Viewing and parsing Variant Call Format files

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Contents

1 Introduction

1.1 1000s genome project

The 1000s Genomes Project, started in 2008, was a large effort by researchers to catalog the genetic variation of different people all over the world via the usage of whole genome sequencing. Its goal was to create a reference of human genetic variation globally. The project was completed in 2015 after characterizing the genomes of 2,504 people from 26 different populations [1]. While the vast majority of the 88 million variants they discovered were single nucleotide polymorphism (SNPs)(84.7 million), the researchers also discovered short insertions/deletions (indels)(3.6 million) and structural variants (60,000). Figure 1 below shows the locations that were collected by the 1000s Genomes Project.

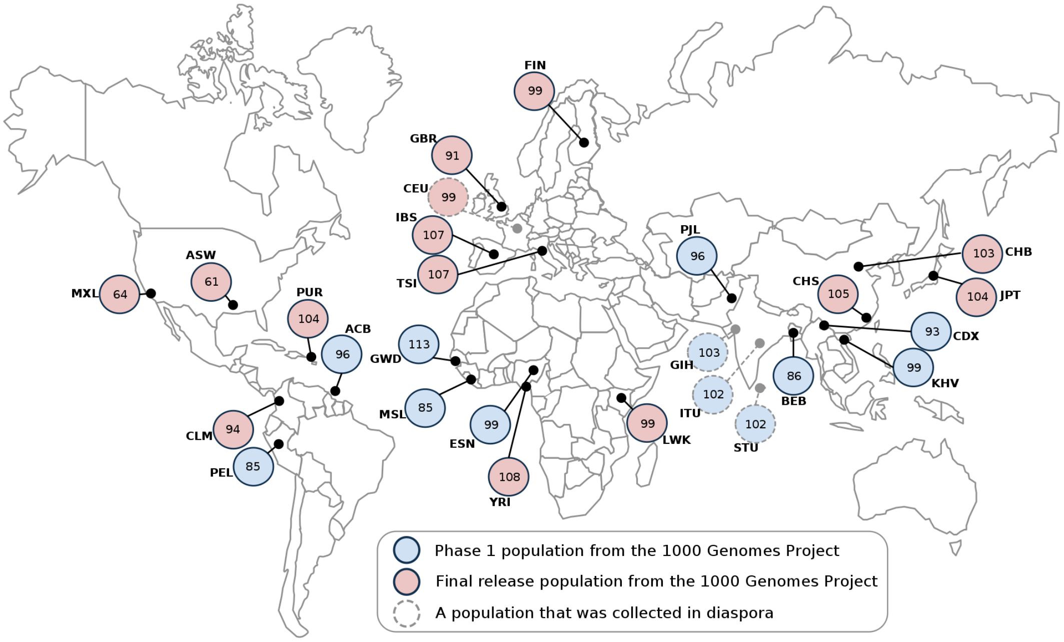


Figure 1: Locations of volunteers for the 1000s Genomes Project. It is noticeable that the project did not collect from various countries around the world, notably among them Russia. Researchers have created the "Genome Russia Project" to fix this [2].

1.2 Variant Call Format

The Variant Call Format (VCF) file type was created by the 1000s Genomes Project so they could store genetic variation data collected in an organized and meaningful way [3]. Using this format, the only necessary thing that needs to be stored is the reference genome and the variations themselves. The VCF file extension is ".vcf", but the file itself is just text, so any text editor can open and display the data inside.

There are a number of steps that are required to go through to end up with a meaningful VCF file. If you send in your samples for sequencing, many companies will complete these steps for you through what is called a pipeline, and they will send you your results of SNPs and indels in VCF form. For example, a sample of DNA is sequenced, which produces a raw FASTA DNA sequence. The sequence is aligned with a reference creating a Sequence Alignment/Map (SAM) file. Processing the SAM file involves IDing where the alignment is different between the reference and the target sequence, and the VCF is built. The term for this is "variant calling" - whereby we are able to ultimately ID the variants from the input of the sequence data. This process has become a standardized way for bioinformaticians to tidy and handle massive amounts of genomic data into parasable files that contain only the variation data. The Global Alliance for Genomics & Health (GA4H) has laid out what they call the Large Scale Genomics work stream, the process mentioned above acting as a pipeline of genomic formatting [4,5].

