Masters Thesis on **Benchmarking Somatic Variant Callers**

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Declaration

I hereby declare, that I am the sole author and composer of my thesis and that no other sources or learning aids, other than those listed, have been used. Furthermore, I declare that I have acknowledged the work of others by providing detailed references of said work.

I hereby also declare, that my Thesis has not been prepared for another examination or assignment, either wholly or excerpts thereof.

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Abstract

Variant calling pipelines consists of two main types, aimed at quantifying different types of variants namely Germline (inheritable variants) and Somatic (uninheritable variants). In terms of benchmarking, many Germline variant calling pipelines were compared and documented by the standards of the Genome in a Bottle (GIAB) Consortium [1]. For Somatic variant calling pipelines, only a few comparisons were achieved because the comparisons are more complex and less well-established. In the diploid human genome, a variant can be found to be either homozygous or heterozygous or not at all. Tumors, on the other hand, are inhomogeneous cells that possibly carry variants with rare mutations thereby making the Somatic variant calling pipelines benchmarking challenging. Due to the diverse natures of these two types of analysis, the goal of this thesis is to benchmark somatic variant callers by selecting few datasets, in order to establish a reliable variant caller for cancer research.

Zusammenfassung

Variantenaufruf-Pipelines bestehen aus zwei Haupttypen, die darauf abzielen, verschiedene Arten von Varianten zu quantifizieren, nämlich Germline (vererbbare Varianten) und Somatic (nicht vererbbare Varianten). In Bezug auf das Benchmarking wurden viele Pipelines mit Keimbahnvarianten verglichen und nach den Standards des Genome in a Bottle (GIAB) -Konsortiums [1] dokumentiert. Für Somatic-Varianten-Calling-Pipelines wurden nur wenige Vergleiche erzielt, da die Vergleiche komplexer und weniger gut etabliert sind. Im diploiden menschlichen Genom kann festgestellt werden, dass eine Variante entweder homozygot oder heterozygot ist oder überhaupt nicht. Tumore hingegen sind inhomogene Zellen, die möglicherweise Varianten mit seltenen Mutationen tragen, wodurch die Somatic-Variante, die Pipelines als Benchmarking bezeichnet, eine Herausforderung darstellt. Aufgrund der unterschiedlichen Natur dieser beiden Analysetypen besteht das Ziel dieser Arbeit darin, somatische Variantenaufrufer durch Auswahl weniger Datensätze zu bewerten, um einen zuverlässigen Variantenaufrufer für die Krebsforschung zu etablieren.

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List of Abbreviations

1. GIAB - Genome in a Bottle
2. INDEL - INsertion/DELetion polymorphism
3. MIRACUM - Medical Informatics in Research and Care in University Medicine
4. SNP - Single Nucleotide Polymorphisms
5. VCF - Variant Calling Format
6. WES - Whole Exome Sequencing
7. WGS - Whole Genome Sequencing

Chapter 1

Introduction

A variant is a type of mutation that differs in some respect from a reference genome/transcriptome standard, where a variant in bioinformatics is an alteration in the most common DNA/RNA nucleotide sequence. It is defined based on the type of DNA/RNA error and can be used to describe an alternation that may be benign, pathogenic or of unknown significance.

Based on the way they occur, variants are of two types i.e. Germline variants and Somatic variants. Germline variants are a gene change in the egg or sperm that are incorporated into the DNA of every cell in the offspring from the parent. These hereditary variants are even referred to as Germline mutations. Somatic variants are alterations in the DNA that occur after conception and can occur in any cell of the body except the germ cells i.e. egg and sperm cells. Therefore, Somatic variants cannot be passed on to children.

The process to identify the variants is called Variant Calling and the variants are identified from the sequence data obtained through sequencing [28]. Sequencing is the operation of determining the precise order of a chain of four bases Adenine (A), Guanine (G), Cytosine (C) and Thymine (T) in a DNA strand.

In terms of sequencing, there is Whole Genome Sequencing (WGS) [29] or Whole Exome Sequencing (WES) [30]. In WGS, also known as full genome sequencing, the DNA sequence of an entire organism's genome is determined at a single time. WES, also known as Exome Sequencing, is a genomic technique for sequencing all of the protein-coding regions of genes in a genome also known as exomes.

Through one of the many different sequencing technologies, a biological sequence and its corresponding quality scores are obtained as FASTQ files, a text-based format for storing both the values. These FASTQ files [3] are then aligned based on a reference genome thereby creating a BAM file [4] which is a compressed binary version of a SAM file or the human-readable text file with biological sequences aligned to a reference sequence.

Using the BAM files, differences between the aligned reads and the reference genome can be written into a Variant Call Format (VCF) file [6]. This process of identifying variants from the sequence data is called Variant Calling and this process is different for both Germline and Somatic variant calling.

In the Germline variant calling, the reference genome is a golden standard sequence for the species of interest and the genomes are diploid. So at any given locus, either all reads have the same base, indicating homozygosity or approximately half the reads have one base and the other half have another indicating heterozygosity with the only exception being the sex chromosomes in the male mammals. In Somatic variant calling, the reference is tissue from the same individual with mosaicism between the cells, where mosaicism is when a person has two or more genetically different sets of cells in their body.

The goal of this thesis is to benchmark Somatic variant callers based on their ability to identify cancer-causing variants. To perform the comparison, we require the Truth Data to which the variant calling outcomes i.e. the VCF files compared, are know. We therefore consider the artificial dataset pair for Somatic variant calling from the GIAB project[1] as the Truth Data and based on the comparisons, the effectiveness of the Somatic variant callers is determined.

There are several variant callers for both Germline and Somatic variant calling. Some commonly-used Germline variant callers are FreeBayes, Strelka2, VarScan, and Beagle. Similarly, some common Somatic variant callers are LoFreq, MuSE, MuTect2, SomaticSniper, Strelka, VarScan and

[1]GIAB project is available at ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/use_cases/mixtures/UMCUTRECHT_NA12878_NA24385_mixture_10052016/

VarDict. The choice of the variant callers that are benchmarked in this thesis is dependent on work done by Dr Wolfgang Maier and Dr Björn Grüning, with data from Use Case 3 of the German MIRACUM initiative [2].

The Medical Informatics in Research and Care in University Medicine (MIRACUM) initiative has three use cases and the referred third use case is "From Knowledge to Action - Support for Molecular Tumor Boards" [2]. A tumor board is a group of doctors and health care providers with different specialities meeting regularly to discuss cancer cases and share knowledge [8]. In contrast to traditional cancer tumor boards, molecular tumor boards are made up of cancer experts across specialities as well as researchers with expertise on a variety of cancer types, gene sequencing technologies and genomic data, who work together to make informed treatment decisions [7].

The MIRACUM consortium aims to support Molecular Tumor Boards with innovative IT solutions by improving the complex processes of quality assurance, data preparation, data analysis, data integration and information retrieval between genetic high-throughput analysis and medical therapy decisions. Additionally, clinicians will be offered decision support through efficient data visualization [2].

Using Galaxy, an open web-based platform that provides accessibility, reproducibility, and transparency, Dr Wolfgang Maier built two workflows that are supported by the MIRACUM partners [15]. A workflow is a series of tools and dataset actions that run in a sequence as a batch operation. The first workflow is the WES tumor/normal sample pair analysis and the second is gene panel data with only the tumor sample.

The major difference between the two workflows is the variant calling software used in them. For the first workflow or the WES tumor/normal sample pair analysis workflow, VarScan Somatic is used and for the second workflow or the gene panel data workflow, LoFreq Call is used.

For this thesis, the WES tumor/normal sample pair analysis workflow is used to obtain VCF files through VarScan Somatic variant caller. Aside from this, the Strelka variant caller is also added to the workflow using Galaxy and the outcomes from both the variant callers are compared to the artificial Truth Data considered from the GIAB FTP site to benchmark.

This report comprises a total of six sections and is outlined as follows. The first section is ‘Introduction’ dealing with the basics needed to approach the thesis. The second section is ‘Literature Study’ encompassing articles, research papers, tools and websites that helped thesis. The third section is ‘Background’ dealing with acquiring workflows, modifying tools, setting parameters to obtain the VCF files etc. The fourth section is ‘Approach’ dealing each step starting from writing ymal files, using Planemo, understanding the tools, reading VCF files etc.

The fifth section is ‘Experiments’ dealing with different comparisons, bias and benchmarking methods. The sixth section is ‘Results’ dealing with the outcomes of the experiments in terms of positions, SNPs, allele frequencies, read depths, variant combinations, and INDELs. Lastly, the seventh section is ‘Conclusion’ dealing with the choice of the variant caller based on the results and scope for future work.

Chapter 2

Literature Study

The first section ‘Background Study’ deals with comprehending the scope of the topic. In the second section, the platform needed to execute the goal i.e. Galaxy [9] and its concepts are dealt with. In the third section, tasks and tools used based on the information obtained from the Galaxy are mentioned and in the last section, research papers related to the thesis are presented.

2.1 Background Study

To begin with, an understanding of the variant calling was developed using the material “Galaxy Training: Introduction to Variant Calling” [25] and the difference between the mutations in Germline and Somatic were comprehended using the resource from BioNinja[1]. In-order to upload and update the progress of the thesis, GitHub was used and as an introduction to the operations as to how to use GitHub, the “Version Control with Git and GitHub” [10] paper was quite useful.

To comprehend information about the third use case of which the thesis is a part, as well as to gain access to the workflows that are used for the variant calling based on MIRACUM [2], the MIRACUM Pipe Galaxy repository [15] was used. Of the two workflows in the MIRACUM Pipe Galaxy repository, the first workflow i.e. WES tumor/normal sample pair analysis workflow [15] is selected for the Somatic variant calling using VarScan Somatic and

[1]https://ib.bioninja.com.au/standard-level/topic-3-genetics/33-meiosis/somatic-vs-germline-mutatio.html

Strelka Somatic variant callers. An understanding about the VarScan and Strelka variant callers was developed using the resources "VarScan 2: Somatic mutation and copy number alterations discovery in cancer by exome sequencing" [13] and "Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs" [14].

To obtain the input data i.e. the forward reads, reverse reads, bed file, and Truth Data VCF file for the selected WES tumor/normal sample pair analysis workflow, access the GIAB FTP[2] resource. For benchmarking the variant calling outcomes, use the Genome In A Bottle (GIAB) consortium standards material [11] as a reference along with the Germline Benchmarking Tools and Standards [5].

2.2 Galaxy

In Galaxy, numerous training materials and tutorials including the variant calling are a part of the "Galaxy Training" material [12]. So to understand the tools used in exome sequencing, the "Exome sequencing data analysis for diagnosing a genetic disease" material [16][17] was used. Similarly, for an introduction to the Somatic Variant Calling, the "Identification of Somatic and Germline Variants from Tumor and Normal Sample Pairs" material [33][34] was used.

To execute the workflows from the command line, a command-line utility resource of the Galaxy named Planemo [20] was used. This resource which helps in developing tools, workflows and training materials for Galaxy is also helpful to execute workflows through the command line. To execute a Galaxy workflow through Planemo, a YAML file [26] is to be created and then using Planemo and a few other details explained in the fourth chapter "Approach", the workflows are executed remotely.

[2]ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/use_cases/mixtures/UMCUTRECHT_NA12878_NA24385_mixture_10052016/

2.3 Tools

After executing the WES tumor/normal sample pair analysis workflow and obtaining the Strelka and VarScan VCF files, there are three tools to analyze, summarize and compare the VCF files. The first tool named vcftools [21] can be used to extract SNPs or INDELs, calculate allele frequencies, and summarize data in a VCF file. The second tool named vcfstats [27] can be used to plot VCF data with specific metrics and focus on variants with certain filters. The third tool named vcftoolz [32] can be used to compare the number of SNPs and positions in two or three VCF files.

An exception in calculating allele frequency using vcftools is the Strelka VCF file. To calculate the allele frequencies in the Strelka VCF file, resources from Strelka User Guide [31] were used and for operations on VCF files such as filtering rows or columns based on a condition, resources from Filtering and Handling VCFs[3] were used.

2.4 Research Papers

For reference, in terms of Germline variants comparison, "Systematic comparison of germline variant calling pipelines cross multiple next-generation sequencers" by Jiayun Chen, et al [18] talks about variant calling performance of 12 combinations in WES datasets testing three variant calling pipelines HaplotypeCaller, Strelka2 and Samtools-Varscan2.

In terms of Somatic variant calling comparison, "Systematic comparison of somatic variant calling performance among different sequencing depth and mutation frequency" by Zixi Chen, et al [19] talks about mutation frequency as a major problem and increasing sequencing depth as a method to improve the mutation calling performance. Using Strelka2 and Mutect2 tools, the performance of 30 combinations of sequencing depth and mutation frequency is observed.

[3]https://speciationgenomics.github.io/filtering_vcfs/

Chapter 3

Background

The VCF files that are to be benchmarked in comparison to the Truth Data are an outcome of the WES analysis workflow. However, there are two more Galaxy workflows involved to calculate the subsampled data that is provided as an input to the WES analysis workflow. These two workflows are the Map and Filter workflows. Of all the three workflows, the WES analysis workflow is supported by the MIRACUM partners and was built and parameterised by Dr Wolfgang Maier in the MIRACUM Pipe Galaxy repository [15].

The Map workflow is a sub-workflow of the WES analysis workflow used to calculate the mapped reads for both Normal and Tumor samples and the Filter workflow built by Dr Mehmet Tekman uses these mapped reads and capture regions to subsample the input data. The reason for these two additional workflows instead of the WES analysis workflow is due to the input size. Using the Map and Filter workflows, the input size is decreased by only selecting the mapped reads in capture regions. This operation decreases the input size extensively thereby decreasing the time for executing the WES analysis workflow and obtaining the Strelka and VarScan VCF files.

3.1 Map Workflow

In the Map workflow, there are three sections. The first section is about the inputs i.e. the forward and reverse reads, the second section is about trimming the unwanted reads and the third section is about mapping the trimmed reads to the reference reads.

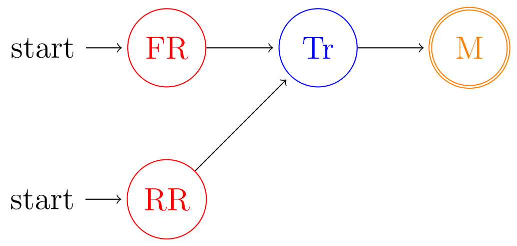


Figure 3.1: Sequence of operations in Map workflow.

In the input section, for the Somatic variant calling, there are two types of samples i.e Normal and Tumor samples. Therefore, there are Normal forward reads, Normal reverse reads, Tumor forward reads and Tumor reverse reads. In the flowchart, the Forward Reads are represented by FR and the Reverse Reads are represented by RR for both Normal and Tumor samples.

In the second section, forward and reverse reads of both Normal and Tumor samples are trimmed based on the minimum quality per base, minimum length of the reads and a few other conditions. Using the 'Trimmomatic tool' in Galaxy, either the paired-end or the single-ended reads are trimmed. In the flowchart, Tr represents all the trimming operations.

In the third section, the trimmed forward and reverse reads of Normal and Tumor samples are mapped with the reference genome i.e. Human Feb. 2009 (GRCh37/hg19)(hg19). Using the 'Map with BWA-MEM tool' in Galaxy, Normal forward and reverse reads are mapped into 'Mapped Normal forward' and 'Mapped Normal reverse' reads. Similarly, the Tumor forward and reverse reads are mapped into 'Mapped Tumor forward' and 'Mapped Tumor reverse' reads. In the flowchart, M represents the mapping operation.

3.2 Filter Workflow

In the Filter workflow, there are three sections. The first section is about the inputs i.e. capture regions, forward, mapped forward, reverse and mapped reverse reads for both Normal and Tumor samples. The second section is about filtering Mapped forward and Mapped reverse reads for both Normal

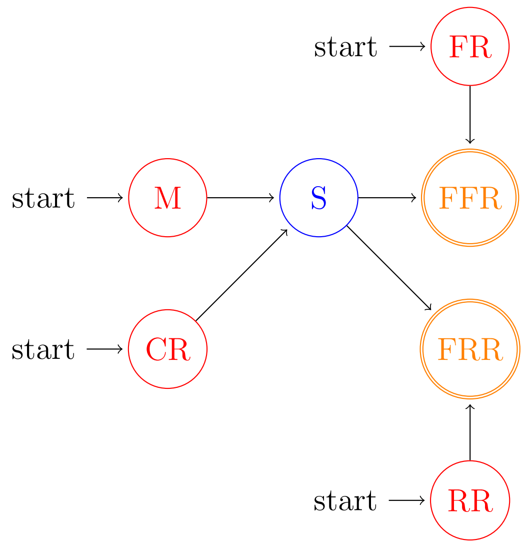


Figure 3.2: Sequence of operations in Filter workflow.

and Tumor samples based on the capture regions and the third section, the forward and reverse reads for both Normal and Tumor samples are filtered based on their mapping with filtered Mapped forward and filtered Mapped reverse reads for both Normal and Tumor samples.

In the first section, the considered inputs are the outcome of the Map workflow or the mapped Normal and Tumor samples for forward and reverse reads, capture regions represented in a BED file, Normal forward reads, Normal reverse reads, Tumor forward reads and Tumor reverse reads. In the flowchart, Mapped forward and reverse reads for both Normal and Tumor samples are represented by M, the Capture Regions are represented by CR, the Forward Reads are represented by FR and the Reverse Reads are represented by RR for both Normal and Tumor samples.

In the second section, based on the capture regions, the mapped Normal and Tumor samples for forward and reverse reads are filtered using the 'Samtools view' from Galaxy. This tool filters and subsamples alignments as per the user requirement and generates a BAM file as the outcome.

In the third section, based on the BAM file from the 'Samtools view', the forward reads and reverse reads for both Normal and Tumor samples represented by FR and RR in the flowchart are mapped to create the Mapped Normal and Tumor samples for forward and reverse reads. Using the 'Filter Sequences by Mapping' tool in Galaxy, the selected reads in the BAM file are mapped with forward and reverse reads for both Normal and Tumor samples. In the flowchart, these outcomes are represented by FFR for Filtered Forward Reads and FRR for Filtered Reverse Reads.

3.3 WES Analysis Workflow

In the WES analysis workflow, there are five sections. The first section is about the inputs i.e. Normal filtered forward reads, Normal filtered reverse reads, Tumor filtered forward reads, and Tumor filtered reverse reads. The second section is about trimming the filtered reads, the third section is about mapping the trimmed filtered reads, the fourth section is about post-processing the mapped trimmed reads and the fifth section is about variant calling.

In the first section, the considered inputs are the outcome of the 'Filter workflow' or the Filtered Normal and Tumor samples for forward and reverse reads, sample names, and purity estimates. In the flowchart, Filtered Normal and Tumor samples for forward and reverse reads are represented by FFR and FRR, sample names are represented by NM, and purity estimates are represented by PR.

In the second section, Filtered forward and reverse reads for both normal and tumor samples are trimmed based on the minimum quality per base, minimum length of the reads and a few other conditions using the 'Trimmomatic tool' in Galaxy. During this operation, either the paired-end or the single-ended reads are trimmed. In the flowchart, Tr represents all the trimming operations.

In the third section, the trimmed Filtered forward and reverse reads for both Normal and Tumor samples are mapped with the reference genome i.e. Human Feb. 2009 (GRCh37/hg19)(hg19). Using the 'Map with BWA-MEM tool' in Galaxy, trimmed filtered normal forward and reverse reads are mapped into 'Filtered Mapped Normal reads' and Tumor forward and reverse

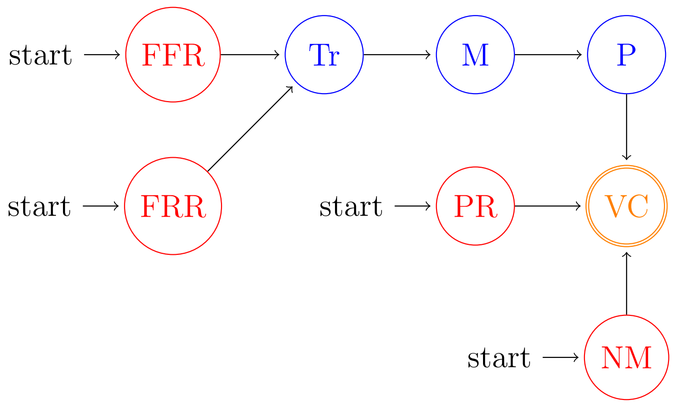


Figure 3.3: Sequence of operations in WES analysis workflow.

reads are mapped into 'Filtered Mapped Tumor reads'. In the flowchart, M represents the mapping operation.

In the fourth section, the filtered mapped Normal reads and filtered mapped Tumor reads are processed to filter the paired-end reads of all samples to retain only those read pairs, for which both the forward and the reverse reads have been mapped to the reference successfully. In this process, data is deduplicated [16][17] and the outcome of this process is a BAM file represented by P for the Post-mapping operation in the flow chart.

In the fifth section, with the BAM input from the Post-mapping operation, sample names and purity estimates for both the Normal and Tumor samples, the variant calling is performed. The choice of the variant caller in the WES workflow is VarScan Somatic with minimum base quality of 28, minimum mapping quality of 1, minimum coverage of 8 and minimum variant allele frequency 0.1. Along with VarScan somatic, Strelka somatic could also be implemented in Galaxy. The outcome of this process is the VCF files and in the flowchart, VC represents Variant Callers.

Chapter 4

Approach

Starting from the input data until performing operations on the VCF files, there are four steps involved. The first step is obtaining the input data and the capture regions, the second step is executing the Map, Filter and WES analysis workflows, the third step is ensuring consistency between the files and selecting the necessary columns for operations and the fourth step is about performing operations like count, sum, comparisons and variant combinations on the VCF files.

4.1 Inputs

The list of inputs needed to obtain the VCF files is based on the Map, Filter and WES Analysis workflows. For the Map workflow, the needed files are Normal forward reads, Normal reverse reads, Tumor forward reads, and Tumor reverse reads. For the Filter workflow, the needed files are Normal forward reads, Normal reverse reads, Tumor forward reads, Tumor reverse reads, Capture Regions, mapped Normal reads, and mapped Tumor reads.

For the WES Analysis workflow, the needed files are Normal sample name, Tumor sample name, Normal purity estimate, Tumor purity estimate, filtered mapped Normal forward reads, filtered mapped Normal reverse reads, filtered mapped Tumor forward reads, filtered mapped Tumor reverse reads and Truth Data VCF file.

The input for the Map workflow and the Truth Data can be downloaded from GIAB FTP[1] and the Capture Regions needed for the Filter workflow can be downloaded from MIRACUM Annotation Data[2]. Except for these inputs, the rest of the inputs are created while executing the workflows.

From the user end, Normal sample name, Tumor sample name, Normal purity estimate, Tumor purity estimate are needed and purity estimates are always between 0.0 to 1.0. In this thesis, the Normal purity estimate is always 1.0 and the Tumor purity estimate which represents the percentage of cancer cells in a solid tumor sample are 0.3, 0.5 and 0.7 in every experiment.

4.2 Execution

The Map, Filter and WES Analysis workflows can be executed either through the command line or through Galaxy. When executing through the command line, choose the Galaxy domain, set up the workflow, create the YAML file and then use the Planemo command to execute the workflow. Example YAML files for the three workflows can be accessed at Appendix A.

```
planemo run workflow.ga
params.yml
--galaxy_url https://usegalaxy.eu/
--galaxy_user_key APIKEY
--engine external_galaxy
--no_shed_install
```

If executing through Galaxy, import the workflow at usegalaxy.eu and run the workflow. In the three workflows, Map workflow should be executed first for the mapped forward and reverse reads for both Normal and Tumor samples. Using the Map workflow outcomes, Filter workflow should be executed to obtain mapped forward and reverse reads for Normal and Tumor samples. Using the Filter workflow outcomes, the WES analysis workflow should be executed.

[1]ftp://ftp-trace.ncbi.nlm.nih.gov/giab/ftp/use_cases/mixtures/UMCUTRECHT_NA12878_NA24385_mixture_10052016/

[2]https://usegalaxy.eu/u/wolfgang-maier/h/miracum-annotation-data

By using Galaxy, the outcomes of one workflow can be dragged and dropped into the new workflow without any processing time. The need for three workflows when the WES analysis workflow alone could produce the VCF files is because using the other workflows, the input data size is reduced by selecting only the reads that map and belong to the capture regions.

4.3 Columns in VCFs

After executing the workflows, VCF outcomes using Strelka and VarScan variant callers with Tumor purity estimate of 0.3, 0.5 and 0.7 are obtained and the columns in all of the VCFs are CHROM, POS, ID, REF, ALT, QUAL, FILTER, INFO, FORMAT, NORMAL, TUMOR.

CHROM is typically a chromosome, POS is the position of a variation in a sequence, ID is the identifier of the variation, REF is the reference base or bases, ALT is the list of alternative alleles, QUAL is the quality score of the alleles, FILTER indicates if a given set of filters passed or not, INFO and FORMAT columns have sub-fields that vary as per the variant caller and SAMPLEs column has NORMAL and TUMOR samples.

Of these columns, ID, QUAL, FILTER and INFO aren't used either in comparison or benchmarking. Of the other columns, CHROM column value is sometimes just a number i.e. 12 and sometimes a string concatenated with a number i.e. chr12. To compare the VCF files, the CHROM values in both of the files should be consistent. To add chr, use the command

```
awk '{
if($0 !~ /^#/)
print "chr"$0;
else if(match($0,/(##contig=<ID=)(.*)/,m))
print m[1]"chr"m[2];
else print $0
}' no_chr.vcf > with_chr.vcf
```

With the chr, CHROM column can be used to segregate reads based on chromosomes. To segregate, use the command and replace chrno to the specific chromosome. For example, chr12

```
grep -w '^#\|^chrno' old_file.vcf > new_file.vcf
```

4.4 Operations

With consistent VCF files, three operations are to be performed to determine the better variant caller for detecting cancer-causing variants. These three operations are comparisons, bias detection and benchmarking. To perform the operations, specific columns from the VCF files should be selected. The command to select the needed columns is

```
cut -f column_numbers old_file.vcf > new_file.vcf
```

Replace the column_numbers with integers based on the column occurance in VCF files starting from 1 and seperate the list with commas if there are couple of columns to be selected i.e 1-3,5.

