Germline SNV and Indel Calling

10/26/20

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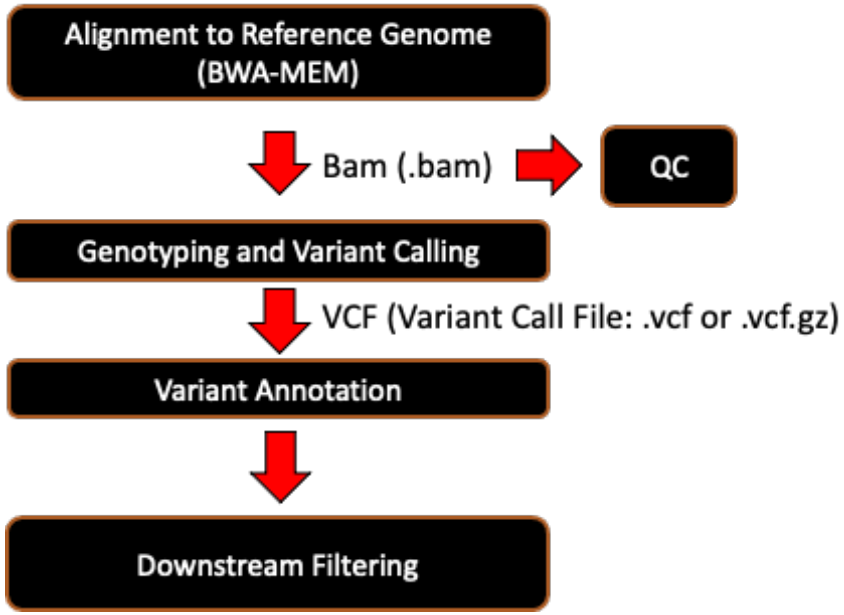
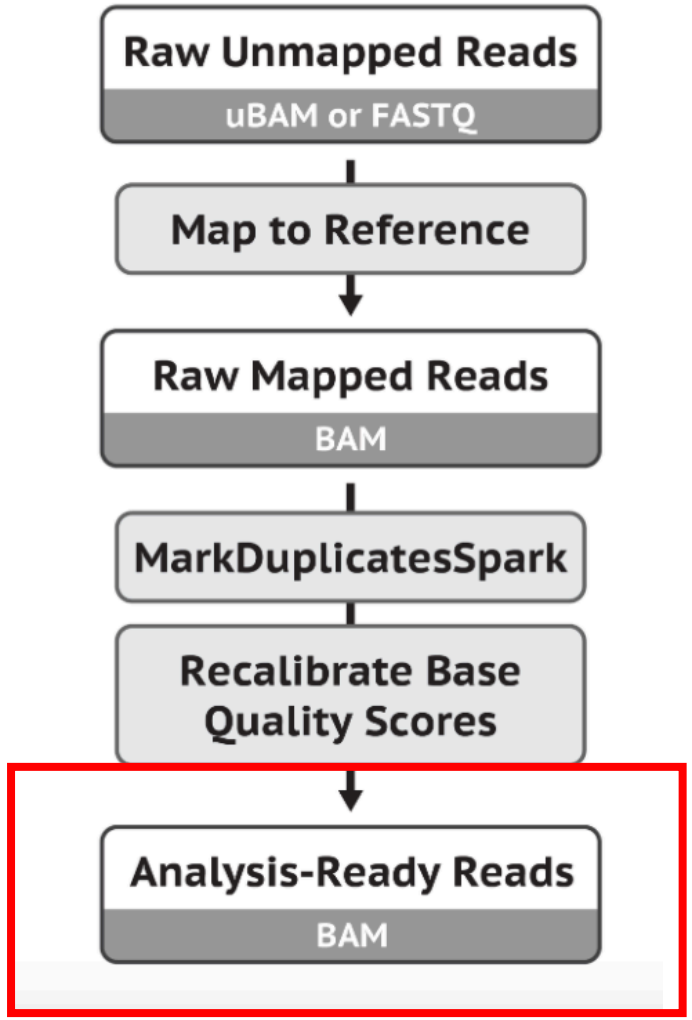
How do we identify Germline SNVs and Indels?

1. Align Reads to Reference
2. Call Genotypes
3. Annotate variants
4. Filter Variants
    1. Annotations
        1. Mapping Quality (MQ)
        2. Read Depth (DP)
        3. Genotype Quality (GQ)
    2. Allele Frequency
    3. Region of Interest
5. Final QC
    1. Manually Check Sequence Context (IGV)
        - Homopolymers, Repeat Regions are hard to sequence



