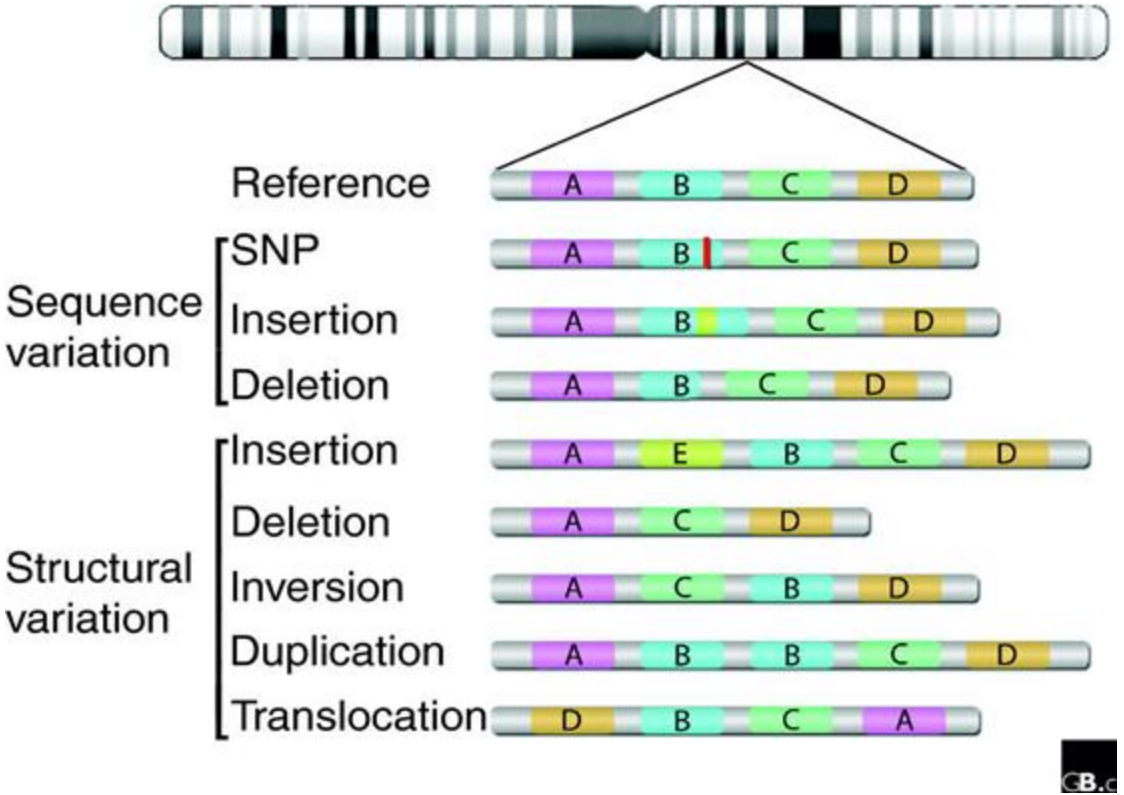
Variant calling

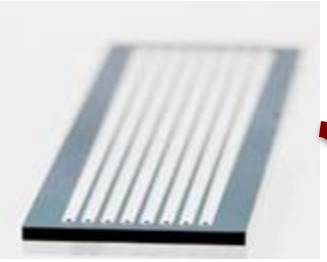
Chris Miller

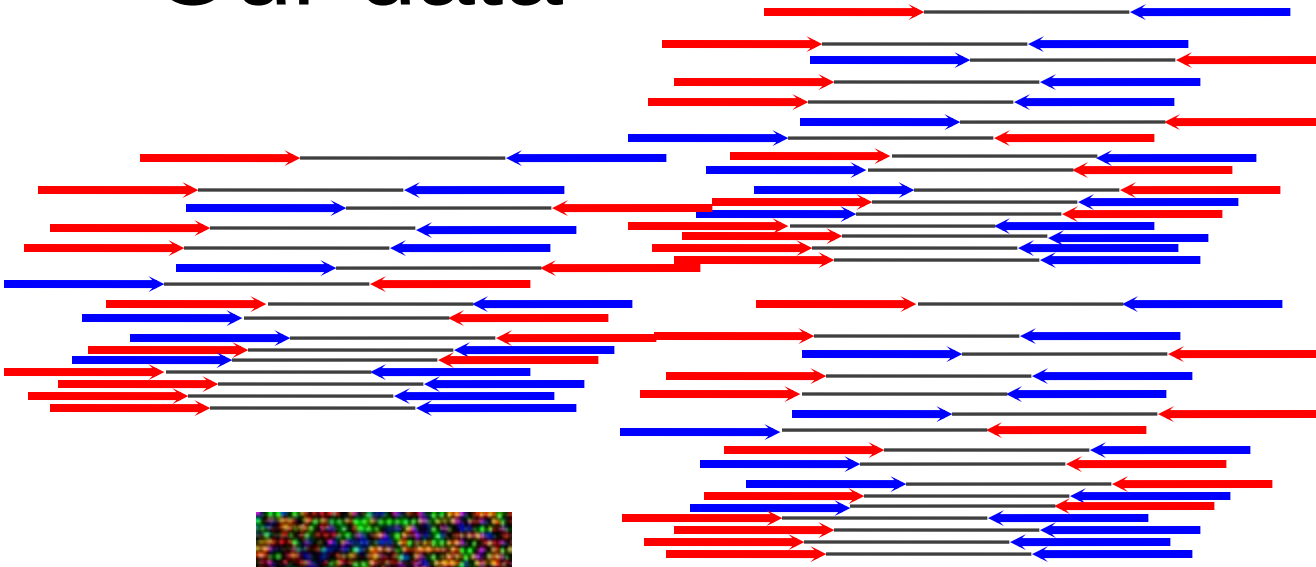
Some slides adapted from Dave Larson, Aaron Quinlan, Sam Peters, and Alex Paul

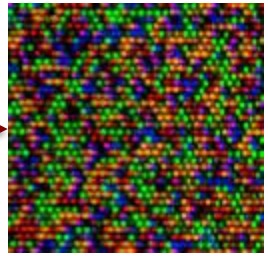
Small Variant Calling



Our data

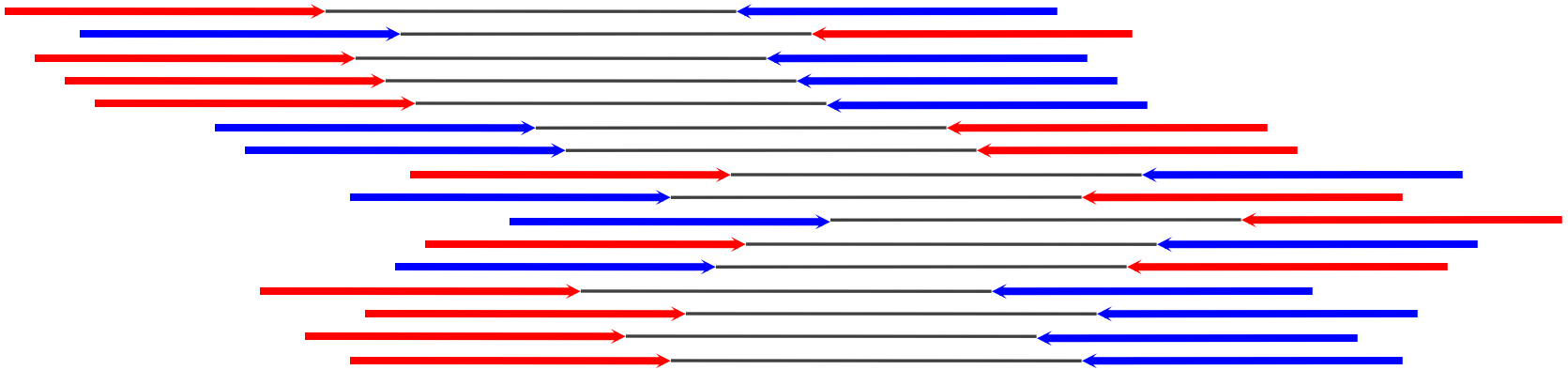






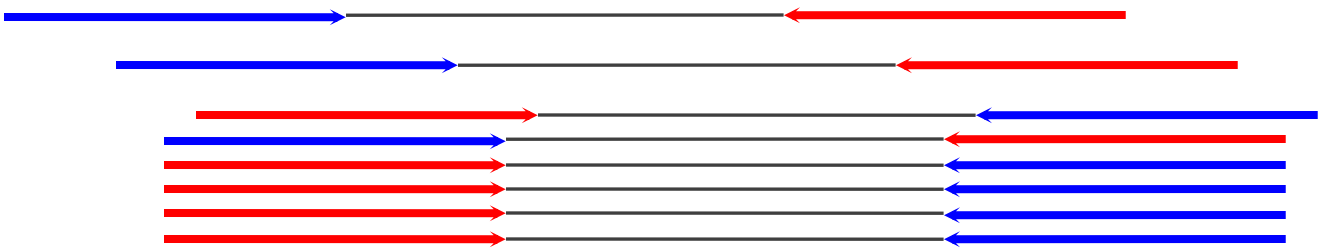
Mapping

Genome Reference Sequence

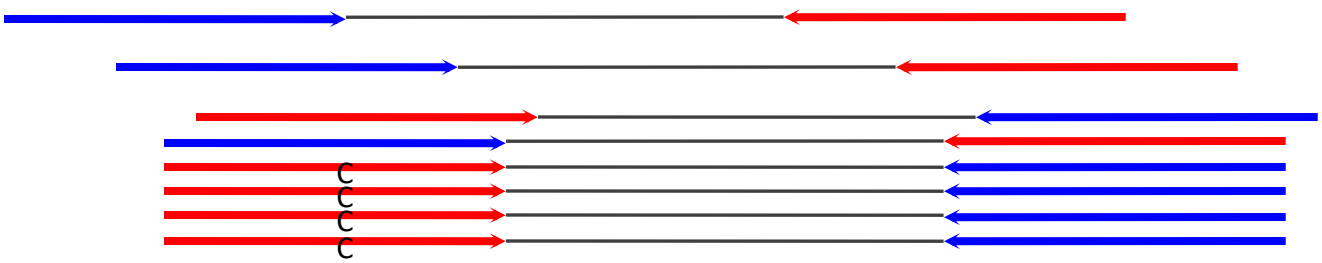


- Single-end reads can be longer, less unique depending on sequence context
- Paired-end reads can span repetitive regions, provide additional information
- Mapping has gotten quite fast, <24 hours for 120 Gbp of sequence
- Split-read alignments are the norm (BWA mem)

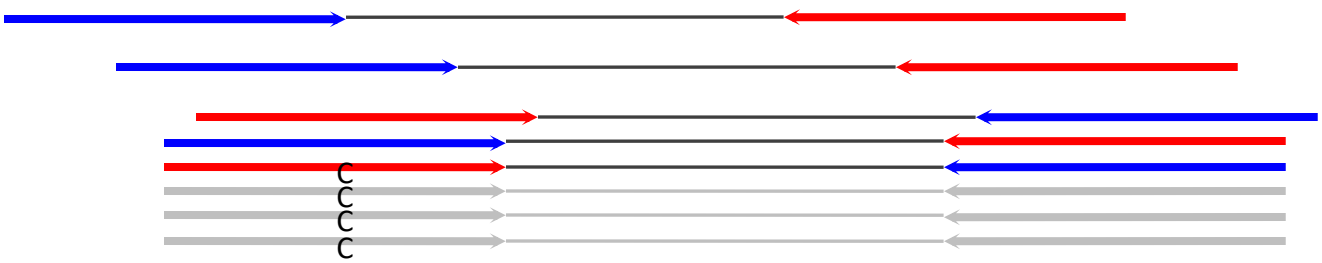
Duplication



Duplication

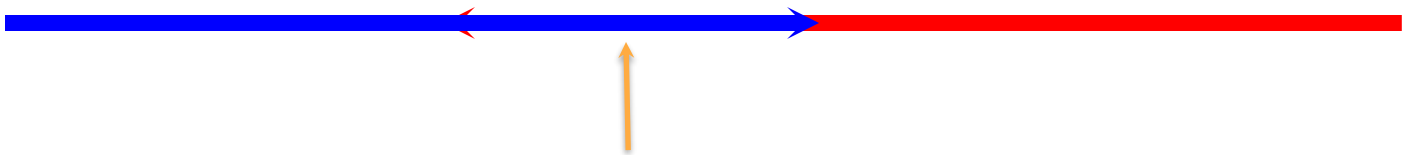


Duplication

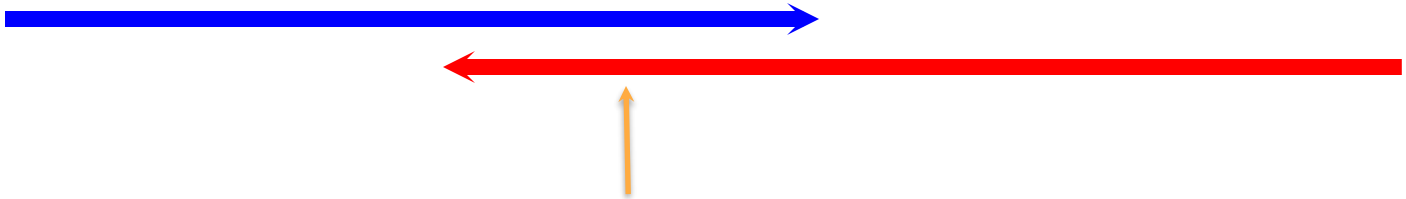


Overlapping reads

Overlapping reads



Overlapping reads



Overlapping reads

Every aspect of this process is fraught with error

- Base calling is not perfect: *0.5% error on average*
- Mapping is not perfect*: the reads are short*
- The reference sequence is not perfect