When performing operations on the selected columns in the VCF files by considering them as csv or tabular files, the header information presented in the VCF files explaining the columns aren't needed. To delete the header information, use the command

```
sed '/^#/d' old_file.vcf > new_file.vcf
```

Chapter 5

Experiments

For the comparison, bias and benchmarking of variant caller outcomes and truth data, there are five experiments to be performed. The first experiment is comparing the positions, SNPs and Indels between the VCF files. The second experiment is comparing the allele frequencies between the VCF files. The third experiment is comparing the read depths between the VCF files. The fourth experiment is comparing the sixteen variant combinations between the VCF files. The fifth experiment would be benchmarking the variant callers based on the True Positives, True Negatives, False Positives and False Negatives in comparison to the truth data. Based on the conclusions of these experiments, the choice of the variant caller between Strelka Somatic and VarScan Somatic is determined for detecting cancer-causing variants.

5.1 Positions, SNPs, & INDELs

A Single-Nucleotide Polymorphism (SNP) is a substitution of a single nucleotide at a specific position in the genome that is present in a sufficiently large fraction of the population and an INDEL is a molecular biology term for an INsertion or DELetion of bases in the genome of an organism. In the first experiment, positions, SNPs and Indels are compared between the variant caller outcomes and the truth data.

This comparison between two or three VCF files can be achieved through the VCFToolz command

```
vcftoolz compare file1.vcf file2.vcf file3.vcf > output.txt
```

This tool notes the differences in a text file alongside plotting Venn diagrams showing the count of the common and mutually exclusive positions and SNPs. However, in the outcome, the number of SNPs in comparison to the number of positions aren't consistent. At least for the input files considered for the thesis and the INDELs aren't even compared by this tool.

So by using the specific code written for this thesis in Appendix D, positions, SNPs and INDELs are compared. To execute the code in Appendix D, files with only SNPs and files with only INDELs are needed. To obtain the file with only SNPs, consider the VCF file as an input and use the command

```
vcftools --vcf input_file.vcf --remove-indels --recode
    --recode-INFO-all --out SNPs_only
```

To obtain the file with only INDELs, consider the VCF file as an input and use the command

```
vcftools --vcf input_file.vcf --keep-only-indels --recode
    --recode-INFO-all --out INDELs_only
```

Using these the VCF, SNP, INDEL files and the code in Appendix D which uses the `len` and `merge` operations along with `mathplotlib` package, the comparison of the positions, SNPs and INDELs in terms of their `count` are listed in tables. The outcomes represented graphically as Venn diagrams and are classified based on the tumor purity of 0.3, 0.5, and 0.7.

5.2 Allele Frequencies

Allele frequency or gene frequency is defined as the relative frequency of an allele at a particular locus in a population, expressed as a fraction or percentage. In the second experiment, the number of positions within specific allele frequencies limits is tabulated and plotted individually per variant caller using the code in Appendix B.

The calculation of the allele frequency varies for VarScan somatic, Truth Data and Strelka variant callers. For VarScan somatic and Truth Data, the allele frequency values are a part of the obtained VCF files. So by using the vcftools, allele frequency for REF and ALT values can be obtained by using the command

```
vcftools --vcf input.vcf --freq --out output
```

For Strelka, the allele frequency values are not a part of the obtained VCF file. So vcftools cannot be used. Instead, the code specifically written for this thesis in the Strelka Allele Frequency section of Appendix B should be used to calculate the allele frequencies for REF and ALT values. In Strelka Somatic, the allele frequency calculation varies between SNPs and INDELs. The formulas and further documentation can be accessed at Strelka User Guide[1].

To calculate the Strelka SNPs allele frequency, the formula is

```
refCounts =
Value of FORMAT column $REF + "U" (e.g. if REF="A" then use the
    value in FOMRAT/AU)

altCounts =
Value of FORMAT column $ALT + "U" (e.g. if ALT="T" then use the
    value in FOMRAT/TU)

tier1RefCounts = First comma-delimited value from $refCounts
tier1AltCounts = First comma-delimited value from $altCounts

Somatic allele frequency =
$tier1AltCounts / ($tier1AltCounts + $tier1RefCounts)
```

To calculate the Strelka INDELs allele frequency, the formula is

```
tier1RefCounts = First comma-delimited value from FORMAT/TAR
```

[1]https://github.com/Illumina/strelka/blob/v2.9.x/docs/userGuide/README.md#somatic

```
tier1AltCounts = First comma-delimited value from FORMAT/TIR

Somatic allele freqeuncy =
$tier1AltCounts / ($tier1AltCounts + $tier1RefCounts)
```

With the allele frequencies of VarScan Somatic, Strelka Somatic, and Truth Data obtained, the values can be classified into four categories.

The first category is the number of positions with less than 0.25 allele frequency value. The second category is the number of positions with allele frequency values between 0.26 and 0.50. The third category is the number of positions with allele frequency values between 0.51 and 0.75 and the fourth category is the number of positions with allele frequency values between 0.76 to 1.00. These four categories are plotted as group bar plot using `mathplotlib` package and the outcomes are classified based on the tumor purity of 0.3, 0.5, and 0.7.

5.3 Read Depths

Read Depth describes the number of times that a given nucleotide in the genome has been read in an experiment and it is also referred to as coverage. A base with 30 read depth or 30x coverage means on average each base has been read by 30 sequences. The third experiment would be comparing the read depths between the variant caller outcomes and the Truth Data using the code in Appendix E.

To calculate the summary statistics and site depth of VCF files, vcftools could be used. To command to obtain the summary statistic is

```
vcftools --vcf input_data.vcf --depth -c > depth_summary.txt
```

To command to calculate the site depth per base is

```
vcftools --vcf input_data.vcf --site-depth --max-missing 1.0 --out
    site_depth_summary
```

However, using neither of the commands, read depth per base or position cannot be obtained. To obtain these values, use the code in Appendix E. In VCF files, read depth is often represented by DP and it is a part of the FORMAT column. For Somatic variant calling, there are both Normal Read Depth and Tumor Read Depth.

For Strelka Somatic, the FORMAT column is represented as DP:FDP:SDP:SUBDP:AU:CU:GU:TU of which DP is the read depth. For VarScan Somatic, the FORMAT column is represented as GT:GQ:DP:AD:ADF:ADR of which DP is the read depth. For Truth Data, the FORMAT column is represented as GT:PS:DP:GQ of which DP is the read depth.

After filtering out the DP values, calculate and compare the minimum value, maximum value, standard deviation, mean of the read depths for Normal and Tumor samples for Strelka, VarScan and Truth Data. This comparison reveals the coverage of the variant callers and the results are documented in a tabular form.

Then by using the mean and standard deviation, a range for read depth that has good coverage for reads is determined and the positions which are between this range are selected and filtered. These filtered positions in comparison to the total number of positions for each variant caller help determine the choice of the variant caller.

5.4 Variant Combination Bias

The REF and ALT columns in VCF files represent the allele in the reference genome and any other allele found at that locus respectively. When only SNPs are considered, then REF and ALT only have four possible bases to occur i.e. A, T, G, C. If the REF and ALT are concatenated, there are in total sixteen combinations that could occur i.e. AA, AT, AG, AC, TA, TT, TG, TC, GA, GT, GG, GC, CA, CT, CG, CC.

The fourth experiment is to find the bias of variant callers in calling a few variant combinations more often than the others in comparison to the Truth Data. Using the code in Appendix F, the count for each of these sixteen combinations from the VCF files are calculated and normalised by division with the total count. The normalization is to calculate the percentage of a variant occurrence in all of the combinations.

Out of the sixteen combinations, AA, TT, GG and CC occur zero times as the variant callers do not add those positions which have the same REF and ALT bases. So out of the twelve normalised counts, bias if any can be identified when a combination's percentage is compared to the Truth Data.

5.5 Benchmarking

Benchmarking is defined as a standard or point of reference against which things may be compared. In this experiment, ALT variants between the different somatic variant callers are compared with the Truth Data using the code in Appendix C. Based on the comparisons, the number of True Positives, True Negatives, False Positives and False Negatives are obtained.

In True Positives, the variant which appears in True Data also appears in variant caller data. In True Negative, the variant is missing in True Data and is also missing in the variant caller data. In False Positive, the variant is missing from the True Data but appears in the variant caller data. In False Negative, the variant appears in True Data but does not appear in variant caller data.

For True Positives, based on the ALTs, there is either True-True Positive or True-False Positive. In True-True Positive, the ALTs match and in True-False Positive, the ALTs don't match but broadly using True Positives, True Negatives, False Positives and False Negatives, Sensitivity and Specificity could be calculated.

Sensitivity is the proportion of patients with the disease who test positive and it can be calculated using the formula where TP is considered to be the number of True Positive ALTs and FN is considered to be the number of False Negative ALTs.

$$P\left[\frac{T^+}{D^+}\right] = \frac{TP}{TP + FN} \tag{5.1}$$

Specificity is the proportion of patients without disease who test negative and it can be calculated using the formula where TN is considered to be the number of True Negative ALTs and FP is considered to be the number of False Positive ALTs.

$$P\left[\frac{T^-}{D^-}\right] = \frac{TN}{TN + FP} \tag{5.2}$$

Based on the Sensitivity and Specificity values of the variant callers, they can be chosen for an application. For tests with high sensitivity, there are a fewer false negative results and for tests with high specificity, there are almost no individuals who aren't affected not ruled out.

Chapter 6

Results

Before analysing the results, it must be noted that the VCF files from the Strelka and VarScan variant callers are subsampled based on their mapping and capture regions through Map and Filter workflows while the Truth Data isn't. Therefore the size of the Strelka and VarScan VCF files are significantly less in comparison to the Truth Data. Given below are the number of positions, SNPs, INDELs in the Truth Data

Positions	**SNPs**	**Indels**
11,04,786	10,07,793	96,993

Table 6.1: Values from Truth Data VCF files

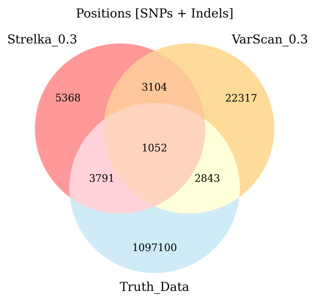
6.1 Positions Comparison

When comparing the number of positions in VCF files, there is an increase in the positions for VarScan VCF when the tumor purity is decreased but the count is unaltered for Strelka VCF. Given below are the comparison results between the positions count.

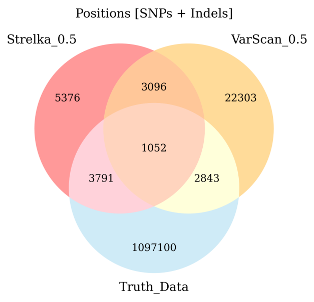
Type/Tumor_Purity	**0.3**	**0.5**	**0.7**
Strelka	13,315	13,315	13,315
VarScan	29,316	29,294	29,171
Truth Data	11,04,786	11,04,786	11,04,786
Strelka & VarScan	3,104	3,096	3,051
VarScan & Truth Data	2,843	2,843	2,843
Strelka & Truth Data	3,791	3,791	3,791
Strelka, VarScan & Truth Data	1,052	1,052	1,052

Table 6.2: Positions count in Strelka and VarScan VCF files

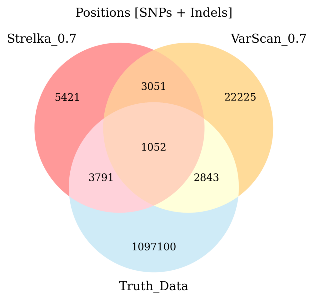
In comparison to the Truth Data, the number of common positions between Strelka VCF and Truth Data VCF is 4,843 and the number of common positions between VarScan VCF and Truth Data VCF is 3,895. Therefore, a total of almost a thousand more common positions could be found between Strelka and Truth Data VCFs than VarScan and Truth Data VCFs.



(a)



(b)



(c)

Figure 6.1: (a) VCF Positions with Tumor Purity 0.3 (b) VCF Positions with Tumor Purity 0.5 (c) VCF Positions with Tumor Purity 0.7

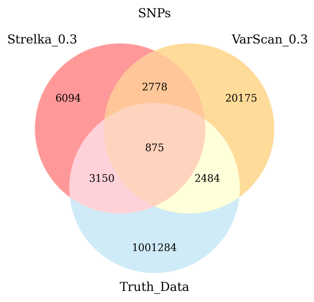
6.2 SNPs Comparison

When comparing the number of SNPs in VCF files, like positions, there is an increase in the SNPs for VarScan VCF when the tumor purity is decreased but the count is unaltered for Strelka VCF. Given below are the comparison results between the SNPs count.

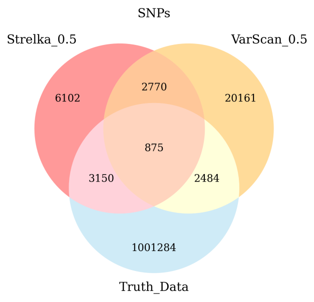
Type/Tumor_Purity	**0.3**	**0.5**	**0.7**
Strelka	12,897	12,897	12,897
VarScan	26,312	26,290	26,185
Truth Data	10,07,793	10,07,793	10,07,793
Strelka & VarScan	2,778	2,770	2,729
VarScan & Truth Data	2,484	2,484	2,484
Strelka & Truth Data	3,150	3,150	3,150
Strelka, VarScan & Truth Data	875	875	875

Table 6.3: SNPs count in Strelka and VarScan VCF files

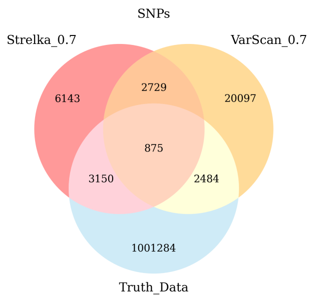
In comparison to the Truth Data, the number of common SNPs between Strelka VCF and Truth Data VCF is 4,025 and the number of common SNPs between VarScan VCF and Truth Data VCF is 3,359. Therefore, a total of almost seven hundred more common SNPs could be found between Strelka and Truth Data VCFs than VarScan and Truth Data VCFs.



(a)



(b)



(c)

Figure 6.2: (a) VCF SNPs with Tumor Purity 0.3 (b) VCF SNPs with Tumor Purity 0.5 (c) VCF SNPs with Tumor Purity 0.7

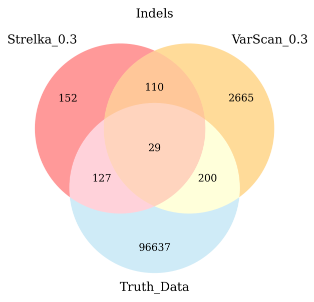
6.3 INDELs Comparison

When comparing the number of INDELs in VCF files, there is a small increase in the INDELs for VarScan VCF when the tumor purity is decreased but the count is unaltered for Strelka VCF. Given below are the comparison results between the INDELs count.

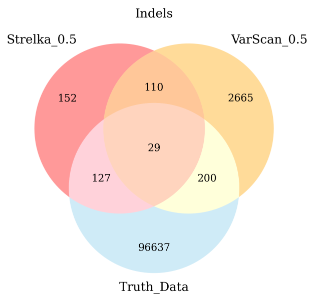
Type/Tumor_Purity	**0.3**	**0.5**	**0.7**
Strelka	418	418	418
VarScan	3,004	3,004	2,986
Truth Data	96,993	96,993	96,993
Strelka & VarScan	110	110	107
VarScan & Truth Data	200	200	200
Strelka & Truth Data	127	127	127
Strelka, VarScan & Truth Data	29	29	29

Table 6.4: INDELs count in Strelka and VarScan VCF files

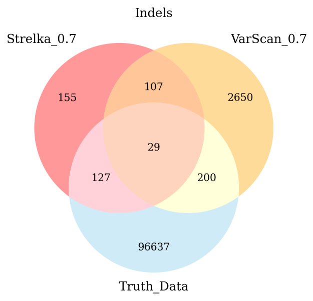
In comparison to the Truth Data, the number of common INDELs between Strelka VCF and Truth Data VCF is 156 and the number of common INDELs between VarScan VCF and Truth Data VCF is 229. Therefore, there are more common INDELs found between VarScan and Truth Data VCFs than Strelka and Truth Data VCFs.



(a)



(b)



(c)

Figure 6.3: (a) VCF INDELs with Tumor Purity 0.3 (b) VCF INDELs with Tumor Purity 0.5 (c) VCF INDELs with Tumor Purity 0.7

6.4 Variants Comparison

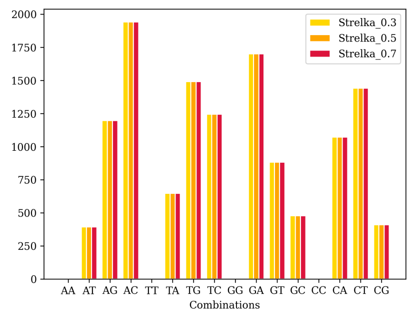
In comparing the SNPs as combinations, the REF and ALT bases are concatenated creating sixteen combinations i.e. AA, AT, AG, AC, TA, TT, TG, TC, GA, GT, GG, GC, CA, CT, CG, CC. When comparing their count, it can be noted that the combinations of AT, AG, TA, TC, GA, GT, GC, CT, CG are higher in the count for the VarScan SNP file compared to the Strelka SNP file.

For the combinations of AC, TG, and CA, the Strelka SNP file has a higher count compared to the VarScan SNP. Though this comparison is an outline of the idea, in comparison to the Truth Data, both Strelka and VarScan SNP files are less in the count. To compare, count data should be normalised.

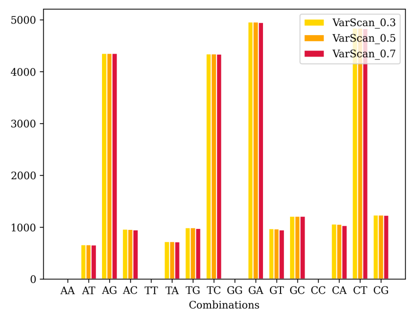
Comb	**S3**	**S5**	**S7**	**V3**	**V5**	**V7**	**TD**
AA	0	0	0	0	0	0	0
AT	393	393	393	662	662	662	32,639
AG	1,196	1,196	1,196	4,354	4,354	4,350	1,52,935
AC	1,942	1,942	1,942	962	958	947	38,384
TT	0	0	0	0	0	0	0
TA	647	647	647	722	720	715	32,817
TG	1,491	1,491	1,491	990	989	976	37,707
TC	1,245	1,245	1,245	4,343	4,342	4,336	1,53,474
GG	0	0	0	0	0	0	0
GA	1,700	1,700	1,700	4,960	4,959	4,950	1,91,603
GT	882	882	882	969	966	949	44,538
GC	478	478	478	1,212	1,212	1,210	43,851
CC	0	0	0	0	0	0	0
CA	1,072	1,072	1,072	1,059	1,054	1,033	44,253
CT	1,441	1,441	1,441	4,843	4,840	4,829	1,91,442
CG	409	409	409	1,235	1,233	1,231	44,049

Table 6.5: SNPs counts in Strelka, VarScan, Truth Data SNP files with Tumor Purity of 0.3, 0.5 and 0.7 where Comb means Combinations, S3 means Strelka SNP file with Tumor Purity of 0.3, S5 means Strelka SNP file with Tumor Purity of 0.5, S7 means Strelka SNP file with Tumor Purity of 0.7, V3 means VarScan SNP file with Tumor Purity of 0.3, V5 means for VarScan SNP file with Tumor Purity of 0.5, V7 means for VarScan SNP file with Tumor Purity of 0.7, & TD means Truth Data SNP file.

Except for a few more SNPs with decreasing Tumor Purity in VarScan SNP files, all SNP comparisons remain the same in every combination.



(a)



(b)

Figure 6.4: (a) Strelka SNPs Counts with Tumor Purity of 0.3, 0.5 and 0.7 (b) VarScan SNPs Counts with Tumor Purity of 0.3, 0.5 and 0.7

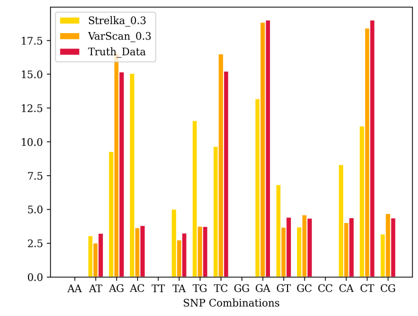
By dividing individual counts by the total numbers of counts, the normalised values for each SNP combination can be calculated. In normalised counts, it can be noted that the combinations of AT, AC, TA, TG, GT, CA are higher in the count for the Strelka SNP file compared to the VarScan SNP file.

For the combinations of AG, TC, GA, GC, CT, CG the count is higher for the VarScan SNP file compared to the Strelka SNP file. In comparison, the Strelka SNP file has AT combination while the VarScan SNP file has AG, AC, TG, TC, GA, GC, CA, CT, and CG near to the Truth Data normalised data values.

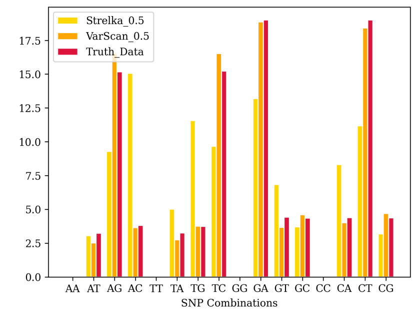
Comb	**S3**	**V3**	**S5**	**V5**	**S7**	**V7**	**TD**
AA	0	0	0	0	0	0	0
AT	3.04	2.51	3.04	2.51	3.04	2.51	3.23
AG	9.27	16.54	9.27	16.56	9.27	16.61	15.17
AC	15.05	3.65	15.05	3.64	15.05	3.61	3.80
TT	0	0	0	0	0	0	0
TA	5.01	2.74	5.01	2.73	5.01	2.73	3.25
TG	11.56	3.76	11.56	3.76	11.56	3.72	3.74
TC	9.65	16.50	9.65	16.51	9.65	16.55	15.22
GG	0	0	0	0	0	0	0
GA	13.18	18.85	13.18	18.86	13.18	18.90	19.01
GT	6.83	3.68	6.83	3.67	6.83	3.62	4.41
GC	3.70	4.60	3.70	4.61	3.70	4.62	4.35
CC	0	0	0	0	0	0	0
CA	8.31	4.02	8.31	4.00	8.31	3.94	4.39
CT	11.17	18.40	11.17	18.41	11.17	18.44	18.99
CG	3.17	4.69	3.17	4.69	3.17	4.70	4.37

Table 6.6: Normalised SNPs counts in Strelka, VarScan, Truth Data SNP files with Tumor Purity of 0.3, 0.5 and 0.7 where Comb means Combinations, S3 means Strelka SNP file with Tumor Purity of 0.3, S5 means Strelka SNP file with Tumor Purity of 0.5, S7 means Strelka SNP file with Tumor Purity of 0.7, V3 means VarScan SNP file with Tumor Purity of 0.3, V5 means for VarScan SNP file with Tumor Purity of 0.5, V7 means for VarScan SNP file with Tumor Purity of 0.7, & TD means Truth Data SNP file.

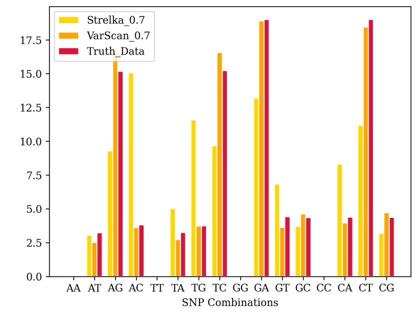
In terms of the bias, Strelka variant caller calls AC and TG combinations far more times and TC combination far fewer times in comparison to both Truth Data and VarScan Somatic variant caller.



(a)



(b)

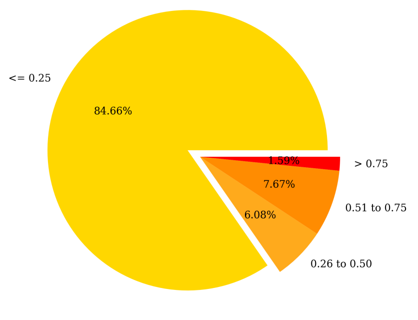


(c)

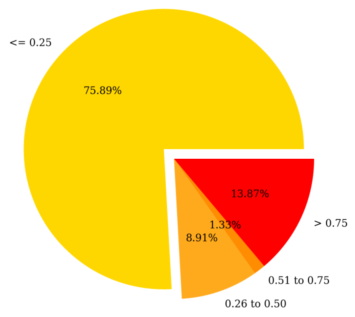
Figure 6.5: (a) Normalised SNPs Counts with Tumor Purity of 0.3 (b) Normalised SNPs Counts with Tumor Purity of 0.5 (c) Normalised SNPs Counts with Tumor Purity of 0.7

6.5 Allele Frequencies

In comparing the allele frequencies, the values are divided into one of the four categories. The first category is ≤ 0.25, the second category is > 0.25 and less than ≤ 0.50, the third category is > 0.50 and less than ≤ 0.75 and the fourth category is > 0.75.



(a)



(b)

Figure 6.6: (a) Percentage of Strelka Normal allele frequency counts (b) Percentage of Strelka Tumor allele frequency counts

In Strelka VCF files, both Normal and Tumor allele frequencies have the highest number of REF base allele frequencies in the first category i.e. ≤ 0.25. There is also a significant increase in the Tumor sample reads when compared to the Strelka Normal sample in the fourth category i.e. > 0.75.

Format	**Purity**	**$\leq$ 0.25**	**0.25 $>$ & $\leq$ 0.50**	**0.50 $>$ & $\leq$ 0.75**	**$>$ 0.75**
Normal	0.3	11,266	809	1,020	212
Tumor	0.3	10,095	1,185	177	1,845
Normal	0.5	11,266	809	1,020	212
Tumor	0.5	10,095	1,185	177	1,845
Normal	0.7	11,266	809	1,020	212
Tumor	0.7	10,095	1,185	177	1,845

Table 6.7: Allele Frequencies count from Strelka somatic variant caller VCF files

Another change is the significant decrease in REF bases in Strelka Normal sample in comparison to the Strelka Tumor sample in the third category i.e. > 0.50 and less than ≤ 0.75.

In VarScan allele frequencies, the highest number of REF base allele frequencies are in the second category i.e. > 0.25 and less than ≤ 0.50.

