NGS – variant analysis

Variant calling

GATK workflow

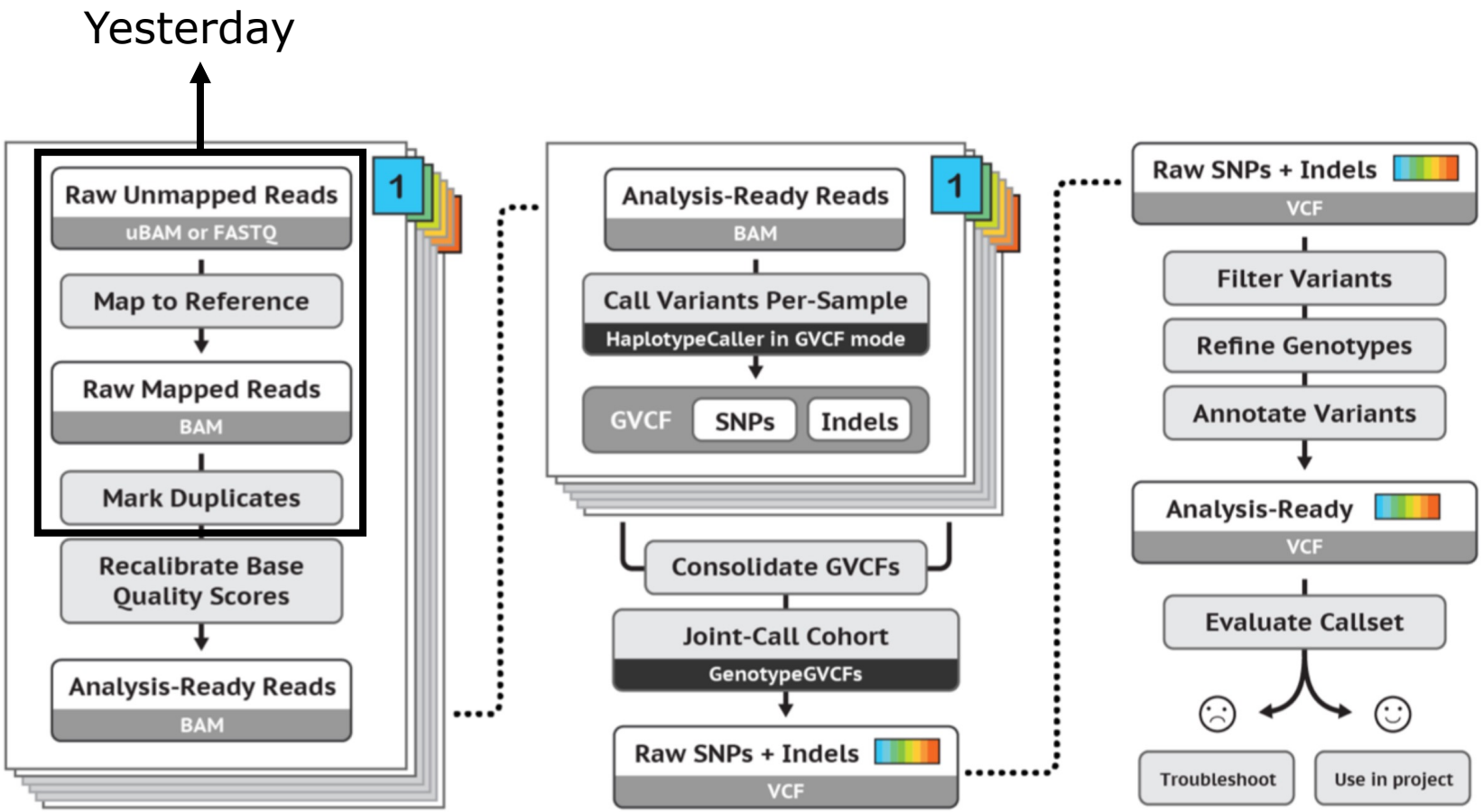
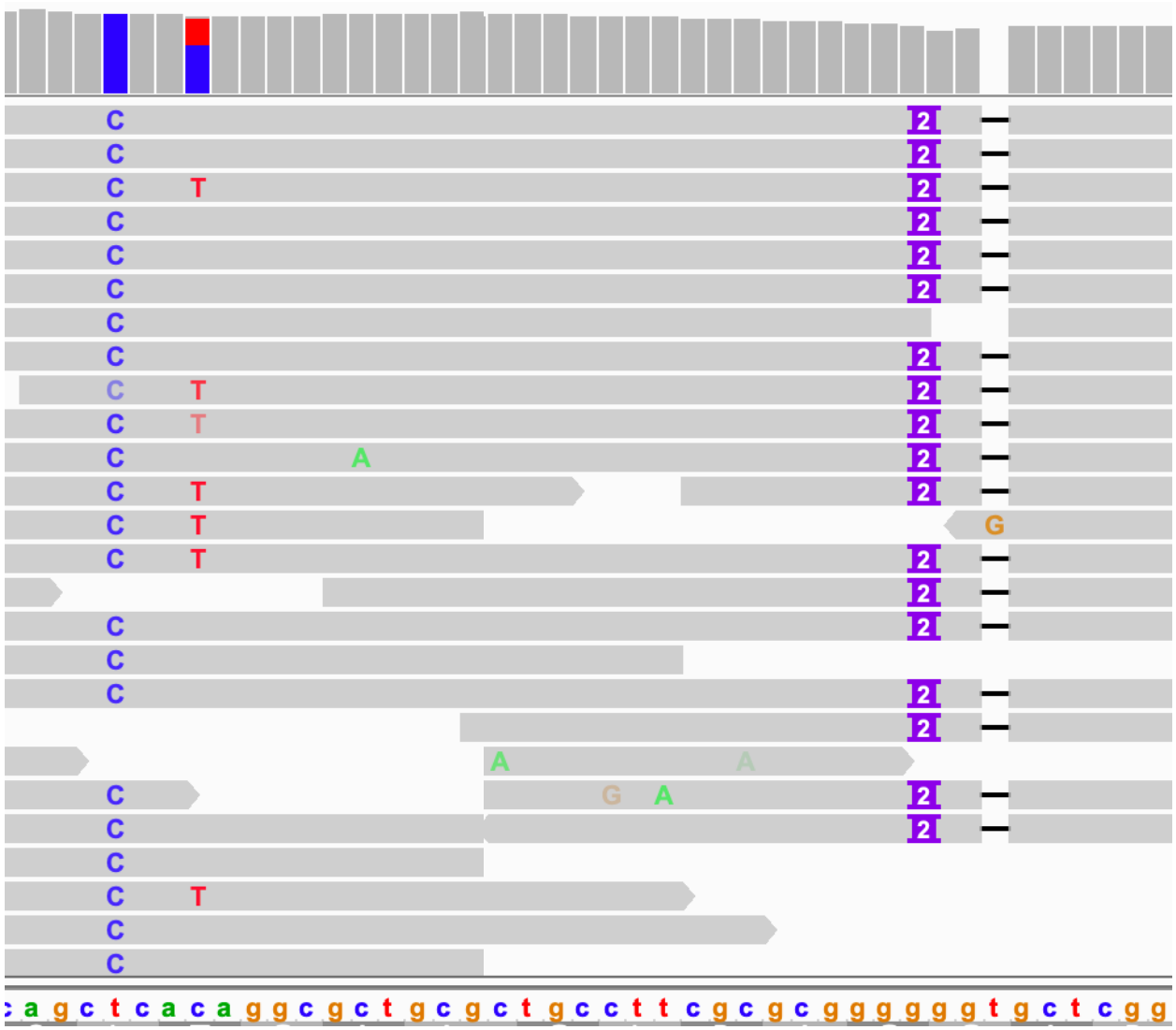


