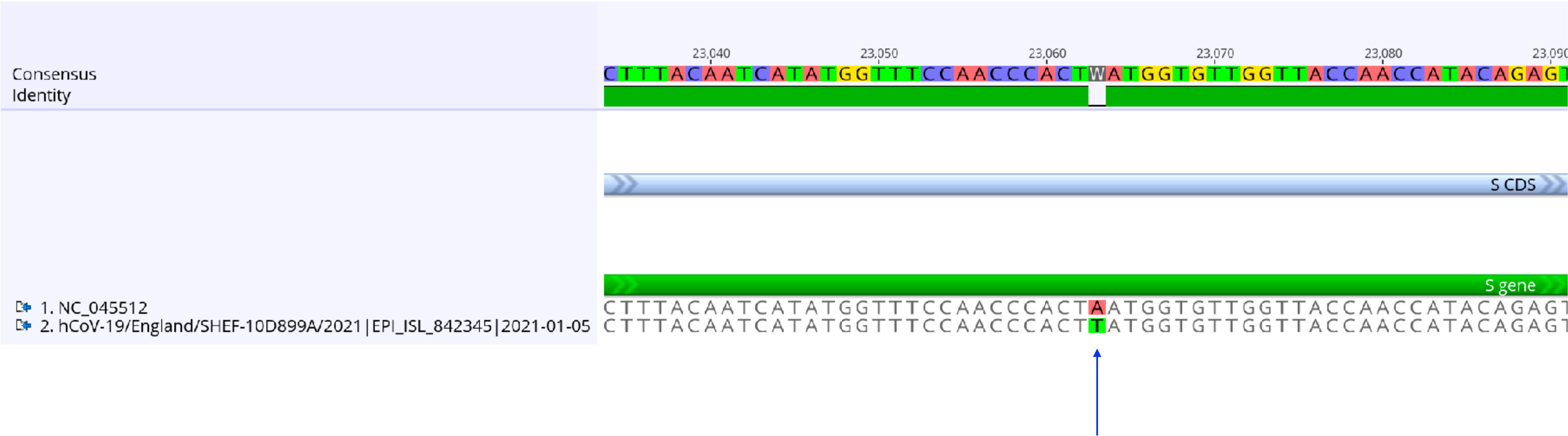


# Detecting and quantifying variants

Mark Stenglein, GDW Workshop



A variant is a difference between a sequence and some reference sequence



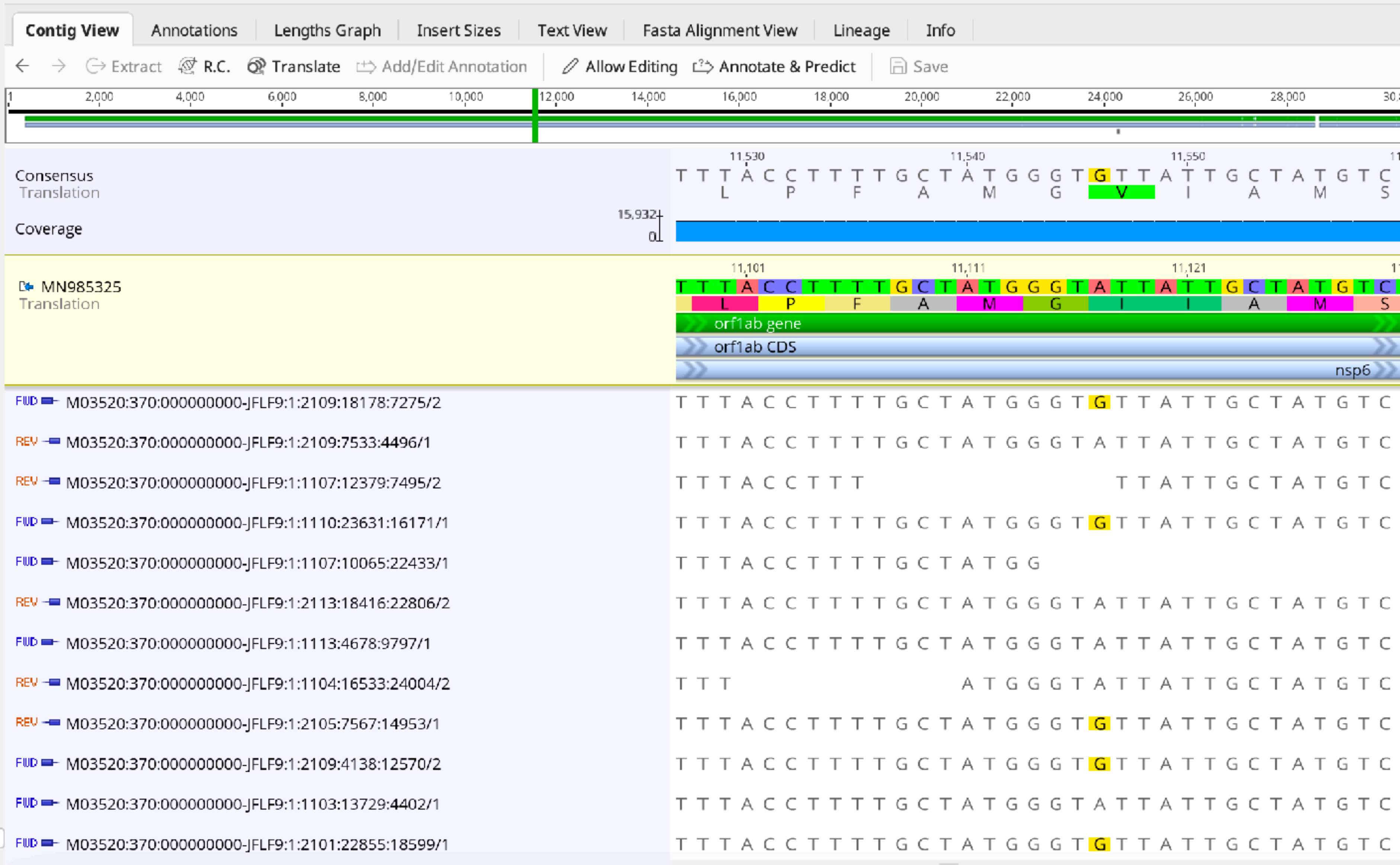
A variant in the SARS-CoV-2 spike sequence relative to the original Wuhan reference sequence

