04. Variant Calling

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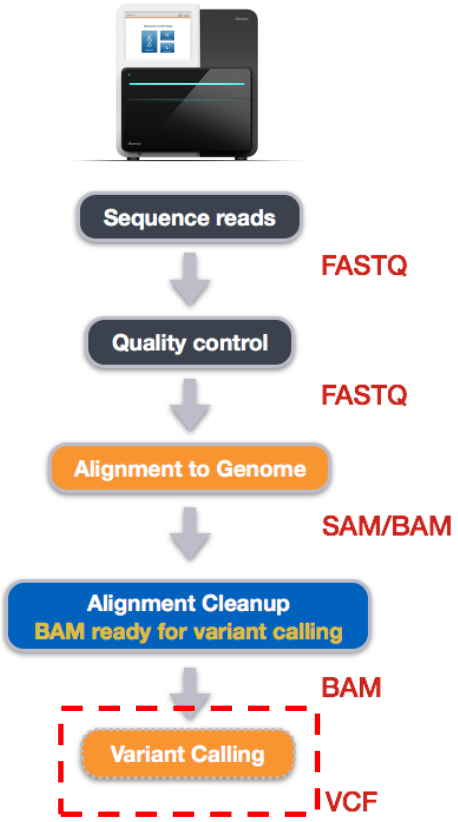
A Quick Recap

In the last lecture, we aligned the reads on the reference genome, and we obtained a **BAM** file

At this point, we are ready to move on and perform what is called **variant calling**



Variant Calling

Variant calling is a computational process that identifies differences between a sample's DNA sequence and a reference genome. Its applications are broad:

- **Disease** Identification: Determines genetic mutations causing genetic disorders.
- Personalized **Treatment**: Tailors treatments to individual genetic makeups.
- **Cancer** Insights: Identifies cancer-driving somatic mutations.

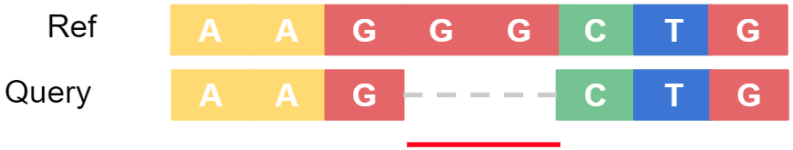
Variant Calling

Genetic variants are often separated into two categories: sequence variants, and structural variants.

Sequence Variants

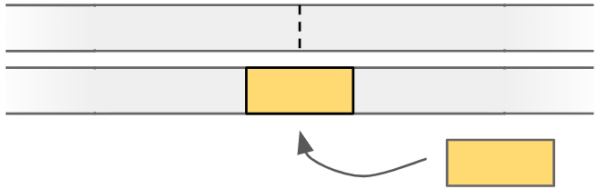
SNV (**S**ingle **N**ucleotide **V**ariant)

INDEL (**In**sertion or **Del**etion)