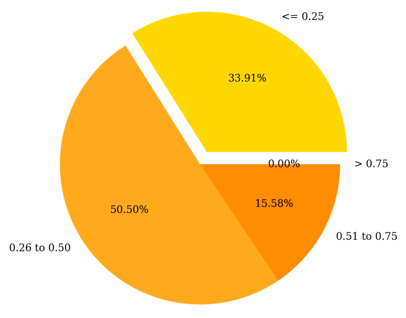
Purity	**$\leq$ 0.25**	**0.25 $>$ & $\leq$ 0.50**	**0.50 $>$ & $\leq$ 0.75**	**$>$ 0.75**
0.3	9,893	14,732	4,546	0
0.5	9,893	14,732	4,546	0
0.7	9,893	14,732	4,546	0

Table 6.8: Allele Frequencies count from VarScan somatic variant caller VCF files

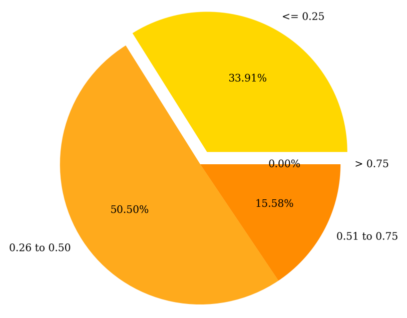
Unlike Strelka allele frequencies, there are no REF base allele frequencies in the fourth category i.e. $>$ 0.75 and less than $\leq$ 0.50 for VarScan allele frequencies.

In comparison to the Strelka, VarScan has less than the number of REF base allele frequencies in the first category i.e. $\leq$ 0.25, more number of REF base allele frequencies in the second category i.e. $>$ 0.25 and less than $\leq$ 0.50.

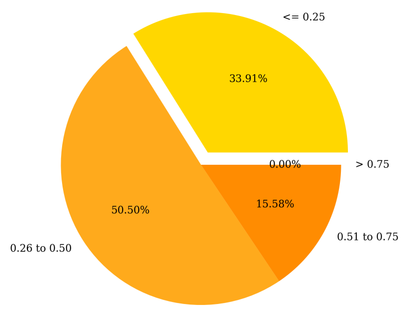
More number of REF base allele frequencies in the third category i.e. $>$ 0.50 and less than $\leq$ 0.75 and less number of REF base allele frequencies in the fourth category i.e. $>$ 0.75.



(a)



(b)



(c)

Figure 6.7: (a) Percentage of VarScan allele frequency with Tumor Purity of 0.3 (b) Percentage of VarScan allele frequency with Tumor Purity of 0.5 (c) Percentage of VarScan allele frequency with Tumor Purity of 0.7

In Truth Data, the majority of REF base allele frequencies are in the first category i.e. ≤ 0.25 and all the counts in the other categories are as follows

$\leq$ **0.25**	**0.25** $>$ **&** $\leq$ **0.50**	**0.50** $>$ **&** $\leq$ **0.75**	$>$ **0.75**
11,266	809	1,020	212

Table 6.9: Allele Frequencies count from Truth Data VCF file

When the allele frequencies of the common positions between the files are compared, the outcomes are distinctive.

Type	**≤ 0.25**	**$0.25 >$ & ≤ 0.50**	**$0.50 >$ & ≤ 0.75**	**> 0.75**
Strelka Normal	469	561	2	0
Strelka Tumor	1,032	0	0	0
VarScan	0	0	1,032	0
Truth Data	0	1,032	0	0

Table 6.10: Allele Frequencies count with Tumor Purity of 0.3, 0.5 & 0.7

All of the Truth Data allele frequencies are in the third category i.e. > 0.50 and less than ≤ 0.75, VarScan allele frequencies are in the fourth category i.e. > 0.75 and Strelka allele frequencies are in the first two categories.

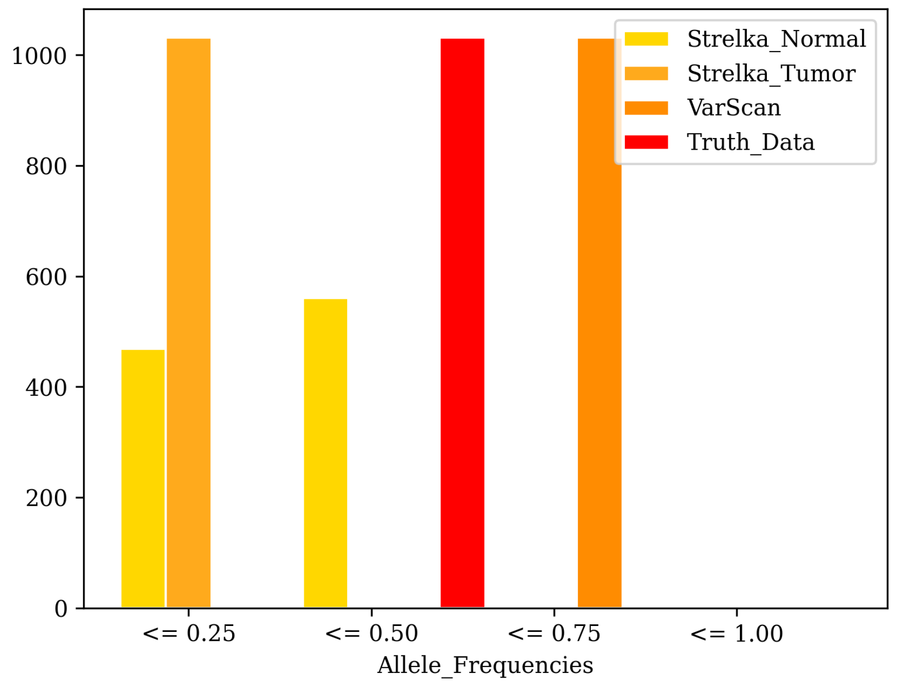


Figure 6.8: Allele Frequencies count with Tumor Purity of 0.3, 0.5 & 0.7

These distinct results aren't particularly helpful in selecting a variant caller. However, they mention the most commonly found allele frequencies occurring in the population are from the VarScan Somatic variant caller and a few of the Strelka Normal allele frequencies and Truth Data allele frequencies are in the same second category i.e. > 0.25 and less than ≤ 0.50.

6.6 Read Depth

To choose a variant caller based on Read Depth, a test would be to compare the number positions with maximum coverage. To find out these positions based on their DP or read depth values, the minimum, maximum, mean, median, mode and standard deviation are calculated using the pandas functions. Given below are the outcome for Normal and Tumor samples for Strelka variant caller with tumor purity of 0.3, 0.5 and 0.7.

Format	**Purity**	**Min**	**Max**	**Mean**	**Median**	**Mode**	**SD**
Normal	0.3	1	299	58.32	53	0 43	32.81
Normal	0.3	0	114	20.71	19	0 17	12.09
Normal	0.5	1	299	58.32	53	0 43	32.81
Tumor	0.5	0	114	20.71	19	0 17	12.09
Normal	0.7	1	299	58.32	53	0 43	32.81
Tumor	0.7	0	114	20.71	19	0 17	12.09

Table 6.11: Read Depth statistics from Strelka variant caller VCF files

In absolute comparison, the statistical values for Strelka and VarScan variant callers are nearby. Given below are the outcome for Normal and Tumor samples for VarScan variant caller with different tumor purities.

Format	**Purity**	**Min**	**Max**	**Mean**	**Median**	**Mode**	**SD**
Normal	0.3	8	250	57.48	55	0 38	25.78
Tumor	0.3	8	98	20.74	19	0 11	9.27
Normal	0.5	8	250	57.48	55	0 38	25.78
Tumor	0.5	8	98	20.74	19	0 11	9.27
Normal	0.7	8	250	57.48	55	0 38	25.78
Tumor	0.7	8	98	20.74	19	0 11	9.27

Table 6.12: Read Depth statistics from VarScan variant caller VCF files

With the statistical values for Strelka, VarScan and Truth Data, the range for read depth that offers the maximum coverage should be calculated. For this, subtract the standard deviation once from the mean to get the lower depth and add it once to get the higher depth. With these ranges for Strelka, VarScan and Truth Data, filter the rows that have the read depth values within this range.

Name	Type	Mean	SD	Lower Limit	Upper Limit
Strelka	Normal	58.32	32.81	25.51	91.13
Strelka	Tumor	20.71	12.09	8.62	32.8
VarScan	Normal	57.48	25.78	31.7	83.26
VarScan	Tumor	20.74	9.27	11.47	30.01

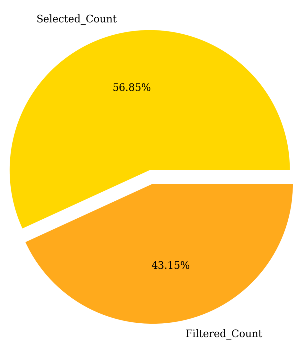
Table 6.13: Read Depth ranges for all tumor purities.

Even with this data, a conclusion cannot be drawn based on the absolute comparison. To normalise the data by dividing it by the total number of reads to notice the selected and filtered reads. The variant caller with the maximum selected percentage can be selected.

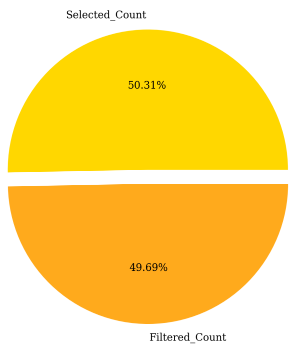
Name	Total Positions	Selected Positions	Filtered Positions
Strelka	13,315	7,589	5,726
VarScan	29,316	14,748	14,568

Table 6.14: Selected positions from variant callers with Tumor purity 0.3.

Between the variant callers, the number of positions for VarScan somatic variant caller change with the decrease in the tumor purity. However, the number of positions isn't significant enough to change the number of selected positions extensively.



(a)



(b)

Figure 6.9: (a) Percentage of selected and filtered reads in Strelka VCF file (b) Percentage of selected and filtered reads in VarScan VCF file

With the observed results considering this input data, Strelka somatic has larger better coverage positions in comparison to the VarScan somatic. More than six percent difference can be noted in the selected positions.

6.7 Benchmarking

There is a significant difference between the number of positions between the Strelka somatic, VarScan somatic VCF files and Truth Data VCF file because both the variant caller's input data have been subsampled. Due to this, there is a disproportionate difference between the number of True Positive ALTs, True Negative ALTs, False Positive ALTs and False Negative ALTs.

The True Negative ALTs are zero for both Strelka somatic and VarScan somatic benchmarking because the VCF files only call variants that have different REF and ALT bases. Therefore when benchmarking the variant callers with the Truth Data, the number of True Negatives will be zero.

Type	**Pur**	**Total**	**TP**	**TTP**	**TFP**	**TN**	**FP**	**FN**
Strelka	0.3	11,14,341	3,791	3,292	499	0	9,555	11,00,995
Strelka	0.5	11,14,341	3,791	3,292	499	0	9,555	11,00,995
Strelka	0.7	11,14,341	3,791	3,292	499	0	9,555	11,00,995

Table 6.15: Benchmarking Strelka ALTs with respect to the Truth Data ALTs where Pur represents Tumor Purity, TP represents True Positive ALTs, TTP represents True True Positive ALTs, TFP represents True False Positive ALTs, TNA represents True Negative ALTs, FP represents False Positive ALTs, and FN represents False Negative ALTs.

In comparison, the number of True Positive ALTs in Strelka somatic are higher compared to VarScan somatic. Even the True True Positive ALTs and True False Positive ALTs are higher in Strelka somatic compared to the VarScan somatic. The number of False Positive ALTs and False Negative ALTs are higher in VarScan somatic in comparison to Strelka somatic.

Type	**Pur**	**Total**	**TP**	**TTP**	**TFP**	**TN**	**FP**	**FN**
VarScan	0.3	11,31,279	2,843	2,699	144	0	26,493	11,01,943
VarScan	0.5	11,31,257	2,843	2,699	144	0	26,471	11,01,943
VarScan	0.7	11,31,134	2,843	2,699	144	0	26,348	11,01,943

Table 6.16: Benchmarking VarScan ALTs with respect to the Truth Data ALTs where Pur represents Tumor Purity, TP represents True Positive ALTs, TTP represents True True Positive ALTs, TFP represents True False Positive ALTs, TNA represents True Negative ALTs, FP represents False Positive ALTs, and FN represents False Negative ALTs.

Based on the benchmarking results, Strelka somatic variant caller can be chosen for detecting cancer-causing variant compared to the VarScan somatic variant caller considering this Truth Data and these set of inputs.

Chapter 7

Conclusions

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Appendix A

Map Workflow YAML

```
NORMAL forward reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

NORMAL reverse reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

TUMOR forward reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

TUMOR reverse reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz
```

Filter Workflow YAML

```
NORMAL forward reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

NORMAL reverse reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz
```

```
NORMAL mapped reads:
     class: File
     location: https://usegalaxy.eu/...display?to_ext=bam

Capture regions:
     class: File
     location: https://usegalaxy.eu/...display?to_ext=bed
```

WES Analysis Workflow YAML

```
Normal sample name: "Normal sample name"
Normal purity estimate: 1.0
Tumor sample name: "Tumor sample name"
Tumor purity estimate: 0.7

NORMAL forward reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

NORMAL reverse reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

TUMOR forward reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

TUMOR reverse reads:
     class: File
     location: https://zenodo.org/record/....fastq.gz

Capture regions:
     class: File
     location: https://usegalaxy.eu/...display?to_ext=bed
```

