



Genome pattern matching using regular expressions

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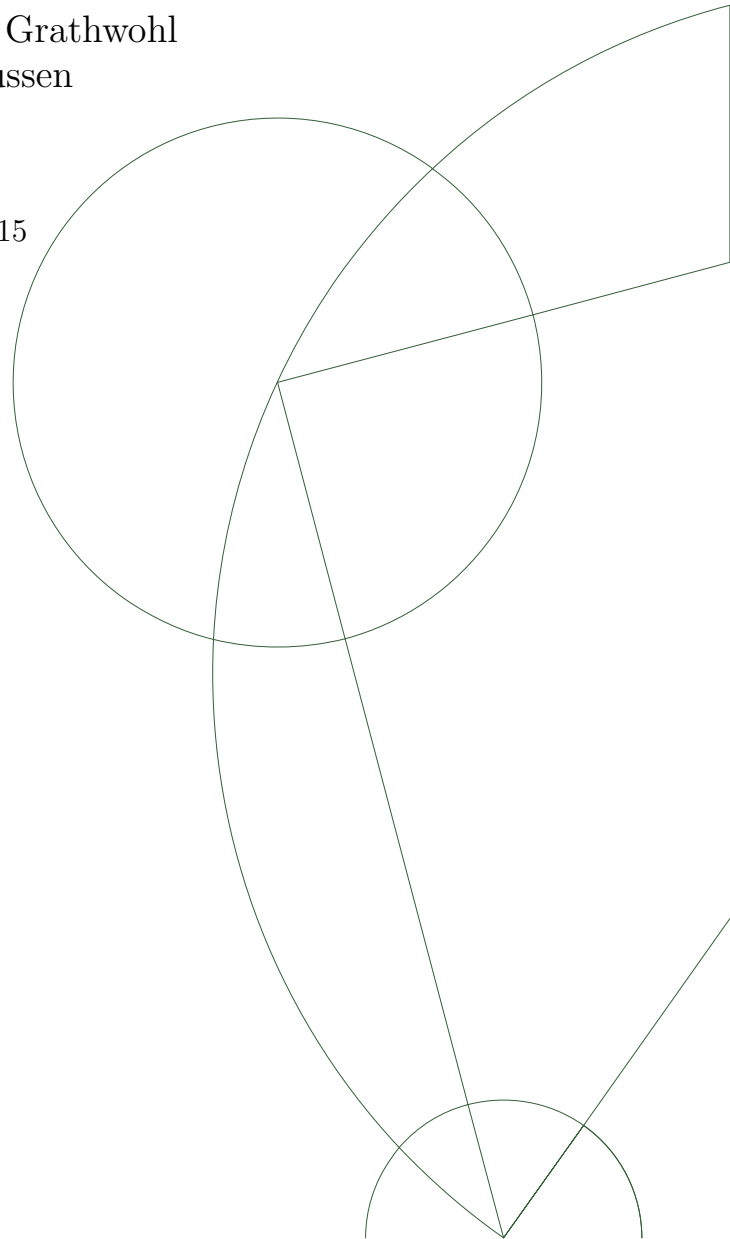
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May 31, 2015



Abstract

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1 Motivation

When the human genome project (a project which had the goal of sequencing all 22 chromosomes of the human genome) was launched in 1990, the project was budgeted to cost 3 billion dollars and was estimated to take fifteen years to complete. However as technology progressed, the project managed to complete its goal two years earlier than expected, in 2003. This was made possible because of the rapid advancements in genome sequencing, and the advancement hasn't stopped since. This has led to decreasing costs of sequencing RNA and DNA, meaning biologists have access to greater amounts of data than ever before. However the technology to process these amounts of data haven't progressed at the same pace as sequencing has. A tool for this is `scan_for_matches`, a pattern-matching tool that searches through text files to match a pattern specified by a user. While `scan_for_matches` has proven to be a fast and reliable tool, because of the amount of data it shifts through, a faster alternative is desired.

After hearing about this problem, we thought that there must be a better way of searching through text that is also theoretically sound. Our first thought was using automata-based searching methods, since this provides a calculable best- and worst-case run time while being theoretically sound. Since regular expressions use an automata-based way of searching, we hypothesized that implementing regular expressions that would have the same functions as `scan_for_matches` would lead to faster run times.

