NGS – variant analysis

Variant calling

GATK workflow

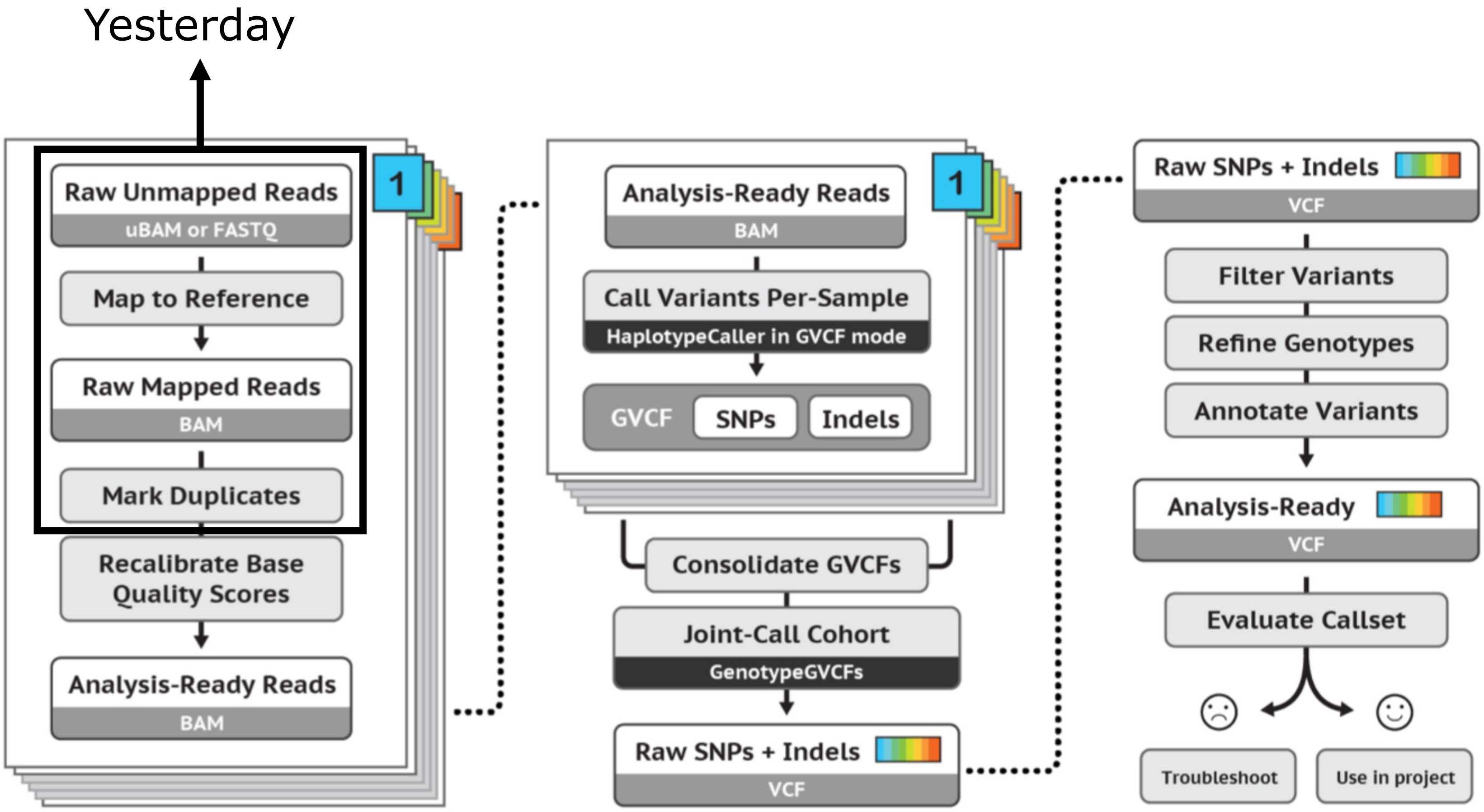
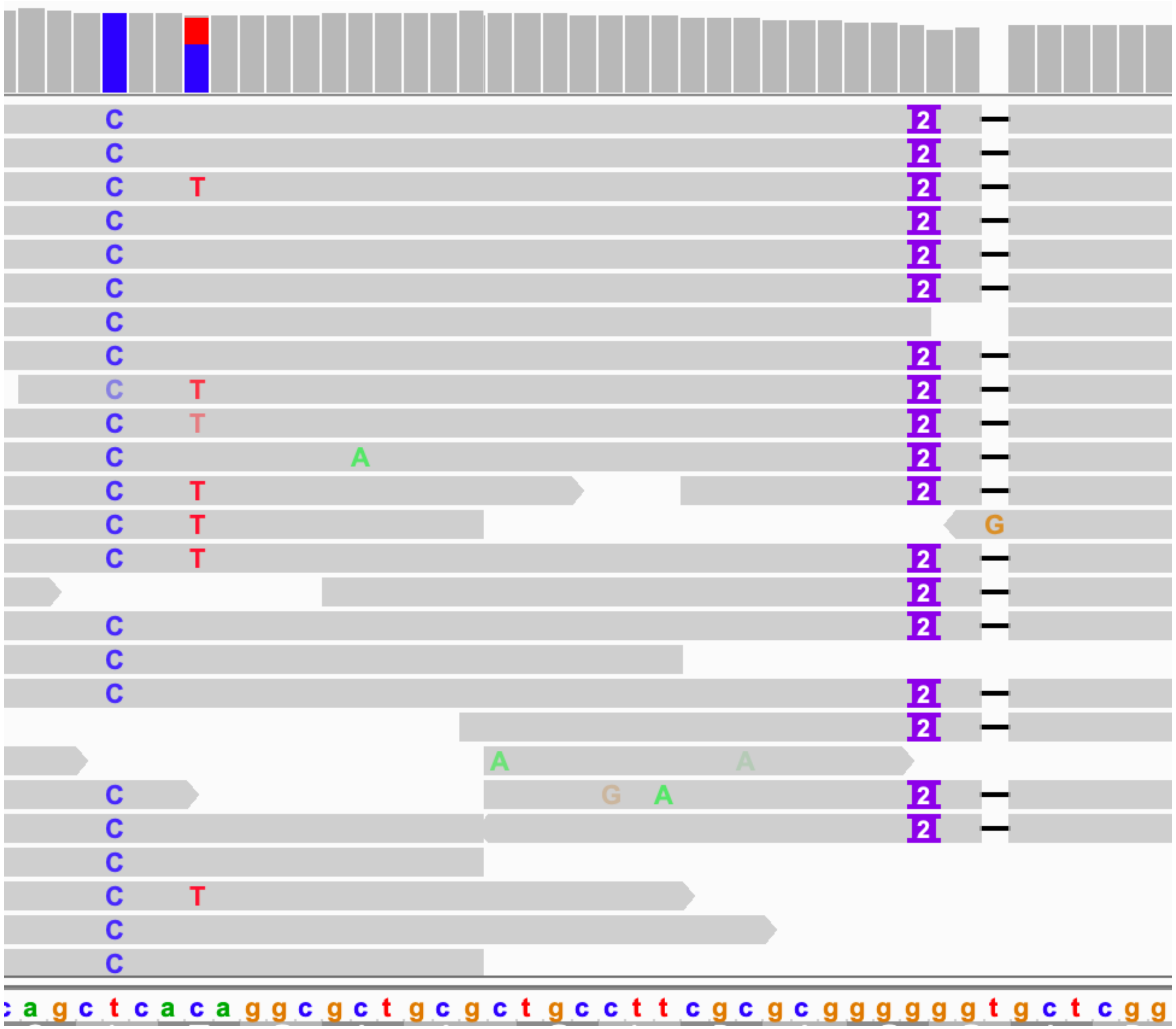