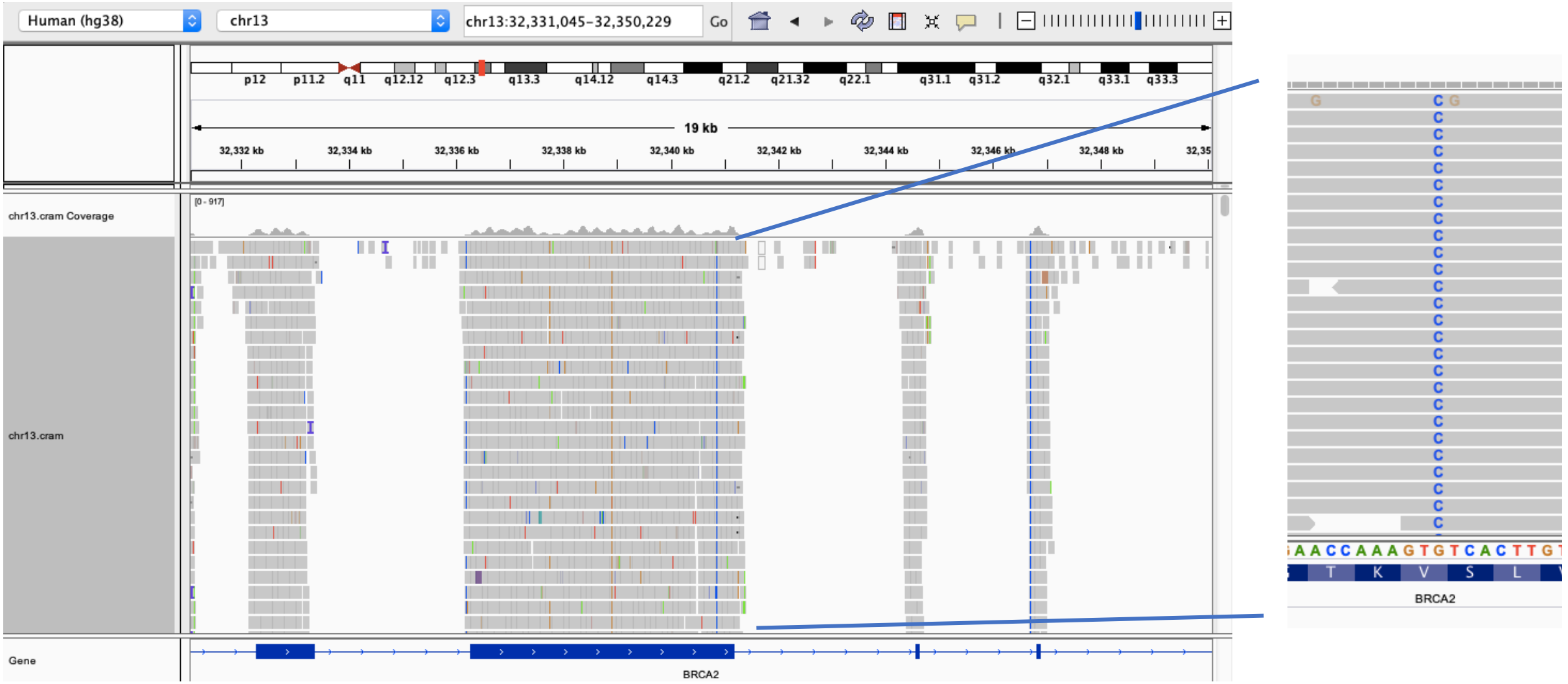
Start with Analysis-Ready Reads



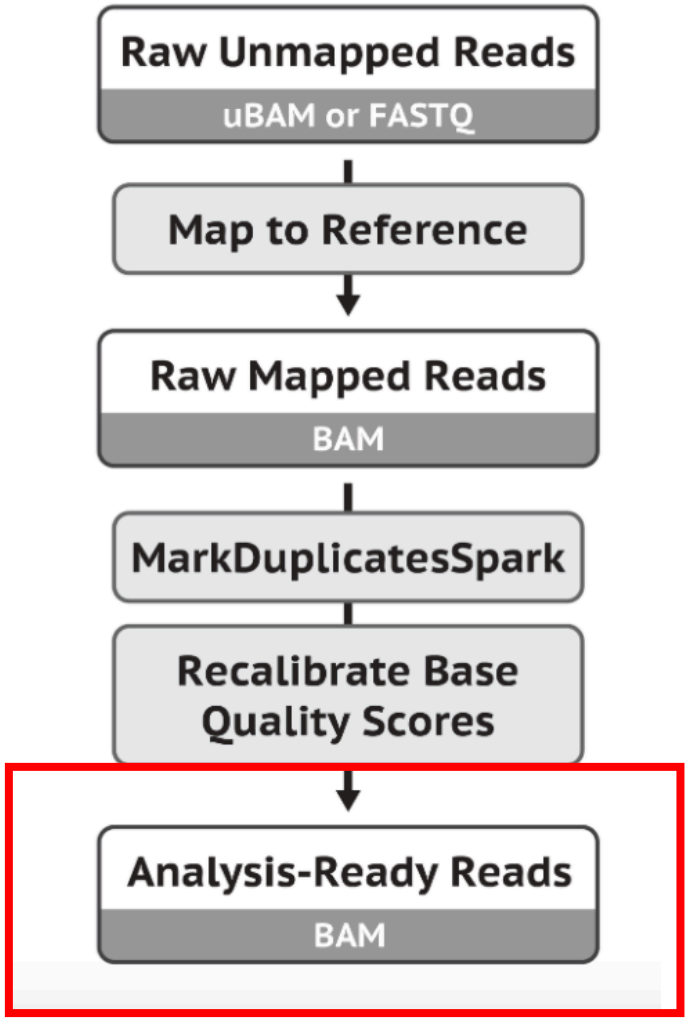
https://gatk.broadinstitute.org/hc/en-us/articles/360035535932-Germline-short-variant-discovery-SNPs-Indels-

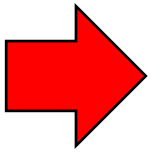
Read Alignment Visualization

Exome Sequencing Data from NA12878



Genotype NGS samples with GATK HaplotypeCaller





GATK HaplotypeCaller

https://gatk.broadinstitute.org/hc/en-us/articles/360035535932-Germline-short-variant-discovery-SNPs-Indels-