2 Problem Analysis

The functionality of `scan_for_matches` dictates what our solution to the problem must be able to do. While a more in-depth analysis of the functionality of `scan_for_matches` can be found in section 7, the requirements for our solution are as follows:

1. Read a text file,
2. Match
 - (a) with errors allowed,
 - (b) a previously found match,
 - (c) a modified pattern, and
3. Return matches with their position

While some of the functionality (items 1 and 3) will be trivial to implement since they are standard functions of most programming languages, there are some challenges to be found in regards of what we must match. Matching with errors allowed are not supported natively in regular expressions, and matching a modified text, which may first be determined at runtime, will be challenging to implement with automata.

3 State-of-the-Art

3.1 RE2

RE2 is Google's regular expression engine written in C++. It supports backtracking, but not backreferencing since backreferencing can't be implemented efficiently according to [1]. Since RE2 doesn't support matching with errors or backreferencing, it would be unable to properly reproduce `scan_for_matches`' functionality.

3.2 TRE

TRE was created by Ville Laurikari for his master's thesis in 2001, and is a regular expression engine supporting backtracking, backreferencing and matching with errors. Because of this, TRE is the best candidate for modification in order to make it work with patterns. We expand on this in section 8.

4 Nucleic Acids

Nucleic acids are one of the building blocks of life, and are composed of a backbone made of a type of sugar, with bases that can bond with one another. A series of these bases on a backbone is called a sequence. The type of sugar as well as the bases depends on the nucleic acid.

4.1 Ribonucleic acid

Ribonucleic acid (RNA) is a large molecule composed of nitrogenous bases nested on a ribose-phosphate backbone. The possible nitrogenous bases, or bases for short, that can be nested on the backbone are guanine (G), adenine (A), uracil (U) and cytosine (C). In nature, the predominant form of RNA are as a singlestranded chain that can fold in on itself, bundled with other chains to form a structure. This flexibility of the backbone that allows for the chain to fold in on itself is possible because the RNA uses a sugar called ribose in its backbone, which is unstable compared to its other form, deoxyribose, used in deoxyribonucleic acid (DNA), but is more flexible, allowing the RNA chain to bend in ways that DNA can not.

The bases found in RNA can form hydrogen bonds with each other, though not all bases can form bonds with each other. Bases that can bond with each other are guanine with cytosine, and adenine with uracil. These bonds are complementary of each other, and forms the structure of each RNA. When two bases bond with each other, they will stick to each other which changes the form of the RNA chain. However sometimes a base will have no complementary base to bond with, causing the base to stick out, giving rise to certain characteristic forms. This will be elaborated on later.

4.2 Deoxyribonucleic acid

Deoxyribonucleic acid (DNA) is a large molecule composed of nitrogenous bases nested on a deoxyribose-phosphate. DNA is mostly found in nature as helixes, where two strands has bonded. Similarly to RNA, DNA has four nitrogenous bases, and shares three of the four that RNA have, (guanine, adenine and cytosine). However instead of uracil, the fourth base is thymine (T).

4.3 Secondary Structures of Nucleic Acids

The secondary structure of a nucleic acid describes how the bases of the nucleic acid has bonded. The secondary structure of nucleic acids can change if the nucleic acid is damaged or has mutated, causing it to gain or lose bases. When two bases bonds they hold onto each other, causing bases that have no complementary base to bond with to stick out. Below are examples of three common secondary structures as well as a brief analysis on how to identify them.

Bulge

A bulge occurs when one or more bases have no base to bond with and these bases are surrounded by bases that have bonded. This causes the bases to get pushed out slightly, resembling a bulging growth. This type of structure occurs when one or more bases has been inserted or deleted, since if a base has been inserted then it will have no base to bond with, and if a base has been deleted then the previously-bonded base will have no base to bond with. Figure 1 shows a bulge.

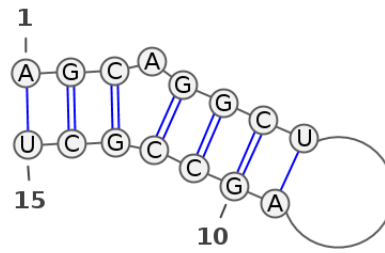


Figure 1: The RNA sequence AGCAGGCUAGCCGCU. Note the bulging A.

Interior Loop

An interior loop is when two or more opposing bases aren't complementary and can't bond, causing them both to bulge. This occurs when one or more consecutive bases mutate to another base. Figure 2 shows an interior loop.

