

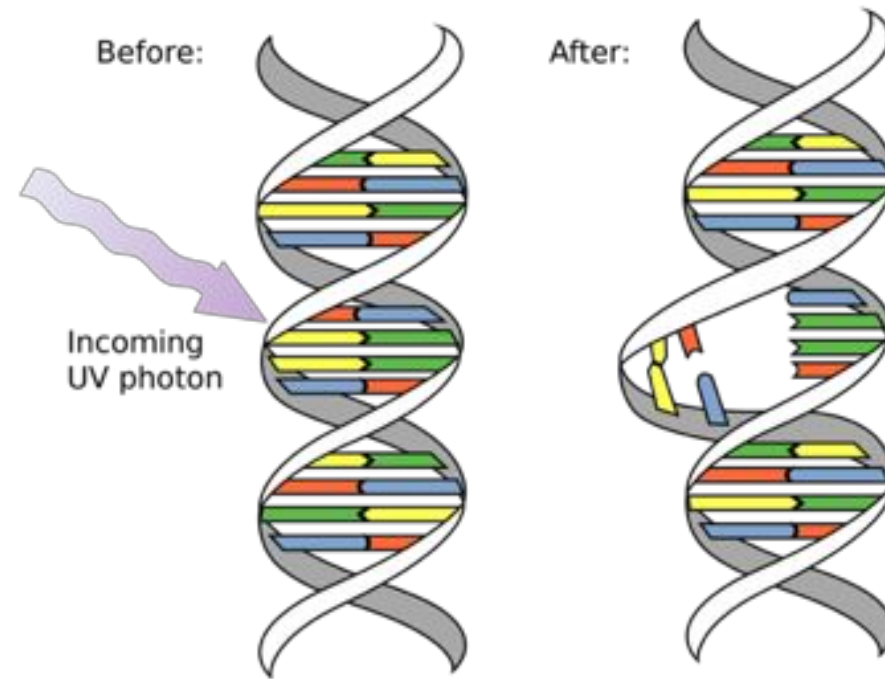
Somatic Mutation Calling

BFX Workshop Week 08

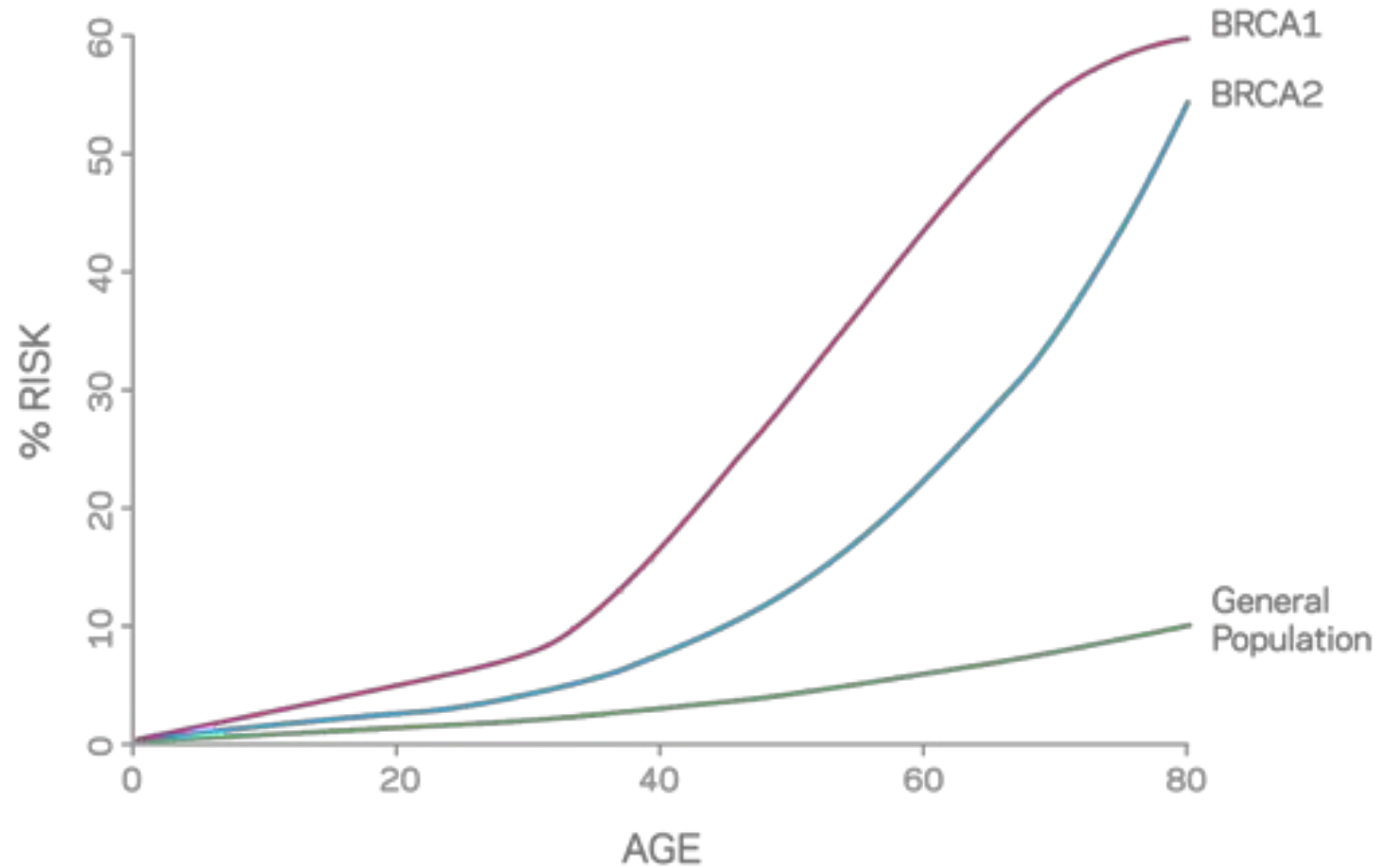
Chris Miller

Cancer is a disease of the genome

- Cancer is caused by **somatic** mutations
- These mutations are introduced into the genome of a cell (errors in DNA copying, UV light, chemicals)
- Most cancers require around 3 driver mutations