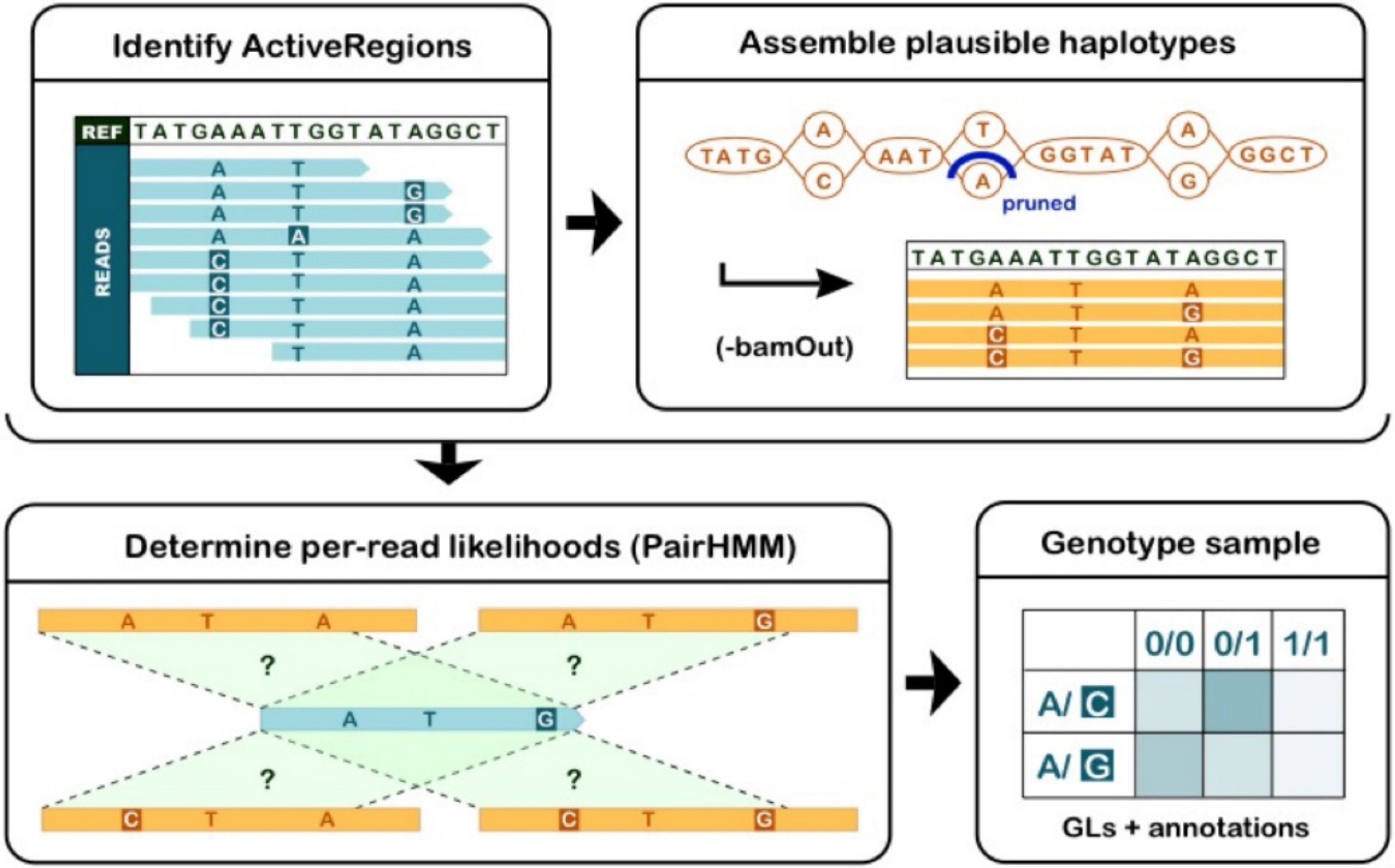
image source: https://gatk.broadinstitute.org


