



Cairo University

OPTIMIZING VARIANT CALLING PERFORMANCE FOR HOTSPOT CASES BASED ON ION TORRENT SEQUENCING TECHNOLOGY

Basma Nasser Abd Elsalam Abd Elfattah

A Thesis Submitted to the
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in Partial Fulfillment of the
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MASTER OF SCIENCE
in
Biomedical Engineering & Systems

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Under the Supervision of

Prof.Dr. Ayman Eldeib

Dr. Mohamed I. Abouelhoda

.....
Professor of Biomedical Engineering
Systems & Biomedical Engineering
Faculty of Engineering, Cairo University

.....
Associate Professor
Systems & Biomedical Engineering
Faculty of Engineering, Cairo University

FACULTY OF ENGINEERING, CAIRO UNIVERSITY
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Dedication

Table of Contents

Acknowledgments	i
List of Tables	vi
List of Figures	vii
Abbreviations	viii
Abstract	ix
1 Introduction	1
1.1 Problem Definition	1
1.2 Thesis Objective	2
1.3 Thesis Organization	2
2 Biological Background	3
2.1 Genetic Background	3
2.2 DNA sequencing	4
2.3 1000 Genome projects	5
2.4 HapMap project	5
2.5 BRCA genes	5
2.6 Genetic variation	6
2.6.1 Single-nucleotide polymorphism	6
2.6.2 Insertion/Deletion polymorphism	6
2.7 Data Sequences Format	7
2.7.1 Fastq	7
2.7.2 FASTA	8
2.7.3 BAM/SAM File Format	8
2.7.4 VCF Format	10
2.7.5 Ion torrent Hotspot File	13
2.7.6 Target File	14
2.8 DNA Sequencing Generations	14
2.9 Next Generation Sequencing	15
2.9.1 NGS Strength	16
2.9.2 NGS Limitations	16
2.9.3 NGS Workflow Overview	16
2.10 Next Generation Sequencing Technologies	19

2.10.1	Roche 454 sequencing	20
2.10.2	Illumina Sequencing	20
2.10.3	Ion torrent: Proton / PGM sequencing	21
2.10.4	SOLiD Sequencing	22
3	Recent Variant Calling Pipeline	23
3.1	Overview of variant caller pipeline	23
3.2	Read Mapping/Alignment tools	24
3.2.1	Burrows-Wheeler Aligner (BWA)	25
3.2.2	TMAP	25
3.3	Variant Calling	26
3.3.1	GATK	26
	GATK Features	26
	GATK workflow	27
	GATK Releases	32
3.3.2	Torrent Variant Caller	33
	TVC Workflow	33
	Torrent Variant Detection Algorithms	35
3.4	GATK and TVC Summary	37
4	Contribution and Methods	39
4.1	Sequencing implementation	41
4.2	Parallel Computing	42
4.2.1	Overview	42
4.2.2	Advantages of Parallel Computing	43
4.3	Parallelism Implementation Types	44
4.4	Parallel Implementation	45
4.5	Methods	46
4.5.1	Datasets	46
4.5.2	Pipelines	47
4.5.3	Aligning Tools	47
	BWA Mem	47
	TMAP	47
	Alignment File Processing	48
4.5.4	Variant Calling Tools	48
	HaplotypeCaller	48
	Torrent Variant Caller (TVC)	48
	Hotspot Preparation	49
	Modified Hotspot (mHotspot) Preparation	49
	Modified TVC (mTVC)	49
4.5.5	Selected Parameters	50
4.5.6	Parallel Algorithm	51
4.5.7	Hardware Specs	51
5	Results and Discussion	52
5.1	Sequence Data Sets and Variant Calling Pipelines	52
5.2	Variant Callers Comparison	52
5.3	Shared Variants Validation	53
5.4	Variants selection and filters	56

5.5	Performance Results	56
6	Conclusion and Future Work	58
6.1	Conclusion	58
6.2	Future work	59
	References	60
	Appendix A	62
	Appendix B	64
	Appendix C	67

List of Tables

2.1	Summary of next generation sequencing technologies	22
4.1	Summary of Data Used	47
5.1	Variant callers pipeline results.	53
5.2	Results of NA12878 using TVC with different hotspot files	55
5.3	Results of BRCA2 using TVC with different hotspot files	55