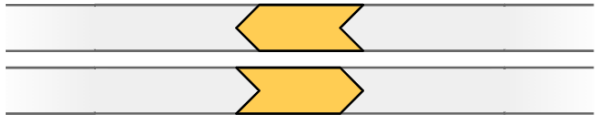


Structural Variants

Insertion



Inversion

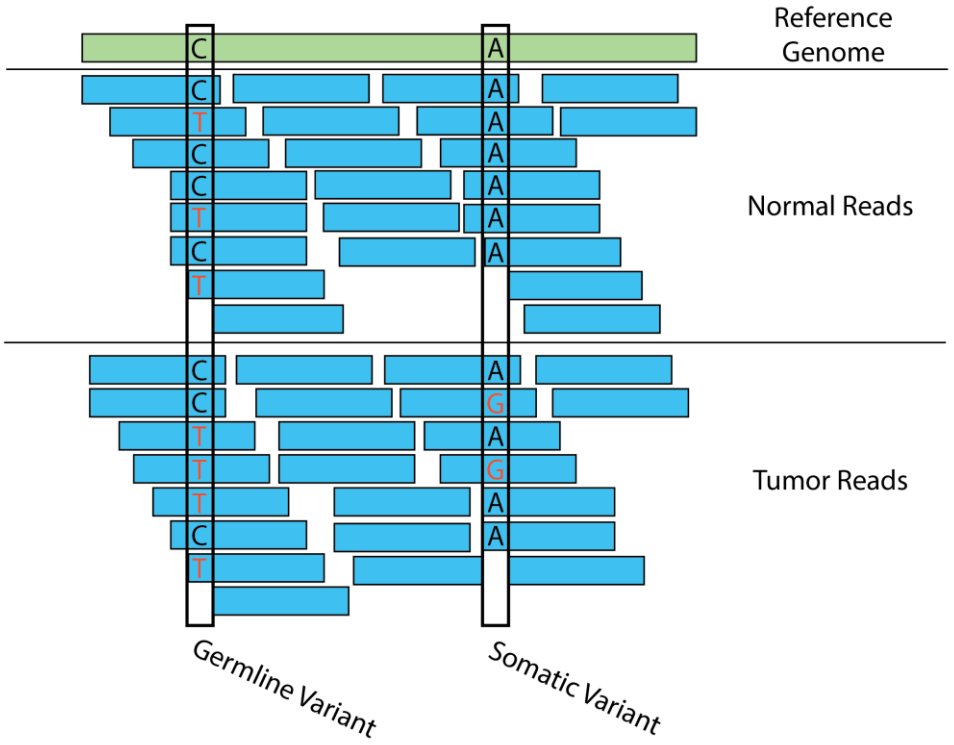


We will mainly focus on SNVs

Germline vs Somatic Variants

- A **germline variant** is a genetic change present in the reproductive cells, which means it can be inherited by offspring and potentially passed down through generations.

- **Somatic variants** are the most common cause of cancer; some of these genetic alterations produce noticeable traits or physical changes (phenotypes), while others do not. These variants are not hereditary.



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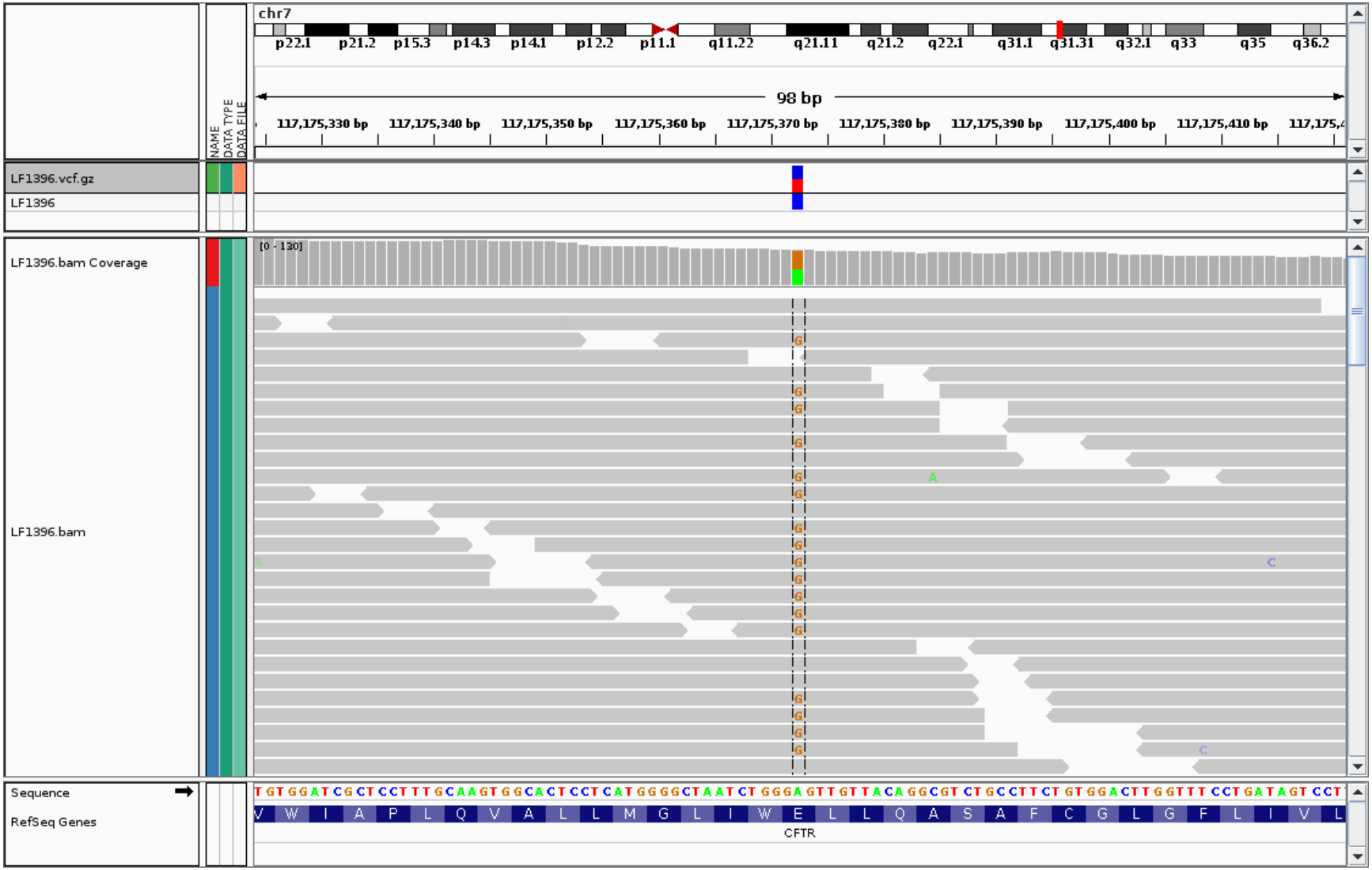
In IGV



How do we find sequence variants?