image source: https://gatk.broadinstitute.org

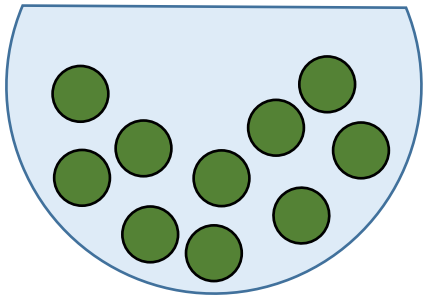


Three important questions

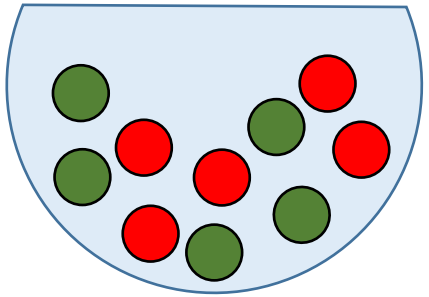
- Is there a variant at location X?
  - Deviation from REF in the alignments
- What are the alleles?
  - The variation in sequence in these deviations
- What is the genotype (HomRef, Heterozygote or HomAlt)?
  - Estimating the allele counts in the sample

Estimating genotype

What are the likely genotypes?



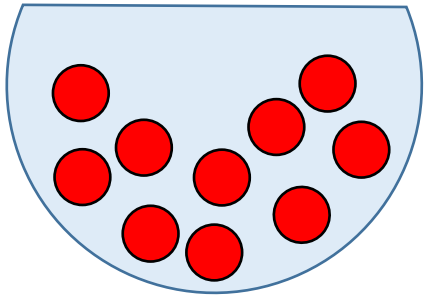
At site X in sample Y we count 9 bases:



5 REF and 4 ALT

$\Pr(X=4) = 0.25$ if heterozygous

so: $\mathcal{L}(p=0.5 \mid X=4) = 0.25$



0 REF and 9 ALT

$\mathcal{L}(p=0.5 \mid X=9) = 0.002$

$\mathcal{L}(p=1 \mid X=9) = 1$

Quiz Question 9

Estimating genotype

What are the likely genotypes?:

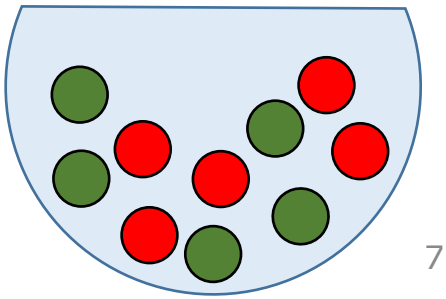
At site X in sample Y we count 9 bases:

8 REF and 1 ALT

$\mathcal{L}(\mathrm{p}{=}0.5 \mid \mathrm{X}{=}1) = 0.017$

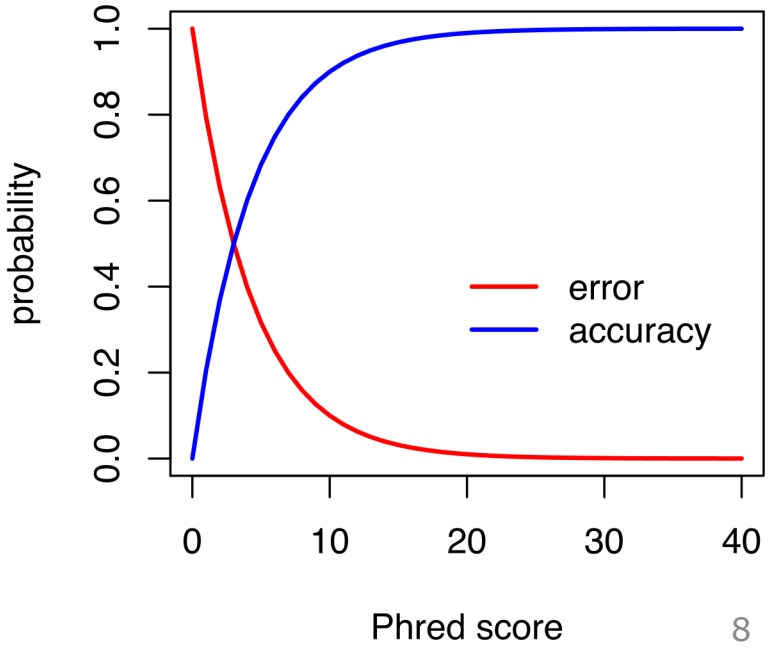
$\mathcal{L}(\mathrm{p}{=}0 \mid \mathrm{X}{=}1) = 0$

Strict binomial distribution would only work with error-free data



Base quality and error

- Base quality: 20 = error probability 0.01
- 100 samples with 40x coverage
- In total 40 errors expected



Estimating the genotype

Genotype likelihood (simplified):

$$\mathcal{L}(g)=\frac{1}{m^k}\prod_{j=1}^{l}\left[(m-g)\epsilon_j+g(1-\epsilon_j)\right]\prod_{j=l+1}^{k}\left[(m-g)(1-\epsilon_j)+g\epsilon_j\right]$$

g: genotype (i.e. 0, 1 or 2)
m: ploidy (2 for human)
ϵ: base error
k: number of bases at the site
l: number of bases that equal reference

In GATK:
$PL = -10*\log10(\mathcal{L}(g))$

Li H. Bioinformatics. 2011;27:2987–93.