Appendix B

Strelka Allele Frequency

```
# Importing the needed packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import csv

matplotlib.rcParams['font.sans-serif'] = ['Computer Modern Roman',
    'sans-serif']

# The first step is to selected the neccessary columns.
# Step 1 - 'cut -f 1-2,4-5,9-11 Input.vcf > Output.vcf'

# Removing all the rows starting with a #
# Step 2 - 'sed '/^#/d' Output.vcf > Updated_Output.vcf'

# Consider the Updated_Output.vcf as input.
dff = pd.read_csv("Selected_Strelka_0.3_Indels.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Selected_Strelka_0.5_Indels.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Selected_Strelka_0.7_Indels.vcf", sep = '\t',
    index_col= False)
```

```
# Renaming the columns after importing the input.
dff.columns = ['CHROM', 'POS', 'REF', 'ALT', 'FORMAT', 'NORMAL',
    'TUMOR']
dff1.columns = ['CHROM', 'POS', 'REF', 'ALT', 'FORMAT', 'NORMAL',
    'TUMOR']
dff2.columns = ['CHROM', 'POS', 'REF', 'ALT', 'FORMAT', 'NORMAL',
    'TUMOR']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff1['CHROM'].astype(str) + '-' +
    dff1['POS'].astype(str)
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising them.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]

dff1 = dff1.drop(['CHROM', 'POS'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]

dff2 = dff2.drop(['CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "DP:FDP:SDP:SUBDP:AU:CU:GU:TU"
dff[['Normal_DP', 'Normal_DP2', 'Normal_TAR', 'Normal_TIR',
    'Normal_TOR', 'Normal_DP50', 'Normal_FDP50', 'Normal_SUBDP50',
    'Normal_BCN50']] = dff['NORMAL'].str.split(':',expand=True)
dff[['Tumor_DP', 'Tumor_DP2', 'Tumor_TAR', 'Tumor_TIR',
    'Tumor_TOR', 'Tumor_DP50', 'Tumor_FDP50', 'Tumor_SUBDP50',
    'Tumor_BCN50']] = dff['TUMOR'].str.split(':',expand=True)

dff1[['Normal_DP', 'Normal_DP2', 'Normal_TAR', 'Normal_TIR',
    'Normal_TOR', 'Normal_DP50', 'Normal_FDP50', 'Normal_SUBDP50',
    'Normal_BCN50']] = dff1['NORMAL'].str.split(':',expand=True)
dff1[['Tumor_DP', 'Tumor_DP2', 'Tumor_TAR', 'Tumor_TIR',
    'Tumor_TOR', 'Tumor_DP50', 'Tumor_FDP50', 'Tumor_SUBDP50',
    'Tumor_BCN50']] = dff1['TUMOR'].str.split(':',expand=True)

dff2[['Normal_DP', 'Normal_DP2', 'Normal_TAR', 'Normal_TIR',
    'Normal_TOR', 'Normal_DP50', 'Normal_FDP50', 'Normal_SUBDP50',
    'Normal_BCN50']] = dff2['NORMAL'].str.split(':',expand=True)
dff2[['Tumor_DP', 'Tumor_DP2', 'Tumor_TAR', 'Tumor_TIR',
    'Tumor_TOR', 'Tumor_DP50', 'Tumor_FDP50', 'Tumor_SUBDP50',
    'Tumor_BCN50']] = dff2['TUMOR'].str.split(':',expand=True)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['FORMAT', 'NORMAL', 'TUMOR', 'Normal_DP',
    'Normal_DP2', 'Normal_TOR', 'Normal_DP50', 'Normal_FDP50',
    'Normal_SUBDP50', 'Normal_BCN50', 'Tumor_DP', 'Tumor_DP2',
    'Tumor_DP2', 'Tumor_TOR', 'Tumor_DP50', 'Tumor_FDP50',
    'Tumor_SUBDP50', 'Tumor_BCN50'], axis=1)

dff1 = dff1.drop(['FORMAT', 'NORMAL', 'TUMOR', 'Normal_DP',
    'Normal_DP2', 'Normal_TOR', 'Normal_DP50', 'Normal_FDP50',
    'Normal_SUBDP50', 'Normal_BCN50', 'Tumor_DP', 'Tumor_DP2',
    'Tumor_DP2', 'Tumor_TOR', 'Tumor_DP50', 'Tumor_FDP50',
    'Tumor_SUBDP50', 'Tumor_BCN50'], axis=1)

dff2 = dff2.drop(['FORMAT', 'NORMAL', 'TUMOR', 'Normal_DP',
    'Normal_DP2', 'Normal_TOR', 'Normal_DP50', 'Normal_FDP50',
    'Normal_SUBDP50', 'Normal_BCN50', 'Tumor_DP', 'Tumor_DP2',
    'Tumor_DP2', 'Tumor_TOR', 'Tumor_DP50', 'Tumor_FDP50',
    'Tumor_SUBDP50', 'Tumor_BCN50'], axis=1)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "DP:FDP:SDP:SUBDP:AU:CU:GU:TU"
dff[['Normal_TAR_First', 'Normal_TAR_Second']] =
    dff['Normal_TAR'].str.split(',',expand=True)
```

```
dff[['Normal_TIR_First', 'Normal_TIR_Second']] =
    dff['Normal_TIR'].str.split(',',expand=True)
dff[['Tumor_TAR_First', 'Tumor_TAR_Second']] =
    dff['Tumor_TAR'].str.split(',',expand=True)
dff[['Tumor_TIR_First', 'Tumor_TIR_Second']] =
    dff['Tumor_TIR'].str.split(',',expand=True)

dff1[['Normal_TAR_First', 'Normal_TAR_Second']] =
    dff1['Normal_TAR'].str.split(',',expand=True)
dff1[['Normal_TIR_First', 'Normal_TIR_Second']] =
    dff1['Normal_TIR'].str.split(',',expand=True)
dff1[['Tumor_TAR_First', 'Tumor_TAR_Second']] =
    dff1['Tumor_TAR'].str.split(',',expand=True)
dff1[['Tumor_TIR_First', 'Tumor_TIR_Second']] =
    dff1['Tumor_TIR'].str.split(',',expand=True)

dff2[['Normal_TAR_First', 'Normal_TAR_Second']] =
    dff2['Normal_TAR'].str.split(',',expand=True)
dff2[['Normal_TIR_First', 'Normal_TIR_Second']] =
    dff2['Normal_TIR'].str.split(',',expand=True)
dff2[['Tumor_TAR_First', 'Tumor_TAR_Second']] =
    dff2['Tumor_TAR'].str.split(',',expand=True)
dff2[['Tumor_TIR_First', 'Tumor_TIR_Second']] =
    dff2['Tumor_TIR'].str.split(',',expand=True)

# Renaming the new table with column names.
dff.columns = ['CHROM_POS', 'REF', 'ALT', 'Normal_TAR',
    'Normal_TIR', 'Tumor_TAR', 'Tumor_TAR', 'Normal_TAR_First',
    'Normal_TAR_Second', 'Normal_TIR_First', 'Normal_TIR_Second',
    'Tumor_TAR_First', 'Tumor_TAR_Second', 'Tumor_TIR_First',
    'Tumor_TIR_Second']

dff1.columns = ['CHROM_POS', 'REF', 'ALT', 'Normal_TAR',
    'Normal_TIR', 'Tumor_TAR', 'Tumor_TAR', 'Normal_TAR_First',
    'Normal_TAR_Second', 'Normal_TIR_First', 'Normal_TIR_Second',
    'Tumor_TAR_First', 'Tumor_TAR_Second', 'Tumor_TIR_First',
    'Tumor_TIR_Second']

dff2.columns = ['CHROM_POS', 'REF', 'ALT', 'Normal_TAR',
    'Normal_TIR', 'Tumor_TAR', 'Tumor_TAR', 'Normal_TAR_First',
    'Normal_TAR_Second', 'Normal_TIR_First', 'Normal_TIR_Second',
    'Tumor_TAR_First', 'Tumor_TAR_Second', 'Tumor_TIR_First',
    'Tumor_TIR_Second']

# Dropping of the unnecessary columns and reorganising them.
dff = dff.drop(['Normal_TAR_Second', 'Normal_TIR_Second',
    'Tumor_TAR_Second', 'Tumor_TIR_Second'], axis=1)
print(dff)

dff1 = dff1.drop(['Normal_TAR_Second', 'Normal_TIR_Second',
    'Tumor_TAR_Second', 'Tumor_TIR_Second'], axis=1)
print(dff1)

dff2 = dff2.drop(['Normal_TAR_Second', 'Normal_TIR_Second',
    'Tumor_TAR_Second', 'Tumor_TIR_Second'], axis=1)
print(dff2)

# Converting string values columns to int for calculations.
dff['Normal_TAR_First'] = dff['Normal_TAR_First'].astype(int)
dff['Normal_TIR_First'] = dff['Normal_TIR_First'].astype(int)
dff['Tumor_TAR_First'] = dff['Tumor_TAR_First'].astype(int)
dff['Tumor_TIR_First'] = dff['Tumor_TIR_First'].astype(int)

dff1['Normal_TAR_First'] = dff1['Normal_TAR_First'].astype(int)
dff1['Normal_TIR_First'] = dff1['Normal_TIR_First'].astype(int)
dff1['Tumor_TAR_First'] = dff1['Tumor_TAR_First'].astype(int)
dff1['Tumor_TIR_First'] = dff1['Tumor_TIR_First'].astype(int)

dff2['Normal_TAR_First'] = dff2['Normal_TAR_First'].astype(int)
dff2['Normal_TIR_First'] = dff2['Normal_TIR_First'].astype(int)
dff2['Tumor_TAR_First'] = dff2['Tumor_TAR_First'].astype(int)
dff2['Tumor_TIR_First'] = dff2['Tumor_TIR_First'].astype(int)

# Adding the values for the formula.
dff['SUM'] = dff["Normal_TAR_First"] + dff["Normal_TIR_First"]
dff['COMMON'] = dff["Tumor_TAR_First"] + dff["Tumor_TIR_First"]
print(dff)

dff1['SUM'] = dff1["Normal_TAR_First"] + dff1["Normal_TIR_First"]
dff1['COMMON'] = dff1["Tumor_TAR_First"] + dff1["Tumor_TIR_First"]
```

```
print(dff1)

dff2['SUM'] = dff2["Normal_TAR_First"] + dff2["Normal_TIR_First"]
dff2['COMMON'] = dff2["Tumor_TAR_First"] + dff2["Tumor_TIR_First"]
print(dff2)

# Getting Allele Frequency
dff['Normal_Allele_Frequency'] = dff['Normal_TIR_First']/dff['SUM']
dff['Tumor_Allele_Frequency'] =
    dff['Tumor_TIR_First']/dff['COMMON']

dff1['Normal_Allele_Frequency'] =
    dff1['Normal_TIR_First']/dff1['SUM']
dff1['Tumor_Allele_Frequency'] =
    dff1['Tumor_TIR_First']/dff1['COMMON']

dff2['Normal_Allele_Frequency'] =
    dff2['Normal_TIR_First']/dff2['SUM']
dff2['Tumor_Allele_Frequency'] =
    dff2['Tumor_TIR_First']/dff2['COMMON']

# Converting string values columans to int.
dff['Normal_Allele_Frequency'] =
    dff['Normal_Allele_Frequency'].astype(float).round(2)
dff['Tumor_Allele_Frequency'] =
    dff['Tumor_Allele_Frequency'].astype(float).round(2)

dff1['Normal_Allele_Frequency'] =
    dff1['Normal_Allele_Frequency'].astype(float).round(2)
dff1['Tumor_Allele_Frequency'] =
    dff1['Tumor_Allele_Frequency'].astype(float).round(2)

dff2['Normal_Allele_Frequency'] =
    dff2['Normal_Allele_Frequency'].astype(float).round(2)
dff2['Tumor_Allele_Frequency'] =
    dff2['Tumor_Allele_Frequency'].astype(float).round(2)

# Concatinating the "CHROM" and "POS"
dff["Normal_Allele_Frequency"] = dff['REF'].astype(str) + ':' +
    dff['Normal_Allele_Frequency'].astype(str)
```

```
dff["Tumor_Allele_Frequency"] = dff['REF'].astype(str) + ':' +
    dff['Tumor_Allele_Frequency'].astype(str)

dff1["Normal_Allele_Frequency"] = dff1['REF'].astype(str) + ':' +
    dff1['Normal_Allele_Frequency'].astype(str)
dff1["Tumor_Allele_Frequency"] = dff1['REF'].astype(str) + ':' +
    dff1['Tumor_Allele_Frequency'].astype(str)

dff2["Normal_Allele_Frequency"] = dff2['REF'].astype(str) + ':' +
    dff2['Normal_Allele_Frequency'].astype(str)
dff2["Tumor_Allele_Frequency"] = dff2['REF'].astype(str) + ':' +
    dff2['Tumor_Allele_Frequency'].astype(str)

# Dropping of the unnecessary columns and reorganising them.
dff = dff.drop(['REF', 'ALT', 'Normal_TAR', 'Normal_TIR',
    'Tumor_TAR', 'Tumor_TAR', 'Normal_TAR_First',
    'Normal_TIR_First', 'Tumor_TAR_First', 'Tumor_TIR_First',
    'SUM', 'COMMON'], axis=1)
print(dff)

dff1 = dff1.drop(['REF', 'ALT', 'Normal_TAR', 'Normal_TIR',
    'Tumor_TAR', 'Tumor_TAR', 'Normal_TAR_First',
    'Normal_TIR_First', 'Tumor_TAR_First', 'Tumor_TIR_First',
    'SUM', 'COMMON'], axis=1)
print(dff1)

dff2 = dff2.drop(['REF', 'ALT', 'Normal_TAR', 'Normal_TIR',
    'Tumor_TAR', 'Tumor_TAR', 'Normal_TAR_First',
    'Normal_TIR_First', 'Tumor_TAR_First', 'Tumor_TIR_First',
    'SUM', 'COMMON'], axis=1)
print(dff2)

# Merging columns based on "CHROM-POS"
First = pd.merge(dff, dff1, on=['CHROM_POS'])
Second = pd.merge(First, dff2, on=['CHROM_POS'])

# Renaming Columns
Second.columns = ['CHROM_POS', 'Strelka_Normal_0.3',
    'Strelka_Tumor_0.3', 'Strelka_0.5_Normal', 'Strelka_0.5_Tumor',
    'Strelka_0.7_Normal', 'Strelka_0.7_Tumor']
```

```
print(Second)

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Selected_Strelka_0.3_SNP.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Selected_Strelka_0.5_SNP.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Selected_Strelka_0.7_SNP.vcf", sep = '\t',
    index_col= False)

# Naming the columns after importing the csv file.
dff.columns = ['CHROM', 'POS', 'REF', 'ALT', 'FORMAT', 'NORMAL',
    'TUMOR']
dff1.columns = ['CHROM', 'POS', 'REF', 'ALT', 'FORMAT', 'NORMAL',
    'TUMOR']
dff2.columns = ['CHROM', 'POS', 'REF', 'ALT', 'FORMAT', 'NORMAL',
    'TUMOR']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff1['CHROM'].astype(str) + '-' +
    dff1['POS'].astype(str)
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]

dff1 = dff1.drop(['CHROM', 'POS'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]

dff2 = dff2.drop(['CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]

# Adding columns for single read depth value.
dff['REF_U'] = dff["REF"] + "U"
dff['ALT_U'] = dff["ALT"] + "U"

dff1['REF_U'] = dff1["REF"] + "U"
dff1['ALT_U'] = dff1["ALT"] + "U"

dff2['REF_U'] = dff2["REF"] + "U"
dff2['ALT_U'] = dff2["ALT"] + "U"

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "DP:FDP:SDP:SUBDP:AU:CU:GU:TU"
dff[['Normal_DP', 'Normal_FDP', 'Normal_SDP', 'Normal_SUBDP',
    'Normal_AU','Normal_CU', 'Normal_GU', 'Normal_TU']] =
    dff['NORMAL'].str.split(':',expand=True)
dff[['Tumor_DP', 'Tumor_FDP', 'Tumor_SDP', 'Tumor_SUBDP',
    'Tumor_AU', 'Tumor_CU', 'Tumor_GU', 'Tumor_TU']] =
    dff['TUMOR'].str.split(':',expand=True)

dff1[['Normal_DP', 'Normal_FDP', 'Normal_SDP', 'Normal_SUBDP',
    'Normal_AU','Normal_CU', 'Normal_GU', 'Normal_TU']] =
    dff1['NORMAL'].str.split(':',expand=True)
dff1[['Tumor_DP', 'Tumor_FDP', 'Tumor_SDP', 'Tumor_SUBDP',
    'Tumor_AU', 'Tumor_CU', 'Tumor_GU', 'Tumor_TU']] =
    dff1['TUMOR'].str.split(':',expand=True)

dff2[['Normal_DP', 'Normal_FDP', 'Normal_SDP', 'Normal_SUBDP',
    'Normal_AU','Normal_CU', 'Normal_GU', 'Normal_TU']] =
    dff2['NORMAL'].str.split(':',expand=True)
dff2[['Tumor_DP', 'Tumor_FDP', 'Tumor_SDP', 'Tumor_SUBDP',
    'Tumor_AU', 'Tumor_CU', 'Tumor_GU', 'Tumor_TU']] =
    dff2['TUMOR'].str.split(':',expand=True)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['FORMAT', 'NORMAL', 'TUMOR', 'Normal_DP',
    'Normal_FDP', 'Normal_SDP', 'Normal_SUBDP', 'Tumor_DP',
    'Tumor_FDP', 'Tumor_SDP', 'Tumor_SUBDP'], axis=1)
dff1 = dff1.drop(['FORMAT', 'NORMAL', 'TUMOR', 'Normal_DP',
    'Normal_FDP', 'Normal_SDP', 'Normal_SUBDP', 'Tumor_DP',
    'Tumor_FDP', 'Tumor_SDP', 'Tumor_SUBDP'], axis=1)
dff2 = dff2.drop(['FORMAT', 'NORMAL', 'TUMOR', 'Normal_DP',
    'Normal_FDP', 'Normal_SDP', 'Normal_SUBDP', 'Tumor_DP',
    'Tumor_FDP', 'Tumor_SDP', 'Tumor_SUBDP'], axis=1)

for i in dff['CHROM_POS']:
    dff.loc[dff['REF_U'] == 'AU', 'REF_Normal'] = dff.Normal_AU
    dff.loc[dff['REF_U'] == 'CU', 'REF_Normal'] = dff.Normal_CU
    dff.loc[dff['REF_U'] == 'GU', 'REF_Normal'] = dff.Normal_GU
    dff.loc[dff['REF_U'] == 'TU', 'REF_Normal'] = dff.Normal_TU
    dff.loc[dff['ALT_U'] == 'AU', 'ALT_Normal'] = dff.Normal_AU
    dff.loc[dff['ALT_U'] == 'CU', 'ALT_Normal'] = dff.Normal_CU
    dff.loc[dff['ALT_U'] == 'GU', 'ALT_Normal'] = dff.Normal_GU
    dff.loc[dff['ALT_U'] == 'TU', 'ALT_Normal'] = dff.Normal_TU
    dff.loc[dff['REF_U'] == 'AU', 'REF_Tumor'] = dff.Tumor_AU
    dff.loc[dff['REF_U'] == 'CU', 'REF_Tumor'] = dff.Tumor_CU
    dff.loc[dff['REF_U'] == 'GU', 'REF_Tumor'] = dff.Tumor_GU
    dff.loc[dff['REF_U'] == 'TU', 'REF_Tumor'] = dff.Tumor_TU
    dff.loc[dff['ALT_U'] == 'AU', 'ALT_Tumor'] = dff.Tumor_AU
    dff.loc[dff['ALT_U'] == 'CU', 'ALT_Tumor'] = dff.Tumor_CU
    dff.loc[dff['ALT_U'] == 'GU', 'ALT_Tumor'] = dff.Tumor_GU
    dff.loc[dff['ALT_U'] == 'TU', 'ALT_Tumor'] = dff.Tumor_TU
print(dff)

for i in dff1['CHROM_POS']:
    dff1.loc[dff1['REF_U'] == 'AU', 'REF_Normal'] = dff1.Normal_AU
    dff1.loc[dff1['REF_U'] == 'CU', 'REF_Normal'] = dff1.Normal_CU
    dff1.loc[dff1['REF_U'] == 'GU', 'REF_Normal'] = dff1.Normal_GU
    dff1.loc[dff1['REF_U'] == 'TU', 'REF_Normal'] = dff1.Normal_TU
    dff1.loc[dff1['ALT_U'] == 'AU', 'ALT_Normal'] = dff1.Normal_AU
    dff1.loc[dff1['ALT_U'] == 'CU', 'ALT_Normal'] = dff1.Normal_CU
    dff1.loc[dff1['ALT_U'] == 'GU', 'ALT_Normal'] = dff1.Normal_GU
    dff1.loc[dff1['ALT_U'] == 'TU', 'ALT_Normal'] = dff1.Normal_TU
    dff1.loc[dff1['REF_U'] == 'AU', 'REF_Tumor'] = dff1.Tumor_AU
    dff1.loc[dff1['REF_U'] == 'CU', 'REF_Tumor'] = dff1.Tumor_CU
    dff1.loc[dff1['REF_U'] == 'GU', 'REF_Tumor'] = dff1.Tumor_GU
    dff1.loc[dff1['REF_U'] == 'TU', 'REF_Tumor'] = dff1.Tumor_TU
    dff1.loc[dff1['ALT_U'] == 'AU', 'ALT_Tumor'] = dff1.Tumor_AU
    dff1.loc[dff1['ALT_U'] == 'CU', 'ALT_Tumor'] = dff1.Tumor_CU
    dff1.loc[dff1['ALT_U'] == 'GU', 'ALT_Tumor'] = dff1.Tumor_GU
    dff1.loc[dff1['ALT_U'] == 'TU', 'ALT_Tumor'] = dff1.Tumor_TU
print(dff1)

for i in dff2['CHROM_POS']:
    dff2.loc[dff2['REF_U'] == 'AU', 'REF_Normal'] = dff2.Normal_AU
    dff2.loc[dff2['REF_U'] == 'CU', 'REF_Normal'] = dff2.Normal_CU
    dff2.loc[dff2['REF_U'] == 'GU', 'REF_Normal'] = dff2.Normal_GU
    dff2.loc[dff2['REF_U'] == 'TU', 'REF_Normal'] = dff2.Normal_TU
    dff2.loc[dff2['ALT_U'] == 'AU', 'ALT_Normal'] = dff2.Normal_AU
    dff2.loc[dff2['ALT_U'] == 'CU', 'ALT_Normal'] = dff2.Normal_CU
    dff2.loc[dff2['ALT_U'] == 'GU', 'ALT_Normal'] = dff2.Normal_GU
    dff2.loc[dff2['ALT_U'] == 'TU', 'ALT_Normal'] = dff2.Normal_TU
    dff2.loc[dff2['REF_U'] == 'AU', 'REF_Tumor'] = dff2.Tumor_AU
    dff2.loc[dff2['REF_U'] == 'CU', 'REF_Tumor'] = dff2.Tumor_CU
    dff2.loc[dff2['REF_U'] == 'GU', 'REF_Tumor'] = dff2.Tumor_GU
    dff2.loc[dff2['REF_U'] == 'TU', 'REF_Tumor'] = dff2.Tumor_TU
    dff2.loc[dff2['ALT_U'] == 'AU', 'ALT_Tumor'] = dff2.Tumor_AU
    dff2.loc[dff2['ALT_U'] == 'CU', 'ALT_Tumor'] = dff2.Tumor_CU
    dff2.loc[dff2['ALT_U'] == 'GU', 'ALT_Tumor'] = dff2.Tumor_GU
    dff2.loc[dff2['ALT_U'] == 'TU', 'ALT_Tumor'] = dff2.Tumor_TU
print(dff2)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "DP:FDP:SDP:SUBDP:AU:CU:GU:TU"
dff[['REF_Normal_First', 'REF_Normal_Second']] =
    dff['REF_Normal'].str.split(',',expand=True)
dff[['ALT_Normal_First', 'ALT_Normal_Second']] =
    dff['ALT_Normal'].str.split(',',expand=True)
dff[['REF_Tumor_First', 'REF_Tumor_Second']] =
    dff['REF_Tumor'].str.split(',',expand=True)
dff[['ALT_Tumor_First', 'ALT_Tumor_Second']] =
    dff['ALT_Tumor'].str.split(',',expand=True)
print(dff)

dff1[['REF_Normal_First', 'REF_Normal_Second']] =
    dff1['REF_Normal'].str.split(',',expand=True)
```

```
dff1[['ALT_Normal_First', 'ALT_Normal_Second']] =
    dff1['ALT_Normal'].str.split(',',expand=True)
dff1[['REF_Tumor_First', 'REF_Tumor_Second']] =
    dff1['REF_Tumor'].str.split(',',expand=True)
dff1[['ALT_Tumor_First', 'ALT_Tumor_Second']] =
    dff1['ALT_Tumor'].str.split(',',expand=True)
print(dff1)

dff2[['REF_Normal_First', 'REF_Normal_Second']] =
    dff2['REF_Normal'].str.split(',',expand=True)
dff2[['ALT_Normal_First', 'ALT_Normal_Second']] =
    dff2['ALT_Normal'].str.split(',',expand=True)
dff2[['REF_Tumor_First', 'REF_Tumor_Second']] =
    dff2['REF_Tumor'].str.split(',',expand=True)
dff2[['ALT_Tumor_First', 'ALT_Tumor_Second']] =
    dff2['ALT_Tumor'].str.split(',',expand=True)
print(dff2)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['REF_U', 'ALT_U', 'Normal_AU', 'Normal_CU',
    'Normal_GU', 'Normal_TU', 'Tumor_AU', 'Tumor_CU', 'Tumor_GU',
    'Tumor_TU', 'REF_Normal_Second', 'ALT_Normal_Second',
    'Normal_AU', 'Normal_CU', 'Normal_GU', 'Normal_TU', 'Tumor_AU',
    'Tumor_CU', 'Tumor_GU', 'Tumor_TU', 'REF_Tumor_Second',
    'ALT_Tumor_Second'], axis=1)
print(dff)

dff1 = dff1.drop(['REF_U', 'ALT_U', 'Normal_AU', 'Normal_CU',
    'Normal_GU', 'Normal_TU', 'Tumor_AU', 'Tumor_CU', 'Tumor_GU',
    'Tumor_TU', 'REF_Normal_Second', 'ALT_Normal_Second',
    'Normal_AU', 'Normal_CU', 'Normal_GU', 'Normal_TU', 'Tumor_AU',
    'Tumor_CU', 'Tumor_GU', 'Tumor_TU', 'REF_Tumor_Second',
    'ALT_Tumor_Second'], axis=1)
print(dff1)

dff2 = dff2.drop(['REF_U', 'ALT_U', 'Normal_AU', 'Normal_CU',
    'Normal_GU', 'Normal_TU', 'Tumor_AU', 'Tumor_CU', 'Tumor_GU',
    'Tumor_TU', 'REF_Normal_Second', 'ALT_Normal_Second',
    'Normal_AU', 'Normal_CU', 'Normal_GU', 'Normal_TU', 'Tumor_AU',
    'Tumor_CU', 'Tumor_GU', 'Tumor_TU', 'REF_Tumor_Second',
    'ALT_Tumor_Second'], axis=1)
print(dff2)

# Naming the columns after importing the csv file.
dff.columns = ['CHROM_POS', 'REF', 'ALT', 'REF_Normal',
    'ALT_Normal', 'REF_Tumor', 'ALT_Tumor', 'REF_Normal_First',
    'ALT_Normal_First', 'REF_Tumor_First', 'ALT_Tumor_First']
print(dff)

dff1.columns = ['CHROM_POS', 'REF', 'ALT', 'REF_Normal',
    'ALT_Normal', 'REF_Tumor', 'ALT_Tumor', 'REF_Normal_First',
    'ALT_Normal_First', 'REF_Tumor_First', 'ALT_Tumor_First']
print(dff1)

dff2.columns = ['CHROM_POS', 'REF', 'ALT', 'REF_Normal',
    'ALT_Normal', 'REF_Tumor', 'ALT_Tumor', 'REF_Normal_First',
    'ALT_Normal_First', 'REF_Tumor_First', 'ALT_Tumor_First']
print(dff2)

# Converting string values columns to int.
dff['REF_Normal_First'] = dff['REF_Normal_First'].astype(int)
dff['ALT_Normal_First'] = dff['ALT_Normal_First'].astype(int)
dff['REF_Tumor_First'] = dff['REF_Tumor_First'].astype(int)
dff['ALT_Tumor_First'] = dff['ALT_Tumor_First'].astype(int)
print(dff)

dff1['REF_Normal_First'] = dff1['REF_Normal_First'].astype(int)
dff1['ALT_Normal_First'] = dff1['ALT_Normal_First'].astype(int)
dff1['REF_Tumor_First'] = dff1['REF_Tumor_First'].astype(int)
dff1['ALT_Tumor_First'] = dff1['ALT_Tumor_First'].astype(int)
print(dff1)

dff2['REF_Normal_First'] = dff2['REF_Normal_First'].astype(int)
dff2['ALT_Normal_First'] = dff2['ALT_Normal_First'].astype(int)
dff2['REF_Tumor_First'] = dff2['REF_Tumor_First'].astype(int)
dff2['ALT_Tumor_First'] = dff2['ALT_Tumor_First'].astype(int)
print(dff2)

# Adding the values for formula.
dff['SUM'] = dff["REF_Normal_First"] + dff["ALT_Normal_First"]
```

```
dff['COMMON'] = dff["REF_Tumor_First"] + dff["ALT_Tumor_First"]
print(dff)

dff1['SUM'] = dff1["REF_Normal_First"] + dff1["ALT_Normal_First"]
dff1['COMMON'] = dff1["REF_Tumor_First"] + dff1["ALT_Tumor_First"]
print(dff1)

dff2['SUM'] = dff2["REF_Normal_First"] + dff2["ALT_Normal_First"]
dff2['COMMON'] = dff2["REF_Tumor_First"] + dff2["ALT_Tumor_First"]
print(dff2)

# Getting Allele Frequency
dff['Normal'] = dff['ALT_Normal_First']/dff['SUM']
dff['Tumor'] = dff['ALT_Tumor_First']/dff['COMMON']
print(dff)

dff1['Normal'] = dff1['ALT_Normal_First']/dff1['SUM']
dff1['Tumor'] = dff1['ALT_Tumor_First']/dff1['COMMON']
print(dff1)

dff2['Normal'] = dff2['ALT_Normal_First']/dff2['SUM']
dff2['Tumor'] = dff2['ALT_Tumor_First']/dff2['COMMON']
print(dff2)

# Converting string values columans to int.
dff['Normal'] = dff['Normal'].astype(float).round(2)
dff['Tumor'] = dff['Tumor'].astype(float).round(2)
print(dff)

dff1['Normal'] = dff1['Normal'].astype(float).round(2)
dff1['Tumor'] = dff1['Tumor'].astype(float).round(2)
print(dff1)

dff2['Normal'] = dff2['Normal'].astype(float).round(2)
dff2['Tumor'] = dff2['Tumor'].astype(float).round(2)
print(dff2)

# Concatinating the "CHROM" and "POS"
dff["Normal"] = dff['REF'].astype(str) + ':' +
    dff['Normal'].astype(str)
```

```
dff["Tumor"] = dff['REF'].astype(str) + ':' +
    dff['Tumor'].astype(str)
print(dff)

dff1["Normal"] = dff1['REF'].astype(str) + ':' +
    dff1['Normal'].astype(str)
dff1["Tumor"] = dff1['REF'].astype(str) + ':' +
    dff1['Tumor'].astype(str)
print(dff1)

dff2["Normal"] = dff2['REF'].astype(str) + ':' +
    dff2['Normal'].astype(str)
dff2["Tumor"] = dff2['REF'].astype(str) + ':' +
    dff2['Tumor'].astype(str)
print(dff2)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['REF', 'ALT', 'REF_Normal', 'ALT_Normal',
    'REF_Tumor', 'ALT_Tumor', 'REF_Normal_First',
    'ALT_Normal_First', 'REF_Tumor_First', 'ALT_Tumor_First',
    'SUM', 'COMMON'], axis=1)
print(dff)

dff1 = dff1.drop(['REF', 'ALT', 'REF_Normal', 'ALT_Normal',
    'REF_Tumor', 'ALT_Tumor', 'REF_Normal_First',
    'ALT_Normal_First', 'REF_Tumor_First', 'ALT_Tumor_First',
    'SUM', 'COMMON'], axis=1)
print(dff1)

dff2 = dff2.drop(['REF', 'ALT', 'REF_Normal', 'ALT_Normal',
    'REF_Tumor', 'ALT_Tumor', 'REF_Normal_First',
    'ALT_Normal_First', 'REF_Tumor_First', 'ALT_Tumor_First',
    'SUM', 'COMMON'], axis=1)
print(dff2)

# Merging columns based on "CHROM-POS"
First = pd.merge(dff, dff1, on=['CHROM_POS'])
Third = pd.merge(First, dff2, on=['CHROM_POS'])

# Renaming Columns
```

```
Third.columns = ['CHROM_POS', 'Strelka_Normal_0.3',
    'Strelka_Tumor_0.3', 'Strelka_0.5_Normal', 'Strelka_0.5_Tumor',
    'Strelka_0.7_Normal', 'Strelka_0.7_Tumor']
print(Third)

# Assigning column names.
Second.columns = ['CHROM_POS', 'Indel_Normal_0.3',
    'Indel_Tumor_0.3', 'Indel_Normal_0.5', 'Indel_Tumor_0.5',
    'Indel_Normal_0.7', 'Indel_Tumor_0.7']
Third.columns = ['CHROM_POS', 'SNP_Normal_0.3', 'SNP_Tumor_0.3',
    'SNP_Normal_0.5', 'SNP_Tumor_0.5', 'SNP_Normal_0.7',
    'SNP_Tumor_0.7']
print(Second)
print(Third)

# Using merge function by setting how='inner'
df = pd.merge(Second, Third, on='CHROM_POS', how='outer')
df['Normal_0.3_AF'] =
    df['Indel_Normal_0.3'].combine_first(df['SNP_Normal_0.3'])
df['Tumor_0.3_AF'] =
    df['Indel_Tumor_0.3'].combine_first(df['SNP_Tumor_0.3'])
df['Normal_0.5_AF'] =
    df['Indel_Normal_0.5'].combine_first(df['SNP_Normal_0.5'])
df['Tumor_0.5_AF'] =
    df['Indel_Tumor_0.5'].combine_first(df['SNP_Tumor_0.5'])
df['Normal_0.7_AF'] =
    df['Indel_Normal_0.7'].combine_first(df['SNP_Normal_0.7'])
df['Tumor_0.7_AF'] =
    df['Indel_Tumor_0.7'].combine_first(df['SNP_Tumor_0.7'])
print(df)

# Dropping unneeded columns.
df = df.drop(['Indel_Normal_0.3', 'Indel_Tumor_0.3',
    'Indel_Normal_0.5', 'Indel_Tumor_0.5', 'Indel_Normal_0.7',
    'Indel_Tumor_0.7', 'SNP_Normal_0.3', 'SNP_Tumor_0.3',
    'SNP_Normal_0.5', 'SNP_Tumor_0.5', 'SNP_Normal_0.7',
    'SNP_Tumor_0.7'], axis=1)
print(df)

# Saving the result into a csv file for plotting.
```

```
df.to_csv('Strelka_Allele_Frequency.csv', sep=',', index = False)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "GT:GQ:DP:AD:ADF:ADR".
df[['Normal_0.3_Allele', 'Normal_0.3_Value']] =
    df['Normal_0.3_AF'].str.split(':',expand=True)
df[['Tumor_0.3_Allele', 'Tumor_0.3_Value']] =
    df['Tumor_0.3_AF'].str.split(':',expand=True)
df[['Normal_0.5_Allele', 'Normal_0.5_Value']] =
    df['Normal_0.5_AF'].str.split(':',expand=True)
df[['Tumor_0.5_Allele', 'Tumor_0.5_Value']] =
    df['Tumor_0.5_AF'].str.split(':',expand=True)
df[['Normal_0.7_Allele', 'Normal_0.7_Value']] =
    df['Normal_0.7_AF'].str.split(':',expand=True)
df[['Tumor_0.7_Allele', 'Tumor_0.7_Value']] =
    df['Tumor_0.7_AF'].str.split(':',expand=True)
print(df)

# Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
dff = df.drop(['CHROM_POS', 'Normal_0.3_AF', 'Tumor_0.3_AF',
    'Normal_0.5_AF', 'Tumor_0.5_AF', 'Normal_0.7_AF',
    'Tumor_0.7_AF', 'Normal_0.3_Allele', 'Tumor_0.3_Allele',
    'Normal_0.5_Allele', 'Tumor_0.5_Allele', 'Normal_0.7_Allele',
    'Tumor_0.7_Allele'], axis=1)

# Renaming the columns.
dff.columns = ['Normal_0.3', 'Tumor_0.3', 'Normal_0.5',
    'Tumor_0.5', 'Normal_0.7', 'Tumor_0.7']
print(dff)

# Converting string values columns to float.
dff['Normal_0.3'] = dff['Normal_0.3'].astype(float)
dff['Tumor_0.3'] = dff['Tumor_0.3'].astype(float)
dff['Normal_0.5'] = dff['Normal_0.5'].astype(float)
dff['Tumor_0.5'] = dff['Tumor_0.5'].astype(float)
dff['Normal_0.7'] = dff['Normal_0.7'].astype(float)
dff['Tumor_0.7'] = dff['Tumor_0.7'].astype(float)
```

```
print(dff)

# Getting a count based on allele frequency values.
dff1 = dff[dff < 0.26].count()
dff2 = dff[dff < 0.51].count()
dff3 = dff[dff < 0.76].count()
dff4 = dff[dff < 1.01].count()

# Getting the final values
dff5 = (dff1 - dff2).abs()
dff6 = (dff2 - dff3).abs()
dff7 = (dff3 - dff4).abs()
print(dff1)
print(dff5)
print(dff6)
print(dff7)

# Converting into list.
First_Column = dff1.tolist()
Second_Column = dff5.tolist()
Third_Column = dff6.tolist()
Fourth_Column = dff7.tolist()
Fifth_Column =['Normal_0.3', 'Tumor_0.3', 'Normal_0.5',
    'Tumor_0.5', 'Normal_0.7', 'Tumor_0.7']
print(First_Column)
print(Second_Column)
print(Third_Column)
print(Fourth_Column)

# Declaring new columns.
dff8 = pd.DataFrame(Fifth_Column, columns = ['Type'])
dff8['Less than 0.25'] = First_Column
dff8['Between 0.25 & 0.50'] = Second_Column
dff8['Between 0.50 & 0.75'] = Third_Column
dff8['Between 0.75 & 1.00'] = Fourth_Column
print(dff8)

# Saving the results in csv.
dff8.to_csv('Strelka_Allele_Frequency_Counts.csv', sep=',', index
    = None)
```

```
dff9 = dff8.drop(['Type'], axis=1)
print(dff9)

# Converting the values to a list
List = dff9.values.tolist()
a1, a2, a3, a4, a5, a6 = List
print(a1)
print(a2)
print(a3)
print(a4)
print(a5)

# set width of bar
width = 0.10

# Columns from the file
# a1 = First_Column
# a2 = Second_Column
# a3 = Third_Column
# a4 = Fourth_Column

# Set position of bar on X axis
r1 = np.arange(len(a1))
r2 = [x + width for x in r1]
r3 = [x + width for x in r2]
r4 = [x + width for x in r3]
r5 = [x + width for x in r4]
r6 = [x + width for x in r5]

# Make the plot
plt.bar(r1, a1, color='#ff0000', width=width, edgecolor='white',
    label='Normal_0.3')
plt.bar(r2, a2, color='#ffa07a', width=width, edgecolor='white',
    label='Tumor_0.3')
plt.bar(r3, a3, color='#f08080', width=width, edgecolor='white',
    label='Normal_0.5')
plt.bar(r4, a4, color='#fa8072', width=width, edgecolor='white',
    label='Tumor_0.5')
```

```
plt.bar(r5, a5, color='#b22222', width=width, edgecolor='white',
    label='Normal_0.7')
plt.bar(r6, a6, color='#800000', width=width, edgecolor='white',
    label='Tumor_0.7')

csfont = {'fontname':'Comic Sans MS'}
hfont = {'fontname':'Helvetica'}

# Add xticks on the middle of the group bars
plt.xlabel('Strelka_Allele_Frequencies')
plt.xticks([r + width for r in range(len(a1))], ['<= 0.25', '<=
    0.50', '<= 0.75', '<= 1.00'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('Strelka_Allele_Frequency_Plot.pdf')
plt.savefig('Strelka_Allele_Frequency_Plot.png', dpi = 300)
```

