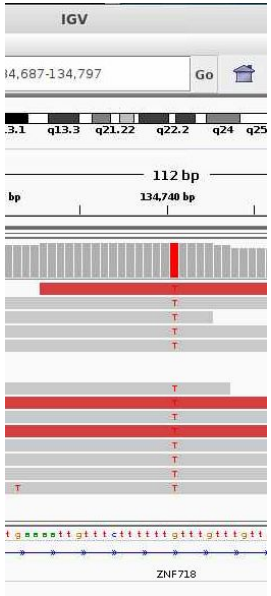
BCB 716 - Sequence Analysis

- Lots of problem set issues requires a change in due date for PS#1/#2 to 11/23
- Will discuss issues at end of lecture
- More schedule changes coming

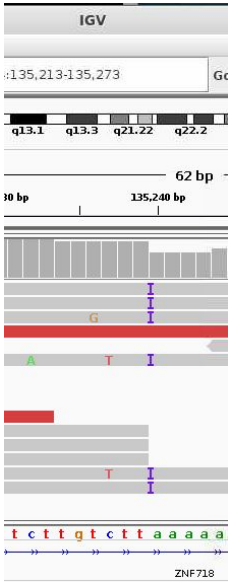
DNA Variant Calling

From last time

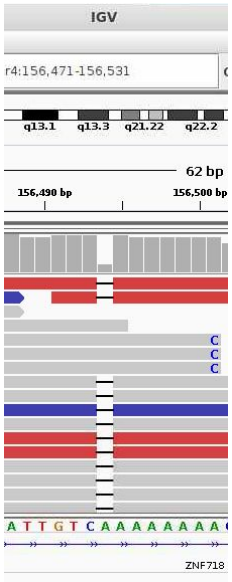
- **We noticed using IGV that there are many variants in Genomes**



g/T SNP



Insertion of an A



Deletion of an A

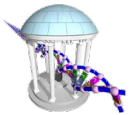
Structural variant (large 11kb deletion)

One of the most popular variant callers

- GATK is a toolkit developed at the Broad Institute for variant calling
- Includes a wide array of tools, many overlapping functions in SAMtools
- Tools for calling variants in a single sample relative to a reference
- Tools for calling variants in a population of multiple sequenced samples
- Tools for calling variants in cases/controls from same sample/organism

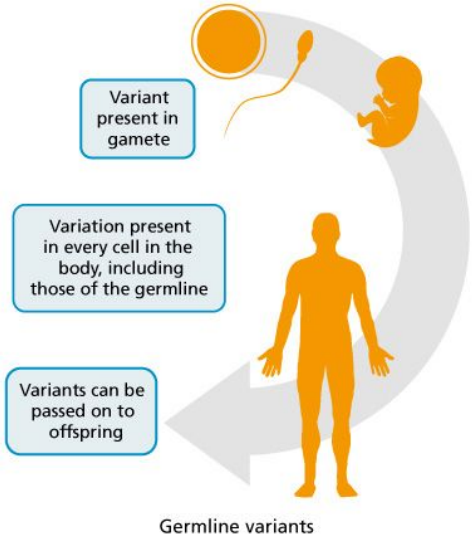
	GERMLINE	SOMATIC
SNPs & INDELs	HaplotypeCaller GVCF	MuTect2
Copy Number	GATK gCNV	GATK CNV + aCNV
Structural Variation	GATK SVDiscovery (beta)	(planned)

We will look for Germline Variants

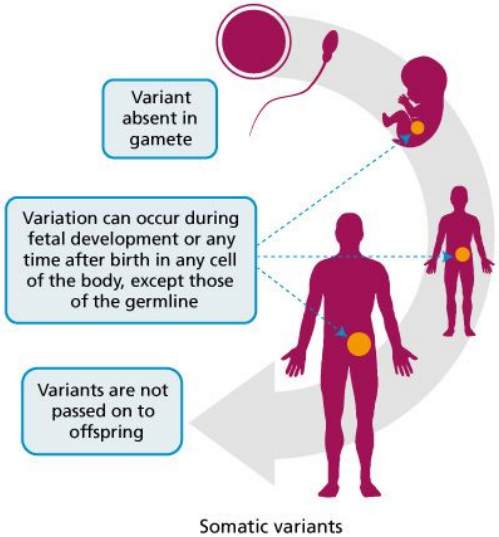


Inherited genome

(basis genome of the germ cells from which an organism is derived)