We have a little help

- Some uncertainty is encapsulated in quality scores
  - the rate at which the data is expected to be wrong
- Each base call (ACTGN) comes with a quality
  - Phred-scaled (-10 * $\log_{10}$ of quality)
  - A base call with quality of 20 is wrong 1 out of every 100 times.
- Read mapping has quality too
  - These are also Phred-scaled

Phred quality score calculation

$$Q = -10*\log_{10}(P_{err})$$

Error probability (P_{err})	$\log_{10}(P_{err})$	Phred quality score
1	0	0
0.1	-1	10
0.01	-2	20
0.001	-3	30
0.0001	-4	40

Goals of a Variant Caller

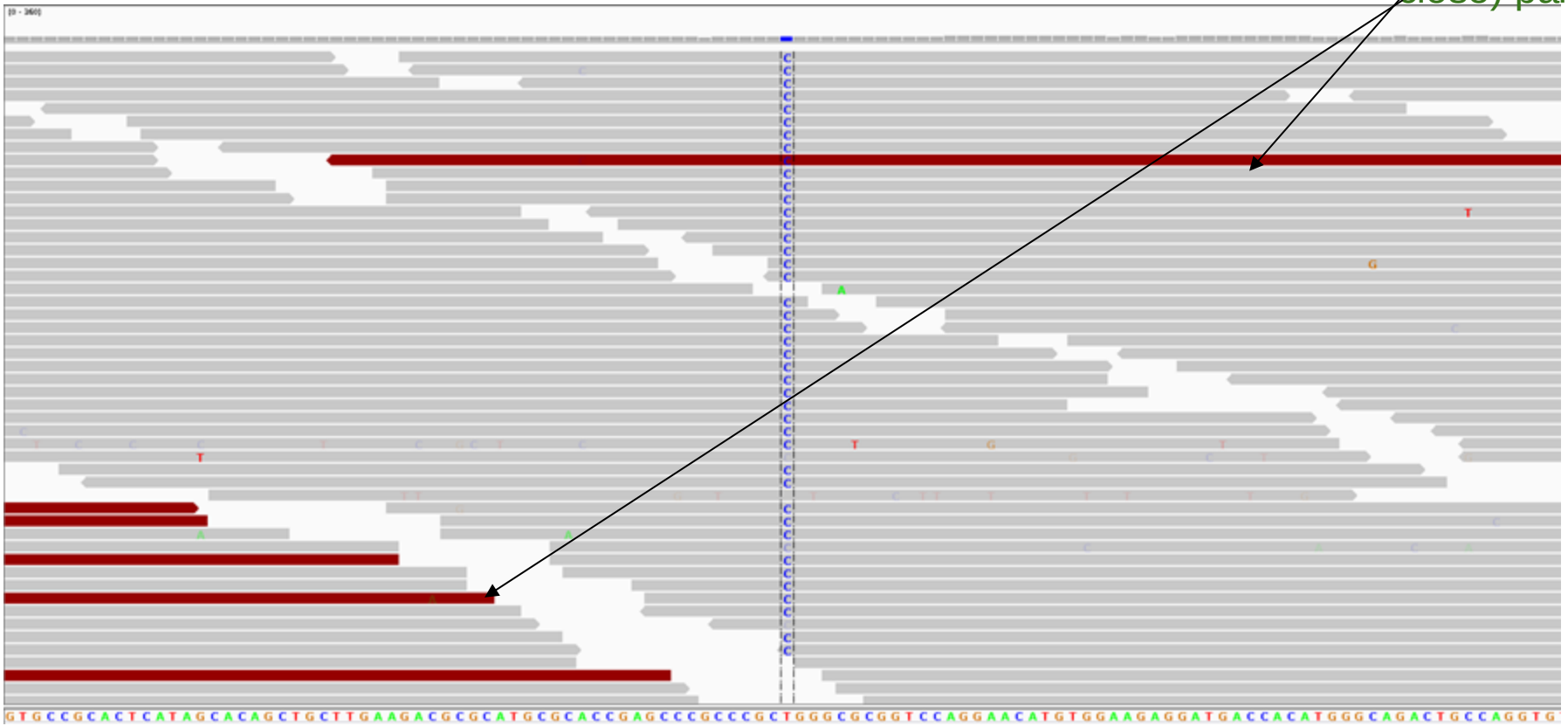
- Sensitively detect mutations
- Precisely detect mutations
  - Confounded by the error we just talked about
  - FDR must be very low as we're looking across a very large space!

Goals of a Variant Caller

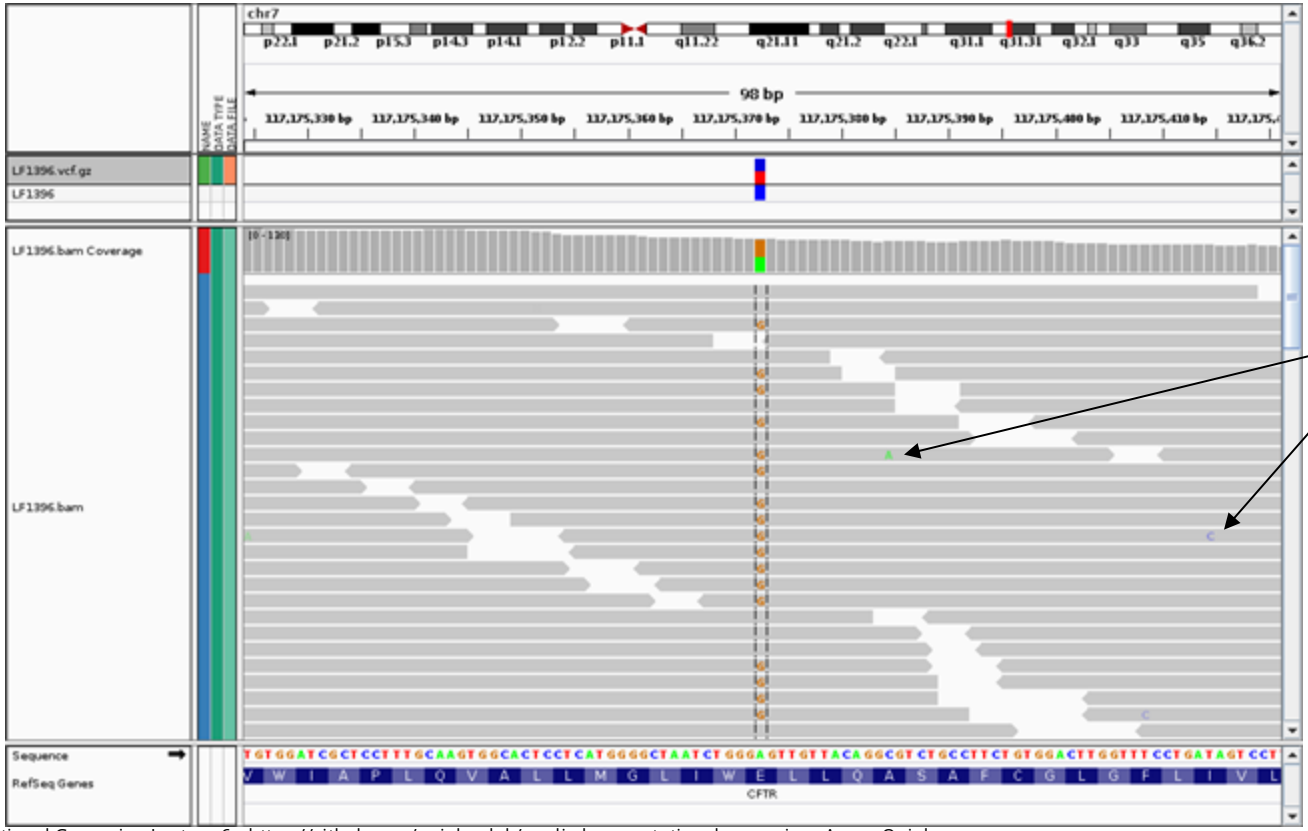
- Sensitively detect mutations
- Precisely detect mutations
  - Confounded by the error we just talked about
  - FDR must be very low as we're looking across a very large space!

- **An FDR of 0.001 = 3.2 million false positives!**

Homozygous for the "C" allele



Sequencing errors fall out as noise (most of the time)



Sequencing errors

adapted from: Applied Computational Genomics, Lecture 6 - https://github.com/quinlan-lab/applied-computational-genomics - Aaron Quinlan