C
T
A
G
2
a g c t c a c a g g c g c t g c g c t g c c t t c g c g c g g g g g g t g c t c g g

Three important questions

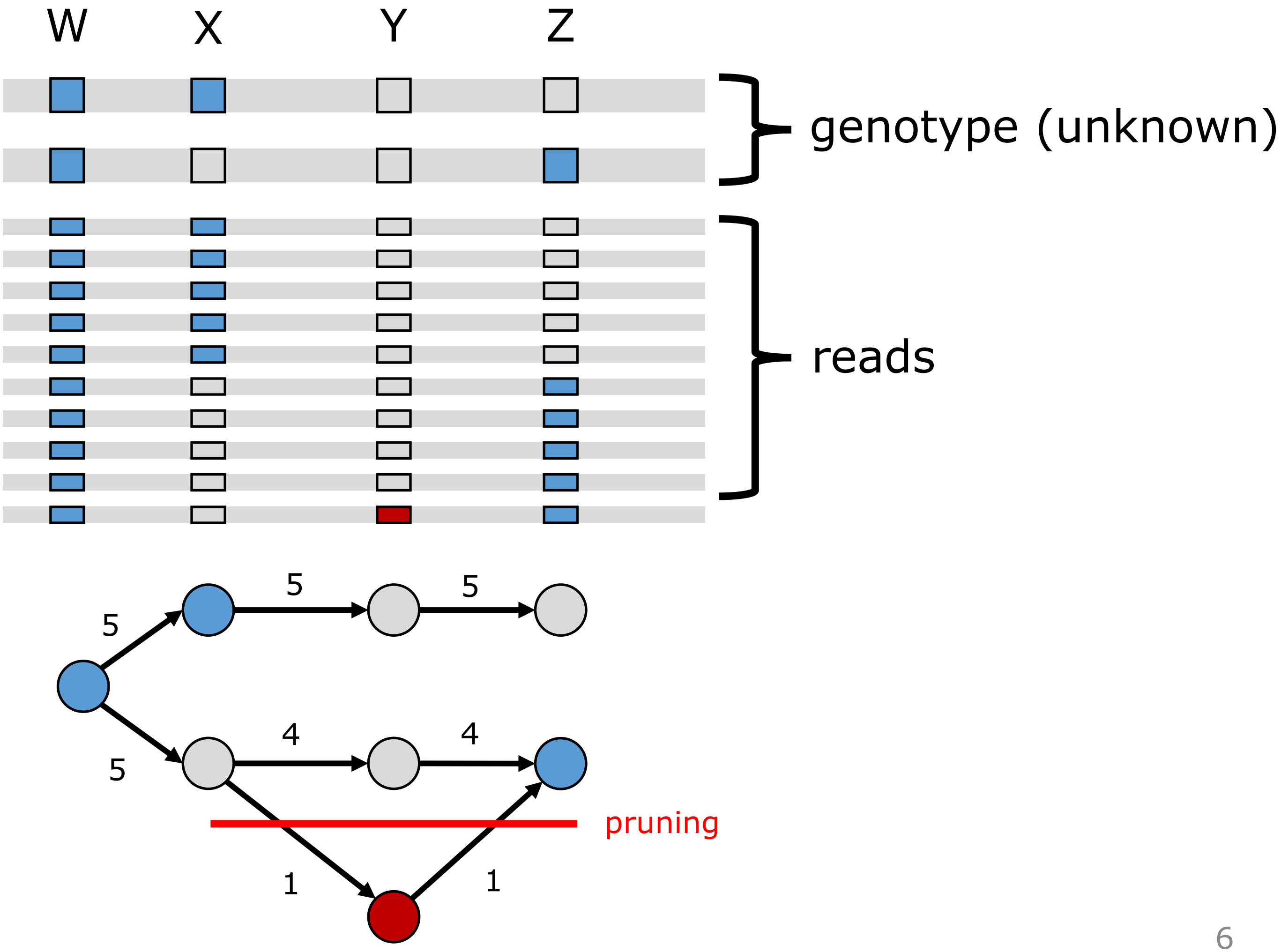
- Is there a variant at location X?
  - Deviation from REF in the alignments
- What are the alleles?
  - The variation in sequence in these deviations
- What is the genotype (HomRef, Heterozygote or HomAlt)?
  - Estimating the allele counts in the sample

