Variant calling

What are genetic variants

- **SNP (single-nucleotide polymorphism)**
  - a mutation or substitution of a single nucleotide that occurs at a specific position in the genome with at least certain frequency (e.g. > 1%).
- **SNV (single-nucleotide variant)**
  - Similar like SNP, but no frequency requirement
- **InDel**
  - Insertion or deletion of one or multiple nucleotides in the genome
- **Structural variation**
  - the variation in structure of the genome, including duplication, insertion, deletion, inverision, copy-number variation, etc.

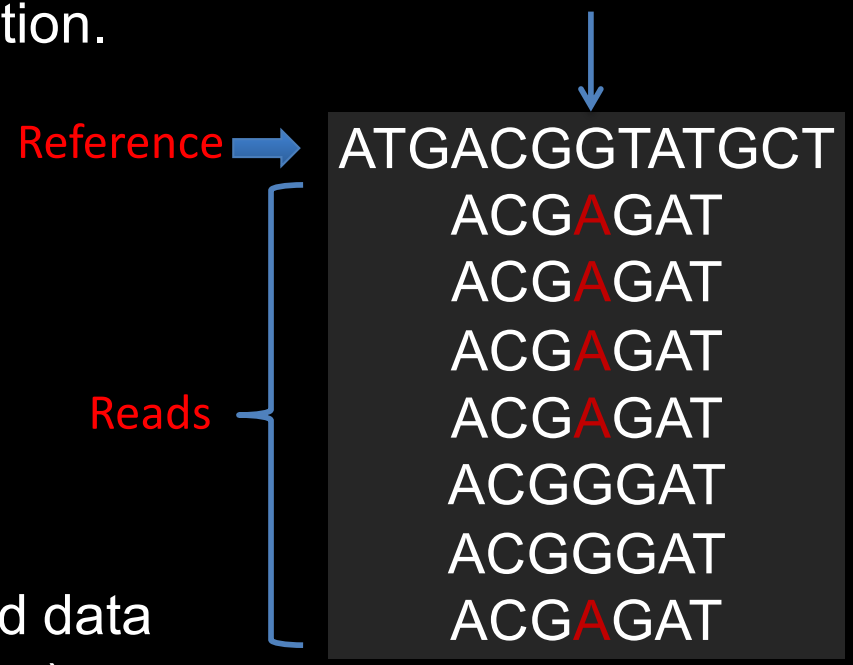
Identification of SNPs (SNP calling)

Single-nucleotide polymorphism (SNP) is the most common genetic variation among individuals. Next-generation sequencing technology provide a cost-effective tool for SNP detection.