Germline variants

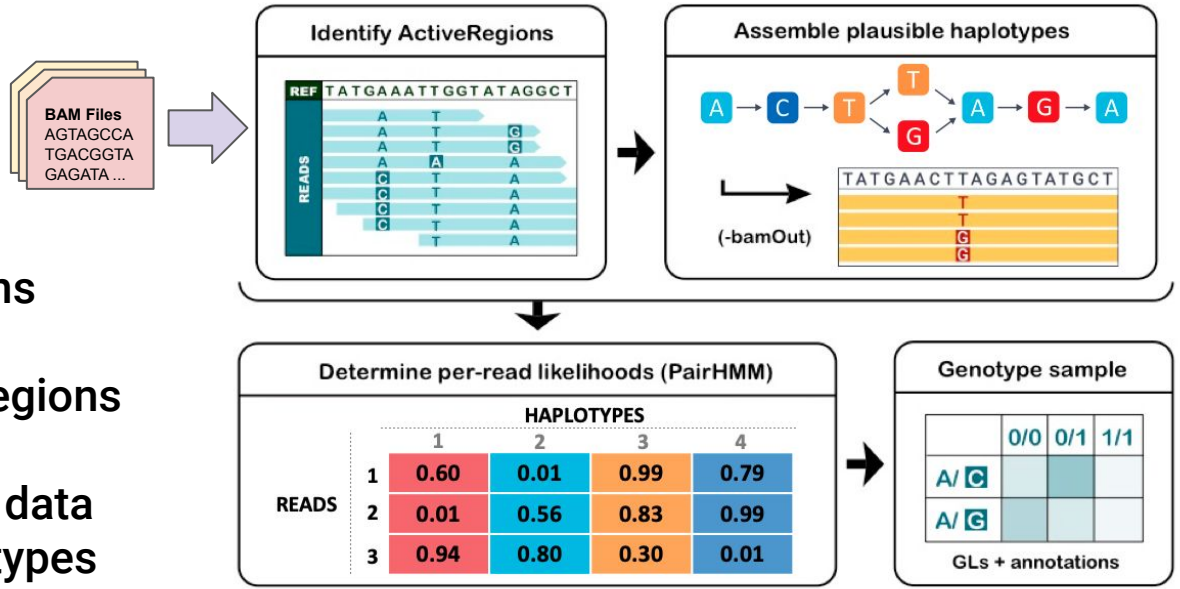


Somatic variants

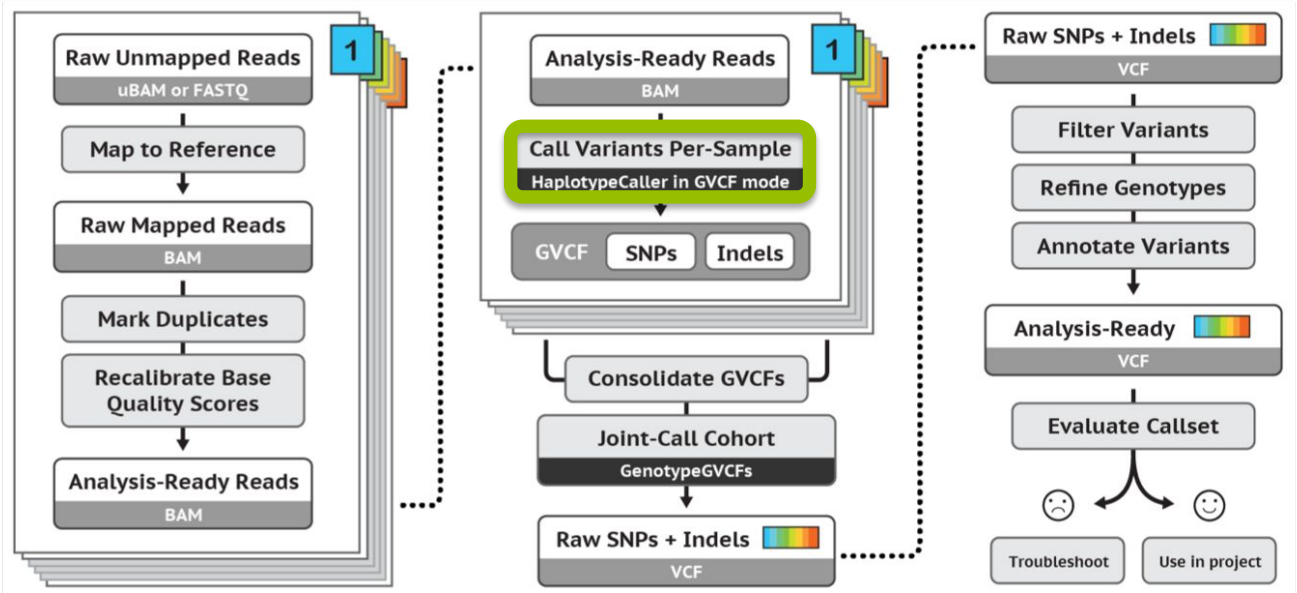
HaplotypeCaller

Four Steps:

1. Identifies active regions
2. Finds haplotypes by reassembling active regions