Call Genotypes Using GATK HaplotypeCaller

How HaplotypeCaller works

1. Define active regions

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

2. Determine haplotypes by assembly of the active region

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

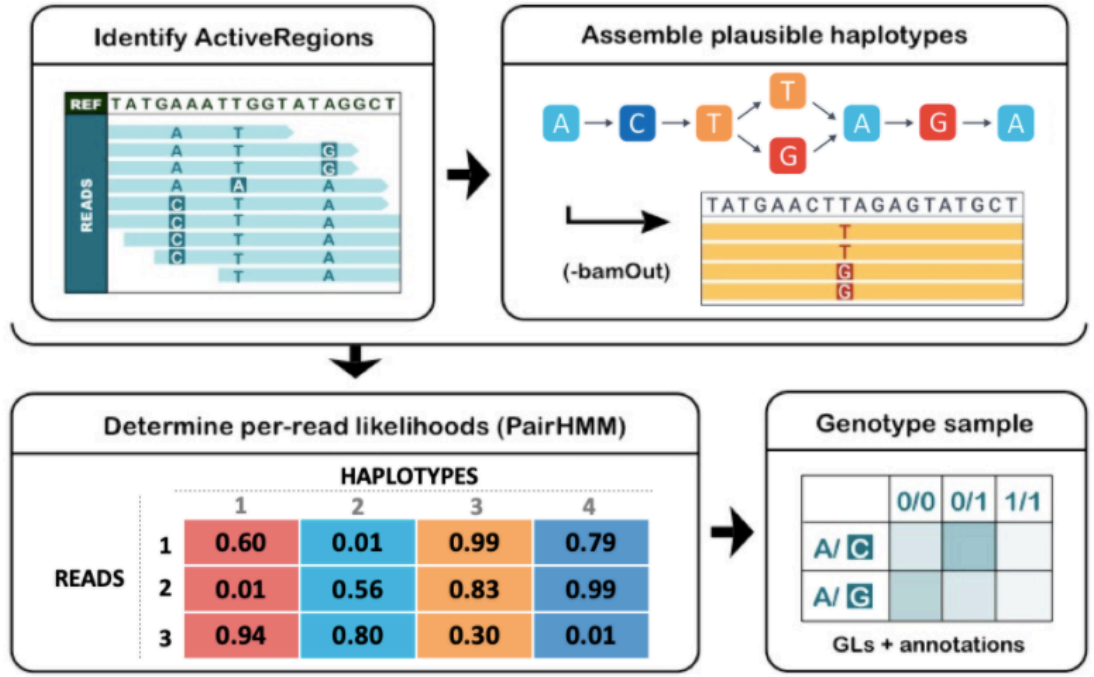
3. Determine likelihoods of the haplotypes given the read data

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

4. Assign sample genotypes

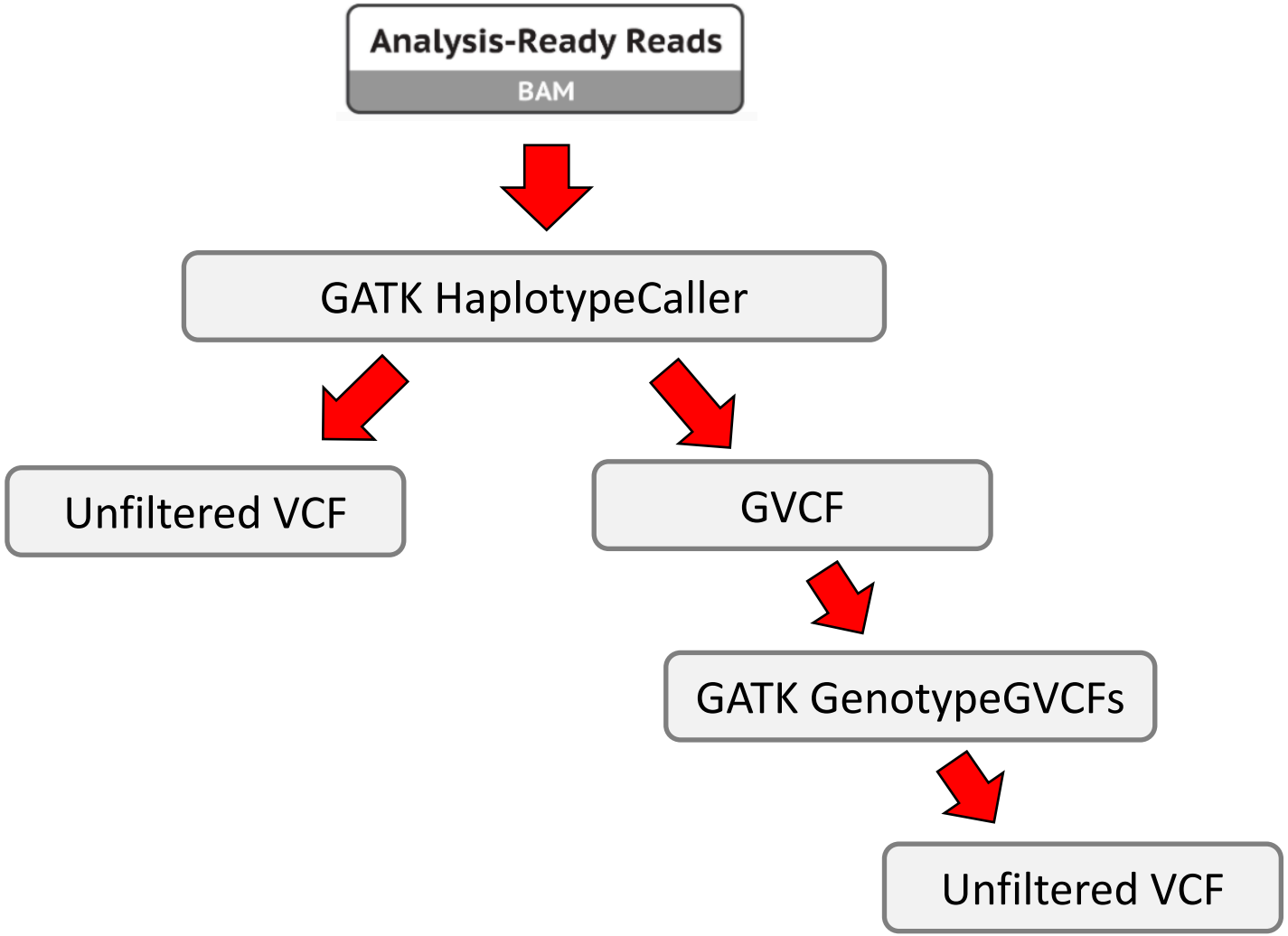
For each potentially variant site, the program applies Bayes' rule, using the likelihoods of alleles given the read data to calculate the likelihoods of each genotype per sample given the read data observed for that sample. The most likely genotype is then assigned to the sample.