HaplotypeCaller



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

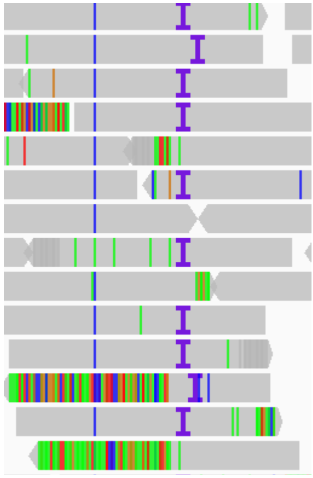
What are the alleles?



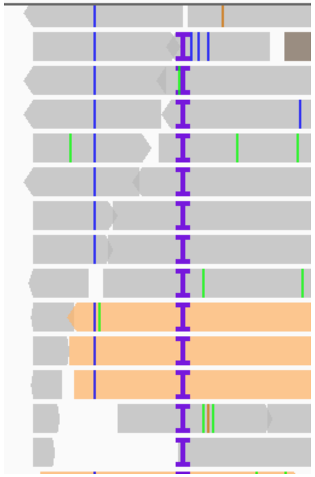
What are the alleles?

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

bwa alignment



re-aligned



What is the genotype?

At a site we count 9 bases

5 REF and 4 ALT

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

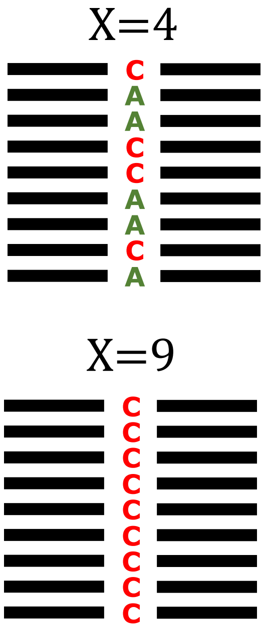
so: $\mathcal{L}(p=0.5 \mid X=4) = 0.25$

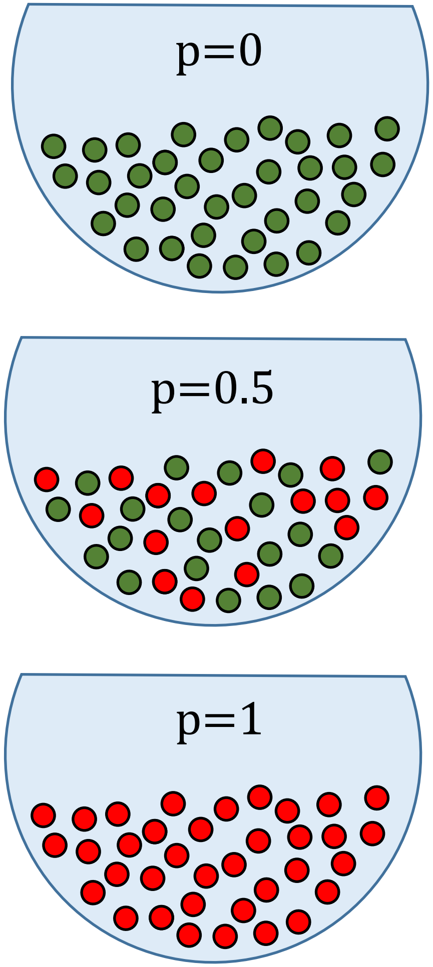
0 REF and 9 ALT

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

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

$\mathcal{L}(p=0 \mid X=9) = 0$





Question

Estimating genotype

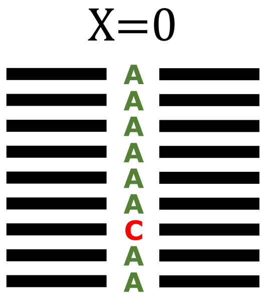
What are the likely genotypes?