3. Assesses haplotype likelihoods given read data
4. Assigns sample genotypes



Workflow for germline short variant discovery

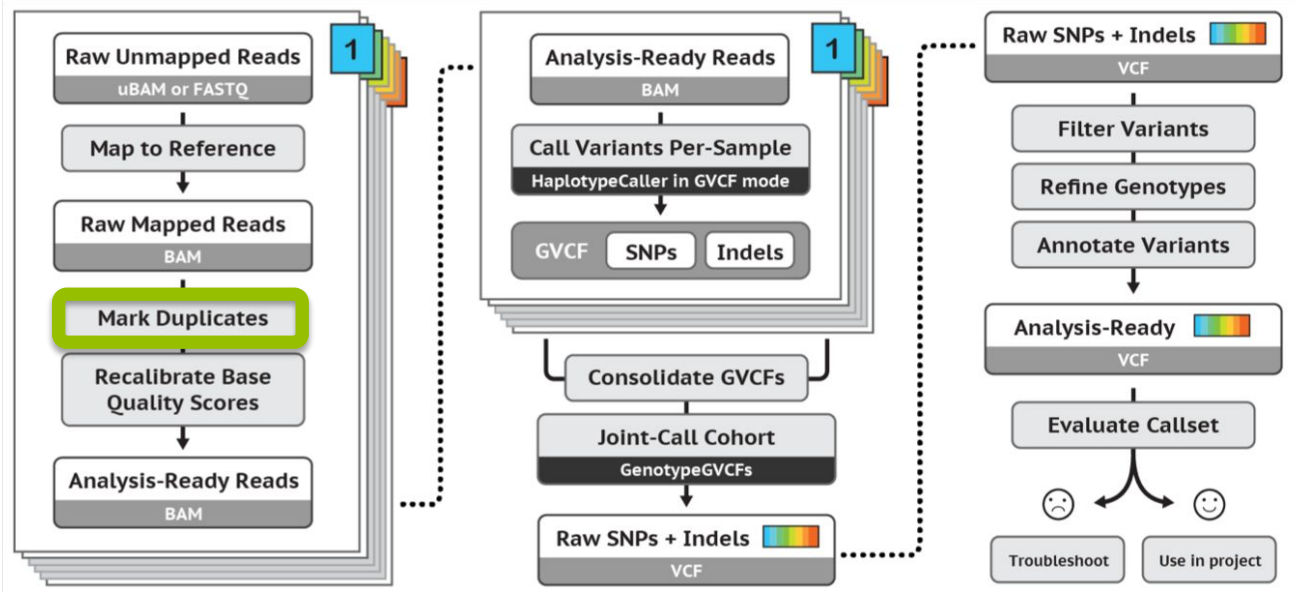


But you don't just run GATK on your BAM file!

