Modern systems for large-scale genomics data processing in the cloud

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Chapter 1

Introduction

1.1 Context and Motivation

In the 17 years since the publication of the first draft human genome[67] the fields of genomics and molecular biology have undergone a major shift. The direction of this shift is towards an increasing adoption of computational approaches alongside experimental methods, bringing both of these fields of study into the realm of information science. This transition has been facilitated by two major factors - the advent of next generation sequencing[114], and the development of the Internet and cloud computing[12]. Next generation sequencing has been responsible for bringing down the cost of DNA sequencing to the point where it has become possible to sequence and study entire populations of individuals[51], while the Internet and cloud computing are democratising access to large-scale computational resources such that computation on big datasets, which was previously only accessible to large institutions, is becoming tractable to a growing group of researchers and citizen scientists.

The continued appetite for sequencing of larger and larger cohorts of individuals by the research community is driven by the desire to better understand the evolutionary history of the human species[63], to identify causes and mechanisms of action of rare genetic diseases that affect a very small proportion of the population[10], and to elucidate and potentially target the genetic component of more common diseases such as cancer[137], heart disease[11], or dementia[116] that place a heavy burden on our society. All of these factors together mean that the need for the generation and interpretation of genomic data is growing at an unprecedented scale.

Yet, the analysis of DNA sequencing data to study human genomes remains a largely unsolved problem. The protein coding sequence of the human genome, its *exome*, constitutes roughly 1% of the human DNA and successful studies have carried out exome-based analyses on cohorts at the scale of tens of thousands of individuals[72]. However, the other 99% of the human genome, it's non-coding regions, contain crucial information such as gene regulatory elements[18] that are essential to our full understanding of the mechanisms and processes that are underlying the human genetic landscape. Given current technologies, Whole Genome Sequencing

(WGS) is considerably more expensive and generates data-set sizes at the petabyte (PB) scale that are challenging for even the largest international consortia to tackle [122]. WGS studies at 100,000 participants scale that are planned for the coming years[32] will further increase data-set size and complexity by several orders of magnitude, a challenge that is presently unanswered by the current generation of bioinformatics infrastructures and algorithms.

A bigger and more distant challenge is the development of clinical sequencing and genomics which will truly bring whole-genome sequencing applications to population scale. Currently DNA sequencing has limited adoption within the clinical practice with applications limited to rare Mendelian disorders[71] and certain types of cancers[109] where a small set of genomic loci is interrogated via a gene panel[6] with a set of well-delineated disease sub-types based on these genetic markers. The use of whole-genome sequencing for clinical applications is presently nearly non-existent due to its high cost compared to the clinical utility of its findings, yet the potential for the impact of this approach remains substantial as certain genomic variants such as Structural Variations (SVs) typically have a large effect on an individual's phenotype due to their size[105], but are generally not amenable to interrogation via gene panels.

The magnitude of the opportunity for improvement in the space of DNA sequencing and genomics is thus clear to us - we seek a way to improve the current methods of DNA data analysis such that it becomes tractable and cost-effective to undertake whole-genome sequencing studies within research and clinical contexts at the scale of hundreds of thousands to millions of human genomes.

1.2 Challenges and Problem Statement

Let's examine the key challenges that need to be addressed in order to enable efficient genomic data analysis at the scale that is desired by the research and clinical communities.

Several broad groups of challenges are identified below and further examined throughout this thesis:

Data Set Size - The size of the raw genomic data generated by population-scale studies will be hundreds to thousands of petabytes making it impractical to move and make copies of the data[123].

Data Retention - The cost of generating the data is significantly higher than the cost of storing the data, thus making it impractical to throw away the raw data after initial analysis[94].

Data Formats - The data formats used for storing genomic data are primarily large size character and binary files (FASTA, SAM, BAM, VCF)[80, 24] that have loose specifications and scale poorly to large cohort sizes. File indexing

structures typically support indexing by genomic coordinate only, thus limiting queryability.

Data Fragmentation - The data will be generated at multiple sequencing centres located in different jurisdictions with a wide variety of genomic data handling requirements. Data processing must proceed at multiple locations that respect the requirements of each jurisdiction[93].

Data Type Diversity - Comprehensive characterization of a person's genome that is useful in a clinical setting implies the collection and integrative analysis of many diverse data types - including germline[83] and somatic[48] genomic variants, transcriptomics[135], epigenomics[64], metabolomics[132], and clinical information. Uniform collection, processing, and integration of these data types is required to successfully associate the role of this genomic variation on disease phenotypes[109].

Data Processing Stages - Data processing for genomics analysis proceeds through a sequence of stages from base-calling, to quality-control, to genome alignment, to variant calling, to annotation, to downstream analysis[28]. Each stage typically has non-trivial computational requirements needing several days on a multi-core machine to complete with increased failure risk as a function of data set size. Intermediate results from one stage are often required as input for downstream stages. Fully sequential processing makes inefficient use of the data by redundantly loading and interrogating the data in memory over a series of passes through the sample.

Toolset Fragmentation - Although comprehensive genomic characterisation of each sample is typically of interest to researchers, specific bioinformatics tools only provide solutions to a limited subspace of the overall problem, thus requiring integration of multiple tools that may produce incongruent outputs and compete for resources producing computational bottlenecks.

Having listed these challenges we attempt to restate the problem in simpler terms before providing a high level overview of the types of approaches and solutions that will be developed and considered in detail in the body of this thesis in order to deliver a conceptual and practical framework for the effective management of genomic data at the desired scale.

Our problem statement is then as follows:

Human genomic data sets will, in the future, be generated for analysis in various locations throughout the world, at the aggregate rate of multiple petabytes of data per day in the context of disease and clinical practice. The desired outcome of these analyses is the comprehensive characterisation of genomic features and their association with phenotypic variables of interest[139]. The goal of the research community is in capturing the maximum number of samples – N, with high accuracy – A, to increase statistical power of studies[59], while the interest of clinicians is to capture specific individuals with high accuracy – A, and in the shortest possible time – T, in order to inform clinical decision making[133]. Both parties wish to do so at

minimal possible total cost – $C = c_g + c_s + c_a + c_r$, taking into account the cost of data generation, cost of data storage, cost of data analysis, and cost of subsequent data retrieval. Because of the high cost of generating this data each time, the data, once generated, will need to be stored for the foreseeable future. The overwhelming data set size prevents data movement between locations, requiring analysis algorithms to be colocated with the data.

The analysis is hampered by reliance on data formats that have not been designed for operation at such large scale and the necessity to execute a variety of computational algorithms[77, 147, 5, 90] on the data that have been individually developed by different authors within an academic context, using different technologies that compete with each other for computational resources, and at-times produce contradictory results that require human intervention to integrate. The underlying assumption of genomic coordinate-sorted ordering and traversal of the data made by most algorithms limits the modes of reasoning about the dataset to a series of pre-processing steps, followed by another series of coordinate-wise traversals through the data, which impose severe processing time costs, such as the requirement to have generated, seen and sorted all of the data, before an analysis can proceed as well as the inability to stop and interpret analysis results mid-processing.

The optimization problem of maximising N, and A, while minimizing C for research purposes remains unsolved for values of N above 3000 samples when it comes to high-coverage whole genome sequencing, while the problem of maximizing A, and minimising T, and C is presently not solved in the clinical setting for any sample size. It is our proposed solution for tackling these issues that we turn to next.

1.3 Proposed Solution

We assume that N, the number of samples that can be successfully sequenced will depend almost entirely on the total cost C, which itself, among other factors, is determined by the desired accuracy and processing time. We thus focus most of our efforts on the joint optimization of cost, accuracy, and time as necessary conditions for the maximisation of effective sample size N and enablement of whole genome sequencing for clinical practice.

We note that the cost of data generation C is dependent on the sequencing technology used, the underlying chemistry, and the cost of the reagents[92]. Improving these characteristics falls outside the scope of our discussion, and we assume the cost of the data generation component c_g of C to be constant throughout this thesis.

We establish and discuss at length the characteristics of the optimization function in the body of the thesis but here note briefly that analysis accuracy A is evaluated along the usual dimensions of sensitivity and specificity and can generally be improved by generating more data for a given sample up to a theoretical maximum inherent in the sequencing technology used and the nature of the analysis algorithms employed. Generation of more data naturally leads to increased analysis time T and cost C. The time to accomplish the analysis can be reduced by either giving up ac-

curacy (by looking at less data, or using faster but less accurate algorithms[78]), by increasing the level of parallelisation within the computational pipeline i.e. parallelising steps that are currently sequential[68], or by utilising additional computational resources, thereby increasing costs. The various components of cost, in turn, can be optimized by improved data storage and retrieval structures[100] (via multi-level caches and hybrid storage media, for example), by improved-efficiency analysis algorithms, and by reduction of analysis accuracy and increase of analysis time (via cheaper hardware).

It is clear from the discussion above that cost, accuracy, and processing time are not orthogonal concerns i.e. changes in one may lead to changes in the other two. It thus appears that no optimization effort is likely to simultaneously satisfy the requirements of all parties that are interested in large scale genomic analysis, and a successful computational framework for delivering such analyses must allow efficient and dynamic optimization of these parameters to fit the needs of the end user. This is typically not the case for present day genomics frameworks because of the sequential way they look at data[89, 129] i.e. all of the data is generated before it is processed by downstream tools, and accuracy and processing time need to be decided on before launching a set of tools because they step through the genome in coordinate-wise manner.

To address these challenges we develop and describe within this thesis a new computational framework, called Rheos, that is based on the concepts of data streaming, cloud computing, and service orientation to provide a comprehensive toolset for genomic data analysis that can potentially scale to processing of millions of genomes while arming its users with the capability to make timely, responsive, and principled decisions about the tradeoffs between analysis cost, accuracy, and duration.

Three distinct characteristics set Rheos apart from current generation genomic analysis frameworks and each of these allows us tackle some of the issues and challenges described in Section **??**. These are:

- Service Orientation
- Event and Data Streaming
- Random Data Ordering

Service orientation[33] allows us to decompose the overall problem of comprehensively reasoning about genomic data into a set of small loosely-coupled components, each of which is optimized to tackle a particular well-defined subset of the complete set of requirements of the system. Each service has a contract that it makes with its clients, it has an explicit set of inputs that it knows how to process, it has an interface that defines the modes of communication it supports, it has a set of outputs that it produces according to its capabilities, and it has a set of operational characteristics that makes explicit commitments about the service's reliability, speed, etc[101]. This has a number of benefits - a service can be small enough that it optimizes the solution to a particular problem without being subject to the same

competing constraints that larger tools are subject to, which provides opportunities for improved performance and hardware utilization. As long as the service respects its input and output commitments it is free to maintain arbitrary internal representations of the data enabling optimization of data storage and query costs (c_s and c_r). A service can be monitored such that hardware is allocated elastically up and down based on demand to ensure optimal utilization, as well as providing a continued measure of whether the service is meeting its operational reliability requirements to its clients[21]. This is especially useful in contexts where demand for certain calculations is highly variable.

The issue of inter-service communication is of major importance because of the large size of the data-set and the potential for various difficult-to-debug run-time race and error conditions inherent in a distributed system[44]. Currently, most bioinformatics tools do not communicate with each other directly via an API, instead they use popular file formats such as SAM/BAM/CRAM[80, 42], and VCF[24], as well as a myriad of more esoteric file formats not only as a storage medium but also as a means of communicating information between each other. This paradigm hurts the ultimate scalability of the entire system because of the necessity to write data to disk and possibly move it over the network in order to enable communication across tools. Furthermore, a file-based information exchange mechanism forces a coarse-grained, sample-level, communication between components that wish to avoid tight coupling between each other, even though most of the reasoning about genomic data occurs at locus, or small locus-neighbourhood, levels[31].

Rheos adopts a data and event stream approach to accomplish scalable fine-grained communication between services[95]. This approach allows each service to listen to and produce data at the level of granularity that it needs to make decisions, and that its downstream dependencies are interested in (for instance at read, locus, or breakpoint levels). When primary data is ingested into the Rheos system (from a sequencer, or a data repository) the data stream can start to be analyzed immediately[52], unlike file-based systems that need to wait for the entire sample to transmit before beginning. This approach can potentially enable real-time analysis given sufficient allocation of computational resources[3]. Since the raw data is extremely large, it is advantageous to move this data between machines, and between disk and RAM as little as possible, thus instead of passing the raw data around the network various services pass around events of interest about the raw data amongst themselves[34]. When a particular service needs the raw data (rather than the corresponding events) for its decision-making it can be shipped this data as necessary, or it can be instructed to run on the host that has already cached this data in memory. Data streaming allows for extreme scalability, but a key challenge when dealing with data streams is that one is no longer guaranteed to ever be able to see "all of the data" for a particular sample, at least in any meaningful amount of time[43]. Because genomic algorithms frequently make use of various summary statistics accumulated over the data-set[102, 74], not being able reason over all the data at once means that approximations for these summary statistics are required. Rheos uses approximations calculated within time windows over the data stream[26, 7] and we consider their properties in detail in the body of this thesis.

A key assumption made by nearly all algorithms in the genomics space that

participate in variant calling and reason over sequence reads is that the reads are coordinate-sorted with respect to the reference genome to which they are aligned[80, 45, 15, 107]. The algorithms then proceed by traversing the genome in coordinate-wise fashion from the beginning of chromosome 1 to the end of chromosome Y interrogating each locus in turn by examining the set of reads that overlap that locus (a read pileup)[80]. Getting the reads into a state that is usable by these algorithms then requires, at a minimum, that all the reads for a given sample have been generated, have gone through QC[140], have been aligned[78], have been investigated for PCR duplicates[129], and have been sorted[129]. Each of these steps can take hours or even days to complete, especially on high coverage whole genome samples. We take a different approach with Rheos by relaxing the requirement for the reads to have been sorted before any variant calling can take place, and instead develop a set of variant calling algorithms that do not assume any particular order within the data that they observe. This allows Rheos to make use of sequence data as soon as it comes off the sequencing machine, thereby dramatically reducing the total time T required to process genomic data compared to the current generation of algorithms. Rheos accomplishes this by employing the service- and stream-based approaches discussed above to process each read on-the-fly as it moves through the system. The read is first assessed for quality, then aligned to a reference genome by the alignment service. This service emits an event with a coordinate that corresponds to the alignment. Variant calling services listen to this event stream and incorporate the evidence for genomic variation supplied by this read into their models of the genomic features that exist at that particular locus for that sample via a statistical framework based on an iterated application of Bayes' rule[145, 9].

Because current generation tools can see all of the data for a particular locus at once they can incorporate all of the evidence supplied by this data in a minimal number of calculations, corresponding to each particular algorithm[80, 45]. Rheos, on the other hand, to incorporate the same amount of evidence will need to perform a larger number of calculations in a redundant manner, incorporating the data as it is observed. This cost is compensated for, however, by the fact that Rheos can immediately incorporate new data about a particular locus when it becomes available without the need to have accumulated all of the data for all of the loci, generating significant time savings. Furthermore, because data arrives in no particular order the set of variant calls produced by Rheos at any given point in time represent a comprehensive characterisation of the sample as if the sample was sequenced at an average coverage consistent with the amount of data that has been observed so far. Observing more data is equivalent to raising the average coverage uniformly throughout the genome, thereby improving call accuracy[4]. This provides us with a framework to actively and dynamically trade off call-set accuracy A for processing time T and cost C as actual data is being observed thereby enabling novel applications whereby sequencing is abandoned early when issues such as sample-swap[36], or contamination[14] are detected. In addition, when sufficient accuracy is reached based on observed data at a particular locus, the framework may choose to stop looking at further data, whereas current generation approaches necessitate committing to a particular sequencing depth a-priori. Furthermore, because current methods iterate through the data in a coordinate-wise manner, their partial results are not really usable until the entire data-set has been traversed (as they represent only

a particular region of the genome), whereas Rheos call-sets represent progressive elaboration of a complete genomic characterisation and are thus usable at any level of accuracy that is fit for the purposes of the underlying analysis. We develop the details of the statistical framework used by Rheos and compare its theoretical and real performance to current generation frameworks in the body of this thesis.

We conceived of Rheos as a modern bioinformatics framework that aims to enable the large scale genomics studies of the future[32, 65, 86] in both research and clinical contexts by providing a toolset that allows for interpretation and comprehensive characterisation of high coverage human whole genome samples at the scale of millions of samples. In order to meet the diverse requirements of its users the framework allows users to make informed and dynamic tradeoffs along the optimization dimensions of of cost, accuracy, and time. Rheos unique abilities rest upon three characteristics that set it apart from current generation tools, these are: service orientation, data streaming, and random data ordering. Taken together these characteristics enable Rheos to perform at unprecedented levels of scale while retaining call-set accuracy and reducing per-sample processing time. We dedicate the main body of this thesis to the development of the theoretical framework underlying Rheos, exploring its characteristics, benefits and tradeoffs, discussing its implementation, and evaluating and comparing Rheos' performance to the current best practices in genomics algorithms on real data.

1.4 Thesis Outline

This thesis focuses on the development of the conceptual framework behind the Rheos platform, the theoretical properties of the various algorithms employed by Rheos, and the implementation and experimental validation of the framework on real data. Chapter 2 provides an introduction to the fields of genomics, including cancer genomics and clinical genomics, a survey of the main tools and algorithms that are commonly used in genomics is provided including the details of the underlying statistical models and operational characteristics. The chapter concludes with a look at workflow frameworks that tie individual algorithms together into computational pipelines. Chapter 5 sets up and describes the conceptual framework underlying Rheos based on the approaches of Service Orientation, Data Streaming, and Random Data Ordering, mentioned above. We describe the overall architecture as well as the model behind individual services that comprise Rheos and investigate the theoretical properties of the algorithms that underlie Rheos-based genomic analysis. Chapter **??** describes the actual implementation of the Rheos framework's components and investigates their operational characteristics. Chapter **??** is dedicated to the experimental evaluation of Rheos in comparison to other extant frameworks and algorithms using real genomic data. We conclude this work in Chapter 6 with a discussion of the results and an examination of the future direction of Rheos development.

Chapter 2

Background and Related Work

2.1 Genomics

The field of genomics is closely related to, yet distinct, from the field of genetics, which itself stems from the work of such seminal figures as Charles Darwin[25] and Gregor Mendel[49]. While genetics largely focuses on the study of single (or relatively small numbers of) genes - the *genotype*, and how genetic variation and mutation affect the physical traits of a given cell or organism - its *phenotype*, genomics focuses on larger scale events and mechanisms that tend to act on the entirety of an organism's genome, shaping its architecture and ultimately affecting its survival.

2.1.1 History of Genomics

Each living cell is a bio-chemical machine that carries out a number of complex behaviours such as interactions with the surrounding environment, motility, metabolism, and reproduction, that are necessary for its survival and proliferation, based on a genetic program that is encoded within the cell's DNA. The DNA is nominally subdivided into functionally distinct areas known as *genes*. The cell utilizes the program within each gene by first *transcribing* the DNA into an intermediary information-carrier molecule called RNA, and then *translating* this RNA into molecules called *proteins* that are utilized by the cell to carry out the majority of its functions. Understanding and interpretation of the underlying genetic program thus underpins our ability to comprehend the entirety of the different behaviours that each cell undertakes.