Variants can be single nucleotide variants (SNVs/SNPs)  
 or insertions/deletions and other types of “structural variation



A 2 codon deletion in the SARS-CoV-2 spike sequence relative to the original Wuhan reference sequence

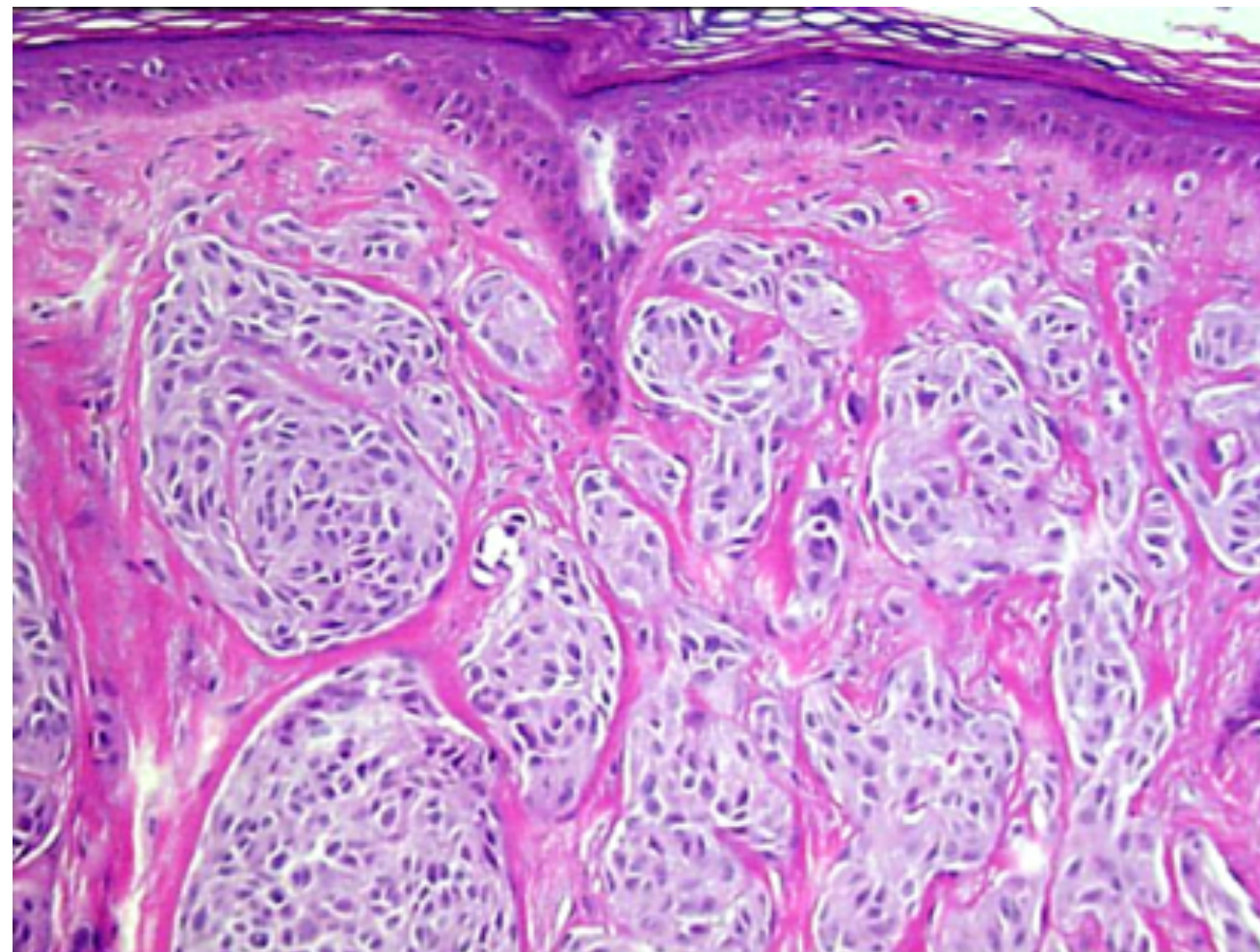
Variants can be detected in reads as mismatches relative to the reference sequence. The fraction of reads with the variant estimates the variant's frequency in a population