https://gatk.broadinstitute.org/hc/en-us/articles/360037225632-HaplotypeCaller



https://gatk.broadinstitute.org/hc/en-us/articles/360035531412

Two Methods for Variant Calling with HaplotypeCaller



Unfiltered VCF Output

header

```
#contig=<ID=HLA-DRB1*01:02:01,length=11229>
#contig=<ID=HLA-DRB1*03:01:01:01,length=13908>
#contig=<ID=HLA-DRB1*03:01:01:02,length=13426>
#contig=<ID=HLA-DRB1*04:03:01,length=15246>
#contig=<ID=HLA-DRB1*07:01:01:01,length=16110>
#contig=<ID=HLA-DRB1*07:01:01:02,length=16120>
#contig=<ID=HLA-DRB1*08:03:02,length=13562>
#contig=<ID=HLA-DRB1*09:21,length=16039>
#contig=<ID=HLA-DRB1*10:01:01,length=13501>
#contig=<ID=HLA-DRB1*11:01:01,length=13921>
#contig=<ID=HLA-DRB1*11:01:02,length=13931>
#contig=<ID=HLA-DRB1*11:04:01,length=13919>
#contig=<ID=HLA-DRB1*12:01:01,length=13404>
#contig=<ID=HLA-DRB1*12:17,length=11260>
#contig=<ID=HLA-DRB1*13:01:01,length=13935>
#contig=<ID=HLA-DRB1*13:02:01,length=13941>
#contig=<ID=HLA-DRB1*14:05:01,length=13933>
#contig=<ID=HLA-DRB1*14:54:01,length=13936>
#contig=<ID=HLA-DRB1*15:01:01:01,length=11080>
#contig=<ID=HLA-DRB1*15:01:01:02,length=11571>
#contig=<ID=HLA-DRB1*15:01:01:03,length=11056>
#contig=<ID=HLA-DRB1*15:01:01:04,length=11056>
#contig=<ID=HLA-DRB1*15:02:01,length=10313>
#contig=<ID=HLA-DRB1*15:03:01:01,length=11567>
#contig=<ID=HLA-DRB1*15:03:01:02,length=11569>
#contig=<ID=HLA-DRB1*16:02:01,length=11005>
#source=HaplotypeCaller
#bcftools_viewVersion=1.10.2-91-g365d117+htslib-1.10.2-109-gdcd4b73
#bcftools_viewCommand=view -r chr13 NA12878-HG001-merged.vcf.gz; Date=Tue Oct 20 08:55:27 2020
```