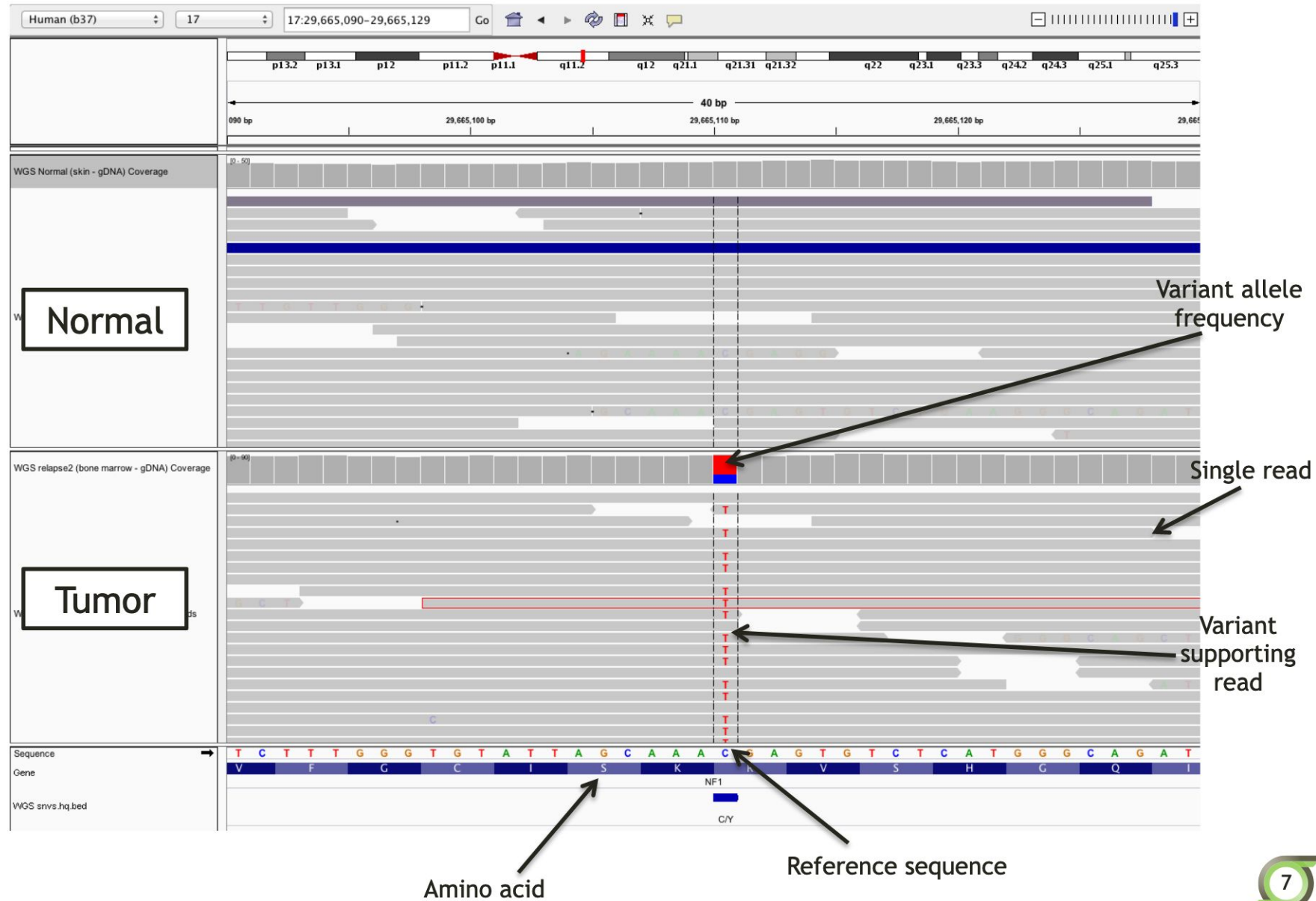
Germline Predisposition



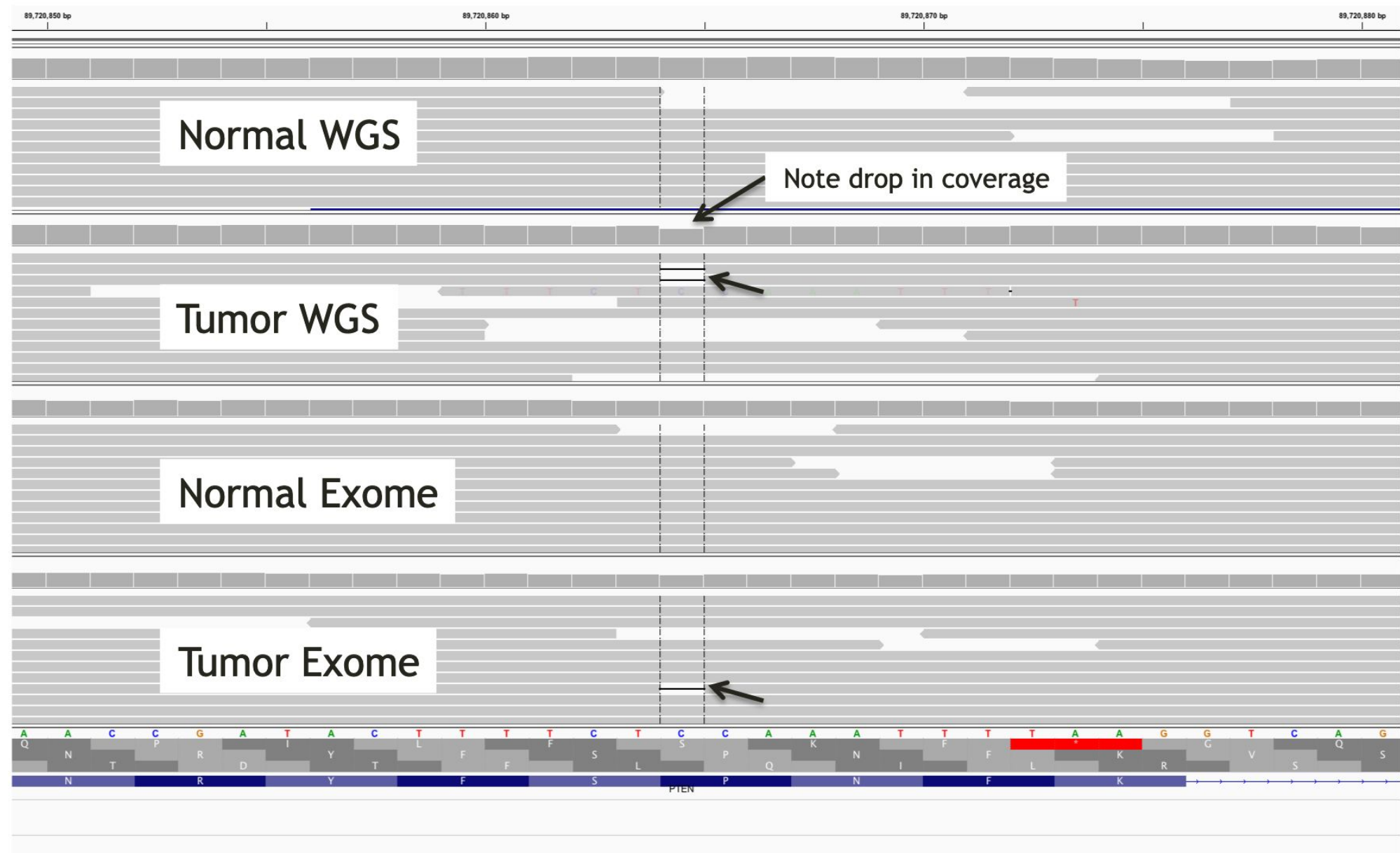
Cancer Sequencing

- In cancer, we have to (at least) double sequencing costs
- Uses both a tumor sample and a matched normal
- We compare them to find somatic mutations