You should do a series of preprocessing steps called "best-practices"

https://software.broadinstitute.org/gatk/best-practices/

Mark Duplicates

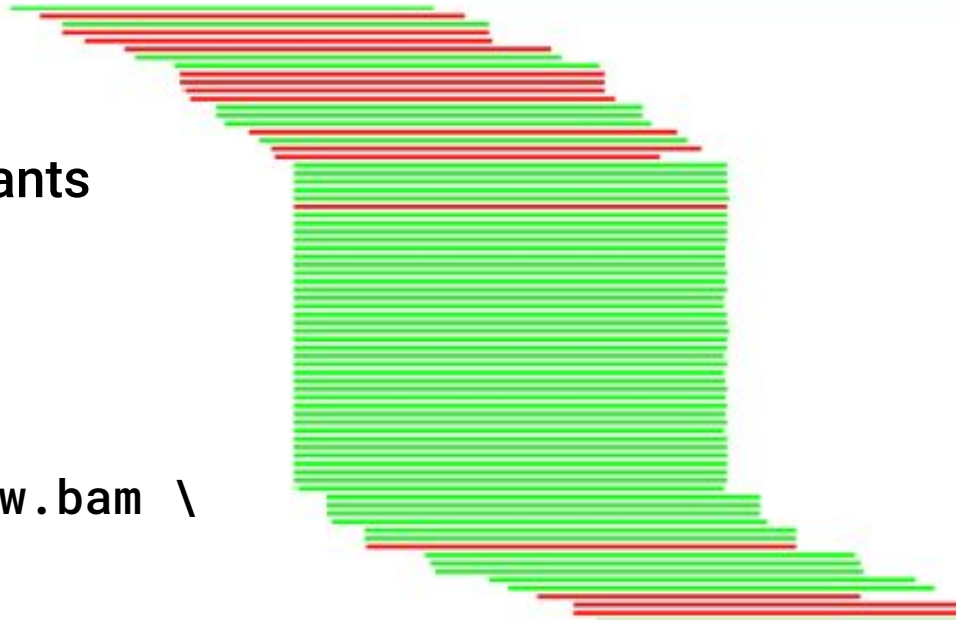


A considerable number of reads are "identical" technical artifacts

Both mated reads have the same exact sequence

Duplicate reads

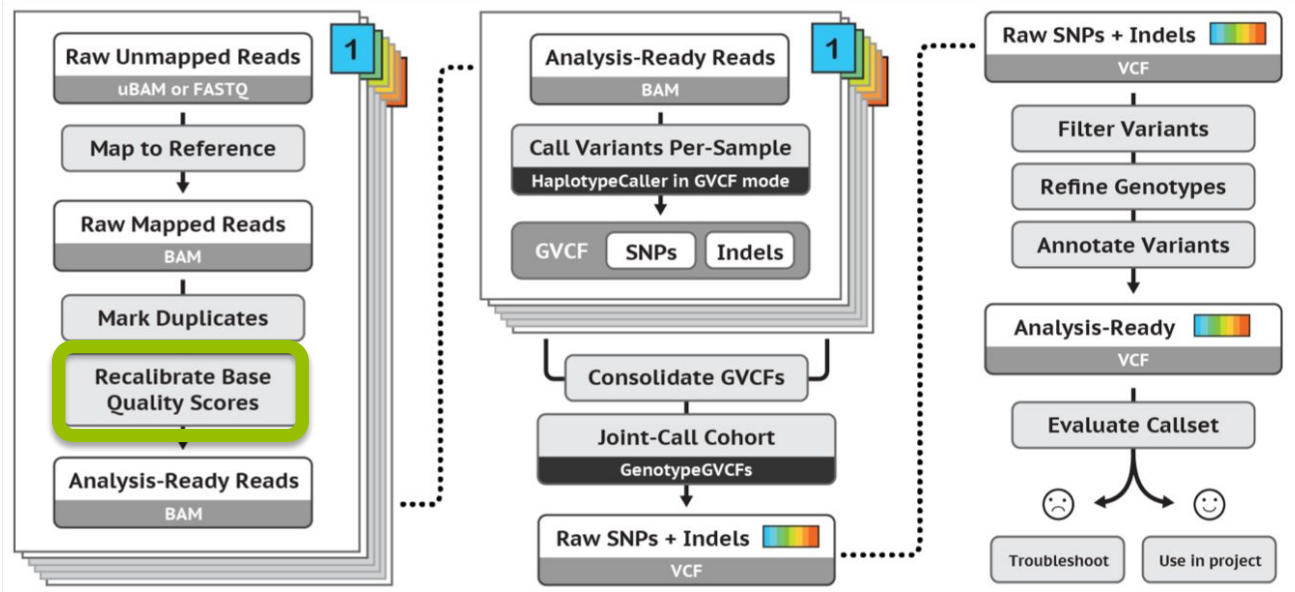
- **PCR duplicates - library preparation**
- **Optical duplicates - sequencing**
  - Multiple optical clusters in random flow cells
- **Don’t add unique information**
- **Gives false allelic ratios of variants**
- **Should be removed/marked**