Truth Data Allele Frequency

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
import matplotlib.pyplot as plt
import csv

# Reading csv files and concatinating "CHROM" and "POS"
dff = pd.read_csv("Somatic_Truth.frq", sep = '\t', index_col=
    False, error_bad_lines=False)
dff.columns = ['CHROM', 'POS', 'N_ALLELES', 'N_CHR', 'ALLELE:FREQ']
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)

# Reorganising columns.
dff = dff.drop(['N_ALLELES', 'N_CHR', 'CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

# Saving the results in csv.
dff.to_csv('Truth_Data.csv', sep=',', index = False)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "GT:GQ:DP:AD:ADF:ADR".
dff[['Allele', 'Freq']] =
    dff['ALLELE:FREQ'].str.split(':',expand=True)
print(dff)

#  Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
dff = dff.drop(['CHROM_POS', 'ALLELE:FREQ', 'Allele'], axis=1)
print(dff)

# Renaming the columns.
dff.columns = ['Freq']
print(dff)

# Converting string values columns to float.
dff['Freq'] = dff['Freq'].astype(float)
print(dff)

# Getting a count based on allele frequency values.
dff1 = dff[dff < 0.26].count()
dff2 = dff[dff < 0.51].count()
dff3 = dff[dff < 0.76].count()
dff4 = dff[dff < 1.01].count()

# Getting the final values
dff5 = (dff1 - dff2).abs()
dff6 = (dff2 - dff3).abs()
dff7 = (dff3 - dff4).abs()
print(dff1)
print(dff5)
print(dff6)
print(dff7)

# Converting into list.
First_Column = dff1.tolist()
Second_Column = dff5.tolist()
Third_Column = dff6.tolist()
Fourth_Column = dff7.tolist()
Fifth_Column =['Truth_Data']
print(First_Column)
print(Second_Column)
print(Third_Column)
print(Fourth_Column)

# Declaring new columns.
dff8 = pd.DataFrame(Fifth_Column, columns = ['Type'])
dff8['Less than 0.25'] = First_Column
dff8['Between 0.25 & 0.50'] = Second_Column
dff8['Between 0.50 & 0.75'] = Third_Column
dff8['Between 0.75 & 1.00'] = Fourth_Column
print(dff8)

# Saving the results in csv.
dff8.to_csv('Truth_Data_Allele_Frequency_Counts.csv', sep=',',
    index = None)
```

VarScan Allele Frequency

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import csv
```

```
# Reading csv files and concatinating "CHROM" and "POS"
dff = pd.read_csv("VarScan_0.3.frq", sep = '\t', index_col= False)
dff.columns = ['CHROM', 'POS', 'N_ALLELES', 'N_CHR', 'ALLELE:FREQ']
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)

dff2 = pd.read_csv("VarScan_0.5.frq", sep = '\t', index_col= False)
dff2.columns = ['CHROM', 'POS', 'N_ALLELES', 'N_CHR',
    'ALLELE:FREQ']
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

dff3 = pd.read_csv("VarScan_0.7.frq", sep = '\t', index_col= False)
dff3.columns = ['CHROM', 'POS', 'N_ALLELES', 'N_CHR',
    'ALLELE:FREQ']
dff3["CHROM_POS"] = dff3['CHROM'].astype(str) + '-' +
    dff3['POS'].astype(str)

# Reorganising columns.
dff = dff.drop(['N_ALLELES', 'N_CHR', 'CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

dff2 = dff2.drop(['N_ALLELES', 'N_CHR', 'CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]
print(dff2)

dff3 = dff3.drop(['N_ALLELES', 'N_CHR', 'CHROM', 'POS'], axis=1)
cols = dff3.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff3 = dff3[cols]
print(dff3)

# Merging columns based on "CHROM_POS"
Result = pd.merge(dff, dff2, on="CHROM_POS")
Merge = pd.merge(Result, dff3, on="CHROM_POS")
```

```
Merge.columns = ['CHROM_POS', 'VarScan_0.3_AF', 'VarScan_0.5_AF',
    'VarScan_0.7_AF']

# Saving the results in csv.
Merge.to_csv('VarScan_Allele_Frequencies.csv', sep=',', index =
    False)

# Creating new columns by splitting the "Allele" and "Value" by
    ':'.
Merge[['VarScan_0.3_Allele', 'VarScan_0.3_Value']] =
    Merge['VarScan_0.3_AF'].str.split(':',expand=True)
Merge[['VarScan_0.5_Allele', 'VarScan_0.5_Value']] =
    Merge['VarScan_0.5_AF'].str.split(':',expand=True)
Merge[['VarScan_0.7_Allele', 'VarScan_0.7_Value']] =
    Merge['VarScan_0.7_AF'].str.split(':',expand=True)
print(Merge)

# Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
dff4 = Merge.drop(['CHROM_POS', 'VarScan_0.3_AF',
    'VarScan_0.5_AF', 'VarScan_0.7_AF', 'VarScan_0.3_Allele',
    'VarScan_0.5_Allele', 'VarScan_0.7_Allele'], axis=1)

# Renaming the columns.
dff4.columns = ['VarScan_0.3', 'VarScan_0.5', 'VarScan_0.7']
print(dff)

# Converting string values columns to float.
dff4['VarScan_0.3'] = dff4['VarScan_0.3'].astype(float)
dff4['VarScan_0.5'] = dff4['VarScan_0.5'].astype(float)
dff4['VarScan_0.7'] = dff4['VarScan_0.7'].astype(float)
print(dff4)

# Getting a count based on allele frequency values.
dff5 = dff4[dff4 < 0.26].count()
dff6 = dff4[dff4 < 0.51].count()
dff7 = dff4[dff4 < 0.76].count()
dff8 = dff4[dff4 < 1.01].count()
```

```
# Getting the final values
dff9 = (dff5 - dff6).abs()
dff10 = (dff6 - dff7).abs()
dff11 = (dff7 - dff8).abs()
print(dff5)
print(dff9)
print(dff10)
print(dff11)

# Converting into list.
First_Column = dff5.tolist()
Second_Column = dff9.tolist()
Third_Column = dff10.tolist()
Fourth_Column = dff11.tolist()
Fifth_Column =['VarScan_0.3', 'VarScan_0.5', 'VarScan_0.7']
print(First_Column)
print(Second_Column)
print(Third_Column)
print(Fourth_Column)

# Declaring new columns.
dff8 = pd.DataFrame(Fifth_Column, columns = ['Type'])
dff8['Less than 0.25'] = First_Column
dff8['Between 0.25 & 0.50'] = Second_Column
dff8['Between 0.50 & 0.75'] = Third_Column
dff8['Between 0.75 & 1.00'] = Fourth_Column
print(dff8)

# Saving the results in csv.
dff8.to_csv('VarScan_Allele_Frequency_Counts.csv', sep=',', index
    = None)

dff9 = dff8.drop(['Type'], axis=1)
print(dff9)

# Converting the values to a list
List = dff9.values.tolist()
a1, a2, a3 = List
print(a1)
print(a2)
print(a3)

# set width of bar
width = 0.15

# Set position of bar on X axis
r1 = np.arange(len(a1))
r2 = [x + width for x in r1]
r3 = [x + width for x in r2]

# Make the plot
plt.bar(r1, a1, color='#ff0000', width=width, edgecolor='white',
    label='VarScan_0.3')
plt.bar(r2, a2, color='#ffa07a', width=width, edgecolor='white',
    label='VarScan_0.5')
plt.bar(r3, a3, color='#f08080', width=width, edgecolor='white',
    label='VarScan_0.7')

# Add xticks on the middle of the group bars
plt.xlabel('VarScan_Allele_Frequencies')
plt.xticks([r + width for r in range(len(a1))], ['<= 0.25', '<=
    0.50', '<= 0.75', '<= 1.00'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('VarScan_Allele_Frequency_Plot.pdf')
plt.savefig('VarScan_Allele_Frequency_Plot.png', dpi = 300)
```

Allele Frequency Comparison

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
```

```
import matplotlib.pyplot as plt
import csv

# Reading csv files and concatinating "CHROM" and "POS"
df = pd.read_csv("Strelka_0.3.csv", sep = '\t', index_col= False)
df1 = pd.read_csv("VarScan_0.3.csv", sep = '\t', index_col= False)
df2 = pd.read_csv("Somatic_Truth.csv", sep = '\t', index_col=
    False)

# Merging columns based on "CHROM-POS"
First = pd.merge(df, df1, on=['CHROM_POS'])
Second = pd.merge(First, df2, on=['CHROM_POS'])

# Renaming Columns
Second.columns = ['CHROM_POS', 'Strelka_Normal', 'Strelka_Tumor',
    'VarScan', 'Truth_Data']
print(Second)

# Saving the results in csv.
Second.to_csv('Tumor_Purity_0.3.csv', sep=',', index = None)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "GT:GQ:DP:AD:ADF:ADR".
Second[['Strelka_Normal_Allele', 'Strelka_Normal_Value']] =
    Second['Strelka_Normal'].str.split(':',expand=True)
Second[['Strelka_Tumor_Allele', 'Strelka_Tumor_Value']] =
    Second['Strelka_Tumor'].str.split(':',expand=True)
Second[['VarScan_Normal_Allele', 'VarScan_Normal_Value']] =
    Second['VarScan'].str.split(':',expand=True)
Second[['Truth_Data_Allele', 'Truth_Data_Value']] =
    Second['Truth_Data'].str.split(':',expand=True)
print(Second)

# Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
dff = Second.drop(['CHROM_POS', 'Strelka_Normal', 'Strelka_Tumor',
    'VarScan', 'Truth_Data', 'Strelka_Normal_Allele',
    'Strelka_Tumor_Allele', 'VarScan_Normal_Allele',
    'Truth_Data_Allele'], axis=1)
print(dff)

# Renaming the columns.
dff.columns = ['Strelka_Normal', 'Strelka_Tumor', 'VarScan',
    'Truth_Data']
print(dff)

# Converting string values columns to float.
dff['Strelka_Normal'] = dff['Strelka_Normal'].astype(float)
dff['Strelka_Tumor'] = dff['Strelka_Tumor'].astype(float)
dff['VarScan'] = dff['VarScan'].astype(float)
dff['Truth_Data'] = dff['Truth_Data'].astype(float)
print(dff)

# Getting a count based on allele frequency values.
dff1 = dff[dff < 0.26].count()
dff2 = dff[dff < 0.51].count()
dff3 = dff[dff < 0.76].count()
dff4 = dff[dff < 1.01].count()

# Getting the final values
dff5 = (dff1 - dff2).abs()
dff6 = (dff2 - dff3).abs()
dff7 = (dff3 - dff4).abs()
print(dff1)
print(dff5)
print(dff6)
print(dff7)

# Converting into list.
First_Column = dff1.tolist()
Second_Column = dff5.tolist()
Third_Column = dff6.tolist()
Fourth_Column = dff7.tolist()
Fifth_Column =['Strelka_Normal', 'Strelka_Tumor', 'VarScan',
    'Truth_Data']
print(First_Column)
print(Second_Column)
print(Third_Column)
print(Fourth_Column)

# Declaring new columns.
dff8 = pd.DataFrame(Fifth_Column, columns = ['Type'])
dff8['Less than 0.25'] = First_Column
dff8['Between 0.25 & 0.50'] = Second_Column
dff8['Between 0.50 & 0.75'] = Third_Column
dff8['Between 0.75 & 1.00'] = Fourth_Column
print(dff8)

# Saving the results in csv.
dff8.to_csv('Tumor_Purity_0.3_AF_Counts.csv', sep=',', index =
    None)

# set width of bar
width = 0.25

# Columns from the file
a1 = First_Column
a2 = Second_Column
a3 = Third_Column
a4 = Fourth_Column

# Set position of bar on X axis
r1 = np.arange(len(a1))
r2 = [x + width for x in r1]
r3 = [x + width for x in r2]
r4 = [x + width for x in r3]

# Make the plot
plt.bar(r1, a1, color='#FFD700', width=width, edgecolor='white',
    label='Strelka_Normal')
plt.bar(r2, a2, color='#FFAA1C', width=width, edgecolor='white',
    label='Strelka_Tumor')
plt.bar(r3, a3, color='#FF8C01', width=width, edgecolor='white',
    label='VarScan')
plt.bar(r4, a4, color='#FF0000', width=width, edgecolor='white',
    label='Truth_Data')

# Add xticks on the middle of the group bars
```

```
plt.xlabel('Tumor_0.3_Allele_Frequencies')
plt.xticks([r + width for r in range(len(a1))], ['<= 0.25', '<=
    0.50', '<= 0.75', '<= 1.00'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('Tumor_Purity_0.3_AF_Plot.pdf')
plt.savefig('Tumor_Purity_0.3_AF_Plot.png', dpi = 300)
```

Appendix C

Strelka Bechmarking

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import csv

# Reading csv files and concatinating "CHROM" and "POS"
df1 = pd.read_csv("Updated_Truth.vcf", sep = '\t', index_col=
    False)
df2 = pd.read_csv("Updated_Strelka_0.3.vcf", sep = '\t',
    index_col= False)
df3 = pd.read_csv("Updated_Strelka_0.5.vcf", sep = '\t',
    index_col= False)
df4 = pd.read_csv("Updated_Strelka_0.7.vcf", sep = '\t',
    index_col= False)

# Merging columns based on "Positions"
dff1 = pd.merge(df1, df2, how="outer", on=['POS'])
dff2 = pd.merge(df1, df3, how="outer", on=['POS'])
dff3 = pd.merge(df1, df4, how="outer", on=['POS'])

# Renaming the columns after importing the input.
dff1.columns = ['POS', 'Truth_Data', 'Strelka_Three']
```

```
dff2.columns = ['POS', 'Truth_Data', 'Strelka_Five']
dff3.columns = ['POS', 'Truth_Data', 'Strelka_Seven']
print(dff1)
print(dff2)
print(dff3)

# Total number of reads
dff4 = len(dff1)
print('Total lenght is')
print(dff4)

dff47 = len(dff2)
print('Total lenght is')
print(dff47)

dff48 = len(dff3)
print('Total lenght is')
print(dff48)

# Comparison column
dff1["TP"] = np.where(dff1["Truth_Data"].notnull() &
   dff1["Strelka_Three"].notnull(), 0, 1)
dff1["TN"] = np.where(dff1["Truth_Data"].isnull() &
   dff1["Strelka_Three"].isnull(), 0, 1)
dff1["FP"] = np.where(dff1["Truth_Data"].isnull() &
   dff1["Strelka_Three"].notnull(), 0, 1)
dff1["FN"] = np.where(dff1["Truth_Data"].notnull() &
   dff1["Strelka_Three"].isnull(), 0, 1)

# Selecting the columns
dff5 = dff1.loc[dff1['TP'] == 0]
dff6 = dff1.loc[dff1['TN'] == 0]
dff7 = dff1.loc[dff1['FP'] == 0]
dff8 = dff1.loc[dff1['FN'] == 0]

# Total numbers of Comparisons
dff9 = len(dff5)
dff10 = len(dff6)
dff11 = len(dff7)
dff12 = len(dff8)
```

```
print("Total number of True Positives:")
print(dff9)
print("Total number of True Negatives:")
print(dff10)
print("Total number of False Postivies:")
print(dff11)
print("Total number of False Negatives:")
print(dff12)

# Comparison column
dff2["TP"] = np.where(dff2["Truth_Data"].notnull() &
   dff2["Strelka_Five"].notnull(), 0, 1)
dff2["TN"] = np.where(dff2["Truth_Data"].isnull() &
   dff2["Strelka_Five"].isnull(), 0, 1)
dff2["FP"] = np.where(dff2["Truth_Data"].isnull() &
   dff2["Strelka_Five"].notnull(), 0, 1)
dff2["FN"] = np.where(dff2["Truth_Data"].notnull() &
   dff2["Strelka_Five"].isnull(), 0, 1)

# Selecting the columns
dff13 = dff2.loc[dff2['TP'] == 0]
dff14 = dff2.loc[dff2['TN'] == 0]
dff15 = dff2.loc[dff2['FP'] == 0]
dff16 = dff2.loc[dff2['FN'] == 0]

# Total numbers of Comparisons
dff17 = len(dff13)
dff18 = len(dff14)
dff19 = len(dff15)
dff20 = len(dff16)

print("Total number of True Positives:")
print(dff17)
print("Total number of True Negatives:")
print(dff18)
print("Total number of False Postivies:")
print(dff19)
print("Total number of False Negatives:")
print(dff20)
```

```
# Comparison column
dff3["TP"] = np.where(dff3["Truth_Data"].notnull() &
    dff3["Strelka_Seven"].notnull(), 0, 1)
dff3["TN"] = np.where(dff3["Truth_Data"].isnull() &
    dff3["Strelka_Seven"].isnull(), 0, 1)
dff3["FP"] = np.where(dff3["Truth_Data"].isnull() &
    dff3["Strelka_Seven"].notnull(), 0, 1)
dff3["FN"] = np.where(dff3["Truth_Data"].notnull() &
    dff3["Strelka_Seven"].isnull(), 0, 1)

# Selecting the columns
dff21 = dff3.loc[dff3['TP'] == 0]
dff22 = dff3.loc[dff3['TN'] == 0]
dff23 = dff3.loc[dff3['FP'] == 0]
dff24 = dff3.loc[dff3['FN'] == 0]

# Total numbers of Comparisons
dff25 = len(dff21)
dff26 = len(dff22)
dff27 = len(dff23)
dff28 = len(dff24)

print("Total number of True Positives:")
print(dff25)
print("Total number of True Negatives:")
print(dff26)
print("Total number of False Postivies:")
print(dff27)
print("Total number of False Negatives:")
print(dff28)

# Merging columns based on "Positions"
dff29 = pd.merge(df1, df2, on=['POS'])
dff30 = pd.merge(df1, df3, on=['POS'])
dff31 = pd.merge(df1, df4, on=['POS'])
dff29.columns = ['POS', 'Truth_Data', 'Strelka_Three']
dff30.columns = ['POS', 'Truth_Data', 'Strelka_Five']
dff31.columns = ['POS', 'Truth_Data', 'Strelka_Seven']
print(dff29)
print(dff30)
print(dff31)

# Comparison column
dff29["Comparison"] = np.where((dff29["Truth_Data"] ==
    dff29["Strelka_Three"]), 0, 1)
dff30["Comparison"] = np.where((dff30["Truth_Data"] ==
    dff30["Strelka_Five"]), 0, 1)
dff31["Comparison"] = np.where((dff31["Truth_Data"] ==
    dff31["Strelka_Seven"]), 0, 1)

# Selecting Positive Comparison
dff35 = dff29.loc[dff29['Comparison'] == 0]
dff36 = dff30.loc[dff30['Comparison'] == 0]
dff37 = dff31.loc[dff31['Comparison'] == 0]

# Total number of Positive Comparison
dff38 = len(dff35)
dff39 = len(dff36)
dff40 = len(dff37)

# Selecting Negative Comparison
dff41 = dff29.loc[dff29['Comparison'] == 1]
dff42 = dff30.loc[dff30['Comparison'] == 1]
dff43 = dff31.loc[dff31['Comparison'] == 1]

# Total number of Negative Comparison
dff44 = len(dff41)
dff45 = len(dff42)
dff46 = len(dff43)

# Delcaring a new dataframe.
df = pd.DataFrame()

# Taking all combinations as a list.
Type = ['Strelka_0.3', 'Strelka_0.5', 'Strelka_0.7']
Total = [dff4, dff47, dff48]
True_Positive = [dff9, dff17, dff25]
True_Negative = [dff10, dff18, dff26]
False_Positives = [dff11, dff19, dff27]
```

```
False_Negatives = [dff12, dff20, dff28]
True_True_Postive = [dff38, dff39, dff40]
True_False_Postive = [dff44, dff45, dff46]

# Adding columns
df['Type'] = Type
df['Total'] = Total
df['True_Positives_ALTs'] = True_Positive
df['True_True_Positives_ALTs'] = True_True_Postive
df['True_False_Positives_ALTs'] = True_False_Postive
df['True_Negatives_ALTs'] = True_Negative
df['False_Positives_ALTs'] = False_Positives
df['False_Negatives_ALTs'] = False_Negatives

# Collecting it into a dataframe.
print(df)

# Saving the results in csv.
df.to_csv('Strelka_Benchmarking.csv', sep=',', index = False)
```

VarScan Benchmarking

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import csv

# Reading csv files and concatinating "CHROM" and "POS"
df1 = pd.read_csv("Updated_Truth.vcf", sep = '\t', index_col=
    False)
df2 = pd.read_csv("Updated_VarScan_0.3.vcf", sep = '\t',
    index_col= False)
```

```
df3 = pd.read_csv("Updated_VarScan_0.5.vcf", sep = '\t',
    index_col= False)
df4 = pd.read_csv("Updated_VarScan_0.7.vcf", sep = '\t',
    index_col= False)

# Merging columns based on "Positions"
dff1 = pd.merge(df1, df2, how="outer", on=['POS'])
dff2 = pd.merge(df1, df3, how="outer", on=['POS'])
dff3 = pd.merge(df1, df4, how="outer", on=['POS'])

# Renaming the columns after importing the input.
dff1.columns = ['POS', 'Truth_Data', 'VarScan_Three']
dff2.columns = ['POS', 'Truth_Data', 'VarScan_Five']
dff3.columns = ['POS', 'Truth_Data', 'VarScan_Seven']
print(dff1)
print(dff2)
print(dff3)

# Total number of reads
dff4 = len(dff1)
print('Total lenght is')
print(dff4)

dff47 = len(dff2)
print('Total lenght is')
print(dff47)

dff48 = len(dff3)
print('Total lenght is')
print(dff48)

# Comparison column
dff1["TP"] = np.where(dff1["Truth_Data"].notnull() &
    dff1["VarScan_Three"].notnull(), 0, 1)
dff1["TN"] = np.where(dff1["Truth_Data"].isnull() &
    dff1["VarScan_Three"].isnull(), 0, 1)
dff1["FP"] = np.where(dff1["Truth_Data"].isnull() &
    dff1["VarScan_Three"].notnull(), 0, 1)
dff1["FN"] = np.where(dff1["Truth_Data"].notnull() &
    dff1["VarScan_Three"].isnull(), 0, 1)
```

```
# Selecting the columns
dff5 = dff1.loc[dff1['TP'] == 0]
dff6 = dff1.loc[dff1['TN'] == 0]
dff7 = dff1.loc[dff1['FP'] == 0]
dff8 = dff1.loc[dff1['FN'] == 0]

# Total numbers of Comparisons
dff9 = len(dff5)
dff10 = len(dff6)
dff11 = len(dff7)
dff12 = len(dff8)

print("Total number of True Positives:")
print(dff9)
print("Total number of True Negatives:")
print(dff10)
print("Total number of False Postivies:")
print(dff11)
print("Total number of False Negatives:")
print(dff12)

# Comparison column
dff2["TP"] = np.where(dff2["Truth_Data"].notnull() &
   dff2["VarScan_Five"].notnull(), 0, 1)
dff2["TN"] = np.where(dff2["Truth_Data"].isnull() &
   dff2["VarScan_Five"].isnull(), 0, 1)
dff2["FP"] = np.where(dff2["Truth_Data"].isnull() &
   dff2["VarScan_Five"].notnull(), 0, 1)
dff2["FN"] = np.where(dff2["Truth_Data"].notnull() &
   dff2["VarScan_Five"].isnull(), 0, 1)

# Selecting the columns
dff13 = dff2.loc[dff2['TP'] == 0]
dff14 = dff2.loc[dff2['TN'] == 0]
dff15 = dff2.loc[dff2['FP'] == 0]
dff16 = dff2.loc[dff2['FN'] == 0]

# Total numbers of Comparisons
dff17 = len(dff13)
```

```
dff18 = len(dff14)
dff19 = len(dff15)
dff20 = len(dff16)

print("Total number of True Positives:")
print(dff17)
print("Total number of True Negatives:")
print(dff18)
print("Total number of False Postivies:")
print(dff19)
print("Total number of False Negatives:")
print(dff20)

# Comparison column
dff3["TP"] = np.where(dff3["Truth_Data"].notnull() &
   dff3["VarScan_Seven"].notnull(), 0, 1)
dff3["TN"] = np.where(dff3["Truth_Data"].isnull() &
   dff3["VarScan_Seven"].isnull(), 0, 1)
dff3["FP"] = np.where(dff3["Truth_Data"].isnull() &
   dff3["VarScan_Seven"].notnull(), 0, 1)
dff3["FN"] = np.where(dff3["Truth_Data"].notnull() &
   dff3["VarScan_Seven"].isnull(), 0, 1)