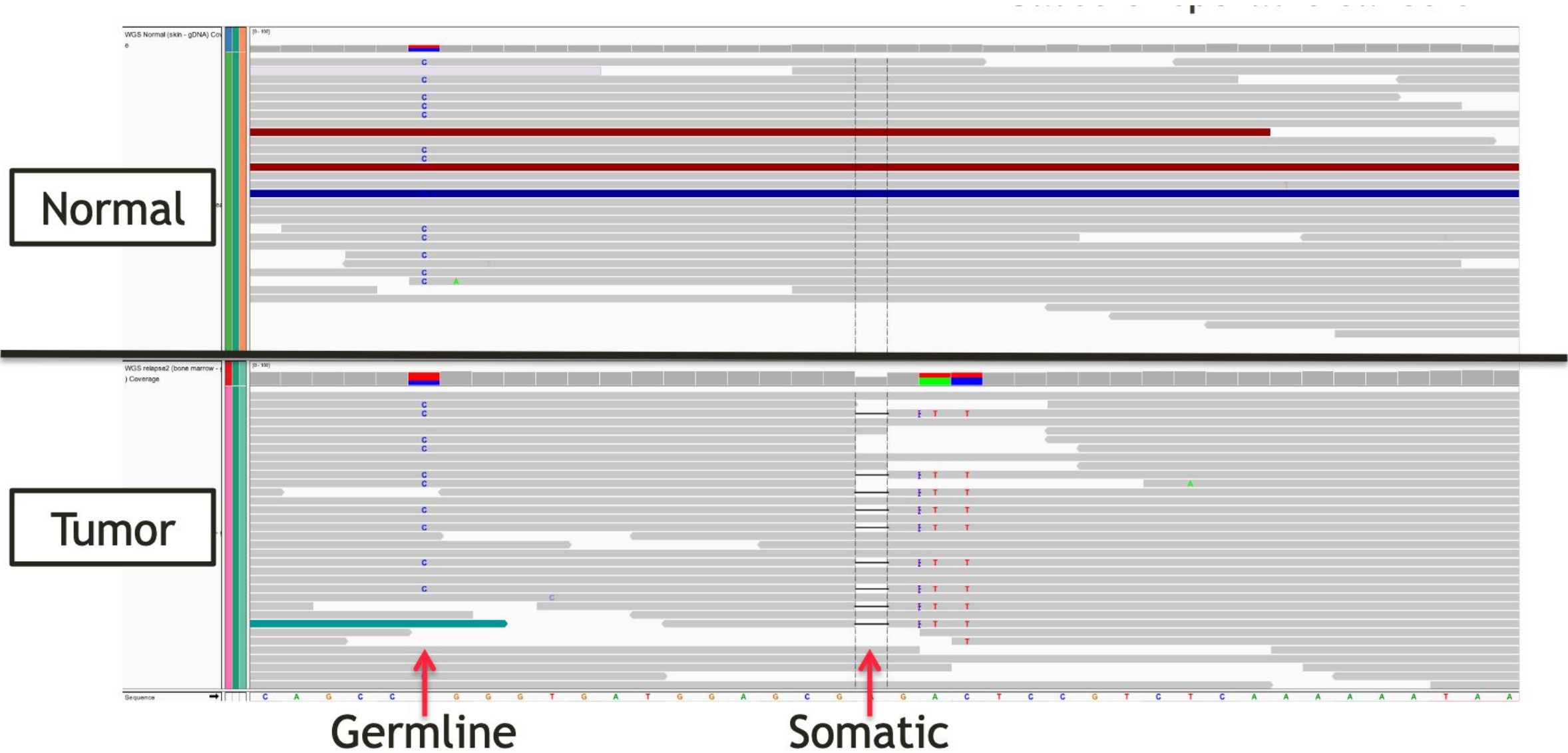
What do somatic variants look like?