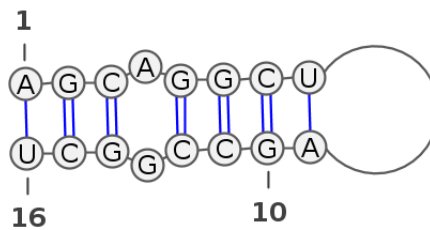


Figure 2: The RNA sequence AGCAGGCUAGCCGGCU. Note the bulging A and G creating a loop inside the bonded strand.

These interior loops vary in size, and can have differing amount of bases on either side of the strands, making them hard to generalize.

Stem Loop

A stem loop, also known as a hairpin loop, occurs when a strand bonds with itself, but leaves a sequence of bases sticking out that doesn't bond with anything. This kind of loop occurs typically in RNA as they are single-stranded, but may happen in DNA if the two strands of the DNA has been separated. Figure 3 shows a stem loop.

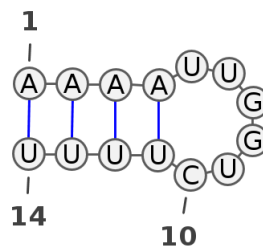


Figure 3: A stem loop of the RNA sequence AAAAAUUGGUCUUUU.

An important thing to take note of is how the sequence can be seen as one long strand that starts from the adenine bases that binds with the uracil bases, loops around without binding to anything and finally become the uracil bases that the adenine bases from the start binds with. This means that the stem loop can be written as one continuous sequence of bases; **AAAAUUGGUCUUUU**. Since we can define a stem loop we can, with the right tools, search through a file documenting the bases of a nucleic acid and find all stem loops the nucleic acid has.

5 Regular expressions

To explain what a regular expression is, we must first introduce languages and alphabets. All literals will be written using the typewriter font, to distinguish between literals and other text.

Definition 1. An alphabet Σ is a finite non-empty set of letters.

Definition 2. A language L is a subset of all strings formed over Σ : $L \subseteq \Sigma^*$

Example 5.1. If we have a DNA sequence string, the alphabet Σ consists of the literals $\{\mathbf{t}, \mathbf{g}, \mathbf{c}, \mathbf{a}\}$. and the language contains strings formed by the literals from this alphabet. For example "gtcaaa" or "gtcaaat".

Definition 3. A regular expression is described by the following grammar:

$$E ::= a|0|1|E_1 + E_2|E_1E_2|E^*$$

where E_1 and E_2 are RE's and $a \in \Sigma$

Definition 4. The language interpretation $L(E)$ of a regular expression is:

$$\begin{aligned} L(0) &= \emptyset \\ L(1) &= \{\epsilon\} \\ L(\mathbf{a}) &= \{\mathbf{a}\} \\ L(E_0 + E_1) &= L(E_0) \cup L(E_1) \\ L(E_1E_2) &= \{w_1w_2 | w_1 \in L(E_1), w_2 \in L(E_2)\} = L(E_1)L(E_2) \\ L(E^*) &= \bigcup_{n=0}^{\infty} L(E)^n \\ L(E)^0 &= \{\epsilon\} \\ L(E)^n &= \underbrace{L(E)L(E)\dots L(E)}_{n \text{ times}}. \end{aligned}$$

[2, p.5 def. 3]

With definition 4, we can now form regular languages.

Example 5.2. Natural numbers can be described as a regular expression. Natural numbers have the alphabet $\Sigma = \{0, 1, 2, 3, 4, 5, 6, 7, 8, 9\}$, the regular expression for natural numbers would look like:

$$E_{nat} = (1+2+3+4+5+6+7+8+9)(0+1+2+3+4+5+6+7+8+9)^*$$

Example 5.3. Given the alphabet $\Sigma = \{\mathbf{a}, \mathbf{b}, \mathbf{c}\}$. With this alphabet, one could form a regular expression that could look like $E = \mathbf{c}(\mathbf{a}+\mathbf{b})^*$. The language L that is produced from this expression, would consist of strings that is a c followed by 0 or more a or b s for example "caaaa", "cbb", "cababba" and so forth. The language interpretation of E is

$$\begin{aligned}
L(E) &= L(c(a+b)^*) \\
&= \{w_1 w_2 | w_1 \in L(c), w_2 \in L((a+b)^*)\} \\
&= \{w_1 w_2 | w_1 \in c, w_2 \in \bigcup_{n=0}^{\infty} L(a+b)^n\} \\
&= \{w_1 w_2 | w_1 \in c, w_2 \in \bigcup_{n=0}^{\infty} (L(a) \cup L(b))^n\} \\
&= \{w_1 w_2 | w_1 \in c, w_2 \in \bigcup_{n=0}^{\infty} (a \cup L(b))^n\} \\
&= \{w_1 w_2 | w_1 \in c, w_2 \in \bigcup_{n=0}^{\infty} (a \cup b)^n\} \\
&= c \bigcup_{n=0}^{\infty} (a \cup b)^n
\end{aligned}$$

