CI server before being deployed to the production server. This has ensured that any modifications to the code base won't break the core functionality of the production build.

3.5.5 User Testing

Once the site has been developed to a stage where the users can get a real sense of how it is looking and how it will function, they have been invited to suggest changes that need to be made, in line with the agile practices that this project has been developed with.

User testing was invaluable as the researchers were able to spot mistakes in the biological data that were invisible to the developer. An instance of this is when they noted that one of the UniprotID's was linking to a gene found in the Flu virus, something that shouldn't be present at all in the yeast genome. After investigating it was found that some of the UniprotID's has been truncated which caused this anomaly. Writing tests for these kinds of checks would have been nigh on impossible, thankfully user tests were able to pick up these kinds of issues.

Chapter 4

Results and Conclusions

I can't really do this section.

Maybe talk about deployment to the Uni servers, and how the researchers will use it?

Chapter 5

Evaluation

- Were the requirements correctly identified?

  Part of the difficulty of this project was going through several iterations of understandings of the data, and how it needed to be interpreted. This meant that the requirements only really became clear very late in the project. However once they were apparent, development from then on was very straight forward.

- Were the design decisions correct?

  The project is functional, and hasn't suffered any bugs, and meets all of the achievable criteria so I believe the design decisions were correct.

- Could a more suitable set of tools have been chosen?

  The use of NodeJS and MongoDB does set this project apart from older similar projects, that use Perl and PostgreSQL. However there is a clear shift in the technologies used for web development, as Perl is on the decline [?], and newer technologies like Node JS are taking it's place. The advantages presented to a web developer of NodeJS are clear and outlined in the design choices section.

- How well did the software meet the needs of those who were expecting to use it?

  Hopefully will find out on Friday!

- If you were starting again, what would you do differently?

  Try to talk to the source of the data sooner and ideally schedule a session with the researchers to look over the data and hopefully unpick what it all meant a lot sooner than we did.

I'm very pleased with the end result of the project, it is a nicely developed clean piece of software that meets it's basic functional requirements. The area that really could have been improved upon was the amount of time it took to get to grips with the data. If I had had the understanding of it much earlier in the project, there could have been more features developed that would have helped the researchers a lot more than what I was able to produce. One area that I would have loved to be able to explore was creating a comparison tool, which would allow them to compare two or more genes directly.

This would have added a lot more complexity to the project, but considering how smoothly the development of the application went, once the data was understood correctly, I think there

would have been plenty of time. The actual development of the database and website did only take around a week, so there was a lot more room for new features, had the data been understood earlier.

The application was built with very robust software engineering principles, and the code is a testament to how cleanly it was developed, there are very few code smells [40] throughout the code. This is mostly thanks to the discipline enforced by having linters and automated testing. The only area that falls down in this regard is the amount of unit tests. As the project stands there are only a handful of unit tests to check the finding of coding sequences. Ideally it would be good to expand the testing to cover the Pug templates, the controller logic, and the search functionality.

Unfortunately by the time the data was understood enough to develop the application fully, there was not enough time to test these features and write up this report.

In the future this will be deployed to the university servers, and I will offer to help maintain and develop new features for the researchers if they need it, as I am keen for their project to go well, which what provided the motivation for this project to be a success, which overall I feel that it has been.

Appendices

Appendix A

Third-Party Code and Libraries

1.1   Nodestack

- https://github.com/bag-man/nodestack

This project uses boilerplate code developed by myself for previous projects. The code sets up a basic Node JS project, with access to to third party services. During the development of this project some features were merged back into the boilerplate, such as the mongoose integration. This boilerplate makes use of several third party libraries, which are all listed in the package.json. Some extra modules have also been added specific to this project such as fasta2json and the clipboard module.