The success of this undertaking is contingent, first and foremost, on our ability to effectively read off the information encoded in the DNA, an activity known as *sequencing*. We are able to sequence DNA thanks to the pioneering work of researchers Rosalind Franklin[41], James Watson, and Francis Crick[136] who first elucidated the physical structure of DNA, then followed by the work of Fred Sanger[112, 111] who devised the first effective DNA sequencing method. The sequencing method

allows us to transform information that is physically encoded on the DNA molecule via a sequence of four distinct types of *basepairs* - Adenine, Cytosine, Guanine, and Thymine into a string stored on a computer using a four-letter alphabet - A,C,T, and G, thus turning DNA interpretation into a digital information processing problem.

While the entire length of the DNA of an organism ranges from several hundred thousand basepairs for simple organisms like viruses and bacteria, to about 3,000,000,000 basepairs for a human, to over 150,000,000,000 for certain plants[103] the limitations of Sanger DNA sequencing technology are such that the sequencing machine can only produce DNA fragment strings, known as *reads* that are 800 - 1,000 basepairs long[112]. Reconstituting the original complete DNA sequence from partial overlaps between reads is thus a costly, time consuming, and computationally intensive problem known as *de-novo assembly*[146]. Once one such full sequence (known as a *reference* sequnce) is assembled however, sequencing other individuals of the same (or closely related) species becomes a significantly easier undertaking. Rather than assembling the sequence *de-novo* one can search for a position on the reference sequence that provides the best matching *alignment* between the reference and each read obtained for the specimen under study. This technique is known as *genomic alignment*[77] or *mapping* and yields for each fragment a coordinate that represents where on the reference sequence the fragment maps to. Furthermore, because the DNA of any two organisms of the same species is largely identical, with differences occurring at about 0.1% of all sites (although this depends on DNA mutation rate)[96] researchers are able to significantly reduce the amount of information that is required to fully represent the genome of a specimen by retaining only the information that describes the sites where that specimen is different from the reference sequence for that species.

A general approach has thus emerged, where each new species of interest undergoes a relatively costly *de-novo* assembly process for the first genome, which then becomes the reference genome for that species. The sequencing of further individuals of that species utilizes, relatively cheaper, *alignment* and identification of *variants* (sites where the individual differs from the reference) to investigate the effect these variants may have on different phenotypes of interest such as disease susceptibility and survival[84].

Although genomicists study many different types of organisms the study of human genomes garners by far the most attention and research funding[**needcitation**] due to the natural desire of humans to better understand ourselves and influence, where possible, genetic factors impacting human longevity and health. Subsequent to the development of DNA sequencing methods by Fred Sanger one of the most audacious and crucial projects for the development of genomics as a branch of science has been The Human Genome Project[67] - an international effort to sequence and *de-novo* assemble the first complete human genome consisting of chromosomes 1-22, X, and Y (as well as mitochondrial DNA) and totalling approximately 3 billion basepairs. The project ran for over 10 years, completing in 2001, and cost more than $3 billion USD. Although the main project effort was completed using the Sanger sequencing method, a competing version of the human genome was simultaneously published by a commercial company led by JC Venter[130], using a new sequencing method called shotgun sequencing[131], a method that formed the basis for a new revolution

in sequencing technology, now termed Next Generation Sequencing[114].

2.1.2 Next Generation Sequencing

The Next Generation Sequencing methodology[85] relies on fragmenting the DNA of a subject into millions of fragments that are between 100-500 basepairs (bp) in length, then sequencing all of the short fragments and aligning all the reads to the reference with the aid of a relatively fast algorithm[78]. Because NGS sequencing methods are prone to certain errors and biases[30], it is necessary to sequence enough DNA fragments to overlap (or cover) every location in the genome several times (typically 10-30), in order to build a statistical model that will be able to determine the underlying sequence, known as *genotyping*[97], with a high degree of confidence. Thus, at present, a single sequenced DNA sample will typically contain 1 billion reads with a file size of 150GB when compressed.

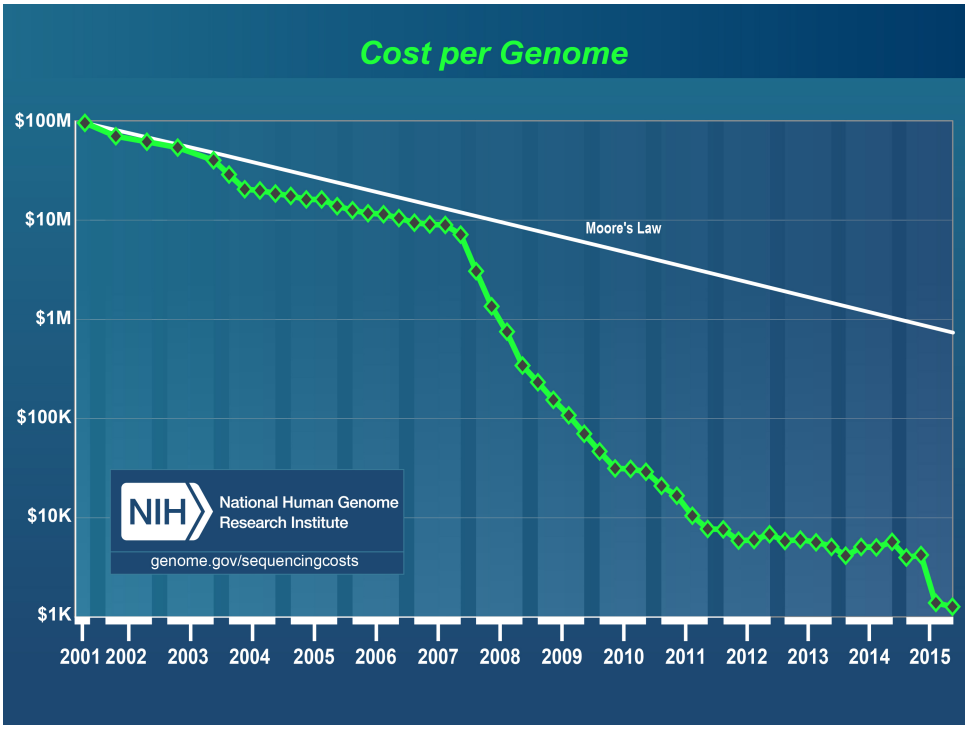

Figure 2.1: Cost of DNA sequencing[127]

Figure 2.1 shows the change in the cost of DNA sequencing over the course of the past 15 years. The precipitous drop in sequencing cost observed since 2008 coincides with wide adoption of NGS methodologies. This drop in price has made tractable a new set of large scale genomics sequencing projects that aim to characterize human genetic diversity at population scale, projects such as the 1000 Genomes Project[17], and the large scale sequencing of the Icelandic population[51].

2.1.3 Genomics Studies

2.1.4 Cancer Genomics

Cancer is a genetic disease that has an extremely high burden on the human population. In 2012, the global incidence of new cases worldwide has been estimated as 14.1 million, and deaths at 8.2 million[128]. The economic cost of cancer to the European Union has been estimated at 126 billion euro in 2009[82], and in the US $124.5 billion USD in 2010[141]. Because of the genetic nature of the disease studying genomes of cancer patients helps uncover the mechanisms behind the development and evolution of cancer[124].

Cancerous tumours arise from a single cell which over time accumulates a series of somatic mutations that cause it to exhibit properties such as: increased mutation rate, increased proliferation, anchorage independent growth, and resisting cell death[53] . Only certain mutations, however, contribute to the development of cancer, while others are benign. Cancer genomics studies aim to identify and characterize those mutations that are cancer drivers and play a role in the formation or progression of tumours[124].

Studying cancer genomes is more complex and expensive than studying the genomes of healthy individuals because each patient requires that two DNA samples are collected - that of the normal tissue, and that of the tumour. This is necessary to identify those mutations that are somatic - i.e. only occur in the tumour cell population[108].

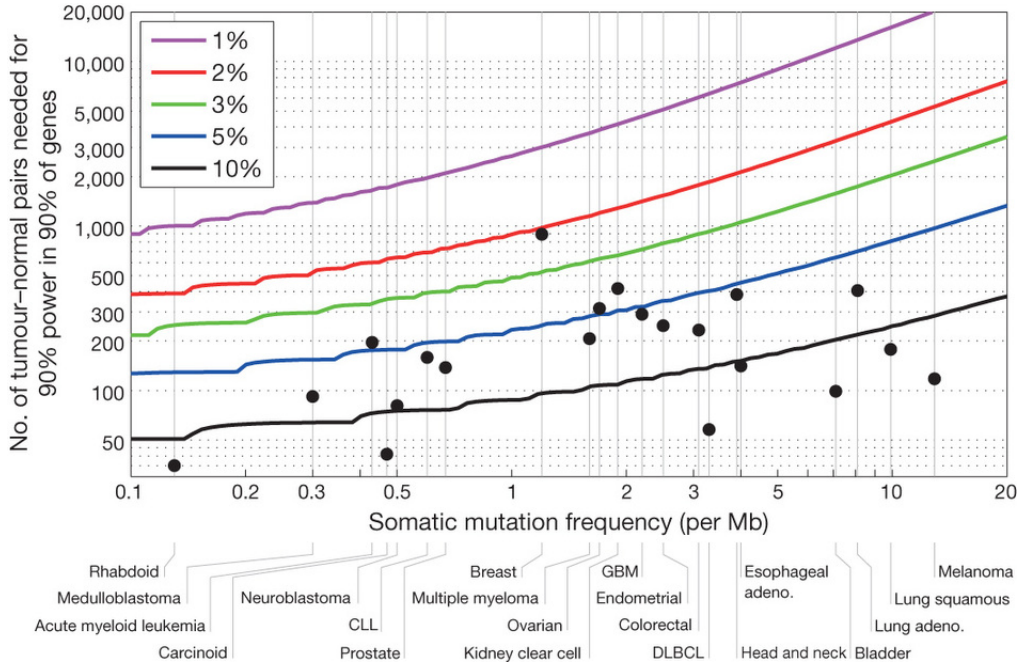

Figure 2.2: Sequencing sample size required by mutation rate[69].

Although there is a large number of identified mutations that are implicated in

cancer (2,002,811 SNV, 10,534 gene fusions, 61,299 genome rearrangements, 695,504 CNV segments in COSMIC v70; August 2014)[39], each mutation has a low chance of being present in any given tumour. Figure 2.2 demonstrates the sample size required to have 90% statistical power to identify 90% of the variants that occur with a set frequency in tumours with varying background mutation rates. Thus, identifying 90% of the mutations occurring with a frequency of at most 1% in Lung Adenocarcinoma requires a sample size of at least 10,000 patients. The necessity to sequence large cohorts of patients in order to be able to comprehensively detect cancer related genomic variants has led to the creation of several large scale cancer sequencing studies.

2.1.5 Clinical Genomics

2.2 Computational Methods for Next Generation Sequencing

Because the size of a typical genome is millions to billions of basepairs long, and current DNA sequencing technology frequently generates errors during the sequencing process, requiring multiple samples of each genomic location to be generated, the amount of data required to be examined in order to characterize even a single sample is well beyond the capabilities of any human. Thus, a multitude of computational approaches are required in order to make the task tractable for individual samples as well as cohorts, and entire populations.

The task of comprehensive characterization of genomic data for an individual is typically decomposed into a series of computational steps, each with its own data representation, and typically developed by a separate research group, which are then assembled into computational pipelines and executed by workflow engines on diverse computing environments. Our goal in this section is to enumerate and describe the individual steps and to provide a survey of the key computational tools and data formats that presently form the set of best practices in this rapidly evolving branch of science. Since Rheos is designed to improve upon these best practices we identify in each section the key mathematical and algorithmic ideas that underpin each approach in order to adapt and translate them into the Rheos framework.

The data that is used in virtually all modern genomics studies is generated on a next generation DNA sequencing machine. Several types of sequencers have been developed but the most frequently used ones are made by Illumina. The raw data produced by such a sequencer is a set of image files, where the color of each pixel represents the corresponding nucleotide base in a DNA strand that is being sequenced in each micro-well of a flowcell, representing the sample of interest. The succession of images produced by each cycle of sequencing then results in a set of reads, a collection of randomly ordered DNA fragments that are further analyzed by downstream tools. The first challenge in generating these reads is the accurate interpretation of pixel colors and mapping them to the corresponding nucleotide

bases, known as base-calling. Because all of the currently available DNA sequencing methodologies are imperfect at reading the underlying DNA sequence a number of errors is introduced into the process at various stages and special QA software is required in order to detect and assess the location and severity of the errors. A typical output of the QA process is a filtered set of reads where the lowest quality reads have been filtered out and each base within each read is assigned a quality score which represents the best current estimate of the probability that the base has been called incorrectly. The currently most frequently used file format for storing DNA sequence reads along with their read qualities is a text file known as fastq.

Depending on whether the organism under study has previously been sequenced there may already exist a reference sequence for it i.e. a file that for each genomic location describes the most frequently occurring nucleotide for that species at that location. Humans, and many other species of organisms already have reference sequences available. If the reference sequence for the organism under study is available then the next processing step involves searching for the position in the reference sequence that best matches each read that has been generated for the sample under study in the previous step. The coordinate of the best match is then assumed to be the location in the genome where that particular read has originated from. This process is known as genome alignment and it is very resource intensive for species with large genomes such as humans (3 billion bases) because a typical sequencing effort will generate at least 1 billion reads for a single sample, and each read needs to be mapped to the reference genome. This problem is made more difficult by the fact that an organism's genome typically has a large proportion of repeated sequence fragments and thus the generated reads do not uniquely align to a single location on the reference. A list of matching positions is generated instead, where each match needs to be scored and the highest scoring match is assumed to be the true origin of the read. Many alignment algorithms exist but the most accurate and fast ones use a two step process of indexing, implemented via hash tables or prefix/suffix tries, to generate a short list of promising match locations, followed by a more exact local alignment that uses dynamic programming to generate a best match. The alignment process is further complicated by the presence of sequencing errors, various genomic variants, and disease state such as cancer, all of which generate significant (and sometimes drastic) differences between the obtained reads and the reference genome, thus necessitating inexact matching approaches. The best algorithms that are currently available have a typical runtime of 24-48 hours on a modern 8-core machine. The most widely adopted standard for storing the alignment data on disk is the SAM[80] (and its binary and indexed counterpart BAM) format developed in the context of the 1000 Genomes Project. In addition to the sequence data and base qualities that are already available in fastq, the SAM format adds a reference coordinate to each read, an overall mapping quality for the read, and whether each position in the read matches the reference sequence, along with other useful metadata.

When a reference sequence does not exist, or when it is undesirable to use one, genome alignment tools are inapplicable and a different approach, called de-novo assembly, is used. Under this approach each read is broken into smaller subsequences called k-mers (of length k), these k-mers are then used to build a graph structure

called a de Bruijn graph. Unique paths through the graph represent possible arrangements of reads that correspond to the underlying sequence and the highest scoring path is chosen as the true sequence. Using the de-novo assembly approach has some advantages over alignment-based methods because it models the structure of the organism's genome directly as it is observed rather than in relation to a reference. This is because no reference is perfect, but instead each reference has its own set of errors that were introduced in its construction. Furthermore, genomic structural variants, which represent large (hundreds to millions of basepairs long) sequences that may be deleted, duplicated, or inverted within a given genome challenge alignment software because of the alignment errors that they introduce and require sophisticated algorithms to later detect, whereas in the de-novo assembly approach these variants are directly modelled as they occur in the underlying sequence and are thus easier to identify. De-novo assembly has its own set of challenges however related to difficulties dealing with repetitive sequences that are found within the genome, as well as the extremely high resource requirements of de-novo assembly algorithms, especially when it comes to memory. The de Bruijn graph is typically built in memory and can be multiple terabytes in size, thus requiring computers with extremely high memory to process. Since, even when using in-memory graph construction the runtime for a single sample is typically several days, it is impractical to move the graph representation to disk without dramatically increasing the algorithm runtime to the point where its duration becomes unreasonable. In practice whole genome de-novo assembly is currently rarely used for processing human genomic data because of the challenges described above. Instead, modern algorithms supplement read alignment with local assembly of particular genomic regions of interest in order to reap some of the benefits offered by assembly-based methods without incurring all of the costs.

Once the reads have been aligned they are typically sorted by genomic coordinate so that all of the reads that overlap a given coordinate can be examined together at once. This is an expensive sortation step that does not lend itself well to parallelization and takes several hours to complete per sample. Subsequent to the sortation step is another round of data QA which aims to throw out low quality reads that poorly align to the reference. Care must be taken however, because these low quality reads may not only signal underlying data or sequencing issues like sample contamination, or lane-swap, but may also signal the presence of structural variants or integration of retrovirus DNA into the host under study, both of which are of high interest to properly identify. Thus, it is common to split the sample into reads of high quality that are further assessed with one set of algorithms and a set of reads that map with low quality, or fail to map at all, to be assessed with a different set of algorithms.

At this point the data is ready to begin the process of variant calling, that is, identifying the genomic features of the sample that are different from the reference sequence for that organism (i.e. mutations). It is important to distinguish germline variant calling from somatic variant calling at this time. In germline variant calling we are trying to identify the set of variants that have been passed to the individuals under study from their parents and are thus present in every cell of the organism forming the underlying genetic background of that individual where some variants

may be neutral to the organism's survival, some may be beneficial, and some may be deleterious. Comprehensively identifying and classifying these is of significant research and clinical interest as they confer susceptibility or resistance to certain disease vectors as well as potential medical remedies and may act as biomarkers to predict disease prognosis or response to treatment within the groups of patients that harbour them.

Somatic mutations are those that each individual cell accumulates over its lifetime and they are of especial interest in the context of cancer where a certain set of mutations accumulated in a particular sequence and over a period of time disrupt the normal cell lifecycle and result in the formation of a malignant tumour. In this context researchers typically sequence both healthy cells (such as those drawn from the patient's blood) and cancerous cells. Mutations are identified in both and the difference between these sets of mutations is then stipulated to be the set of somatic mutations present within that tumour. Just like in the germline case, not all of the somatic mutations contribute to the formation of the cancer and the appropriate identification and classification of those mutations that do (so-called cancer drivers) is an important question of significant clinical and research importance which we consider further below. From a technical standpoint calling somatic variants is significantly more complex than calling germline variants because healthy cells generally conform to the underlying genetic characteristics of the organism, such as the number of chromosomes and ploidy (23 chromosomes, diploid, for humans), whereas in the cancer cells these characteristics can be severely disrupted with entire chromosomes missing or present in amplified copy number, requiring different and more complex statistical models to accurately identify. An additional complexity that is unique to somatic variant calling is the concept of sub-clonal mutations. These are mutations that have been acquired only by some of the cells within a tumour. Since sequencing samples data from a large number of cells within a tumour the reads from which are all pooled together, only a comparatively low number of reads will contain information about sub-clonal mutations, thus making them more difficult to detect, even though such mutations may have a significant impact on the tumour phenotype and thus would be very important to properly identify.