https://jchoigt.files.wordpress.com/2012/07/igv_e217g_snapshot.p

It is not always so easy

Random versus systematic error

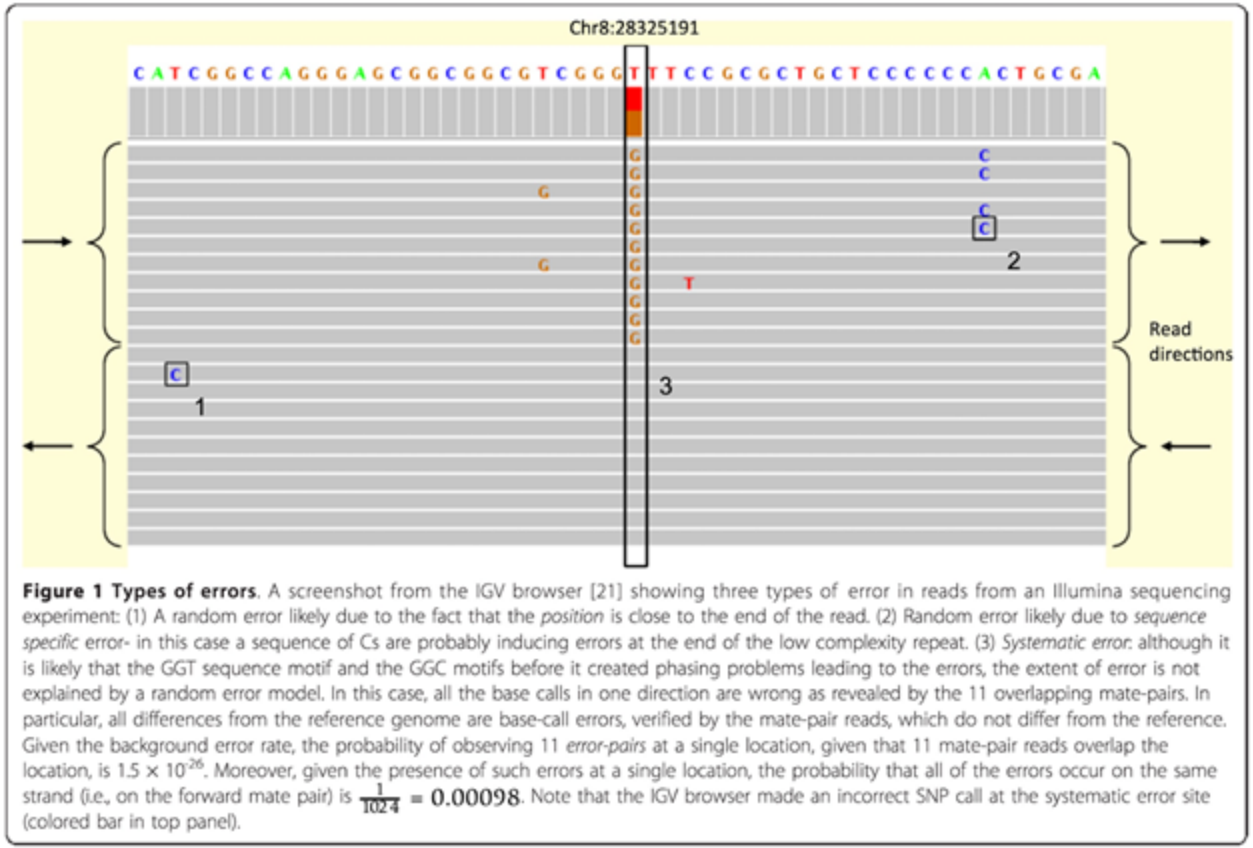
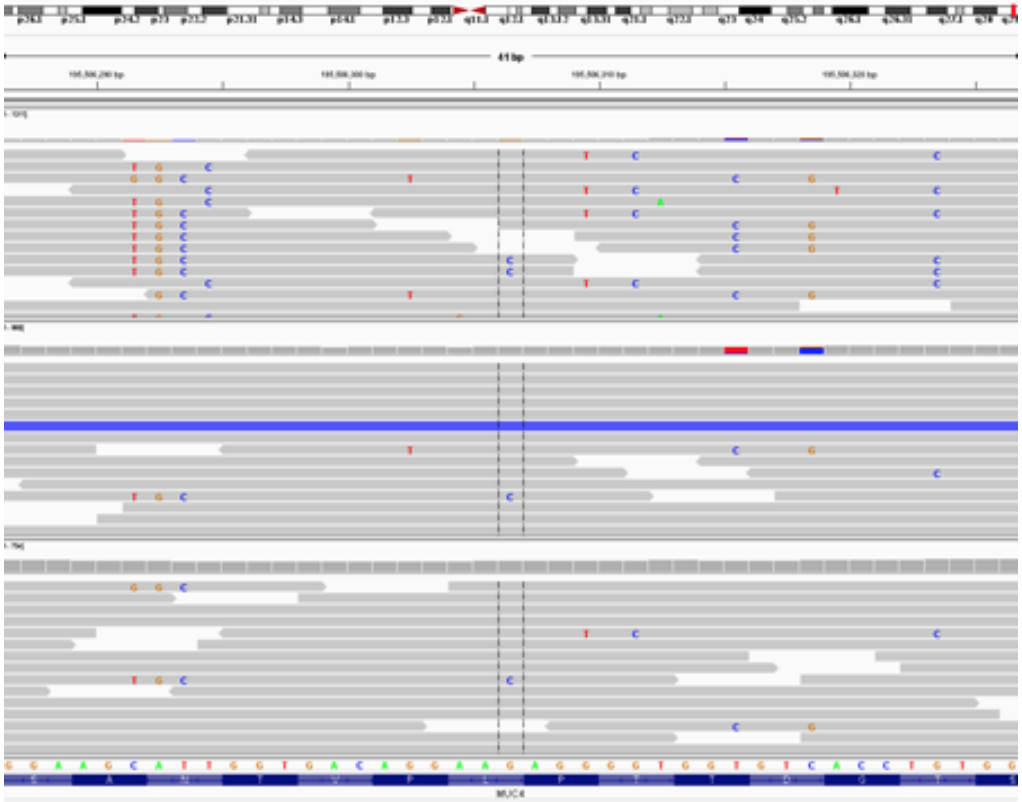


Figure 1 Types of errors. A screenshot from the IGV browser [21] showing three types of error in reads from an Illumina sequencing experiment: (1) A random error likely due to the fact that the *position* is close to the end of the read. (2) Random error likely due to *sequence specific* error- in this case a sequence of Cs are probably inducing errors at the end of the low complexity repeat. (3) *Systematic error*: although it is likely that the GGT sequence motif and the GGC motifs before it created phasing problems leading to the errors, the extent of error is not explained by a random error model. In this case, all the base calls in one direction are wrong as revealed by the 11 overlapping mate-pairs. In particular, all differences from the reference genome are base-call errors, verified by the mate-pair reads, which do not differ from the reference. Given the background error rate, the probability of observing 11 *error-pairs* at a single location, given that 11 mate-pair reads overlap the location, is 1.5×10^{-26}. Moreover, given the presence of such errors at a single location, the probability that all of the errors occur on the same strand (i.e., on the forward mate pair) is $\frac{1}{1024} = 0.00098$. Note that the IGV browser made an incorrect SNP call at the systematic error site (colored bar in top panel).

adapted from: Applied Computational Genomics, Lecture 6 - https://github.com/quinlan-lab/applied-computational-genomics - Aaron Quinlan

https://www.researchgate.net/publication/51814240_Identification_and_correction_of_systematic_error_in_high-

Pileups of many differences from paralogy



RESEARCH ARTICLE | OPEN ACCESS

FLAGS, frequently mutated genes in public exomes

Casper Shyr, Maja Tarailo-Graovac, Michael Gottlieb, Jessica JY Lee, Clara van Karnebeek and Wyeth W Wasserman

BMC Medical Genomics 2014 7:64 | DOI: 10.1186/s12920-014-0064-y | © Shyr et al.; licensee BioMed Central Ltd. 2014

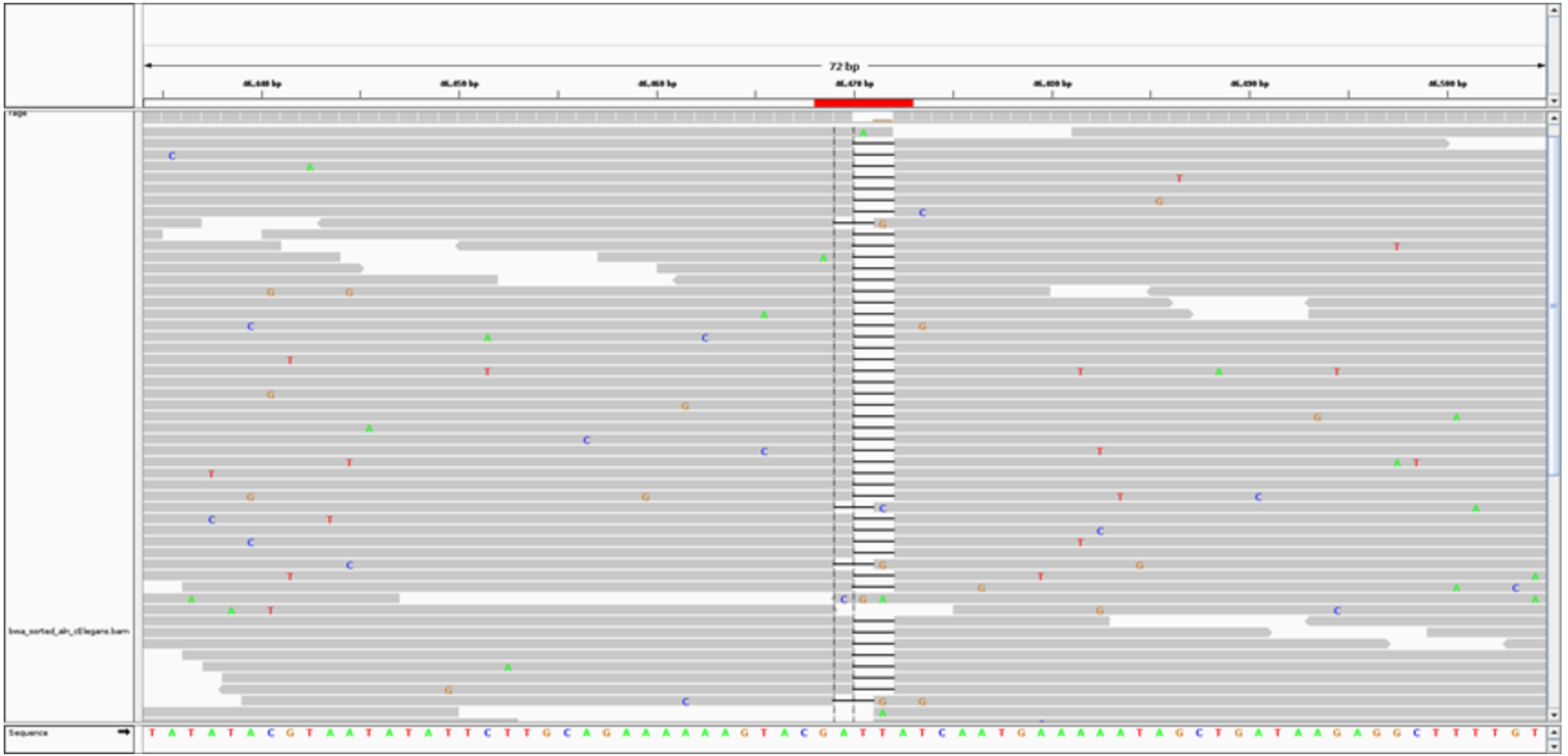
Received: 16 June 2014 | Accepted: 24 October 2014 | Published: 3 December 2014

Open Peer Review reports

adapted from: Applied Computational Genomics, Lecture 6 - https://github.com/quinlan-lab/applied-computational-genomics - Aaron Quinlan

http://massgenomics.org/2013/06/ngs-false-positives.html

Calling INDELs is *much* harder than SNPs

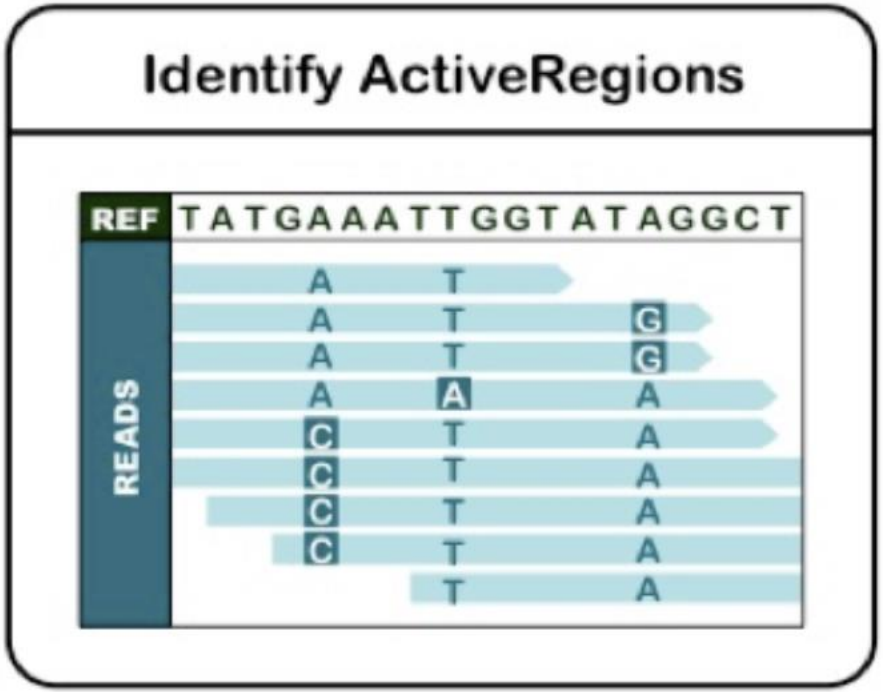


adapted from: Applied Computational Genomics, Lecture 6 - https://github.com/quinlan-lab/applied-computational-genomics - Aaron Quinlan