calls

```
##bcftools_viewCommand=view -r chr13 NA12878-HG001-merged.vcf.gz; Date=Tue Oct 20 08:55:27 2020
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  NA12878-HG001
chr13   16002338        .       G       A       61.65   .       AC=1;AF=0.5;AN=2;BaseQRankSum=-0.674;DP=4;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=25.51;MQRankSum=0.319;QD=15.41;ReadPosRankSum=-0.319;SOR
chr13   16003734        .       A       G       64.64   .       AC=1;AF=0.5;AN=2;BaseQRankSum=-0.842;DP=6;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=48.15;MQRankSum=0.842;QD=10.77;ReadPosRankSum=-0.842;SOR
chr13   16003747        .       G       T       61.64   .       AC=1;AF=0.5;AN=2;BaseQRankSum=-0.366;DP=7;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=47.07;MQRankSum=1.068;QD=8.81;ReadPosRankSum=0.566;SOR=0
chr13   16003803        .       C       T       113.64  .       AC=1;AF=0.5;AN=2;BaseQRankSum=1.834;DP=6;ExcessHet=3.0103;FS=4.771;MLEAC=1;MLEAF=0.5;MQ=44.96;MQRankSum=-1.834;QD=18.94;ReadPosRankSum=0.842;
chr13   16006318        .       G       A       37.32   .       AC=2;AF=1;AN=2;DP=2;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=22.55;QD=18.66;SOR=0.693 GT:AD:DP:GQ:PL  1/1:0,2:2:6:49,6,0
chr13   16007398        .       G       A       37.32   .       AC=2;AF=1;AN=2;DP=2;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=50;QD=18.66;SOR=0.693     GT:AD:DP:GQ:PL  1/1:0,2:2:6:49,6,0
chr13   16008737        .       T       A       90.84   .       AC=2;AF=1;AN=2;DP=3;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=39.03;QD=30.28;SOR=1.179 GT:AD:DP:GQ:PL  1/1:0,3:3:9:104,9,
chr13   16009860        .       A       G       151.64  .       AC=1;AF=0.5;AN=2;BaseQRankSum=-0.792;DP=7;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=48.97;MQRankSum=0.328;QD=21.66;ReadPosRankSum=1.204;SOR=
chr13   16009875        .       A       G,AT    252.06  .       AC=1,1;AF=0.5,0.5;AN=2;DP=7;ExcessHet=3.0103;FS=0;MLEAC=1,1;MLEAF=0.5,0.5;MQ=48.97;QD=25.36;SOR=0.941   GT:AD:DP:GQ:PL  1/2:0,4,3:7:99:269,10
chr13   16009887        .       CAG     C       61.6    .       AC=1;AF=0.5;AN=2;BaseQRankSum=-0.366;DP=7;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=48.97;MQRankSum=-1.465;QD=8.8;ReadPosRankSum=0;SOR=0.446
chr13   16011213        .       G       A       166.14  .       AC=2;AF=1;AN=2;DP=4;ExcessHet=3.0103;FS=0;MLEAC=2;MLEAF=1;MQ=54.78;QD=28.73;SOR=1.609    GT:AD:DP:GQ:PL  1/1:0,4:4:12:180,12,0
chr13   16011228        .       G       A       166.14  .       AC=2;AF=1;AN=2;DP=4;ExcessHet=3.0103;FS=0;MLEAC=2;MLEAF=1;MQ=54.78;QD=30.97;SOR=1.609    GT:AD:DP:GQ:PL  1/1:0,4:4:12:180,12,0
chr13   16012620        .       C       A       85.14   .       AC=2;AF=1;AN=2;DP=4;ExcessHet=3.0103;FS=0;MLEAC=2;MLEAF=1;MQ=39.56;QD=21.29;SOR=0.693    GT:AD:DP:GQ:PL  1/1:0,4:4:12:99,12,0
chr13   16015532        .       G       GA      67.28   .       AC=2;AF=1;AN=2;DP=3;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=29.58;QD=33.64;SOR=2.303 GT:AD:DP:GQ:PL  1/1:0,2:2:6:79,6,0
chr13   16015554        .       G       A       35.48   .       AC=2;AF=1;AN=2;DP=1;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=40;QD=27.24;SOR=1.609     GT:AD:DP:GQ:PL  1/1:0,1:1:3:45,3,0
chr13   16021251        .       A       G       70.64   .       AC=1;AF=0.5;AN=2;BaseQRankSum=0;DP=4;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=51.66;MQRankSum=-0.674;QD=17.66;ReadPosRankSum=0;SOR=0.69GT:A
chr13   16021268        .       T       A       70.64   .       AC=1;AF=0.5;AN=2;BaseQRankSum=0;DP=5;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=47.54;MQRankSum=-0.674;QD=17.66;ReadPosRankSum=0;SOR=0.69GT:A
chr13   16021728        .       A       G       37.32   .       AC=2;AF=1;AN=2;DP=2;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=27;QD=18.66;SOR=0.693     GT:AD:DP:GQ:PL  1/1:0,2:2:6:49,6,0
chr13   16023773        .       A       T       119.96  .       AC=2;AF=1;AN=2;DP=5;ExcessHet=3.0103;FS=0;MLEAC=2;MLEAF=1;MQ=42.69;QD=23.99;SOR=1.022    GT:AD:DP:GQ:PL  1/1:0,5:5:15:134,15,0
chr13   16042755        .       G       A       70.84   .       AC=2;AF=1;AN=2;DP=3;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=31.93;QD=23.61;SOR=1.179 GT:AD:DP:GQ:PL  1/1:0,3:3:9:84,9,0
chr13   16048306        .       T       A       58.32   .       AC=2;AF=1;AN=2;DP=2;ExcessHet=3.0103;FS=0;MLEAC=1;MLEAF=0.5;MQ=60;QD=29.16;SOR=2.303     GT:AD:DP:GQ:PL  1/1:0,2:2:6:70,6,0
```