6 Nondeterministic Finite Automaton

Definition 5. An nondeterministic finite automaton (NFA) is a 5-tuple $(Q, \Sigma, \Delta, q^s, q^a)$. Where Q is a finite set of states, Σ is the input alphabet, the initial state $q^s \in Q$, the accepting state $q^a \in Q$ and Δ that is a set of 3 tuples containing all the transitions in Q , with the type of $\Delta \subseteq Q \times (\Sigma \cup \{\epsilon\}) \times Q$.

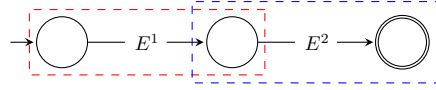
6.1 Conversion from RE to NFA

Each expression can be converted to an NFA, and vice versa. Table 1 shows how each RE can be converted into a NFA. The states in Q is represented as circles. The initial state q^s is shown by adding a small arrow pointing to it, and the accepting state q^a is shown as a double circle. Transitions in Δ is represented as $(q, a, q') \in \Delta$ or $(q, \epsilon, q') \in \Delta$, we write these transitions as $q \xrightarrow{a} q'$ or $q \xrightarrow{\epsilon} q'$. For subexpressions we use boxes and split the transition arrow, marking it with an E , to denote the NFA resulted from converting the expression.

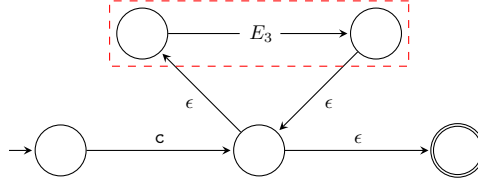
Table 1: Translation table from regular expressions to NFA

0	
1	
a	
$E^1 E^2$	
$E^1 + E^2$	
E^*	

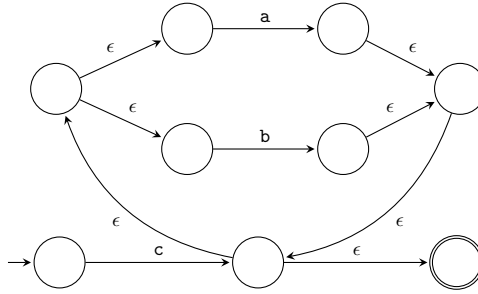
Example 6.1. In example ??, the expression $c(a + b)^*$ is used. The NFA buildup is done like the language interpretation in the example. We first construct the $E_1 E_2$ expression, where $E_1 = c$ and $E_2 = (a + b)^*$.



Now we construct the subexpressions, E_1 and E_2 . E_2 results in a new subexpression $E_3 = a + b$.



The last part is constructing E_3 , we skip ahead and construct the literals **a** and **b** as well.



7 Scan_For_Matches

((TODO: UNCHANGED))

Scan_for_matches is a string-searching tool created by Ross Overbeek, David Joerg and Morgan Price in C which searches through text files. Users specify what they wish to search for by defining a pattern, and scan_for_matches returns all matches that corresponds to the specified pattern.

Definition 6. Let E be any pattern that's in the alphabet Σ as defined in definition 7. Let 0 be the empty string. Let A be a string that we are processing to see if the pattern is valid. A pattern may then be constructed as such:

Definition 7. Let Σ denote an alphabet. Then we can define a pattern as follows:

h	Match the sequence h , where $h \in \Sigma$
$n \dots m$	Match n to m characters where $n \leq m$
$x=n \dots m$	Match n to m characters, and call the sequence x
$x \mid y$	Match either x or y
$x[n,m,l]$	Match x , allowing for n mismatches, m deletions and insertions where $n,m,l \geq 0$
$\text{length}(x+y) < n$	The length of patterns $x+y < n$ where $n > 0$
$z=\{hl, lh\}$	Create a pattern rule where h is the complement of l , and l is the complement of h , where $h \in \Sigma$, $l \in \Sigma$, and call it z
$<x$	Match the reverse of pattern x
$\sim x$	Match the reverse complement of pattern x using the G-C, C-G, A-T and T-A pairing rule
$z \sim x$	Match the reverse complement of pattern x using pattern rule z
$\sim x$	Match only pattern x if it is at the start of a string
$x \$$	Match only pattern x if it is at the end of a string

Definition 6 states that a pattern may be any combination of the alphabet defined in definition 7. Using these patterns, it is possible to make very specific or very broad searches in a text file.