The **GATK suite** is a group of software to perform variant calling and all the tasks needed to obtain high quality SNVs.

Mutect v2

Mutect2 employs a Bayesian statistical framework based on the **Allelic Fraction**

$$\boldsymbol{Allelic\ Fraction\ (AF)} = \frac{\boldsymbol{Number\ of\ variant\ reads}}{\boldsymbol{Total\ reads\ at\ loci}}$$

The allelic fraction is used as a **filter** criterion. SNVs with a very low allelic fraction may be likely due to sequencing errors or very rare subclonal mutations

Must do!

Before using GATK, we need to run this command to configure it:

```
source /usr/local/GenomicsTechnologies/setup_env.sh
```

How to run Mutect 2

- Before actually callling the variants, we need to create a sequence dictionary, which is simply an index that Mutect2 needs to run.

- You can generate this dictionary by typing on the shell:

```
gatk CreateSequenceDictionary -R <fasta filename>
```

<fasta filename>: is the fasta file with the reference genome that you used for the alignment index generation

How to run Mutect 2

- At this point, you can run Mutect2 by typing:

```
gatk Mutect2 --reference <fasta filename> --input <sample_name.sorted.bam> --output <sample_name.unfiltered.vcf.gz>
```

- Note that the input is the sorted bam filed that you obtained with samtools

The VCF file

https://samtools.github.io/hts-specs/VCFv4.2.pdf

VCF (Variant Call Format) is a text file format.
It contains:

- meta-information lines
- header line
- data lines each containing information about a position in the genome.

```
##fileformat=VCFv4.2
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number of Samples With Data">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Total Depth">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency">
##INFO=<ID=AA,Number=1,Type=String,Description="Ancestral Allele">
##INFO=<ID=DB,Number=0,Type=Flag,Description="dbSNP membership, build 129">
##INFO=<ID=H2,Number=0,Type=Flag,Description="HapMap2 membership">
##FILTER=<ID=q10,Description="Quality below 10">
##FILTER=<ID=s50,Description="Less than 50% of samples have data">
##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">
##FORMAT=<ID=HQ,Number=2,Type=Integer,Description="Haplotype Quality">
#CHROM POS     ID        REF    ALT     QUAL FILTER INFO                              FORMAT      NA00001        NA00002        NA00003
20     14370   rs6054257 G      A       29   PASS   NS=3;DP=14;AF=0.5;DB;H2           GT:GQ:DP:HQ 0|0:48:1:51,51 1|0:48:8:51,51 1/1:43:5:.,.
20     17330   .         T      A       3    q10    NS=3;DP=11;AF=0.017               GT:GQ:DP:HQ 0|0:49:3:58,50 0|1:3:5:65,3   0/0:41:3
20     1110696 rs6040355 A      G,T     67   PASS   NS=2;DP=10;AF=0.333,0.667;AA=T;DB GT:GQ:DP:HQ 1|2:21:6:23,27 2|1:2:0:18,2   2/2:35:4
20     1230237 .         T      .       47   PASS   NS=3;DP=13;AA=T                   GT:GQ:DP:HQ 0|0:54:7:56,60 0|0:48:4:51,51 0/0:61:2
20     1234567 microsat1 GTC    G,GTCT  50   PASS   NS=3;DP=9;AA=G                    GT:GQ:DP    0/1:35:4       0/2:17:2       1/1:40:3
```

Description

Metadata

Data

Filter the VCF

The output from Mutect2 is a raw variant calling output and the calls need to be **filtered** to ensure against errors such as:

- Technical artifacts
- Germline mutations
- Sequencing Errors

FilterMutectCalls will annotate the FILTER field in the VCF file with whether the variant is passing with **PASS** or the reasons why it failed filtering.

```
gatk FilterMutectCalls –reference <fasta filename> --variant
<sample_name.unfiltered.vcf.gz> --output <sample_name.filtered.vcf.gz>
```

Filter the VCF

FilterMutectCalls contains a set of filters, divided into three categories: technical artifacts, non-somatic, and sequencing error.

Filter	Threshold	Explanation
clustered_events	max-events-in-region	mutations sharing an assembly region
duplicate_evidence	unique-alt-read-count	unique insert start/end pairs of alt reads
multiallelic	max-alt-alleles-count	passing alt alleles at a site
base_qual	min-median-base-quality	median base quality of alt reads
map_qual	min-median-mapping-quality	median mapping quality of alt reads
fragment	max-median-fragment-length-difference	difference of alt and ref reads' median fragment lengths
position	min-median-read-position	median distance of alt mutations from end of read
panel_of_normals	panel-of-normals	presence in panel of normals

For more details on the filtering steps see the second chapter of Mutect manual:

https://github.com/broadinstitute/gatk/blob/master/docs/mutect/mutect.pdf

Questions ?