Next Step: Filter Raw (Unfiltered) VCF file

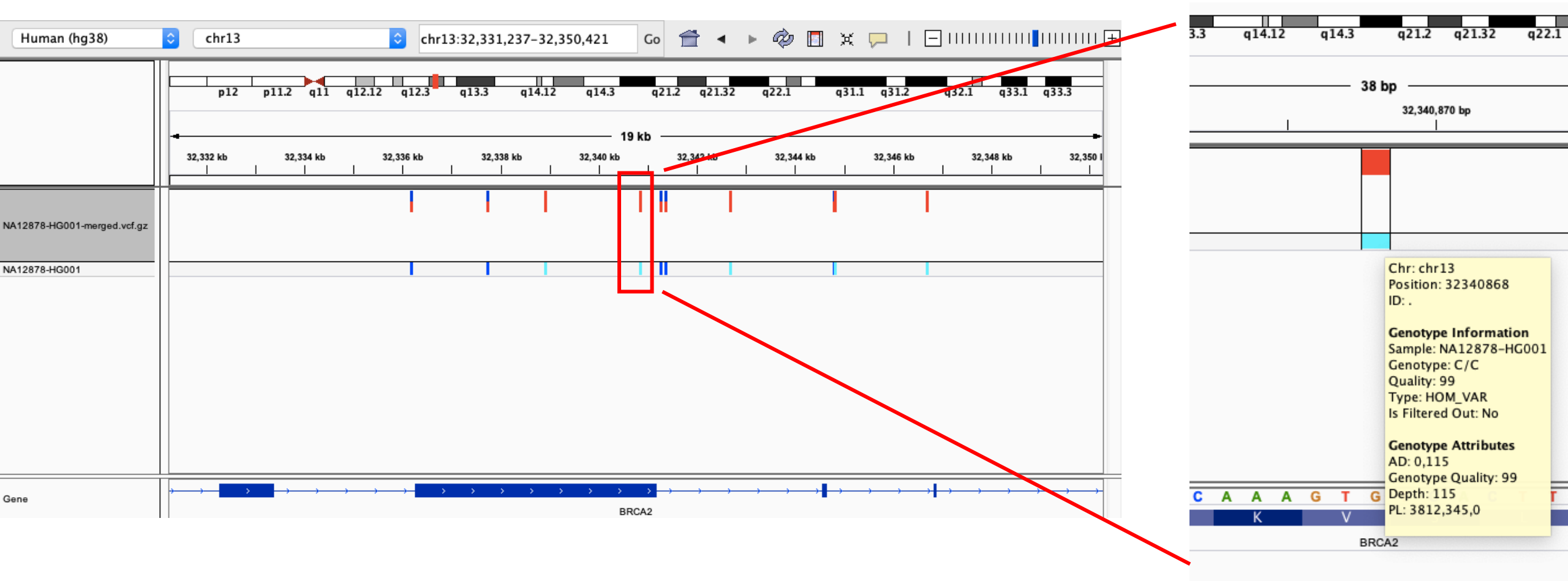
- **Problem**: **Too many Variants**
  - Non-related Human's differ by 0.1% --> 3Million SNPs (haploid genome)

- Solution: Filter Low Quality and Common Variants
  - Common Variants > 5%
  - Low Quality
    - GQ < 20, DP < 8, MQ < 40 or GATK VQSR

Filtering Criteria

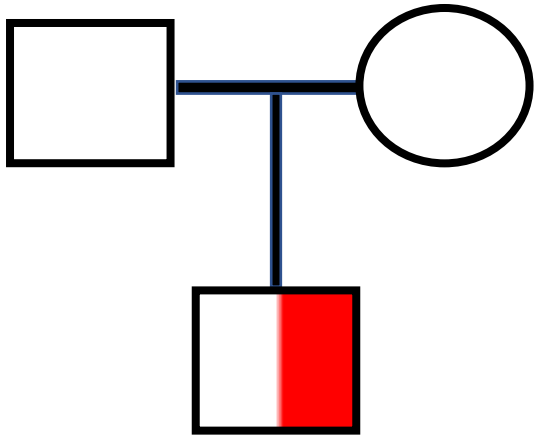
Low Quality	Variant Quality Score Filtering (VQSR) or Quality Annotations	GQ ≥ 20, DP ≥ 8, MQ ≥ 40
Remove Common Variants	GNOMAD Allele Frequency	GNOMAD_freq < .05
*Select Region	CDS (Whole Exome Sequencing)	chr13