1.2   package.json

Below is the modules listed in the package.json for the project, all third party software is listed here along with it's version. This is how node modules are packaged, this automates the management of all modules, and locks the versions. For this project I was using yarn [**?**] to manage the packages rather than the default node package manager (npm) [**?**]. Yarn has a couple of advantages, mainly that it is a lot faster than npm3 and that it automatically creates a lock file for modules and their dependencies.

```
1    "devDependencies": {
2      "codecov": "^1.0.1",
3      "eslint": "^2.3.0",
4      "eslint-config-clock": "^1.2.0",
5      "eslint-config-standard": "^5.1.0",
6      "eslint-plugin-promise": "^3.3.0",
7      "eslint-plugin-standard": "^1.3.1",
8      "husky": "^0.13.2",
9      "istanbul": "^1.0.0-alpha.2",
10     "mocha": "^3.1.2",
11     "nodemon": "^1.11.0"
12   },
13   "dependencies": {
14     "babel": "^6.5.2",
15     "babel-core": "^6.18.0",
```

```
16      "babel-loader": "^6.2.5",
17      "babel-preset-es2015": "^6.18.0",
18      "clipboard": "^1.6.1",
19      "express": "^4.14.0",
20      "fasta2json": "^0.1.1",
21      "mongoose": "^4.8.6",
22      "morgan": "^1.7.0",
23      "path": "^0.12.7",
24      "pug": "^2.0.0-beta11",
25      "stylus": "^0.54.5",
26      "webpack": "^2.2.1"
27    }
```

1.2.1   Development Dependencies

This section of third party modules are not installed on the production builds of the application, they are only for use when developing the application, they don't change the functionality of it at all, just make it easier to work on.

1.2.1.1   codecov

This provides integration with codecov's services, to provide interactive test coverage information.

- https://www.npmjs.com/package/codecov @ 1.0.1

1.2.1.2   eslint

This is the linting engine that is used to check the source code for mistakes.

- https://www.npmjs.com/package/eslint @ 2.3.0

1.2.1.3   eslint-config-clock

This is a set of configurations for eslint that describe the code formatting that is preferred for this project.

- https://www.npmjs.com/package/eslint-config-clock 1.2.0

1.2.1.4   eslint-config-standard

The clock config is built on top of this standard configuration

- https://www.npmjs.com/package/eslint-config-standard @ 5.1.0

1.2.1.5   eslint-plugin-promise

Eslint didn't support promises natively at the time of writing, so this was used to detect promises in the code.

- https://www.npmjs.com/package/eslint-plugin-promise @ 3.3.0

1.2.1.6   eslint-plugin-standard

Add's a few extra rules to eslints configuration options.

- https://www.npmjs.com/package/eslint-plugin-standard: 1.3.1

1.2.1.7   husky

Simply runs the test suite before code is pushed to remote repositories.

- https://www.npmjs.com/package/husky @ 0.13.2

1.2.1.8   istanbul

Provides coverage information at the end of the test suite, and for codecov's usage.

- https://www.npmjs.com/package/istanbul @ 1.0.0-alpha.2

1.2.1.9   mocha

Testing framework for javascript.

- https://www.npmjs.com/package/mocha @ 3.1.2

1.2.1.10   nodemon

Monitors source files for changes, and relaunches the application when files are changed.

- https://www.npmjs.com/package/nodemon @ 1.11.0

1.2.2   Build Dependencies

These third party modules are the frameworks libraries and modules that are actually used to by the application to function.

1.2.2.1   babel

Transpiler for javascript. Used for converting ES6 code into ES5 for browser compatibility.

- https://www.npmjs.com/package/babel @ 6.5.2

1.2.2.2   babel-core

Core configurations for babel.

- https://www.npmjs.com/package/babel-core @ 6.18.0

1.2.2.3   babel-loader

Allows for transpiling to be done from webpack build manager.

- https://www.npmjs.com/package/babel-loader @ 6.2.5

1.2.2.4   babel-preset-es2015

The standard ES5 configuration.

- https://www.npmjs.com/package/babel-preset-es2015 @ 6.18.0

1.2.2.5   clipboard

Clipboard module used for copying text to a users system clipboard from the browser.

- https://www.npmjs.com/package/clipboard @ 1.6.1

1.2.2.6   express

The web framework that powers the node application.

- https://www.npmjs.com/package/express @ 4.14.0

1.2.2.7   fasta2json

Module that reads fasta files into JSON objects.

- https://www.npmjs.com/package/fasta2json @ 0.1.1

1.2.2.8   mongoose

MongoDB object modelling framework.

- https://www.npmjs.com/package/mongoose @ 4.8.6

1.2.2.9   morgan

Cleaner and clearer logging output.