Command line:

```
$ picard MarkDuplicates -I raw.bam \
      -O marked.bam \
      -M markedMetrics.txt
```

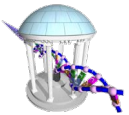
Base Quality Score Recalibration (BQSR)



The base confidence (quality string) of an individual read does not account for other reads.

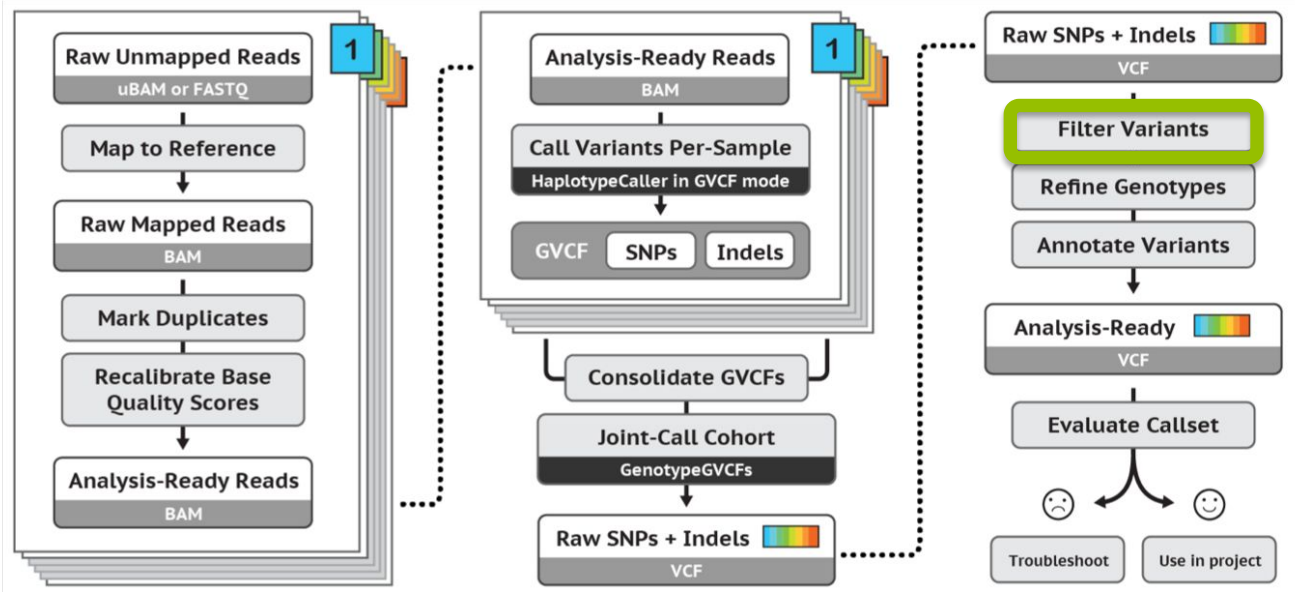
Post-alignment we can revisit the "optical" quality in the context of supporting evidence

Base Quality Score Recalibration (BQSR)



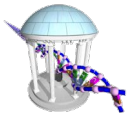
- **During base calling, the sequencer estimates a quality score for each base. This is the quality scores present in the fastq files.**
- **Systematic (non-random) errors in the base quality score estimation can occur.**
  - due to the physics or chemistry of the sequencing reaction
  - manufacturing flaws in the equipment
  - etc
- **Can cause bias in variant calling**
- **Base Quality Score Recalibration helps to calibrate the scores so that they correspond to the real per-base sequencing error rate (phred scores)**

Filter variants



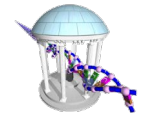
This is a post-variant calling step that selects takes a VCF file, which we will discuss shortly and identifies that high-confidence calls