Example 7.1. Say we want to write a pattern that finds the sequence GUUC, allowing one mismatch, followed by a random sequence which has a length between 3 and 5, followed by the reverse complement of the first sequence that we found. We can then write this as

$$p1=GUUC[1,0,0] \ 3 \dots 5 \ \sim p1$$

Example 7.1 will match a stem loop as described in section 4.3. Note that if we wanted to find all stem loops in a file where the bonded bases are of length 4, we would replace GUUC[1,0,0] with an arbitrary sequence of characters by writing $p1=4 \dots 4 \ 3 \dots 5 \ \sim p1$.

8 TRE

Recall that in section 2, we determined that the challenge of our solution would lie in matching

1. with errors allowed,
2. a previously found match, and
3. a modified pattern.

An implementation based on TRE would address two of the three previously mentioned problems. Item 1 would require approximate matching support, and TRE fully supports approximate matching. Item 2 could be resolved with backreferences (even if backreferences are computationally inefficient) and TRE also supports this. Item 3 means that sometimes we want to match a modification of a pattern found previously - like the reverse complement of a pattern. This would have to be implemented in TRE's parser (to denote a symbol for the modified patterns, e.g. the reverse or the complement of a pattern) as well as in TRE's basic functionality.

When analysing TRE to see how it worked so we could start modifying the program, we discovered that TRE would define every newline as a delimiter. The delimiter specifies how the data should be broken up, so for every new line the current line would be loaded into the buffer and be processed. This in itself would not be a problem if not because TRE would discard any match that was currently being processed when it reached a delimiter, causing matches that wrapped around two lines to be discarded. The fix to this was easy to make however; if a wrapper was created which would feed the text data to TRE, ignoring all newlines, then TRE would load the entire file into its buffer and would no longer cause it to discard potential matches that continued to the next line.

When we then tried to run a file through TRE which had no newlines, we discovered another feature of TRE which would not work for our project; TRE would only match one match per delimiter - the earliest, best-matching match (where a best-matching match is a match with the least amount of errors). Since we had to trim the text files for TRE so there were no newlines, TRE would only return one match. TRE was built around this feature, which has lead to the following design choices;

- the program runs through the data once to determine how many errors the best-matching match has, and then runs through the text file again, stopping when the best-matching match has been found,
- TRE ignores any matches that are not best-matching, meaning there's no way for TRE to identify and output acceptable matches.

This coupled with little documentation of how the code works meant that it would take a long time to properly analyse TRE in order to find out how we could modify it to suit our project. At this point we decided that creating our own solution would be less time-consuming, while also allowing us to design our solution ourselves.

9 Our Implementation

We constructed a simple program, which would take a regular expression and use the methods description in section 6.1 in to construct a modified NFA structure and use it search for matches using a given pattern.

Our implementation supports a series of regular expression symbols, including $+$, $*$, $|$, $?$ along with concatenation of characters. This allows for construction of simple NFA's from regular expressions, for example the regular expression "(GAT)+" would produce a structure as shown in figure 4

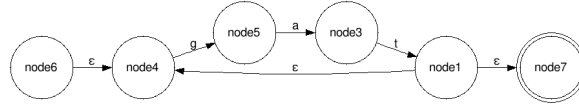


Figure 4: Example of how our implementation builds NFA got regular expression (GAT)+

Each node in the figure has a number corresponding to the time it was created in the code, examining figure 4 we can see that the NFA part having GAT was constructed first, and then the surrounding nodes responsible for the $+$ were added onto that, much like one would expect from the description in section 6.1.

When a NFA is constructed, our implementation will try each possible option when matching, meaning that if there's an epsilon transition it will take it, as well as any other available path. If it cannot match a character directly, but it has the option to handle an insertion or mutation at once, it will take both of them, causing the unmatched state to spawn two new states. This way we can guarantee that we will find every possible match for a given pattern. However it also causes an increased runtime given an increase in mismatches.