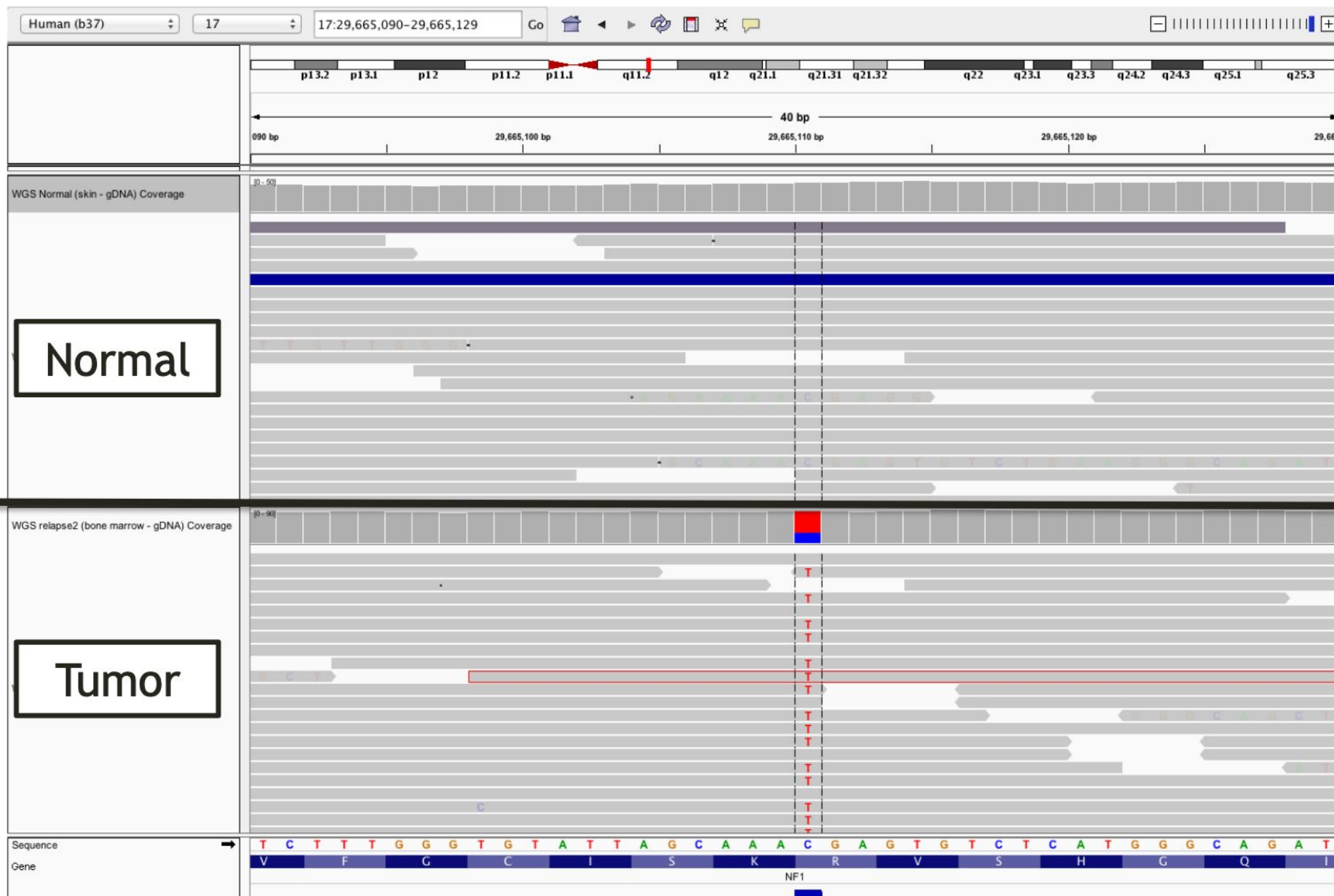
Indels



Germline vs Somatic



VAF = Variant reads / Total reads



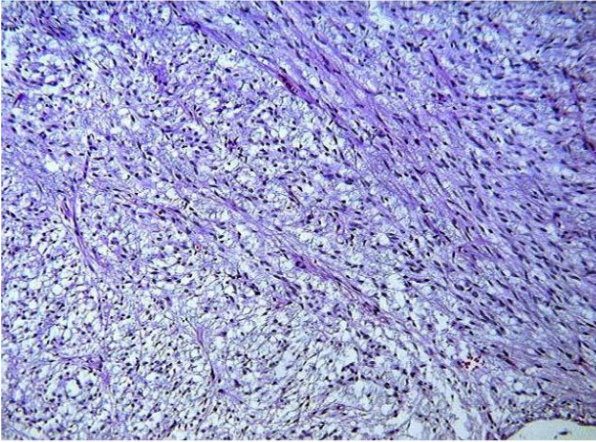
Normal

$$\text{VAF} = 0/20 = 0\%$$

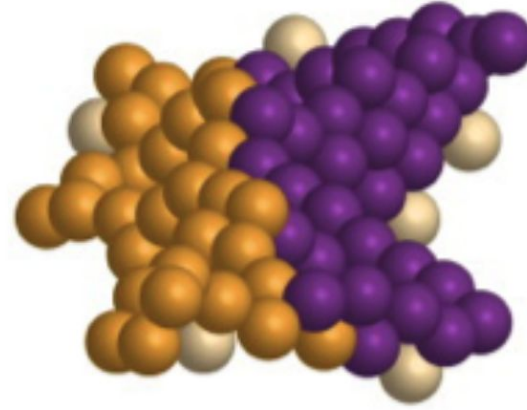
Tumor

$$\text{VAF} = 14/20 = 70\%$$

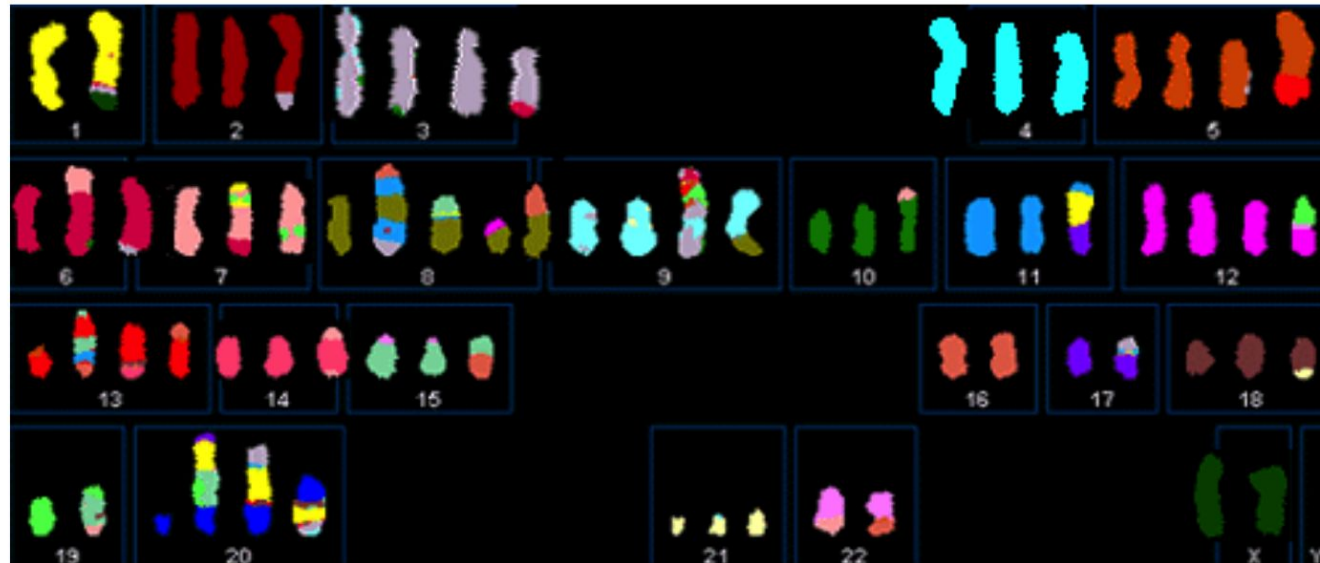
Tumors are often impure, heterogeneous, and aneuploid



Tumors are often impure
(contain normal cells)



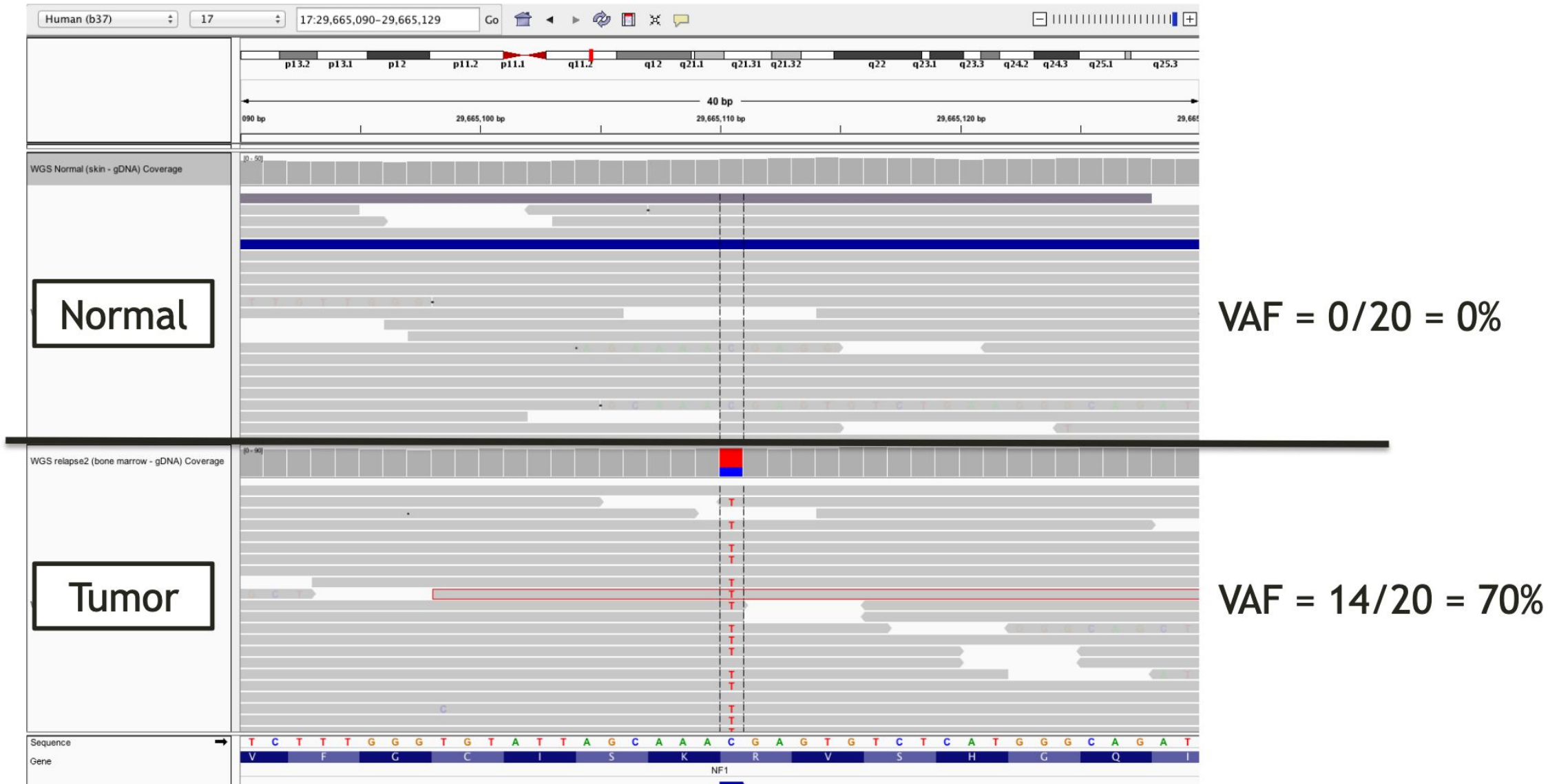
Tumors are often genetically
diverse collections of cells