Exercise

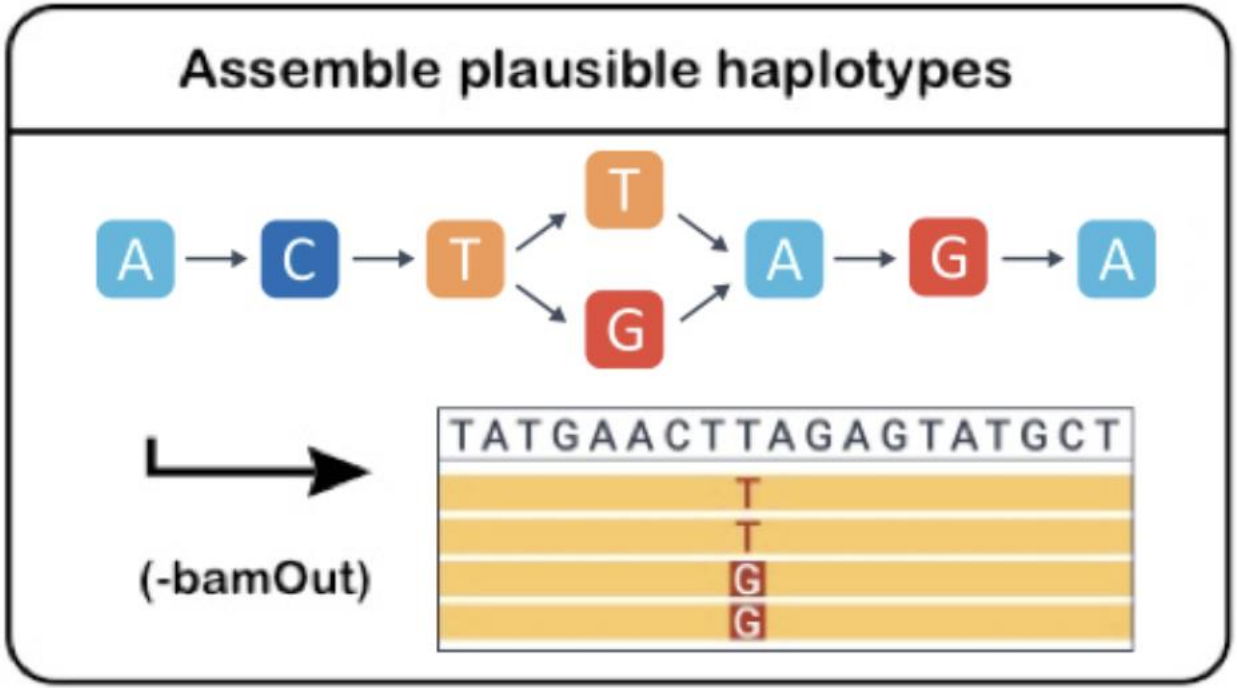
Germline SNV and Indel Calling

Call Genotypes Using GATK HaplotypeCaller



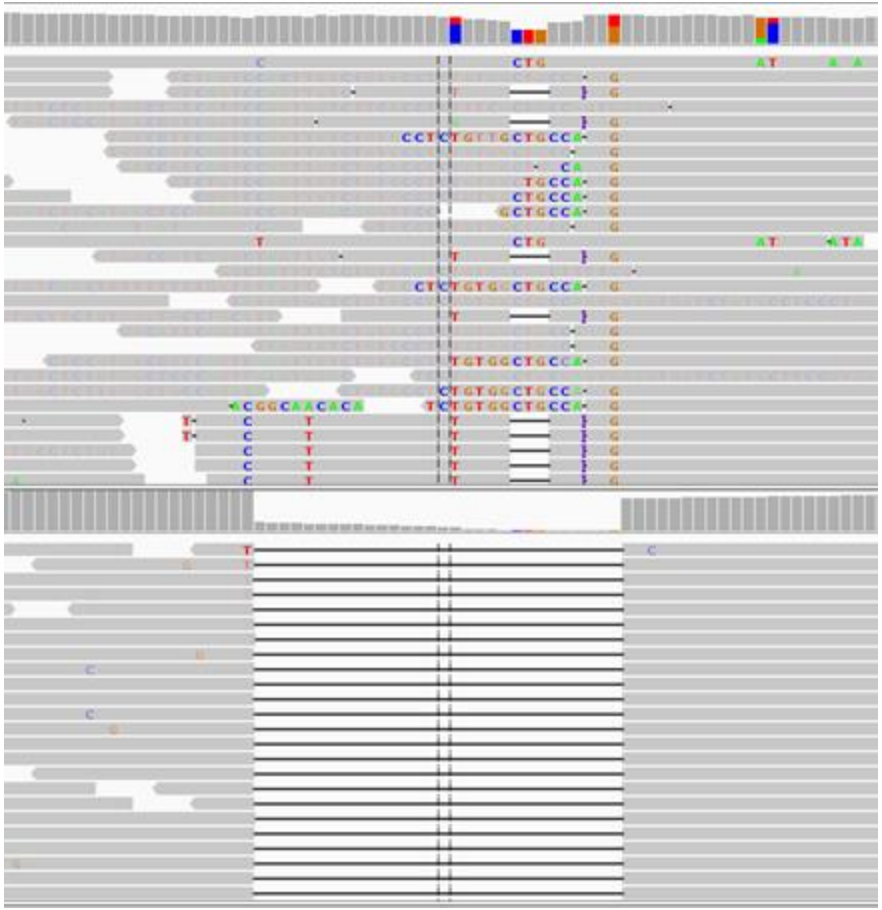
https://gatk.broadinstitute.org/hc/en-us/articles/360035531412-HaplotypeCaller-in-a-nutshell

Call Genotypes Using GATK HaplotypeCaller



https://gatk.broadinstitute.org/hc/en-us/articles/360035531412-HaplotypeCaller-in-a-nutshell

Indel "realignment"

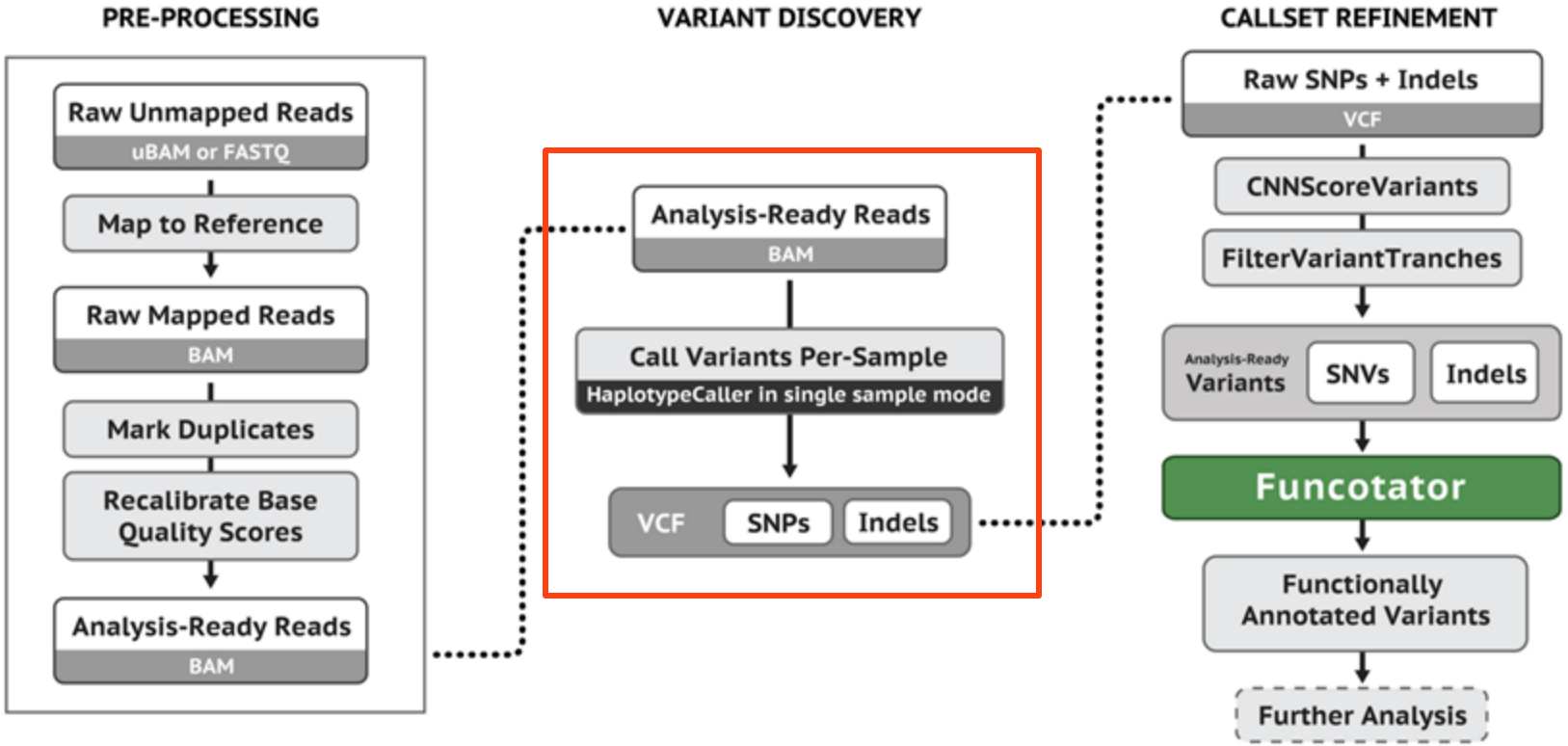


https://github.com/mozack/abra

Call Genotypes Using GATK HaplotypeCaller

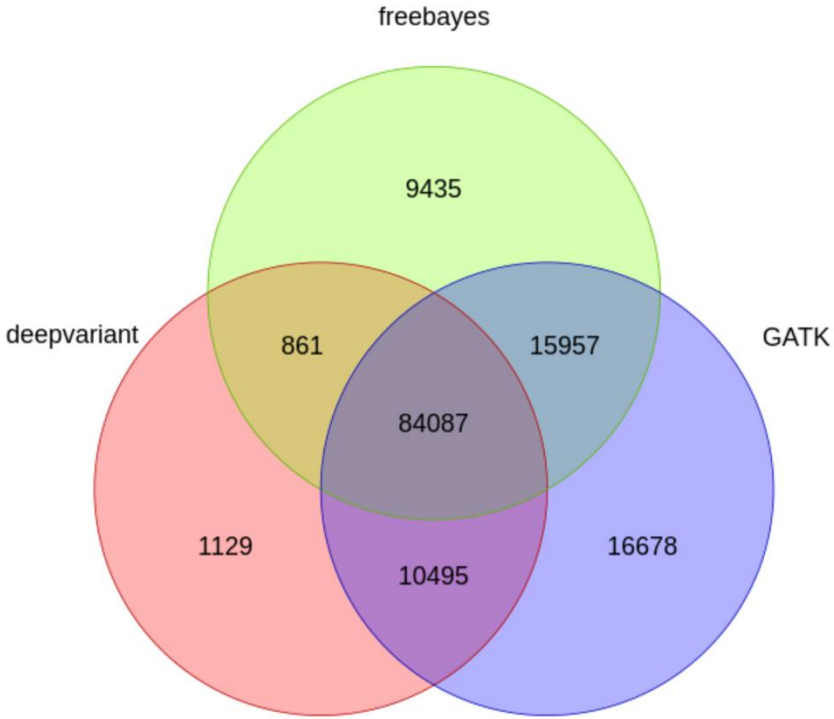
https://gatk.broadinstitute.org/hc/en-us/articles/360035531412-HaplotypeCaller-in-a-nutshell