We typically think of three classes of genomic variants that are identified by different methods and oftentimes by separate tools. The simplest to accurately detect, and most frequently occurring are Single Nucleotide Polymorphisms (SNPs), in the germline case, and Single Nucleotide Variants (SNVs), in the somatic case. These are single basepair substitutions where the germline genome differs from the reference sequence by a single letter (for SNPs), or the somatic genome differs from the germline genome by a single letter (for SNVs). SNPs are quite common in humans and occur at the rate of approximately 1 per 1,000 bases on average, or, equivalently, 3 million per individual. Somatic SNVs have a widely varying incidence rate depending on the type of cancer involved with typical rates between (INSERT RATES HERE). For humans, which are diploid (i.e. have two copies of each of the chromosomes, except for the sex chromosomes X and Y), we classify SNPs and SNVs as being either heterozygous (with one reference allele and one variant allele) or homozygous (with both alleles being variant). Methods to detect and accurately genotype SNPs and SNVs typically rely on counting the reads that overlap a given

genomic position and evaluating a statistical model that contrasts the probability of the site being reference versus the probability of the site being variant in the face of potential sequencing errors which are expressed as base quality scores and mapping quality scores (as previously described). The models employed for somatic SNV detection and genotyping are significantly more complex than the models for germline variant detection because of the possibility of sub-clonal mutations (as previously described) as well as regions of amplified copy number (i.e. regions where the organism is no longer diploid but can have any number of additional copies of a chromosomal region, or an entire chromosome). More advanced methods output not only lists of variant sites for a sample but calculate a distribution of genotype likelihoods, i.e. all the possible genotypes at a given variant site along with their relative probabilities so that these can be integrated into the models of downstream statistical analyses in a comprehensive manner.

Indels represent sequence insertions and deletions that are anywhere from 1 base-pair (bp) to about 50 basepairs long. There is no strict upper bound on the length of an indel and individual tools typically decide on their own cutoffs for length although pretty much all tools place their cutoff at a length that is smaller than the typical read length (150 - 500 bp presently). Indel callers typically look for several mismatched bases in a row between the reference and the sample under study and classify the entire length of the mismatched sequence as an insertion or deletion correspondingly. Other indel callers borrow some of the methodology from structural variant callers which are similar to indels, only typically bigger in size, and are potentially more complex.

Structural variants (SVs) are more large scale genomic rearrangements that occur in both germline and somatic genomes and can have a very drastic effect on the organism's phenotype because they can affect a large number of genes at once, resulting in the loss of function of particular important genes, or the creation of gene fusions where, because of a rearrangement, one gene comes under the programmatic control of another gene thereby disrupting important cellular processes. The most common types of structural variants include insertions, deletions, segmental duplications, inversions, and translocations. Simpler structural variants sometimes combine to produce more complex events that are especially difficult to detect properly. The methods for calling and genotyping of structural variants typically rely on looking at the reads that are deemed low quality for the SNP calling process. These are reads that fail to map to the reference genome, split-reads, which are reads where one part of the read maps to one location on the reference and another part of the read maps to another location, and divergently mapped reads (sequencing is frequently done on read pairs where two ends of a DNA fragment of standard size are sequenced in the opposite directions generating a pair of reads with a standard distance, called insert size, inbetween them), with a shorter or longer than expected insert size. SV callers break down these reads into smaller fragments (k-mers) and attempt to map these k-mers to the reference sequence. The goal is to determine the location of breakpoints, which are positions on the sample genome where a DNA strand break is thought to have occurred as part of the genomic rearrangement that has taken place. Once a list of breakpoints is obtained the algorithm attempts to reconstruct the most likely event sequence that these breakpoints could have arisen

from, pairing up adjacent breakpoints that are the result of a sequence deletion, for example. Thus, each pair of breakpoints typically gives rise to a single SV call in the final output of the caller. SV calling is a complex and error-prone process that generates double-digit false-positive and false-negative rate, especially in the somatic case, where patient genomes can undergo drastic rearrangements as a result of cancer-related processes such as chromothripsis and are thus extremely difficult to resolve with accuracy.

Once variants (SNPs/SNVs, Indels, and SVs) have been comprehensively called, a filtering step is necessary because callers are typically initially tuned for highest sensitivity in order to detect the most variants, thus admitting an increased number of false positive calls. Additionally, because calling of SNPs and SVs typically occurs separately by different tools there can be significant call-set overlap where the SNP caller sees a region as a group of SNPs, whereas the SV caller will see it as a single breakpoint. These overlaps need to be resolved in order to avoid redundant calls. A number of filtering approaches exist, some of which rely on heuristics such as strand bias, or read support to filter out low quality variants. Other filtering approaches rely on curated variant databases or machine learning methods in order to reduce the number of false positive calls. One popular filtering approach involves ensemble calling where several different variant calling methods are used on the same dataset and a variant is excluded unless it is called by multiple tools. These methods are typically able to reduce the false positive rate of the call-set by 5-10% while only nominally affecting the false negative rate.

When a filtered high quality call-set has been prepared it is of interest to determine which of the variants are likely to have an effect on the organism's phenotype and which variants are likely to have no consequence. This is accomplished via variant annotation. The annotation process consults a database of known genes and other genomic elements (promoters, enhancers, etc.) to determine the likely consequence of each variant based on the type of mutation that it represents i.e. a synonymous mutation (that doesn't change the underlying amino acid) is likely to have no phenotypic effect, whereas a stop gain mutation inside the coding region of a known gene may indicate a potential loss of function of that gene and may thus have a considerable effect on the observed phenotype. When annotating somatic mutations it is important to consider known cancer genes and delineate whether mutations are "passengers" or "drivers" depending on whether they are thought to be driving the carcinogenesis process by constitutively activating a cancer gene or deactivating a tumour suppressor, or they are simply acquired as part of the genomic instability that is induced by carcinogenesis. An outcome of the variant annotation process then, is a list of somatic or germline variants accompanied by a designation of the known genomic features that they fall in, along with an assigned functional impact. This is typically the last step of an NGS analysis pipeline after which the variant call-set is considered completed and can be used for any number of downstream analyses depending on the particular research question or clinical application being considered. For instance, the variants may be used as input into a Genome Wide Association Study (GWAS), a Quantitative Trait Locus (QTL) analysis, a rare variant association study, or as input into the computation of a clinical biomarker.

2.2.1 File Formats

FASTQ - BAM/SAM - CRAM - VCF -

2.2.2 Base-calling

2.2.3 Alignment

BWA/MEM - Bowtie - Gem -

2.2.4 Raw Data QA

2.2.5 Germline SNP Calling

Single Nucleotide Polymorphisms or SNPs are locations in an individual's genome where that individual differs from the reference sequence at a single position. The reference sequence is haploid i.e. it provides a single base (for instance T) at every genomic location, whereas the human genome is diploid (there are two copies of each chromosome, and thus two bases at each location) for chromosomes 1-22, and chromosome X for females, while being haploid for chromosomes X and Y for males. Thus, at each genomic location, the human genome may be:

Homozygous Reference - When both alleles carried by the individual at that location match the reference.

Heterozygous - When one allele matches the reference and one is different from the reference.

Homozygous Alternate - When both alleles are the same and different from the reference.

Multiallelic - When both alleles are different from the reference and are different from each other[57].

SNPs are the most common type of genomic variant, with every individual carrying over 3 million SNPs on average[119]. Furthermore, the presence of certain SNPs is strongly associated with disease[19], where some SNPs are known to be causative[61], while others, are merely associated with a disease phenotype[113]. A large number of scientific studies[56] and clinical practice[142] is thus enabled by efficient and comprehensive characterization of the gamut of human SNPs to assess their contribution to disease risk, see Figure 2.3.

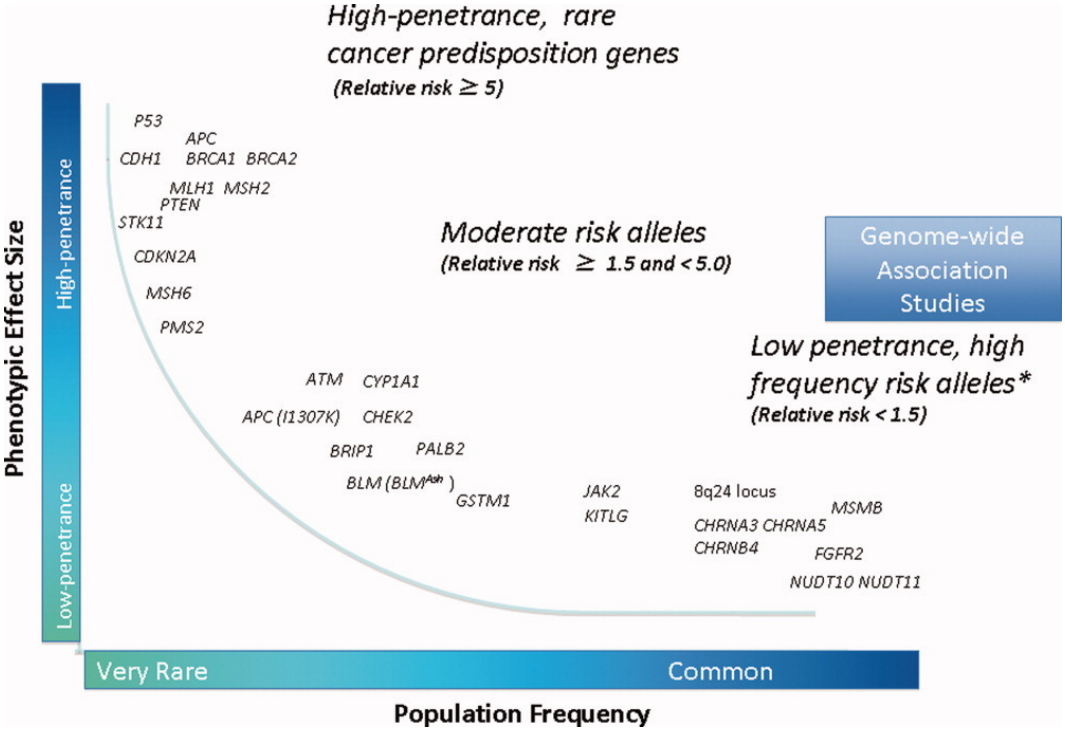

Figure 2.3: Distribution of mutations by population frequency against phenotypic effect size.[138].

There are a number of methods that have been used for assessing SNPs with the aid of microarry technology[54], but here we focus on methods that make use of Next Generation Sequencing (NGS). Since the primary data type generated by NGS is a sequencing read, most presently used methods for SNP detection rely on investigating the collection of sequencing reads that overlap each genomic locus and comparing the observed data to the reference sequence. It is important to distinguish two typically separate activities that take place as part of SNP calling - variant calling, and genotyping. Variant calling attempts to locate positions in the sample genome where that sample is different from the reference, whereas genotyping attempts to assign an actual genotype (e.g. homozygous-alternate), along with a measure of confidence, to each putative variant. We present several of the key computational methods currently used in SNP calling with additional detail. These are:

- samtools
- freebayes
- GATK
- platypus

These tools have been selected because they have been developed independently, at different institutions, and have been repeatedly demonstrated to produce consistent and high-quality results. See Figure 2.4 for a recent comparison.

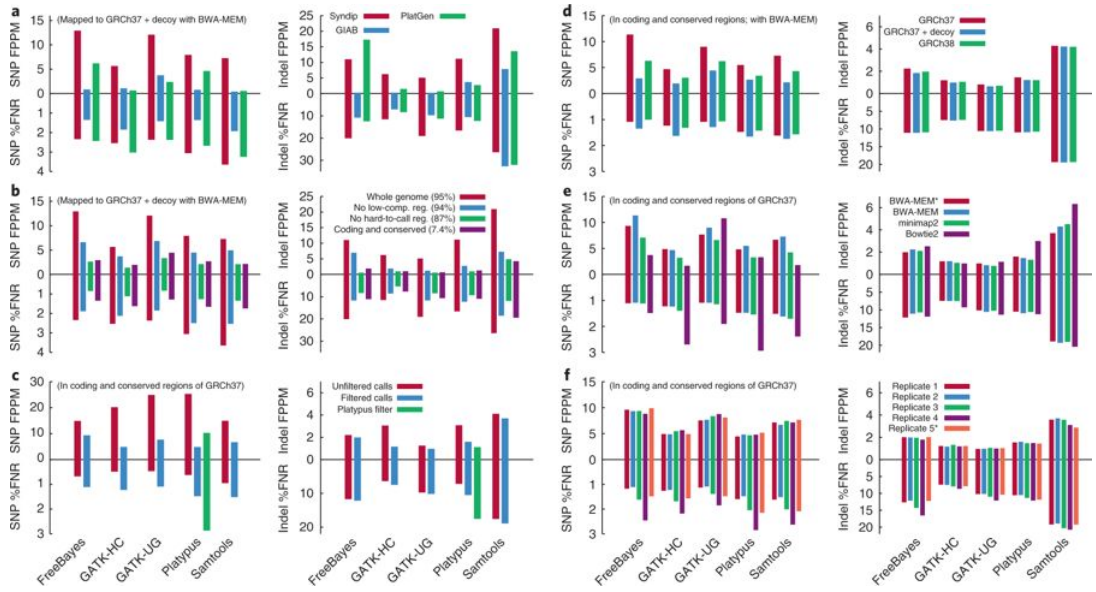

Figure 2.4: Comparison of samtools,freebayes, GATK, and platypus on three benchmark data sets - Syndip, GIAB, and PlatGen. Here FPPM - number of false positives per megabase of sequence, and FNR - false negative rate = 100xFN/(TP+FN).[79].

samtools

Samtools[80] is a software package for genomic data processing developed by Heng Li et al. in the context of the 1000 Genomes Project[17] and implemented as a C program with a CLI interface. This tool has enjoyed continued and widespread use in the bioinformatics community for the purposes of small variant calling (including SNPs). All of the mathematical results in this section are reproduced from the 2011 paper by Li[73] that describes the method, as well as a set of mathematical notes made available separately by Li[75] in 2010.

Although the samtools framework could be extended to support calling multi-allelic sites, the framework, as-published, has been developed for calling only bi-allelic variants. Table 2.1 contains commonly used definitions.

It is additionally assumed that there are n individuals being sequenced with the i-th individual having ploidy m_i (typically 2 in practice). At a particular genomic locus, the sequence read data for the i-th individual is d_i and the genotype is g_i, an integer in $[0, m_i]$, counting the number of reference alleles in the individual at that locus. Furthermore, it is assumed for simplicity that data at individual genomic loci are independent (which isn't necessarily true), as are sequencing and mapping errors between loci and individuals.

Because of the above independence assumptions the joint likelihood function of the data observed for all individuals factors as a product of individual likelihood

Table 2.1: Samtools common definitions

Symbol	Description
n	Number of samples
m_i	Ploidy of the i-th sample ($1 \leq i \leq n$)
M	Total number of chromosomes in samples: $M = \sum_i m_i$
d_i	Sequencing data (bases and qualities) for the i-th sample
g_i	Genotype (the number of reference alleles) of the i-th sample ($0 \leq g_i \leq m_i$)[1]
ϕ_k	Probability of observing k reference alleles ($\sum_{k=0}^{M} \phi_k = 1$)
$P(A)$	Probability of an event A
$\mathcal{L}_i(\theta)$	Likelihood function for the i-th sample: $\mathcal{L}_i(\theta) = P(d_i\|\theta)$

functions:

$$\mathcal{L}(\theta) = \prod_{i=1}^{n} \mathcal{L}_i(\theta) \tag{2.1}$$

Suppose that a single sample i represents an individual of ploidy m_i and a given locus is covered by k reads. The sequencing data d_i is composed of an array of bases where each element has value 1 representing the reference allele and is 0 otherwise.

$$d_i = (b_1, \ldots, b_k) = (\underbrace{1, \ldots, 1}_{l}, \underbrace{0, \ldots, 0}_{k-l})$$

The error probability of the j-th base is ϵ_j, which is taken to be the larger between sequencing and mapping errors for that read. Under the independence assumptions above:

$$\mathcal{L}_i(0) = P(d_i|0) = \prod_{j=1}^{l} \epsilon_j \prod_{j=l+1}^{k} (1 - \epsilon_j) = \left(1 - \sum_{j=l+1}^{k} \epsilon_j + o(\epsilon^2)\right) \prod_{j=1}^{l} \epsilon_j \tag{2.2}$$

$$\mathcal{L}_i(m_i) = P(d_i|m_i) = \left(1 - \sum_{j=1}^{l} \epsilon_j + o(\epsilon^2)\right) \prod_{j=l+1}^{k} \epsilon_j \tag{2.3}$$

For $0 < g_i < m_i$:

$$\begin{aligned}
\mathcal{L}_i(g_i) = P(d_i|g_i) &= \sum_{a_1=0}^{1} \cdots \sum_{a_k=0}^{1} \Pr\{d_i|B_1 = a_1, \ldots, B_k = a_k\} \Pr\{B_1 = a_1, \ldots, B_k = a_k|g\} \\
&= \sum_{\vec{a}} \left(\frac{g}{m}\right)^{\sum_j a_j} \left(1 - \frac{g}{m}\right)^{k - \sum_j a_j} \cdot \prod_j p_j(a_j) \\
&= \left(1 - \frac{g}{m}\right)^k \prod_j \sum_{a=0}^{1} p_j(a) \left(\frac{g}{m-g}\right)^a \\
&= \left(1 - \frac{g}{m}\right)^k \prod_{j=1}^{l} \left(\epsilon_j + \frac{g}{m-g}(1-\epsilon_j)\right) \prod_{j=l+1}^{k} \left(1 - \epsilon_j + \frac{\epsilon_j g}{m-g}\right) \\
&= \left(1 - \frac{g}{m}\right)^k \left\{ \left(\frac{g}{m-g}\right)^l + \left(1 - \frac{g}{m-g}\right) \left(\sum_{j=1}^{l} \epsilon_j - \sum_{j=l+1}^{k} \epsilon_j \right) + o(\epsilon^2) \right\}
\end{aligned}$$

where $a = \sum_j a_j$ and

$$p_j(a) = \begin{cases} \epsilon_j & a = 1 \\ 1 - \epsilon_j & a = 0 \end{cases}$$

In particular, for a diploid sample ($m = 2$), the likelihoods for $g = 0, 1, 2$ are

$$\mathcal{L}(0) = \prod_{j=1}^{l} \epsilon_j \prod_{j=l+1}^{k} (1 - \epsilon_j) \tag{2.4}$$

$$\mathcal{L}(1) = \frac{1}{2^k} \tag{2.5}$$

$$\mathcal{L}(2) = \prod_{j=1}^{l} (1 - \epsilon_j) \prod_{j=l+1}^{k} \epsilon_j \tag{2.6}$$

For instance, taking $g_i = 2$ (i.e. the true genotype is homozygous-reference) as an example, and under above independence assumptions, the likelihood of observing the data d_i is the likelihood of sampling l reads without error (the reads match the reference) and $k - l$ reads with error (the reads do not match the reference).