Tumors may be aneuploid

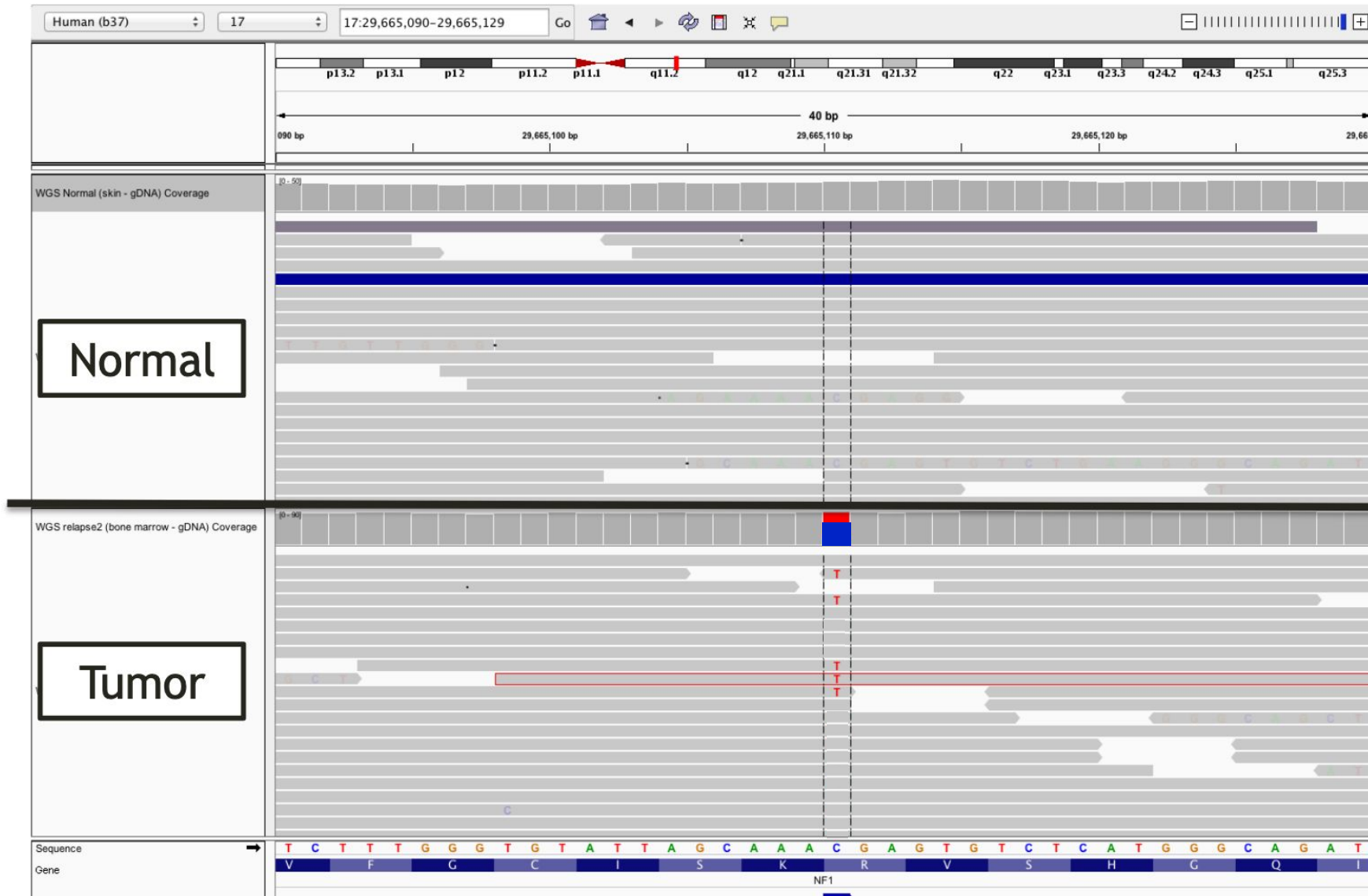
How does purity influence VAF?

VAF = Variant reads / Total reads



How does purity influence VAF?