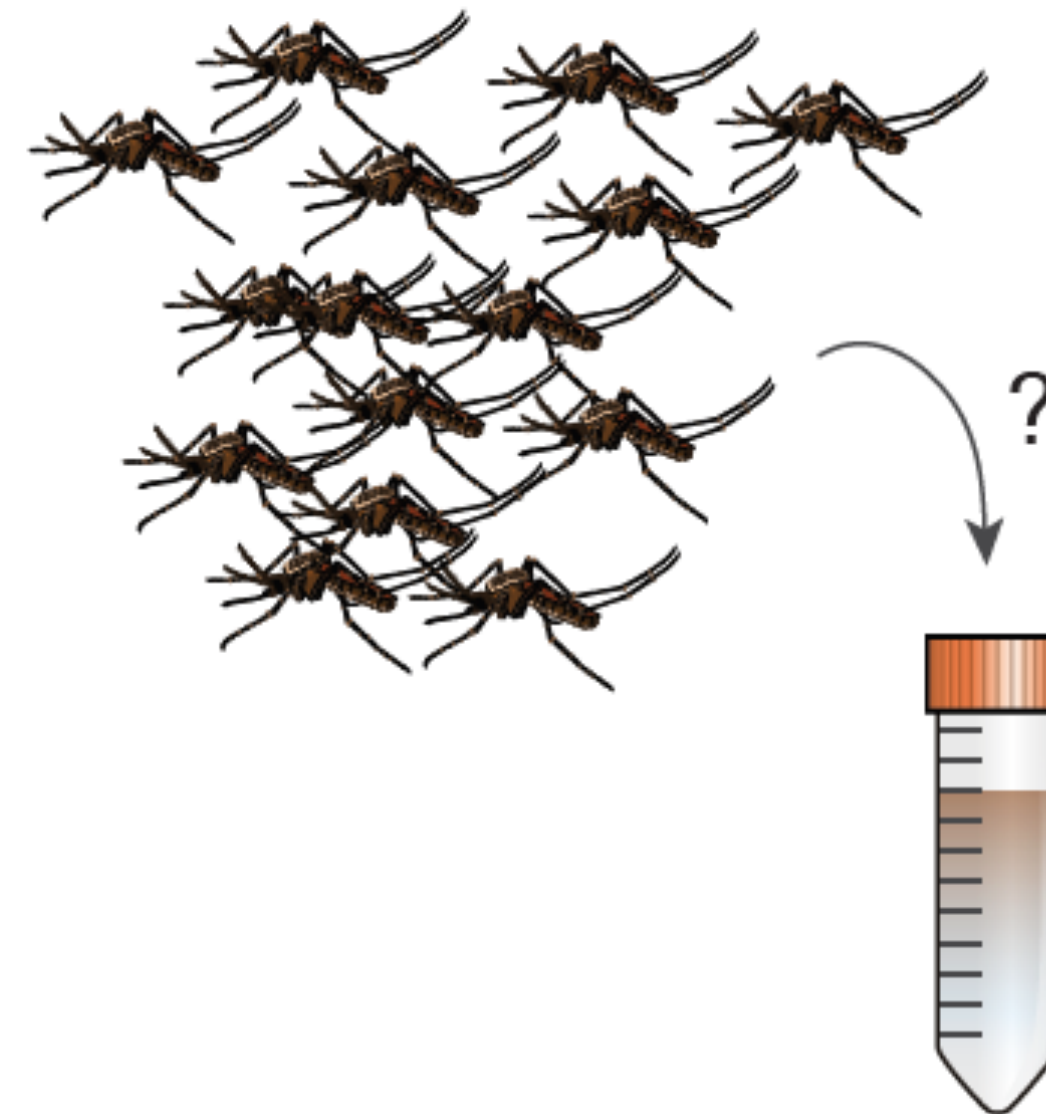


# Sub-consensus genomic variation is important in a variety of