1.3 Anatomy of a VCF file

While the text in a VCF file in itself is not immediately very helpful to a human reader, I will go over the basics of the format itself before we learn how to use tools that have been created to parse and manipulate them. The anatomy of a VCF file is extremely well documented, and the full specification of the newest standard (VCFv4.3) is freely available online [6].

Figure 2 below shows the standardized anatomy of a VCF file. It can be a lot to parse if you have never seen one before, so I will go over the most important parts below.

There are three standard parts of a VCF File:

1. Meta data rows that begin with “##”
2. Field definition line that begins with “#” and is always the last row of the header. This row has 8 mandatory fields, which contain further information.
3. Variation data itself, with each row representing one variant. Data is filled according to the column.

`"." is used in place of known data`

VCF example (From Figure 2a) with only one variant row:

```
##fileformat=VCFv4.1
##fileDate=20110413
##source=VCFtools
##reference=file:///refs/human_NCBI36.fasta
#CHROM  POS ID    REF   ALT QUAL  FILTER    INFO
 1        5  rs12 A     G    67   PASS      .
```

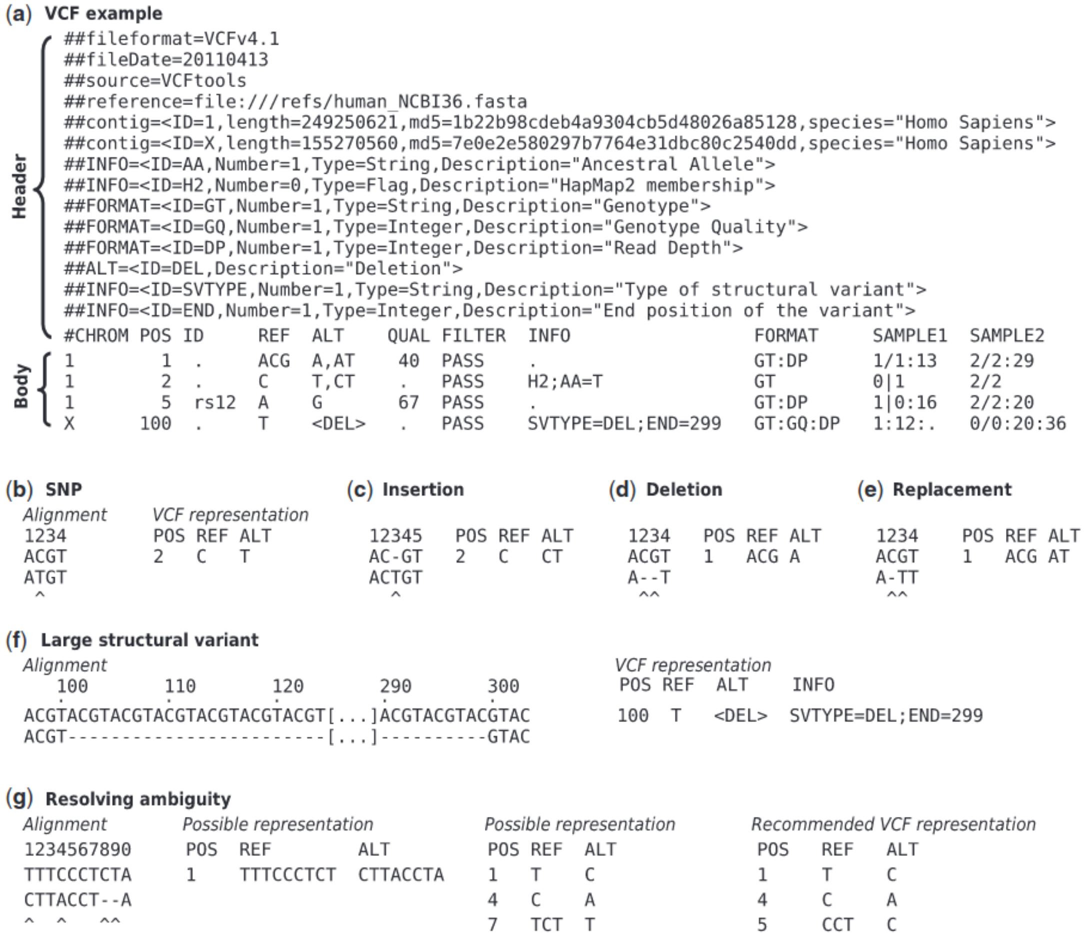
This small VCF example, taken from Figure 2a, show the first four rows of the VCFfile, the 8 mandatory fields, and one variant. While `fileformat` is mandatory as the first header row, the other rows are not. Yet, it is clear that presenting data such as the reference genome is useful. This metadata provides a standardized way to share information in the VCF file about various things like version of reference sequence, software that has already been used on the file, and is featured to fit the dataset itself. In this VCF you can see the reference human_NCBI36.fasta was used.