Main steps for Germline Single-Sample Data



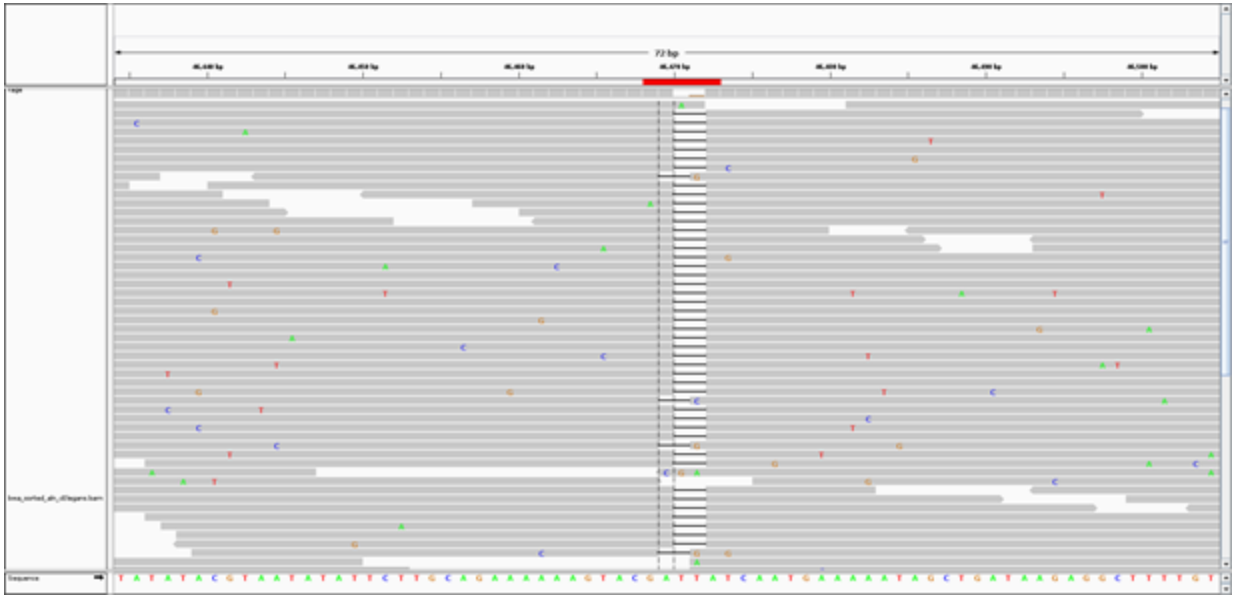
Other Germline Variant callers

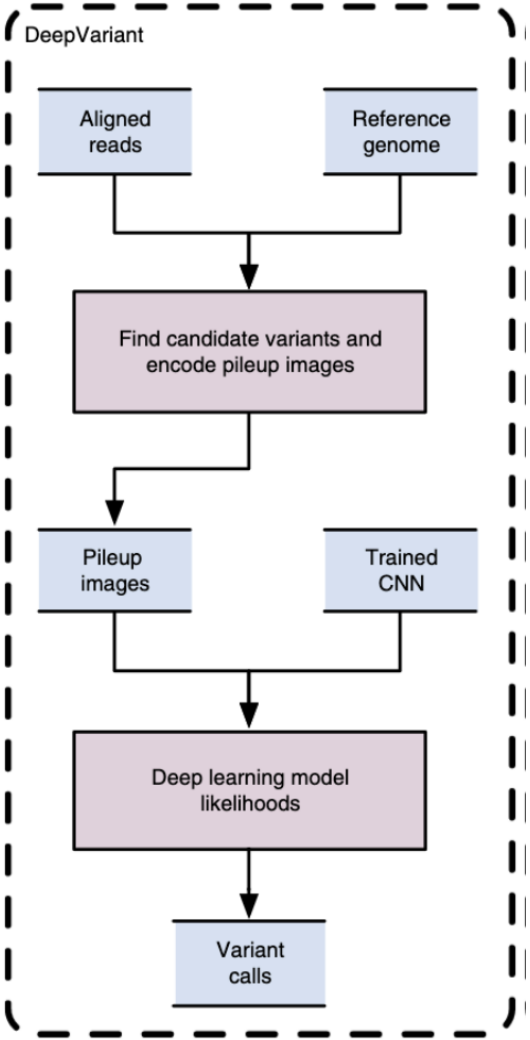
- Freebayes: lighter weight, faster
- DeepVariant: Neural-network based caller from Google ML



https://ucdavis-bioinformatics-training.github.io/2021-July-Genome-Wide-Association-Studies/data_reduction/comparison

DeepVariant

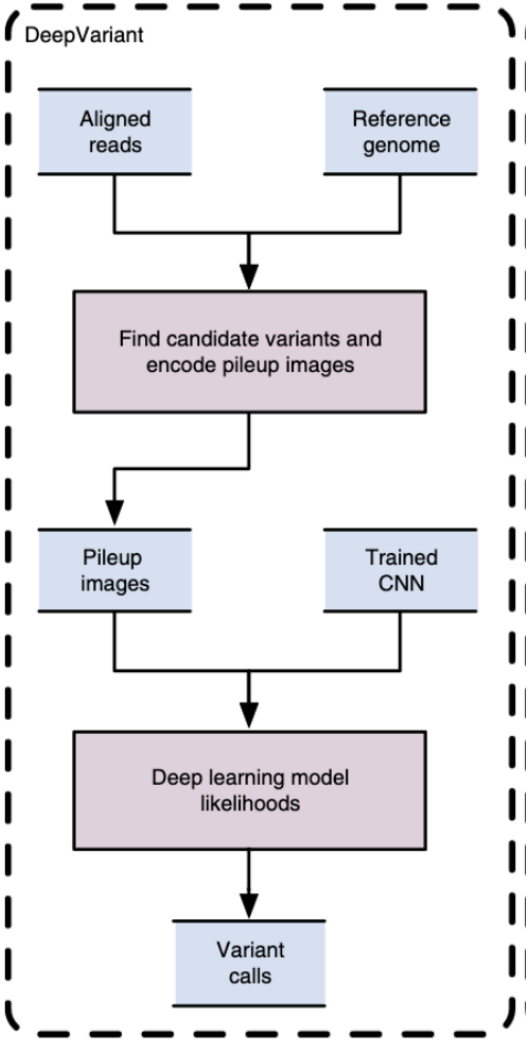




https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6450397/

https://www.nature.com/articles/nbt.4235

DeepVariant



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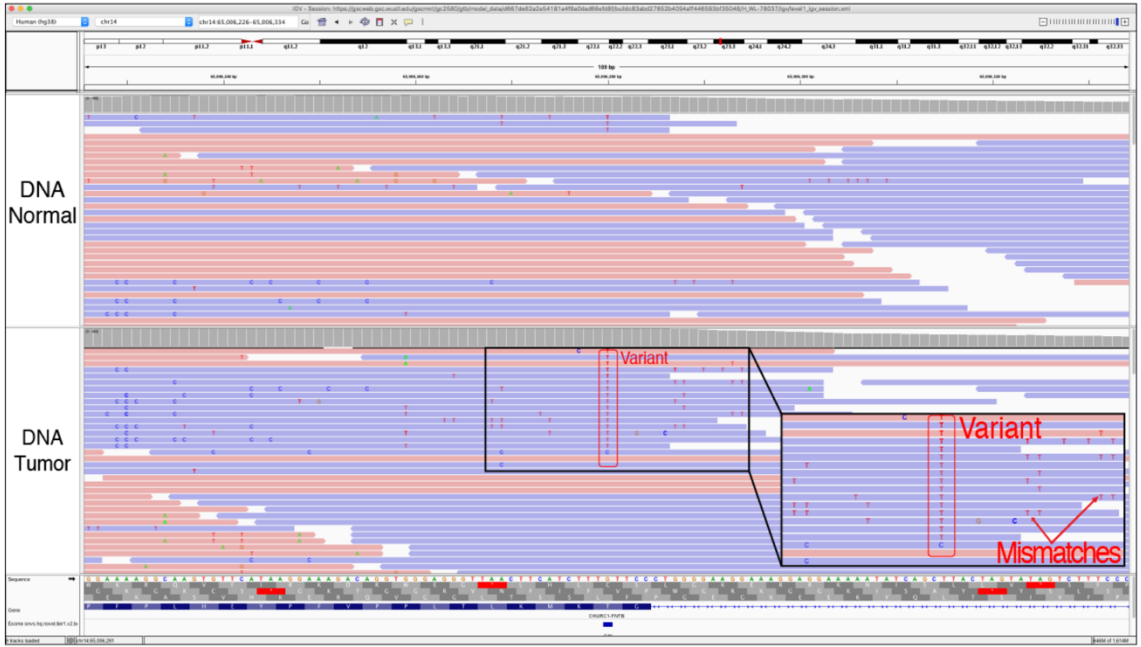
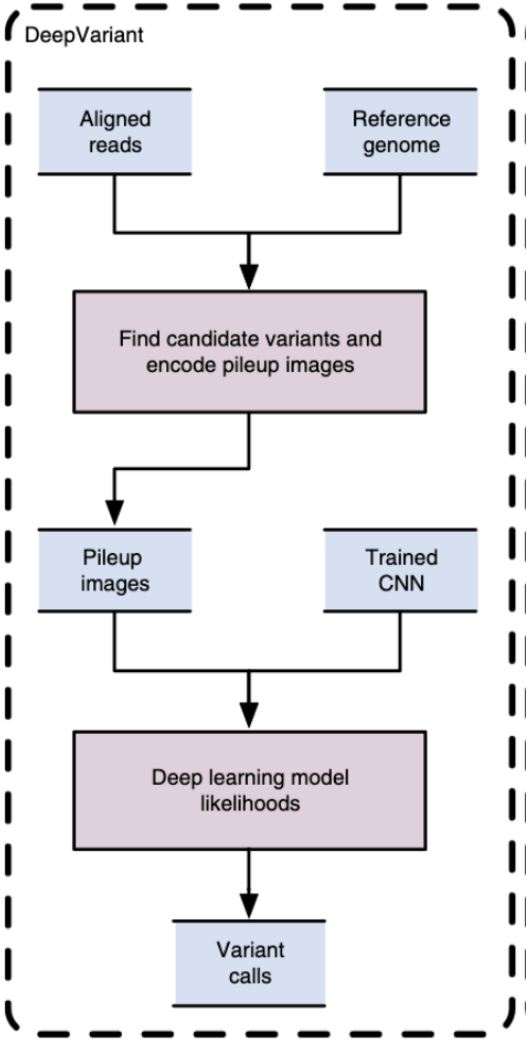
DeepVariant





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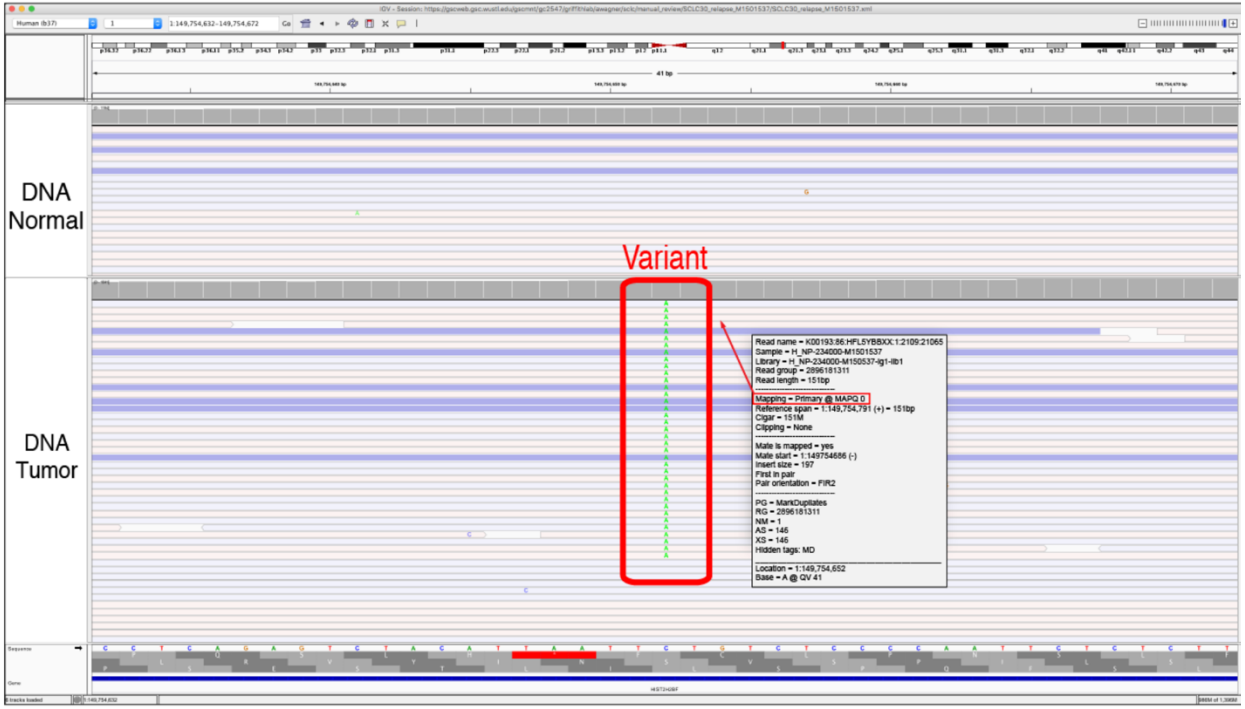
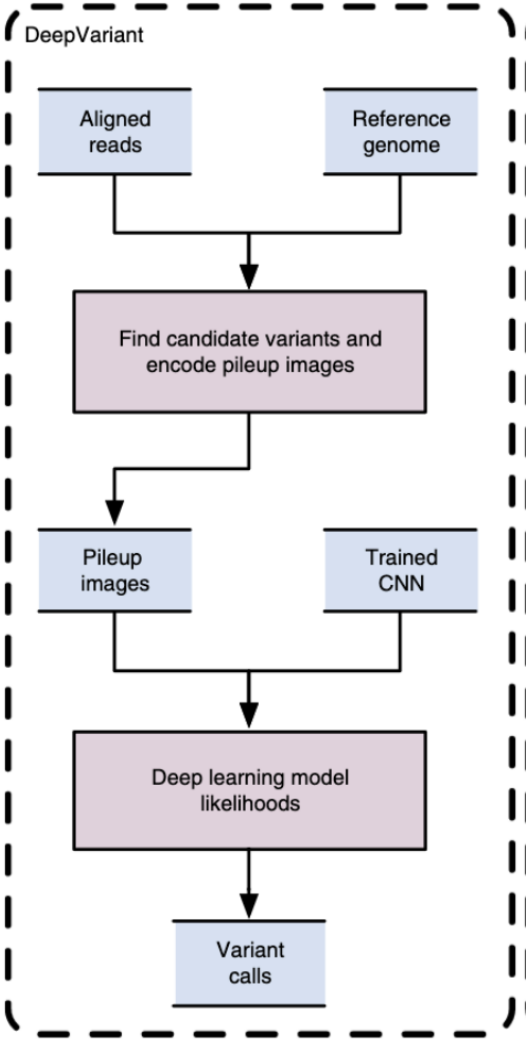
DeepVariant





https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6450397/

https://www.nature.com/articles/nbt.4235

DeepVariant

Here are 3 examples that we would consider canonical easy-to-classify loci, and that DeepVariant calls confidently and correctly:

The variant above is a “2”, which means both chromosomes match the variant allele, so this locus represents a homozygous alternate locus.