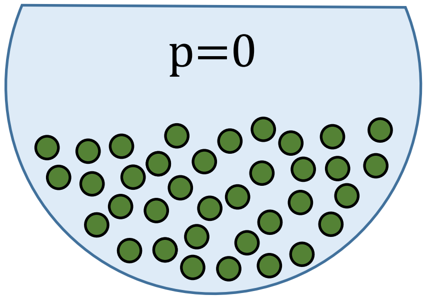
At a site we count 9 bases

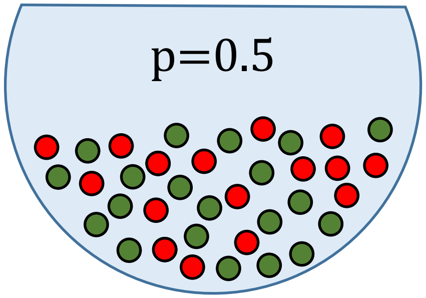
8 REF and 1 ALT

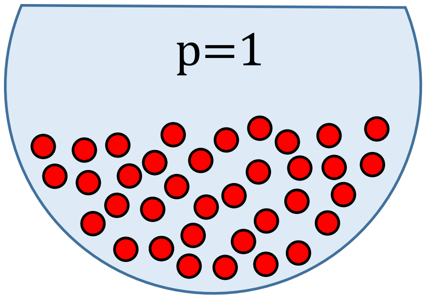
$\mathcal{L}(\mathrm{p}=0.5 \mid \mathrm{X}=1) = 0.017$

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





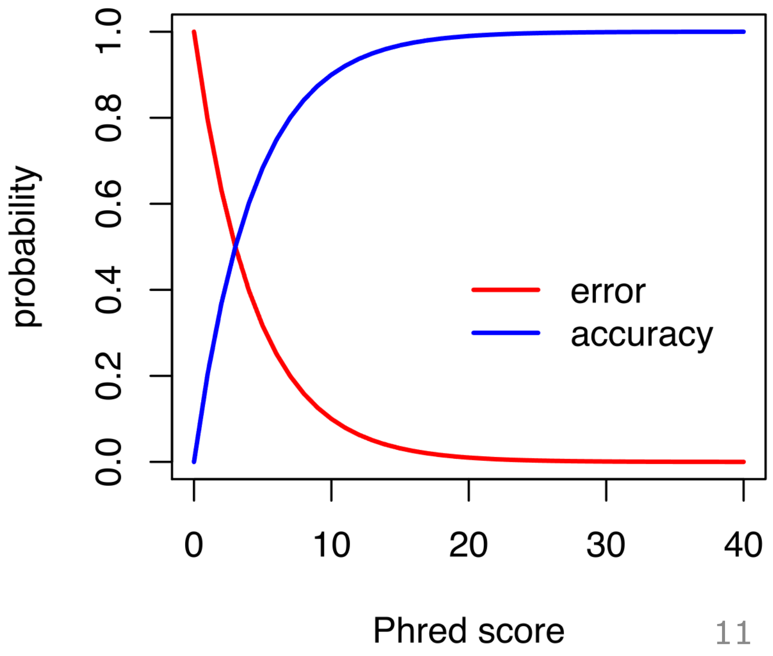




Strict binomial distribution would only works with error-free data

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)
∊: 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 $\in = 0.01$

