Building a hardware accelerator for DNA sequence alignment using an MPSoC
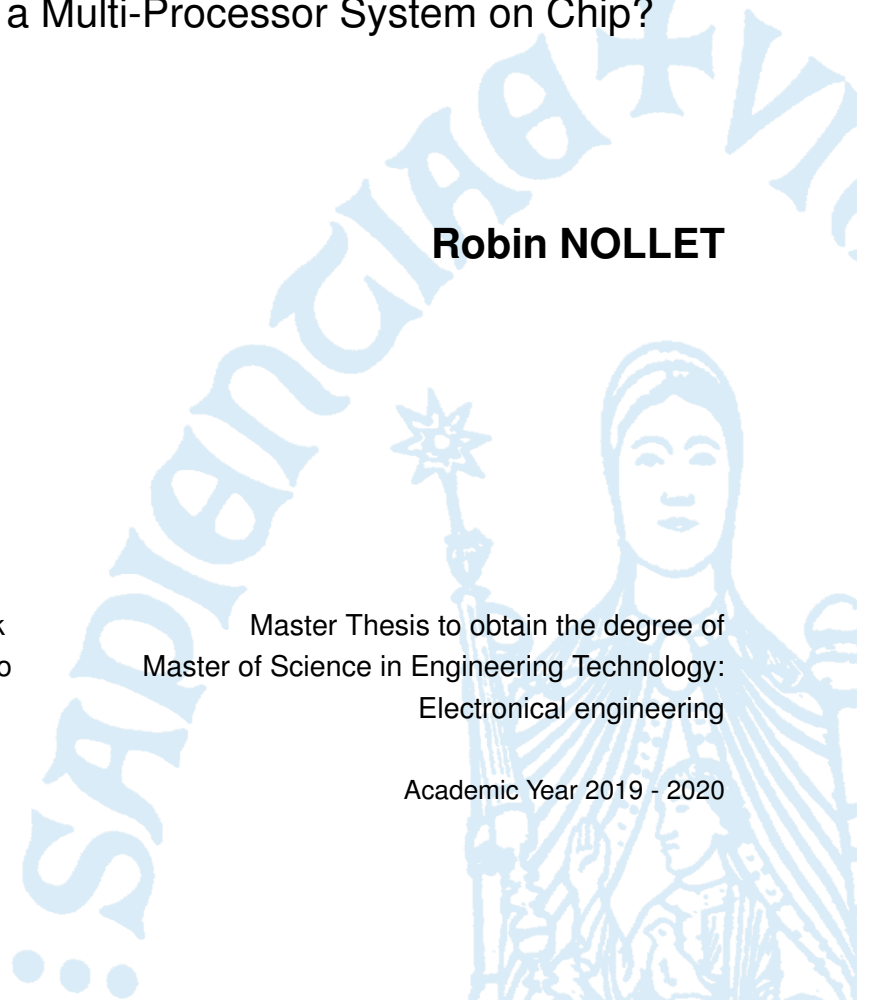
acceleration of the Smith-Waterman algorithm for reference genome mapping with a Multi-Processor System on Chip?

Robin NOLLET

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Summary

De (korte) samenvatting, toegankelijk voor een breed publiek, wordt in het Nederlands geschreven en bevat **maximum 3500 tekens**. Deze samenvatting moet ook verplicht opgeladen worden in KU Loket.

Abstract

Het extended abstract of de wetenschappelijke samenvatting wordt in het Engels geschreven en bevat **500 tot 1.500 woorden**. Dit abstract moet **niet** in KU Loket opgeladen worden (vanwege de beperkte beschikbare ruimte daar).

Keywords: Voeg een vijftal keywords in (bv: Latex-template, thesis, ...)

Contents

List of Figures

List of Tables

List of symbols

Maak een lijst van de gebruikte symbolen. Geef het symbool, naam en eenheid. Gebruik steeds SI-eenheden en gebruik de symbolen en namen zoals deze voorkomen in de hedendaagse literatuur en normen. De symbolen worden alfabetisch gerangschikt in opeenvolgende lijsten: kleine letters, hoofdletters, Griekse kleine letters, Griekse hoofdletters. Onderstaande tabel geeft het format dat kan ingevuld en uitgebreid worden. Wanneer het symbool een eerste maal in de tekst of in een formule wordt gebruikt, moet het symbool verklaard worden. Verwijder deze tekst wanneer je je thesis maakt.

b	Breedte	$[mm]$
A	Oppervlakte van de dwarsdoorsnede	$[mm^2]$
c	Lichtsnelheid	$[m/s]$

List of abbreviations

Chapter 1

Introduction

Chapter 2

Background information on DNA and DNA sequencing

2.1 Biology and DNA

2.1.1 History of genetics and DNA

Genetics   For thousands of years, humans have observed the effects of heredity and implemented their knowledge to domesticate plants and animals. However, the science behind heredity was only started to be understood since 1859 with the publication of *on the origin of species* by Charles Darwin.

Around 1865, an Austrian monk and botanist Gregor Mendel, who studied at the university in Brno in the current Czech Republic, published his results on the hybridization studies of pea plants. He is often credited as being the father of modern genetics. In his findings, he implemented the role of *factors* that influence the expression of traits. These factors later became known as *genes*.

Gregor Mendel

DNA   In 1869, Swiss physician Friedrich Miescher discovered a microscopic substance in the pus of discarded surgical bandages. Later, in 1909, Phoebus Levene named this substance DeoxyriboNucleic Acid (DNA) since it is found in the nucleus of a cell and has acidic properties.

The full structure of DNA was discovered by Francis Crick and James Watson at the Cavendish Laboratory at the University of Cambridge.

2.1.2 Structure of DNA

DNA, or Deoxyribonucleic Acid, is the molecule that stores the genetic information of all living organisms. It is the information that programs all of the activities in a cell.

Structurally, DNA is a polymer, which means each molecule is built up out of small repeating molecular units. In DNA, these units are called *nucleotides*.
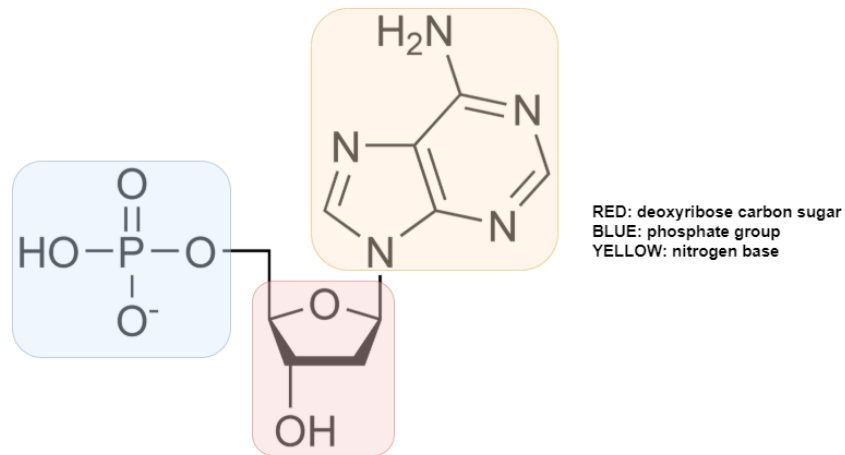
Figure 2.1: The structure of one nucleotide

Each nucleotide consists of 3 parts:

1. A carbon sugar molecule called *Deoxyribose*.

2. A phosphate group to connect the Deoxyribose molecules.

3. One of four possible nitrogen bases: Adenine (A), Thymine (T), Cytosine (C) or Guanine (G).

It is important to note that in most living organisms DNA does not exist as a single polymer, but rather a pair of molecules that are held tightly together. This is the famous *double helix*.

Figure 2.2: The famous double helix

Like in any good structure, there is a need for the main support. In DNA, the sugars and phosphates bond together to form twin backbones. These sugar-phosphate bonds run down each side of the helix, but chemically in opposite directions.

The first phosphate group, at the start of the molecule, connects to the sugar group's 5th carbon. At the end of the structure, the 3rd carbon of the sugar group is unconnected. This makes a

pattern typically noted as $[5' \rightarrow 3']$. Now, since the other molecule in the helix goes in the opposite direction, the pattern of the other backbone is typically noted as $[3' \rightarrow 5']$.
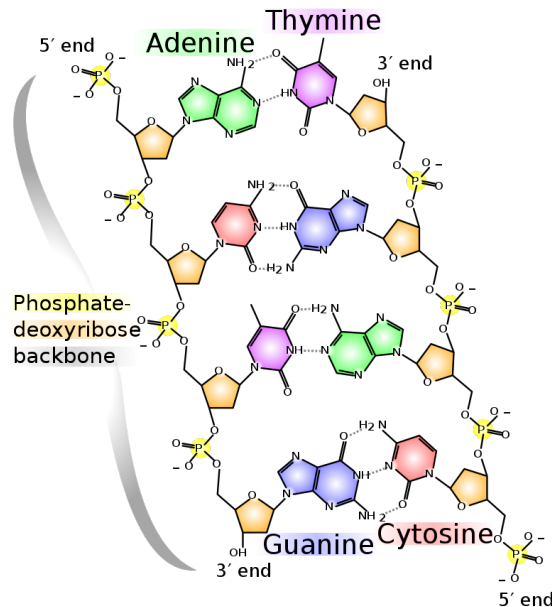
Figure 2.3: A short part of a DNA molecule containing a $[5' - ACTG - 3']$ (left) and a complementary strand of $[3' - TGAC - 5']$ (right). These 2 strands are interconnected by hydrogen bonds

These two long chains are linked together by the nitrogen bases via their relatively weak hydrogen bonds, but there can't just be any pair of nitrogen bases. Adenine can only make hydrogen bonds with Thymine. Likewise, Guanine can only bond with Cytosine. These bonded nitrogen bases are called *base paires*.