One option for optimization could be to make the transition from NFA to Deterministic finite automaton or DFA, which we will not describe in detail here. But as an argument against this, our implementation was made to primarily focus on matching straightforward RNA sequences with insertions, mutations and deletions, which would result in simple linear NFA's, which little to no epsilon transitions.

10 Experimental Results & Tests

Using our implementation, TRE and scan_for_matches, we were able to do some benchmarking in order to compare performances.

For this a virtual machine is created, using Oracle VirtualBox¹. The machine running the virtualbox is running Windows 8.1 Pro x64, with 8,00 GB RAM, an AMD FX 4300 Quad-Core Processor 3.80 GHz, of which 1 core and 4096MB RAM was given to the virtual machine, which would run Ubuntu 14.04.2 LTS 64 bit.

For the benchmarking some data was required:

Figure 5 shows a series of fasta files, these files include nucleotide sequences, which all differ in size, decreasing from chr1 to chr22. These fasta files are the kind of data which scan_for_matches are expected to run, and thus excellent for benchmark testing, it's worth noting however, that each of these files are 50 characters long, as TRE matches searches through lines separately, and longer or shorter line sizes might affect the performance of TRE, this was not tested.

For testing, a suitable pattern is required. As our implementation has primarily been focused on supporting insertion, deletion and mutation on a sequence, a simple RNA sequence *TGCAAGCGTTAAT* with variable insertions is chosen as the search pattern.

¹virtualbox.org

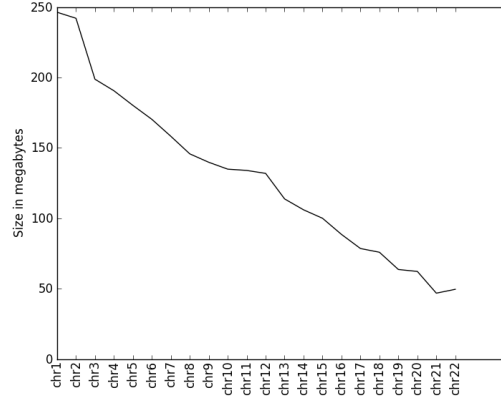


Figure 5: Plot of sample files used for benchmarking, these files range from 246.3 Megabytes to 46.8 Megabytes in size

Each test would be executed on each of the mentioned fasta files a total of 10 times, given an average runtime which was used in the following results.

First test using said sequence with 1 insertions:

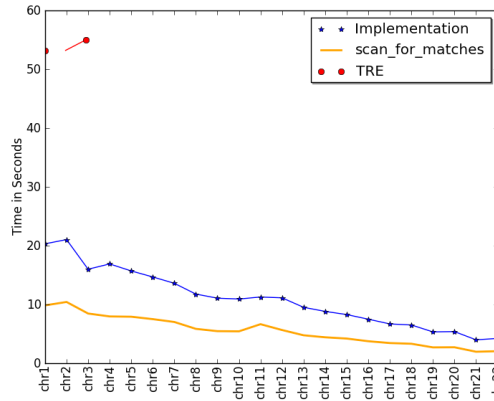


Figure 6: Running time of search through fasta files mentioned in figure 5, allowing one insertions on pattern TGCAAGCGTTAAT

From Figure 6, it's clear that given the 22 files, scan_for_matches runs faster than both our implementation, at roughly at half the time, and TRE which is about 5 times slower than scan_for_matches on this simple pattern.

Next test uses the same sequence but with two insertions instead of one.

While scan_for_matches did increase its runtime slightly, the second insertion really affected our implementation, resulting in it running at about the same speed as TRE. And while TRE also had its runtime slightly increased, it's almost unchanged from one insertion.

Lastly there's three insertions:

In Figure 8 our implementation really pulls away from scan_for_matches and TRE, which remains almost unchanged, relative to our implementation, which more than doubles its runtime.

From these three tests its clear to see that our implementation has a problem with increasing number of insertions, and as deletions and mutations are handled in a similar fashion we can conclude that our current implementation has a major flaw, should it be used with more advanced patterns.