contexts

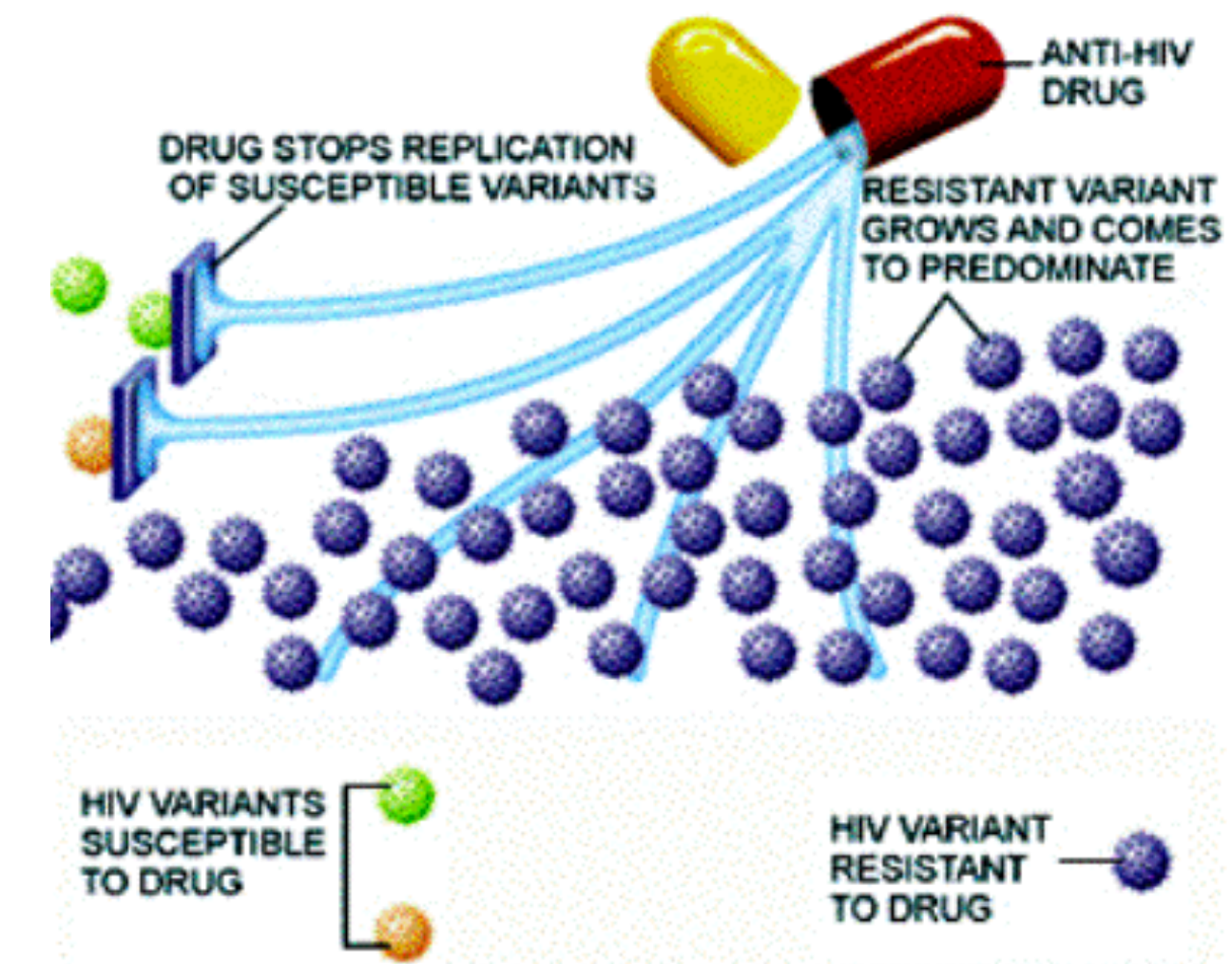
Rare somatic variants in cancer  
(cancer subclones)