It is the order of these bases, which is also called the *sequence*, that allows this DNA to store useful information. In this way, e.g. $AGGTCCATG$ means something completely different as a base sequence than e.g. $TTCCAGATC$.

Since each of the bases in the sequence has only one possible counterpart, you can predict what its matching counterpart will be in the opposite string. For example:

If the following sequence is known

$$[5' - AGGTCCG - 3']$$

we can deduce the sequence in the other direction as

$$[3' - TCCAGGC - 5']$$

2.1.3 DNA in the human body

In human cells, DNA molecules can be found in the nucleus of all cells in the body. It consists of 46 very long molecules, which during cell division condense in what we call *chromosomes*.

The only exception is in reproductive cells (the egg cell and the sperm cell), which only have 23 chromosomes. These chromosomes are packed tightly together in the nucleus of the cell. If all of these 46 chromosomes are put together, this makes about two times 3 billion base pairs. These 3 billion base pairs provide the assembly instructions for pretty much everything inside the cell.

These 46 chromosomes, which make up our whole DNA, are always present in pairs in the cells. Each time, the pair consists of one chromosome from the father and the other one from the mother.

The 23 chromosome pairs are classified in:

- 22 pairs of autosomal chromosomes, marked 1 to 22 according to the length of the sequence. The longest chromosome (chromosome number-1) is 248,956,422 bases long. The shortest (chromosome number-22) is 50,818,468 bases long.

- In each cell, there is also an X chromosome plus an X or Y chromosome, dependent on the gender (XY for male, XX for female).
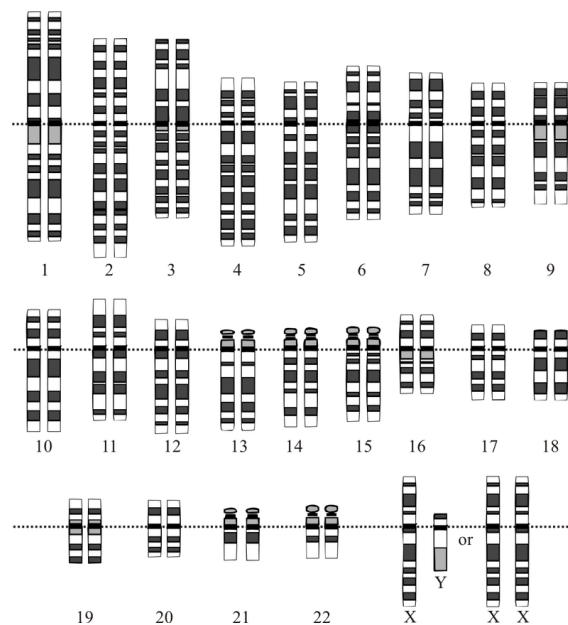
Figure 2.4: the 46 human chromosomes

2.2   The Human Genome Project

In the field of Bioinformatics, an important dataset is the *Human Genome*. This is the full DNA sequence found in the Nucleus, ordered from chromosome 1 to 22, followed by the X and Y chromosome.

In October 1990, biologists in the relatively new field of molecular biology started the Human Genome Project. The goal of this project was to determine the sequence of the 3 billion base pairs that make up human DNA. This project was completed and published in 2003, So nowadays we have a good idea of how the human genome is built up.

The Human Genome is easily found on the internet since it is publically available. One of the most often used is *hg19*, which was published in 2009. Since DNA has only 4 possible bases (A, T, C or G), this can be encoded in a 2-bit representation. If this encoding is used, the Human Genome is approximately 750 megabytes.
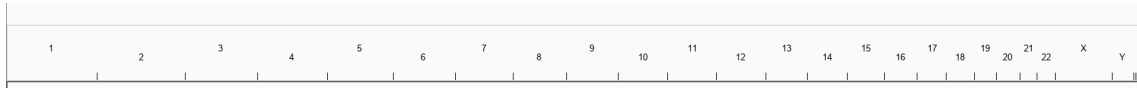
Figure 2.5: The order of the human genome as depicted in the IGV software

2.3 Sequencing

2.3.1 The sequencing technology

The term *Sequencing* is used for all techniques to read and decipher the DNA code from a given snippet of DNA. During the last years, the techniques that sequence human DNA has changed quite a lot. For about 15 years the *Next Generation Sequencing (NGS)* is the technique most often used. The biggest advantage of NGS, in comparison with other techniques, is the speed of the sequencing since it can sequence billions of short DNA molecules in parallel. In practice, this sequencing is most often done by the instruments of the company Illumina, which dominates the market (around 90% market share).

How whole-genome NGS works

1. The DNA to sequence is isolated from the cells. Most often this is the whole genome.

2. In some cases, The isolated DNA can now be copied enzymatically. This step is repeated until there are enough copies of the same DNA. Usually, this is in the millions or billions of copies.
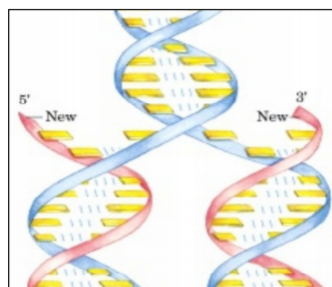
Figure 2.6: the enzymatic copying of a string of DNA. The original is unzipped, thus allowing new nucleotide bases to attach to the exposed bases.

3. the full DNA sequence is now broken apart into small DNA molecules (100 to 1000 bases long). This is done using enzymes or high-frequency sound waves.

4. Now the sequencing can start: a *flow cell* is used where these small DNA molecules can bind to a glass surface.

5. Different enzymatic and chemical reactions can now be done on this flow cell through an automatic flow of reagents. The following steps are iterated until the full read has been filled in:

   (a) The entire flowcell is filled with nucleotides, all with different nitrogen bases. Important is that at each of these nucleotides there is a fluorescent group attached to the phosphor group. This makes sure no other nucleotide can bind.

   (b) The fluorescent groups have a different color, dependent on the nitrogen base attached (A, G, T of C). At this time a camera picture of the flowcell is taken and stored.

   (c) after the flowcell is emptied of the loose nucleotides, another reagent flows in this flowcell. This reagent splits the fluorescent group from the phosphor group so that in the next iteration a new nucleotide group can bind with the read.
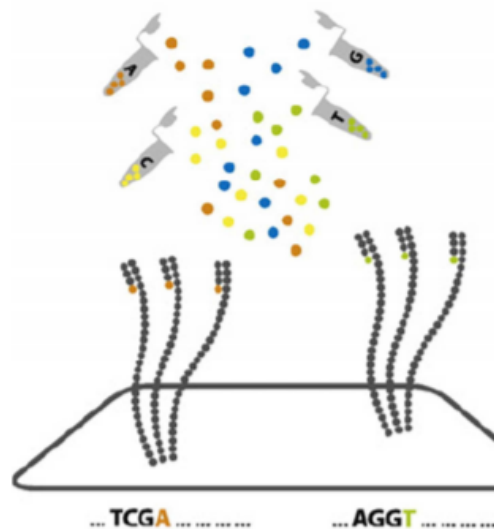
Figure 2.7: Sequencing technology used by Illumina attaches a nucleotide with a fluorescent tag to the next base in the read, captures a picture the read to determine the base, and removes the fluorescent tag so a new nucleotide group can bind in the next iteration.

6. After the whole DNA snippets have been filled in, the machine deduces the sequence in the DNA snippet. The pictures that were taken in order during the operation show the colors released in a specific spot, and by extent the attached nitrogen base. By the means of some image processing techniques, it is quite easy to get all the sequences from all the molecules bound on the flowcell. this is called the *Primary processing*.

Figure 2.8: From left to right is the pictures taken at each iteration in the flowcell. The color at that specific spot marks which nucleotide has been bound. With the use of some image processing techniques the exact sequence in that spot can be identified.

7. In the *secondary processing*, the sequence is trimmed by quality, etc. The operations that are done on the read in this step are outside the scope of this thesis.

As a result of the NGS, we get a (large) file in the FASTQ format.

2.3.2 The FASTQ file format

Since the color of each spot observed in the camera pictures in the primary processing can have a light shift, there is a specific "uncertainty" about the correct base is in that spot. This is called the *quality* of the base.

The *FASTQ* file format has become the de-facto standard as output from sequencing instruments. It is a text-based format for storing both the bases in the sequence and their corresponding quality.

A FASTQ file uses four lines per sequenced DNA fragment:

1. a '@' character followed by a sequence ID, plus an optional description. This description mostly contains the coordinate of the spot on the flowcell.