PL and GQ

Our example: 8 REF and 1 ALT

Assuming base error probability $\epsilon = 0.01$

$\mathrm{PL} = \text{-}10^{*}\log10(\mathcal{L}(\mathrm{g}))$

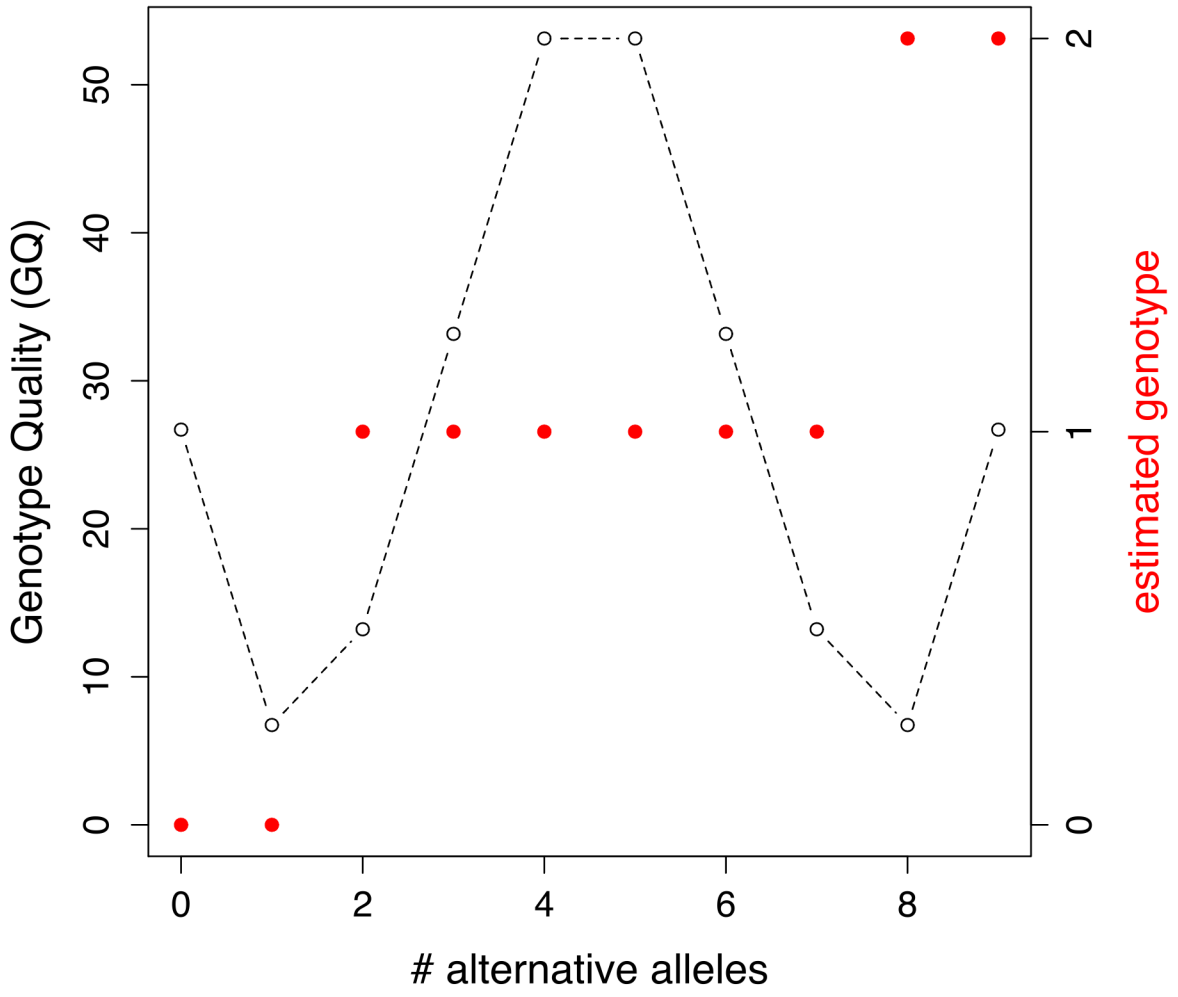
Genotype	HomRef	Heterozygous	HomAlt
$\mathcal{L}$(g)	0.0092	0.0020	9.9E-17
PL	20	27	160