Population genomics using  
pools of individuals (Pool-Seq)

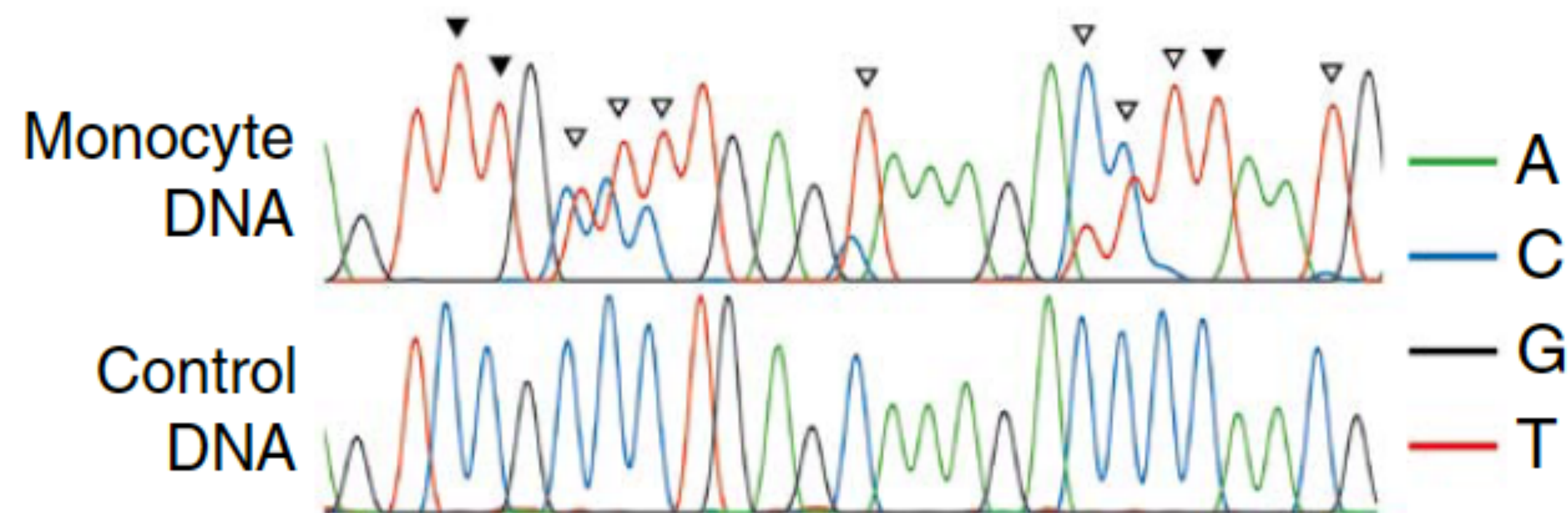
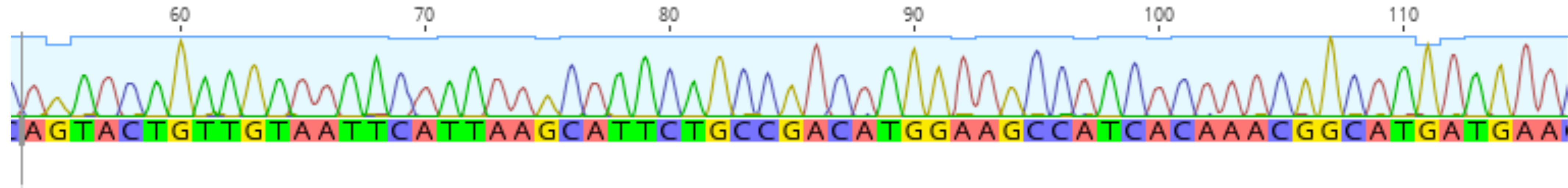