- https://www.npmjs.com/package/morgan @ 1.7.0

1.2.2.10   pug

HTML templating language.

- https://www.npmjs.com/package/pug @ 2.0.0-beta11

1.2.2.11   stylus

CSS templating language.

- https://www.npmjs.com/package/stylus @ 0.54.5

1.2.2.12   webpack

Javascript build manager.

- https://www.npmjs.com/package/webpack @ 2.2.1

Appendix B

Ethics Submission

Appendix C

Code Examples

Annotated Bibliography

[1] Unkown, "Data displaying number of packages available to different platforms," https: //modulecounts.com, 2017, accessed April 29th 2017.

[2] M. B. Arnaud, M. C. Costanzo, M. S. Skrzypek, G. Binkley, C. Lane, S. R. Miyasato, and G. Sherlock, "The candida genome database (cgd), a community resource for candida albicans gene and protein information," *Nucleic acids research*, vol. 33, no. suppl 1, pp. D358– D363, 2005.

[3] P. Zhou, D. Emmert, and P. Zhang, "Using chado to store genome annotation data," *Current Protocols in Bioinformatics*, pp. 9–6, 2006.

[4] L. S. Gramates, S. J. Marygold, G. dos Santos, J.-M. Urbano, G. Antonazzo, B. B. Matthews, A. J. Rey, C. J. Tabone, M. A. Crosby, D. B. Emmert, *et al.*, "Flybase at 25: looking to the future," *Nucleic Acids Research*, p. gkw1016, 2016.

[5] B. PostgreSQL, "Postgresql," *Web resource: http://www. PostgreSQL. org/about*, 2010.

[6] L. Wall *et al.*, "The perl programming language," 1994.

[7] M. F. Sanner *et al.*, "Python: a programming language for software integration and development," *J Mol Graph Model*, vol. 17, no. 1, pp. 57–61, 1999.

[8] S. Tilkov and S. Vinoski, "Node. js: Using javascript to build high-performance network programs," *IEEE Internet Computing*, vol. 14, no. 6, pp. 80–83, 2010.

[9] S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, and D. J. Lipman, "Gapped blast and psi-blast: a new generation of protein database search programs," *Nucleic acids research*, vol. 25, no. 17, pp. 3389–3402, 1997.

[10] B. Buchfink, C. Xie, and D. H. Huson, "Fast and sensitive protein alignment using diamond," *Nature methods*, vol. 12, no. 1, pp. 59–60, 2015.

[11] S. T. Sherry, M.-H. Ward, M. Kholodov, J. Baker, L. Phan, E. M. Smigielski, and K. Sirotkin, "dbsnp: the ncbi database of genetic variation," *Nucleic acids research*, vol. 29, no. 1, pp. 308–311, 2001.

[12] K. D. Pruitt, T. Tatusova, and D. R. Maglott, "Ncbi reference sequences (refseq): a curated non-redundant sequence database of genomes, transcripts and proteins," *Nucleic acids research*, vol. 35, no. suppl 1, pp. D61–D65, 2007.

[13] P. Klus, S. Lam, D. Lyberg, M. S. Cheung, G. Pullan, I. McFarlane, G. S. Yeo, and B. Y. Lam, "Barracuda-a fast short read sequence aligner using graphics processing units," *BMC research notes*, vol. 5, no. 1, p. 27, 2012.

[14] M. Yano, H. Mori, Y. Akiyama, T. Yamada, and K. Kurokawa, "Clast: Cuda implemented large-scale alignment search tool," *BMC bioinformatics*, vol. 15, no. 1, p. 406, 2014.

[15] Y. Liu, D. L. Maskell, and B. Schmidt, "Cudasw++: optimizing smith-waterman sequence database searches for cuda-enabled graphics processing units," *BMC research notes*, vol. 2, no. 1, p. 73, 2009.

[16] Various, "Biostars - a discussion on splitting a fasta file, on the biostars forum," https://www.biostars.org/p/13270/, 2012, accessed April 2017.

[17] G. Gremme, S. Steinbiss, and S. Kurtz, "Genometools: a comprehensive software library for efficient processing of structured genome annotations," *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, vol. 10, no. 3, pp. 645–656, 2013.