VAF = Variant reads / Total reads

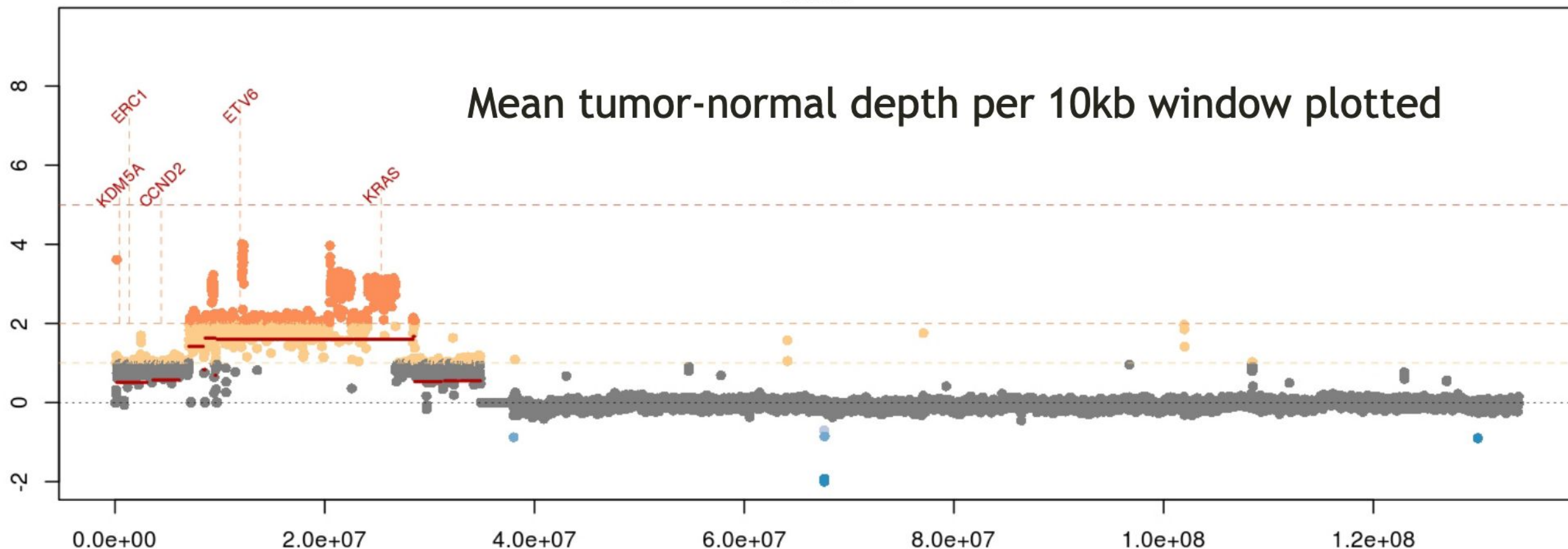


$$\text{VAF} = 0/20 = 0\%$$

$$\text{VAF} = 5/20 = 25\%$$

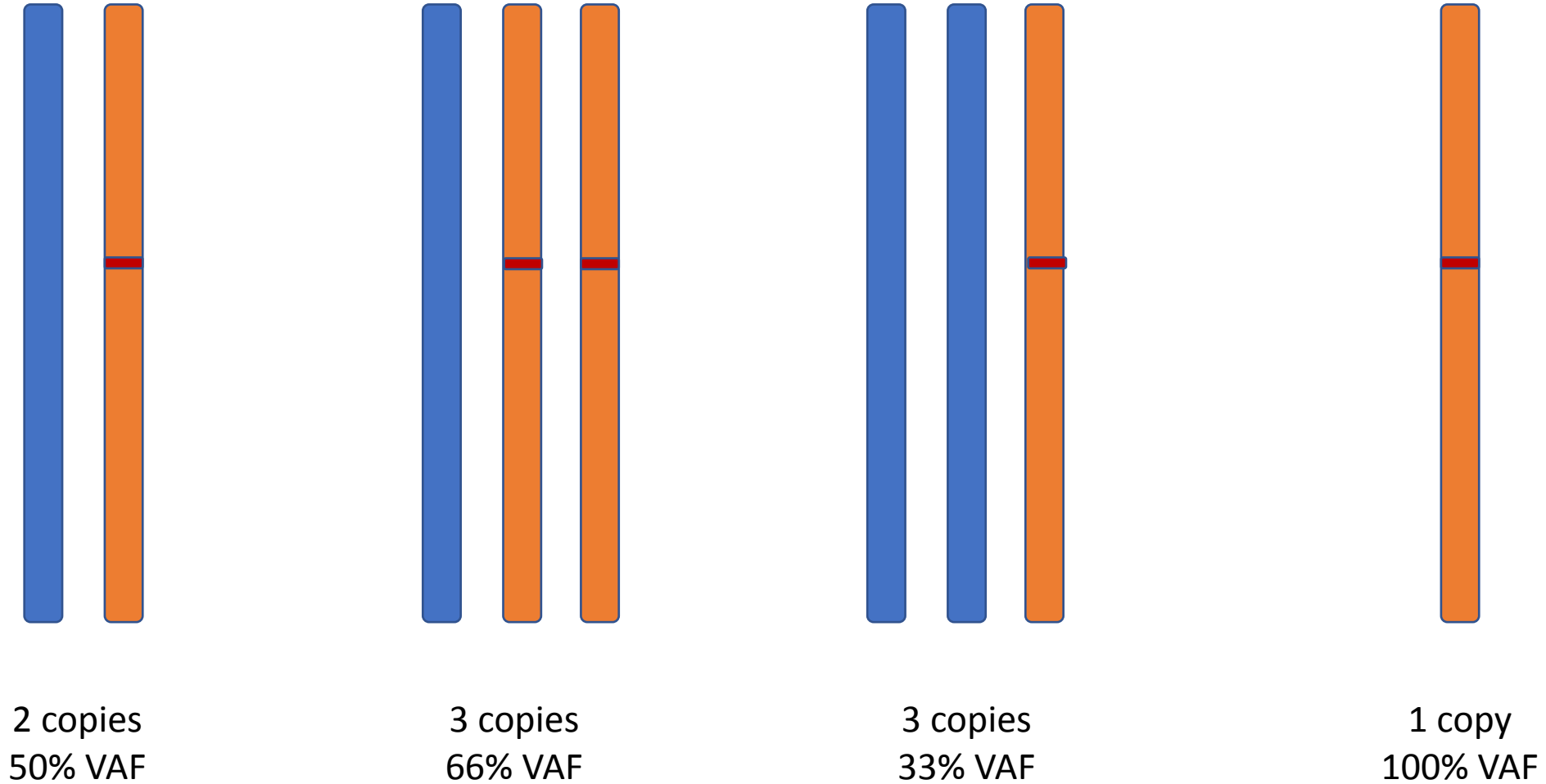


Gains