Lowest PL = most likely genotype

$\mathrm{GQ} =$ Second lowest PL – Lowest PL $= 27 - 20 = 7$

$$p(genotype\ error) = 10^{\frac{-7}{10}} = 0.2$$

Estimating the genotype

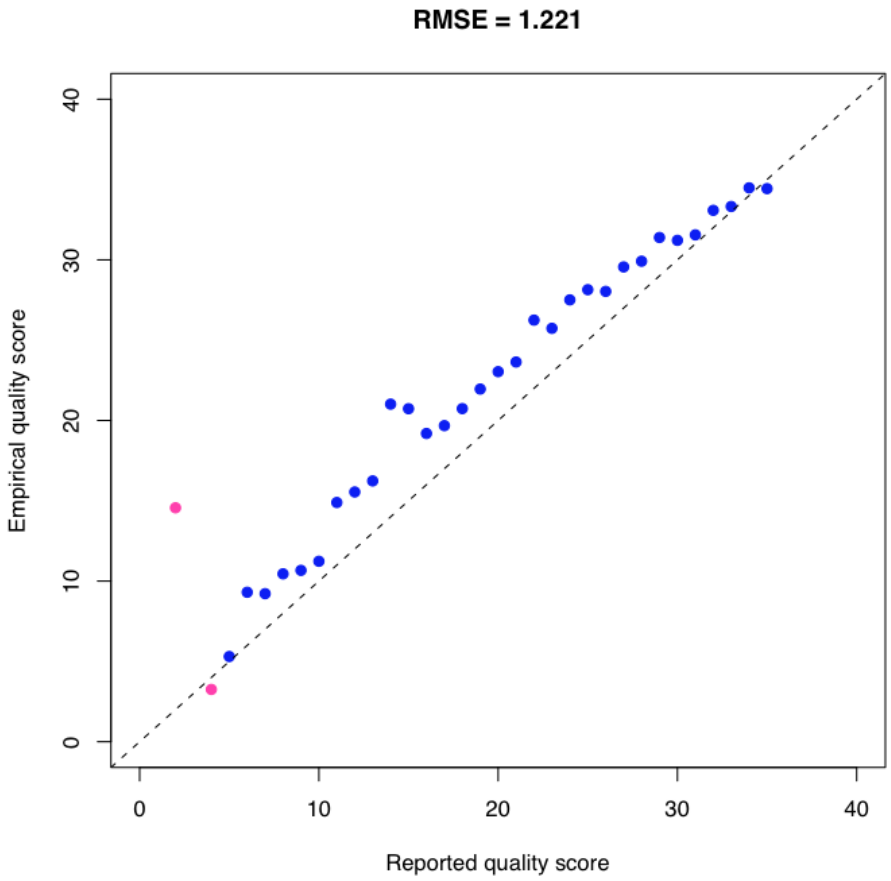


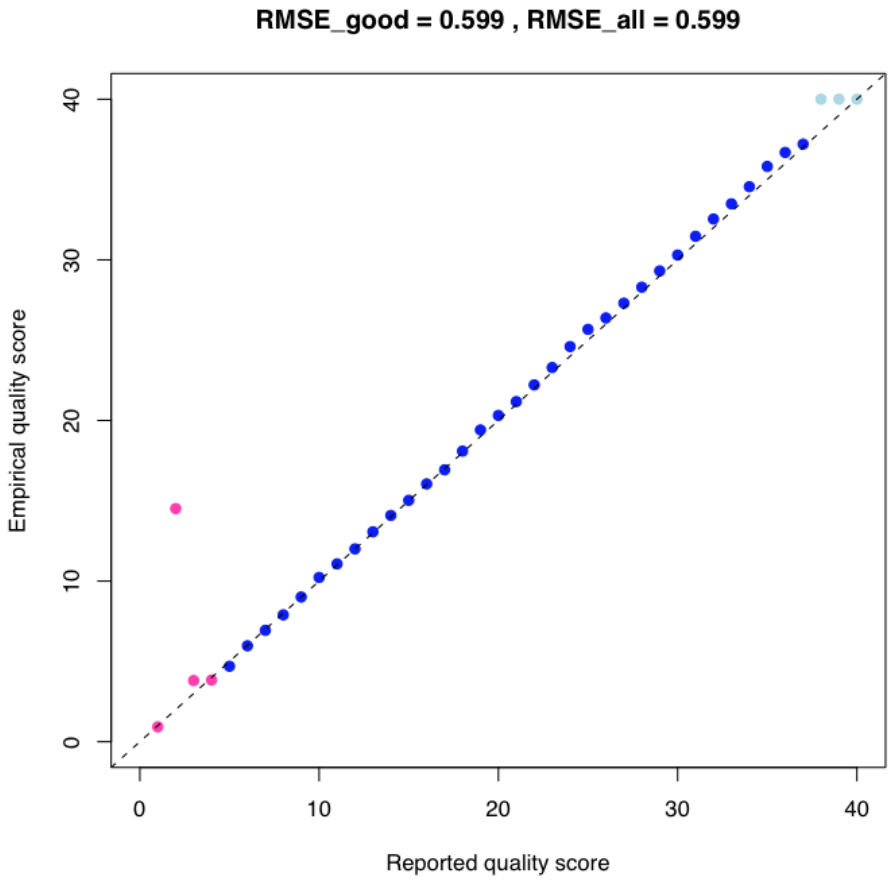
g = 0,1 or 2
m = 2
∈ = 0.01 (BQ=20)
k = 9

Base quality correction

- Essential for estimating genotype likelihood
- Context can affect base quality, e.g.:
  - homopolymers
  - cycle
- estimated error rate ≠ 'real' error rate
- Base quality score recalibration (BQSR) takes this context into account