The field definition lines contains the following fields:

Field name	VCF Example value	Description
CHROM	1	Chromosome (in order)
POS	5	Position of the starting base pair variant
ID	rs12	Unique variant identifier
REF	A	reference allele (one BP or sequence) at the same position
ALT	G	alleles from the sample

Field name	VCF Example value	Description
QUAL	67	quality score that is phred-scaled
FILTER	PASS	information of site filtering
INFO	.	semicolon separated and has user annotations that correspond to meta data above

Each row below the headers represents one variant. As a reminder, VCF files exist to show the variants and this is represented by SNPs (Figure 2b), indels (Figure 2c,d), and structural variants (Figure 2e,f).


Header
Body

(a) VCF example

```
##fileformat=VCFv4.1
##fileDate=20110413
##source=VCFtools
##reference=file:///refs/human_NCBI36.fasta
##contig=<ID=1,length=249250621,md5=1b22b98cdeb4a9304cb5d48026a85128,species="Homo Sapiens">
##contig=<ID=X,length=155270560,md5=7e0e2e580297b7764e31dbc80c2540dd,species="Homo Sapiens">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Read Depth">
##ALT=<ID=DEL,Description="Deletion">
##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant">
##INFO=<ID=END,Number=1,Type=Integer,Description="End position of the variant">
#CHROM POS ID     REF  ALT    QUAL FILTER  INFO                FORMAT     SAMPLE1   SAMPLE2
1        1 .      ACG  A,AT    40  PASS    .                   GT:DP      1/1:13    2/2:29
1        2 .      C    T,CT    .   PASS    H2;AA=T             GT         0|1       2/2
1        5 rs12   A    G       67  PASS    .                   GT:DP      1|0:16    2/2:20
X      100 .      T    <DEL>   .   PASS    SVTYPE=DEL;END=299  GT:GQ:DP   1:12:.    0/0:20:36
```

(b) SNP

```
Alignment   VCF representation
1234        POS REF ALT
ACGT        2   C   T
ATGT
 ^
```

(c) Insertion

```
12345   POS REF ALT
AC-GT   2   C   CT
ACTGT
  ^
```

(d) Deletion

```
1234   POS REF ALT
ACGT   1   ACG A
A--T
 ^^
```

(e) Replacement

```
1234   POS REF ALT
ACGT   1   ACG AT
A-TT
 ^^
```

(f) Large structural variant

```
Alignment                                                   VCF representation
  100       110       120       290       300               POS REF  ALT    INFO
   .         .         .         .         .
ACGTACGTACGTACGTACGTACGTACGT[...]ACGTACGTACGTAC            100  T   <DEL>  SVTYPE=DEL;END=299
ACGT------------------------[...]----------GTAC
```

(g) Resolving ambiguity

```
Alignment    Possible representation      Possible representation   Recommended VCF representation
1234567890   POS  REF        ALT          POS REF  ALT              POS   REF   ALT
TTTCCCTCTA   1    TTTCCCTCT  CTTACCTA     1   T    C                1     T     C
CTTACCT--A                                4   C    A                4     C     A
^  ^   ^^                                 7   TCT  T                5     CCT   C
```

Figure 2: a) Anatomy of a VCF file. b-f) Further explanation of the BAM alignment representation of a genome and its VCF representation. g) Recommended VCF representation of alignment data that could be ambiguous [1].