Filtering



- **Remove low quality variants**
- **Variant quality score recalibration (VQSR):**
  - For large data sets (>1 WGS or >30WES samples)
  - GATK has a machine learning algorithm that can be trained to recognise ”likely false” variants
  - Use VQSR when possible!
- **Hard filters:**
  - For smaller data sets
  - Hard filters on information in the VCF file
  - For example: Flag variants with ”QD < 2” and ”MQ< 40.0”
  - GATK recommendations on hard filters: https://gatkforums.broadinstitute.org/gatk/discussion/2806/howto-apply-hard-filters-to-a-call-set

For One Sample

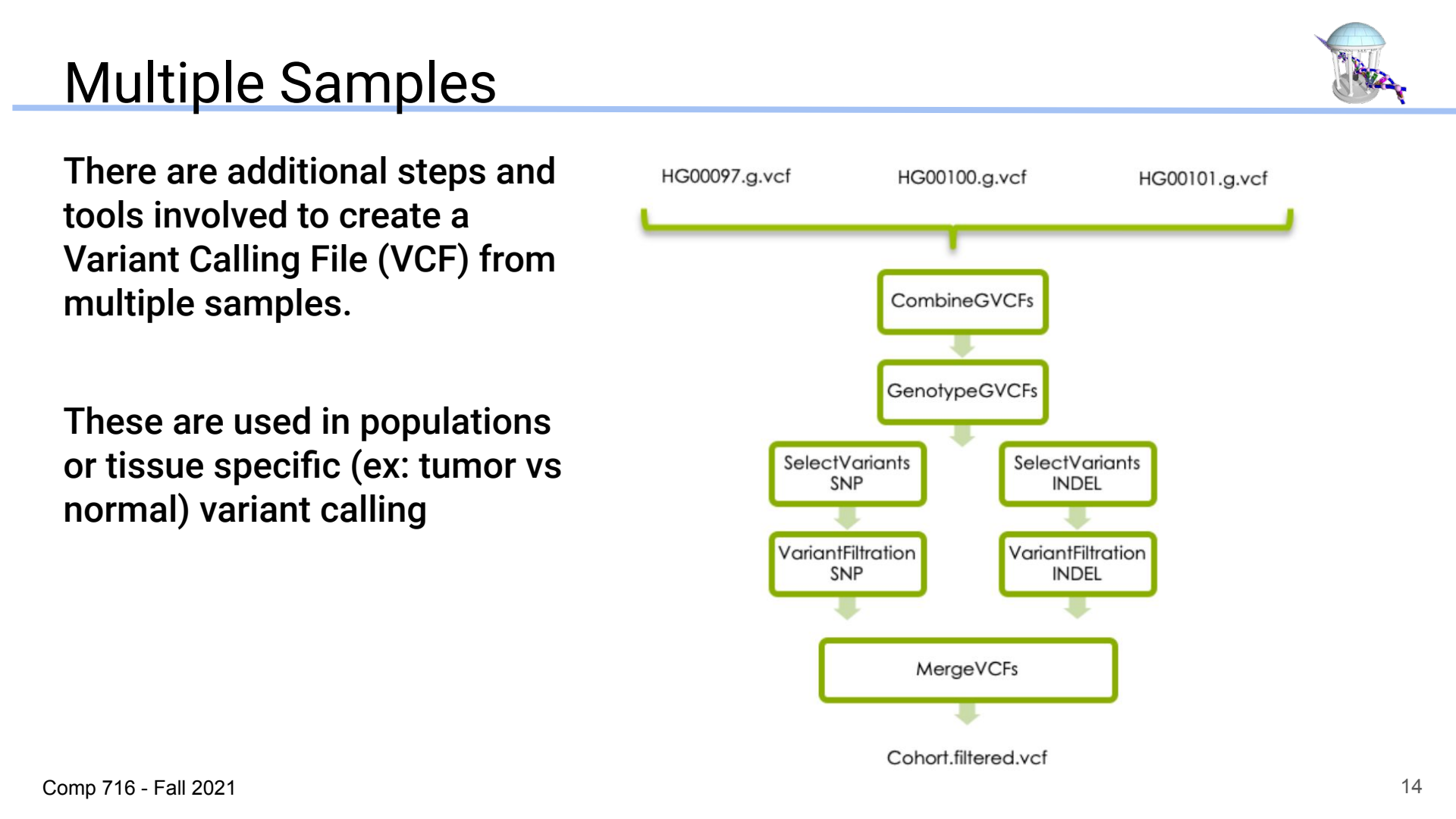


Here is a typical GATK pipeline for variant calling

1. **Align**
2. **Mark duplicates**
3. **Recalibrate Quality strings**
4. **Call Variants**