# Selecting the columns
dff21 = dff3.loc[dff3['TP'] == 0]
dff22 = dff3.loc[dff3['TN'] == 0]
dff23 = dff3.loc[dff3['FP'] == 0]
dff24 = dff3.loc[dff3['FN'] == 0]

# Total numbers of Comparisons
dff25 = len(dff21)
dff26 = len(dff22)
dff27 = len(dff23)
dff28 = len(dff24)

print("Total number of True Positives:")
print(dff25)
print("Total number of True Negatives:")
print(dff26)
print("Total number of False Postivies:")
print(dff27)
print("Total number of False Negatives:")
print(dff28)

# Merging columns based on "Positions"
dff29 = pd.merge(df1, df2, on=['POS'])
dff30 = pd.merge(df1, df3, on=['POS'])
dff31 = pd.merge(df1, df4, on=['POS'])
dff29.columns = ['POS', 'Truth_Data', 'VarScan_Three']
dff30.columns = ['POS', 'Truth_Data', 'VarScan_Five']
dff31.columns = ['POS', 'Truth_Data', 'VarScan_Seven']
print(dff29)
print(dff30)
print(dff31)

# Comparison column
dff29["Comparison"] = np.where((dff29["Truth_Data"] ==
   dff29["VarScan_Three"]), 0, 1)
dff30["Comparison"] = np.where((dff30["Truth_Data"] ==
   dff30["VarScan_Five"]), 0, 1)
dff31["Comparison"] = np.where((dff31["Truth_Data"] ==
   dff31["VarScan_Seven"]), 0, 1)

# Selecting Positive Comparison
dff35 = dff29.loc[dff29['Comparison'] == 0]
dff36 = dff30.loc[dff30['Comparison'] == 0]
dff37 = dff31.loc[dff31['Comparison'] == 0]

# Total number of Positive Comparison
dff38 = len(dff35)
dff39 = len(dff36)
dff40 = len(dff37)

# Selecting Negative Comparison
dff41 = dff29.loc[dff29['Comparison'] == 1]
dff42 = dff30.loc[dff30['Comparison'] == 1]
dff43 = dff31.loc[dff31['Comparison'] == 1]

# Total number of Negative Comparison
dff44 = len(dff41)
```

```
dff45 = len(dff42)
dff46 = len(dff43)

# Delcaring a new dataframe.
df = pd.DataFrame()

# Taking all combinations as a list.
Type = ['VarScan_0.3', 'VarScan_0.5', 'VarScan_0.7']
Total = [dff4, dff47, dff48]
True_Positive = [dff9, dff17, dff25]
True_Negative = [dff10, dff18, dff26]
False_Positives = [dff11, dff19, dff27]
False_Negatives = [dff12, dff20, dff28]
True_True_Postive = [dff38, dff39, dff40]
True_False_Postive = [dff44, dff45, dff46]

# Adding columns
df['Type'] = Type
df['Total'] = Total
df['True_Positives_ALTs'] = True_Positive
df['True_True_Positives_ALTs'] = True_True_Postive
df['True_False_Positives_ALTs'] = True_False_Postive
df['True_Negatives_ALTs'] = True_Negative
df['False_Positives_ALTs'] = False_Positives
df['False_Negatives_ALTs'] = False_Negatives

# Collecting it into a dataframe.
print(df)

# Saving the results in csv.
df.to_csv('VarScan_Benchmarking.csv', sep=',', index = False)
```

Appendix D

Strelka Positions, SNPs, Indels

```
# Importing packages.
import numpy as np
import pandas as pd

# Inputing vcf files for positions.
df = pd.read_csv("Updated_Strelka_0.3.vcf", sep = '\t', index_col=
    False)
df1 = pd.read_csv("Updated_Strelka_0.5.vcf", sep = '\t',
    index_col= False)
df2 = pd.read_csv("Updated_Strelka_0.7.vcf", sep = '\t',
    index_col= False)

# Inputing vcf files for SNPs.
df3 = pd.read_csv("Updated_Strelka_0.3_SNPs.vcf", sep = '\t',
    index_col= False)
df4 = pd.read_csv("Updated_Strelka_0.5_SNPs.vcf", sep = '\t',
    index_col= False)
df5 = pd.read_csv("Updated_Strelka_0.7_SNPs.vcf", sep = '\t',
    index_col= False)

# Inputing vcf files for Indels.
df6 = pd.read_csv("Updated_Strelka_0.3_Indels.vcf", sep = '\t',
    index_col= False)
df7 = pd.read_csv("Updated_Strelka_0.5_Indels.vcf", sep = '\t',
    index_col= False)
df8 = pd.read_csv("Updated_Strelka_0.7_Indels.vcf", sep = '\t',
    index_col= False)
```

```
# Outcome for positions.
Strelka3_Positions = len(df)
Strelka5_Positions = len(df1)
Strelka7_Positions = len(df2)

print("Number of positions in Strelka 0.3:")
print(Strelka3_Positions)
print("Number of positions in Strelka 0.5:")
print(Strelka5_Positions)
print("Number of positions in Strelka 0.7:")
print(Strelka7_Positions)

# Outcome for SNPs.
Strelka3_SNPs = len(df3)
Strelka5_SNPs = len(df4)
Strelka7_SNPs = len(df5)

print("Number of SNPs in Strelka 0.3:")
print(Strelka3_SNPs)
print("Number of SNPs in Strelka 0.5:")
print(Strelka5_SNPs)
print("Number of SNPs in Strelka 0.7:")
print(Strelka7_SNPs)

# Outcome for SNPs.
Strelka3_Indels = len(df6)
Strelka5_Indels = len(df7)
Strelka7_Indels = len(df8)

print("Number of Indels in Strelka 0.3:")
print(Strelka3_Indels)
print("Number of Indels in Strelka 0.5:")
print(Strelka5_Indels)
print("Number of Indels in Strelka 0.7:")
print(Strelka7_Indels)

# Delcaring a new dataframe.
df = []
```

```
# Taking all combinations as a list.
data = {'Type': ['Strelka_Tumor_Purity_0.3',
    'Strelka_Tumor_Purity_0.5', 'Strelka_Tumor_Purity_0.7'],
    'Strelka_Positions': [Strelka3_Positions, Strelka5_Positions,
    Strelka7_Positions], 'Strelka_SNPs': [Strelka3_SNPs,
    Strelka5_SNPs, Strelka7_SNPs], 'Strelka_INDELs':
    [Strelka3_Indels, Strelka5_Indels, Strelka7_Indels]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
print(df)

# Saving the results in csv.
df.to_csv('Strelka_Counts.csv', sep=',', index = None)
```

Truth Data Positions, SNPs, Indels

```
# Importing packages.
import numpy as np
import pandas as pd

# Inputing vcf files for positions.
df1 = pd.read_csv("Updated_Somatic_Truth.vcf", sep = '\t',
    index_col= False)

# Inputing vcf files for SNPs.
df2 = pd.read_csv("Updated_Somatic_Truth_SNPs.vcf", sep = '\t',
    index_col= False)

# Inputing vcf files for Indels.
df3 = pd.read_csv("Updated_Somatic_Truth_Indels.vcf", sep = '\t',
    index_col= False)

# Outcome for positions.
Truth_Positions = len(df1)

print("Number of positions in Somatic Truth:")
print(Truth_Positions)
```

```
# Outcome for SNPs.
Truth_SNPs = len(df2)

print("Number of SNPs in Somatic Truth:")
print(Truth_SNPs)

# Outcome for SNPs.
Truth_Indels = len(df3)

print("Number of Indels in Somatic Truth:")
print(Truth_Indels)

# Delcaring a new dataframe.
df = []

# Taking all combinations as a list.
data = {'Type': ['Somatic_Truth'], 'Truth_Positions':
    [Truth_Positions], 'Truth_SNPs': [Truth_SNPs], 'Truth_INDELs':
    [Truth_Indels]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
print(df)

# Saving the results in csv.
df.to_csv('Truth_Counts.csv', sep=',', index = None)
```

VarScan Positions, SNPs, Indels

```
# Importing packages.
import numpy as np
import pandas as pd

# Inputing vcf files for positions.
df = pd.read_csv("Updated_VarScan_0.3.vcf", sep = '\t', index_col=
    False)
```

```
df1 = pd.read_csv("Updated_VarScan_0.5.vcf", sep = '\t',
    index_col= False)
df2 = pd.read_csv("Updated_VarScan_0.7.vcf", sep = '\t',
    index_col= False)

# Inputing vcf files for SNPs.
df3 = pd.read_csv("Updated_VarScan_0.3_SNPs.vcf", sep = '\t',
    index_col= False)
df4 = pd.read_csv("Updated_VarScan_0.5_SNPs.vcf", sep = '\t',
    index_col= False)
df5 = pd.read_csv("Updated_VarScan_0.7_SNPs.vcf", sep = '\t',
    index_col= False)

# Inputing vcf files for Indels.
df6 = pd.read_csv("Updated_VarScan_0.3_Indels.vcf", sep = '\t',
    index_col= False)
df7 = pd.read_csv("Updated_VarScan_0.5_Indels.vcf", sep = '\t',
    index_col= False)
df8 = pd.read_csv("Updated_VarScan_0.7_Indels.vcf", sep = '\t',
    index_col= False)

# Outcome for positions.
VarScan3_Positions = len(df)
VarScan5_Positions = len(df1)
VarScan7_Positions = len(df2)

print("Number of positions in VarScan 0.3:")
print(VarScan3_Positions)
print("Number of positions in VarScan 0.5:")
print(VarScan5_Positions)
print("Number of positions in VarScan 0.7:")
print(VarScan7_Positions)

# Outcome for SNPs.
VarScan3_SNPs = len(df3)
VarScan5_SNPs = len(df4)
VarScan7_SNPs = len(df5)

print("Number of SNPs in VarScan 0.3:")
print(VarScan3_SNPs)
print("Number of SNPs in VarScan 0.5:")
print(VarScan5_SNPs)
print("Number of SNPs in VarScan 0.7:")
print(VarScan7_SNPs)

# Outcome for SNPs.
VarScan3_Indels = len(df6)
VarScan5_Indels = len(df7)
VarScan7_Indels = len(df8)

print("Number of Indels in VarScan 0.3:")
print(VarScan3_Indels)
print("Number of Indels in VarScan 0.5:")
print(VarScan5_Indels)
print("Number of Indels in VarScan 0.7:")
print(VarScan7_Indels)

# Delcaring a new dataframe.
df = []

# Taking all combinations as a list.
data = {'Type': ['VarScan_Tumor_Purity_0.3',
    'VarScan_Tumor_Purity_0.5', 'VarScan_Tumor_Purity_0.7'],
    'VarScan_Positions': [VarScan3_Positions, VarScan5_Positions,
    VarScan7_Positions], 'VarScan_SNPs': [VarScan3_SNPs,
    VarScan5_SNPs, VarScan7_SNPs], 'VarScan_INDELs':
    [VarScan3_Indels, VarScan5_Indels, VarScan7_Indels]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
print(df)

# Saving the results in csv.
df.to_csv('VarScan_Counts.csv', sep=',', index = None)
```

Positions Comparison

```
# Importing packages.
```

```
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import pyplot as plt
from matplotlib_venn import venn3_circles, venn3_unweighted
from matplotlib_venn import _common, _venn3
from matplotlib.patches import Circle
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'

# Reading csv files and concatinating "CHROM" and "POS"
df = pd.read_csv("Updated_Strelka_0.3.vcf", sep = '\t', index_col=
    False)
df1 = pd.read_csv("Updated_VarScan_0.3.vcf", sep = '\t',
    index_col= False)
df2 = pd.read_csv("Updated_Somatic_Truth.vcf", sep = '\t',
    index_col= False)

# Merging columns based on "POS"
First = pd.merge(df, df1, on=['POS'])
Second = pd.merge(df1, df2, on=['POS'])
Third = pd.merge(df, df2, on=['POS'])
Fourth = pd.merge(First, df2, on=['POS'])

# Position outcomes.
print("Number of Strelka Positions:")
Strelka_Positions = len(df)
print(Strelka_Positions)

print("Number of VarScan Positions:")
VarScan_Positions = len(df1)
print(VarScan_Positions)

print("Number of Truth Data Positions:")
Truth_Positions = len(df2)
print(Truth_Positions)

print("Number of Positions in Strelka and VarScan:")
Strelka_VarScan_Positions = len(First)
```

```
print(Strelka_VarScan_Positions)

print("Number of Positions in VarScan and Truth Data:")
VarScan_Truth_Positions = len(Second)
print(VarScan_Truth_Positions)

print("Number of Positions in Truth Data and Strelka:")
Strelka_Truth_Positions = len(Third)
print(Strelka_Truth_Positions)

print("Number of Positions in Strelka, VarScan & Truth Data:")
Strelka_VarScan_Truth_Positions = len(Fourth)
print(Strelka_VarScan_Truth_Positions)

# Delcaring a new dataframe.
df3 = []

# Taking all combinations as a list.
data = {'Type': ['Strelka', 'VarScan', 'Truth_Data',
    'Strelka_and_VarScan', 'VarScan_and_Truth_Data',
    'Truth_Data_and_Strelka',
    'Strelka_and_VarScan_and_Truth_Data'], 'Positions':
    [Strelka_Positions, VarScan_Positions, Truth_Positions,
    Strelka_VarScan_Positions, VarScan_Truth_Positions,
    Strelka_Truth_Positions, Strelka_VarScan_Truth_Positions]}

# Collecting it into a dataframe.
df3 = pd.DataFrame(data)
print(df3)

# Saving the results in csv.
df3.to_csv('Positions_Count.csv', sep=',', index = None)

# Formulas
Strelka_Exclude = Strelka_Positions - (Strelka_VarScan_Positions +
    Strelka_Truth_Positions + Strelka_VarScan_Truth_Positions)
VarScan_Exclude = VarScan_Positions - (Strelka_VarScan_Positions +
    VarScan_Truth_Positions + Strelka_VarScan_Truth_Positions)
Truth_Exclude = Truth_Positions - (VarScan_Truth_Positions +
    Strelka_Truth_Positions + Strelka_VarScan_Truth_Positions)
```

```
# Set of values.
subsets = (Strelka_Exclude, VarScan_Exclude,
    Strelka_VarScan_Positions, Truth_Exclude,
    Strelka_Truth_Positions, VarScan_Truth_Positions,
    Strelka_VarScan_Truth_Positions)

# Adding venn diagram.
v = venn3_unweighted(subsets, set_labels = ('Strelka_0.3',
    'VarScan_0.3', 'Truth_Data'), set_colors=('red', 'orange',
    'skyblue'))
areas = (1, 1, 1, 1, 1, 1, 1)
centers, radii = _venn3.solve_venn3_circles(areas)

# Saving the values.
plt.title("Positions [SNPs + Indels]")
plt.show()
plt.savefig('Tumor_Purity_0.3_Positions_Plot.pdf')
plt.savefig('Tumor_Purity_0.3_Positions_Plot.png', dpi = 300)
```

SNPs Comparison

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
from matplotlib import pyplot as plt
from matplotlib_venn import venn3_circles, venn3_unweighted
from matplotlib_venn import _common, _venn3
from matplotlib.patches import Circle

# Reading csv files and concatinating "CHROM" and "POS"
df = pd.read_csv("Updated_Strelka_0.3_SNPs.vcf", sep = '\t',
    index_col= False)
```

```
df1 = pd.read_csv("Updated_VarScan_0.3_SNPs.vcf", sep = '\t',
    index_col= False)
df2 = pd.read_csv("Updated_Somatic_Truth_SNVs.vcf", sep = '\t',
    index_col= False)
print("Length of Strelka SNPs:")
Strelka_SNPs = len(df)
print(Strelka_SNPs)
print("Length of VarScan SNPs:")
VarScan_SNPs = len(df1)
print(VarScan_SNPs)
print("Length of Truth SNPs:")
Truth_SNPs = len(df2)
print(Truth_SNPs)

# Adding REF and ALT
df["REF_ALT"] = df["REF"] + df["ALT"]
df1["REF_ALT"] = df1["REF"] + df1["ALT"]
df2["REF_ALT"] = df2["REF"] + df2["ALT"]

# Merging columns based on "POS"
First = pd.merge(df, df1, on=['POS'])
Second = pd.merge(df1, df2, on=['POS'])
Third = pd.merge(df, df2, on=['POS'])
Fourth = pd.merge(First, df2, on=['POS'])

# Dropping of the unnecessary columns.
First = First.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y'], axis=1)
Second = Second.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y'], axis=1)
Third = Third.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y'], axis=1)
Fourth = Fourth.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y', 'REF',
    'ALT'], axis=1)
print(First)
print(Second)
print(Third)
print(Fourth)

# Conditional replacement.
First['Result'] = np.where(First["REF_ALT_x"] ==
    First["REF_ALT_y"], 0, 1)
First = First[First["Result"] == 0]
```

```
print(First)

Second['Result'] = np.where(Second["REF_ALT_x"] ==
    Second["REF_ALT_y"], 0, 1)
Second = Second[Second["Result"] == 0]
print(Second)

Third['Result'] = np.where(Third["REF_ALT_x"] ==
    Third["REF_ALT_y"], 0, 1)
Third = Third[Third["Result"] == 0]
print(Third)

Fourth['Result'] = np.where(((Fourth["REF_ALT_x"] ==
    Fourth["REF_ALT_y"]) & (Fourth["REF_ALT_x"] ==
    Fourth["REF_ALT"]) & (Fourth["REF_ALT_y"] ==
    Fourth["REF_ALT"])), 0, 1)
Fourth = Fourth[Fourth["Result"] == 0]
print(Fourth)

# Position outcomes.
print("Number of SNPs in Strelka and VarScan:")
Strelka_VarScan_SNPs = len(First)
print(Strelka_VarScan_SNPs)

print("Number of SNPs in VarScan and Truth Data:")
VarScan_Truth_SNPs = len(Second)
print(VarScan_Truth_SNPs)

print("Number of SNPs in Truth Data and Strelka:")
Strelka_Truth_SNPs = len(Third)
print(Strelka_Truth_SNPs)

print("Number of SNPs in Strelka, VarScan & Truth Data:")
Strelka_VarScan_Truth_SNPs = len(Fourth)
print(Strelka_VarScan_Truth_SNPs)

# Delcaring a new dataframe.
df3 = []

# Taking all combinations as a list.
```

```
data = {'Type': ['Strelka', 'VarScan', 'Truth_Data',
    'Strelka_and_VarScan', 'VarScan_and_Truth_Data',
    'Truth_Data_and_Strelka',
    'Strelka_and_VarScan_and_Truth_Data'], 'SNPs': [Strelka_SNPs,
    VarScan_SNPs, Truth_SNPs, Strelka_VarScan_SNPs,
    VarScan_Truth_SNPs, Strelka_Truth_SNPs,
    Strelka_VarScan_Truth_SNPs]}

# Collecting it into a dataframe.
df3 = pd.DataFrame(data)
print(df3)

# Saving the results in csv.
df3.to_csv('SNPs_Count.csv', sep=',', index = None)

# Formulas
Strelka_Exclude = Strelka_SNPs - Strelka_VarScan_SNPs -
    Strelka_Truth_SNPs - Strelka_VarScan_Truth_SNPs
VarScan_Exclude = VarScan_SNPs - Strelka_VarScan_SNPs -
    VarScan_Truth_SNPs - Strelka_VarScan_Truth_SNPs
Truth_Exclude = Truth_SNPs - VarScan_Truth_SNPs -
    Strelka_Truth_SNPs - Strelka_VarScan_Truth_SNPs

# Set of values.
subsets = (Strelka_Exclude, VarScan_Exclude, Strelka_VarScan_SNPs,
    Truth_Exclude, Strelka_Truth_SNPs, VarScan_Truth_SNPs,
    Strelka_VarScan_Truth_SNPs)

# Adding venn diagram.
v = venn3_unweighted(subsets, set_labels = ('Strelka_0.3',
    'VarScan_0.3', 'Truth_Data'), set_colors=('red', 'orange',
    'skyblue'))
areas = (1, 1, 1, 1, 1, 1, 1)
centers, radii = _venn3.solve_venn3_circles(areas)

# Saving the values.
plt.title("SNPs")
plt.show()
plt.savefig('Tumor_Purity_0.3_SNPs_Plot.pdf')
plt.savefig('Tumor_Purity_0.3_SNPs_Plot.png', dpi = 300)
```

Indels Comparison

```
# Importing packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
from matplotlib import pyplot as plt
from matplotlib_venn import venn3_circles, venn3_unweighted
from matplotlib_venn import _common, _venn3
from matplotlib.patches import Circle

# Reading csv files and concatinating "CHROM" and "POS"
df = pd.read_csv("Updated_Strelka_0.3_Indels.vcf", sep = '\t',
    index_col= False)
df1 = pd.read_csv("Updated_VarScan_0.3_Indels.vcf", sep = '\t',
    index_col= False)
df2 = pd.read_csv("Updated_Somatic_Truth_Indels.vcf", sep = '\t',
    index_col= False)
print("Length of Strelka Indels:")
Strelka_Indels = len(df)
print(Strelka_Indels)
print("Length of VarScan Indels:")
VarScan_Indels = len(df1)
print(VarScan_Indels)
print("Length of Truth Indels:")
Truth_Indels = len(df2)
print(Truth_Indels)

# Adding REF and ALT
df["REF_ALT"] = df["REF"] + df["ALT"]
df1["REF_ALT"] = df1["REF"] + df1["ALT"]
df2["REF_ALT"] = df2["REF"] + df2["ALT"]

# Merging columns based on "POS"
First = pd.merge(df, df1, on=['POS'])
Second = pd.merge(df1, df2, on=['POS'])
```

```
Third = pd.merge(df, df2, on=['POS'])
Fourth = pd.merge(First, df2, on=['POS'])

# Dropping of the unnecessary columns.
First = First.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y'], axis=1)
Second = Second.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y'], axis=1)
Third = Third.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y'], axis=1)
Fourth = Fourth.drop(['REF_x', 'ALT_x', 'REF_y', 'ALT_y', 'REF',
    'ALT'], axis=1)
print(First)
print(Second)
print(Third)
print(Fourth)

# Conditional replacement.
First['Result'] = np.where(First["REF_ALT_x"] ==
    First["REF_ALT_y"], 0, 1)
First = First[First["Result"] == 0]
print(First)

Second['Result'] = np.where(Second["REF_ALT_x"] ==
    Second["REF_ALT_y"], 0, 1)
Second = Second[Second["Result"] == 0]
print(Second)

Third['Result'] = np.where(Third["REF_ALT_x"] ==
    Third["REF_ALT_y"], 0, 1)
Third = Third[Third["Result"] == 0]
print(Third)

Fourth['Result'] = np.where(((Fourth["REF_ALT_x"] ==
    Fourth["REF_ALT_y"]) & (Fourth["REF_ALT_x"] ==
    Fourth["REF_ALT"]) & (Fourth["REF_ALT_y"] ==
    Fourth["REF_ALT"])), 0, 1)
Fourth = Fourth[Fourth["Result"] == 0]
print(Fourth)

# Position outcomes.
print("Number of Indels in Strelka and VarScan:")
Strelka_VarScan_Indels = len(First)
```

```
print(Strelka_VarScan_Indels)

print("Number of Indels in VarScan and Truth Data:")
VarScan_Truth_Indels = len(Second)
print(VarScan_Truth_Indels)

print("Number of SNPs in Truth Data and Strelka:")
Strelka_Truth_Indels = len(Third)
print(Strelka_Truth_Indels)

print("Number of SNPs in Strelka, VarScan & Truth Data:")
Strelka_VarScan_Truth_Indels = len(Fourth)
print(Strelka_VarScan_Truth_Indels)

# Delcaring a new dataframe.
df3 = []

# Taking all combinations as a list.
data = {'Type': ['Strelka', 'VarScan', 'Truth_Data',
    'Strelka_and_VarScan', 'VarScan_and_Truth_Data',
    'Truth_Data_and_Strelka',
    'Strelka_and_VarScan_and_Truth_Data'], 'INDELs':
    [Strelka_Indels, VarScan_Indels, Truth_Indels,
    Strelka_VarScan_Indels, VarScan_Truth_Indels,
    Strelka_Truth_Indels, Strelka_VarScan_Truth_Indels]}

# Collecting it into a dataframe.
df3 = pd.DataFrame(data)
print(df3)

# Saving the results in csv.
df3.to_csv('Indels_Count.csv', sep=',', index = None)

# Formulas
Strelka_Exclude = Strelka_Indels - Strelka_VarScan_Indels -
    Strelka_Truth_Indels - Strelka_VarScan_Truth_Indels
VarScan_Exclude = VarScan_Indels - Strelka_VarScan_Indels -
    VarScan_Truth_Indels - Strelka_VarScan_Truth_Indels
Truth_Exclude = Truth_Indels - VarScan_Truth_Indels -
    Strelka_Truth_Indels - Strelka_VarScan_Truth_Indels
```

```
# Set of values.
subsets = (Strelka_Exclude, VarScan_Exclude,
    Strelka_VarScan_Indels, Truth_Exclude, Strelka_Truth_Indels,
    VarScan_Truth_Indels, Strelka_VarScan_Truth_Indels)

# Adding venn diagram.
v = venn3_unweighted(subsets, set_labels = ('Strelka_0.3',
    'VarScan_0.3', 'Truth_Data'), set_colors=('red', 'orange',
    'skyblue'))
areas = (1, 1, 1, 1, 1, 1, 1)
centers, radii = _venn3.solve_venn3_circles(areas)

# Saving the values.
plt.title("Indels")
plt.show()
plt.savefig('Tumor_Purity_0.3_Indels_Plot.pdf')
plt.savefig('Tumor_Purity_0.3_Indels_Plot.png', dpi = 300)
```