$\mathrm{PL} = -10 * \log 10(\mathcal{L}(\mathrm{g}))$

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

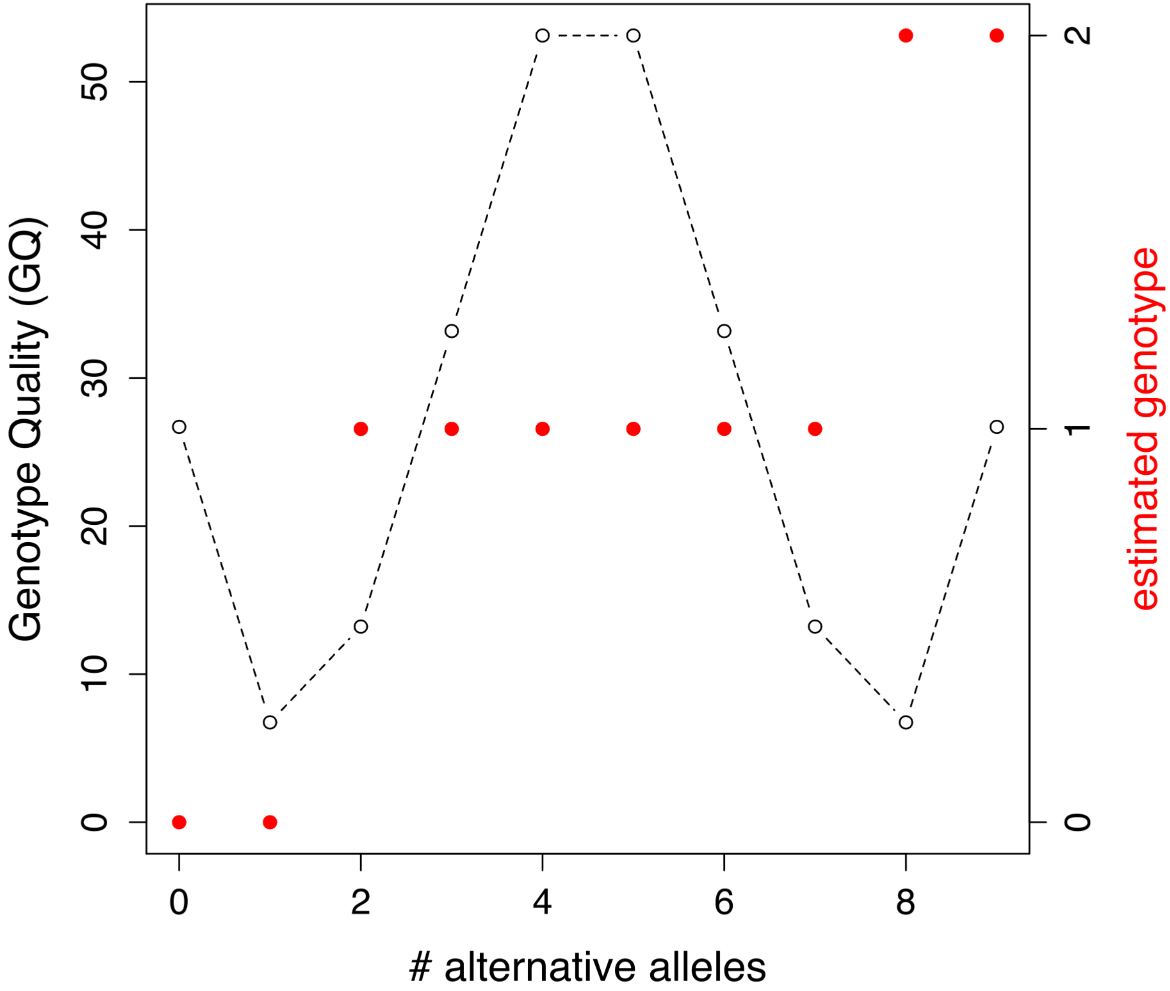
Lowest PL = most likely genotype

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

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

Question

Estimating the genotype

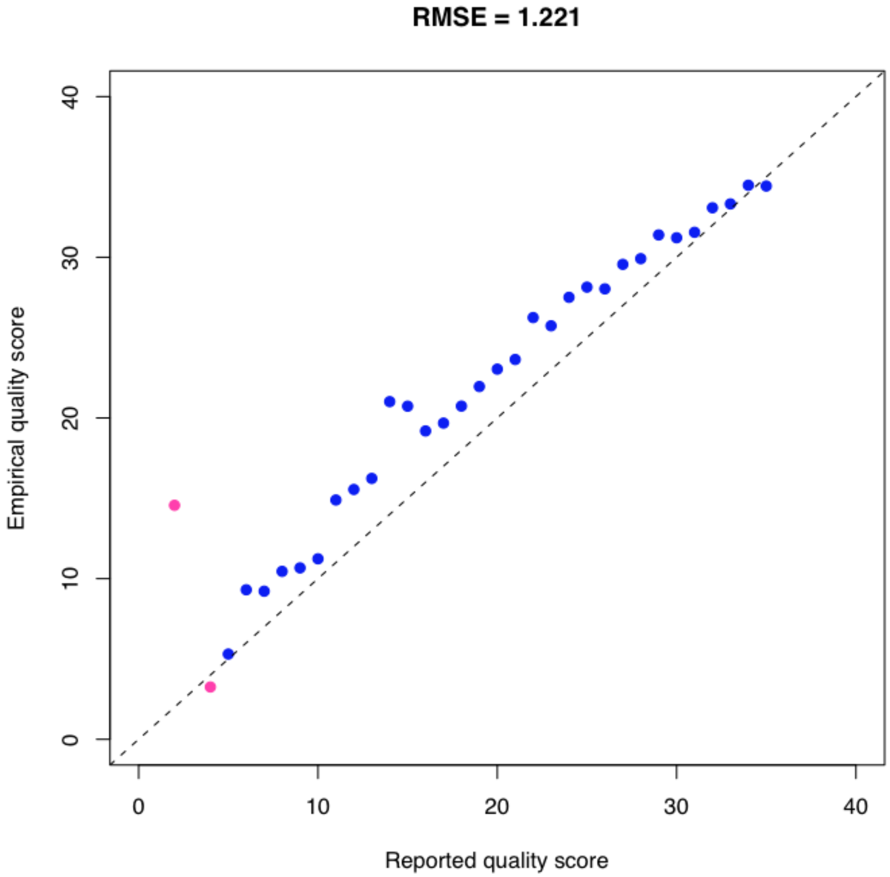


```
g = 0,1 or 2
m = 2
∈ = 0.01 (BQ=20)
k = 9
```