Repeat for multiple samples

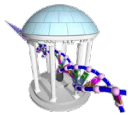
Multiple Samples



There are additional steps and tools involved to create a Variant Calling File (VCF) from multiple samples.

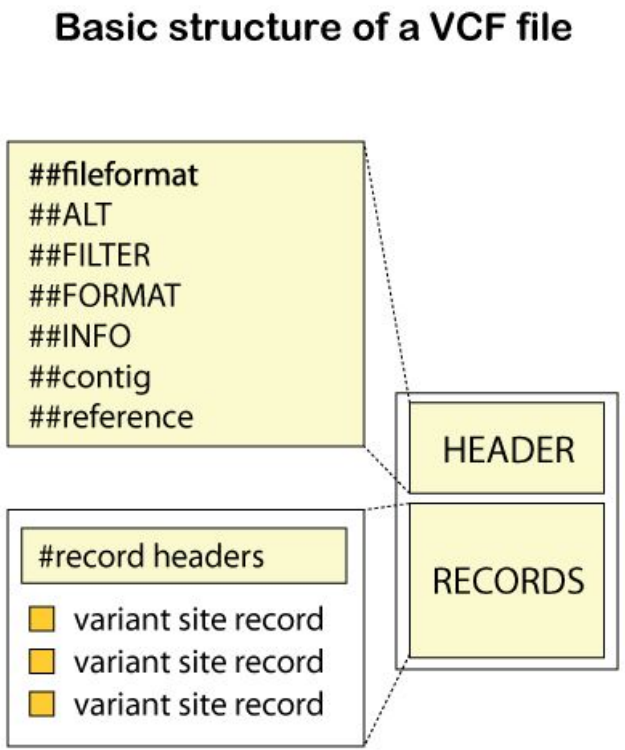
These are used in populations or tissue specific (ex: tumor vs normal) variant calling

Variant Calling Files (VCF)

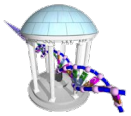


- **Multiple Headers ("##")**
  - Information about dataset
  - Reference used
  - Annotations of options and filters
  - Parameter settings
  - Command line used
  - Header column formats
  - Variant header starts with single "#"

- **Variant Calls**
  - One line for each variant
  - Annotates supporting evidence