The variant ID is a unique value that is useful when looking to search for information about the variant. For example, in our VCF example above, the one row that exists has an ID of `rs12`. This SNP ID is assigned by the NCBI's database of SNPs, called dbSNP or the European Variation Archive (EVA). The European Bioinformatics Institute (EMBL-EBI) has a great resource listing all of the varying ID values and where they come from [7].

You may have noticed that there is some meta data information in Figure 2a that I did not introduce. The specifics of what each line means can become quite convoluted. The NIH has an excellent resource on INFO tags and their meaning. Likewise, the 1000s Genome Project created a poster with a VCF example as well.

2 Tutorial

Obtaining a VCF file
We will be using this VCF file from the NIH repo here. It is a VCF that represents all human variants that are of clinical significance and has been mapped to GRCh37 assemblies.

This file is inside of the VCF folder attached to this PDF.

Running the Unix command `wc -l clinvar_20221211.vcf.gz`

shows that this file is 240,695 lines long, so it will be a mistake to open this in a text file or in Excel. Luckily, there are many freely available programs that can open and parse this data in an efficient manner.

As is common in Computer Science, and perhaps an example of not invented here syndrome, there are about a million different tools that various people and organizations have created to deal with managing VCF files.

2.1 Viewing a VCF file with IGV

So, you have a VCF file. The variants in this file can be viewed in many different biologically meaningful ways. It would be nice to see a visual representation of all of the genetic variants on each chromosome. Fortunately, the Broad Institute has created a program, Integrated Genomics Viewer (IGV) that can do exactly that.

Steps for IGV

1. IGV can be installed here. There is also a web app, but it is not as functional as the desktop app.
2. If you are familiar with Java and know you have Java Runtime Environment 11 (JRE) install on your computer, you can install the app that says "Separate Java 11 required", otherwise install the "Java included" version of your OS.
3. Unzip the file installed onto your computer. Open a terminal and `cd` to the directory and execute `\.igv-launcher.bat` if you are on Windows or `./igv.sh` if you are on Linux or Mac OS.

It would also be nice to see what effects they have on them.

2.2 Doing anything useful with a VCF file

In their 2011 paper highlighting the creation of the VCF file format [2], the 1000s Genome Project created their on application that can be used to parse and annotate VCF files called "VCF Tools".

How to read manually

How to parse manually

People have written programs that will do this for you Command line programs There are many of them List one that is good because it is documented and seems to be well tested

3 Other available programs

This tutorial has examined two programs, IGV and VCFTools to visualize and parse data respectively. However, there are a large number of other programs that have been created to do this as well. I have listed them here as a resource should you wish to view them. They vary from very well documented to not.

- sgkit provides a Python API, with parsing performed by cyvcf2
- https://alimanfoo.github.io/2017/06/14/read-vcf.html

4 Conclusion

The pipeline from sequencing DNA to determining the variation in the DNA sample compared to a reference involves a lot of steps and a massive amount of data. There exists a standardized way in which bioinformaticians use to process this data. One of the steps is the output, a VCF file containing the variation. Yet, this file still contains a large amount of data that can make it difficult to immediately be meaningful. There are programs and tools that we can use to help parse the information out of these VCF files into a more human readable format, so that we can extract biologically meaningful conclusions, results, etc.

5 References

1. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature. 2015 Oct 1;526(7571):68-74. doi: 10.1038/nature15393. PMID: 26432245; PMCID: PMC4750478.

2. Oleksyk TK, Brukhin V, O'Brien SJ. The Genome Russia project: closing the largest remaining omission on the world Genome map. Gigascience. 2015 Nov 13;4:53. doi: 10.1186/s13742-015-0095-0. PMID: 26568821; PMCID: PMC4644275.

3. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R; 1000 Genomes Project Analysis Group.

The variant call format and VCFtools. Bioinformatics. 2011 Aug 1;27(15):2156-8. doi: 10.1093/bioinformatics/btr330. Epub 2011 Jun 7. PMID: 21653522; PMCID: PMC3137218.

4. https://samtools.github.io/hts-specs/

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6. https://samtools.github.io/hts-specs/VCFv4.3.pdf

7. https://www.ebi.ac.uk/training/online/courses/human-genetic-variation-introduction/variant-identification-and-analysis/variant-identifiers/