Appendix E

Strelka Read Depth

```
# Importing the needed packages.
import numpy as np
import pandas as pd

# Reading the csv input file that is obtained after performing the
    following operations on the vcf file.
# Step 1 - 'cut -f 1-2, 9-11 Input-VCF-File > Output-VCF-File'
# Step 2 - 'sed '/^#/d' Output-VCF-File > Updated_Output-VCF-File'

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Updated_Strelka_0.3.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Updated_Strelka_0.5.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Updated_Strelka_0.7.vcf", sep = '\t',
    index_col= False)

# Naming the columns after importing the csv file.
dff.columns = ['CHROM', 'POS', 'NORMAL', 'TUMOR', '2:NORMAL',
    '2:TUMOR']
dff1.columns = ['CHROM', 'POS', 'NORMAL', 'TUMOR', '2:NORMAL',
    '2:TUMOR']
dff2.columns = ['CHROM', 'POS', 'NORMAL', 'TUMOR', '2:NORMAL',
    '2:TUMOR']

# Concatinating the "CHROM" and "POS"
```

```
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff2["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

dff1 = dff1.drop(['CHROM', 'POS'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]
print(dff1)

dff2 = dff2.drop(['CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]
print(dff2)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "DP:FDP:SDP:SUBDP:AU:CU:GU:TU"
dff[['Normal_Read_Depth', 'NORMAL-FDP', 'NORMAL-SDP',
    'NORMAL-SUBDP', 'NORMAL-AU', 'NORMAL-CU', 'NORMAL-GU',
    'NORMAL-TU', 'NORMAL-Last']] =
    dff['NORMAL'].str.split(':',expand=True)
dff[['Tumor_Read_Depth', 'TUMOR-FDP', 'TUMOR-SDP', 'TUMOR-SUBDP',
    'TUMOR-AU', 'TUMOR-CU', 'TUMOR-GU', 'TUMOR-TU', 'TUMOR-Last']]
    = dff['TUMOR'].str.split(':',expand=True)
dff[['2:Normal_Read_Depth', 'NORMAL1-FDP', 'NORMAL1-SDP',
    'NORMAL1-SUBDP', 'NORMAL1-AU', 'NORMAL1-CU', 'NORMAL1-GU',
    'NORMAL1-TU', 'NORMAL1-Last']] =
    dff['2:NORMAL'].str.split(':',expand=True)
```

```
dff[['2:Tumor_Read_Depth', 'TUMOR1-FDP', 'TUMOR1-SDP',
    'TUMOR1-SUBDP', 'TUMOR1-AU', 'TUMOR1-CU', 'TUMOR1-GU',
    'TUMOR1-TU', 'TUMOR1-Last']] =
    dff['2:TUMOR'].str.split(':',expand=True)

dff1[['Normal_Read_Depth', 'NORMAL-FDP', 'NORMAL-SDP',
    'NORMAL-SUBDP', 'NORMAL-AU', 'NORMAL-CU', 'NORMAL-GU',
    'NORMAL-TU', 'NORMAL-Last']] =
    dff1['NORMAL'].str.split(':',expand=True)
dff1[['Tumor_Read_Depth', 'TUMOR-FDP', 'TUMOR-SDP', 'TUMOR-SUBDP',
    'TUMOR-AU', 'TUMOR-CU', 'TUMOR-GU', 'TUMOR-TU', 'TUMOR-Last']]
    = dff1['TUMOR'].str.split(':',expand=True)
dff1[['2:Normal_Read_Depth', 'NORMAL1-FDP', 'NORMAL1-SDP',
    'NORMAL1-SUBDP', 'NORMAL1-AU', 'NORMAL1-CU', 'NORMAL1-GU',
    'NORMAL1-TU', 'NORMAL1-Last']] =
    dff1['2:NORMAL'].str.split(':',expand=True)
dff1[['2:Tumor_Read_Depth', 'TUMOR1-FDP', 'TUMOR1-SDP',
    'TUMOR1-SUBDP', 'TUMOR1-AU', 'TUMOR1-CU', 'TUMOR1-GU',
    'TUMOR1-TU', 'TUMOR1-Last']] =
    dff1['2:TUMOR'].str.split(':',expand=True)

dff2[['Normal_Read_Depth', 'NORMAL-FDP', 'NORMAL-SDP',
    'NORMAL-SUBDP', 'NORMAL-AU', 'NORMAL-CU', 'NORMAL-GU',
    'NORMAL-TU', 'NORMAL-Last']] =
    dff2['NORMAL'].str.split(':',expand=True)
dff2[['Tumor_Read_Depth', 'TUMOR-FDP', 'TUMOR-SDP', 'TUMOR-SUBDP',
    'TUMOR-AU', 'TUMOR-CU', 'TUMOR-GU', 'TUMOR-TU', 'TUMOR-Last']]
    = dff2['TUMOR'].str.split(':',expand=True)
dff2[['2:Normal_Read_Depth', 'NORMAL1-FDP', 'NORMAL1-SDP',
    'NORMAL1-SUBDP', 'NORMAL1-AU', 'NORMAL1-CU', 'NORMAL1-GU',
    'NORMAL1-TU', 'NORMAL1-Last']] =
    dff2['2:NORMAL'].str.split(':',expand=True)
dff2[['2:Tumor_Read_Depth', 'TUMOR1-FDP', 'TUMOR1-SDP',
    'TUMOR1-SUBDP', 'TUMOR1-AU', 'TUMOR1-CU', 'TUMOR1-GU',
    'TUMOR1-TU', 'TUMOR1-Last']] =
    dff2['2:TUMOR'].str.split(':',expand=True)

# Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
```

```
dff = dff.drop(['NORMAL', 'TUMOR', '2:NORMAL', '2:TUMOR',
    'NORMAL-FDP', 'NORMAL-SDP', 'NORMAL-SUBDP', 'NORMAL-AU',
    'NORMAL-CU', 'NORMAL-GU', 'NORMAL-TU', 'NORMAL-Last',
    'TUMOR-FDP', 'TUMOR-SDP', 'TUMOR-SUBDP', 'TUMOR-AU',
    'TUMOR-CU', 'TUMOR-GU', 'TUMOR-TU', 'TUMOR-Last',
    'NORMAL1-FDP', 'NORMAL1-SDP', 'NORMAL1-SUBDP', 'NORMAL1-AU',
    'NORMAL1-CU', 'NORMAL1-GU', 'NORMAL1-TU', 'NORMAL1-Last',
    'TUMOR1-FDP', 'TUMOR1-SDP', 'TUMOR1-SUBDP', 'TUMOR1-AU',
    'TUMOR1-CU', 'TUMOR1-GU', 'TUMOR1-TU', 'TUMOR1-Last'], axis=1)

dff1 = dff1.drop(['NORMAL', 'TUMOR', '2:NORMAL', '2:TUMOR',
    'NORMAL-FDP', 'NORMAL-SDP', 'NORMAL-SUBDP', 'NORMAL-AU',
    'NORMAL-CU', 'NORMAL-GU', 'NORMAL-TU', 'NORMAL-Last',
    'TUMOR-FDP', 'TUMOR-SDP', 'TUMOR-SUBDP', 'TUMOR-AU',
    'TUMOR-CU', 'TUMOR-GU', 'TUMOR-TU', 'TUMOR-Last',
    'NORMAL1-FDP', 'NORMAL1-SDP', 'NORMAL1-SUBDP', 'NORMAL1-AU',
    'NORMAL1-CU', 'NORMAL1-GU', 'NORMAL1-TU', 'NORMAL1-Last',
    'TUMOR1-FDP', 'TUMOR1-SDP', 'TUMOR1-SUBDP', 'TUMOR1-AU',
    'TUMOR1-CU', 'TUMOR1-GU', 'TUMOR1-TU', 'TUMOR1-Last'], axis=1)

dff2 = dff2.drop(['NORMAL', 'TUMOR', '2:NORMAL', '2:TUMOR',
    'NORMAL-FDP', 'NORMAL-SDP', 'NORMAL-SUBDP', 'NORMAL-AU',
    'NORMAL-CU', 'NORMAL-GU', 'NORMAL-TU', 'NORMAL-Last',
    'TUMOR-FDP', 'TUMOR-SDP', 'TUMOR-SUBDP', 'TUMOR-AU',
    'TUMOR-CU', 'TUMOR-GU', 'TUMOR-TU', 'TUMOR-Last',
    'NORMAL1-FDP', 'NORMAL1-SDP', 'NORMAL1-SUBDP', 'NORMAL1-AU',
    'NORMAL1-CU', 'NORMAL1-GU', 'NORMAL1-TU', 'NORMAL1-Last',
    'TUMOR1-FDP', 'TUMOR1-SDP', 'TUMOR1-SUBDP', 'TUMOR1-AU',
    'TUMOR1-CU', 'TUMOR1-GU', 'TUMOR1-TU', 'TUMOR1-Last'], axis=1)

# Replacing '.' values with '0'
dff.replace('.', '0', inplace=True)
dff1.replace('.', '0', inplace=True)
dff2.replace('.', '0', inplace=True)

# Converting string values columans to int.
dff['Normal_Read_Depth'] = dff['Normal_Read_Depth'].astype(int)
dff['2:Normal_Read_Depth'] = dff['2:Normal_Read_Depth'].astype(int)
dff['Tumor_Read_Depth'] = dff['Tumor_Read_Depth'].astype(int)
dff['2:Tumor_Read_Depth'] = dff['2:Tumor_Read_Depth'].astype(int)
```

```
dff1['Normal_Read_Depth'] = dff1['Normal_Read_Depth'].astype(int)
dff1['2:Normal_Read_Depth'] =
    dff1['2:Normal_Read_Depth'].astype(int)
dff1['Tumor_Read_Depth'] = dff1['Tumor_Read_Depth'].astype(int)
dff1['2:Tumor_Read_Depth'] = dff1['2:Tumor_Read_Depth'].astype(int)

dff2['Normal_Read_Depth'] = dff2['Normal_Read_Depth'].astype(int)
dff2['2:Normal_Read_Depth'] =
    dff2['2:Normal_Read_Depth'].astype(int)
dff2['Tumor_Read_Depth'] = dff2['Tumor_Read_Depth'].astype(int)
dff2['2:Tumor_Read_Depth'] = dff2['2:Tumor_Read_Depth'].astype(int)

# Adding columns for single read depth value.
dff['Normal_RD'] = dff["Normal_Read_Depth"] +
    dff["2:Normal_Read_Depth"]
dff['Tumor_RD'] = dff["Tumor_Read_Depth"] +
    dff["2:Tumor_Read_Depth"]

dff1['Normal_RD'] = dff1["Normal_Read_Depth"] +
    dff1["2:Normal_Read_Depth"]
dff1['Tumor_RD'] = dff1["Tumor_Read_Depth"] +
    dff1["2:Tumor_Read_Depth"]

dff2['Normal_RD'] = dff2["Normal_Read_Depth"] +
    dff2["2:Normal_Read_Depth"]
dff2['Tumor_RD'] = dff2["Tumor_Read_Depth"] +
    dff2["2:Tumor_Read_Depth"]

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['Normal_Read_Depth', 'Tumor_Read_Depth',
    '2:Normal_Read_Depth', '2:Tumor_Read_Depth'], axis=1)
dff.columns = ['CHROM_POS', 'Normal_Read_Depth',
    'Tumor_Read_Depth']
print(dff)

dff1 = dff1.drop(['Normal_Read_Depth', 'Tumor_Read_Depth',
    '2:Normal_Read_Depth', '2:Tumor_Read_Depth'], axis=1)
dff1.columns = ['CHROM_POS', 'Normal_Read_Depth',
    'Tumor_Read_Depth']
```

```
print(dff1)

dff2 = dff2.drop(['Normal_Read_Depth', 'Tumor_Read_Depth',
    '2:Normal_Read_Depth', '2:Tumor_Read_Depth'], axis=1)
dff2.columns = ['CHROM_POS', 'Normal_Read_Depth',
    'Tumor_Read_Depth']
print(dff2)

# Merging columns based on "CHROM-POS"
First = pd.merge(dff, dff1, on=['CHROM_POS'])
Second = pd.merge(First, dff2, on=['CHROM_POS'])

# Renaming Columns
Second.columns = ['CHROM_POS', 'Strelka_Normal_0.3',
    'Strelka_Tumor_0.3', 'Strelka_Normal_0.5', 'Strelka_Tumor_0.5',
    'Strelka_Normal_0.7', 'Strelka_Tumor_0.7']
print(Second)

# Saving the results in csv.
Second.to_csv('Strelka_Read_Depth.csv', sep=',', index = None)

# Finding the minimum values
min1 = Second['Strelka_Normal_0.3'].min()
min2 = Second['Strelka_Tumor_0.3'].min()
min3 = Second['Strelka_Normal_0.5'].min()
min4 = Second['Strelka_Tumor_0.5'].min()
min5 = Second['Strelka_Normal_0.7'].min()
min6 = Second['Strelka_Tumor_0.7'].min()

# Finding the maximum values
max1 = Second['Strelka_Normal_0.3'].max()
max2 = Second['Strelka_Tumor_0.3'].max()
max3 = Second['Strelka_Normal_0.5'].max()
max4 = Second['Strelka_Tumor_0.5'].max()
max5 = Second['Strelka_Normal_0.7'].max()
max6 = Second['Strelka_Tumor_0.7'].max()

# Finding the mean values
mean1 = Second['Strelka_Normal_0.3'].mean()
mean2 = Second['Strelka_Tumor_0.3'].mean()
```

```
mean3 = Second['Strelka_Normal_0.5'].mean()
mean4 = Second['Strelka_Tumor_0.5'].mean()
mean5 = Second['Strelka_Normal_0.7'].mean()
mean6 = Second['Strelka_Tumor_0.7'].mean()

# Finding the minimum values
median1 = Second['Strelka_Normal_0.3'].median()
median2 = Second['Strelka_Tumor_0.3'].median()
median3 = Second['Strelka_Normal_0.5'].median()
median4 = Second['Strelka_Tumor_0.5'].median()
median5 = Second['Strelka_Normal_0.7'].median()
median6 = Second['Strelka_Tumor_0.7'].median()
print(median6)

# Finding the minimum values
mode1 = Second['Strelka_Normal_0.3'].mode()
mode2 = Second['Strelka_Tumor_0.3'].mode()
mode3 = Second['Strelka_Normal_0.5'].mode()
mode4 = Second['Strelka_Tumor_0.5'].mode()
mode5 = Second['Strelka_Normal_0.7'].mode()
mode6 = Second['Strelka_Tumor_0.7'].mode()
print(mode6)

# Delcaring a new dataframe.
df = pd.DataFrame()

# Taking all combinations as a list.
Type = ['Strelka_Normal_0.3', 'Strelka_Tumor_0.3',
    'Strelka_Normal_0.5', 'Strelka_Tumor_0.5',
    'Strelka_Normal_0.7', 'Strelka_Tumor_0.7']
Minimum_Value = [min1, min2, min3, min4, min5, min6]
Maximum_Value = [max1, max2, max3, max4, max5, max6]
Mean_Value = [mean1, mean2, mean3, mean4, mean5, mean6]
Median_Value = [median1, median2, median3, median4, median5,
    median6]
Mode_Value = [mode1, mode2, mode3, mode4, mode5, mode6]

# Adding columns
df['Type'] = Type
df['Minimum_Value'] = Minimum_Value
```

```
df['Maximum_Value'] = Maximum_Value
df['Mean_Value'] = Mean_Value
df['Median_Value'] = Median_Value
df['Mode_Value'] = Mode_Value
# Collecting it into a dataframe.
print(df)

# Saving the results in csv.
df.to_csv('Strelka_Read_Depth_Statistics.csv', sep=',', index =
    False)
```

Truth Data Read Depth

```
# Importing the needed packages.
import numpy as np
import pandas as pd

# Reading the csv input file that is obtained after performing the
    following operations on the vcf file.
# Step 1 - 'cut -f 1-2,9-10 Input.vcf > Output.vcf'
# Step 2 - 'sed '/^#/d' Output.vcf > Updated.vcf'

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Updated_Somatic_Truth.vcf", sep = '\t',
    index_col= False)

# Naming the columns after importing the csv file.
dff.columns = ['CHROM', 'POS', 'FORMAT', 'VALUE']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS', 'FORMAT'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
```

```
dff = dff[cols]
print(dff)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "GT:PS:DP:GQ".
dff[['VALUE-GT','VALUE-PS','Read_Depth', 'VALUE-GQ']] =
    dff['VALUE'].str.split(':',expand=True)

# Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
dff = dff.drop(['VALUE', 'VALUE-GT', 'VALUE-PS', 'VALUE-GQ'],
    axis=1)
print(dff)

# Saving the result into a csv file for plotting.
dff.to_csv('Truth_Data_Read_Depth.csv', sep=',', index = None)

# Finding the minimum values
min1 = dff['Read_Depth'].min()

# Finding the maximum values
max1 = dff['Read_Depth'].max()

# Finding the mean values
mean1 = dff['Read_Depth'].mean()

# Finding the minimum values
median1 = dff['Read_Depth'].median()

# Finding the minimum values
mode1 = dff['Read_Depth'].mode()

# Delcaring a new dataframe.
df = pd.DataFrame()

# Taking all combinations as a list.
Type = ['Truth_Data']
Minimum_Value = [min1]
```

```
Maximum_Value = [max1]
Mean_Value = [mean1]
Median_Value = [median1]
Mode_Value = [mode1]

# Adding columns
df['Type'] = Type
df['Minimum_Value'] = Minimum_Value
df['Maximum_Value'] = Maximum_Value
df['Mean_Value'] = Mean_Value
df['Median_Value'] = Median_Value
df['Mode_Value'] = Mode_Value
# Collecting it into a dataframe.
print(df)

# Saving the results in csv.
df.to_csv('Truth_Data_Read_Depth_Statistics.csv', sep=',', index =
    False)
```

VarScan Read Depth

```
# Importing the needed packages.
import numpy as np
import pandas as pd

# Reading the csv input file that is obtained after performing the
    following operations on the vcf file.
# Step 1 - 'cut -f 1-2,9-11 Input-VCF-File > Output-VCF-File'
# Step 2 - 'sed '/^#/d' Output-VCF-File > Updated_Output-VCF-File'

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Updated_VarScan_0.3.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Updated_VarScan_0.5.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Updated_VarScan_0.7.vcf", sep = '\t',
    index_col= False)
```

```
# Naming the columns after importing the csv file.
dff.columns = ['CHROM', 'POS', 'FORMAT', 'NORMAL', 'TUMOR']
dff1.columns = ['CHROM', 'POS', 'FORMAT', 'NORMAL', 'TUMOR']
dff2.columns = ['CHROM', 'POS', 'FORMAT', 'NORMAL', 'TUMOR']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff1['CHROM'].astype(str) + '-' +
    dff1['POS'].astype(str)
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS', 'FORMAT'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

dff1 = dff1.drop(['CHROM', 'POS', 'FORMAT'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]
print(dff1)

dff2 = dff2.drop(['CHROM', 'POS', 'FORMAT'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]
print(dff2)

# Creating new columns by splitting the "NORMAL" and "TUMOR"
    columns by ':' and renaming the new columns based on the format
    "GT:GQ:DP:AD:ADF:ADR".
dff[['NORMAL-GT','NORMAL-GQ','Normal_Read_Depth',
    'NORMAL-AD','NORMAL-ADF', 'NORMAL-ADR']] =
    dff['NORMAL'].str.split(':',expand=True)
```

```
dff[['TUMOR-GT','TUMOR-GQ','Tumor_Read_Depth',
    'TUMOR-AD','TUMOR-ADF', 'TUMOR-ADR']] =
    dff['TUMOR'].str.split(':',expand=True)

dff1[['NORMAL-GT','NORMAL-GQ','Normal_Read_Depth',
    'NORMAL-AD','NORMAL-ADF', 'NORMAL-ADR']] =
    dff1['NORMAL'].str.split(':',expand=True)
dff1[['TUMOR-GT','TUMOR-GQ','Tumor_Read_Depth',
    'TUMOR-AD','TUMOR-ADF', 'TUMOR-ADR']] =
    dff1['TUMOR'].str.split(':',expand=True)

dff2[['NORMAL-GT','NORMAL-GQ','Normal_Read_Depth',
    'NORMAL-AD','NORMAL-ADF', 'NORMAL-ADR']] =
    dff2['NORMAL'].str.split(':',expand=True)
dff2[['TUMOR-GT','TUMOR-GQ','Tumor_Read_Depth',
    'TUMOR-AD','TUMOR-ADF', 'TUMOR-ADR']] =
    dff2['TUMOR'].str.split(':',expand=True)

# Dropping of the unnecessary columns and only choosing the
    "NORMAL Depth" i.e. "NORMAL-DP" and "TUMOR Depth" i.e.
    "TUMOR-DP"
dff = dff.drop(['NORMAL', 'TUMOR', 'NORMAL-GT', 'NORMAL-GQ',
    'NORMAL-AD', 'NORMAL-ADF', 'NORMAL-ADR', 'TUMOR-GT',
    'TUMOR-GQ', 'TUMOR-AD', 'TUMOR-ADF', 'TUMOR-ADR'], axis=1)
print(dff)

dff1 = dff1.drop(['NORMAL', 'TUMOR', 'NORMAL-GT', 'NORMAL-GQ',
    'NORMAL-AD', 'NORMAL-ADF', 'NORMAL-ADR', 'TUMOR-GT',
    'TUMOR-GQ', 'TUMOR-AD', 'TUMOR-ADF', 'TUMOR-ADR'], axis=1)
print(dff1)

dff2 = dff2.drop(['NORMAL', 'TUMOR', 'NORMAL-GT', 'NORMAL-GQ',
    'NORMAL-AD', 'NORMAL-ADF', 'NORMAL-ADR', 'TUMOR-GT',
    'TUMOR-GQ', 'TUMOR-AD', 'TUMOR-ADF', 'TUMOR-ADR'], axis=1)
print(dff2)

# Merging columns based on "CHROM-POS"
First = pd.merge(dff, dff1, on=['CHROM_POS'])
Second = pd.merge(First, dff2, on=['CHROM_POS'])
```

```
# Renaming Columns
Second.columns = ['CHROM_POS', 'VarScan_Normal_0.3',
    'VarScan_Tumor_0.3', 'VarScan_Normal_0.5', 'VarScan_Tumor_0.5',
    'VarScan_Normal_0.7', 'VarScan_Tumor_0.7']
print(Second)

# Saving the results in csv.
Second.to_csv('VarScan_Read_Depth_Counts.csv', sep=',', index =
    None)

# Finding the minimum values
min1 = Second['VarScan_Normal_0.3'].min()
min2 = Second['VarScan_Tumor_0.3'].min()
min3 = Second['VarScan_Normal_0.5'].min()
min4 = Second['VarScan_Tumor_0.5'].min()
min5 = Second['VarScan_Normal_0.7'].min()
min6 = Second['VarScan_Tumor_0.7'].min()

# Finding the maximum values
max1 = Second['VarScan_Normal_0.3'].max()
max2 = Second['VarScan_Tumor_0.3'].max()
max3 = Second['VarScan_Normal_0.5'].max()
max4 = Second['VarScan_Tumor_0.5'].max()
max5 = Second['VarScan_Normal_0.7'].max()
max6 = Second['VarScan_Tumor_0.7'].max()

# Finding the mean values
mean1 = Second['VarScan_Normal_0.3'].mean()
mean2 = Second['VarScan_Tumor_0.3'].mean()
mean3 = Second['VarScan_Normal_0.5'].mean()
mean4 = Second['VarScan_Tumor_0.5'].mean()
mean5 = Second['VarScan_Normal_0.7'].mean()
mean6 = Second['VarScan_Tumor_0.7'].mean()

# Finding the minimum values
median1 = Second['VarScan_Normal_0.3'].median()
median2 = Second['VarScan_Tumor_0.3'].median()
median3 = Second['VarScan_Normal_0.5'].median()
median4 = Second['VarScan_Tumor_0.5'].median()
median5 = Second['VarScan_Normal_0.7'].median()
```

```
median6 = Second['VarScan_Tumor_0.7'].median()

# Finding the minimum values
mode1 = Second['VarScan_Normal_0.3'].mode()
mode2 = Second['VarScan_Tumor_0.3'].mode()
mode3 = Second['VarScan_Normal_0.5'].mode()
mode4 = Second['VarScan_Tumor_0.5'].mode()
mode5 = Second['VarScan_Normal_0.7'].mode()
mode6 = Second['VarScan_Tumor_0.7'].mode()

# Delcaring a new dataframe.
df = pd.DataFrame()

# Taking all combinations as a list.
Type = ['VarScan_Normal_0.3', 'VarScan_Tumor_0.3',
    'VarScan_Normal_0.5', 'VarScan_Tumor_0.5',
    'VarScan_Normal_0.7', 'VarScan_Tumor_0.7']
Minimum_Value = [min1, min2, min3, min4, min5, min6]
Maximum_Value = [max1, max2, max3, max4, max5, max6]
Mean_Value = [mean1, mean2, mean3, mean4, mean5, mean6]
Median_Value = [median1, median2, median3, median4, median5,
    median6]
Mode_Value = [mode1, mode2, mode3, mode4, mode5, mode6]

# Adding columns
df['Type'] = Type
df['Minimum_Value'] = Minimum_Value
df['Maximum_Value'] = Maximum_Value
df['Mean_Value'] = Mean_Value
df['Median_Value'] = Median_Value
df['Mode_Value'] = Mode_Value
# Collecting it into a dataframe.
print(df)

# Saving the results in csv.
df.to_csv('VarScan_Read_Depth_Statistics.csv', sep=',', index =
    False)
```