DeepVariant correctly classifies the variant above as a “1”, which means that one of the two alleles matches the variant allele, i.e. it is heterozygous.

https://google.github.io/deepvariant/posts/2020-02-20-looking-through-deepvariants-eyes/

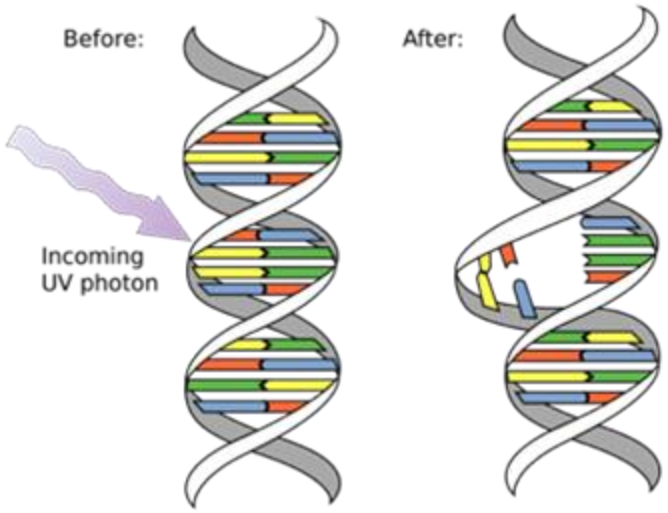
Somatic Mutation Calling

Cancer is a disease of the genome

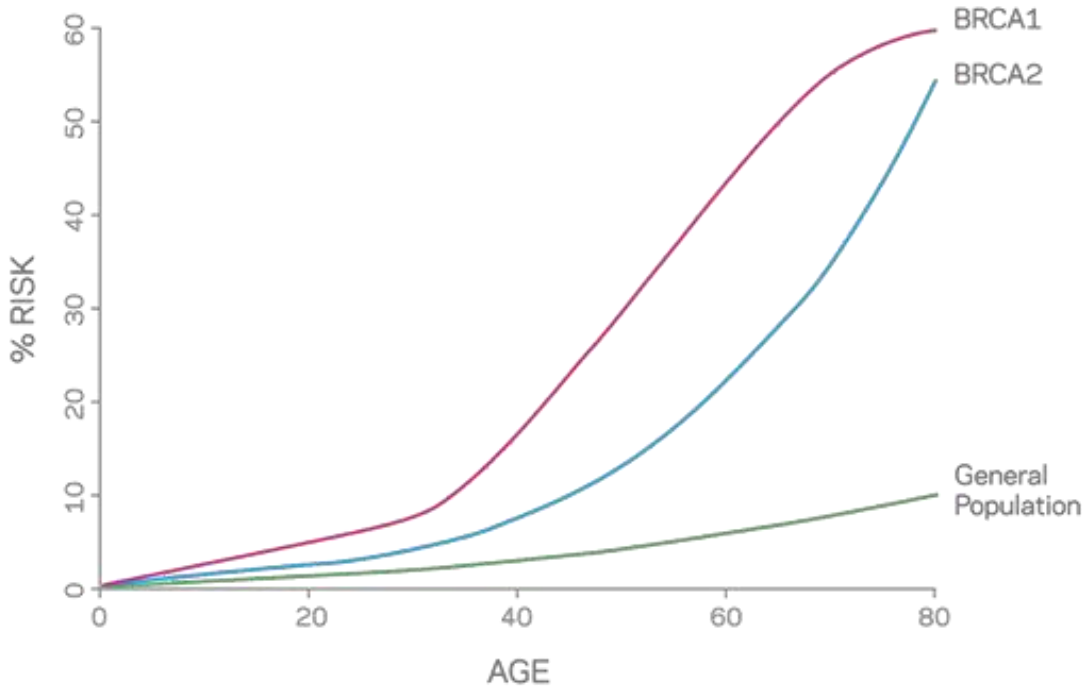
- Cancer is caused by **somatic** mutations