Let $\Phi = \{\phi_k\}$ for $k \in [0, m_i]$ be a prior distribution of genotype probabilities (a model from population genetics, such as Wright-Fisher, can be used, or an empirical distribution from antoher study), the actual genotype for individual i at the given locus is estimated via Bayes' Rule as:

$$\hat{g}_i = \underset{g_i}{\operatorname{argmax}} \Pr\{G_i = g_i|d_i, \Phi\} = \underset{g_i}{\operatorname{argmax}} \frac{P(d_i|g_i)\phi_k}{\sum_{h_i} P(d_i|h_i)\phi_h} = \underset{g_i}{\operatorname{argmax}} \frac{\mathcal{L}(g_i)\phi_k}{\sum_{h_i} \mathcal{L}(h_i)\phi_h} \tag{2.7}$$

The variant quality is defined as:

$$Q_{\text{var}} = -10 \log_{10} \Pr\{G = m_i|d_i, \Phi\} \tag{2.8}$$

i.e. the Phred-scaled probability that the locus is homozygous reference given the observed data. The locus is called *variant* if the variant quality exceeds a certain pre-defined threshold.

Equations 2.7 and 2.8 thus represent the key computations corresponding to the activities of SNP genotyping and variant calling that are performed by samtools for each genomic locus in a sample.

freebayes

freebayes[45] is a C software package implemented by E. Garrison for discovery and genotyping of SNPs, indels, and other small variants that builds on a previous method by G. Marth[87]. Where small means that the variant length is smaller than the size of a sequencing read. Unlike samtools, which looks at all genomic loci independently, freebayes uses local sequence context to guide detection and genotyping of variants, additionally freebayes builds variant haplotypes[23] i.e. groups of variants that are inherited together on the same DNA molecule. All of the figures and methematical formuls in this section are reproduced from the 2012 preprint by Garrison et al[45].

Table 2.2: Freebayes common definitions

Symbol	Description
n	Number of samples
m_i	Copy number of the i-th sample ($1 \leq i \leq n$)
M	Total number of copies of each locus in all samples: $M = \sum_i m_i$
$\{b_j : j \in [1, K]\}$	Set of K distinct alleles present among M copies
$\{C_j : j \in [1, K]\}$	Corresponding set of allele counts for each b_K
$\{f_j : j \in [1, K]\}$	Corresponding set of allele frequencies for each b_K
G_i	Unphased genotype of individual i
$\{b_{i_j} : i \in [1, n], j \in [1, k_i]\}$	Set of k_i distinct alleles of G_i
$\{c_{i_j} : i \in [1, n], j \in [1, k_i]\}$	Set of k_i allele counts for each b_{i_j}
$\{f_{i_j} : i \in [1, n], j \in [1, k_i], f_i = c_i/k_i\}$	Set of allele frequencies of G_i
$B_i : \lvert B_i \rvert = m_i$	Multiset of alleles equivalent to G_i
$R_i = \{r_j : j \in [1, s_i]\}$	Set of s_i sequencing observations for each sample i s.t. there are $\sum_{i=1}^{n} \lvert R_i \rvert$ reads at a given locus
$\{q_j : j \in [1, s_i]\}$	Mapping quality, i.e. probability that read r_j is mis-mapped against the reference

For a set of n individuals the conditional probability of a combination of genotypes

given the observed data is assessed simultaneously as:

$$P(G_1, \ldots, G_n | R_1, \ldots, R_n) = \frac{P(G_1, \ldots, G_n) P(R_1, \ldots, R_n | G_1, \ldots, G_n)}{P(R_1, \ldots, R_n)} \tag{2.9}$$

$$P(G_1, \ldots, G_n | R_1, \ldots, R_n) = \frac{P(G_1, \ldots, G_n) \prod_{i=1}^{n} P(R_i | G_i)}{\sum_{\forall G_1, \ldots, G_n} P(G_1, \ldots, G_n) \prod_{i=1}^{n} P(R_i | G_i)} \tag{2.10}$$

For a single sample at a particular locus there are R_i reads, and k_i observed alleles - $B'_i = b'_1, \ldots, b'_{k_i}$, which correspond to $b_1, \ldots, b_i$ underlying alleles represented at the given locus. For each observed allele b'_i there is a corresponding count of observations o_f s.t. : $\sum_{j=1}^{k_i} o_j = s_i$ and each b'_i corresponds to a true allele b_i. The probability of a single observation b'_i given a genotype in a single sample is:

$$P(b'_i | G) = \sum_{\forall (b_i \in G)} f_i P(b'_i | b_i) \tag{2.11}$$

where f_i is the allele frequency of b_i in G. The authors introduce the following approximation:

$$P(b' | b) = \begin{cases} 1 & \text{if } b' = b \\ P(error) & \text{if } b' \neq b \end{cases} \tag{2.12}$$

where $P(error)$ is derived from the base quality score from the sequencing read. Using the above approximation the probability of observing the data R_i given the genotype is estimated as:

$$P(R_i | G) \approx \binom{s_i}{o_1, \ldots, o_{k_i}} \prod_{j=1}^{k_i} f_{i_j}^{o_j} \prod_{l=1}^{s_i} P(b'_l | b_l) \tag{2.13}$$

In order to evaluate the posterior probability of a particular combination of genotypes given the data, the authors derive:

$$P(G_1, \ldots, G_n | f_1, \ldots, f_k) = \binom{M}{c_1, \ldots, c_k}^{-1} \prod_{i=1}^{n} \binom{m_i}{c_{i_1}, \ldots, c_{i_{k_i}}} \tag{2.14}$$

counting the number of ways of selecting a set of unphased genotypes G_i given the allele frequency spectrum f_k, and

$$P(f_1, \ldots, f_k) = P(a_1, \ldots, a_M) = \frac{M!}{\theta \prod_{z=1}^{M-1} (\theta + z)} \prod_{j=1}^{M} \frac{\theta^{a_j}}{j^{a_j} a_j!} \tag{2.15}$$

using Ewens' sampling formula[37], where θ is the population mutation rate and allele frequencies f_i are transformed to frequency counts $a_1, \ldots, a_M : \sum_{c=1}^{M} ca_c = M$ where each a_f is the number of alleles in $b_1, \ldots, b_k$ whose allele count in the sample set is c.

Once variants are initially detected using Equation 2.10, the method continues to build local haplotypes grouping variants that are inherited together in dynamically-sized windows based on a distance threshold between successive variants. Each group of variants is combined into a haplotype observation H_i, with an assigned quality score that is the minimum of the supporting reads' mapping quality and the minimum base quality of the variant allele observations (bases that span the variants). The size of the window is determined via an iterative process where an initial variant is used as the seed, and all of the reads that overlap it are added. If these reads overlap any other variants, then these are added, along with any reads that overlap them, and so on, until no new variants can be added.

Once the window is determined, the method uses a gradient descent algorithm to find a MAP estimate of the genotype for each sample. It starts with the maximum likelihood estimate for each sample's genotype given the observed data, and then attempts to find a genotype assignment that has a higher posteriror probability across all samples.

GATK

The GATK[28] is not actually a tool that's built solely for SNP calling, but is instead a comprehensive framework for genomic data analysis that includes tools for data pre-processing and QA, SNP and indel calling, CNV calling, and SV calling, as well as post-processing and filtering. It is implemented as a Java program and the latest version makes use of the in-memory computing engine Apache Spark for efficient computation over large data sets. Here we focus on the SNP calling aspects of the framework. There are two components in the GATK that deal with SNP calling, the UnifiedGenotyper, and the HaplotypeCaller. The UnifiedGenotyper is an older component that has been superceded by the HaplotypeCaller for all practical purposes and we focus our attention on it. All of the mathematical formulas and figures, as well as some of the descriptions, in this section have been reproduced from the 2010 manuscript by McKenna et al.[89], the 2011 manuscript by DePristo et al.[28], and the GATK website[1]

The GATK Best Practices pipeline (see Figure 2.5) is a set of best practices published by The Broad Institute that describe how to best use their software. In the context of this section we are primarily interested in the processes that occur in the middle panel of this figure that deal with processing aligned reads to call and genotype variants that will then be used for post-processing and filtering.

The HaplotypeCaller which is the tool that is primarily used for SNP calling takes a continued refinement approach, where the data is processed in the following sequential steps:

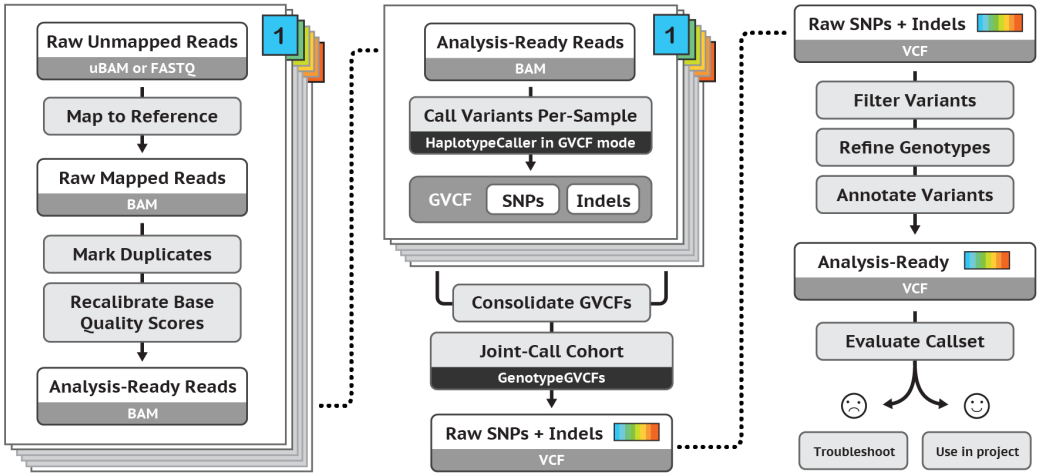

Figure 2.5: GATK Best Practices pipeline[1]

Define active regions - The program determines which regions of the genome it needs to operate on (active regions), based on the presence of evidence for variation.

Determine haplotypes by assembly of the active region - For each active region, the program builds a De Bruijn-like graph to reassemble the active region and identifies what are the possible haplotypes present in the data. The program then realigns each haplotype against the reference haplotype using the Smith-Waterman algorithm in order to identify potentially variant sites.

Determine likelihoods of the haplotypes given the read data - For each active region, the program performs a pairwise alignment of each read against each haplotype using the PairHMM algorithm. This produces a matrix of likelihoods of haplotypes given the read data. These likelihoods are then marginalized to obtain the likelihoods of alleles for each potentially variant site given the read data.

Assign sample genotypes - For each potentially variant site, the program applies Bayes' rule, using the likelihoods of alleles given the read data to calculate the likelihoods of each genotype per sample given the read data observed for that sample. The most likely genotype is then assigned to the sample.

Active regions are defined by targeting loci with high quality reads that are different from the refernce and surrounded by soft-clipped bases (bases in the read that couldn't be aligned by the alignment algorithm). The region is then smoothed by a Gaussian kernel and passed onto variant calling if its profile score exceeds a pre-defined threshold.

For each active region the GATK performs local assembly by first building a de-Bruijn graph[16] using just the reference sequence for the active region. The graph is enhanced by comparing each read from the active region and creating nodes in the graph where the read differs from the graph. Edge weights between pairs of nodes are increased when a read passes through that edge. The resulting graph must

meet complexity requirements based on the ratio of non-unique to unique kmers and presence of cycles (that are a sign of repetitive sequence). When the graph does not meet complexity requirements it is automatically rebuilt with successively increased kmer sizes. If a sufficiently complex graph cannot be built, the region is discarded because the method is not able to produce a high quality variant call in this case. Once the graph is built it is refined by pruning out paths that are not supported by a sufficient number of reads. By traversing the paths in the graph and selecting those with a high aggregate weight the algorithm builds a list of possible local haplotypes within the active region. Each candidate haplotype is then re-aligned to the reference sequence using Smith-Waterman[121] alignment to produce a list of potential variant sites that includes SNPs and other potential variants.

Reads from the active region are aligned to each haplotype using a Hidden Markov Model via the PairHMM algorithm[31] to produce a likelihood of each read given a haplotype. The set of haplotype likelihoods is transformed to a set of likelihoods per allele where for each allele at a given site the the highest scoring likelihood for a given read and a given haplotype that supports that allele is selected. Under a read independence assumption the total likelihood of the allele is computed as the product of read likelihoods.

For each variant locus the probability of each genotype is calculated using:

$$P(G|D) = \frac{P(G)P(D|G)}{\sum_i P(G_i)P(D|G_i)} \tag{2.16}$$

where D is the set of reads, G_i is the set of possible genotypes, and under the assumptions of a diploid sample, independent read observations, and independent errors. The prior probability of genotypes $P(G)$ is assumed to be uniform.

Based on the diploid and independence assumptions:

$$P(D|G) = \prod_j \left(\frac{P(D_j|H_1)}{2} + \frac{P(Dj|H_2)}{2} \right) \tag{2.17}$$

where $P(D_j|H_n)$ is the likelihood of read given the haplotype perviously obtained.

The genotype with the highest $P(G|D)$ is emitted as the genotype for that variant.

Platypus

Platypus[107] is a Python and C package by Rimmer et al., that provides SNP, indel, and other small and medium (upt to 1kb) variant calling capabilities utilizing a Bayesian statistical framework built on top of reference-free local assembly of haplotypes. All of the figures and methematical formuls in this section are reproduced from the 2014 Nature Genetics manuscript by Rimmer et al. See Figure 2.6 for the general sequence of data processing steps in platypus.

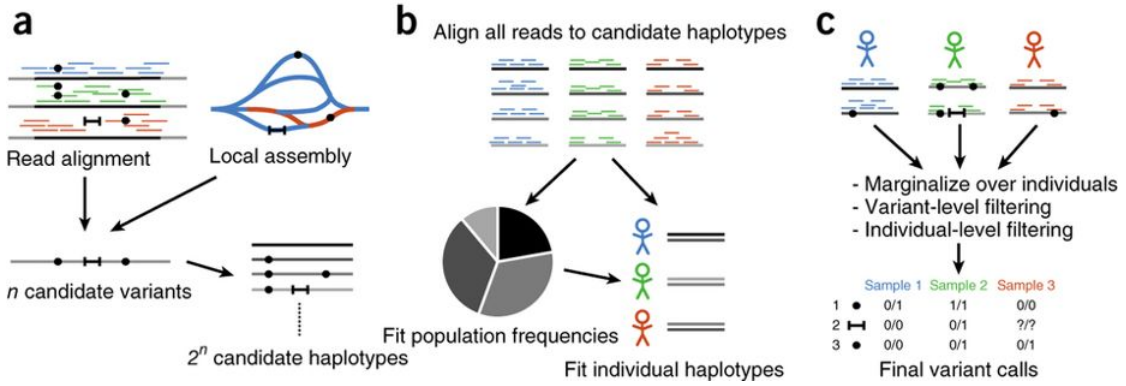

Figure 2.6: Three stages of data processing in Platypus[107]

Platypus works by loading batches of reads into memory from a BAM file. 100 kb (kilobases) are loaded at a time. Low quality reads are filtered out. Sites that are flagged as potential variants by the aligned become candidate variants. The local assembly step operates on successive windows of 1.5 kb in size, including all reads that map to the window as well as their mates, irrespective of where or if they map. As a first step, the algorithm builds a colored de Bruijn graph[62] from the reads and the reference sequence. Candidate variant haplotypes are produced by finding paths that diverge from the reference and come back to the reference by performing a Depth First Search from the graph node that diverges and until the reference is reached again. All such paths are recorded and contain putative SNPs, MNVs, indels, and larger rearrangements that are up to the window-size in length. A prior of 0.33×10^{-3} is used for SNPs under the assumption that SNPs occur in humans at a density of 10^{-3} per base and every non-reference allele is equally likely. Smaller windows are created by combining groups of candidate variants that are within 15 basepairs of each other, as long as window size is smaller than read-length and there are fewer than 8 variants in a group.

Candidate haplotypes are generated by taking all possible combinations of candidate variants (assuming diploid sample), resulting in 2^n haplotypes. For a window with 8 variants there are 256 possible haplotypes. The haplotype likelihood $P(r|h)$ is calculated by aligning reads to each haplotype using a Hidden Markov Model. Platypus uses the Viterbi algorithm[40] to compute the most likely path through the HMM as an approximation of the actual likelihood as a matter of optimization. After haplotype likelihoods are computed for all combinations of reads and haplotypes Platypus uses Expectation Maximization to estimate the frequency of each haplotype under the following model:

$$\mathcal{L}(R|\{h_i, f_i\}_{i=1...a}) = \prod_{\substack{samples,\\ s}} \sum_{\substack{haplotypes,\\ i,j}} f_i f_j \prod_{\substack{reads,\\ r \in R_s}} \left(\frac{1}{2} p(r|h_i) + \frac{1}{2} p(r|h_j) \right) \tag{2.18}$$

where f_i is frequency of haplotype h_i, a is the number of considered alleles, R is all reads, R_s reads in sample s.

The posterior probability of a variant v given the data is computed as:

$$P(v|R) = \frac{P(v)\mathcal{L}(R|\{h_i, f_i\}_{i=1...a})}{P(v)\mathcal{L}(R|\{h_i, f_i\}_{i=1...a}) + (1 - P(v))\mathcal{L}(R|\{h_i, \frac{f_i}{1-F_v}\}_{i \in I_v})} \tag{2.19}$$

here the likelihood of data R given all haplotypes is compared to the likelihood of R given those haplotypes that do not include v. I_v is the set of haplotype indices such that h_i does not contain v, and $F_v = \sum_{i \in I_v} f_i$. A variant is called when its posterior exceeds a pre-defined threshold. Genotype likelihoods for a variant are calculated by marginalizing over the genotypes at other variants within the window, and the best likelihood is selected as the genotype.

Remarks

Although samtools, freebayes, GATK, and Platypus are not the only tools that have been successfully used for germline SNP calling in the past 10 years, they have enjoyed sustained popularity and continued use in large scale genomics projects such as 1000 Genomes Project, ExAC[72], PCAWG[122] and others. They thus can serve as primary examples of how the problem of calling germline SNPs is currently approached within the field, as well as providing a benchmark for new methods that attempt to solve similar problems. There are several key distinctions in the approaches that these tools take to the overall problem, yet a number of key similarities exist that we outline here and adopt as a general framework for tackling the SNP calling problem in our work.

Site Selection The problem of selecting sites in the genome for detecting variants has variety of approaches from evaluating every single site independently as one that potentially harbors a variant (as in samtools) to the varied windowing approaches used by the other variant callers. Looking at sites independently has the benefit of simplicity, while the windowing approach, at the expense of additional computation, allows a more comprehensive evaluation of a genomic region that is not limited to SNPs but can also support the detection of other types of variants. Thus, even though an independent site approach may be acceptable in an initial implementation, some form of windowing is desired in order to benefit from knowledge about the surrounding sequence context. In general, the interestingness of a site for the purposes of detecting a potential variant is universally linked to the presence of high quality reads spanning a particular locus that disagree with the reference sequence at that locus.