2. The sequence of DNA bases identified by the machine. This is either A, G, C, T, or n when the base cannot be identified with a specific threshold certainty.

3. a '+' character, optionally followed by the sequence ID (again) and an optional description.

4. the quality values for each respective base in line 2. The length of this line must be the same as the number of bases in line 2

The quality score in memory is a value in the range $0x21$ (lowest quality) to $0x7e$ (highest quality). Since this value is represented in ASCII in the file format, this ranges from the '!' character to the '~' character. Hereunder is a complete list of the possible values of the quality score:

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNOPQRSTUVWXYZ
[\]^_`abcdefghijklmnopqrstuvwxyz{|}~
```

Important to note is that this quality score is logarithmic. Also, the '@' and '+' character is a possible value for the quality, so this will be something to look out for when implementing the interpreter for this file.

A FASTQ file containing a single sequence of a DNA framgent of 60 bases might look like this:

```
@*((((***+))%%%++)(%%%%).1***-+*''))**55CCF>>>>>>CCCCCCC65
```

Keep in mind that a FASTQ file consists of multiple of these sequences, all stacked under each other.

Chapter 3

Methods for DNA sequence alignment

3.1 DNA sequence aligning

The human genome (e.g. $hg19$) is used as a reference genome for all sequenced human DNA. However, the genetic code of all humans is slightly different. Genetic sequence alignment is the science where you try to align 2 sequences with each other so that the amount of differences is minimal. In this chapter, the most frequently used algorithms are discussed.

3.1.1 Alignment in general

In genetic codes, there are 3 types of differences between the given sequence and the reference:

- Insertion: one or more bases have been added in the genetic code in a specific spot.

- Deletion: one or more bases have been removed from the genetic code in a specific spot.

- Substitution: one or more bases have been substituted by other bases.

Inserts and deletions are often described by a single term, *indel*.

For example: if we want to align the following sequences:

```
Seq1: ATATCGGC
Seq2: ATCG
```

The alignment itself can now be done in different ways. Possible alignments are:

```
Alignment 1
Seq1: AtaTCgGc
Seq2: A--TC-G-
Alignment 2
Seq1: atATCGgc
Seq2: --ATCG--
```

Which alignment that is the actual output, depends on the algorithm and the given parameters. The '-' character represents a base that is not present.

Keep in mind, there is no one "correct" alignment. The core of the alignment algorithms is the same each time, but the parameters of these algorithms are changed depending on the application.

3.2 Local versus global alignment

To explain the difference between local and global alignment, we can take a look at the following example:

```
The 2 DNA sequences:
Seq1: TCCCAGTTTGTGTCAGGGGACACGAG
Seq2: CGCCTCGTTTTCAGCAGTTATGTGCAGATC


Alignment 1 :
Seq1: -----------tccCAGTT-TGTGTCAGgggacacgag
Seq2: cgcctcgttttcagCAGTTATGTG-CAGatc-------


Alignment 2 :
Seq1 : tcCCa-GTTTgt-GtCAGggg-acaC-GA-g
Seq2 : cgCCtcGTTTtcaG-CAGttatgtgCaGAtc
```

Both alignments are valid but different. The first alignment is *locally aligned*. This means that the similarities are prioritized in the same region, with the similarity as high as possible. On the other hand, the second alignment is *globally aligned*. Here the similarities over the full length of the sequences are used for the alignment.

In practice, the local alignment is used most often, since it can give you information of 2 sequences that do not have (approximately) the same length.

3.3 Commonly used algorithms

In this section, we will take a look at some algorithms that are used most often for DNA sequence alignment.

The most used algorithms are often categorized in 2 ways:

- local alignment versus global alignments (see 3.2)

- dynamic algorithms versus heuristic algorithms: dynamic algorithms are exact but slow and computationally demanding, whereas heuristic algorithms are faster but are approximations and the best alignment is not guaranteed.

Hereunder is a schematic view of some algorithms that are used in practice:

	Dynamic programming	Heuristic programming
Local alignment	Smith-waterman	FASTA, BLAST
Global alignment	Needleman-Wunsch	X

Table 3.1 Classification of DNA alignment algorithms

Keep in mind, a lot of other claimed "algorithms" (for example BFAST, ...), are accelerated versions of the Smith-Waterman algorithm.

3.3.1   Needleman-Wunsch

Needleman and Wunch proposed a new algorithm for genetic sequence alignment in 1970, now known as the *Needleman-Wunsch* (N-W) algorithm. Since this algorithm is meant for global alignment, which is seldomly used in practice, further discussion of the algorithm will not be done. However, N-W has a lot of similarities with the Smith-Waterman algorithm, discussed in the next section.

3.3.2   Smith-Waterman

The *Smith-Waterman* (S-W) algorithm was first proposed by Temple F. Smith and Michael S. Waterman in 1981. It is a variation on the N-W algorithm, adapted for local alignment. It is a dynamic programming technique, so an optimal local alignment is guaranteed.

The core of the algorithm is a matrix fillup, with data dependencies on the previous cells. Hereunder an analysis of the algorithm:

1. Symbols used in the analysis:

   Let sequences $A = a_1a_2a_3\ldots a_n$ and $B = b_1b_2b_3\ldots b_m$ be the sequences that need to be locally aligned. Here n and m are the lengths of the sequences A and B

2. Define the parameters:

   - Define $s(a,b)$ be the *similarity matrix* (sometimes also called the *substitution matrix*) for the two sequences. It is used for "rewarding" when $a_i = b_j$ and "punishing" when $a_i \neq b_j$.
     In the most general way, we define the similarity score as a matrix of values, e.g.:

	A	C	G	T
A	3	-3	-3	-3
C	-3	3	-3	-3
G	-3	-3	3	-3
T	-3	-3	-3	3

Table 3.2 Similarity matrix example

Often, there are only 2 scores used (equal or not equal). In this case, the similarity matrix can be condensed as follows:

$$s(a_i, b_j) = \begin{cases} +3, & a_i = b_j \\ -3, & a_i \neq b_j \end{cases}$$

- Define d as the *gap penalty* which regulates the score for an insertion or a deletion. This parameter can be:

  - *Linear*: The penalty is constant. So, in this case, it doesn't matter e.g. the previous was also a gap.

  - *Affine*: An affine gap penalty considers gap opening and extension separately. For the sake of simplicity, my further implementation will not include this refinement of the algorithm. The algorithm can be extended to include this affine gap penalty, but this would make the algorithm more complex and we would limit our ability to develop possible accelerations.

3. The initialization: We construct a scoring matrix H with dimensions $(n+1) \times (m+1)$. The first column and first row we initialize with 0.

   For example: if we want to align the sequences A = $TGTTACGG$ and B = $GGTTGACTA$:

		T	G	T	T	A	C	G	G
	0	0	0	0	0	0	0	0	0
G	0								
G	0								
T	0								
T	0								
G	0								
A	0								
C	0								
T	0								
A	0								

Table 3.3 Example of the initialization of the scoring matrix

4. Matrix fill in: We fill in the matrix using the following formula:

$$H_{ij} = max \begin{cases} H_{i-1,j-1} + s(a_i, b_j), \\ H_{i-1,j} - d, \\ H_{i,j-1} - d, \\ 0 \end{cases}$$

If we keep in mind that the value of a cell may never be lower than 0, we can represent the data dependencies in the following schematic:
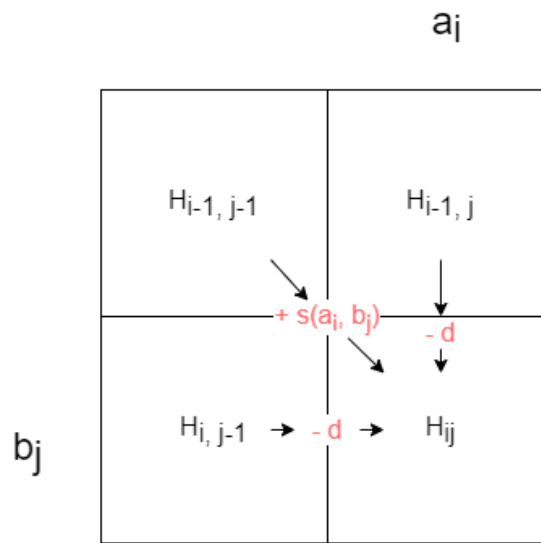
Figure 3.1: Data dependencies in the H matrix

Where $s(a, b)$ and d are the parameters of the algorithm. If we use the following values as an example:

$$s(a_i, b_j) = \begin{cases} +3, & a_i = b_j \\ -3, & a_i \neq b_j \end{cases} \quad \text{and} \quad d = 2$$

We can now fill up the scoring matrix H:

	T	G	T	T	A	C	G	G
	0	0	0	0	0	0	0	0	0
G	0	0	3	1	0	0	0	3	3
G	0	0	3	1	0	0	0	3	6
T	0	3	1	6	4	2	0	1	4
T	0	3	1	4	9	7	5	3	2
G	0	1	6	4	7	6	4	8	6
A	0	0	4	3	5	10	8	6	5
C	0	0	2	1	3	8	13	11	9
T	0	3	1	5	4	6	11	10	8
A	0	1	0	3	2	7	9	8	7

Table 3.4 Example of a populated scoring matrix

5. Traceback: We start at the cell with the highest score in the matrix H. Starting here we only move left, up or diagonally (left-up) to the cell on which the value in the cell was based until we hit a cell with value 0.

	T	G	T	T	A	C	G	G
	0	0	0	0	0	0	0	0	0
G	0	0	3	1	0	0	0	3	3
G	0	0	3	1	0	0	0	3	6
T	0	3	1	6	4	2	0	1	4
T	0	3	1	4	9	7	5	3	2
G	0	1	6	4	7	6	4	8	6
A	0	0	4	3	5	10	8	6	5
C	0	0	2	1	3	8	13	11	9
T	0	3	1	5	4	6	11	10	8
A	0	1	0	3	2	7	9	8	7

Table 3.5 Example of a traceback in S-W

From this traceback we can now deduce the following alignment:

```
GTT-AC
||||||
GTTGAC
```

This alignment is the output of our algorithm.

3.4   Problem definition

3.4.1   Mapping to a reference genome

From the DNA sequencing machines, we get a big amount of reads in the FASTQ format. We should note that all these reads are worthless without a proper interpretation.

In most cases, the first step in the analysis of the reads is knowing from which part of the genome it's derived. Typically, the read is compared with the whole genome in a local alignment, for example with the Smith-Waterman algorithm. As an output, we would get the position in the human genome and an alignment with its score (how well the sequence fits in that spot). This practice is commonly referred to as *Mapping to reference genome*.

Since the reads from DNA sequencing machines are 75 to 300 bases long, and the whole human genome is approximately 3 billion bases, this comparison is computationally a very intensive task. If we analyze the S-W algorithm (as we have done in 3.3.2), we can see that the value of each cell in the matrix is only dependent on the left-upmost 3 cells. Therefore, It leads us to believe that this algorithm can be accelerated on other hardware solutions such as an FPGA (which will be discussed in chapter 4) since S-W is heavily parallelizable.

In most clinical applications where mapping to a reference genome is used, the number of reads to be compared with the genome is in the millions.
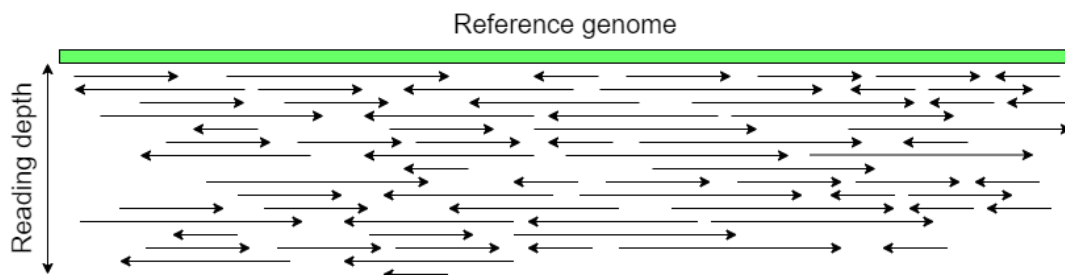
Figure 3.2: Mapping to a reference genome. The direction of the read is represented by arrows

Since the read can be from the complementary DNA molecule in the double helix, the sequences can be in forward $[5' \rightarrow 3']$ direction, or in complementary $[3' \rightarrow 5']$ direction. To transform that read to the used reference genome direction we need to perform the following changes to the read to make its reverse complementary:

1. The bases should be changed to their corresponding base in the base pair;

2. The sequence should be reversed.

We have no way of knowing in which direction the read is taken, both the forward and the backward possibility should be compared with the reference and the best-aligned version of these two should be chosen.

Please note, in most normal cases we can assume the distribution of reads is practically uniform. Therefore, each base in the human genome will be covered by a statistically expected amount of reads. This amount is referred to as the *reading depth*.

3.4.2 The sam and bam file format

As a convention, the output of mapping algorithms is in a *SAM* (Sequence Alignment Map) or *BAM* (Binary Alignment Map) file format. The BAM file format is just a compressed version of the SAM format, but the SAM format is more readable, which makes it easier for troubleshooting. There exist a lot of tools to transform a SAM to BAM file already, so in this thesis, we will focus on the SAM format. A SAM file consists of two sections: a header and an alignment section.

3.4.2.1 Header

The header is used for information that is independent of the alignments, such as the name of the used algorithm, reference genome, used commands during generation, etc. The header must be at the beginning of the file, before the alignment section. Each line of the header field must start with an '@' character, so these lines are easily distinguished from lines the alignment section.

3.4.2.2 Alignment Section

Each line in the alignment section represents one mapped sequence and consists of 11 mandatory and some optional fields, which are separated with a tab.

The 11 mandatory fields are:

1. **Qname**: this is the name of the query (the sequence to match) and can be found on the first line of the FASTQ file, which is the input to the mapping algorithm.

2. **FLAG**: a combination of bitwise flags where each bit has a specific interpretation. It consists of 11 bits, but for basic alignments only 2 fields are important:

   - bit 2 (binary: $00000000X00$, integer value 4) is set to 1 if the sequence is unmapped or the map is not found

   - bit 4 (binary: $000000X0000$, integer value 16) is set to 1 if the sequence has been mapped as its reverse complement.

3. **Rname**: the name of the reference genome. This can be found on the first line of the FASTA file, where the reference genome is stored. If the read is unmapped, this field contains a '*' character

4. **Pos**: the position of the leftmost base in the alignment. Keep in mind that the indexing of the reference starts with 1 for the first base.

5. **MapQ**: the mapping quality, which indicates how good the sequence fits in the specific position. This can be any value between 0 and 254. Value 255 is a reserved value to represent an unavailable quality.

6. **CIGAR**, which stands for *Concise Idiosyncratic Gapped Alignment Report*. It is a string that indicates where the matches (M), insertions (I), and deletions (D) occur. For example, if the CIGAR states $3M1I6M2D10M$ this means from left to right: 3 matches, then an insertion, followed by 6 matches, 2 deletions, and finally 10 matches. In case the sequence is unmapped, this field should be filled with a '*' character

7. **Rnext**: reference sequence name of the primary alignment of the text read in the template. For this thesis, we will fill this field with a '*' character.

8. **Pnext**: the position of the primary alignment of the next read in the template. For this thesis, we will fill this field with a '0' character.

9. **Tlen**: the observed template length, from the first till last mapped base. For this thesis, we will fill this field with a '0' character.

10. **Seq**: the full sequence. This can be found in the FASTQ file on the second line.

11. **Qual**: the qualities of the sequence, also given by the FASTQ file on the fourth line.

An example of one line in a SAM file:

```
SRR11    0    MN98    25    254    7M    0    0    GTTAAAG    BBBBBCB
```

In this example:

- The name of the sequence is $SRR11$

- The read is matched in the $[5'->3']$ direction

- The match is found at the 25th base of the reference genome

- The mapping quality is 254

- CIGAR = $7M$, so a perfect match

- The sequence is $GTTAAAG$ with quality $BBBBBCB$

3.4.3 Clinical application

We will discuss as examples two clinical applications of mapping to a reference genome.

1. ***NIPT (non-invasive prenatal testing), a test for detecting genetic defects in a foetus.***
   During or after conception, DNA can be lost or gained in the fertilized egg cell. This can result

in a severe syndrome of the child. For example, Down syndrome is caused by a trisomy of chromosome 21. Normally all chromosomes are present twice in each cell, one from the mother and the other from the father. In Down syndrome patients something went wrong during cell division at the very early stage of development, and the fetus has in its cells three times chromosome 21. Because chromosome 21 is quite small and does not contain that many genes, the child can survive, though with typical mental and clinical problems.

Figure 3.3: Trisomy 21 karyotype. The trisomy of chromosome-21 is indicated by an arrow

Before high throughput DNA sequencing technologies were available like they are today, testing if a fetus has a trisomy-21 could only be done by taking a small amount of amniotic fluid (fluid around the fetus). However, to obtain this fluid there was a need for a risky invasive procedure (called *amniocentesis*) leading sometimes to termination of the pregnancy.

It is known that small amounts of DNA of the fetus are present in the blood of the mother, in the cell-free DNA (*cfDNA*) which we find in the blood plasma (the clear, aqueous part of the blood). The blood plasma is used by our body to transport 'waste', including DNA from cells that were broken down. When fetal cells die, which is a normal process, the building blocks of these cells are transported in the plasma of the blood from the mother, included small DNA fragments from the fetus.

NIPT (non-invasive prenatal testing) is used to analyze DNA derived from the mother's blood. A large number of short cfDNA fragments are sequenced at random. Then, each sequence is mapped to the whole human genome to find out where it comes from. Finally, the distribution of these reads is calculated. If we observed that a higher frequency of reads as compared to normal individuals coming from chromosome-21, it is almost certain that the fetus has Down's syndrome.
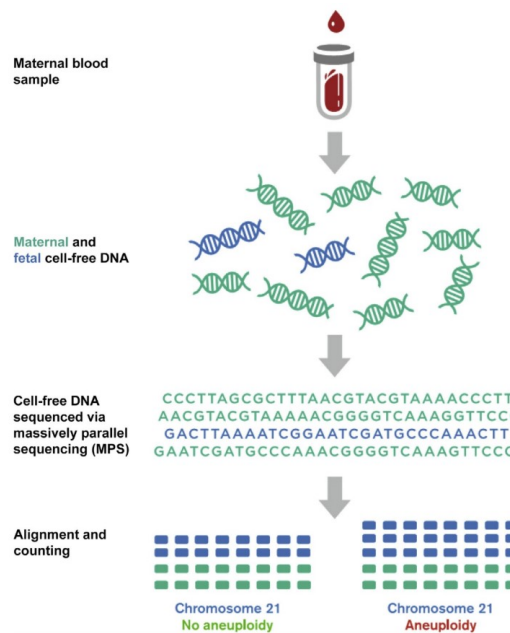
Figure 3.4: A schematic overview of the NIPT test

Using the same method, we can also find other defects in the number of chromosomes. For example trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), or even in the sex chromosomes, such as XXY (Klinefelter syndrome) or lack of a second X or a Y chromosome (Turner syndrome).

2. **Shallow whole-genome sequencing of tumor DNA.**

   It is a known fact that damaged DNA can lead to tumor development. This damage can be single bases changes but can also be loss or gain of large DNA sequences where important genes are located. When someone is diagnosed with cancer, the knowledge of which DNA regions are lost or gained can be important to decide on treatment.

   A relatively new technique to detect all gains and losses of DNA material in one single experiment is *sWGS* (shallow whole-genome sequencing). The technique is performed as follows: DNA from the tumor is fragmented (it is broken in small pieces, eg. by a fragmentase enzyme or by high-frequency sound). These pieces are sequenced randomly, and with a mapping algorithm to the reference genome, the over- or underrepresentation of reads (as compared with a normal sample) indicates if regions of the DNA have changed, and which regions these are.

Chapter 4

Platforms for accelerating Smith-Waterman

As we saw in the analysis of the Smith-Waterman algorithm in 3.3.2, we can see that the value of each cell in the matrix is only dependent on the left-upmost 3 cells. Therefore, It leads us to believe that this algorithm can be accelerated on other hardware solutions which are better equipped for parrallellism than a normal CPU.

4.1 Overview of possible hardware

When designing a processing unit based on semiconductor technologies, it is always a tradeoff between efficiency and flexibility. For example, the CPU in a PC should be flexible so that it can run any set of instructions without a lot of intervention between changes. On the other hand, if a certain algorithm or instructionset should be executed as fast as possible, the FPGA's and ASICs are the best choice. At the extreme, an ASIC or *Application Specific Integrated Circuit* can be chosen, but this means a complete chip should be designed from the ground up just for this specific application. This means that an ASIC is extremely expensive to design and build, especially for small production quantities. In figure 4.1 an overview of the different options can be found.

Figure 4.1: An overview of the different semiconductor technologies

4.1.1 CPU

The power of the CPU is it's flexibility: it can very easily be programmed with new instructions in very few time. However, if the CPU would only run one specific algorithm for its whole lifetime, it would be terribly inefficient. Also, CPU's work sequentially, which is less suitable for algorithms which demand a large amount of computations which could be done in parallel.
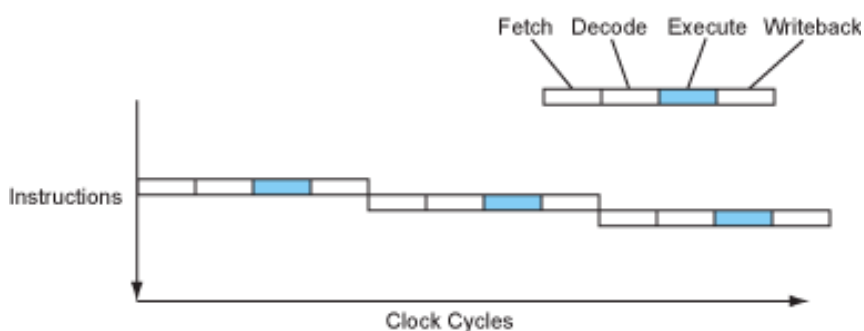
Figure 4.2: A cpu processes instructions sequentially. It could be accelerated using pipelining, but the maximum stays 1 instruction per clock cycle

Since the original implementations on CPU, it became clear that a CPU was not the most suitable platform for the S-W algorithm, since it consists of a lot of operations which can be done in parallel. However, since the rise SIMD, CPU's have made a comeback. *SIMD* stands for *Single Instruction Multiple Data* and makes it possible to manipulate more than 1 attribute of data with one single instruction, although be it the same instruction on all the data. CLC bio, a Danish company specialised in bioinformatics, has been able to achieve impressive speedups with a software implementation using SIMD, closing in on 200x.

Nowadays, MIT has published *diagonalsw*, which an implementation of S-W using the SIMD instruction set, and is licensed under the open-source MIT license.

4.1.2 GPU

a GPU, or *Graphical Processing Unit* is a semiconductor technology that is specialized in video encoding and decoding, which means it is especially built for matrix manipulations. Since the Smith-Waterman algorithm is basically a big matrix manipulation, it lead researchers to believe that it could be accelerated on a GPU. In 1997, an implementation of S-W on a GPU was published which achieved a speedup of 2x over all previous software implementations.

At the time of writing, 11 different implementations for Smith Waterman have been reported, 3 of which reporting a speedup over 30 times.

4.1.3 FPGA

an FPGA, or *Field Programmable Gate Array*, is an integrated circuit consisting of programmable logic components. These logic components can be programmed as any logic function, such as AND, XOR, etc. In most FPGA other elements are also found, such as memory blocks, DSP blocks, etc.

In the most basic FPGA's, the following items are definitely present:

1. CLB's, or *Complex Logic Blocks*, consisting of a *LookUp Table* (LUT) and a flipflop. A LUT can be programmable so that it contains any type of logic function.

2. Programmable Interconnects have to connect the CLB's into a bigger circuit, which is also called *routing*. This routing has the most influence on delays, and are also responsible for most errors.

3. I/O blocks, which connect the internal logic inside with the outside pins of the FPGA. Most can be configured as input, output or bidirectional.
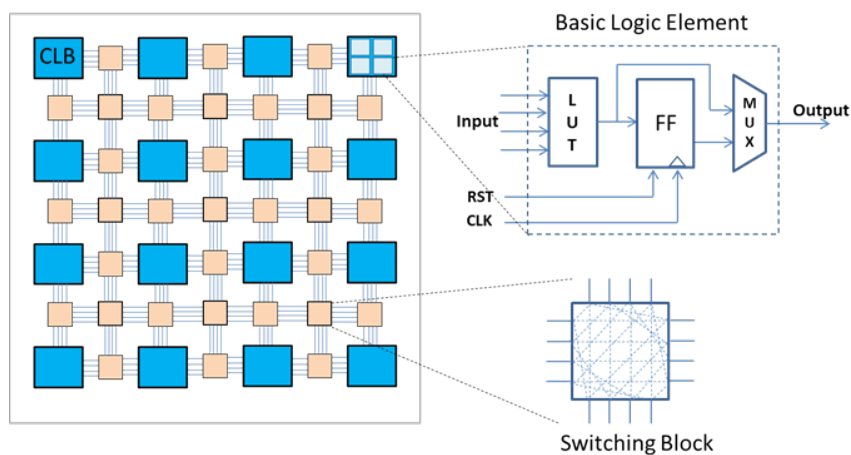
Figure 4.3: The basic layout of an FPGA

For an implementation or design of a circuit which should be loaded in an FPGA, a *Hardware Description Language* (HDL) is often the only practical choise to implement such a system, since drawing the circuits with a CAD program or by hand would take a very long time.

In a paper form 2007, an implementation of S-W with an FPGA (virtex-4) achieved a speedup up to 100x in comparison with a 2.2GHz Opteron processor. A few companies also have made some implementations of FPGA in the past, e.g. Cray Inc., TimeLogics, ...

A master's thesis from 2011 by Vermij E. has a detailed analysis of an FPGA based Smith-Waterman implementation. In other papers, it was found that the performance per Watt level for an FPGA implementation is better than a GPU or CPU by a factor of 12-21 times.

4.2 Hardware selection

4.2.1 Recent advances in High Level Synthesis

4.2.2 Platform communication

niet belangrijk via SD ev. Ethernet: FTP

reading from sd card so not a lot of information exchange needed if necessary =¿ FTP if needed

Chapter 5

Initial Difficulties during the implementation

Disclaimer: The purpose of this chapter is more to show the learning process I had to go through in order to achieve a suitable implementation for the genome mapping problem. I had to learn some new programming techniques such as HLS, SDSoC ... especially for this thesis, since I did not have any experience in it yet. This chapter might be less applicable if the reader is interested in the science and implementation approach of the genome mapping problem, but can be of interest if the reader is also new to the mentioned programming techniques.

5.1 Using Vivado HLS and Xilinx SDK

5.1.1 learning HLS in examples

Ik moet nog starten met HLS, nog nooit gedaan.

learning HLS

lab1: BASIC WORKFLOW: How to make project, difference between sources and test bench, GUI and it's components, simulation, synthesis (=¿ estimate and Gantt chart), viewing resulting VHDL code, co-simulation, view simulation waveforms, IP Core export, creating multiple solutions and comparing, setting a directive (like unroll a loop).

lab2: DATA TYPES adhv FIR filter: floating point VS fixed point, saturation with fixed point, fixed point with larger internal bit-width (LSB laten vallen),

lab3: INTERFACES: - basic argument-level interfaces (ap_vld, ap_none, ap_hs) - block-level interface of pipelined component (pipeline on the top function)

lab4: ARRAYS: Sequential, pipelining internal loop, rewind parameter, top function pipelining, array map, array partition (cyclic and complete), array reshape technique

5.1.2   Learning how to program target board

hello world via Xilinx SDK =¿ baremetal -¿ zelf kiezen waar uitgevoerd + dataverplaatsingen zelf implementeren

5.2   SDSoC

Op een Linuxdistributie.

Snel functie verplaatsen van PS naar PL.

gebruikt ook delen van HLS

5.2.1   learning process on matrix multiplication example in SDSoC

Eerste test: matrixmultiplicatie

lab1: INTRODUCTION: basic workflow, working with hardware accelerators, running the project, ...

lab2: PERFORMANCE ESTIMATION: rescource utilization, comparing software and hardware implementations, overall speedup comparison (all using LINUX hosted)

lab3: diff between ACP, AFI and GP ACP Hardware functions have cache coherent access to DDR via the PS L2 cache. AFI (HP) Hardware functions have fast non-cache coherent access to DDR via the PS memory controller. GP Processor directly writes/reads data to/from hardware function. Inefficient for large data transfers. + error reporting (opening log files to see more information). Additional: the hardware functions and DMAs can only access the physical address space (not virtual) =¿ DMA that can handle a list of pages for a single array is known as Scatter-Gather DMA. (malloc(), sds_alloc()) Typically sds_alloc = best (area and performance) but uses physical memory (minder van beschikbaar dan virtueel).

lab4: directives to optimize the accelerator

lab5: task level pipelining =¿ speedup x3 theoretical, probably I make some mistakes

lab6: DEBUG

lab7: HARDWARE DEBUG: trace feature: what is the application doing? (software, hardware, transfer, recieve)

lab8: EMULATION

Chapter 6

Software implementation with pure Smith-Waterman

This chapter will cover an approach to reference genome mapping using pure smith-waterman. This is a computationally very demanding task, but is more precise than heuristic methods such as FASTA, ...

6.1 The concept

For reference genome mapping a sequence of 75 to 300 bases in length should be mapped to the reference genome. The idea behind this mapping and the applications is thoroughly described in 3.

In this implementation, a mapping with "pure" Smith-Waterman was chosen. Commonly, an algorithm is used to select candidate locations where a small local smith-waterman is performed, or no smith waterman is performed at all. These methods are often less precise but computationally less demanding. Here, the alignment of the sequence is done with the full reference genome.
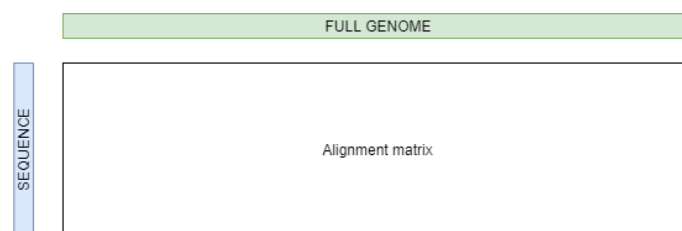
Figure 6.1: The concept of the implementation: the sequences is mapped to the whole genome

6.2 General overview of the implementation

6.2.1 Parameters and Types

Nucleotide base   First of all, It seemed important to define the nucleotide bases. There are only 4 possible bases (A, C, G and T), so 2 bits are enough. The following coding was chosen:

Base	A	C	G	T
Code	00	01	10	11

Table 6.1 Encoding for the nucleotide bases

To store these bases the uint8_t type from the stdint library was used. it is 1 byte in size which is the smallest available type in the C language. If more time would have been available, and an implementation with the full human genome would have been made, it might be a good idea to define a specific type consisting of only 2 bits, which would be a lot more memory efficient.

DNA sequence type   To easily keep track of a sequence in the program, a type was created which can hold a sequence of DNA. It consists of 2 parts: the length of the sequence and a pointer to the first base in memory. Since the genome can have a length of more than 3 billion bases, and the sequence is only a maximum of 300 bases in length, it made sense to use a separate type for the reference and the sequence.
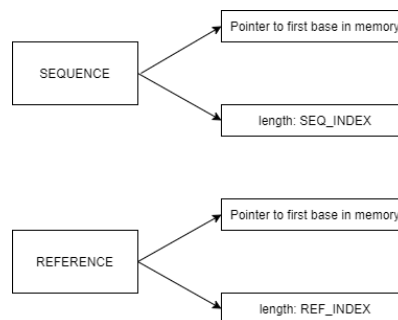
Figure 6.2: The created types to store the sequence and the reference

To index these "arrays" of bases, a new type was created for both the sequence indexing (SEQ_INDEX) and the reference indexing (REF_INDEX). Since an index to the sequence can be as high as the $length - 1$, it made sense to make the length attribute out of these newly created index types.

6.2.2 The code structure

1. Allocate some memory for the following data:

   (a) The reference genome, which can be quite large;

   (b) current read;

   (c) reverse complementary of current read;

   (d) matrix for during the alignment. The amount of memory that should be allocated to this matrix is equal to the size of the sequence times the size of the reference.

2. Initialize the first row and first column of the matrix on 0, as it should be to perform the Smith-Waterman Algorithm;

3. Load the reference genome from the fasta file;

4. Open the FASTQ file for loading unmapped reads, and the SAM file to store the reads once they are mapped;

5. For every read in the FASTQ file, we perform the following operations:

   (a) Load the next read from FASTQ file;

   (b) Perform the alignment. (see 6.3.3);

   (c) write the mapped read to the SAM file;

6. Close the FASTQ and SAM files;

7. free the reserved memory again

6.3   Details of the implementation

As in most implementations in software, it was decided to split up the functionality of the program in multiple blocks. We have 3 big blocks:

1. Interfacing with the memory: reserving memory for the reference, the sequence, and the alignment matrix.

2. Interfacing with the files and the filesystem: Since the FASTA, FASTQ and SAM files are in a specific format, it made sense to build interpreters from and to these formats.

3. The alignment itself, which is the core of the program.
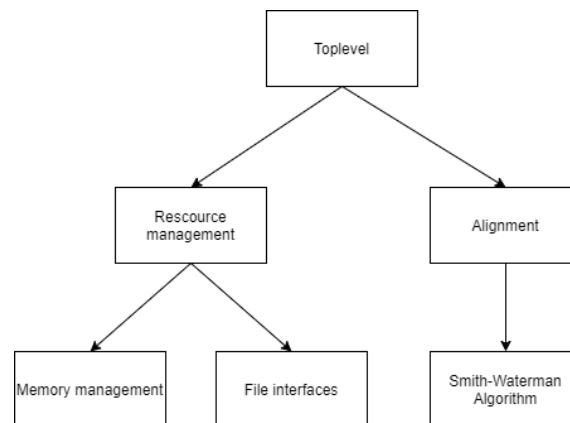
Figure 6.3: an organisation chart of the split up functionalities

6.3.1   File interfaces

6.3.1.1   Parameters and types

Since we need to interface FASTA, FASTQ, and SAM files, it seemed appropriate to create 3 custom types. For the FASTA file, which stores the genome, the type *GENOME* was created. In the case of the FASTQ file and the SAM file, the respective types *READ* and *MAPPED_READ* were created. Notice that the attributes of the types match the information to interface the files.

The information stored in the *GENOME* type is the name of the genome (*Rname*) and the reference.

Figure 6.4: The type created to store the genome information.

In the *READ* type, the current sequence is stored together with its quality string and its name.

Figure 6.5: The type created to store the read information

The *MAPPED_READ* type contains all the information needed to write a full line in the SAM file, which is the output of the program. Mark, the read itself is also stored in this type since all the information in the read will also be written to the SAM file.