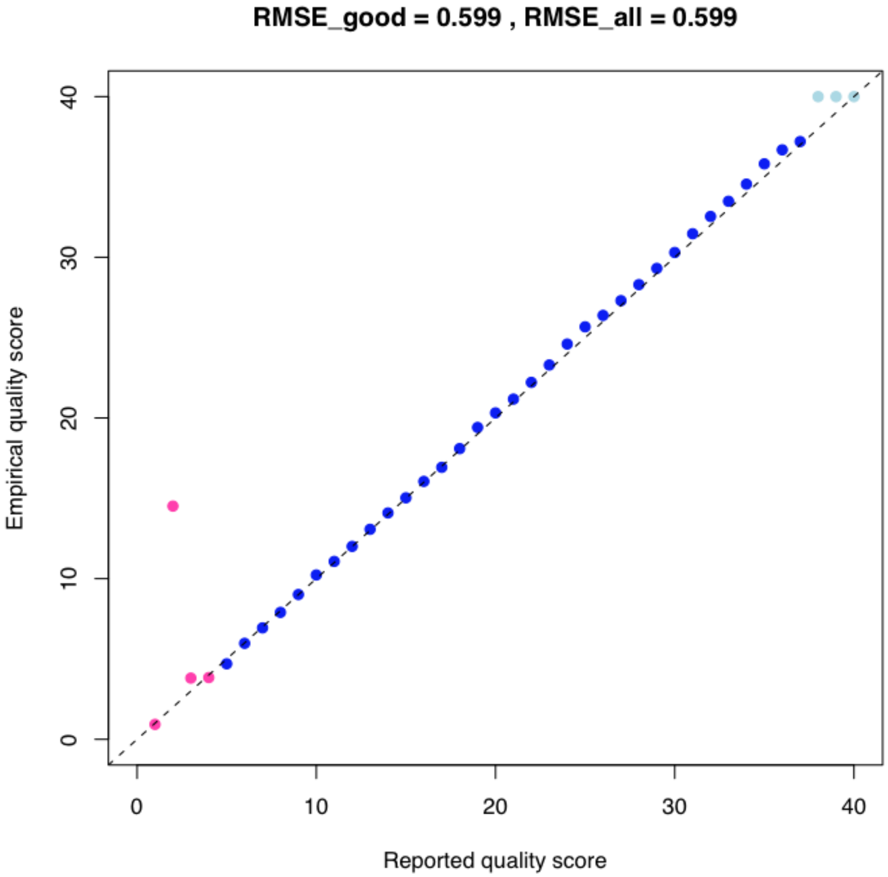
Base quality correction

- 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-

vcf

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##FORMAT=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth (reads with MQ=255 or with bad mates are filtered)">
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##FORMAT=<ID=GT,Number=1,Type=String,Description="Genotype">
##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Normalized, Phred-scaled likelihoods for genotypes as defined in the VCF specs">
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##GATKCommandLine=<ID=HaplotypeCaller,CommandLine="HaplotypeCaller --bam-output
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##INFO=<ID=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have been filtered">
##contig=<ID=chr20,length=64444167>
#CHROM     POS ID REF ALT    QUAL FILTER                     INFO         FORMAT                father                mother
chr20 10019252  .   G   C  134.68      . AC=1;AF=0.167;AN=6;DP=15 GT:AD:DP:GQ:PL     0/1:3,5:8:58:143,0,58        0/0:2,0:2:6:0,6,48
chr20 10019348  .   A ACT 1587.89      . AC=5;AF=0.833;AN=6;DP=45 GT:AD:DP:GQ:PL   0/1:7,6:13:99:231,0,256   1/1:0,13:13:39:573,39,0
chr20 10019469  .   C   T 1792.98      . AC=4;AF=0.667;AN=6;DP=89 GT:AD:DP:GQ:PL 0/1:17,15:32:99:465,0,503 0/1:11,12:23:99:289,0,289
```

vcf

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##FORMAT=<ID=PL,Number=G,Type=Integer,Description="Normalized, Phred-scaled likelihoods for genotypes as defined in the VCF specs">
##GATKCommandLine=<ID=GenotypeGVCFs,CommandLine="GenotypeGVCFs --output
##GATKCommandLine=<ID=HaplotypeCaller,CommandLine="HaplotypeCaller --bam-output
##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=DP,Number=1,Type=Integer,Description="Approximate read depth; some reads may have been filtered">
##contig=<ID=chr20,length=64444167>
#CHROM     POS ID REF ALT    QUAL FILTER                     INFO         FORMAT                    father                    mother
chr20 10019252  .   G   C  134.68      . AC=1;AF=0.167;AN=6;DP=15 GT:AD:DP:GQ:PL     0/1:3,5:8:58:143,0,58        0/0:2,0:2:6:0,6,48
chr20 10019348  .   A ACT 1587.89      . AC=5;AF=0.833;AN=6;DP=45 GT:AD:DP:GQ:PL   0/1:7,6:13:99:231,0,256   1/1:0,13:13:39:573,39,0
chr20 10019469  .   C   T 1792.98      . AC=4;AF=0.667;AN=6;DP=89 GT:AD:DP:GQ:PL 0/1:17,15:32:99:465,0,503 0/1:11,12:23:99:289,0,289
```
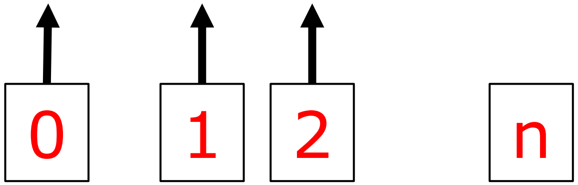
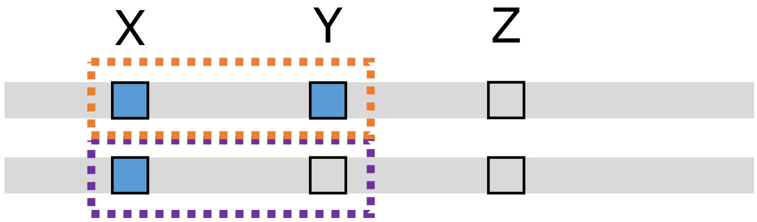

vcf

```
#CHROM POS      ID   REF   ALT    FORMAT   NA00001   NA00002
20     14370    .    G     A      GT:GQ    0|0:48    1|0:48
20     17330    .    T     A      GT:GQ    0|0:49    0|1:99
20     1110696  .    A     G,T    GT:GQ    1|2:21    2|1:27
20     1230237  .    T     .      GT:GQ    0|0:54    0|0:48
20     1234567  .    GTC   G,GTCT GT:GQ    0/1:35    0/2:17
```