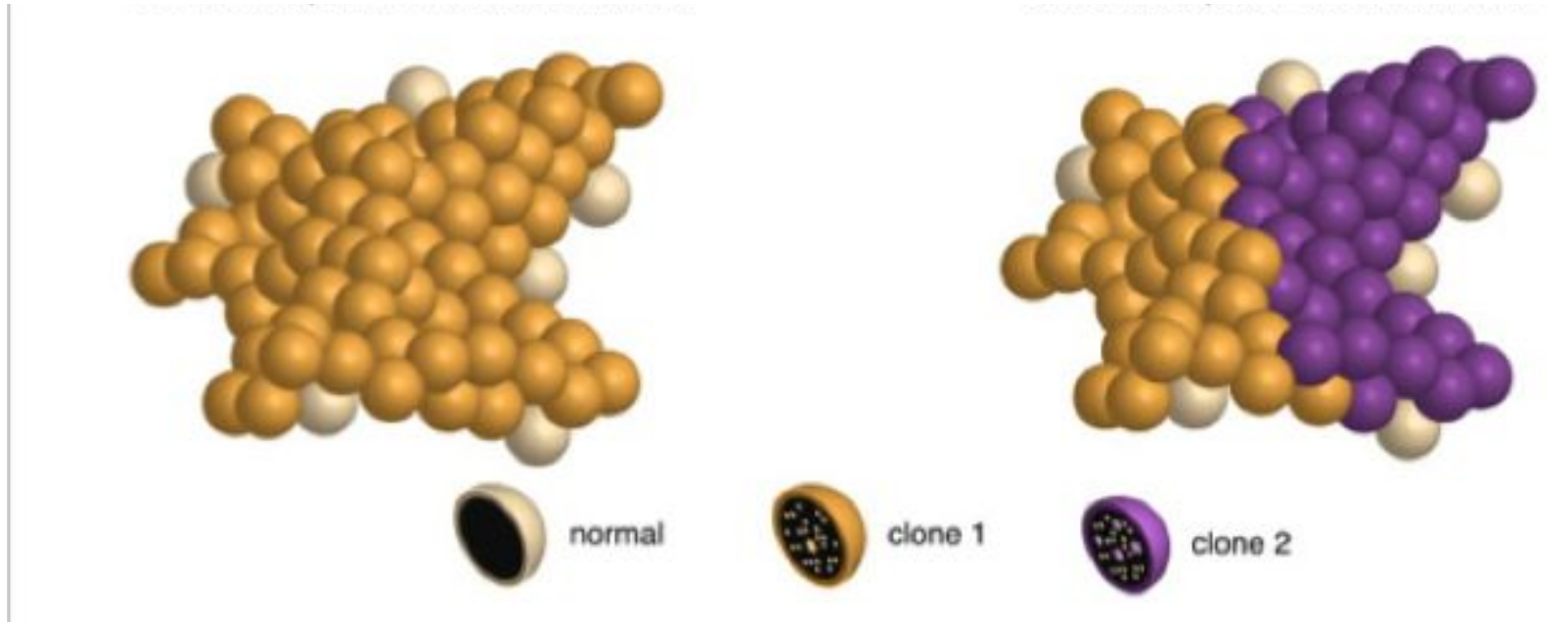


KRAS amplification in a metastatic breast cancer

How does copy number influence VAF?

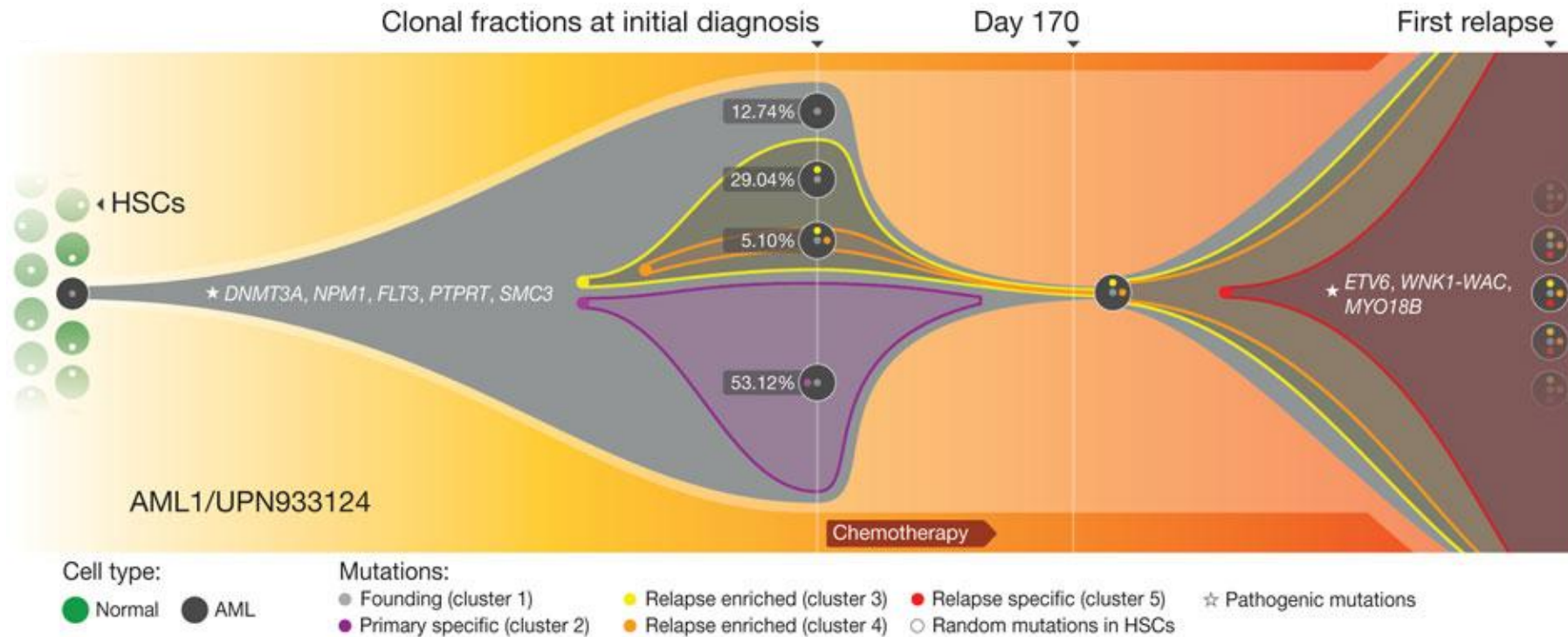


How does clonality influence VAF?