VCF Visualization with IGV

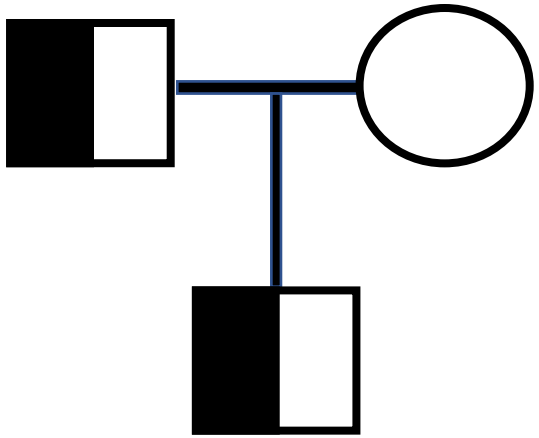


Further Analysis after SNV Calling

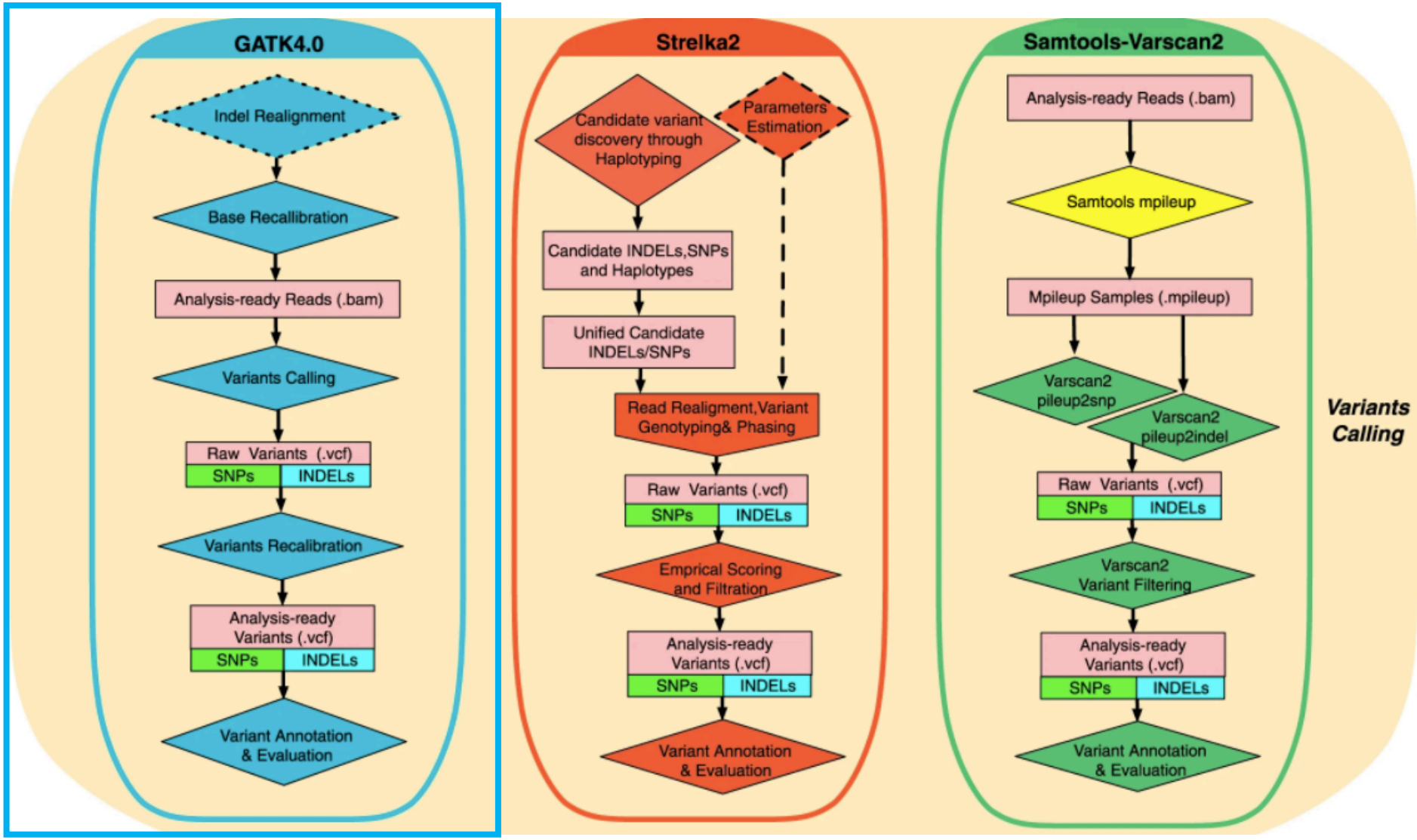
- De-novo Analysis with Trios



- Rare Transmitted Analysis



Germline SNV Calling Tools



Chen, J., Li, X., Zhong, H. *et al.* Systematic comparison of germline variant calling pipelines cross multiple next-generation sequencers. *Sci Rep* **9**, 9345 (2019). https://doi.org/10.1038/s41598-019-45835-3

GATK4 Docker Image

Use Docker Image with GATK4 Already Installed

- Use GATK4 Docker image: https://hub.docker.com/r/broadinstitute/gatk/
- Advantage over local installation: No need to download multiple programs and worry about incompatibilities
  - Easy to use different versions of GATK4

How to use GATK4

- Use commands in same manner as Linux command line
  - Link for syntax: https://gatk.broadinstitute.org/hc/en-us/articles/360035531892
  - Includes Picard tools
- List all tools: /gatk/gatk --list

Extra Information

GATK4 Installation

Download Files

- See here for guide: https://gatk.broadinstitute.org/hc/en-us/articles/360036194592-Getting-started-with-GATK4
- Download GATK4 jar files: https://github.com/broadinstitute/gatk/releases
- Note: You will need to download a few other programs to run full SNV discovery workflows
  - BWA-MEM
  - SAM-Tools

or

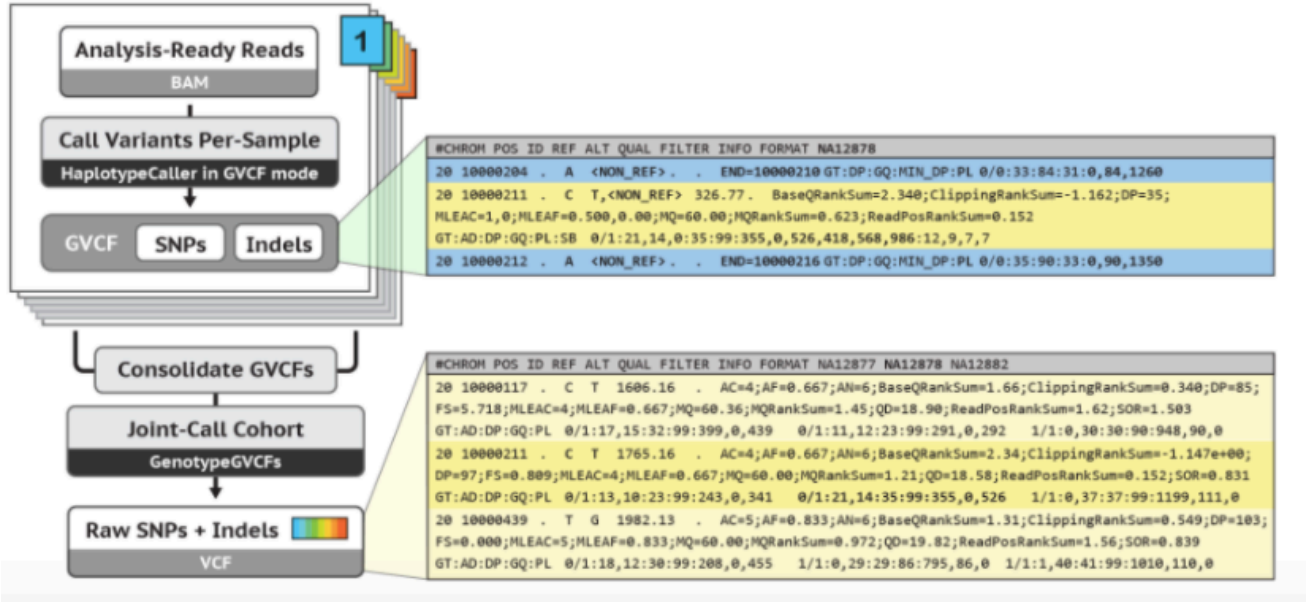
Use Docker Image with GATK4 Already Installed