VCF header column descriptions

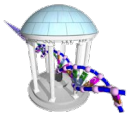


- **Part of header that describes each column of a variant call**

```
##fileformat=VCFv4.2
##ALT=<ID=NON_REF,Description="Represents any possible alternative allele at this location">
##FILTER=<ID=LowQual,Description="Low quality">
##FORMAT=<ID=AD,Number=R,Type=Integer,Description="Allelic depths for the ref and alt alleles i
##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth (reads with MQ=255 or
##FORMAT=<ID=GQ,Number=1,Type=Integer,Description="Genotype Quality">
##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=MIN_DP,Number=1,Type=Integer,Description="Minimum DP observed within the GVCF bloc
##FORMAT=<ID=PGT,Number=1,Type=String,Description="Physical phasing haplotype information, desc
##FORMAT=<ID=PID,Number=1,Type=String,Description="Physical phasing ID information, where each
##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Normalized, Phred-scaled likelihoods for ger
##FORMAT=<ID=RGQ,Number=1,Type=Integer,Description="Unconditional reference genotype confidence
##FORMAT=<ID=SB,Number=4,Type=Integer,Description="Per-sample component statistics which compri
##GATKCommandLine.HaplotypeCaller=<ID=HaplotypeCaller,Version=3.7-0-gcfedb67,Date="Fri Jan 20 1
##GATKCommandLine=<ID=GenotypeGVCFs,CommandLine="[command-line goes here]",Version=4.beta.6-117
##INFO=<ID=AC,Number=A,Type=Integer,Description="Allele count in genotypes, for each ALT allele
##INFO=<ID=AF,Number=A,Type=Float,Description="Allele Frequency, for each ALT allele, in the sa
##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
##INFO=<ID=ClippingRankSum,Number=1,Type=Float,Description="Z-score From Wilcoxon rank sum test
##INFO=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have be
##INFO=<ID=DS,Number=0,Type=Flag,Description="Were any of the samples downsampled?">
##INFO=<ID=END,Number=1,Type=Integer,Description="Stop position of the interval">
##INFO=<ID=ExcessHet,Number=1,Type=Float,Description="Phred-scaled p-value for exact test of ex
##INFO=<ID=FS,Number=1,Type=Float,Description="Phred-scaled p-value using Fisher's exact test t
##INFO=<ID=HaplotypeScore,Number=1,Type=Float,Description="Consistency of the site with at most
##INFO=<ID=InbreedingCoeff,Number=1,Type=Float,Description="Inbreeding coefficient as estimatec
##INFO=<ID=MLEAC,Number=A,Type=Integer,Description="Maximum likelihood expectation (MLE) for th
##INFO=<ID=MLEAF,Number=A,Type=Float,Description="Maximum likelihood expectation (MLE) for the
##INFO=<ID=MQ,Number=1,Type=Float,Description="RMS Mapping Quality">
##INFO=<ID=MQRankSum,Number=1,Type=Float,Description="Z-score From Wilcoxon rank sum test of Al
##INFO=<ID=QD,Number=1,Type=Float,Description="Variant Confidence/Quality by Depth">
##INFO=<ID=RAW_MQ,Number=1,Type=Float,Description="Raw data for RMS Mapping Quality">
##INFO=<ID=ReadPosRankSum,Number=1,Type=Float,Description="Z-score from Wilcoxon rank sum test
##INFO=<ID=SOR,Number=1,Type=Float,Description="Symmetric Odds Ratio of 2x2 contingency table t
```

All of these header elements are describing valid options for the "INFO" column

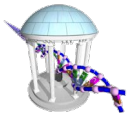
VCF variant calls



- **Tab separated columns**
- **Semicolons separate multivalued column entries**

```
[HEADER LINES]
#CHROM  POS ID      REF ALT QUAL    FILTER  INFO          FORMAT          NA12878
20  10001019    .   T   G   364.77  .   AC=1;AF=0.500;AN=2;BaseQRankSum=0.699;ClippingRankSum=0
20  10001298    .   T   A   884.77  .   AC=2;AF=1.00;AN=2;DP=30;ExcessHet=3.0103;FS=0.000;MLEAC
20  10001436    .   A   AAGGCT  1222.73 .   AC=2;AF=1.00;AN=2;DP=29;ExcessHet=3.0103;FS=0.000;M
20  10001474    .   C   T   843.77  .   AC=2;AF=1.00;AN=2;DP=27;ExcessHet=3.0103;FS=0.000;MLEAC
20  10001617    .   C   A   493.77  .   AC=1;AF=0.500;AN=2;BaseQRankSum=1.63;ClippingRankSum=0.
```

VCF call supporting evidence



Showing the FORMAT and NA12878 columns

GT: 0/0 : homozygous reference; 0/1 : heterozygous; 1/1 : the sample is homozygous alternate

AD: Allele depth data, reads with ref allele, reads with alt allele

DP: "Filtered" depth of coverage at position

GQ: Genotype Quality represents the Phred-scaled confidence

PL: "Normalized" Phred-scaled likelihoods of the possible genotypes,
one number for each genotype 1/1,0/1,0/0

```
20  10001298    .   T   A   884.77  .   [CLIPPED]   GT:AD:DP:GQ:PL  1/1:0,30:30:89:913,89,0
20  10001436    .   A   AAGGCT  1222.73 .   [CLIPPED]   GT:AD:DP:GQ:PL  1/1:0,28:28:84:1260,84,
20  10004769    .   TAAAACTATGC T   622.73  .   [CLIPPED]   GT:AD:DP:GQ:PL  0/1:18,17:35:99:660
```

Next Time

We move to RNA sequence analysis