Haplotype Construction Samtools has the simplest model here as it does not attempt to construct haplotypes at all. This limits the ability of samtools to accurately represent genomic architecture, and prevents it from being able to supply

phasing information for variants, which is of interest. Freebayes has the next simplest model, where putative haplotypes are constructed directly from the observed read sequences with the observation window. GATK and Platypus actually perform local assembly in the window to come up with an arrangement of reads that is free of artifacts[76] associated with alignment to the reference. Although local assembly appears to improve the ability of these callers to accruately represent sequence variants (especially non-SNPs) this processing step introduces a significant impact on the overall processing cost, mostly incurred from the resource-intensive HMM-aided alignment of reads to the putative haplotypes.

Allele Frequency Spectrum Estimation A distribution of allele frequencies in a population is of interest as it can be used as a prior in the calculation of genotype likelihoods for the samples under analysis and local and population-specific allele frequencies can vary significantly from values implied by generic population-genetics models. Most callers provide capabilities for estimating the Allele Frequency Spectrum by Expectation Maximization or similar approaches (Equation (5) in [73], not covered here, equation 2.15 for freebayes above). Alternatively, a non-informative prior (as in GATK) can be used.

Genotyping Pretty universally across the methods, genotyping is set up as a Bayesian inference selecting a Maximum Likelihood Estimate or a Maximum A Posteriori estimate of the genotype (for a single site or a haplotype) given the reads data, and taking into account the probability of errors dervied from read base quality and mapping quality scores supplied by the aligner. See Eq. 2.7 for samtools', Eq. 2.10 frebayes', Eq. 2.16 GATK's, Eq. 2.18 Platypus' model setups. This model thus remains highly relevant for any new development in the space.

2.2.6 Germline Indel Calling

2.2.7 Germline Structural Variant Calling

Structural Variants (SVs) are medium- to large-size alterations (typically >50bp in length) of the genomic sequence that fall into a several broad categories (see Figure 2.7) including insertions, deletions, tandem and interspersed duplications, inversions, translocations, mobile element insertions, as well as complex rearrangements that constitute a combination of the above classes or are otherwise difficult to classify.

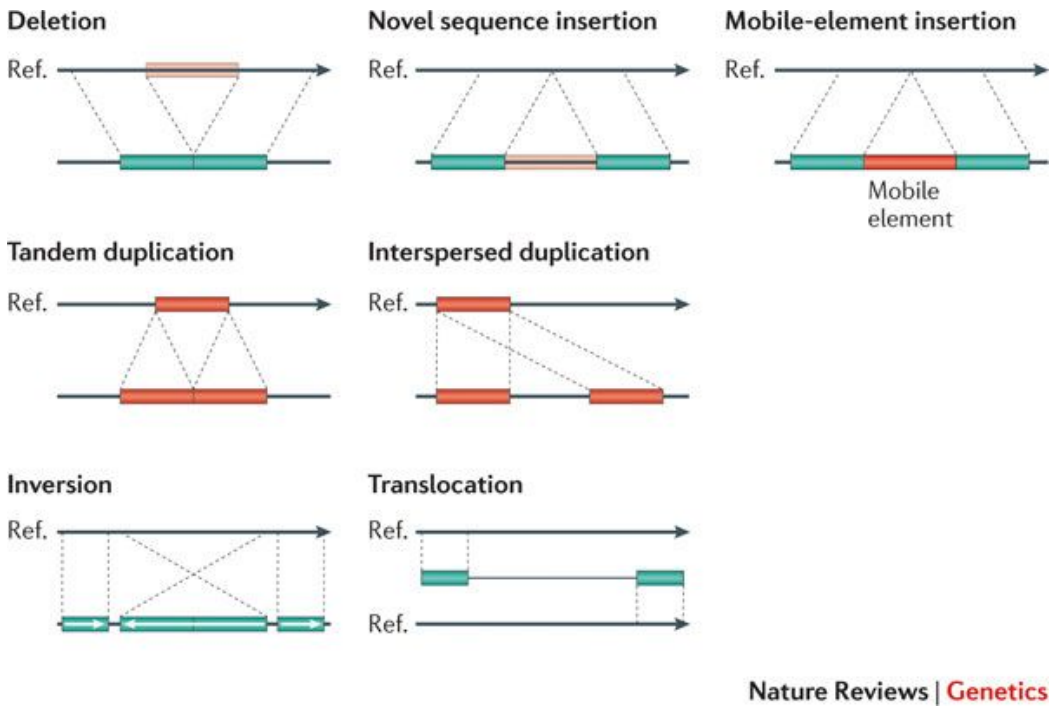

Figure 2.7: Classes of structural variation.[5].

Accurate detection of SVs remains an open challenge since both sensitivity and specificity performance of SV calling is an order of magnitude worse than for SNP calling. For instance, in the latest data release of the 1000 Genomes Project an estimated sensitivity of 88% was achieved for deletions, 65% for duplications, 32% for inversions[125] and False Discovery Rate of up to 89%autocitemills2011mapping. A key challenge that impacts calling accuracy is small read size relative to the size of the variants. Since SVs can be hudreds of kilobases in length and typical Illumina reads are only 150-500 baspeairs long, accurate reconstruction relies on an agglomeration of reads in order to produce the variants. Structural Variant detection approaches typically make use of several sources of evidence (see Figure 2.8) for the detection of "breakpoints" - regions of the genome where a DNA double-strand break is detected and sequence is either inserted or excised. The breakpoints are then interpreted to produce the most likely variants that they represent.

Discordantly-mapped read pairs are pairs of sequencing reads that the alignment algorithm has mapped at a distance that is statistically significantly larger or smaller than the average read-pair distance for that sample, or that have an unusual read orientation, since read-pairs are supposed to be sequencing from two ends of a molecule towards each other. Reads that map closer than they are supposed to are indicative of an insertion, reads that map farther are indicative of a deletion, and reads that map in an unusual orientation are indicative of an inversion.

Split-reads are read-pairs where one of the reads maps properly but the aligner is not able to map the other read because different pieces of the read map to different locations in the genome indicating that this read spans a breakpoint. Distance

between the split read pieces and their mapping orientation are informative of the type of breakpoint that the read spans.

Read-depth Regions have a higher than normal or lower than normal read depth (count of reads spanning a region) are indicative of increased or reduced copy number respectively. Care must be taken to distinguish actual SVs from areas of repetitive sequence where the same simple sequence pattern is repeated many times. Typically aligners are not able to accurately resolve such areas and map many reads to the same set of coordinates making it appear like a duplication.

Assembly-based approaches perform local assembly in a reference-free manner to reconstruct the variants encoded in the sample sequence that the aligner may struggle with resolving because of a large number or increased complexity of rearrangements.

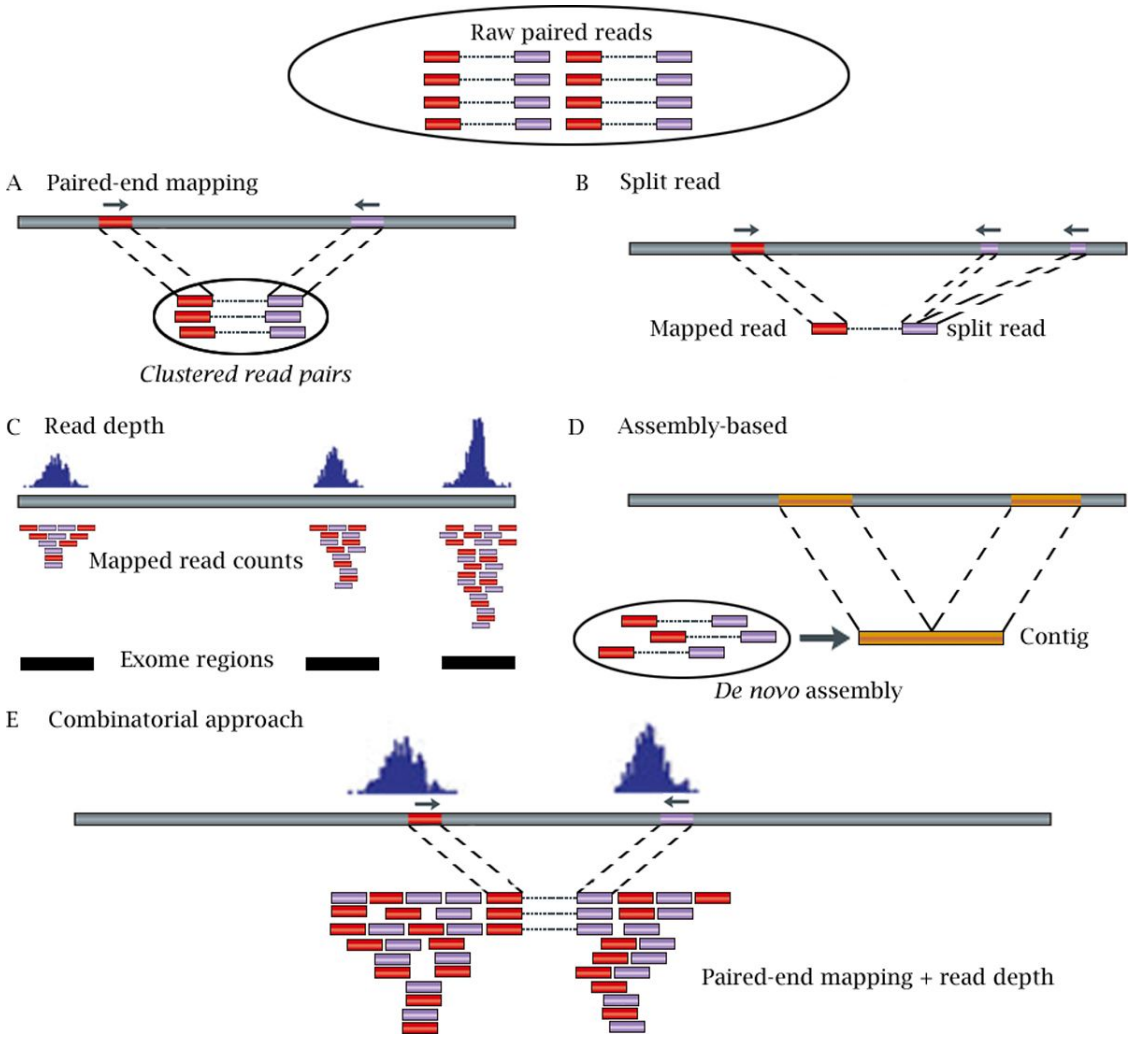

Figure 2.8: Sources of evidence for the presence of Structural Variants.[147].

We look at several SV callers in closer detail.

Delly

Delly[106] is a structural variant calling tool built in 2011 by T. Rausch et al. in the context of the 1000 Genomes Project. Delly uses discordantly-mapped reads and split-reads as sources of evidence for the presence of SVs, is able to characterize a broad set of variants and has been implemented in C++. All of the mathematical formulas and figures in this section are reproduced from the 2012 mauscript by Raush et al.

The discordantly-mapped reads component of Delly begins by computing the median and standard deviation of the read insert size (distance between the ends of the two reads in a pair), as well as the default read orientation by sampling a pre-defined number of reads from the BAM file. Read-pairs that have an insert size farther than 3 standard deviations from the median are considered as evidence for structural variation. This induces a minimum SV size detectable by Delly. See Figure 2.9 for the variant types implied by each insert size deviation and read-pair orientation.

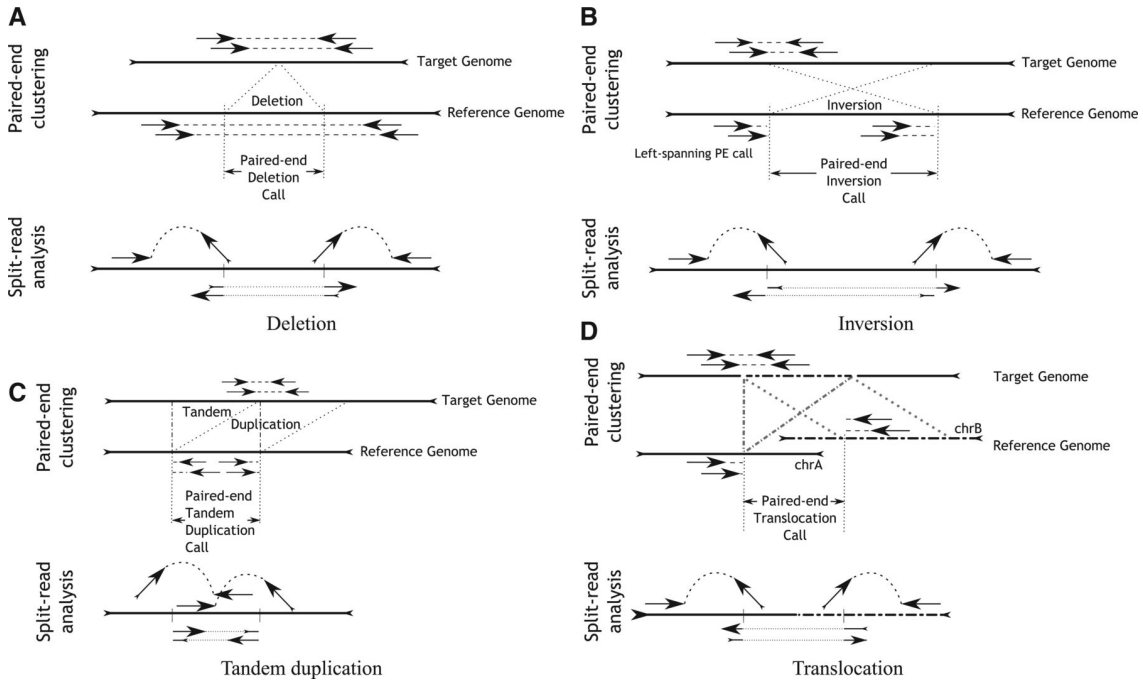

Figure 2.9: Variant classes detectable by Delly based on their read-pair and split-read signatures.[106].

Using the list of discordantly-mapped read-pairs Delly builds an undirected, weighted graph to indicate which read-pairs support the same variant. In the graph $G(V, E)$, a read-pair p_i corresponds to a node $v_i \in V$ and an edge $e_{v_i,v_j} \in E$ connects two nodes that support the same SV. The weight $w(e_{v_i,v_j})$ is the absolute value of the difference between the SV sizes predicted by v_i and v_j. v_i and v_j support the same variant when the corresponding read pairs have the same read orientation and the absolute difference between the left and right ends of the two reads is less than the expected insert size. Variants are identified by computing connected components C_i

of G. When components are not fully connected, Delly sorts the edges in each such component by weight and attempts to find a maximal clique M_i within C_i using edge with the smallest weight as the seed of the clique. The clique is extended by searching for the next smallest edge for which one of the vertices is already in the clique M_i, and requiring that $M_i \cup \{v_l, v_m\}$ is also a clique, until no further vertices can be added. The vertices in M_i are reported as the read pairs supporting that SV. Each rearrangement type is analyzed independently in this manner.

All of the rearrangements identified in the discordantly-mapped read analysis are refined using split-read analysis. The reads used for split-read analysis are those where one read in the pair maps to the reference and the other is unmapped. All such reads within a distance of 2 standard deviations of the median insert size from the breakpoint are considered up to a configurable maximum of 1000. Delly splits each unmapped read into kmers and aligns the kmers to the reference sequence spanning the SV.

Lumpy

Lumpy[70] is an SV caller developed in 2013 by R. Layer et al. and is a package implemented in C++. All of the mathematical formulas and figures presented in this section are reproduced from the 2014 publication by R. Layer et al.

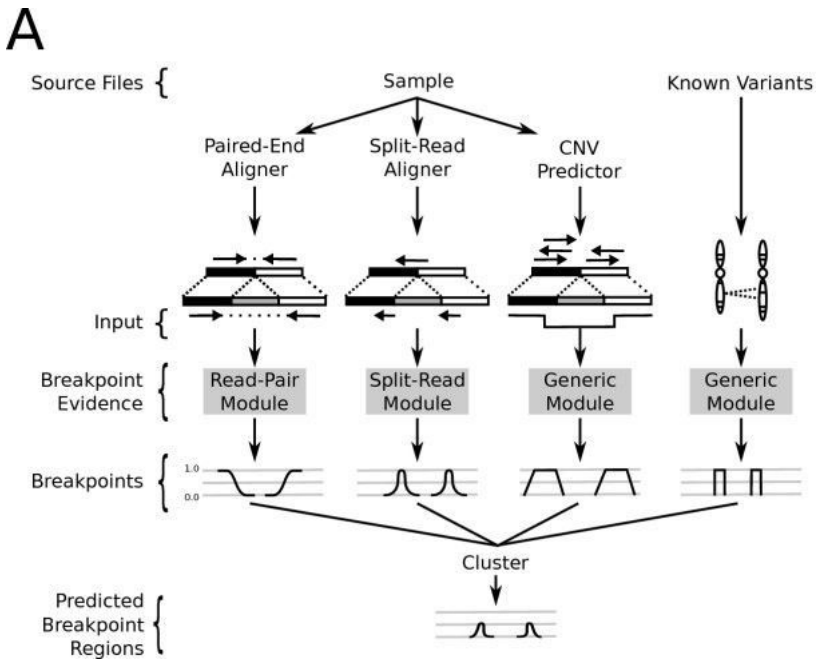

Figure 2.10: Lumpy calling model integrates several signals from a single sample.[70].

Lumpy defines an SV breakpoint as a pair of bases that are adjacent in the sample under study but not in the reference genome. Furthermore, each breakpoint is represented as a pair of probability distributions that span the predicted breakpoint regions and represent the uncertainty about the precise location of the breakpoint.

Lumpy integrates multiple signals (see Figure 2.10) to update the probability distributions that represent each breakpoint based on different kinds of evidence provided. A breakpoint is a tuple $b = \langle E, l, r, v \rangle$ where E is the evidence, $b.l$ and $b.r$ are the left and right intervals each having start and end coordinates, $b.l.s$ and $b.l.e$ for example. $b.l.p$ is a vector of length $b.l.e - b.l.s$ where each $p[i]$ is the probablity that $b.l.s + i$ is the true location of the breakpoint. $b.v$ is the breakpoint class (insertion, deletion, etc.). Overlapping breakpoints are merged together. The intervals that contain 95% of the probability density, as well as the Maximum Likelihood Estimates of the location of each variant are returned.

The paired-end analysis looks at read pairs $\langle x, y \rangle$ where each read is aligned to the reference genome as $R(x) = \langle c, o, s, e \rangle$ where c is the chromosome, $o \in +, -$ indicates alignment orientation, s and e represent the alignment start and end respectively. It is assumed that x and y align uniquely and that $R(x).s < R(x).e < R(y).s < R(y).e$. $S(x) = \langle o, s, e \rangle$ is defined to be the alignment of x with respect to the sample's (unknown) genome. Read pairs are assumed to be aligned with $R(x).o = +, R(y).o = -$ and having $R(y).e - R(x).s$ approximately equal to the library preparation fragment size. Read pairs that align outside the expected parameters for orientation and distance constitute evidence for structural variant breakpoints, in particular reads with the same or switched orientation, and pairs that align at a distance shorter or longer than the fragment size. Expected fragment length is estimated from the mean fragment length in the sample, along with its standard deviation. Breakpoint variety is determined as follows:

When $R(x).c = R(y).c$, the variety is inferred from read orientation. $R(x).o = R(y).o$ implies an inversion. $R(x).o = -, R(y).o = +$ implies a tandem duplication, $R(x).o = +, R(y).o = -$ implies a deletion. Insertions are not presently supported. When $R(x).c \neq R(y).c$ the breakpoint is labelled an interchromosomal rearrangement.