BQSR



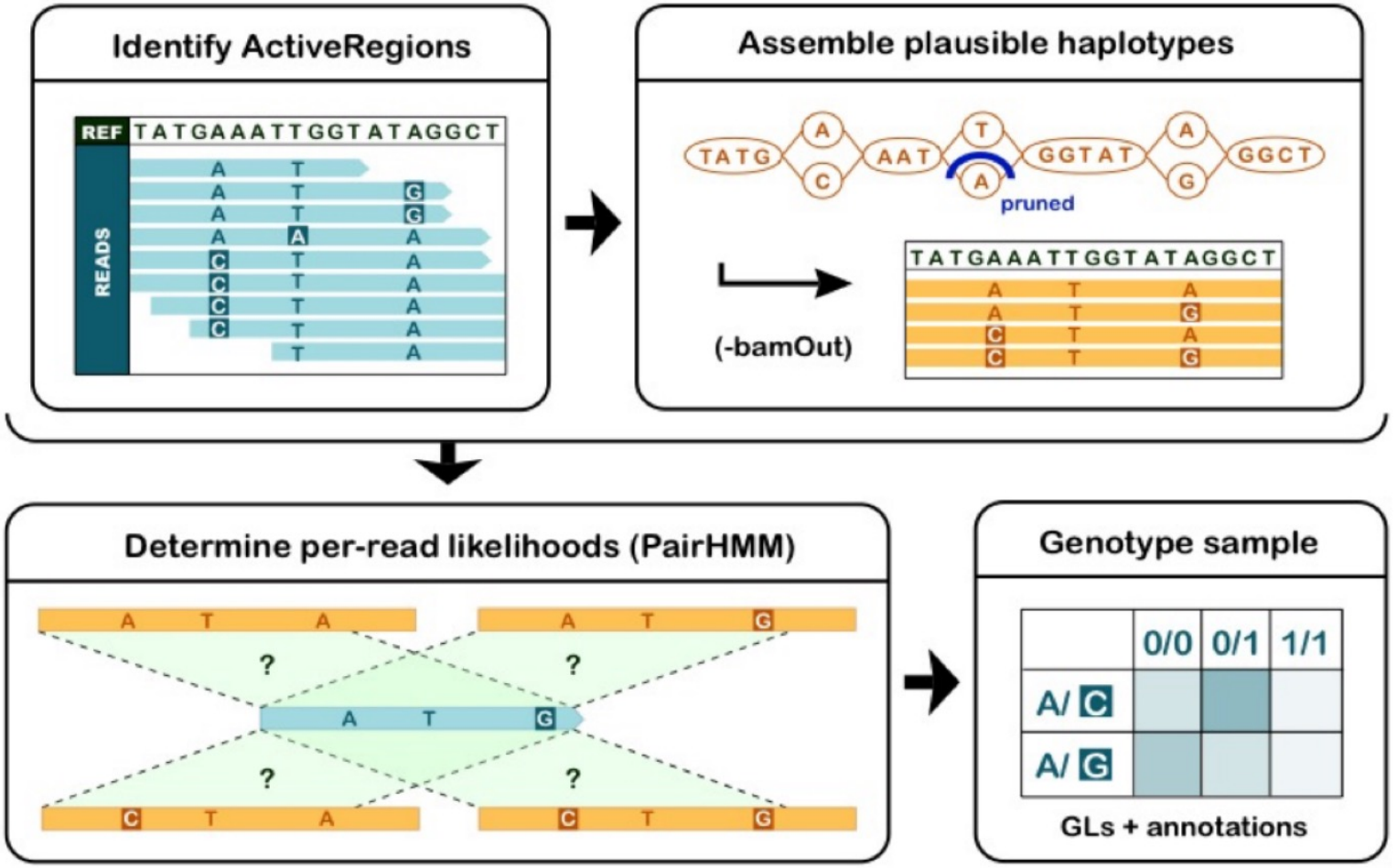


Before BQSR

After BQSR

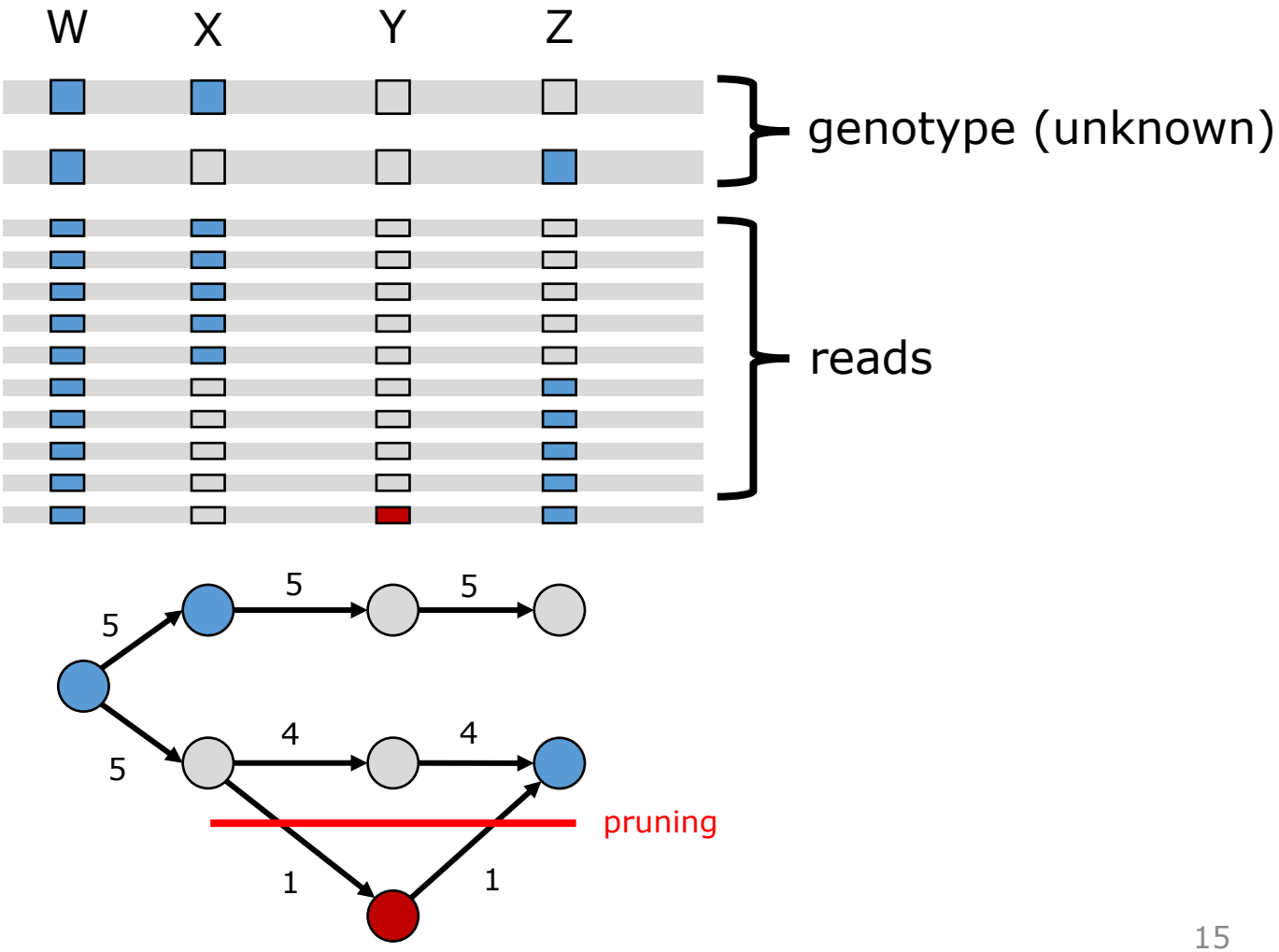
https://gatk.broadinstitute.org/hc/en-us/articles/360035890531-Base-Quality-Score-Recalibration-BQSR-