Intrahost viral  
variation



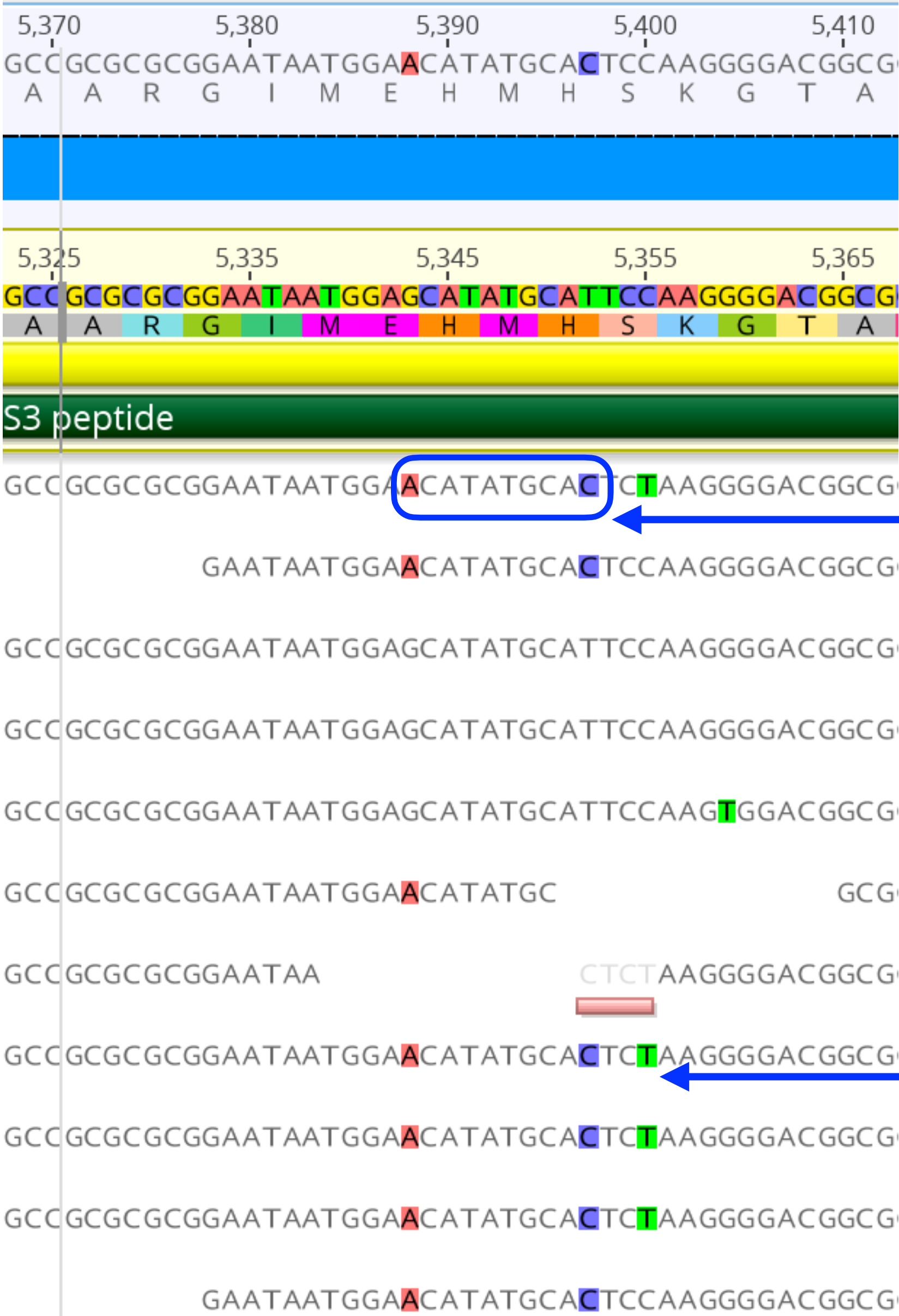


Sanger sequencing typically produces consensus sequence



It is possible to analyze chromatograms to obtain variant frequencies - but it's difficult to quantify and not really done

Goal: identify variants, their frequencies, and potential functional impact



Consensus sequence

Reference sequence

These 2 variants are at a >50% allele frequency and so are consensus changing variants