$\langle x, y \rangle$ are mapped to $b.l$ and $b.r$ as follows. By convention x is assumed to map to l and y to r. It is assumed that there exists a single breakpoint b between x and y. Orientation of x determines whether l is upstream or downstream from x. Thus, if $R(x).s = +$, then the breakpoint begins after $R(x).e$. The length of the breakpoint interval is proportional to expected fragment length and its standard deviation. The distance of one end of the breakpoint from x is assumed to be less than expected insert size L plus $v_f s$ - a configurable number of standard deviations. The probability that position i in the breakpoint interval l is part of the actual breakpoint is estimated as the probability that x and y span i, which is true when the fragment that had produced $\langle x, y \rangle$ is longer than the distance from x to i. Thus, the quantity of interest is $P(S(y).e - S(x).s > i - R(x).s)$ for $R(x).o = +$ and $P(S(y).e - S(x).s > R(x).e - i)$ for $R(x).o = -$. This quantity is estimated from the empirical distribution of fragment sizes collected from the sample.

Lumpy does not perform its own split-read alignment instead relying on the results of a split-read aligner like YAHA[38]. Every split-read is a DNA fragment X that consits of two or more sub-fragments $x_i, ..., x_j$ that do not map to adjacent locations of the reference. Each sub-fragment pair $\langle x_i, x_{i+1} \rangle$ is evaluated independently depending on how it maps with respect to the reference $R(x)$. When

$R(x_i).o = R(x_{i+1}).o$ the event is marked as either a deletion or a tandem duplication, where $R(x_i).s < R(x_{i+1}).s$ implies a deletion, and otherwise a tandem duplication. When orientations do not match, the event is marked an inversion. To account for potential inaccuracies in the location of the breakpoint with respect to the split-read pair, each split-read pair is mapped to two breakpoint intervals l and r that are centered at the split. The probability is modeled to be highest at the split with an exponential decrease towards the edges, with a configurable width parameter.

SvABA

SvABA[134] is a germline and somatic SV caller by J. Walla et al., created in 2016. Svaba is implemented in C++ and relies on genome-wide local assembly for variant detection. Internally SvABA makes use of SGA[120] and BWA-MEM[77] for assembly and read mapping respectively.

Figure 2.11: The SvABA variant callng method[134].

SvABA performs local de-novo assembly in 25-kbp windows tiled across the genome with a 2kb overlap. First reads that may be indicative of variation are extracted from a BAM file, these include reads with high-quality soft-clipped bases, discordant reads (with non-standard orietnation or with insert size greater than four standard deviations away from the sample mean insert size, determined using a sample of 5 million reads with top and bottom 5% of insert sizes truncated), unmapped reads, reads with unmapped mates, and reads with insertions or deletions indicated in the CIGAR string. Low quality reads that are marked as PCR duplicates, have failed QC, and reads with repeats of >20 basepairs are filtered out. Discordant reads are realigned to the reference with BWA-MEM and those with an available nondiscordant alignment of >70% of the maximum alignment score are discarded. Reads with many different (>20) high quality candidate alignments are also discarded. Remaining discordant reads are clustered based on orientation and insert-size and assembled using SGA.

The contigs produced by SGA are aligned to the reference genome using BWA-MEM and examined for potential variants. Contigs with an alignment that has fewer than 30 nonaligned bases and no alignment gaps are considered reference. Indels are called when the alignment has gaps, and SVs called when the resulting alignment is multi-part. In order to evaluate read support for the putative variants, reads from the windows are aligned to both the reference and the assembled contigs using BWA-MEM. Reads are considered matching to the contig when the alignment score for the read to the contig is greater than the alignment of the read to the reference and is >90% of the length of the match. Reads that have an alignment that is up to 8 bases to the left or right of a putative variant are consdered supporing the variant.

Remarks

We looked at Delly, Lumpy, and SvABA in the context of germline SV calling. Although there are some differences in the details of the approaches taken by the tools' authors there are consistent similarities as well, which additionally carry over to other SV callers such as Pindel[143], and Manta[13], that have not been presented here. These approaches rely heavily on paired-end reads and select those that are mapped uncommonly far apart or close together. These are clustered or optionally locally assembled to produce putative breakpoints. Breakpoint locations are further refined by split-read analysis, using those reads that are unmapped by regular read mapping software. These reads are broken down into kmers and each kmer is aligned separately to non-adjacent locations in the genome. Alternative haplotypes may be constructed using these reads and alignments to the reference and the alternative haplotypes scored for genotyping purposes. A new method for structural variant calling should thus focus on making the best use of these two data types (discordant and split reads), while possibly also making use of read depth information to be competitive with the current generation of best callers.

2.2.8 Variant Filtering

2.2.9 Somatic SNP Calling

Mutect - Muse - Pindel -

2.2.10 Somatic Indel Calling

2.2.11 Somatic Structural Variant Calling

2.2.12 Germline Variant Annotation

Annovar - Variant Effect Predictor -

2.2.13 Somatic Variant Annotation

2.2.14 de-novo Assembly

Velvet - Abyss -

2.3 High Performance and High Throughput Computing

The practice of performing large scale scientific computation on supercomputers or clusters of commodity hardware can be split into two notions - High Performance Computing (HPC) and High Throughput Computing (HTC).

The European Grid Infrastructure defines these as follows[46]:

> HPC - A computing paradigm that focuses on the efficient execution of compute intensive, tightly-coupled tasks. Given the high parallel communication requirements, the tasks are typically executed on low latency interconnects which makes it possible to share data very rapidly between a large numbers of processors working on the same problem. HPC systems are delivered through low latency clusters and supercomputers and are typically optimised to maximise the number of operations per seconds. The typical metrics are FLOPS, tasks/s, I/O rates.

> HTC - A computing paradigm that focuses on the efficient execution of a large number of loosely-coupled tasks. Given the minimal parallel communication requirements, the tasks can be executed on clusters or physically distributed resources using grid technologies. HTC systems are typically optimised to maximise the throughput over a long period of time and a typical metric is jobs per month or year.

Although early High Performance Computing efforts (1960's - 1980's) relied on supercomputers with a shared memory model[110], where all of the memory was shared between multiple processors, by the late 1980's machines with a distributed memory model[98], where each processor has its own memory, started gaining ground, forming the basis for the modern HPC cluster.

The software interface that the user has to a HPC/HTC cluster typically takes the shape of a queueing system such as PBS[55] or LSF[148] where the user writes a script that submits a series of jobs to the queueing system. The jobs can invoke software that is installed by the IT department that manages the cluster. The user is not able to install any software and has limited visibility into the runtime performance characteristics of the jobs they submit.

2.4 Cloud Computing

Cloud computing has emerged in the early 2000's enabled by improvements in hardware virtualization which was driven by the adoption of Virtual Private Networks, and the desire to commercialize access to compute capacity as a utility[12].

The National Institute of Standards and Technology provides a standard defini-

tion of cloud computing that encompasses several areas of this domain - Essential Characteristics, Service Models, and Deployment Models[91].

The Essential Characteristics of a cloud are as follows:

On-demand self-service - End-user can independently manage infrastructure without involving the service provider.

Broad network access - Cloud resources are available on the network via a set of standard protocols.

Resource pooling - Service providers dynamically assign virtual infrastructure in a multi-tenant environment based on consumer demand.

Rapid elasticity - Resources can be elastically provisioned and discarded according to customer requirements.

Measured service - Resource usage by end users is measured and transparently provided back to the user by the service provider.

It is the self-service and broad network access characteristics that set cloud computing apart from traditional HPC computing the most.

Service Models include:

Infrastructure as a Service (IaaS) - This service allows the the user to provision and control virtualized infrastructure such as VMs and networks.

Platform as a Service (PaaS) - This service allows the user to deploy their application onto virtualized hardware but not to control the management of the infrastructure.

Software as a Service (SaaS) - This service allows the user to make use of applications that are deployed on virtualized hardware but not to manage the applications or the infrastructure itself.

The Deployment Models covered by the NIST definition are as follows:

Private Cloud - Operated privately by a single organization and not accessible on a public network.

Community Cloud - Established for use by a particular community of users with a common interest.

Public Cloud - Established for general use by the public.

Hybrid Cloud - A collection of cloud entities that use one of the other deployment models but allow application portability.

The first publicly available commercial cloud computing platform has been developed by Amazon.com and launched in August, 2006 in the form of two services - Elastic Compute Cloud (EC2), and Simple Storage Service (S3). Cloud offerings by Microsoft and Google followed in 2010, and 2012. This early lead has allowed Amazon to capture the majority of the public cloud computing market, earning $2.57 billion USD in Q1 2016 revenue.

One of the main drawbacks, however, of using Amazon's or another proprietary cloud solution is the issue of "vendor lock-in" i.e. inability to easily switch infrastructure providers should the customer wish to do so, because of the amount of software relying on the proprietary cloud provider protocols. Another key reason for avoiding public clouds is the necessity to store sensitive data. This issue applies both to the commercial enterprise (with industries such as banking, and payments) and scientific domains (especially genomics and medicine) where handling of sensitive patient data is restricted based on both technical security, as well as ethical considerations[66].

To help alleviate these concerns an open-source cloud platform called Openstack was launched in 2010 jointly by Rackspace Hosting and NASA[115]. Openstack provides most of the same features that are provided by Amazon Web Services and other commercial cloud providers as free open-source tools. These include:

- Infrastructure
- Networking
- Identity Management
- Block Storage
- Object Storage
- Managed Databases
- Queues
- Monitoring

Openstack deployments for the basis for most academic private and community clouds such as EBI Embassy Cloud[20], University of Chicago Open Science Data Cloud[50], Cancer Genome Collaboratory[144], and Helix Nebula[88]. Because these clouds implement the security measures necessary when handling patient data they are a system of choice for large scale bioinformatics analyses.

2.5 Workflow Systems

The focus on workflow stems the work of Frederick Taylor (1856-1915) and Henry Gantt (1861-1919) on the improvement and automation of industrial processes, also

known as "scientific management"[126]. One of the key techniques that were devised at the time and served as the prototype for future workflows were "time and motion studies"[8] where employees were observed as they performed repetitive cycles of work in order to determine standard execution times and sequences of steps. As this field evolved over the course of the 20th century it gave rise to several other related fields such as Operations Management, Business Process Management, and Lean Manufacturing.

In 1993 an international consortium was formed with the purpose of defining the standards related to workflows and workflow management systems. This consortium is called the Workflow Management Coalition (WfMC). One of the key specifications produced by the WMC in 1995 is The Workflow Reference Model[58]. This document provides two basic definitions that illuminate the scope and purpose of workflow systems:

> Workflow - The computerised facilitation or automation of a business process, in whole or part.

> Workflow Management System - A system that completely defines, manages and executes "workflows" through the execution of software whose order of execution is driven by a computer representation of the workflow logic.

A number of standards have been produced for workflow definition, many of them are XML-based[117]. Notable examples include:

XPDL - Was developed by the WfMC, currently at version 2.2, as of 2012. Uses an XML dialect to express process definitions

BPML - Developed by the Object Management Group (OMG) using XML. Deprecated as of 2008 in favour of BPEL.

BPEL/BPEL4WS - Developed by Organization for the Advancement of Structure Information Standard (OASIS). Uses XML format. Adopted by Microsoft and IBM for their workflow products -

Graphically, workflow definitions are typically expressed using a Petri-Net[104] or Business Process Model and Notation (BPMN), the latter borrowing its structure from UML activity diagrams. A set of workflow definition design patterns exists to guide workflow creation[29]. A workflow engine is responsible for ingesting workflow definitions, generating their graphical representation, and allowing the user to execute the workflow definitions on suitable hardware.

As initially the focus of workflow systems research and development has been on process improvement within commercial enterprises there exists a large pool of workflow engine implementations targeted at that sector. Some of these are:

jBPM - An open-source workflow engine that is based on the Java platform and is currently owned by Red Hat.

Activiti - An open-source workflow engine that has been developed by previous jBPM developers.

Oracle BPEL Process Manager - A commercial workflow engine acquired by Oracle from Collaxa in 2004, now integrated into the rest of the Oracle portfolio.

Websphere Process Server - Commercial workflow engine that is part of IBM's Business Process Manager suite.

Although these tools have gained wide adoption in the enterprise community they have had limited success within scientific circles. Instead, several open-source workflow management systems exist that have been purpose-built for the scientific domain, and especially bioinformatics. These include:

Kepler[81] - A Java-based WfMS built on top of the Ptolemy II[27] execution engine.

Taverna[99] - A Java-based WfMS originally built by myGrid, currently under incubation at Apache Software Foundation.

Galaxy[47] - A Python-based WfMS developed specifically for bioinformatics applications with a focus on GUI-driven development of workflows.

Curcin et al[22] provide a head-to-head comparison of six scientific workflow systems including Taverna and Kepler, whereby Taverna is described as primarily being aimed at researchers who wish to build scientific workflows from web services utilizing a proprietary XML dialect called SCUFL which implements a DAG model of workflows. The primary execution environment for a Taverna workflow is on a grid or an HPC cluster. Kepler implemented a different methodology, whereby workflow modelling, which is taken on by Actors, is separated from workflow execution, taken on by Directors. An Actor knows only about its inputs, the computation that it needs to perform, and the output that it needs to produce, while Directors provide different models of execution, such as Synchronous Data Flow, Process Network, Continuous Time, and Discrete Event.

The Galaxy workflow framework has a specific focus on bioinformatics analyses and comes with a large library of community-developed bioinformatics workflows. The user creates and executes workflows via a web-based GUI where pre-installed tools and scripts can be laid out into a pipeline. The primary deployment environment for Galaxy is on an institutional HPC cluster although a separate component allows the deployment of a Galaxy instance on Amazon Web Services[2].

2.6 Service Oriented Architectures

2.7 Stream-based Systems

Chapter 3

The Butler Framework - Requirements and Architecture

Chapter 4

The Butler Framework - Implementation and Experimental Validation

Chapter 5

The Rheos Framework

In this chapter we describe a software framework called Rheos, which demonstrates an approach for reasoning about large genomic datasets utilizing concepts of service-orientation and data streaming in contrast with traditional genomic data analysis frameworks[28] that take a procedural batch-based approach. Rheos' focus on service-orientation and streaming allow the users to make active tradeoff decisions between analysis time, cost, and quality as well as setting up precise operational Service Level Agreements, both between Rheos components, and between Rheos and external systems, as we describe in detail below.

5.1 General Framework Design

As already discussed in Chapters 1 and 2, the general problem consists of collecting DNA samples from a population of individuals under study, sequencing these samples using Next Generation Sequencing techniques, identifying the mutations that are present, annotating their functional impact and utilizing the obtained data in a downstream data analysis with research or clinical decision-making goals. While there is a great variety of possible downstream analyses that may be performed depending on the individual goals of the analyst, there is a fairly well established set of steps for processing of the raw NGS data into a set of annotated variants, and it is these steps that we target with this work. The typical approach that is in widespread use today is to collect a batch of samples and then process each sample individually with a sequence of individual tools, that may be described via a higher-level workflow construct (such as in Figure 2.5, or using a framework like Butler, as described in Chapters 3, 4). There are, however, a number of factors that leave room for improvement in this model. These improvements lie along a set of dimensions that we describe briefly in the Introduction via a utility function $U_i = C_i + T_i + A_i$ for sample $i \in [1, N_s]$ that needs to be optimized, and that we describe in more detail here.

We use the following definitions throughout the text:

Table 5.1: Rheos common definitions

Symbol	Description
N_p	Number of people
$P = \{p_i : i \in [1, N_p]\}$	Set of individuals under study
N_s	Number of samples
$S = \{s_i : i \in [1, N_s]\}$	Set of sequenced DNA samples. Each individual can have one or more samples.
A_i	Accuracy score of analysis for sample i (precise definition of Accuracy TBD)
$C_i = c_{g_i} + c_{s_i} + c_{a_i} + c_{r_i}$	Cost score of data generation, storage, analysis, and retrieval respectively
T_i	Time score to process sample i
$U_i = C_i + T_i + A_i$	A utility function for individual i that penalizes high cost, high processing time, and low accuracy
$U = \sum_{i=1}^{N_s} U_i$	Overall utility of processing N_s samples through Rheos.

5.2 Data Streaming Architecture

The overall technical architecture of the Rheos system is set up as a Service Oriented Architecture (SOA)[118] which is an information system architecture paradigm where the overall problem that the system is trying to solve is broken down into a collection of loosely-coupled components called services. Each service has a well defined interface of inputs that it accepts and outputs that it produces. Services can be combined and orchestrated together to produce the overall desired output for the system. A key distinguishing feature of this architectural approach is that each service can be individually optimized to fulfill its contract most efficiently helping break down some of the performance limitations brought about by the necessity to simultaneously tackle competing constraints in more monolithic information system designs. Additionally, within a services framework, the dependencies between separate services can be negotiated not only in terms of service interfaces, but also in terms Service Level Agreements which constitute Quality of Service promises made by one service to its dependents[60]. Because it is unlikely that a service designer will be able to accurately foresee all of the demands that will be placed on a service during its lifetime the SLAs provide a valuable feedback framework through which the service can be evaluated as it operates in production, as well as serving as a basis for negotiating evolving requirements between dependent services.

While general web services can support any data processing paradigm, in the Rheos framework we adopt a data streaming approach[7]. In this approach we assume that the input to any service is a randomly ordered sequence of messages $M = m_1, m_2, ...$ where each message represents a fact about the underlying domain that the service reasons over, as well as some metadata, including an identifier, and a variety of timestamps of interest. The content of each message may provide a

datum, such as the measurement of a quantity of interest, or signal that a particular event has taken place. It is in general assumed that the data stream is infinite in size, that messages may arrive out of order, and that any message that is placed in the stream is observed at most once, and may, in fact, never be observed. Messages are typically not sent directly from one service to another, instead the transfer of messages is mediated by a queuing system using a publish-subscribe[35] model. Under this model each queue acts as a *topic*. Message producers can publish data to the topic, and message consumers subscribe to receive messages from the topic. A message is consumed from the queue only after all of the subscribed consumers have seen it. End users retrieve information from the system via a set of User Interfaces that support both push (notifications) and pull (querying) models of data retrieval. A more detailed description of the architectural aspects of the system follows:

5.2.1 Service-Oriented Data Streaming Model

A data stream $M_{s,d} = m_1, m_2, ...$ is a sequence of datagrams transmitted over the network with the following properties:

- The stream has a source s and a destination d.
- A message m in the stream is a tuple of the form $(header, payload)$, where:
  - *header* is a tuple of the form $(id, \dots)$ that holds at minimum a unique identifier id for messages, and may hold additional metadata.
  - *payload* is an arbitrary data structure that holds the informational content of the message.
- $|M| = \infty$ by assumption.
- Messages may not arrive at destination d in the same order that they were sent from source s.
- If $t_{i,s}$ is the time message m_i leaves the source s and $t_{i,d}$ is the time of arrival at destination d, then $\sup_i\{t_{i,d} - t_{i,s}\} = \infty$, i.e. a given sent message may never arrive at its destination.