- Use GATK4 Docker image: https://hub.docker.com/r/broadinstitute/gatk/
- Guide for Local Docker use: https://gatk.broadinstitute.org/hc/en-us/articles/360035889991
  - Compute0 or compute1 will use slightly different syntax
- Advantage over local installation: No need to download multiple programs and worry about incompatibilities

HaplotypeCaller Modes

- HaplotypeCaller Modes
  1. VCF
     - For Single Sample Workflow. No need to run GenotypeGVCFs.
     - No reference confidence call for Genotypes
     - Only produces calls at variant sites
  2. GVCF (Default)
     - Genomic VCF File with condensed non-variant block
     - Produces calls at all sites with compressed non-variant “blocks”
     - Scales well: For joint-analysis and joint-genotyping of large cohorts
     - Best Practices Workflow
  3. GVCF (BP_Resolution)
     - Genomic VCF File with no compression
     - Highest Resolution, but Very large file sizes
- GVCF uses reference model to emit confidence in Genotype call
  - Updates GQ and PL annotation
  - ***Intermediate File**: Must be used with GATK GenotypeGVCFs in order to produce a final vcf

GVCF Format and Information

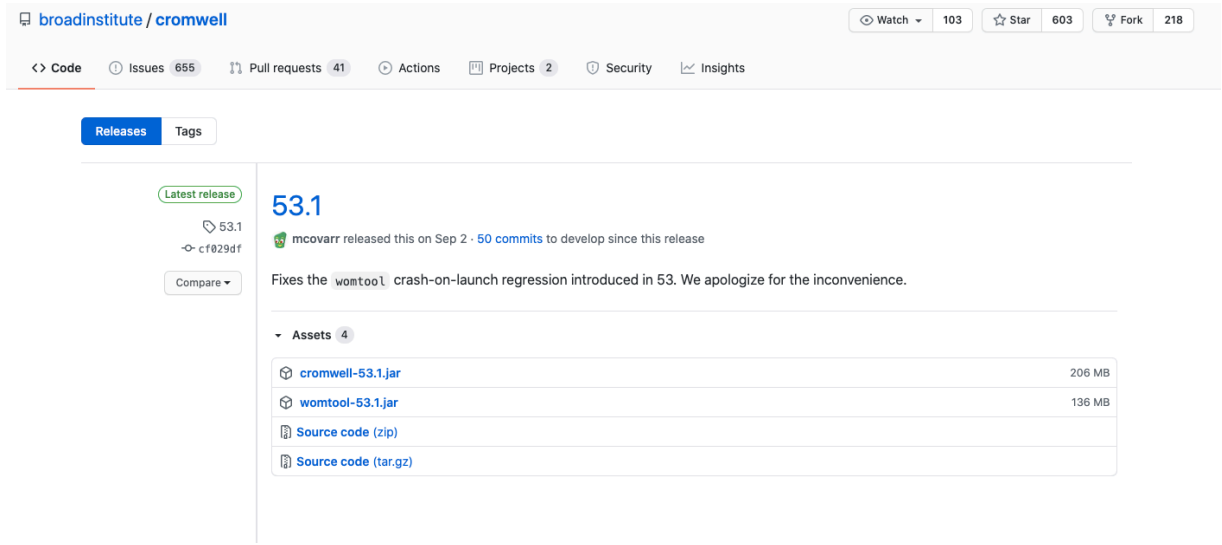


#CHROM	POS	ID	REF	ALT	QUAL	FILTER	INFO	FORMAT	NA12878-HG001
chr13	32315314	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315315	.	T	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315316	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315317	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315318	.	A	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315319	.	G	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:16,1:17:16:0,16,522
chr13	32315320	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315321	.	T	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315322	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:48:0,48,720
chr13	32315323	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:16,0:16:48:0,48,556
chr13	32315324	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:16,0:16:48:0,48,556
chr13	32315325	.	G	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591
chr13	32315326	.	C	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591
chr13	32315327	.	T	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591
chr13	32315328	.	T	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591
chr13	32315329	.	T	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591
chr13	32315330	.	A	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591
chr13	32315331	.	T	<NON_REF>	.	.	.	GT:AD:DP:GQ:PL	0/0:17,0:17:51:0,51,591

https://gatk.broadinstitute.org/hc/en-us/articles/360035531812-GVCF-Genomic-Variant-Call-Format

How to Install Cromwell Jar File

GO TO
https://github.com/broadinstitute/cromwell/releases



Download jar file directly to HPC environment with ‘wget’
wget https://github.com/broadinstitute/cromwell/releases/download/53.1/cromwell-53.1.jar

Next Step Annotation

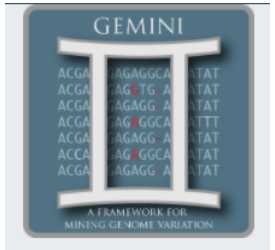
Annotate Variants with known Database Information

Annotation Tools

* Ensembl Variant Effect Predictor (VEP)

ANNOVAR

hail



Databases

gnomAD
genome aggregation database

dbNSFP