List of Figures

2.1	DNA Helix strand.	4
2.2	Single-nucleotide polymorphism example.[7]	6
2.3	Insertion/Deletion polymorphism example.	7
2.4	A chart demonstrating how the speed of DNA sequencing innovations has expanded since the early strategies in the 1980s.	15
2.5	NGS workflow from nucleic acid extraction to variant annotation.	17
2.6	NGS library is prepared by fragmenting a gDNA sample and ligating specialized adaptors to both fragment ends.	18
2.7	library is loaded into a flow cell and the fragments hybridize to the flow cell surface. Each bound fragment amplified into a clonal cluster through bridge amplification.	18
2.8	Reads are aligned to a reference sequence with read aligner software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.	19
3.1	Unified steps of the variant calling pipelines.	24
3.2	GATK workflow.	27
3.3	Preprocessing Steps.	29
3.4	Variant discovery steps.	31
3.5	Evaluation steps.	32
3.6	Flow diagram of torrent variant caller.	35
3.7	Torrent Variant algorithm flow	37
4.1	Updated variant caller pipeline. (mTVC)	40
4.2	Serial implementation diagram	42
4.3	sequential computing, algorithm is constructed and implemented as a serial stream of instructions. These instructions are executed on a CPU on one machine. Only one instruction executes at a time, after that in- struction is finished, the next one is executed.	42
4.4	Parallel computing - breaking the problem into independent parts so that each processing element can execute its part of the algorithm simultane- ously with the others.	43
4.5	Parallel implementation diagram	46
5.1	Intersection of variants from running TVC on NA12878 sample with hapmap 3.3 and mTVC using the same parameters.	54
5.2	Execution time for NA12878 and BRAC2	57

Abbreviations

BAM	Binary Sequence Alignment/Map.
BRCA	BReast CAncer genes.
DNA	Deoxyribonucleic Acid.
ENCODE	Encyclopedia of DNA Elements.
GATK	Genome Analysis Toolkit.
GIAB	Genome in a bottle
Hapmap	Haplotype Map.
HC	HaplotypeCaller
InDel	Insertions and DELetion.
mTVC	Modified torrent variant caller script.
NGS	Next Generation Sequencing.
PCR	Polymerase chain reaction
PE	Paired-end.
PGM	Personal genome machine.
SAM	Sequence Alignment/Map.
SNP	Single nucleotide polymorphism.
TMAP	Torrent Mapping Alignment Program.
TVC	Torrent Variant Caller.
VCF	Variant call Format.
VQSR	Variant Quality Score Recalibration

Abstract

In last few years, Next generation sequencing (NGS) revolutionized in DNA sequencing research. The latest major technologies released are Illumina and Ion Torrent. In this study, we analyze the performance of calling variants using both platforms. In order to compare between both platforms, we used two sequenced data sets from ion community, which contain flow spaces required by Ion Torrent. We are concerned with the execution time of both platforms. Ion torrent detects genome variants faster than Illumina but with low accuracy. Moreover, we found that Ion Torrent called slightly more variants but with higher false positive rate. The hotspot option provided by ion torrent variant caller provides more accurate variant calling positions as the filtration restricted to specific positions in the genome, but this leads to time consumption. Here, we enhanced the execution time to attain the accurate positive variants using torrent variant caller by using the NOCALL variants as hotspot regions to torrent variant caller (TVC). We developed two dependent packages the first one to create the new hotspot file (mHotspot) required by TVC and the second one to rerun the TVC with mHotspot file generated to get the exact positions of variants.

Chapter 1

Introduction

Sequencing technologies are evolving rapidly; in 2011, a lot of sequencing technologies were developed. The advances of sequencing technologies make it possible to detect enormous number of potential disease-causing variants; also, next generation sequencing (NGS) data has been used for diagnostic purposes. This is due to the improvements made to the bioinformatics tools used to analyze the massive data produced by NGS instruments.

NGS technologies use same workflow for searching for mutations in a patient. Align the raw data to human reference genome, and identify single nucleotide variants and INDELs that cause the phenotype of interest. We have to decide the best tools to use for each stage of the pipeline. There are various tools for sample sequencing, but the most important parts are to select the proper aligner and variant caller to achieve the optimal results for SNPs and INDEL variants.

Different pipelines with different combinations between aligners and variant callers identify Gene variants (SNPs and INDELs). The most widely used aligners are BWA. GATK and torrent variant caller (TVC) are popular tools for variant calling [1].

In this thesis, we describe the pipelines and typical tools for read alignment and variant callers. In addition, we will elaborate in details the results of using ion torrent workflow for detecting variants from two different samples (NA12878 and BRCA2). In addition, we will describe new workflow for TVC in order to enhance time of execution using our plugin mTVC.

1.1 Problem Definition

Several studies have conducted comparison between different variant callers. From these studies, quail et al. BMC Genomics 2012 [2] mentioned that ion torrent has poor performance with only 65% of genome is covered with high quality reads compared to other platforms. It was mentioned that the overall rate of SNP Calling was slightly higher in ion torrent than Illumina, 82% of SNPs being called correctly. But far less false positives ion torrent from Illumina for SNPs and InDels. They noted that the inbuilt automatic variant calling in ion torrent calling only 1.4% of variants and to detect small InDels using proton platform we need to develop more accurate variant calling technique [4]. From torrent variant caller [5], using hotspot and target file, TVC evaluates the variants in specific locations and regions in order to achieve high accuracy and increase the true

positive variants. However, using hotspot file takes days to process and generate variants report. Although, TVC takes minutes to generate the variants without using hotspot file.

1.2 Thesis Objective

The main purpose of the thesis is to optimize the processing time of Torrent Variant Caller (TVC) to call variants when using hotspot file. We achieved this by using the NOCALL variants and reuse it as hotspot positions to TVC. Additionally, we present solution to automate the generation of new hotspot file and enhance the run time of torrent variant caller to detect variants.