A service $S = \{o_i\}$ is a collection of operations o_i that act on one or more input data streams $\{M_i\}$ to produce one or more transformed output data streams $\{M_j\}$. Specifically:

An operation O is a tuple of the form $O = (i, o, p, f)$, where:

- $i = \{M_j, j \in [0, K]\}$ is a set of $K \geq 0$ input data streams.
- $o = \{M'_j, j \in [0, L]\}$ is a set of $L \geq 0$ output data streams.
- $f : M^K \mapsto M'^L$ is a transformation function that produces messages m' in the output streams based on messages m observed in the input streams.

- $p = \{p_i\}$ is a set of potentially optional query parameters.

There are several distinct categories of operations that a service can perform on a set of input streams. We describe these here:

Windowing Function - Service S observes a sliding window, which is a sample of size n of messages from stream M_i and computes a summary statistic (see Figure 5.1) over the sample which is meant to be an approximation of the corresponding population parameter.

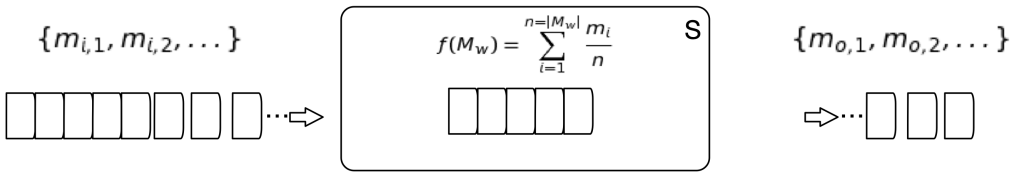

Figure 5.1: Service S computes a summary statistic over a window of messages from stream M

Decorator Function - Service S observes messages m_i and applies a function that augments (decorates) each message with additional attributes (see Figure 5.2) producing augmented messages m_o as output.

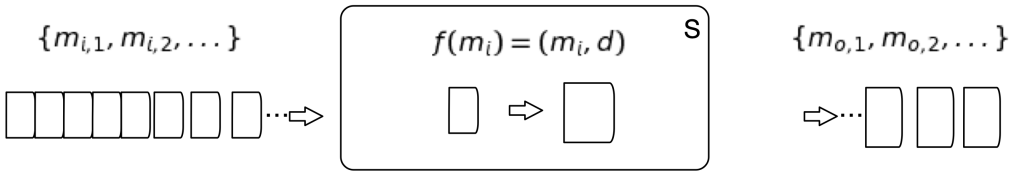

Figure 5.2: Service S computes a summary statistic over a window of messages from stream M

Filter Function - Service S observes messages m_i and applies a function $f : M \mapsto \{True, False\}$ that evaluates to a boolean value (see Figure 5.2). Only messages that map to $True$ are emitted as output.

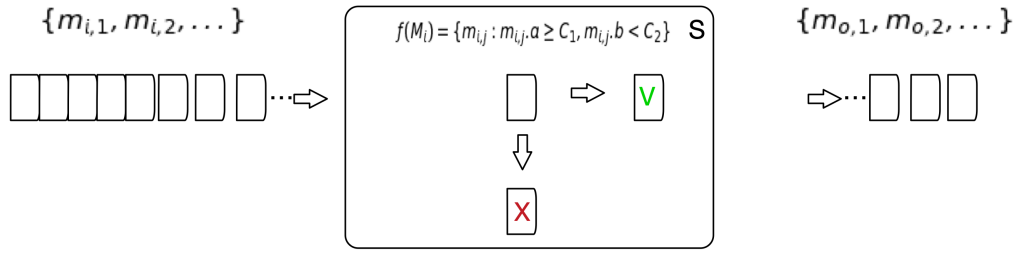

Figure 5.3: Service S filters messages from input stream M and only allows through those that pass the filtering condition.

Aggregator Function - Service S observes messages m_i and applies a function $f : M \mapsto \{True, False\}$ that evaluates to a boolean value (see Figure 5.2). Only messages that map to $True$ are emitted as output.

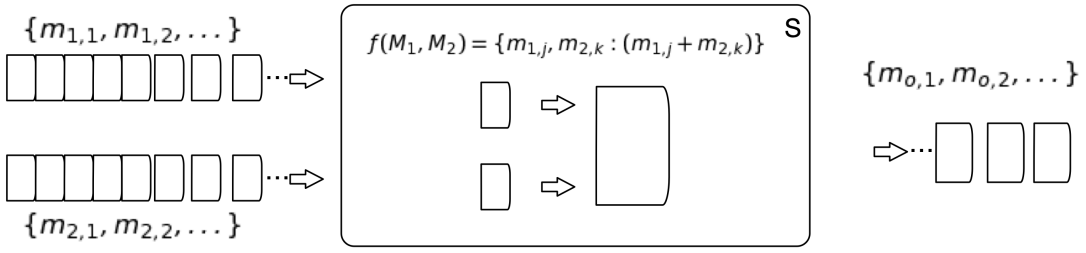

Figure 5.4: Service S filters messages from input stream M and only allows through those that pass the filtering condition.

Local State Aggregator Function - Service S observes messages m_i and applies a function $f : M \mapsto \{True, False\}$ that evaluates to a boolean value (see Figure 5.2). Only messages that map to $True$ are emitted as output.

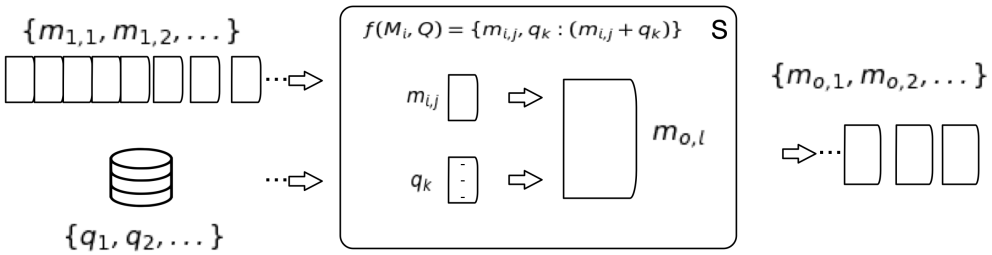

Figure 5.5: Service S filters messages from input stream M and only allows through those that pass the filtering condition.

Persistence Function - Service S observes messages m_i and applies a function $f : M \mapsto \{True, False\}$ that evaluates to a boolean value (see Figure 5.2). Only messages that map to $True$ are emitted as output.

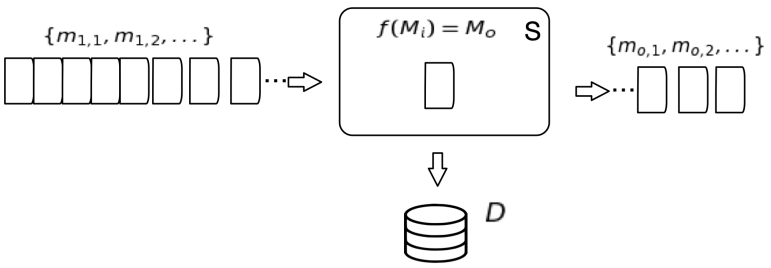

Figure 5.6: Service S filters messages from input stream M and only allows through those that pass the filtering condition.

Query Function - Service S observes messages m_i and applies a function $f : M \mapsto \{True, False\}$ that evaluates to a boolean value (see Figure 5.2). Only messages that map to $True$ are emitted as output.

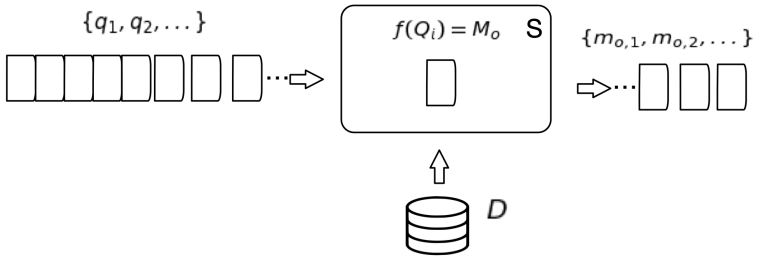

Figure 5.7: Service S filters messages from input stream M and only allows through those that pass the filtering condition.

Aggregator Local State Aggregator Persistence Query

———— Set up the general architecture of information flow through the system - types of entities, communication channels, contracts, SLAs, capabilities:

- Each stream is a collection of randomly ordered datagrams.
- A datagram may constitute data or an event.
- The rate of the stream is understood.
- A service applies a transformation to the stream by virtue of making a decision upon inspecting stream contents.
- A service makes decisions based on:
  - Individual datagrams
  - Sliding windows of datagrams
  - Particular sequences of datagrams
  - Integration of incoming stream signal with a static data store
  - Combinations of multiple streams
- A service emits a new stream as output.
- A service may decorate an existing stream with new info or create new datagrams.
- A service may persist data to a permanent store.
- The rate at which the service can make decisions is understood (along with limiting conditions).
- Communication between services is mediated via queues.

- Services provide an SLA on uptime, latency, throughput, etc.
- Services fail fast.
- System output supports push and pull modes.

5.3 Domain-specific Problems

Describe specific problems that need to be addressed by the system in a stream-based formulation - how to go from a collection of raw reads to a set of annotated variants: map reads, perform QC filtering, model loci, assemble alternative haplotypes, call variants, filter variants, annotate, produce output.

- Perform read QC
- Align reads to reference
- Collect read stream statistics
- Assemble local read contigs
- Model individual loci
- Call variants (maybe need to split by variant types)
- Genotype variants
- Filter variants
- Annotate variants
- Output variants

5.4 Services of Rheos

Provide a mapping from the domain-specific problems onto a particular implementation in the data streaming architecture. List services, their responsibilities, contracts, etc.

Metadata - Take in metadata related to patients, samples, files, etc. In: Metdata records, Out: ingestion confirmation events.

Read Streaming - Take data from outside the system (file, web service, etc) and turn it into a standard stream. In: files, external streams, Out: internal read stream.

Read Persistence - Store reads on disk, index. In: read stream, Out: persistence confirmation events.

Read Statistics - Look at read stream and calculate various approximate stats of interest - insert size, GC-bias, etc. In: read stream, Out: running stats of interest

QC - Compute QC score for reads. In: reads, Out: reads with QC score

Read Filtering - Filter out low quality reads based on configured parameters. In: reads with QC score, Out: filtered reads.

Read Mapping - Align reads to reference genome. In: stream of reads, Out: streams of mapped, unmapped, split reads.

Local Assembly - Local assembly of reads into candidate haplotypes. In: stream of aligned reads, Out: Updated haplotypes event.

Haplotype Persistence - Storage and lookup of candidate haplotypes. In: stream of reads, Out: persistence confirmation events, stream of haplotypes.

Variant Calling - Evaluate candidate haplotypes for presence of variation. In: haplotype update events, Out: variant update events.

Variant Persistence - Storage and lookup of variants. In: stream of reads, Out: persistence confirmation events, stream of variants.

Genotyping - Genotype variant sites. In: variant update stream, Out: genotype update events.

Variant Filtering - Filter out low quality variants. In: stream of variants, Out: filtered stream of variants.

Variant annotation - Annotate variants for functional impact. In: stream of variants, Out: stream of annotated variants.

Output variants - Format variants for external output. In: stream of variants, Out: files, external variant stream.

Notification - Notify the user when events of interest occur. In: any stream, Out: stream of notifications.

5.5 Proof of concept implementation

Describe actual implementation efforts. Focus on very basic use case (take already mapped reads from a file, turn them into a stream, use stream to call SNPs, maybe some Indels/SVs). Demonstrate some comparison metrics compared to other callers. Demonstrate some service-level metrics (throughput etc.)

5.6 Conclusions

Rheos is great!

Chapter 6

Discussion and Conclusion

Appendix A

Code Listings

Listing 1: Terraform configuration of a worker VM

```
provider "openstack" {
        user_name = "${var.user_name}"
        password = "${var.password}"
        tenant_name = "${var.tenant_name}"
        auth_url = "${var.auth_url}"
}

resource "openstack_compute_instance_v2" "worker" {
          image_id = "${var.image_id}"
        flavor_name = "s1.massive"
        security_groups = ["internal"]
        name = "${concat("worker-", count.index)}"
        network = {
                uuid = "${var.main_network_id}"
        }
        connection {
                user = "${var.user}"
                 key_file = "${var.key_file}"
                 bastion_key_file = "${var.bastion_key_file}"
                 bastion_host = "${var.bastion_host}"
                 bastion_user = "${var.bastion_user}"
                 agent = "true"

        }
        count = "175"
        key_pair = "${var.key_pair}"
        provisioner "remote-exec" {
                inline = [
                        "sudo mv /home/centos/saltstack.repo
                        ↪  /etc/yum.repos.d/saltstack.repo",
                        "sudo yum install salt-minion -y",
```

```
31                          "sudo service salt-minion stop",
32                          "echo 'master: ${var.salt_master_ip}' | sudo tee  -a
                            ↪  /etc/salt/minion",
33                          "echo 'id: ${concat("worker-", count.index)}' | sudo tee
                            ↪  -a /etc/salt/minion",
34                          "echo 'roles: [worker, germline, consul-client]' | sudo
                            ↪  tee -a /etc/salt/grains",
35                          "sudo hostname ${concat("worker-", count.index)}",
36                          "sudo service salt-minion start"
37                  ]
38          }
39  }
```

Listing 2: Terraform configuration of a security group

```
1   resource "openstack_compute_secgroup_v2" "internal" {
2           name = "internal"
3           description = "Allows communication between instances"
4           #SSH
5           rule {
6                   from_port = 22
7                   to_port = 22
8                   ip_protocol = "tcp"
9                   self = "true"
10          }
11          #Saltstack
12          rule {
13                  from_port = 4505
14                  to_port = 4506
15                  ip_protocol = "tcp"
16                  self = "true"
17          }
18  }
```

Listing 3: Salt Pillar for specifying test data location.

```
1   test_data_sample_path: /shared/data/samples
2
3   test_data_base_url: http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/data/
4
5   test_samples:
6     NA12874:
7       -
8         - NA12874.chrom11.ILLUMINA.bwa.CEU.low_coverage.20130415.bam
9         - 88a7a346f0db1d3c14e0a300523d0243
```

```
    -
      - NA12874.chrom11.ILLUMINA.bwa.CEU.low_coverage.20130415.bam.bai
      - e61c0668bbaacdea2c66833f9e312bbb
```

Listing 4: Using Salt Mine to look up a server's IP Address.

```
consul-client:
  service.running:
    - enable: True
    - watch:
      - file: /etc/opt/consul.d/*
{%- set servers = salt['mine.get']('roles:(consul-server|consul-bootstrap)',
↪  'network.ip_addrs', 'grain_pcre').values() %}
{%- set node_ip = salt['grains.get']('ip4_interfaces')['eth0'] %}
# Create a list of servers that can be used to join the cluster
{%- set join_server = [] %}
{%- for server in servers if server[0] != node_ip %}
{% do join_server.append(server[0]) %}
{%- endfor %}
join-cluster:
  cmd.run:
    - name: consul join {{ join_server[0] }}
    - watch:
      - service: consul-client
```

Listing 5: Using Top File to map States to Roles.

```
base:
  '*':
    - consul
    - dnsmasq
    - collectd
  'G@roles:monitoring-server':
    - influxdb
    - grafana
  'G@roles:job-queue':
    - rabbitmq
```

Listing 6: Collectd configuration for metrics collection.

```
# Read metrics about cpu usage
[[inputs.cpu]]
  ## Whether to report per-cpu stats or not
  percpu = true
```

```
  ## Whether to report total system cpu stats or not
  totalcpu = true
  ## If true, collect raw CPU time metrics.
  collect_cpu_time = false
  ## If true, compute and report the sum of all non-idle CPU states.
  report_active = false


# Read metrics about disk usage by mount point
[[inputs.disk]]
  ## By default, telegraf gather stats for all mountpoints.
  ## Setting mountpoints will restrict the stats to the specified mountpoints.
  # mount_points = ["/"]

  ## Ignore some mountpoints by filesystem type. For example (dev)tmpfs (usually
  ## present on /run, /var/run, /dev/shm or /dev).
  ignore_fs = ["tmpfs", "devtmpfs", "devfs"]


# Read metrics about disk IO by device
[[inputs.diskio]]
  ## By default, telegraf will gather stats for all devices including
  ## disk partitions.
  ## Setting devices will restrict the stats to the specified devices.
  # devices = ["sda", "sdb"]
  ## Uncomment the following line if you need disk serial numbers.
  # skip_serial_number = false
  #
  ## On systems which support it, device metadata can be added in the form of
  ## tags.
  ## Currently only Linux is supported via udev properties. You can view
  ## available properties for a device by running:
  ## 'udevadm info -q property -n /dev/sda'
  # device_tags = ["ID_FS_TYPE", "ID_FS_USAGE"]
  #
  ## Using the same metadata source as device_tags, you can also customize the
  ## name of the device via templates.
  ## The 'name_templates' parameter is a list of templates to try and apply to
  ## the device. The template may contain variables in the form of '$PROPERTY' or
  ## '${PROPERTY}'. The first template which does not contain any variables not
  ## present for the device is used as the device name tag.
  ## The typical use case is for LVM volumes, to get the VG/LV name instead of
  ## the near-meaningless DM-0 name.
  # name_templates = ["$ID_FS_LABEL","$DM_VG_NAME/$DM_LV_NAME"]


# Get kernel statistics from /proc/stat
[[inputs.kernel]]
```

```
  # no configuration


# Read metrics about memory usage
[[inputs.mem]]
  # no configuration


# Get the number of processes and group them by status
[[inputs.processes]]
  # no configuration


# Read metrics about swap memory usage
[[inputs.swap]]
  # no configuration


# Read metrics about system load & uptime
[[inputs.system]]
  # no configuration
```

Listing 7: Filebeat Prospector configuration.

```
[collectd]
  enabled = true
  bind-address = ":8096"
  database = "metrics"
  retention-policy = ""
  batch-size = 5000
  batch-pending = 10
  batch-timeout = "10s"
  read-buffer = 0
  typesdb = "/usr/share/collectd/types.db"
```

Listing 8: TICKscript for alerting on CPU value.

```
// Parameters
var info = 70
var warn = 80
var crit = 90
var infoSig = 2.5
var warnSig = 3
var critSig = 3.5
var period = 10s
```

```
var every = 10s

// Dataframe
var data = stream
  |from()
    .database('metrics')
    .retentionPolicy('default')
    .measurement('cpu_value')
    .where(lambda: "type" == 'percent' AND "type_instance" == 'idle')
  |eval(lambda: 100 - "value")
    .as('used')
  |window()
    .period(period)
    .every(every)
  |mean('used')
    .as('stat')

// Thresholds
var alert = data
  |eval(lambda: sigma("stat"))
    .as('sigma')
    .keep()
  |alert()
    .id('{{ index .Tags "host"}}/cpu_value')
    .message('{{ .ID }}:{{ index .Fields "stat" }}')
    .info(lambda: "stat" > info OR "sigma" > infoSig)
    .warn(lambda: "stat" > warn OR "sigma" > warnSig)
    .crit(lambda: "stat" > crit OR "sigma" > critSig)

// Alert
alert
  .log('/tmp/cpu_alert_log_2.txt')
```