$$P(g|D) = \text{Prior}(g).P(D|g) \,/\, \Sigma\, \text{Prior}(x).P(D|x)$$

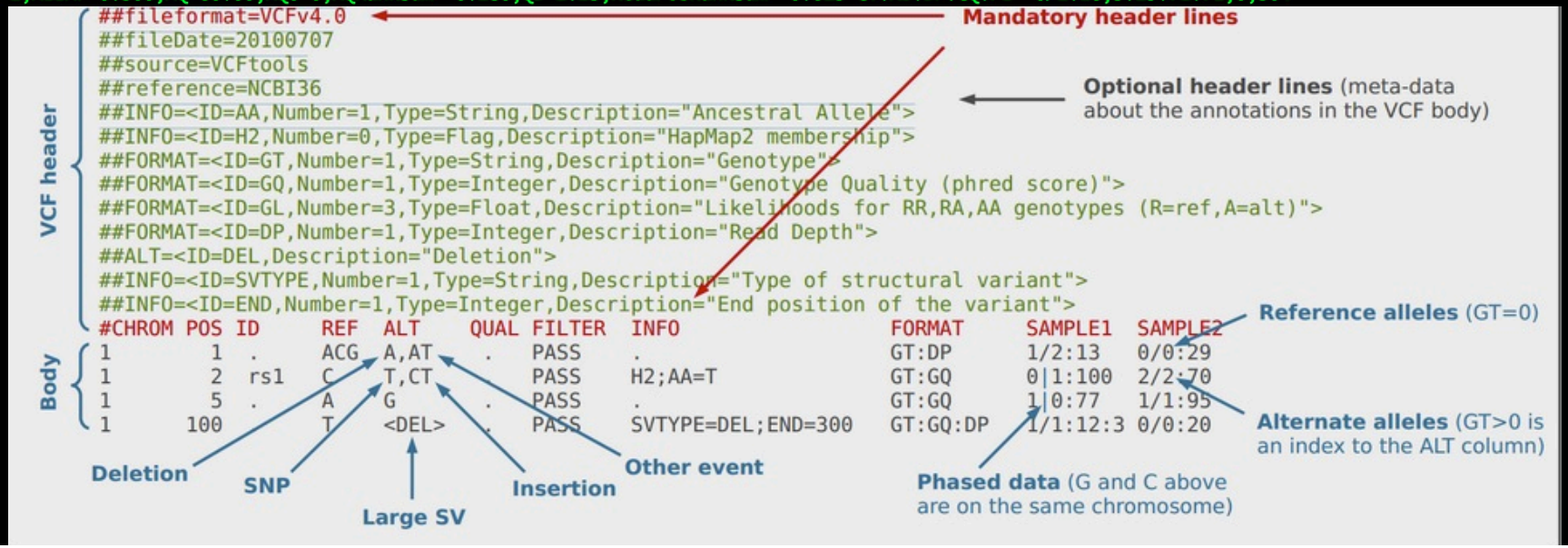
SNP calling algorithm may consider:

- Sequencing quality
- Alignment uniqueness and accuracy
- Likelihood calculation based on observed data
- Prior probability (dbSNP or other resource)



Variant calling format (VCF)

```
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele, in the same order as listed">
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency, for each ALT allele, in the same order as listed">
##INFO=<ID=AN,Number=1,Type=Integer,Description="Total number of alleles in called genotypes">
##INFO=<ID=BaseQRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxon rank sum test of Alt Vs. Ref base qualities">
##INFO=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have been filtered">
##INFO=<ID=DS,Number=0,Type=Flag,Description="Were any of the samples downsampled?">
##INFO=<ID=Dels,Number=1,Type=Float,Description="Fraction of Reads Containing Spanning Deletions">
##INFO=<ID=FS,Number=1,Type=Float,Description="Phred-scaled p-value using Fisher's exact test to detect strand bias">
##INFO=<ID=HaplotypeScore,Number=1,Type=Float,Description="Consistency of the site with at most two segregating haplotypes">
##INFO=<ID=InbreedingCoeff,Number=1,Type=Float,Description="Inbreeding coefficient as estimated from the genotype likelihoods per-sample when compared
 against the Hardy-Weinberg expectation">
##INFO=<ID=MLEAC,Number=A,Type=Integer,Description="Maximum likelihood expectation (MLE) for the allele counts (not necessarily the same as the AC), f
or each ALT allele, in the same order as listed">
##INFO=<ID=MLEAF,Number=A,Type=Float,Description="Maximum likelihood expectation (MLE) for the allele frequency (not necessarily the same as the AF),
for each ALT allele, in the same order as listed">
##INFO=<ID=MQ,Number=1,Type=Float,Description="RMS Mapping Quality">
##INFO=<ID=MQ0,Number=1,Type=Integer,Description="Total Mapping Quality Zero Reads">
##INFO=<ID=MQRankSum,Number=1,Type=Float,Description="Z-score From Wilcoxon rank sum test of Alt vs. Ref read mapping qualities">
##INFO=<ID=QD,Number=1,Type=Float,Description="Variant Confidence/Quality by Depth">
##INFO=<ID=RPA,Number=.,Type=Integer,Description="Number of times tandem repeat unit is repeated, for each allele (including reference)">
##INFO=<ID=RU,Number=1,Type=String,Description="Tandem repeat unit (bases)">
##INFO=<ID=ReadPosRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxon rank sum test of Alt vs. Ref read position bias">
##INFO=<ID=STR,Number=0,Type=Flag,Description="Variant is a short tandem repeat">
##contig=<ID=gb|BK006935.2|,length=230218>
##reference=file:///home/lijun/Day1/Variant_calling/S288C_Chromosome_I.fasta
#CHROM  POS     ID      REF     ALT     QUAL    FILTER  INFO    FORMAT  exomeSM
gb|BK006935.2|  32      .       C       T       42.77   .       AC=1;AF=0.500;AN=2;BaseQRankSum=0.615;DP=19;Dels=0.00;FS=0.000;HaplotypeScore=13.5644;
MLEAC=1;MLEAF=0.500;MQ=60.00;MQ0=0;MQRankSum=-0.280;QD=2.25;ReadPosRankSum=-0.615 GT:AD:DP:GQ:PL  0/1:16,3:19:71:71,0,504
```