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



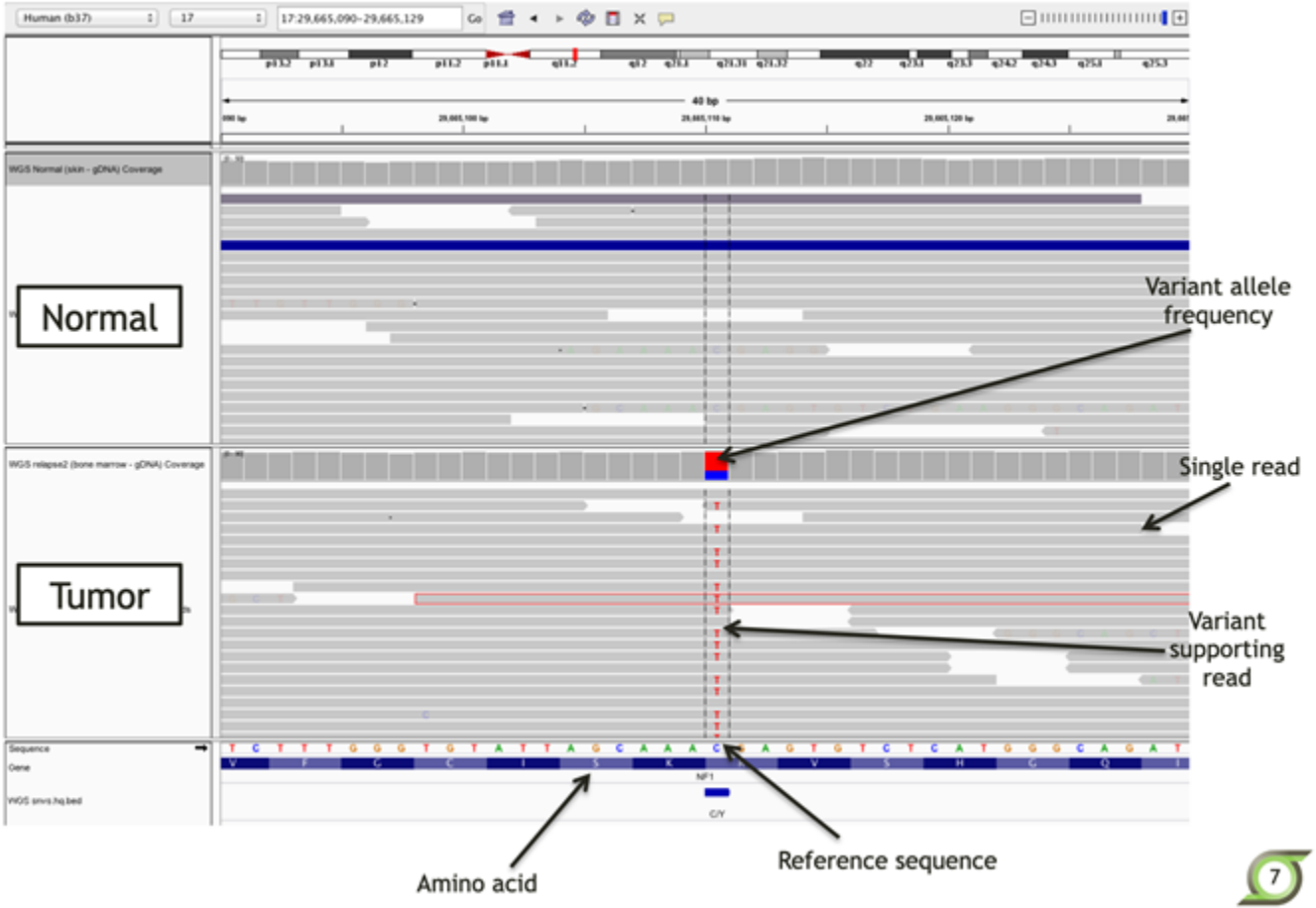
Germline Predisposition



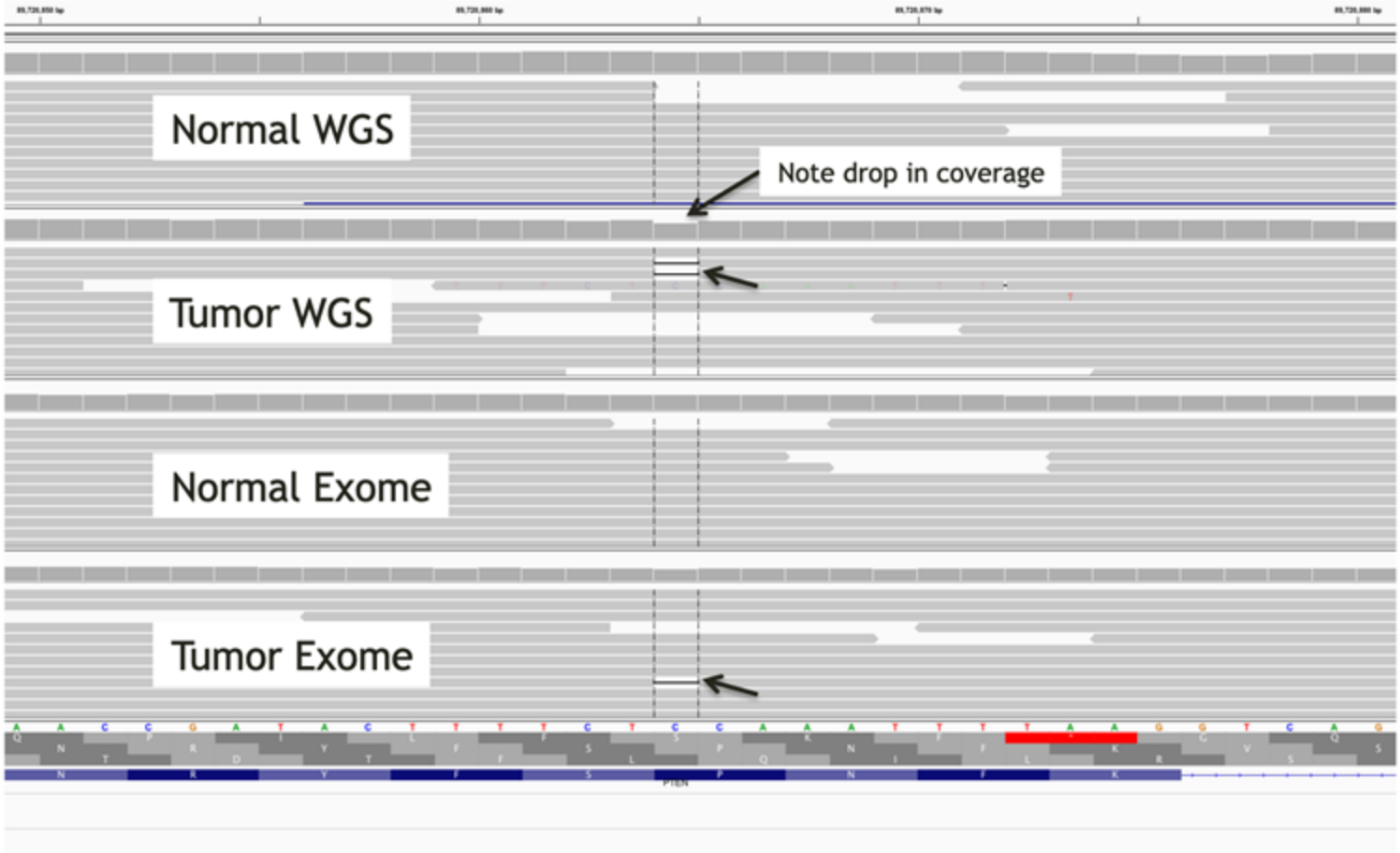
Cancer Sequencing

- 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

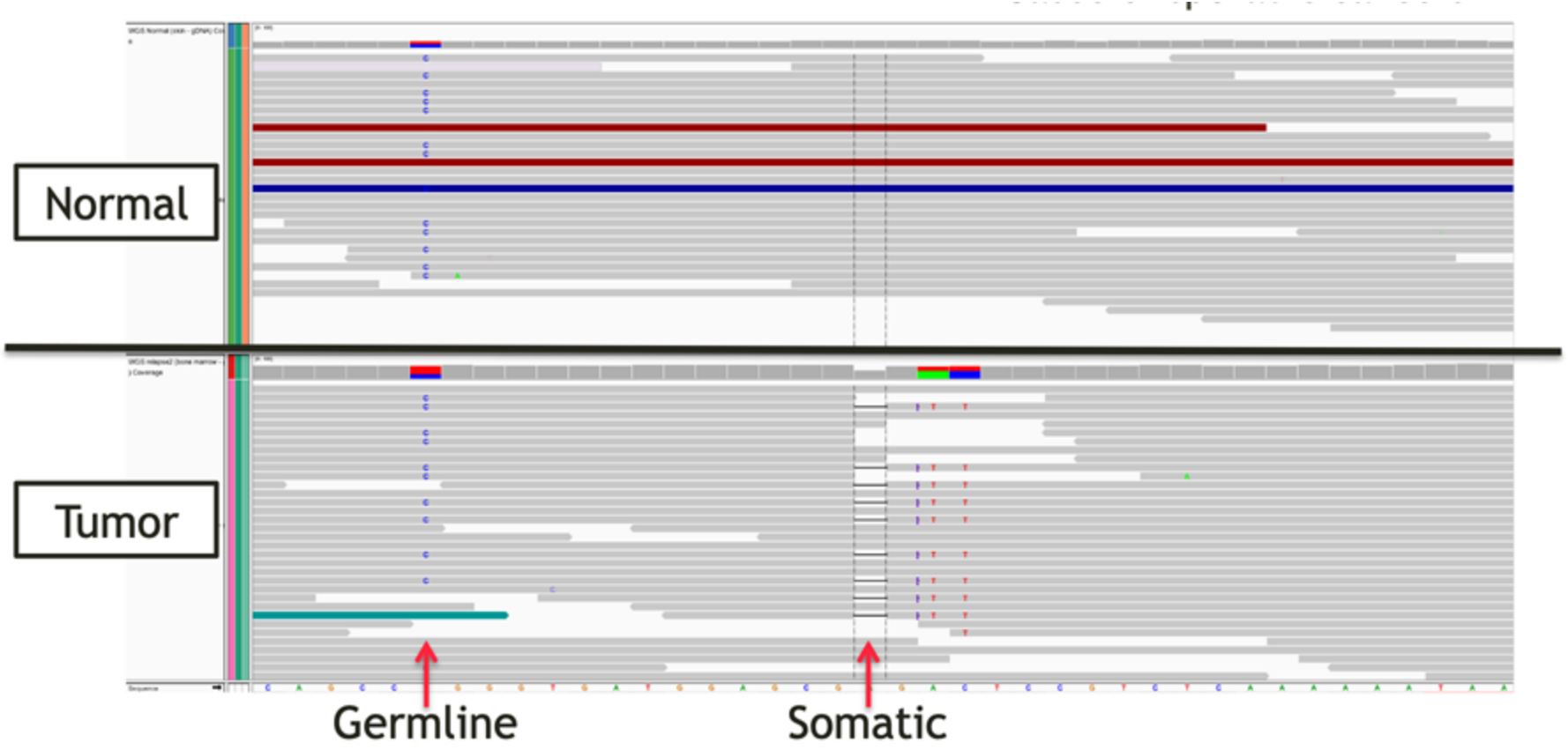
What do somatic variants look like?



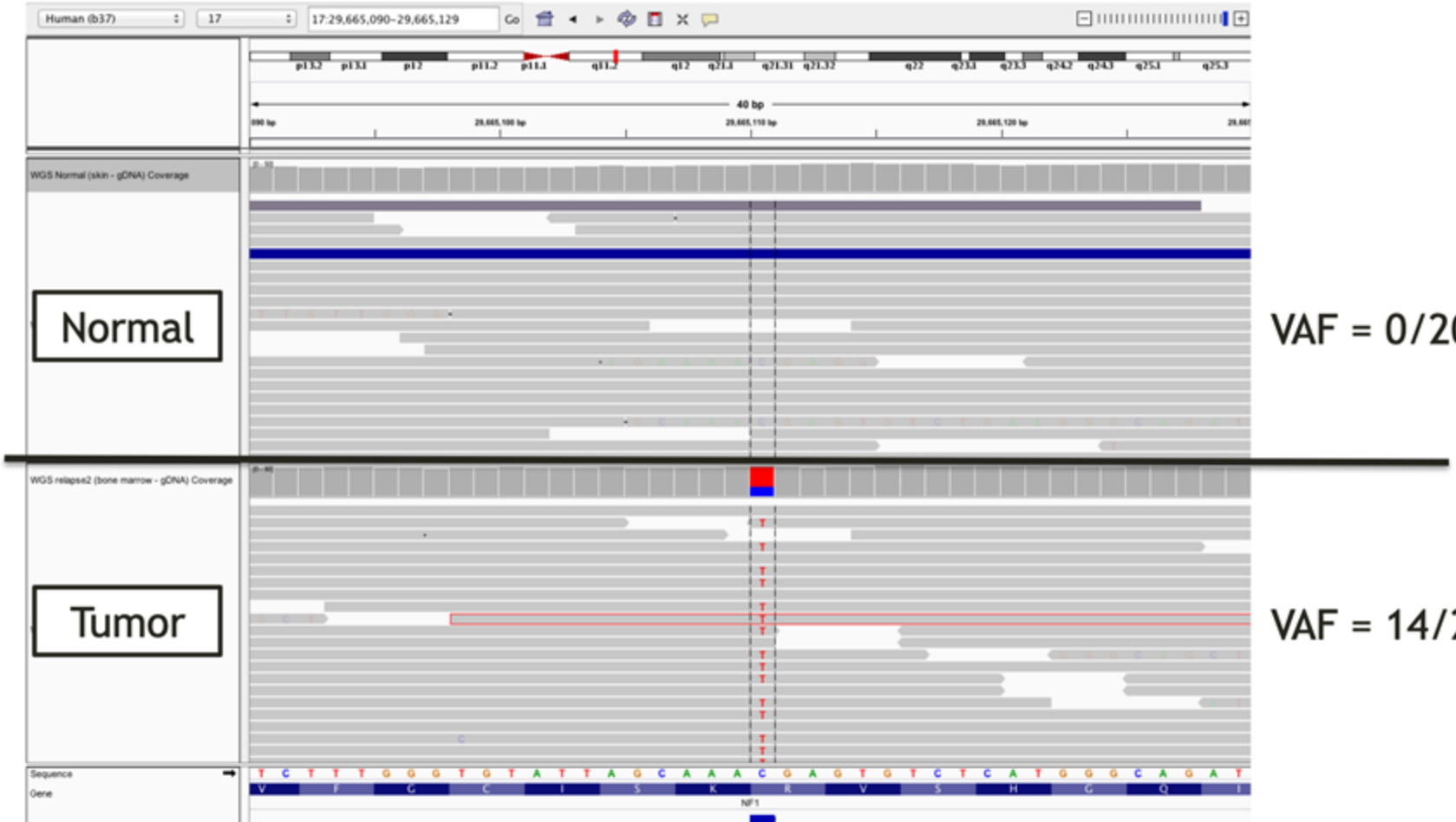
Indels


Normal WGS
Note drop in coverage
Tumor WGS
Normal Exome
Tumor Exome
PTEN

Germline vs Somatic



VAF = Variant reads / Total reads

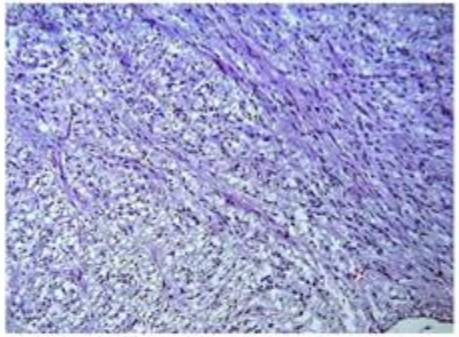


VAF = 0/20 = 0%

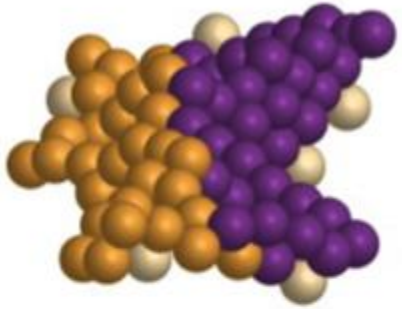
VAF = 14/20 = 70%

Check on Mutect

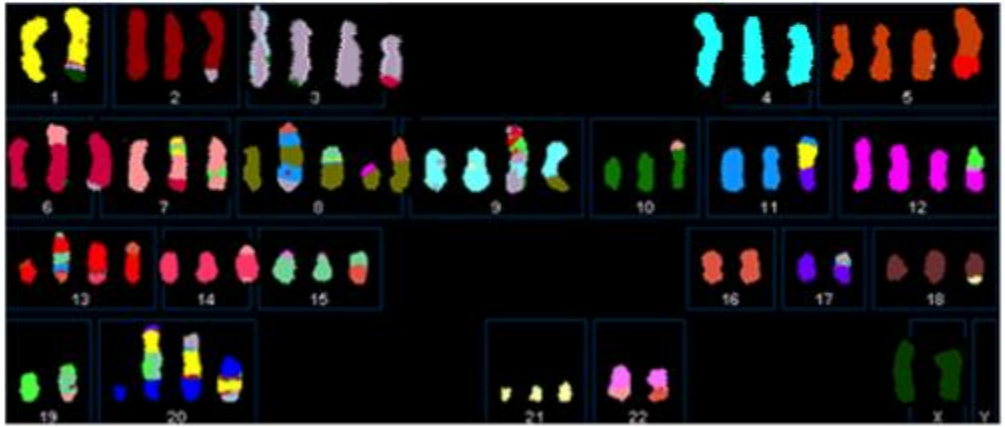
Tumors are often impure, heterogeneous, and aneuploid



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

VAF = 0/20 = 0%

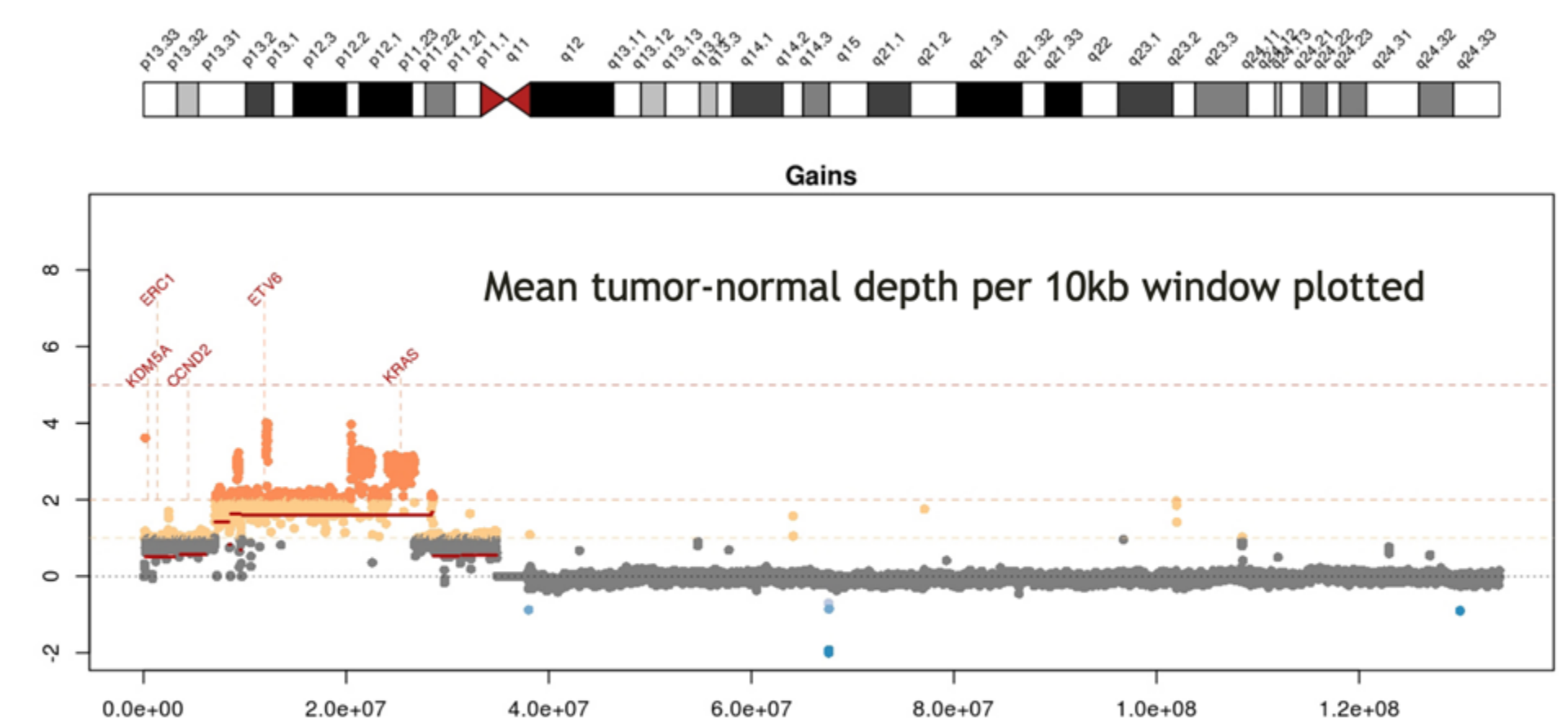
VAF = 14/20 = 70%

How does purity influence VAF?