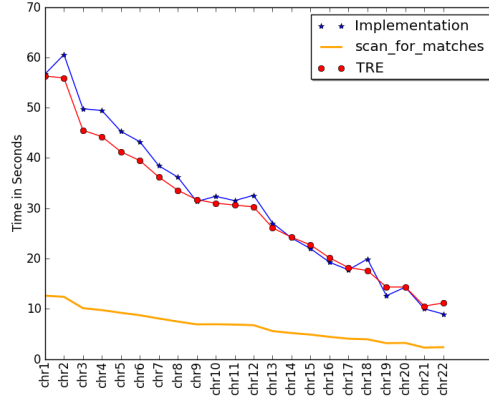


Figure 7: Running time of search through fasta files mentioned in figure 5, allowing two insertions on pattern TGCAAGCGTTAAT

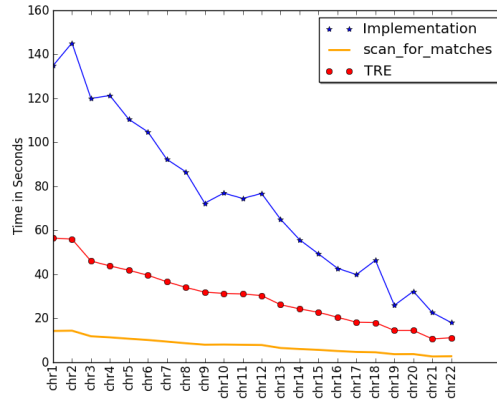


Figure 8: Running time of search through fasta files mentioned in figure 5, allowing three insertions on pattern TGCAAGCGTTAAT

Another interesting thing to test for was the number of hits when searching the files, in table 2 the number of hits which came up when searching on file chr1.fa are shown.

	1 insertion	2 insertions	3 insertions
Our implementation	5	47	235
TRE	1	19	76
scan_for_matches	5	43	192

Table 2: Number of hits in fasta file chr1, using the mentioned benchmark tests.

The primary reason for our implementation to get more results than scan_for_matches is that our implementation finds every single match in the file, including overlapping matches, while scan_for_matches, by default, only finds matches which does not overlap. TRE has the major disadvantage here that it doesn't match across newlines, causing it to miss a lot of matches.

11 Alternative solutions

11.1 Forming patterns using Regular expressions

The initial goal of this project was to use regular expressions to match the sequences. The problem of only using regular expressions, is the amount of patterns explode in size, when adding missmatching. Below is a description of how many patterns are formed from a pattern of length n . When a new pattern is formed, it constructs them into 1 regular expression using the alternation operator separating each of the new expressions.

Mutations are done by having a character is replaced by a wildcard, this is done for every character in the pattern. When adding multiple mutations, characters which is already wildcards are not changed. The formula for the amount of patterns formed from mutations, is the number of combinations that can be formed from the amount of mutations in t , this is the binomial coefficient².

A insertion is a wildcard added between the characters in the pattern, so for each pattern $n-1$ new patterns occur.

A deletion is removing a character from the pattern, it is not allowed to remove a character next to an insertion, as this cannot occur in RNA and DNA strings. Given multiple insertion, they will be spread out throughout the pattern in most cases, so an approximation of how many patterns formed would be $(n - \text{insertions} * 2)$.

So the final formula would look something like $\binom{n}{m} * (n - 1) * (n - i * 2)$, where n is the length of the string, m is amount of mutations and i is amount of insertions.

Example 11.1. *Given a pattern of size 30, with 2 mutations, 1 deletion, 1 insertion. It would produce the following amount of patterns:*

$$\begin{aligned} \text{After mutation: } \binom{30}{2} &= 435 \\ \text{After insertion: } 435 * (30 - 1) &= 12615 \\ \text{After Deletion: } 12615 * (30 - 2) &= 353220 \end{aligned}$$

As shown in example 11.1, the amount of patterns formed from using regular expressions only, would be too large for a regular expression matcher to find in a reasonable time.

11.2 Preprocessing data

Preprocessing data gives certain advantages, it allows for faster lookups into the string that is being searched on. With a structure like suffix tree's³, to store the location of all the substrings. It would be possible to do lookups in constant time given the correct choice of data structures. This would allow using the large regular expressions with mismatches to be run in a reasonable time. The disadvantage of preprocessing data, is the time it takes to construct a indexed structure of the string, and it may produce a tree larger than the original string, which could be an issue in some of the larger data files which are several GB in size. If the file have to be reused many times, it may be justified to create one.

²http://en.wikipedia.org/wiki/Binomial_coefficient

³http://en.wikipedia.org/wiki/Suffix_tree

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