Criteria to filter SNPs (or other variants)

- Hard filter
  - QUAL (Phrep quality) 20
  - DP (Depth) 5
  - QD (QualByDepth) 2
  - AC (Total number of alternate alleles called) 2
  - RPB (Mann-Whitney U test of Read Position Bias (bigger is better))
  - FS (FisherStrand) 60
  - MQ (RMSMappingQuality) 40
  - MQRankSum (MappingQualityRankSumTest) 12.5
  - ReadPosRankSum (ReadPosRankSumTest) 8.0

- Soft filter
  - Variant Quality Score Recalibration (VQSR)
  - Add filter information instead of remove variants

Softwares for SNP calling

- Samtools/bcftools
  - Complete solution for variant discovery
  - Hard and soft filter
  - Multiplody supported

- GATK
  - Complete solution for variant discovery
  - Both hard filter and soft (self-learned) filter (e.g. human data)
  - Multiplody supported

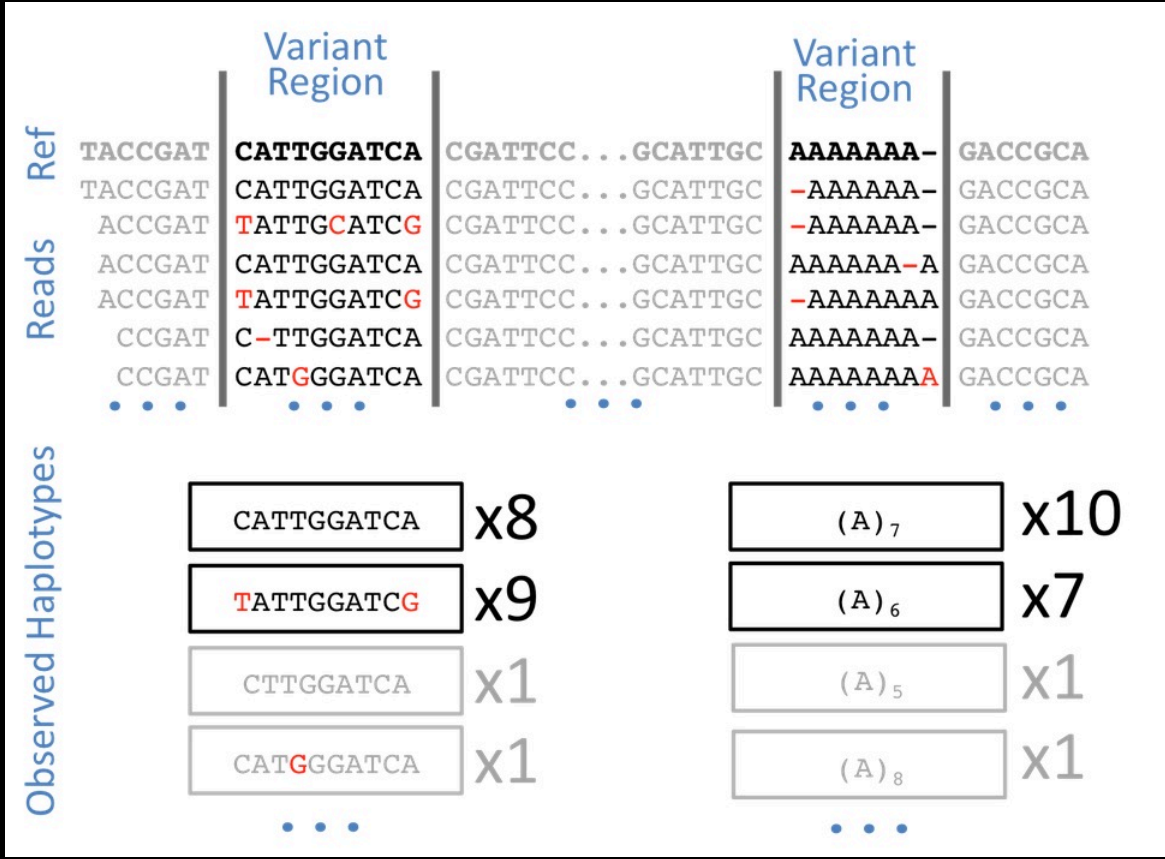
- Freebayes
  - Variant calling
  - Hard filter
  - Multiplody supported, population or pool sample supported