[18] M. J. Donlin, "Using the generic genome browser (gbrowse)," *Current Protocols in Bioinformatics*, pp. 9–9, 2009.

[19] M. E. Skinner, A. V. Uzilov, L. D. Stein, C. J. Mungall, and I. H. Holmes, "Jbrowse: a next-generation genome browser," *Genome research*, vol. 19, no. 9, pp. 1630–1638, 2009.

[20] Various, "Usage statistics and market share of perl for websites," https://w3techs.com/technologies/details/pl-perl/all/all, 2017, accessed April 29th 2017.

[21] R. N. Smith, J. Aleksic, D. Butano, A. Carr, S. Contrino, F. Hu, M. Lyne, R. Lyne, A. Kalderimis, K. Rutherford, *et al.*, "Intermine: a flexible data warehouse system for the integration and analysis of heterogeneous biological data," *Bioinformatics*, vol. 28, no. 23, pp. 3163–3165, 2012.

[22] C. G. Database, "Annotated proteins found in *Candida albicans*," http://www.candidagenome.org/download/sequence/C_albicans_SC5314/Assembly22/current/C_albicans_SC5314_A22_current_default_protein.fasta.gz, 2017, accessed April 29th 2017.

[23] ——, "All annotations and id's for every protein in cgd," http://www.candidagenome.org/download/go/gene_association.cgd.gz, 2017, accessed April 29th 2017.

[24] M. Ashburner, C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, *et al.*, "Gene ontology: tool for the unification of biology," *Nature genetics*, vol. 25, no. 1, pp. 25–29, 2000.

[25] O. Garland, "Vim configuration," https://github.com/bag-man/dotfiles/blob/master/vimrc, 2017, accessed April 29th 2017.

[26] Various, "Github remote git repository," https://github.com/about, 2017, accessed April 29th 2017.

[27] ——, "Husky," https://github.com/typicode/husky, 2017.

[28] ——, "Eslint," https://github.com/eslint/eslint, 2017.

[29] ——, "Mocha," https://github.com/mochajs/mocha, 2017.

[30] ——, "Mocha," https://github.com/gotwarlost/istanbul, 2017.

[31] ——, "Travis ci documentation," https://docs.travis-ci.com/, 2017, accessed April 29th 2017.

[32] ——, "Codecov documentation," https://docs.codecov.io/, 2017, accessed April 29th 2017.

[33] N. Middleton and R. Schneeman, *Heroku: Up and Running*. " O'Reilly Media, Inc.", 2013.

[34] I. K. Chaniotis, K.-I. D. Kyriakou, and N. D. Tselikas, "Is node.js a viable option for building modern web applications? a performance evaluation study," *Computing*, vol. 97, no. 10, pp. 1023–1044, 2015. [Online]. Available: http://dx.doi.org/10.1007/s00607-014-0394-9

[35] O. Garland, "Nodestack," https://github.com/bag-man/nodestack, 2017.

[36] Various, "Nodejs homepage," https://nodejs.org/, 2017, accessed April 29th 2017.

[37] J. Juanes, "fasta2json," https://github.com/jmjuanes/fasta2json, 2017.

[38] J. M. Cherry, E. L. Hong, C. Amundsen, R. Balakrishnan, G. Binkley, E. T. Chan, K. R. Christie, M. C. Costanzo, S. S. Dwight, S. R. Engel, *et al.*, "Saccharomyces genome database: the genomics resource of budding yeast," *Nucleic acids research*, p. gkr1029, 2011.

[39] Various, "Bootstrap," https://github.com/twbs/bootstrap, 2017.

[40] A. Conesa, S. Götz, J. M. García-Gómez, J. Terol, M. Talón, and M. Robles, "Blast2go: a universal tool for annotation, visualization and analysis in functional genomics research," *Bioinformatics*, vol. 21, no. 18, pp. 3674–3676, 2005.

[41] M. Mantyla, J. Vanhanen, and C. Lassenius, "A taxonomy and an initial empirical study of bad smells in code," in *Software Maintenance, 2003. ICSM 2003. Proceedings. International Conference on*. IEEE, 2003, pp. 381–384.