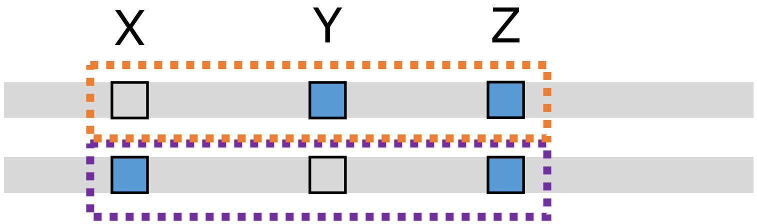


Question

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

Question

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 short and long reads

GATK workflow

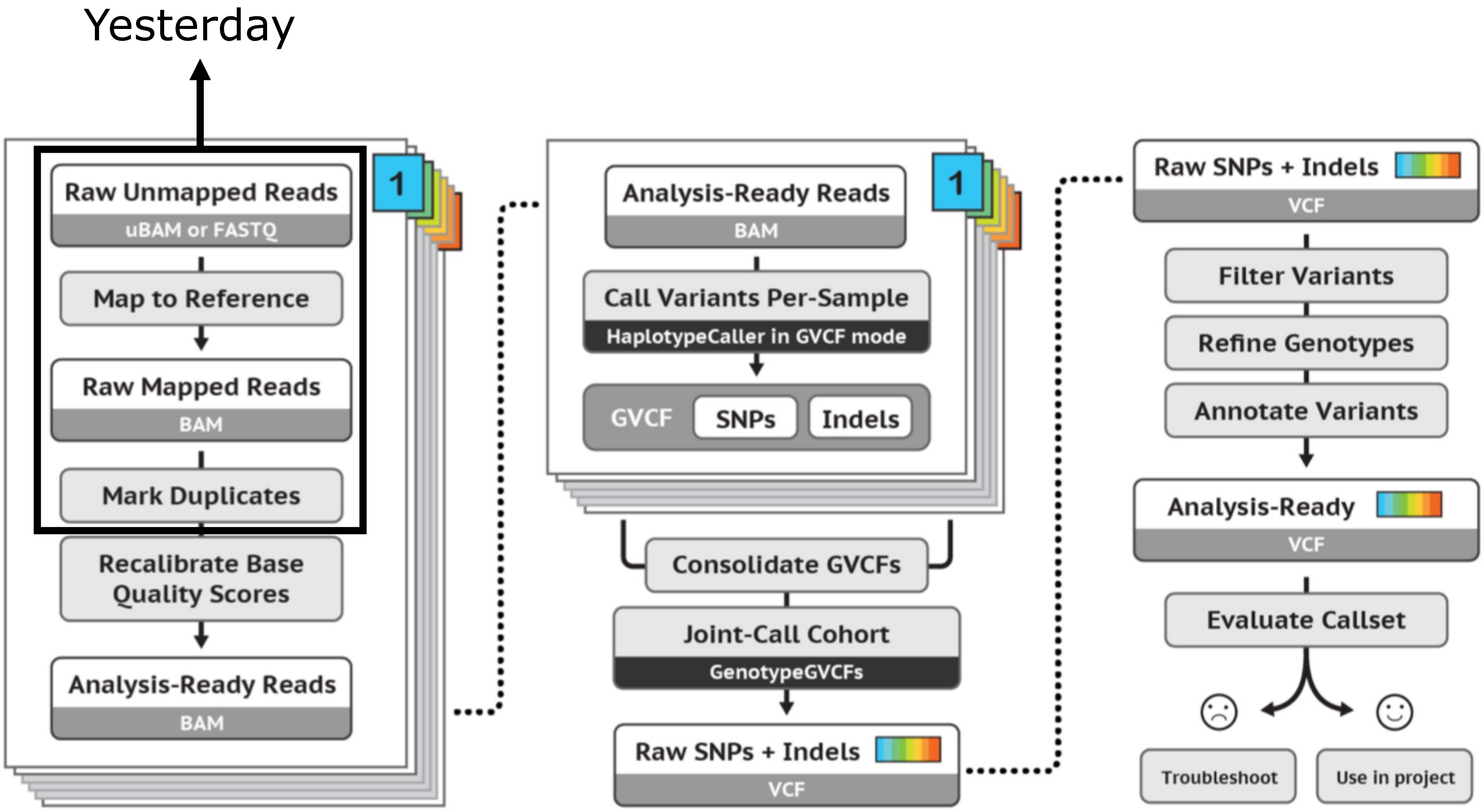


image source: https://gatk.broadinstitute.org