Appendix F

Strelka Variants

```
# Importing the needed packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import pandas as pd
import csv
import numpy as np

# Reading the csv input file that is obtained after performing the
    following operations on the vcf file.
# Step 1 - 'cut -f 1-2,4-5 Input.vcf > Output.vcf'
# Step 2 - 'sed '/^#/d' Output.vcf > Updated.vcf'

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Updated_Strelka_0.3_SNV.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Updated_Strelka_0.5_SNV.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Updated_Strelka_0.7_SNV.vcf", sep = '\t',
    index_col= False)

# Naming the columns after importing the csv file.
```

```
dff.columns = ['CHROM', 'POS', 'REF', 'ALT']
dff1.columns = ['CHROM', 'POS', 'REF', 'ALT']
dff2.columns = ['CHROM', 'POS', 'REF', 'ALT']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff1['CHROM'].astype(str) + '-' +
    dff1['POS'].astype(str)
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

dff1 = dff1.drop(['CHROM', 'POS'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]
print(dff1)

dff2 = dff2.drop(['CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]
print(dff2)

# Mentioning the column names and inputing the csv file.
dff.columns = ['CHROM_POS', 'REF', 'ALT']
dff1.columns = ['CHROM_POS', 'REF', 'ALT']
dff2.columns = ['CHROM_POS', 'REF', 'ALT']

# Printing the list.
dff4 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'A')]
dff5 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'A')]
dff6 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'A')]
```

```
AA1 = len(dff4.index)
AA2 = len(dff5.index)
AA3 = len(dff6.index)

dff7 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'T')]
dff8 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'T')]
dff9 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'T')]
AT1 = len(dff7.index)
AT2 = len(dff8.index)
AT3 = len(dff9.index)

dff10 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'G')]
dff11 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'G')]
dff12 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'G')]
AG1 = len(dff10.index)
AG2 = len(dff11.index)
AG3 = len(dff12.index)

dff13 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'C')]
dff14 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'C')]
dff15 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'C')]
AC1 = len(dff13.index)
AC2 = len(dff14.index)
AC3 = len(dff15.index)

dff16 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'T')]
dff17 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'T')]
dff18 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'T')]
TT1 = len(dff16.index)
TT2 = len(dff17.index)
TT3 = len(dff18.index)

dff19 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'A')]
dff20 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'A')]
dff21 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'A')]
TA1 = len(dff19.index)
TA2 = len(dff20.index)
TA3 = len(dff21.index)

dff22 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'G')]
```

```
dff23 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'G')]
dff24 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'G')]
TG1 = len(dff22.index)
TG2 = len(dff23.index)
TG3 = len(dff24.index)

dff25 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'C')]
dff26 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'C')]
dff27 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'C')]
TC1 = len(dff25.index)
TC2 = len(dff26.index)
TC3 = len(dff27.index)

dff28 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'G')]
dff29 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'G')]
dff30 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'G')]
GG1 = len(dff28.index)
GG2 = len(dff29.index)
GG3 = len(dff30.index)

dff31 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'A')]
dff32 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'A')]
dff33 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'A')]
GA1 = len(dff31.index)
GA2 = len(dff32.index)
GA3 = len(dff33.index)

dff34 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'T')]
dff35 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'T')]
dff36 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'T')]
GT1 = len(dff34.index)
GT2 = len(dff35.index)
GT3 = len(dff36.index)

dff37 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'C')]
dff38 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'C')]
dff39 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'C')]
GC1 = len(dff37.index)
GC2 = len(dff38.index)
GC3 = len(dff39.index)
```

```
dff40 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'C')]
dff41 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'C')]
dff42 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'C')]
CC1 = len(dff40.index)
CC2 = len(dff41.index)
CC3 = len(dff42.index)

dff43 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'A')]
dff44 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'A')]
dff45 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'A')]
CA1 = len(dff43.index)
CA2 = len(dff44.index)
CA3 = len(dff45.index)

dff46 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'T')]
dff47 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'T')]
dff48 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'T')]
CT1 = len(dff46.index)
CT2 = len(dff47.index)
CT3 = len(dff48.index)

dff49 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'G')]
dff50 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'G')]
dff51 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'G')]
CG1 = len(dff49.index)
CG2 = len(dff50.index)
CG3 = len(dff51.index)

# Delcaring a new dataframe.
df = []
df1 = []
df2 = []

# Taking all combinations as a list.
data = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'Strelka_0.3': [AA1, AT1, AG1, AC1, TT1, TA1, TG1, TC1, GG1,
    GA1, GT1, GC1, CC1, CA1, CT1, CG1]}
```

```
data1 = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'Strelka_0.5': [AA2, AT2, AG2, AC2, TT2, TA2, TG2, TC2, GG2,
    GA2, GT2, GC2, CC2, CA2, CT2, CG2]}
data2 = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'Strelka_0.7': [AA3, AT3, AG3, AC3, TT3, TA3, TG3, TC3, GG3,
    GA3, GT3, GC3, CC3, CA3, CT3, CG3]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
df1 = pd.DataFrame(data1)
df2 = pd.DataFrame(data2)

# Merging columns based on "CHROM-POS"
First = pd.merge(df, df1, on=['ALT', 'REF'])
Second = pd.merge(First, df2, on=['ALT', 'REF'])

# Mentioning the column names and inputing the csv file.
Second.columns = ['REF', 'ALT', 'Strelka_0.3', 'Strelka_0.5',
    'Strelka_0.7']
print(Second)

# Saving the results in csv.
Second.to_csv('Strelka_Counts.csv', sep=',', index = None)

# Reading csv files and concatinating "CHROM" and "POS"
dff = pd.read_csv("Strelka_Counts.csv", sep = ',', index_col=
    False, error_bad_lines=False)
dff.columns = ['REF', 'ALT', 'Strelka_One', 'Strelka_Two',
    'Strelka_Three']

# set width of bar
width = 0.25

# Columns from the file
a1 = dff.Strelka_One.to_list()
a2 = dff.Strelka_Two.to_list()
```

```
a3 = dff.Strelka_Three.to_list()

# Set position of bar on X axis
r1 = np.arange(len(a1))
r2 = [x + width for x in r1]
r3 = [x + width for x in r2]

# Make the plot
plt.bar(r1, a1, color='#FFD700', width=width, edgecolor='white',
    label='Strelka_0.3')
plt.bar(r2, a2, color='#FFA500', width=width, edgecolor='white',
    label='Strelka_0.5')
plt.bar(r3, a3, color='#DC143C', width=width, edgecolor='white',
    label='Strelka_0.7')

# Add xticks on the middle of the group bars
plt.xlabel('Combinations')
plt.xticks([r + width for r in range(len(a1))], ['AA', 'AT', 'AG',
    'AC', 'TT', 'TA', 'TG', 'TC', 'GG', 'GA', 'GT', 'GC', 'CC',
    'CA', 'CT', 'CG'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('Strelka_Counts_Plot.pdf')
plt.savefig('Strelka_Counts_Plot.png', dpi = 300)
```

Truth Data Variants

```
# Importing the needed packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import pandas as pd
import csv
import numpy as np

# Importing the csv file.
dff = pd.read_csv("Updated_Somatic_Truth_SNP.vcf", sep = '\t',
    index_col= False)

# Mentioning the column names and inputing the csv file.
dff.columns = ['CHROM', 'POS', 'REF', 'ALT']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

# Mentioning the column names and inputing the csv file.
dff.columns = ['CHROM_POS', 'REF', 'ALT']

# Printing the list.
dff1 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'A')]
AA = len(dff1.index)
print('The number of REF as A and ALT as A is')
print(AA)

dff2 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'T')]
AT = len(dff2.index)
print('The number of REF as A and ALT as T is')
print(AT)

dff3 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'G')]
AG = len(dff3.index)
print('The number of REF as A and ALT as G is')
print(AG)
```

```
dff4 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'C')]
AC = len(dff4.index)
print('The number of REF as A and ALT as C is')
print(AC)

dff5 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'T')]
TT = len(dff5.index)
print('The number of REF as T and ALT as T is')
print(TT)

dff6 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'A')]
TA = len(dff6.index)
print('The number of REF as T and ALT as A is')
print(TA)

dff7 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'G')]
TG = len(dff7.index)
print('The number of REF as T and ALT as G is')
print(TG)

dff8 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'C')]
TC = len(dff8.index)
print('The number of REF as T and ALT as C is')
print(TC)

dff9 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'G')]
GG = len(dff9.index)
print('The number of REF as G and ALT as G is')
print(GG)

dff10 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'A')]
GA = len(dff10.index)
print('The number of REF as G and ALT as A is')
print(GA)

dff11 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'T')]
GT = len(dff11.index)
print('The number of REF as G and ALT as T is')
print(GT)
```

```
dff12 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'C')]
GC = len(dff12.index)
print('The number of REF as G and ALT as C is')
print(GC)

dff13 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'C')]
CC = len(dff13.index)
print('The number of REF as C and ALT as C is')
print(CC)

dff14 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'A')]
CA = len(dff14.index)
print('The number of REF as C and ALT as A is')
print(CA)

dff15 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'T')]
CT = len(dff15.index)
print('The number of REF as C and ALT as T is')
print(CT)

dff16 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'G')]
CG = len(dff16.index)
print('The number of REF as C and ALT as G is')
print(CG)

# Delcaring a new dataframe.
df = []

# Taking all combinations as a list.
data = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'Truth_Data': [AA, AT, AG, AC, TT, TA, TG, TC, GG, GA, GT, GC,
    CC, CA, CT, CG]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
print(df)

# Exporting the outcome into CSV.
```

```
df.to_csv('Truth_Data_Counts.csv', sep=',', index = None)

# Reading csv files and concatinating "CHROM" and "POS"
dff = pd.read_csv("Truth_Data_Counts.csv", sep = ',', index_col=
    False, error_bad_lines=False)
dff.columns = ['REF', 'ALT', 'Truth_Data']

# set width of bar
width = 0.35

# Columns from the file
a1 = dff.Truth_Data.to_list()

# Set position of bar on X axis
r1 = np.arange(len(a1))

# Make the plot
plt.bar(r1, a1, color='#FFD700', width=width, edgecolor='white',
    label='Truth_Data')

# Add xticks on the middle of the group bars
plt.xlabel('Combinations')
plt.xticks([r + width for r in range(len(a1))], ['AA', 'AT', 'AG',
    'AC', 'TT', 'TA', 'TG', 'TC', 'GG', 'GA', 'GT', 'GC', 'CC',
    'CA', 'CT', 'CG'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('Truth_Data_Counts_Plot.pdf')
plt.savefig('Truth_Data_Counts_Plot.png', dpi = 300)
```

VarScan Variants

```
# Importing the needed packages.
import numpy as np
import pandas as pd
import matplotlib
```

```
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import pandas as pd
import csv
import numpy as np

# Reading the csv input file that is obtained after performing the
    following operations on the vcf file.
# Step 1 - 'cut -f 1-2,4-5 Input.vcf > Output.vcf'
# Step 2 - 'sed '/^#/d' Output.vcf > Updated.vcf'

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Updated_VarScan_0.3_SNP.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Updated_VarScan_0.5_SNP.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Updated_VarScan_0.7_SNP.vcf", sep = '\t',
    index_col= False)

# Naming the columns after importing the csv file.
dff.columns = ['CHROM', 'POS', 'REF', 'ALT']
dff1.columns = ['CHROM', 'POS', 'REF', 'ALT']
dff2.columns = ['CHROM', 'POS', 'REF', 'ALT']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff1['CHROM'].astype(str) + '-' +
    dff1['POS'].astype(str)
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
```

```
print(dff)

dff1 = dff1.drop(['CHROM', 'POS'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]
print(dff1)

dff2 = dff2.drop(['CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]
print(dff2)

# Mentioning the column names and inputing the csv file.
dff.columns = ['CHROM_POS', 'REF', 'ALT']
dff1.columns = ['CHROM_POS', 'REF', 'ALT']
dff2.columns = ['CHROM_POS', 'REF', 'ALT']

# Printing the list.
dff4 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'A')]
dff5 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'A')]
dff6 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'A')]
AA1 = len(dff4.index)
AA2 = len(dff5.index)
AA3 = len(dff6.index)

dff7 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'T')]
dff8 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'T')]
dff9 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'T')]
AT1 = len(dff7.index)
AT2 = len(dff8.index)
AT3 = len(dff9.index)

dff10 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'G')]
dff11 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'G')]
dff12 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'G')]
AG1 = len(dff10.index)
AG2 = len(dff11.index)
AG3 = len(dff12.index)
```

```
dff13 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'C')]
dff14 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'C')]
dff15 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'C')]
AC1 = len(dff13.index)
AC2 = len(dff14.index)
AC3 = len(dff15.index)

dff16 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'T')]
dff17 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'T')]
dff18 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'T')]
TT1 = len(dff16.index)
TT2 = len(dff17.index)
TT3 = len(dff18.index)

dff19 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'A')]
dff20 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'A')]
dff21 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'A')]
TA1 = len(dff19.index)
TA2 = len(dff20.index)
TA3 = len(dff21.index)

dff22 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'G')]
dff23 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'G')]
dff24 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'G')]
TG1 = len(dff22.index)
TG2 = len(dff23.index)
TG3 = len(dff24.index)

dff25 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'C')]
dff26 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'C')]
dff27 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'C')]
TC1 = len(dff25.index)
TC2 = len(dff26.index)
TC3 = len(dff27.index)

dff28 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'G')]
dff29 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'G')]
dff30 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'G')]
GG1 = len(dff28.index)
```

```
GG2 = len(dff29.index)
GG3 = len(dff30.index)

dff31 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'A')]
dff32 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'A')]
dff33 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'A')]
GA1 = len(dff31.index)
GA2 = len(dff32.index)
GA3 = len(dff33.index)

dff34 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'T')]
dff35 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'T')]
dff36 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'T')]
GT1 = len(dff34.index)
GT2 = len(dff35.index)
GT3 = len(dff36.index)

dff37 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'C')]
dff38 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'C')]
dff39 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'C')]
GC1 = len(dff37.index)
GC2 = len(dff38.index)
GC3 = len(dff39.index)

dff40 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'C')]
dff41 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'C')]
dff42 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'C')]
CC1 = len(dff40.index)
CC2 = len(dff41.index)
CC3 = len(dff42.index)

dff43 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'A')]
dff44 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'A')]
dff45 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'A')]
CA1 = len(dff43.index)
CA2 = len(dff44.index)
CA3 = len(dff45.index)

dff46 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'T')]
dff47 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'T')]
```

```
dff48 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'T')]
CT1 = len(dff46.index)
CT2 = len(dff47.index)
CT3 = len(dff48.index)

dff49 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'G')]
dff50 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'G')]
dff51 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'G')]
CG1 = len(dff49.index)
CG2 = len(dff50.index)
CG3 = len(dff51.index)

# Delcaring a new dataframe.
df = []
df1 = []
df2 = []

# Taking all combinations as a list.
data = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'VarScan_0.3': [AA1, AT1, AG1, AC1, TT1, TA1, TG1, TC1, GG1,
    GA1, GT1, GC1, CC1, CA1, CT1, CG1]}
data1 = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'VarScan_0.5': [AA2, AT2, AG2, AC2, TT2, TA2, TG2, TC2, GG2,
    GA2, GT2, GC2, CC2, CA2, CT2, CG2]}
data2 = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'VarScan_0.7': [AA3, AT3, AG3, AC3, TT3, TA3, TG3, TC3, GG3,
    GA3, GT3, GC3, CC3, CA3, CT3, CG3]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
df1 = pd.DataFrame(data1)
df2 = pd.DataFrame(data2)

# Merging columns based on "CHROM-POS"
```

```
First = pd.merge(df, df1, on=['ALT', 'REF'])
Second = pd.merge(First, df2, on=['ALT', 'REF'])

# Mentioning the column names and inputing the csv file.
Second.columns = ['REF', 'ALT', 'VarScan_0.3', 'VarScan_0.5',
    'VarScan_0.7']
print(Second)

# Saving the results in csv.
Second.to_csv('VarScan_Counts.csv', sep=',', index = None)

# Reading csv files and concatinating "CHROM" and "POS"
dff = pd.read_csv("VarScan_Counts.csv", sep = ',', index_col=
    False, error_bad_lines=False)
dff.columns = ['REF', 'ALT', 'VarScan_One', 'VarScan_Two',
    'VarScan_Three']

# set width of bar
width = 0.25

# Columns from the file
a1 = dff.VarScan_One.to_list()
a2 = dff.VarScan_Two.to_list()
a3 = dff.VarScan_Three.to_list()

# Set position of bar on X axis
r1 = np.arange(len(a1))
r2 = [x + width for x in r1]
r3 = [x + width for x in r2]

# Make the plot
plt.bar(r1, a1, color='#FFD700', width=width, edgecolor='white',
    label='VarScan_0.3')
plt.bar(r2, a2, color='#FFA500', width=width, edgecolor='white',
    label='VarScan_0.5')
plt.bar(r3, a3, color='#DC143C', width=width, edgecolor='white',
    label='VarScan_0.7')

# Add xticks on the middle of the group bars
plt.xlabel('Combinations')
plt.xticks([r + width for r in range(len(a1))], ['AA', 'AT', 'AG',
    'AC', 'TT', 'TA', 'TG', 'TC', 'GG', 'GA', 'GT', 'GC', 'CC',
    'CA', 'CT', 'CG'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('VarScan_Counts_Plot.pdf')
plt.savefig('VarScan_Counts_Plot.png', dpi = 300)
```

Variants Comparison

```
# Importing the needed packages.
import numpy as np
import pandas as pd
import matplotlib
from matplotlib import rc
matplotlib.rcParams['mathtext.fontset'] = 'cm'
matplotlib.rcParams['font.family'] = 'serif'
import matplotlib.pyplot as plt
import pandas as pd
import csv
import numpy as np

# Reading the csv input file that is obtained after performing the
    following operations on the vcf file.
# Step 1 - 'cut -f 1-2,4-5 Input.vcf > Output.vcf'
# Step 2 - 'sed '/^#/d' Output.vcf > Updated.vcf'

# The first step is to selected the needed columns in the vcf file.
# The second step if to eliminate all lines that start with a '#'
dff = pd.read_csv("Updated_Strelka_0.3_SNV.vcf", sep = '\t',
    index_col= False)
dff1 = pd.read_csv("Updated_VarScan_0.3_SNP.vcf", sep = '\t',
    index_col= False)
dff2 = pd.read_csv("Updated_Somatic_Truth_SNP.vcf", sep = '\t',
    index_col= False)
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# SNP Counts
SSC = len(dff)
VSC = len(dff1)
TSC = len(dff2)

# Naming the columns after importing the csv file.
dff.columns = ['CHROM', 'POS', 'REF', 'ALT']
dff1.columns = ['CHROM', 'POS', 'REF', 'ALT']
dff2.columns = ['CHROM', 'POS', 'REF', 'ALT']

# Concatinating the "CHROM" and "POS"
dff["CHROM_POS"] = dff['CHROM'].astype(str) + '-' +
    dff['POS'].astype(str)
dff1["CHROM_POS"] = dff1['CHROM'].astype(str) + '-' +
    dff1['POS'].astype(str)
dff2["CHROM_POS"] = dff2['CHROM'].astype(str) + '-' +
    dff2['POS'].astype(str)

# Dropping of the unnecessary columns and reorganising the columns.
dff = dff.drop(['CHROM', 'POS'], axis=1)
cols = dff.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff = dff[cols]
print(dff)

dff1 = dff1.drop(['CHROM', 'POS'], axis=1)
cols = dff1.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff1 = dff1[cols]
print(dff1)

dff2 = dff2.drop(['CHROM', 'POS'], axis=1)
cols = dff2.columns.tolist()
cols = cols[-1:] + cols[:-1]
dff2 = dff2[cols]
print(dff2)

# Mentioning the column names and inputing the csv file.
dff.columns = ['CHROM_POS', 'REF', 'ALT']
dff1.columns = ['CHROM_POS', 'REF', 'ALT']
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dff2.columns = ['CHROM_POS', 'REF', 'ALT']

# Printing the list.
dff4 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'A')]
dff5 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'A')]
dff6 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'A')]
AA1 = len(dff4.index)
AA2 = len(dff5.index)
AA3 = len(dff6.index)

dff7 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'T')]
dff8 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'T')]
dff9 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'T')]
AT1 = (len(dff7.index)/SSC) * 100
AT2 = (len(dff8.index)/VSC) * 100
AT3 = (len(dff9.index)/TSC) * 100

dff10 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'G')]
dff11 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'G')]
dff12 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'G')]
AG1 = (len(dff10.index)/SSC) * 100
AG2 = (len(dff11.index)/VSC) * 100
AG3 = (len(dff12.index)/TSC) * 100

dff13 = dff[(dff['REF'] == 'A') & (dff['ALT'] == 'C')]
dff14 = dff1[(dff1['REF'] == 'A') & (dff1['ALT'] == 'C')]
dff15 = dff2[(dff2['REF'] == 'A') & (dff2['ALT'] == 'C')]
AC1 = (len(dff13.index)/SSC) * 100
AC2 = (len(dff14.index)/VSC) * 100
AC3 = (len(dff15.index)/TSC) * 100

dff16 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'T')]
dff17 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'T')]
dff18 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'T')]
TT1 = len(dff16.index)
TT2 = len(dff17.index)
TT3 = len(dff18.index)

dff19 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'A')]
dff20 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'A')]
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dff21 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'A')]
TA1 = (len(dff19.index)/SSC) * 100
TA2 = (len(dff20.index)/VSC) * 100
TA3 = (len(dff21.index)/TSC) * 100

dff22 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'G')]
dff23 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'G')]
dff24 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'G')]
TG1 = (len(dff22.index)/SSC) * 100
TG2 = (len(dff23.index)/VSC) * 100
TG3 = (len(dff24.index)/TSC) * 100

dff25 = dff[(dff['REF'] == 'T') & (dff['ALT'] == 'C')]
dff26 = dff1[(dff1['REF'] == 'T') & (dff1['ALT'] == 'C')]
dff27 = dff2[(dff2['REF'] == 'T') & (dff2['ALT'] == 'C')]
TC1 = (len(dff25.index)/SSC) * 100
TC2 = (len(dff26.index)/VSC) * 100
TC3 = (len(dff27.index)/TSC) * 100

dff28 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'G')]
dff29 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'G')]
dff30 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'G')]
GG1 = len(dff28.index)
GG2 = len(dff29.index)
GG3 = len(dff30.index)

dff31 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'A')]
dff32 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'A')]
dff33 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'A')]
GA1 = (len(dff31.index)/SSC) * 100
GA2 = (len(dff32.index)/VSC) * 100
GA3 = (len(dff33.index)/TSC) * 100

dff34 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'T')]
dff35 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'T')]
dff36 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'T')]
GT1 = (len(dff34.index)/SSC) * 100
GT2 = (len(dff35.index)/VSC) * 100
GT3 = (len(dff36.index)/TSC) * 100
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dff37 = dff[(dff['REF'] == 'G') & (dff['ALT'] == 'C')]
dff38 = dff1[(dff1['REF'] == 'G') & (dff1['ALT'] == 'C')]
dff39 = dff2[(dff2['REF'] == 'G') & (dff2['ALT'] == 'C')]
GC1 = (len(dff37.index)/SSC) * 100
GC2 = (len(dff38.index)/VSC) * 100
GC3 = (len(dff39.index)/TSC) * 100

dff40 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'C')]
dff41 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'C')]
dff42 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'C')]
CC1 = len(dff40.index)
CC2 = len(dff41.index)
CC3 = len(dff42.index)

dff43 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'A')]
dff44 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'A')]
dff45 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'A')]
CA1 = (len(dff43.index)/SSC) * 100
CA2 = (len(dff44.index)/VSC) * 100
CA3 = (len(dff45.index)/TSC) * 100

dff46 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'T')]
dff47 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'T')]
dff48 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'T')]
CT1 = (len(dff46.index)/SSC) * 100
CT2 = (len(dff47.index)/VSC) * 100
CT3 = (len(dff48.index)/TSC) * 100

dff49 = dff[(dff['REF'] == 'C') & (dff['ALT'] == 'G')]
dff50 = dff1[(dff1['REF'] == 'C') & (dff1['ALT'] == 'G')]
dff51 = dff2[(dff2['REF'] == 'C') & (dff2['ALT'] == 'G')]
CG1 = (len(dff49.index)/SSC) * 100
CG2 = (len(dff50.index)/VSC) * 100
CG3 = (len(dff51.index)/TSC) * 100

# Delcaring a new dataframe.
df = []
df1 = []
df2 = []
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# Taking all combinations as a list.
data = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'Strelka_0.3': [AA1, AT1, AG1, AC1, TT1, TA1, TG1, TC1, GG1,
    GA1, GT1, GC1, CC1, CA1, CT1, CG1]}
data1 = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'VarScan_0.3': [AA2, AT2, AG2, AC2, TT2, TA2, TG2, TC2, GG2,
    GA2, GT2, GC2, CC2, CA2, CT2, CG2]}
data2 = {'REF': ['A', 'A', 'A', 'A', 'T', 'T', 'T', 'T', 'G', 'G',
    'G', 'G', 'C', 'C', 'C', 'C'], 'ALT': ['A', 'T', 'G', 'C', 'T',
    'A', 'G', 'C', 'G', 'A', 'T', 'C', 'C', 'A', 'T', 'G'],
    'Truth_Data': [AA3, AT3, AG3, AC3, TT3, TA3, TG3, TC3, GG3,
    GA3, GT3, GC3, CC3, CA3, CT3, CG3]}

# Collecting it into a dataframe.
df = pd.DataFrame(data)
df1 = pd.DataFrame(data1)
df2 = pd.DataFrame(data2)

# Merging columns based on "CHROM-POS"
First = pd.merge(df, df1, on=['ALT', 'REF'])
Second = pd.merge(First, df2, on=['ALT', 'REF'])

# Mentioning the column names and inputing the csv file.
Second.columns = ['REF', 'ALT', 'Strelka_0.3', 'VarScan_0.3',
    'Truth_Data']
print(Second)

# Saving the results in csv.
Second.to_csv('Tumor_Purity_0.3_Counts.csv', sep=',', index = None)

# Reading csv files and concatinating "CHROM" and "POS"
dff = pd.read_csv("Tumor_Purity_0.3_Counts.csv", sep = ',',
    index_col= False, error_bad_lines=False)
dff.columns = ['REF', 'ALT', 'Strelka', 'VarScan', 'Truth']

# set width of bar
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width = 0.25

# Columns from the file
a1 = dff.Strelka.to_list()
a2 = dff.VarScan.to_list()
a3 = dff.Truth.to_list()

# Set position of bar on X axis
r1 = np.arange(len(a1))
r2 = [x + width for x in r1]
r3 = [x + width for x in r2]

# Make the plot
plt.bar(r1, a1, color='#FFD700', width=width, edgecolor='white',
    label='Strelka_0.3')
plt.bar(r2, a2, color='#FFA500', width=width, edgecolor='white',
    label='VarScan_0.3')
plt.bar(r3, a3, color='#DC143C', width=width, edgecolor='white',
    label='Truth_Data')

# Add xticks on the middle of the group bars
plt.xlabel('SNP Combinations')
plt.xticks([r + width for r in range(len(a1))], ['AA', 'AT', 'AG',
    'AC', 'TT', 'TA', 'TG', 'TC', 'GG', 'GA', 'GT', 'GC', 'CC',
    'CA', 'CT', 'CG'])

# Create legend & Show graphic
plt.legend()
plt.show()
plt.savefig('Tumor_Purity_0.3_Plot.pdf')
plt.savefig('Tumor_Purity_0.3_Plot.png', dpi = 300)
```