Variant calling in metagenome

- Difficulty
  - a mixture of genome DNA
  - Highly variable abundance
  - Multiple strains for each species
  - Higher uncertainty in alignment
- Software available
  - Freebayes

Why Freebayes works better for calling variants in metagenomes?

- Not precise alignment based
- Flexible ploidy
- Capable to deal with pool samples
- Population diversity incorporated



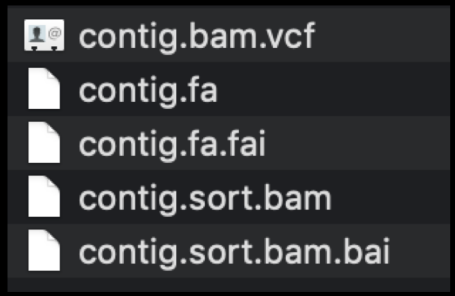
Important parameters in Freebayes

- -T (theta) -- The expected mutation rate or pairwise nucleotide diversity
- -p -- ploidy
- -J -- pooled-discrete
- -K --pooled-continuous

Visualize the variants

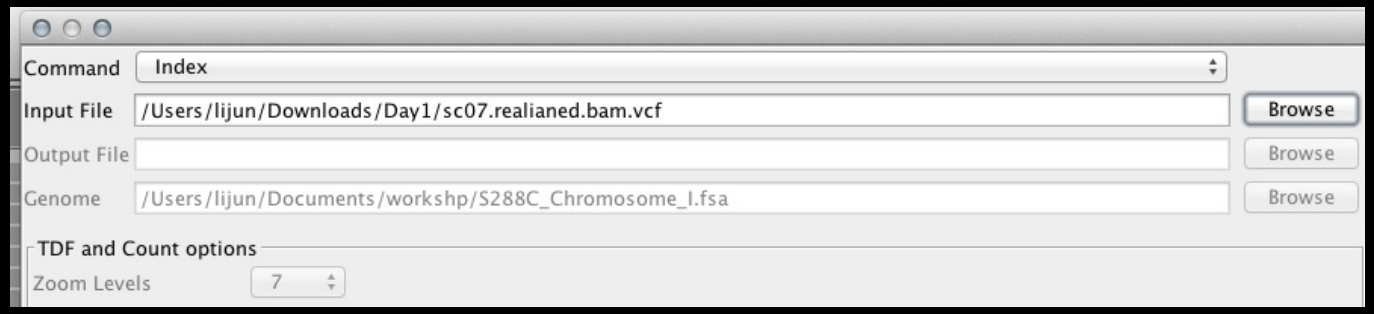
- Open IGV and go to “Genome”-> “load genome from file” , then select “contig.fa” in your pc

Transfer these files to your pc



- Load bam alignment “contig.sort.bam”

- Go to “Tools” -> “Run igvtools”,“Browse” your vcf file ” contig.bam.vcf” and select command “Index”. Click “Run”



- Load vcf file “contig.bam.vcf”

What you see is like

Practice

- Map yeast reads to the reference genome and refine the alignment
- Call variants using bcftools
- Filter the variants
- Call variants in the demo metagenome data
- Filter the variants using vcffilter
- Visualize the variants