HaplotypeCaller



Poplin R et al. Scaling accurate genetic variant discovery to tens of thousands of samples. bioRxiv. 2017;1–22.

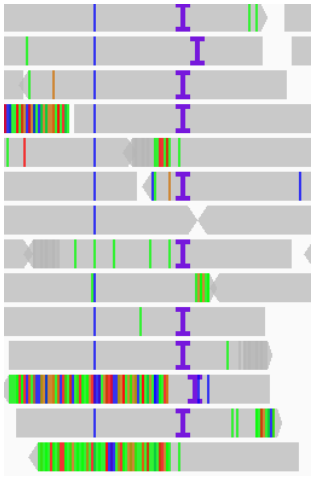
HaplotypeCaller



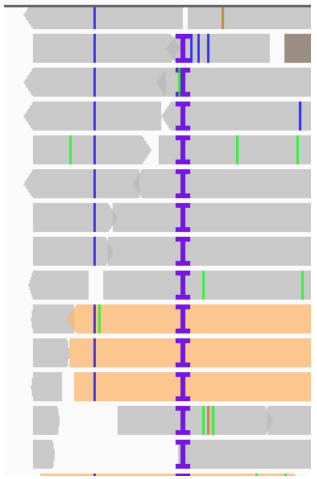
HaplotypeCaller

- Indel realignment
- Expensive process, but only on ‘active’ regions

bwa alignment



re-aligned



vcf

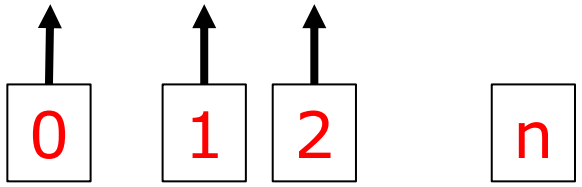
```
##fileformat=VCFv4.3
##fileDate=20090805
##source=myImputationProgramV3.1
##reference=file:///seq/references/1000GenomesPilot-NCBI36.fasta
##contig=<ID=20,length=62435964,assembly=B36,md5=f126cdf8a6e0c7f379d618ff66beb2da,species="Homo sapiens",taxonomy=x>
##phasing=partial
##INFO=<ID=NS,Number=1,Type=Integer,Description="Number 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
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
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
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
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
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

samples

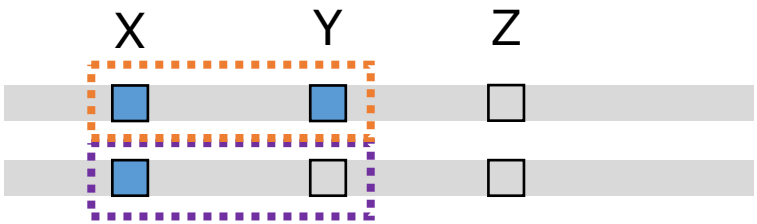
Quiz Question 10

#CHROM	POS	ID	REF	ALT
20	14370	rs6054257	G	A
20	17330	.	T	A
20	1110696	rs6040355	A	G,T