1.3 Thesis Organization

In chapter 2, we will introduce briefly the biological terms related to the problem and DNA sequencing generations and technologies. As well as, we defined an overview of NGS. A detailed review of variant calling pipelines presented in chapter 3. Chapter 4 review parallel computing implementation types, advantages, and the contribution also elaborate the steps and tools used to achieve. The results are discussed in chapter 5. Then finally, chapter 6 concludes and discusses our future work. Appendix A and B summarize the common steps to run variant calling pipelines. Appendix C shows the steps to run the developed package.

Chapter 2

Biological Background

In this chapter, we introduce the basic concepts of biology and bioinformatics that are needed in the thesis. We will show the main topics of bioinformatics such as sequencing technologies, biological sequence databases and sequences format.

2.1 Genetic Background

In 1857, Gregor Mendel performed an experiment with plants that increased the interest in the study of genetics. After that, Friedrich Miescher in 1869 discovered a substance he called "nuclein" or "nucleic acid" that exists only in the chromosome later he isolated sample of material known now as "DNA". James Watson and Francis Crick began to examine the DNA's structure guided by X-ray diffraction photos of DNA fibers taken by Maurice Wilkins. They created double helix model with little bars called nucleotides connecting the two strands.

Our human body is build of billions of cells. Each cell varies according to the organ function. DNA (deoxyribonucleic acid) considered as the blueprint for our life, which exists in the nucleus of the cell. It is present in all living organisms from the smallest bacterium to the largest whale. DNA determines the genetic characteristics and the behavior of organism including the diseases that may develop.

The DNA structure includes four main nucleotide bases, Adenine (A), Cytosine (C), Guanine (G) and Thymine (T), attached to sugar and phosphate string. Each DNA bases bound to each other using hydrogen bonds to form units called base pairs, A with T and C with G. Each base attached to a sugar and a phosphate molecule as shown in figure 2.1. Human DNA contains around 3 billion bases. These bases sequenced according to the organ and information that needs to be transmitted.

The nucleus contains different number of chromosomes for each organism. The set of chromosomes make up a genome. The human genome arranged into 46 chromosomes. The sequence of pieces of DNA called genes that act as instructions to make molecules called proteins. According to Human Genome Project estimation, humans have between 20,000 and 25,000 genes. Every human has pairs of each gene, inherited from each parent.

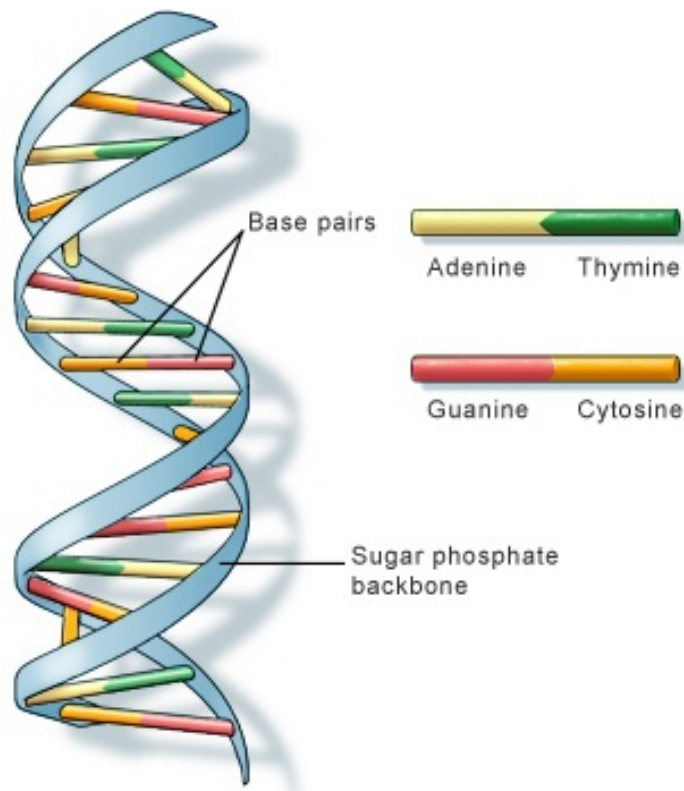


Figure 2.1: DNA Helix strand.

2.2 DNA sequencing

DNA sequencing is a laboratory method to determine the order of the nucleotides 4 bases (Adenine (A), thymine (T), cytosine (C), guanine (G)) in a piece of DNA strand. we cannot sequence a genome from start to end. We have to break the DNA strand into many smaller fragments, then sequencing the pieces and label each base individually with different colors. The detailed flow described in the next chapter. Previously, to sequence one or two genes we may spend several years. DNA sequencing technologies have been developed rapidly since the completion of Human Genome Project. We can sequence the entire genome in few hours and with less money.

DNA sequencing can tell the scientists the genetic information in particular DNA segment.

In addition, sequencing can be used in molecular biology to allow researchers to identify the changes in gene that may cause disease and identify potential drug targets. DNA sequencing can be used to study how different organisms related to each other and how they evolved. Moreover, we can determine the risk of genetic diseases.