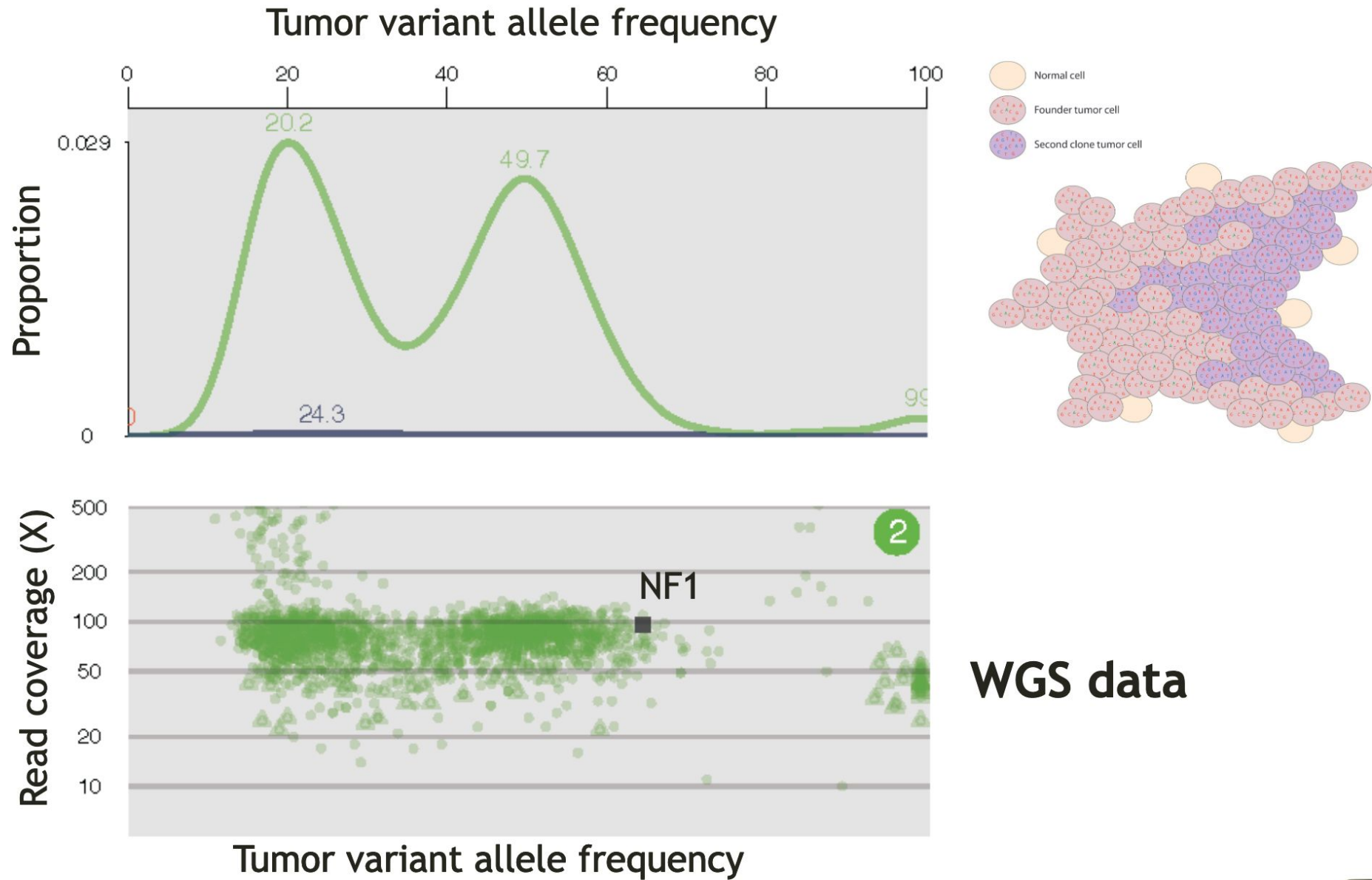


- Subclones contain genetically diverse populations of cells
- Evolution occurs at the molecular and cellular levels
- The growth rates for subclones are often different

Clonal evolution in relapsed AML



Dominant clone vs. sub-clonal (and driver vs. passenger)

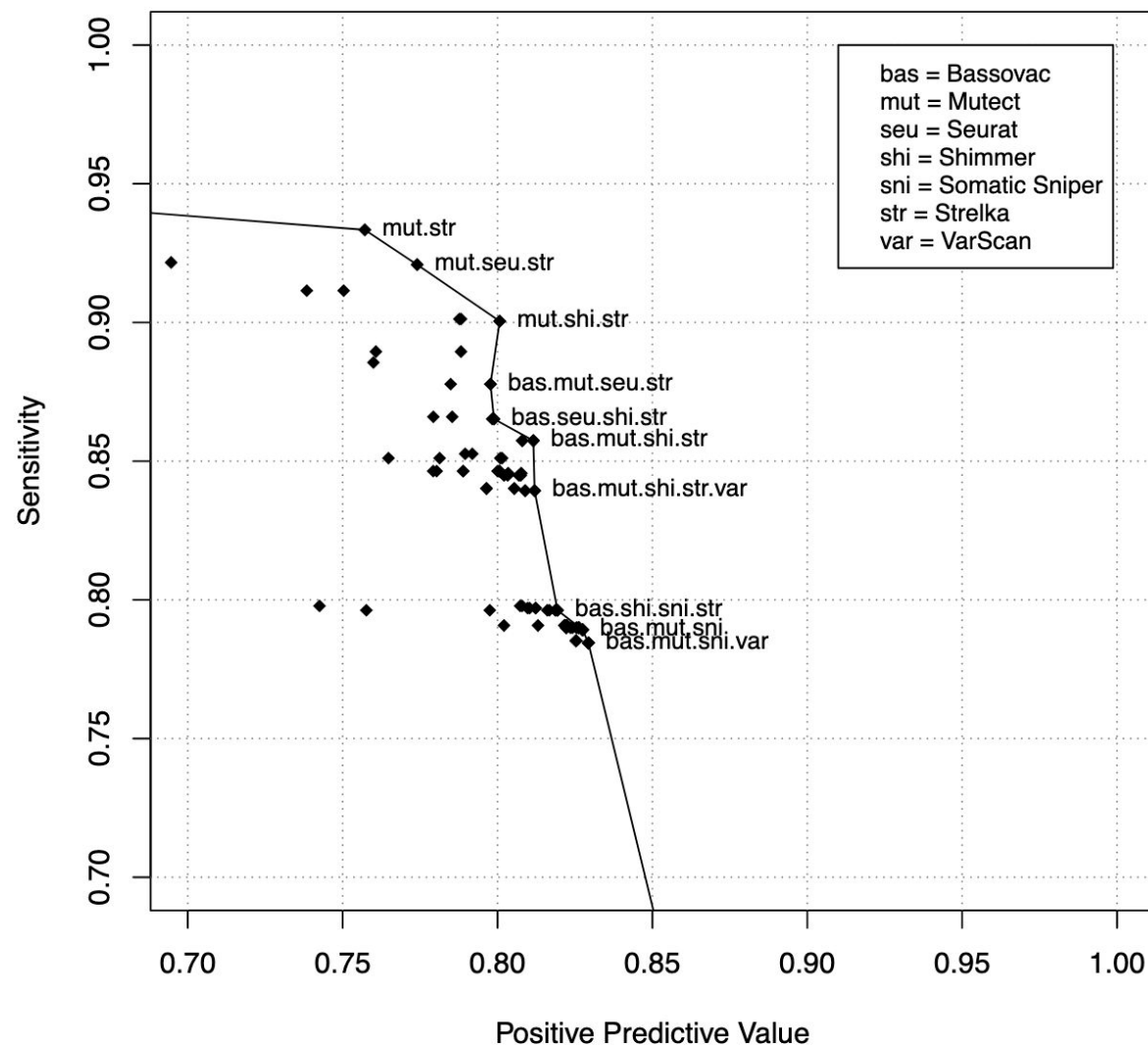


Somatic variant calling is harder

- There are more factors to consider, a wider range of possibilities, and often, more sketchy samples
- One class of callers tries to address this problem with Bayesian statistics

Use of multiple variant callers can improve sensitivity and accuracy

Performance of caller Intersections



Somatic Variant detection workflow

- Step 1 – run the callers
 - Mutect, Strelka, VarScan, Pindel

Somatic Variant detection workflow

- Varscan

- `java -jar VarScan.v2.3.6.jar somatic <(samtools mpileup ... normal.bam) <(samtools mpileup ... tumor.bam)`

- Mutect

- `/gatk/gatk Mutect2 -O output.vcf -R ref.fa -I tumor.bam -tumor TUMORNAME -I normal.bam -normal NORMALNAME -L interval.list`

Somatic Variant detection workflow

- Step 1 – run the callers
 - Mutect, Strelka, VarScan, Pindel
- Step 2 – Merge the VCFs
 - GATK - CombineVariants

Somatic Variant detection workflow

- Combine Variants

```
/usr/bin/java -jar /opt/GenomeAnalysisTK.jar -T  
CombineVariants -genotypeMergeOptions PRIORITIZE  
--rod_priority_list mutect,varsan -o combined.vcf.gz  
--variant:mutect mutect_output.vcf --variant:varsan  
varsan_output.vcf
```

Somatic Variant detection workflow

- Step 1 – run the callers
 - Mutect, Strelka, Varscan, Pindel
- Step 2 – Merge the VCFs
 - GATK - CombineVariants
- Step 3: Annotate and filter

Homework

- Take a tumor and normal bam file and identify somatic variants using multiple callers
- Produce a merged VCF with your results