Listing 9: TICKscript for handling dead VMs.

```
{% raw %}
var db = 'telegraf'
var rp = 'autogen'
var measurement = 'system'
var groupBy = ['host']
var whereFilter = lambda: TRUE
var period = 30s
var name = 'Host Deadman'
var idVar = name + ':{{.Group}}'
var blah = '{{index .Tags "host"}}'
var message = 'The host {{index .Tags "host"}} is offline as of {{.Time}}.'
var messageN = 'The host {{index .Tags "host"}} is back online at {{.Time}}.'
```

```
13 var idTag = 'alertID'
14 var levelTag = 'level'
15 var messageField = 'message'
16 var durationField = 'duration'
17 var outputDB = 'chronograf'
18 var outputRP = 'autogen'
19 var outputMeasurement = 'alerts'
20 var triggerType = 'deadman'
21 var threshold = 0.0
22 var data = stream
23     |from()
24         .database(db)
25         .retentionPolicy(rp)
26         .measurement(measurement)
27         .groupBy(groupBy)
28         .where(whereFilter)
29
30 var trigger = data
31     |deadman(threshold, period)
32         .stateChangesOnly()
33         .message('{{ if eq .Level "CRITICAL" }}' + message + '{{else}}' +
           ↪  messageN + '{{end}}')
34         .id(idVar)
35         .idTag(idTag)
36         .levelTag(levelTag)
37         .messageField(messageField)
38         .durationField(durationField)
39         .slack()
40         .channel('#embassyalerts')
41 {% endraw %}
42         .exec('butler_healing_agent', 'relaunch-worker', '-t', '{{
           ↪  pillar['terraform_files'] }}', '-s', '{{ pillar['terraform_state']
           ↪  }}', '-v', '{{ pillar['terraform_vars'] }}', '-p', '{{
           ↪  pillar['terraform_provider'] }}')
43 {% raw %}
44 trigger
45     |eval(lambda: "emitted")
46         .as('value')
47         .keep('value', messageField, durationField)
48     |influxDBOut()
49         .create()
50         .database(outputDB)
51         .retentionPolicy(outputRP)
52         .measurement(outputMeasurement)
53         .tag('alertName', name)
54         .tag('triggerType', triggerType)
55
56 trigger
```

```
    |httpOut('output')
{% endraw %}
```

Listing 10: Butler healing agent code for restarting the Airflow Scheduler.

```
def call_command(command, cwd=None):
  try:
      logging.debug("About to invoke command: " + command)
      my_output = check_output(command, shell=True, cwd=cwd, stderr=STDOUT)
      logging.debug("Command output is: " + my_output)
      return my_output
  except CalledProcessError as e:
      logging.error("An error occurred! Command output is: " +
      ↪  e.output.decode("utf-8") )
      raise

def is_critical(level):
  return level == "CRITICAL"

def parse_alert_data():
  return json.loads(sys.stdin.read())

def get_host_name(alert_data):
  return alert_data["data"]["series"][0]["tags"]["host"]

def restart_service(host, service_name):
  call_command("pepper {} service.restart {}".format(host, service_name), None)

def parse_args():
  my_parser = argparse.ArgumentParser()

  sub_parsers = my_parser.add_subparsers()

  common_args_parser = argparse.ArgumentParser(
      add_help=False, conflict_handler='resolve')

  restart_airflow_scheduler_parser = sub_parsers.add_parser(
      "restart-airflow-scheduler", parents=[common_args_parser],
      ↪  conflict_handler='resolve')

  ↪  restart_airflow_scheduler_parser.set_defaults(func=restart_airflow_scheduler_command)

def restart_airflow_scheduler_command(args, alert_data):
  if is_critical(alert_data["level"]):
      restart_service("-G 'roles:tracker'", "airflow-scheduler")
```

Listing 11: Butler healing agent code for relaunching a failed VM.

```
def call_command(command, cwd=None):
  try:
      logging.debug("About to invoke command: " + command)
      my_output = check_output(command, shell=True, cwd=cwd, stderr=STDOUT)
      logging.debug("Command output is: " + my_output)
      return my_output
  except CalledProcessError as e:
      logging.error("An error occurred! Command output is: " +
      ↪  e.output.decode("utf-8") )
      raise

def is_critical(level):
  return level == "CRITICAL"

def parse_alert_data():
  return json.loads(sys.stdin.read())

def get_host_name(alert_data):
  return alert_data["data"]["series"][0]["tags"]["host"]

def restart_service(host, service_name):
  call_command("pepper {} service.restart {}".format(host, service_name), None)

def parse_args():
  my_parser = argparse.ArgumentParser()

  sub_parsers = my_parser.add_subparsers()

  common_args_parser = argparse.ArgumentParser(
    add_help=False, conflict_handler='resolve')

  relaunch_worker_parser = sub_parsers.add_parser(
      "relaunch-worker", parents=[common_args_parser],
      ↪  conflict_handler='resolve')
  relaunch_worker_parser.add_argument(
      "-t", "--terraform_location", help="Location of the terraform definition
      ↪  files.",
      dest="terraform_location", required=True)
  relaunch_worker_parser.add_argument(
      "-s", "--terraform_state_location", help="Location of the terraform state
      ↪  file.",
      dest="terraform_state_location", required=True)
  relaunch_worker_parser.add_argument(
      "-v", "--terraform_var_file_location", help="Location of the terraform vars
      ↪  file.",
      dest="terraform_var_file_location", required=True)
  relaunch_worker_parser.add_argument(
      "-p", "--terraform_provider", help="The terraform provider to use.",
```

```
44          choices = provider_list,
45          dest="terraform_provider", required=True)
46      relaunch_worker_parser.set_defaults(func=relaunch_worker_command)
47
48      def is_key_present(key_data, host_name):
49      parsed_key_data = json.loads(key_data)
50      return_data = parsed_key_data["return"][0]["data"]["return"]
51
52      if "minions" in return_data:
53          return_vals = return_data["minions"]
54          for val in return_vals:
55              if val == host_name:
56                  return True
57
58      return False
59
60  def locate_minon_key(host_name):
61      minion_connect_try = 1
62      while minion_connect_try <= MINION_CONNECT_MAX_RETRIES:
63          logging.info("Attempt #{} of {} to retrieve minion key for host {} from the
            ↪  master.".format(minion_connect_try, MINION_CONNECT_MAX_RETRIES,
            ↪  host_name))
64          key_data = call_command("pepper --client=wheel key.name_match
            ↪  match={}".format(host_name))
65          logging.debug("Retrieved key data: " + key_data)
66          if is_key_present(key_data, host_name):
67              return True
68          else:
69              logging.debug("Key data for host {} not found at time {}. Sleeping for
                ↪  {} seconds.".format(host_name, datetime.now(),
                ↪  MINION_CONNECT_SLEEP_PERIOD))
70              time.sleep(MINION_CONNECT_SLEEP_PERIOD)
71              minion_connect_try = minion_connect_try + 1
72
73      return False
74
75  def relaunch_worker_command(args, alert_data):
76      if is_critical(alert_data["level"]):
77          host_name = get_host_name(alert_data)
78
79
80          tf_location = args.terraform_location
81          tf_state_location = args.terraform_state_location
82          tf_var_file_location = args.terraform_var_file_location
83          tf_resource = provider_resource_lookup[args.terraform_provider]
84          worker_number = host_name.split("-")[1]
85
86          call_command("pepper --client=wheel key.delete match={}".format(host_name))
```

```
87        call_command("terraform taint -lock=false -state={}
          ↪  {}.worker.{}".format(tf_state_location, tf_resource, worker_number),
          ↪  tf_location)
88        call_command("terraform apply -lock=false -state={} --var-file {}
          ↪  -auto-approve".format(tf_state_location, tf_var_file_location),
          ↪  tf_location)
89
90        locate_minon_key(host_name)
91
92        call_command("pepper '*' mine.update")
93        call_command("pepper {} state.apply dnsmasq".format(host_name))
94        call_command("pepper {} state.apply consul".format(host_name))
95        call_command("pepper {} state.highstate".format(host_name))
```

Listing 12: Consul service definition for PostgreSQL.

```
1  {
2          "results_base_path": "/shared/data/results/discovery/",
3          "results_local_path": "/tmp/discovery/",
4          "freebayes": {
5                  "mode": "discovery",
6                  "flags": "--min-repeat-entropy 1
                   ↪  --report-genotype-likelihood-max"
7          }
8  }
```

Listing 13: Source code for the freebayes workflow.

```
1  from airflow import DAG
2  from airflow.operators import BashOperator, PythonOperator
3  from datetime import datetime, timedelta
4
5  import os
6  import logging
7  from subprocess import call
8
9  import tracker.model
10 from tracker.model.analysis_run import *
11 from tracker.util.workflow_common import *
12
13
14 def run_freebayes(**kwargs):
15
16     config = get_config(kwargs)
17     logger.debug("Config - {}".format(config))
```

```
18
19      sample = get_sample(kwargs)
20
21      contig_name = kwargs["contig_name"]
22      contig_whitelist = config.get("contig_whitelist")
23
24
25      if not contig_whitelist or contig_name in contig_whitelist:
26
27          sample_id = sample["sample_id"]
28          sample_location = sample["sample_location"]
29
30          result_path_prefix = config["results_local_path"] + "/" + sample_id
31
32          if (not os.path.isdir(result_path_prefix)):
33              logger.info(
34                  "Results directory {} not present,
                    ↪  creating.".format(result_path_prefix))
35              os.makedirs(result_path_prefix)
36
37          result_filename = "{}/{}_{}.vcf".format(
38              result_path_prefix, sample_id, contig_name)
39
40          freebayes_path = config["freebayes"]["path"]
41          freebayes_mode = config["freebayes"]["mode"]
42          freebayes_flags = config["freebayes"]["flags"]
43
44          reference_location = config["reference_location"]
45
46          if freebayes_flags == None:
47              freebayes_flags = ""
48
49          if freebayes_mode == "discovery":
50              freebayes_command = "{} -r {} -f {} {} {} > {}".\
51                  format(freebayes_path,
52                         contig_name,
53                         reference_location,
54                         freebayes_flags,
55                         sample_location,
56                         result_filename)
57          elif freebayes_mode == "regenotyping":
58              variants_location = config["variants_location"]
59
60              freebayes_command = "{} -r {} -f {} -@ {} {} {} > {}".\
61                  format(freebayes_path,
62                         contig_name,
63                         reference_location,
64                         variants_location[contig_name],
```

```
                        freebayes_flags,
                        sample_location,
                        result_filename)
        else:
             raise ValueError("Unknown or missing freebayes_mode -
             ↪  {}".format(freebayes_mode))

        call_command(freebayes_command, "freebayes")

        compressed_sample_filename = compress_sample(result_filename, config)
        generate_tabix(compressed_sample_filename, config)
        copy_result(compressed_sample_filename, sample_id, config)
    else:
        logger.info(
            "Contig {} is not in the contig whitelist,
            ↪  skipping.".format(contig_name))


default_args = {
    'owner': 'airflow',
    'depends_on_past': False,
    'start_date': datetime.datetime(2020, 01, 01),
    'email': ['airflow@airflow.com'],
    'email_on_failure': False,
    'email_on_retry': False,
    'retries': 1,
    'retry_delay': timedelta(minutes=5),
}

dag = DAG("freebayes", default_args=default_args,
          schedule_interval=None, concurrency=10000, max_active_runs=2000)


start_analysis_run_task = PythonOperator(
    task_id="start_analysis_run",
    python_callable=start_analysis_run,
    provide_context=True,
    dag=dag)


validate_sample_task = PythonOperator(
    task_id="validate_sample",
    python_callable=validate_sample,
    provide_context=True,
    dag=dag)

validate_sample_task.set_upstream(start_analysis_run_task)

```

```
complete_analysis_run_task = PythonOperator(
    task_id="complete_analysis_run",
    python_callable=complete_analysis_run,
    provide_context=True,
    dag=dag)

for contig_name in tracker.util.workflow_common.CONTIG_NAMES:
    freebayes_task = PythonOperator(
        task_id="freebayes_" + contig_name,
        python_callable=run_freebayes,
        op_kwargs={"contig_name": contig_name},
        provide_context=True,
        dag=dag)

    freebayes_task.set_upstream(validate_sample_task)

    complete_analysis_run_task.set_upstream(freebayes_task)
```

Listing 14: Saltstack state for workflow deployment.

```
current_runs = run_session.query(Configuration.config[("sample","
↪  sample_id")].astext).\
        join(AnalysisRun, AnalysisRun.config_id == Configuration.config_id).\
        join(Analysis, Analysis.analysis_id == AnalysisRun.analysis_id).\
        filter(and_(Analysis.analysis_id == analysis_id, AnalysisRun.run_status
        ↪  != tracker.model.analysis_run.RUN_STATUS_ERROR)).all()

available_samples = sample_session.query(PCAWGSample.index.label("index"),
↪  sample_id.label("sample_id"), sample_location.label("sample_location")).\
        join(SampleLocation, PCAWGSample.index == SampleLocation.donor_index).\
        filter(and_(sample_location != None, sample_id.notin_(current_runs))).\
        limit(num_runs).all()
```

Listing 15: Butler Analysis configuration for SNP genotyping.

```
{
        "variants_location": {
            "1": "/freebayes.chr_1.sites.snv_indel.annot.final.vcf.gz",
            "2": "/freebayes.chr_2.sites.snv_indel.annot.final.vcf.gz",
            "3": "/freebayes.chr_3.sites.snv_indel.annot.final.vcf.gz",
            "4": "/freebayes.chr_4.sites.snv_indel.annot.final.vcf.gz",
            "5": "/freebayes.chr_5.sites.snv_indel.annot.final.vcf.gz",
            "6": "/freebayes.chr_6.sites.snv_indel.annot.final.vcf.gz",
            "7": "/freebayes.chr_7.sites.snv_indel.annot.final.vcf.gz",
            "8": "/freebayes.chr_8.sites.snv_indel.annot.final.vcf.gz",
```

```
            "9": "/freebayes.chr_8.sites.snv_indel.annot.final.vcf.gz",
            "10": "/freebayes.chr_10.sites.snv_indel.annot.final.vcf.gz",
            "11": "/freebayes.chr_11.sites.snv_indel.annot.final.vcf.gz",
            "12": "/freebayes.chr_12.sites.snv_indel.annot.final.vcf.gz",
            "13": "/freebayes.chr_13.sites.snv_indel.annot.final.vcf.gz",
            "14": "/freebayes.chr_14.sites.snv_indel.annot.final.vcf.gz",
            "15": "/freebayes.chr_15.sites.snv_indel.annot.final.vcf.gz",
            "16": "/freebayes.chr_16.sites.snv_indel.annot.final.vcf.gz",
            "17": "/freebayes.chr_17.sites.snv_indel.annot.final.vcf.gz",
            "18": "/freebayes.chr_18.sites.snv_indel.annot.final.vcf.gz",
            "19": "/freebayes.chr_19.sites.snv_indel.annot.final.vcf.gz",
            "20": "/freebayes.chr_20.sites.snv_indel.annot.final.vcf.gz",
            "21": "/freebayes.chr_21.sites.snv_indel.annot.final.vcf.gz",
            "22": "/freebayes.chr_22.sites.snv_indel.annot.final.vcf.gz",
            "X": "/freebayes.chr_X.sites.snv_indel.annot.final.vcf.gz",
            "Y": "/freebayes.chr_Y.sites.snv_indel.annot.final.vcf.gz"
        },
        "results_base_path":
        ↪  "/shared/data/results/regenotype_freebayes_discovery/",
        "results_local_path": "/tmp/regenotype_freebayes_discovery/",
        "freebayes": {
                "mode": "regenotyping",
                "flags": "-l"
        }
}
```

Listing 16: Butler Workflow configuration for Data Submission.

```
{
        "gnos": {
                "ebi": {
                        "url": "https://gtrepo-ebi.annailabs.com"
                },
                "osdc_icgc": {
                        "url": "https://gtrepo-osdc-icgc.annailabs.com"
                },
                "osdc_tcga": {
                        "url": "https://gtrepo-osdc-tcga.annailabs.com"
                }
        },
        "rsync": {
                "flags": "-a -v --remove-source-files"
        }
}
```

Listing 17: Butler Analysis configuration for Data Submission.

```
{
        "gnos": {
                "ebi": {
                        "key_location":
                        ↪  "/home/airflow/.ssh/sergei_pcawg_gnos_icgc.pem"
                },
                "osdc_icgc": {
                        "key_location":
                        ↪  "/home/airflow/.ssh/sergei_pcawg_gnos_icgc.pem"
                },
                "osdc_tcga": {
                        "key_location":
                        ↪  "/home/airflow/.ssh/sergei_bionimbus_gnos_may.pem"
                }
        },
        "metadata_template_location": "/opt/pcawg-germline/workflows/gtupload-wo
        ↪  rkflow/analysis_template.xml",
        "submission_base_path":
        ↪  "/shared/data/results/freebayes_discovery_gnos_submission/",
        "destination_repo_mapping": {
                "ICGC": "ebi",
                "TCGA": "osdc_tcga"
        }
}
```

Listing 18: Python code for the run_delly function which implements the functionality of the delly_genotype task inside the Butler Delly Workflow.

```
def run_delly(**kwargs):

    config = get_config(kwargs)
    sample = get_sample(kwargs)

    sample_id = sample["sample_id"]
    sample_location = sample["sample_location"]

    result_path_prefix = config["results_local_path"] + "/" + sample_id

    if (not os.path.isdir(result_path_prefix)):
        logger.info(
            "Results directory {} not present,
            ↪  creating.".format(result_path_prefix))
        os.makedirs(result_path_prefix)

    delly_path = config["delly"]["path"]
    reference_location = config["reference_location"]
```

```
    variants_location = config["variants_location"]
    variants_type = config["variants_type"]
    exclude_template_path = config["delly"]["exclude_template_path"]

    result_filename = "{}/{}_{}.bcf".format(
        result_path_prefix, sample_id, variants_type)

    log_filename = "{}/{}_{}.log".format(
        result_path_prefix, sample_id, variants_type)

    delly_command = "{} call -t {} -g {} -v {} -o {} -x {} {} > {}".\
        format(delly_path,
               variants_type,
               reference_location,
               variants_location,
               result_filename,
               exclude_template_path,
               sample_location,
               log_filename)

    call_command(delly_command, "delly")

    copy_result(result_filename, sample_id, config)
```

Listing 19: Butler Delly Workflow analysis configuration to genotype deletions.

```
{
        "variants_location": "/delly_deletion_sites/del.sites.bcf",
        "results_base_path":
        ↪  "/shared/data/results/delly_germline_deletions_14_07_2016/",
        "results_local_path": "/tmp/delly_germline_deletions/",
        "variants_type": "DEL"

}
```

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