20	1230237	.	T	.
20	1234567	microsat1	GTC	G,GTCT

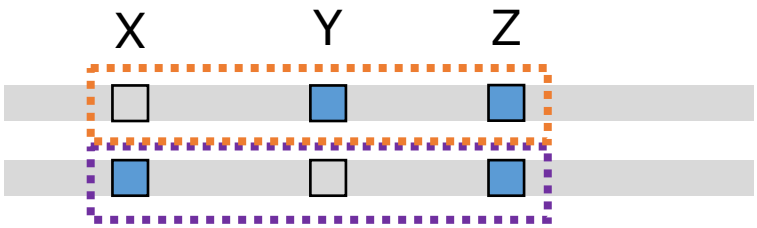


FORMAT	NA00001	NA00002
GT:GQ:DP:HQ	0|0:48:1:51,51	1|0:48:8:51,51
GT:GQ:DP:HQ	0|0:49:3:58,50	0|1:3:5:65,3
GT:GQ:DP:HQ	1|2:21:6:23,27	2|1:2:0:18,2
GT:GQ:DP:HQ	0|0:54:7:56,60	0|0:48:4:51,51
GT:GQ:DP	0/1:35:4	0/2:17:2

sample 1



sample 2



sample1.vcf

CHROM	POS	ID	SAMP1
20	1101	SNPX	1|1
20	1203	SNPY	0|1

sample2.vcf

CHROM	POS	ID	SAMP2
20	1101	SNPX	1|0
20	1203	SNPY	0|1
20	1253	SNPZ	1|1

combined.vcf

CHROM	POS	ID	SAMP1	SAMP2
20	1101	SNPX	1|1	1|0
20	1203	SNPY	0|1	0|1
20	1253	SNPZ	?	1|1

Quiz Question 11

Missing genotype problem

- Most variant callers genotype all samples in one go. But:
  - variant calling process can become very computational intensive
  - new sample? Redo entire variant call
- GATK uses GVCF:
  - Store information on non-variant regions

Other software

- **freebayes**: haplotype-aware variant calling -> good alternative to gatk
- **bcftools**: working with vcfs (part of samtools)
- **vcftools**: working with vcfs
- **whatshap**: haplotyping
- **DeepVariant**: variant calling in long reads

GATK workflow

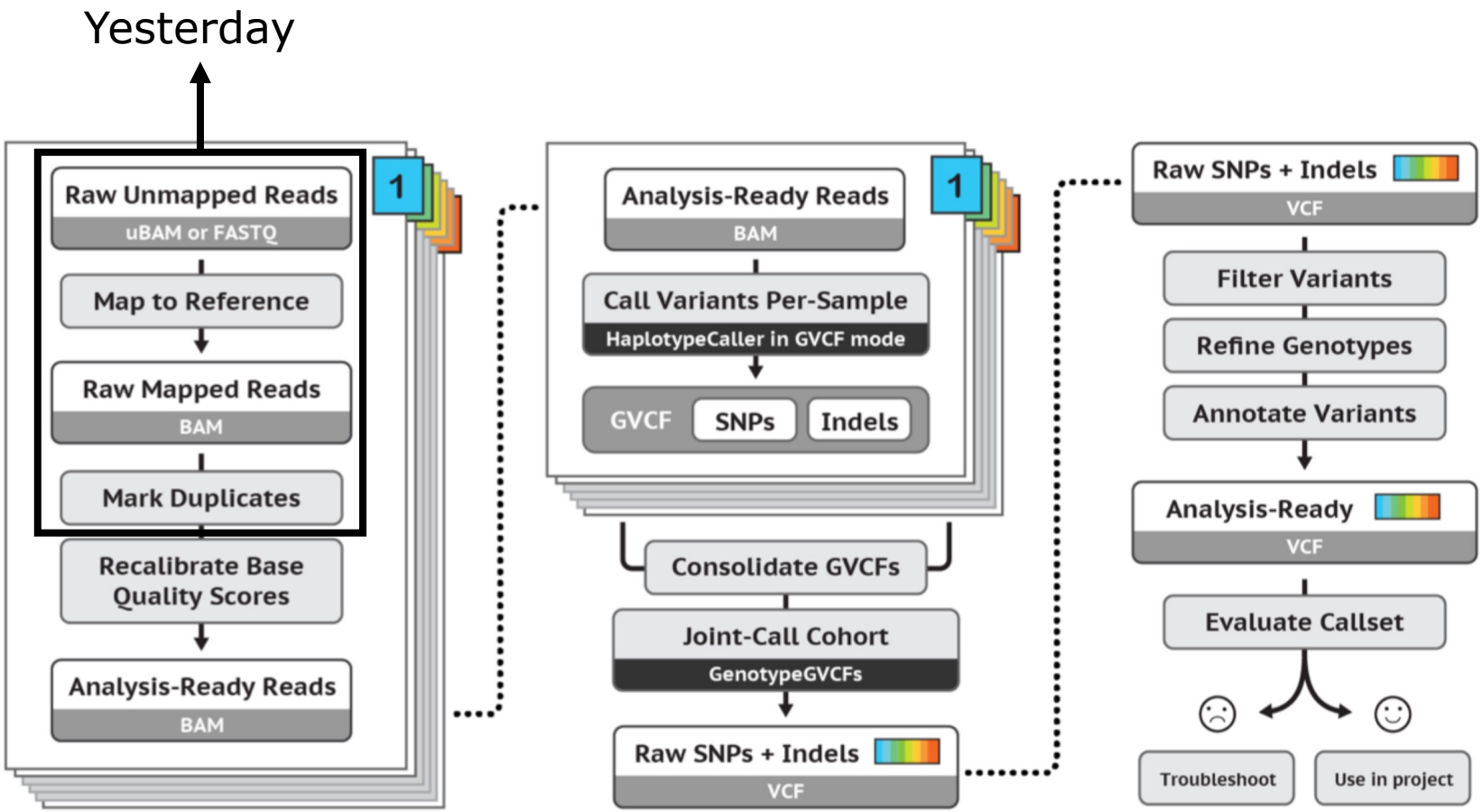


image source: https://gatk.broadinstitute.org