VAF = Variant reads / Total reads

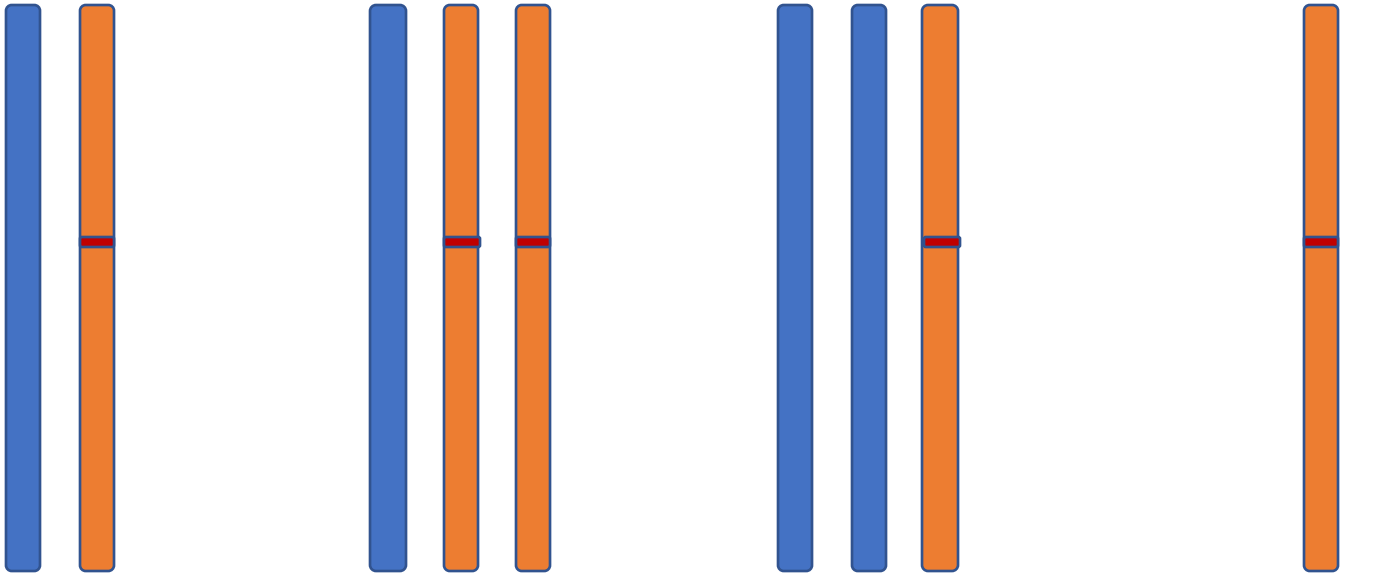
VAF = 0/20 = 0%

VAF = 5/20 = 25%



KRAS amplification in a metastatic breast cancer

How does copy number influence VAF?



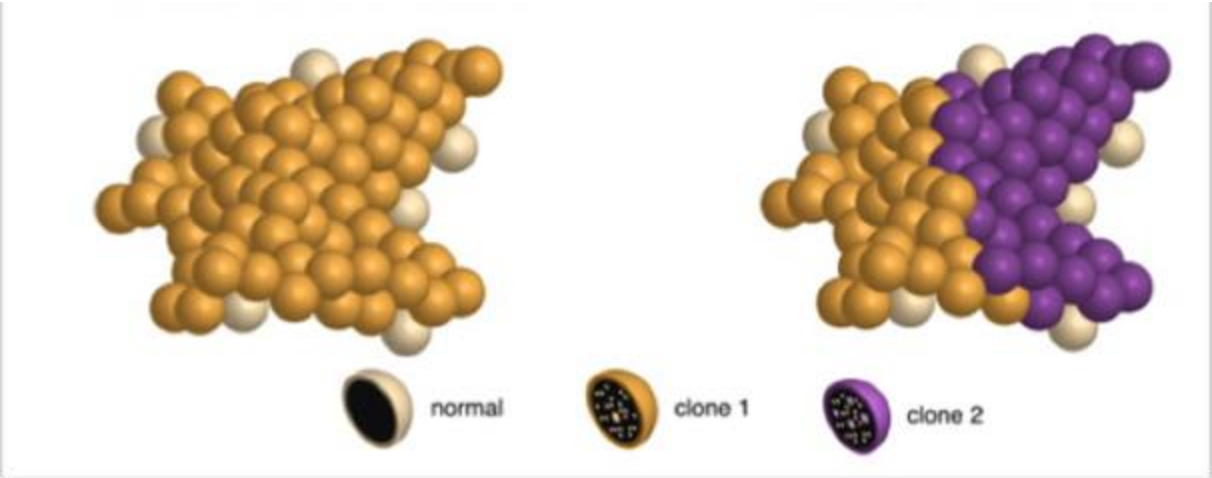
2 copies
50%
VAF

3 copies
66%
VAF

3 copies
33%
VAF

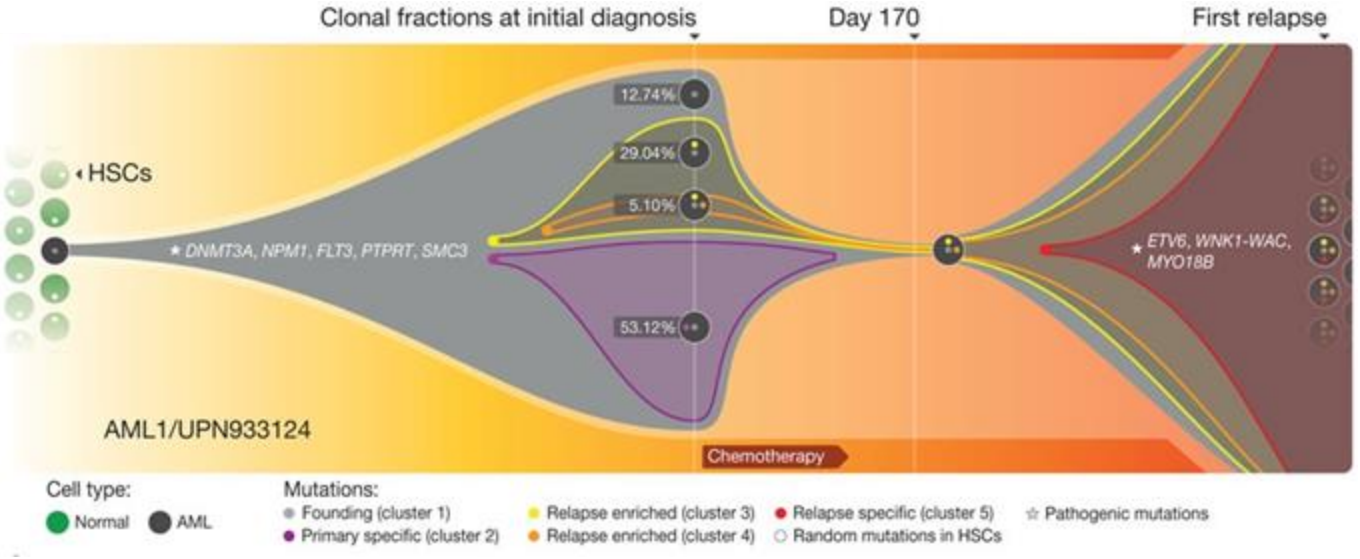
1 copy
100%
VAF

How does clonality influence VAF?



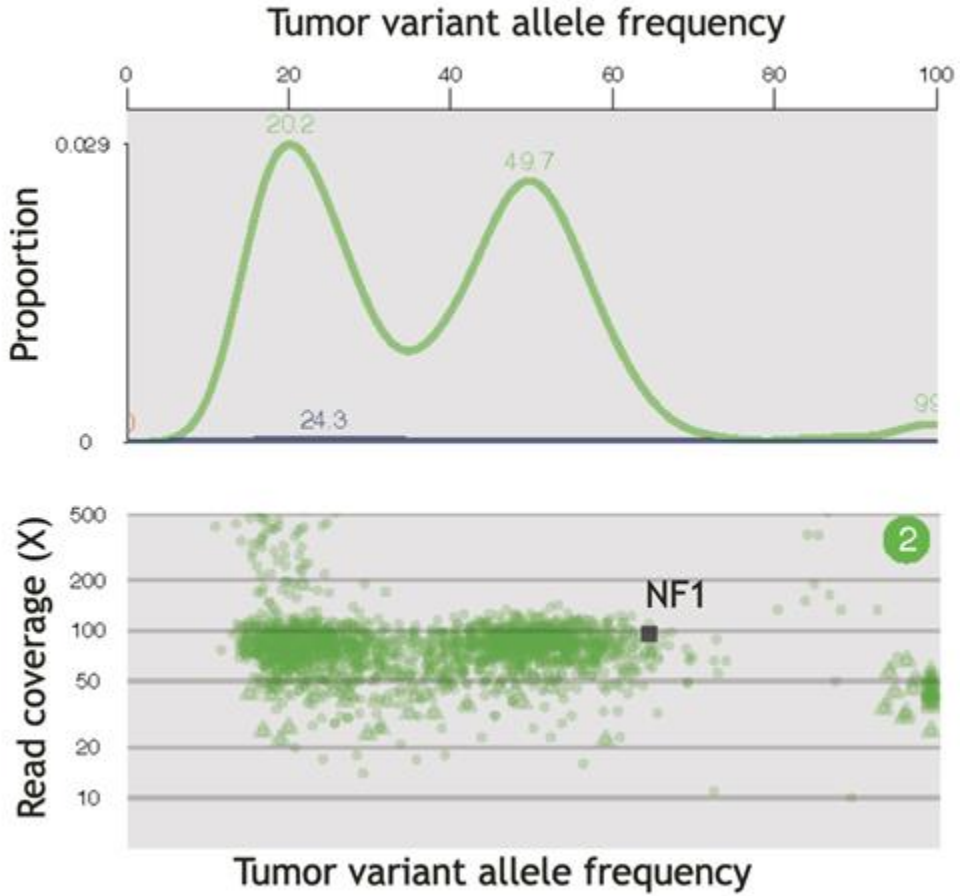
- Subclones contain genetically diverse populations of cells
- Evolution occurs at the molecular and cellular levels
- The growth rates for subclones are often different

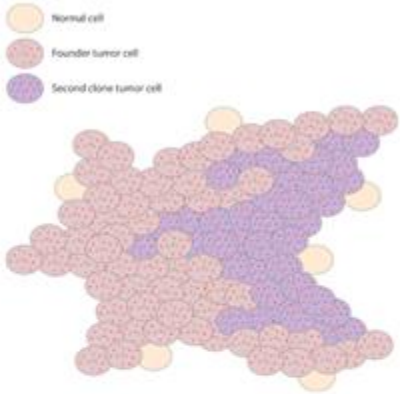
Clonal evolution in relapsed AML



Nature 481, 506–510
doi:10.1038/nature10738

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





WGS data

Somatic variant calling is harder

- There are more factors to consider, a wider range of possibilities, and often, more sketchy samples

Somatic Variant detection callers

- Mutect
- Strelka
- Varscan
- Pindel
- Lancet
- Deep Somatic
- VarDict
- Seurat
- Shimmer
- more...

Lots of choices!

Use of multiple variant callers can improve sensitivity and accuracy