```
┌─────────────────────────┐
│      MAPPED_READ         │
├─────────────────────────┤
│ Read                    │
│ Rname                   │
│ Flag                    │
│ Position                │
│ MapQ                    │
│ CIGAR                   │
│ Rnext                   │
│ Pnext                   │
│ Tlen                    │
└─────────────────────────┘
```

Figure 6.6: The type created to store the mapping information, together with the read

6.3.1.2 Code structure

First of all, the representation of the bases in the files (which is in the ASCII character format) should be transformable to the BASE type in the program. Because of this, conversion functions were created to change the character to base or vice versa.

FASTA interface   The function of this part is to load the genome from the FASTA file into the created *GENOME* type. The FASTA file format is a simple text-based way of storing a genome. On the first line is a '¿' character followed by the name of the genome (*Rname*). Starting from the second line to the end of the file the genome is stored. Often, this genome is split up using spaces or in multiple lines, so when coding the interpreter we need to make sure to skip these whitespace characters.

For better code readability, the genome loading is split into 2 functions that are executed in order:

1. loading the genome info

2. loading the genome into the REFERENCE type.

FASTQ interface   this part of the program is responsible for loading the next read as a stream. The buildup of a FASTA file is thoroughly described in 2.3.2.

In this case, the code was also split up into functions for better readability:

1. Loading the QName, which is found on the first line for every read, behind a '@' character

2. Loading the sequence, which is stored in a text-based format in the FASTQ file, so it should be transformed to the SEQUENCE type. In a sequence, a base may be suddenly marked with an 'N' character, which means after the primary processing the process was unable to identify the base. Because of the way the sequencing machines work, this only happens at the end of the sequence. So, it was decided to only cut the sequence short at the moment an 'N' character is registered.

   For example, if the sequence were $ACGGCGCATTACNNAN$, the interface will only store $ACGGCGCATTAC$. This is justified by the fact that we are statistically certain that this

sequence will be matched correctly from the moment the read is more than 15-20 bases long. The statistical proof itself will not be covered in this thesis.

3. loading the qualities. Found on the fourth line for each read, the qualities for each base are stored. The information is stored in an array with the same length as the sequence.

SAM interface The SAM interface will write the current mapped read to a SAM file, one line for one read. The buildup of a SAM file is thoroughly described in 3.4.2.

For this implementation, some values of the SAM format are not important and should be set to a default value. So it came naturally to create an "init" function, in which these default values are assigned.

- RNext should be set to the '*' character;

- Pnext should be set to 0;

- TLen should be set to 0;

Then, to write the line in the output SAM file, a function was created which accepts an attribute of the *MAPPED_READ* format and writes it as a line in the file.

6.3.2 Memory management

6.3.2.1 malloc and sds_alloc

The allocation of memory in a C program is usually done using the "malloc" function. However, on the used SoC, a distribution of Linux is running. Like in most operating systems, this Linux distribution uses a virtual address space to enlarge the available RAM virtually.

The original idea for the project was to be able to accelerate certain functions using the programmable hardware available in the SoC chip. Since this hardware should be able to access the data in the memory, there two options:

1. Build a copy of the address translator on the hardware. This would require a lot of work and would slow the whole process of looking things up in memory down.

2. Let the software interface the physical memory so that we don't use the virtual addresses. This solution was used in the final implementation

Luckily, Xilinx has published a library with an *sds_alloc* function. This does the same as the malloc function in the C language but in physical memory instead of the virtual memory.

6.3.2.2 Allocating memory

To allocate memory, the following formula was used:

```
memory_pointer = (TYPE*) sds_alloc( length * sizeof(TYPE) );
```

The sds_alloc function returns a pointer to the first address in memory that has been reserved. It turns out that this pointer should be cast explicitly to the type used.

- In case of reserving space for the sequence and the reference, the "TYPE" part in the formula should be filled in by the BASE type. For the length part of the formula, parameters were created named seqMax and refMax respectively.

- When space for the alignment matrix will be reserved, the "TYPE" part in the formula should be filled in by the CELL type (for more explanation on the CELL-type, see 6.3.3.1). As for the length of the space to reserve, this should be equal to refMax times seqMax.

6.3.3   The alignment

6.3.3.1   Parameters and types

A naive approach to implementing the S-W algorithm might be to have the alignment matrix filled in by the values only. If we would use this approach, it would quickly become clear that the backtracking is impossible, since we also need to know where the value originates from. Also, when generating the CIGAR-string, it is good to know which direction it originates from, as it implies a match (M), insertion (I), or a deletion (D). Since all this information should be stored in the alignment matrix, it seemed a good idea to create a new type. This type was called *CELL*.
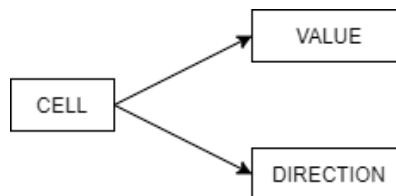
Figure 6.7: The type created to store the information for every cell in the alignment matrix

The cell stores the place it originates from, using a *DIRECTION* attribute. There are 4 possible directions a value can originate from: zero (coded as 0), diagonal (1), up (2), left (3).

Furthermore, it stores the value using a special type *CELL_VALUE*. The size of values this type can store should be greater than the length of the sequence times the similarity score. Keep in mind that to calculate the maximum, this value-type should be able to go negative.

6.3.3.2   Code structure

The alignment layer   The alignment layer does everything in the alignment of the sequence that has nothing to do with the matrix fill in. That functionality is outsourced to a separate function.

Also, for the CIGAR string, a certain threshold was programmed in. The CIGAR string of a matched read consists of multiple parts. For example: $6M1I7M$ consists of 3 parts; $6M$, $1I$ and $7M$. By limiting the number of parts that are allowed we can define a threshold by which a sequence is considered as "aligned". If both the forward and reverse sequences go over this limit, the sequence will be mapped as "unmatched".

1. Fill in the matrix using the forward direction of the sequence.

2. Store the maximum value of the matrix.

3. Generate the CIGAR string for this forward direction. We have to do this so early because you need the full matrix for the CIGAR generation, and we want to reuse the memory for the matrix according to the reverse sequence.

4. Check if the CIGAR limit has been exceeded. If not, then store the position of the map.

5. Reverse the sequence

6. Repeat for the reverse sequence.

7. Check for the limit of the CIGAR. If it was exceeded in both forward and reverse sequence, mark it as unmatched.

8. Check which read fit best (the forward or reverse one) by comparing the maximum values in the mapping. Assign the best one to the MAPPED_READ type.

The fill in layer   This is probably the easiest layer of them all, but will also be one of the most computationally demanding ones. It skims over every cell in the matrix, except for the first row and first column, and uses the cell generation layer to generate the cell on that specific spot. Also, while having an iteration that goes over every cell, it became a good idea to use this iteration to find the maximum cell in the matrix, which is the starting point for the backtracking.

**The cell generation layer**   This layer consists of 3 parts:

1. Generate the 3 values originating from diagonal, up, and left cells, using the formulas described by the S-W algorithm. For this step, the parameters s (for the similarity score) and gp (for the gap penalty) were created. For testing, These were mostly kept on $s = 3$ and $gp = 2$, but can be easily changed according to one's needs.

2. Determine which of these three values is the greatest. If all negative, choose 0.

3. Assign the correct value and direction of the newly generated cell.

This layer must be efficiently coded, and since it runs for every cell (and there are a lot of cells), it can be expected to be the core of the program.

6.4   Implementation results

6.4.1   The IGV software

The free and easy to download program IGV (or Integrative Genomics Viewer) is a visualization tool for exploring the genomic dataset, such as mapped reads. In practice, it is used quite often to examine the results from mapped reads.
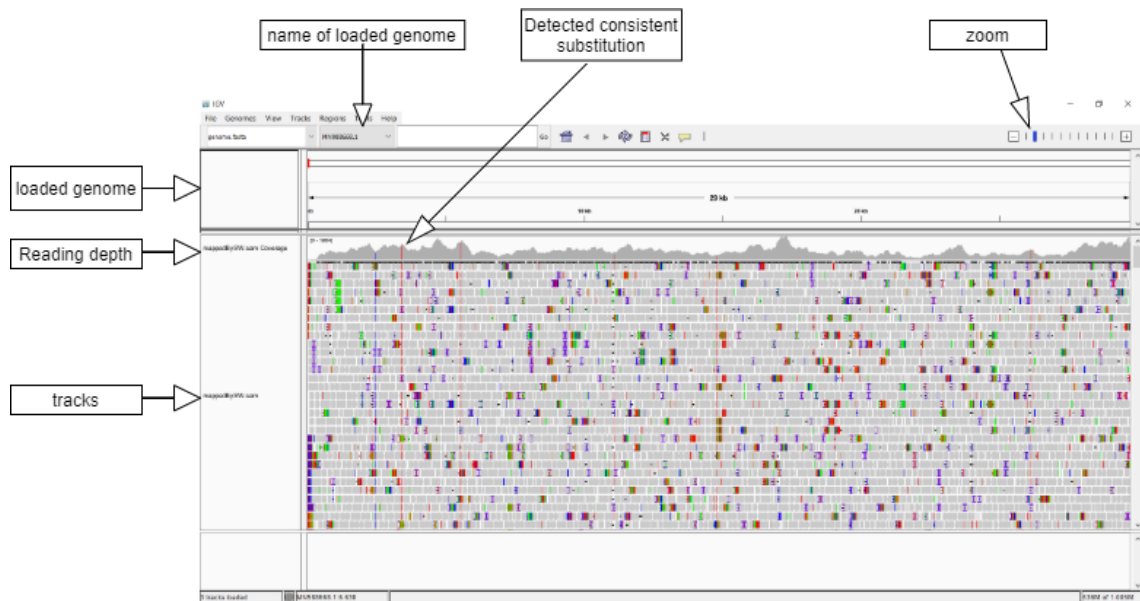
Figure 6.8: The layout of the IGV analysing software

The most important functions in IGV for this thesis are:

- It color-codes insertion, deletions or substitutions, so it is easy to see "from a distance";

- It can display the read depth for every base in the genome;

- If the read depth is sufficient, it can also determine if an indel or substitution is part of the read, or is consistent enough throughout all the reads to show it in the full genome;

- The sequence direction is indicated by an arrow when examining a specific read.

Figure 6.9: A few reads up close in the IGV analysing software. Notice how the color coded bases are substitutions

6.4.2   Sample data

To try the implementation on the human genome directly seemed way too ambitious. Therefore, it seemed appropriate to try the implementation first on an organism that has a genome of only a few thousand bases in length. Since this thesis is performed around the spring of 2020, the "coronavirus" (or SARS-CoV-2) seemed like an appropriate candidate. After a quick google search, it turned out that this virus had a genome consisting of merely 29 thousand bases.

The genome of the coronavirus was found easily online with reference number MN988668.1 in the genome bank of NCBI.

The FASTQ files for the coronavirus used in this theses were found in the SRA (sequence read archive) of the NCBI, submitted by the University of Washington.

Of course, when fitting these fastQ files through the implementation from this thesis, we have to be able to compare it with an implementation which is certainly correct. There consists an online tool called galaxy where a lot of existing bioinformatical algorithms and programs can be performed on given sample data using cloud computing.

So, by converting the fastQ files given by the SRA of the NCBI using one of these available applications, we can get a pretty good reference to match our results against. As the application to compare to, the Bowtie algorithm was chosen, since it is commonly used for reference genome mapping in practice.

6.4.3   Results

We will compare the results from the sample data using the online bowtie application and the implementation described in this chapter.

After running the software implementation overnight with the unmapped sequences from the coronavirus, we obtained a dataset of mapped reads. Both these mapped reads and the dataset obtained by using the Galaxy online tool were imported into IGV for comparison, which can be seen in figure 6.10
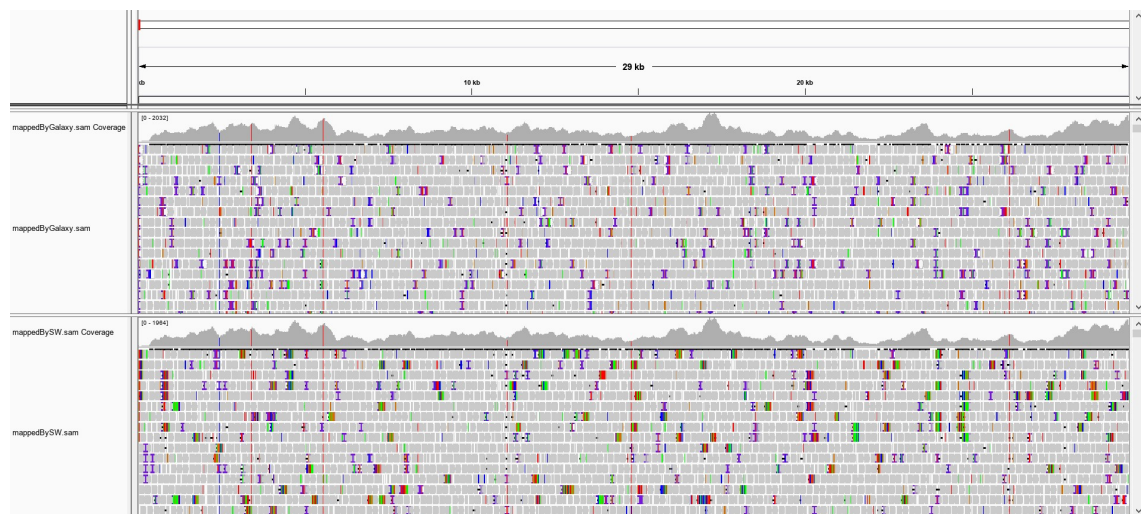
Figure 6.10: A comparison of the data obtained by Galaxy (top) and the data obtained by the implementation discussed in this chapter

It is immediately obvious that the implementation is working correctly since the reading depth graphs are approximately the same. Furthermore, IGV was able to detect and identify consistent substitutions or indels in the reads.

If you look closely to figure, some small differences between the exact reads are visible. This will probably be because both the algorithm and the parameters were a bit different.

The implications for the coronavirus data   The coronavirus is constantly changing because of the mutations of some bases in the genome over time, just like the flu. In this way, it is possible to trace where infections originate. For example, the corona in China can have slightly different bases than from Italy or Spain. We can identify these changes by sequencing the whole genome and looking for consistent substitutions or indels over the whole dataset.

From these results shown by IGV 6.10, some mutations are shown, so we can assume that the coronavirus which was sequenced in Washington (taken from a US patient) has some slight differences with the original Chinese sequenced genome.

Chapter 7

Acccelerating the software implementation using HLS

7.1 Analysing software performance

In the SDSoC environment, it is possible to analyse the software running on the SoC chip using a TCF profiler. After running this analysis, the TCF profiler returns an overview of the functions, sorted on the amount of time spent when running. The analysis is shown in figure 7.1
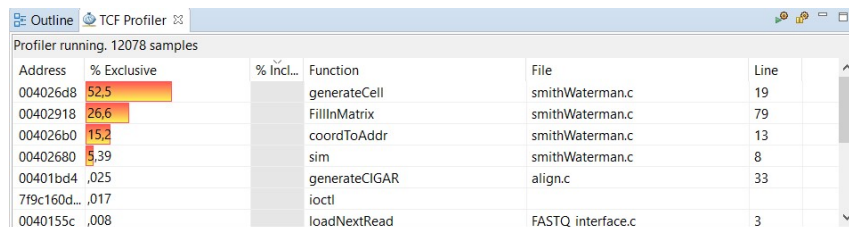

Figure 7.1: a TCF profile of the software implementation

When examining this analysis, we should keep in mind that the generateCell, coordToAddr and sim functions are inline functions used in the FillInMatrix method. Just as suspected the software spends almost all of its time in these methods, so it's worth it to try to accelerate these functions.

7.2 Recoding parts of the software to be more hardware friendly

Back in 2011, Vermij E. did a thesis on RVE (recursive variable expansion). In it, he discusses the most efficient ways to program a processing element to generate one value in the alignment matrix. His results can be found in figure 7.2.
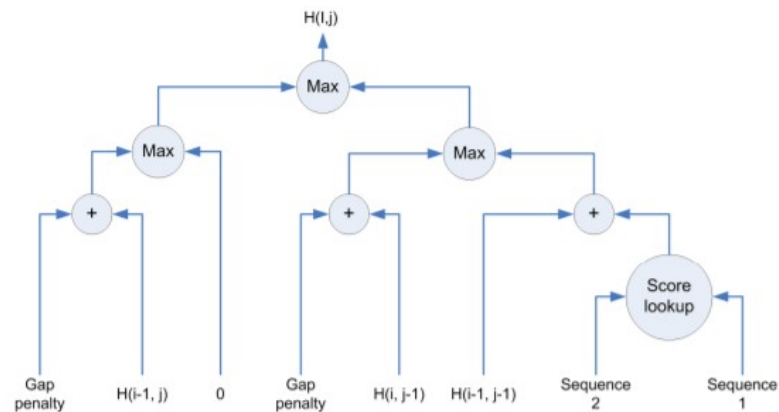
Figure 7.2: The optimal processing found in the thesis from Vermij E.

It seemed like a good idea to reimplement the generatecell function, using this newly found scheme. However, it is also important to keep track of where the value comes from. Therefore, the following (new) code was adopted for generating a cell:

```
//calculate the possible  values
CELL diagonalCELL = { diagonal.value + sim(refVal, seqVal), 1 };
CELL leftCELL = { left.value - gp, 2 };
CELL upCELL = { up.value - gp, 3 };
CELL zeroCELL = { 0, 0 };


CELL upstreamA = (leftCELL.value > upCELL.value) ? leftCELL : upCELL;
CELL upstreamB = (diagonalCELL.value > zeroCELL.value) ?
                                        diagonalCELL : zeroCELL;


CELL newCell = (upstreamA.value > upstreamB.value) ? upstreamA : upstreamB;

//Return the cell:
return newCell;
```

Where the second attribute in the CELL type is the direction.

7.3   Hardware acceleration

7.4   Comparison with the software

Chapter 8

Conclusion and future research

Gap opening and extension

BFAST algorithm

BASE type memory efficient

FTP for easy on and offloading the data

SEQ_INDEX type maken memory efficient (75-300)

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Appendix A

Uitleg over de appendices

Bijlagen worden bij voorkeur enkel elektronisch ter beschikking gesteld. Indien essentieel kunnen in overleg met de promotor bijlagen in de scriptie opgenomen worden of als apart boekdeel voorzien worden.

Er wordt wel steeds een lijst met vermelding van alle bijlagen opgenomen in de scriptie. Bijlagen worden genummerd het een drukletter A, B, C,...

Voorbeelden van bijlagen:

Bijlage A: Detailtekeningen van de proefopstelling

Bijlage B: Meetgegevens (op USB)

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