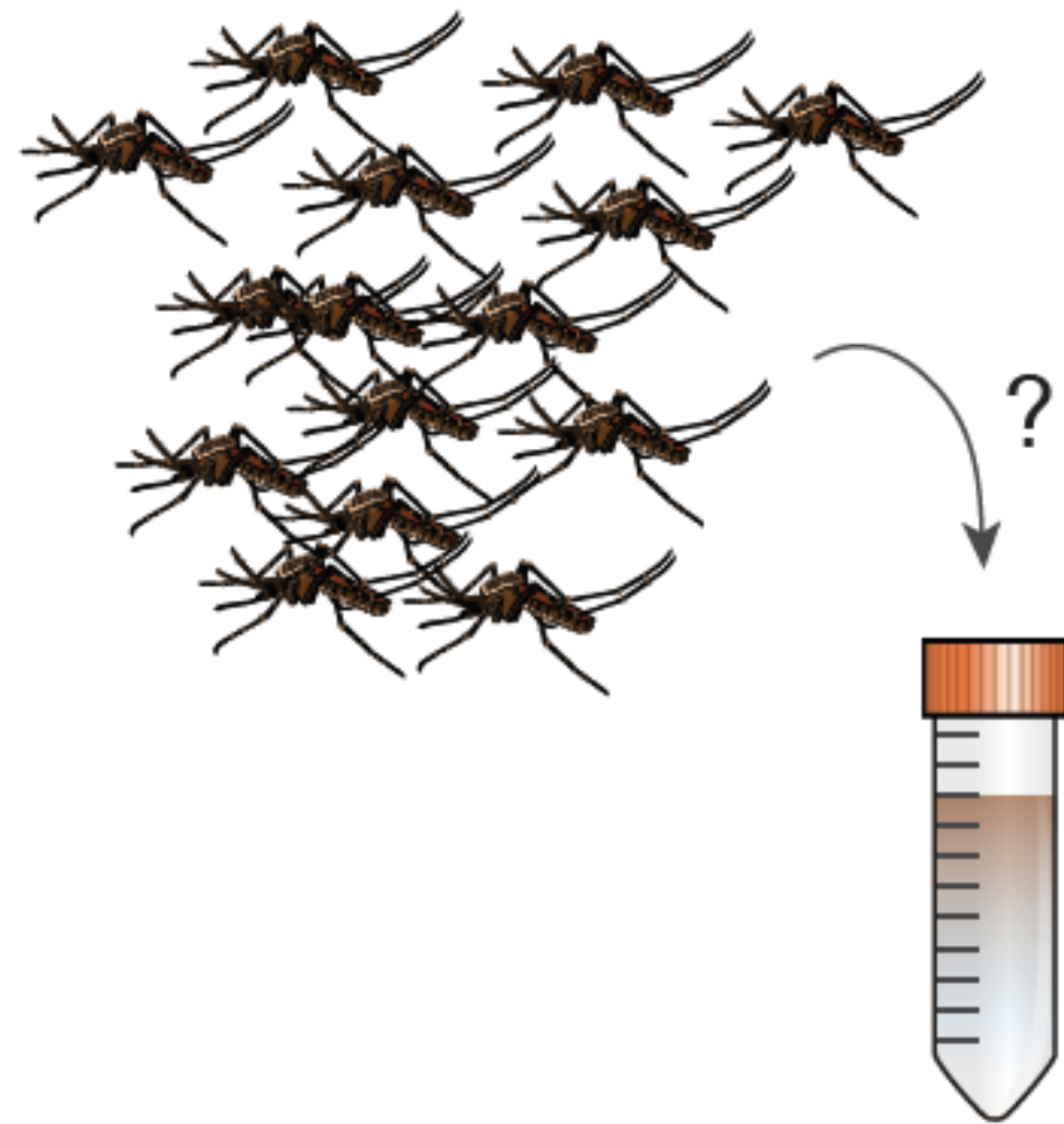
They are also both synonymous mutations

This T's allele frequency is 42% -> no consensus change

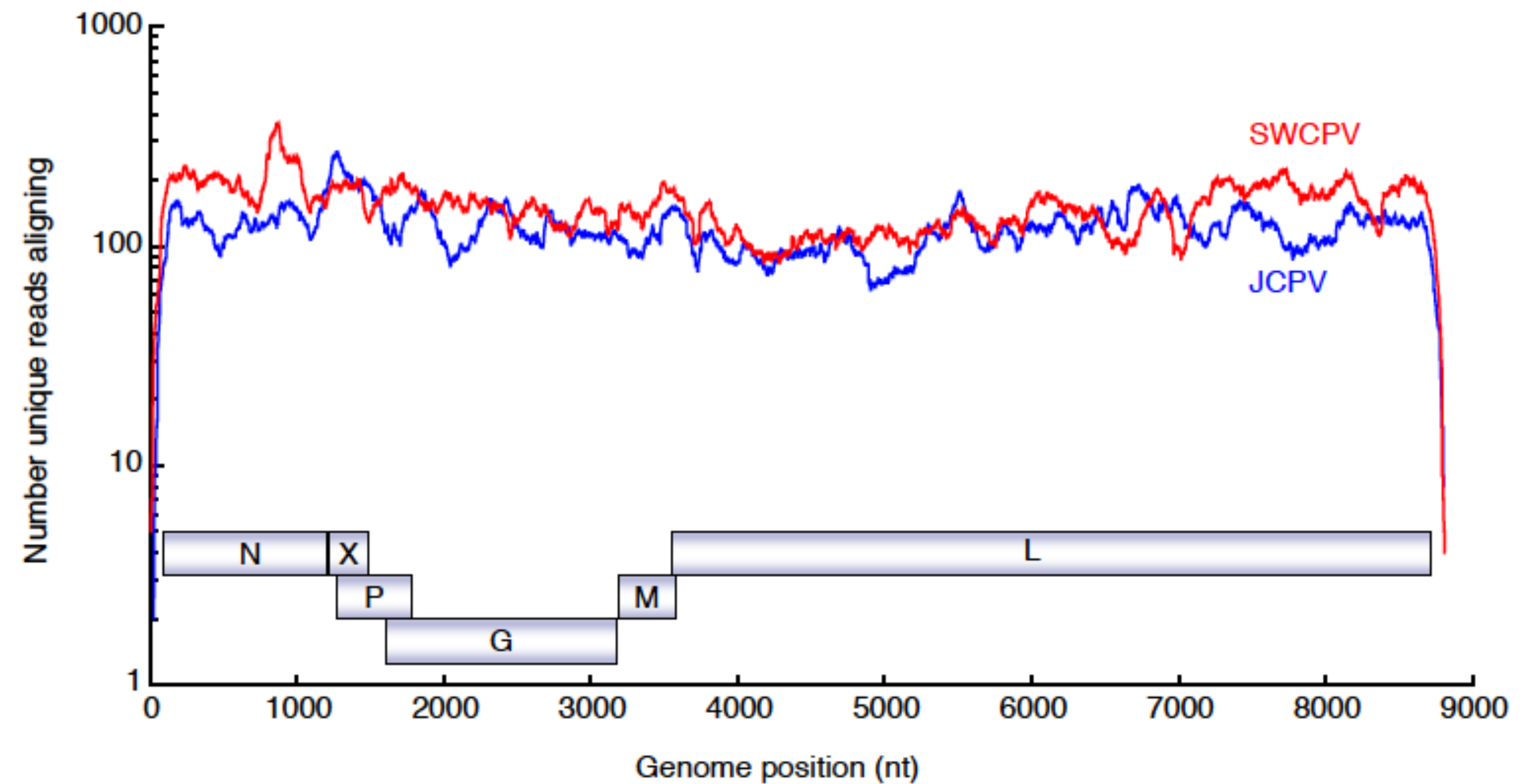


# Biological and technical limitations to the ability to detect rare variants

Pool size could limit the ability to detect rare variants



unlikely to observe variants with frequency  $< 1\%$  in these datasets



carpet python bornaviruses





# Distinguishing sequencing errors from true rare variants can be a challenge

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GAATAATGGAACATATGCACCTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGTGGACGGCGCTCATGTAT

GGAATAATGGAACATATGC GCGCTCATGTAT

GGAATAA CTCTAAGGGGACGGCGCTCATGTAT



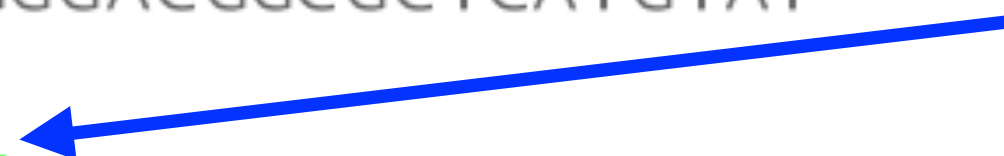
GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GAATAATGGAACATATGCACCTCCAAGGGGACGGCGCTCATGTAT

sequencing error, or real low frequency variant?



# Variant calling is also sensitive to mapping

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GAATAATGGAACATATGCACCTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGTGGACGGCGCTCATGTAT

GGAATAATGGAACATATGC GCGCTCATGTAT

GGAATAA CTCTAAGGGGACGGCGCTCATGTAT

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

GGAATAATGGAACATATGCACCTCTAAGGGGACGGCGCTCATGTAT

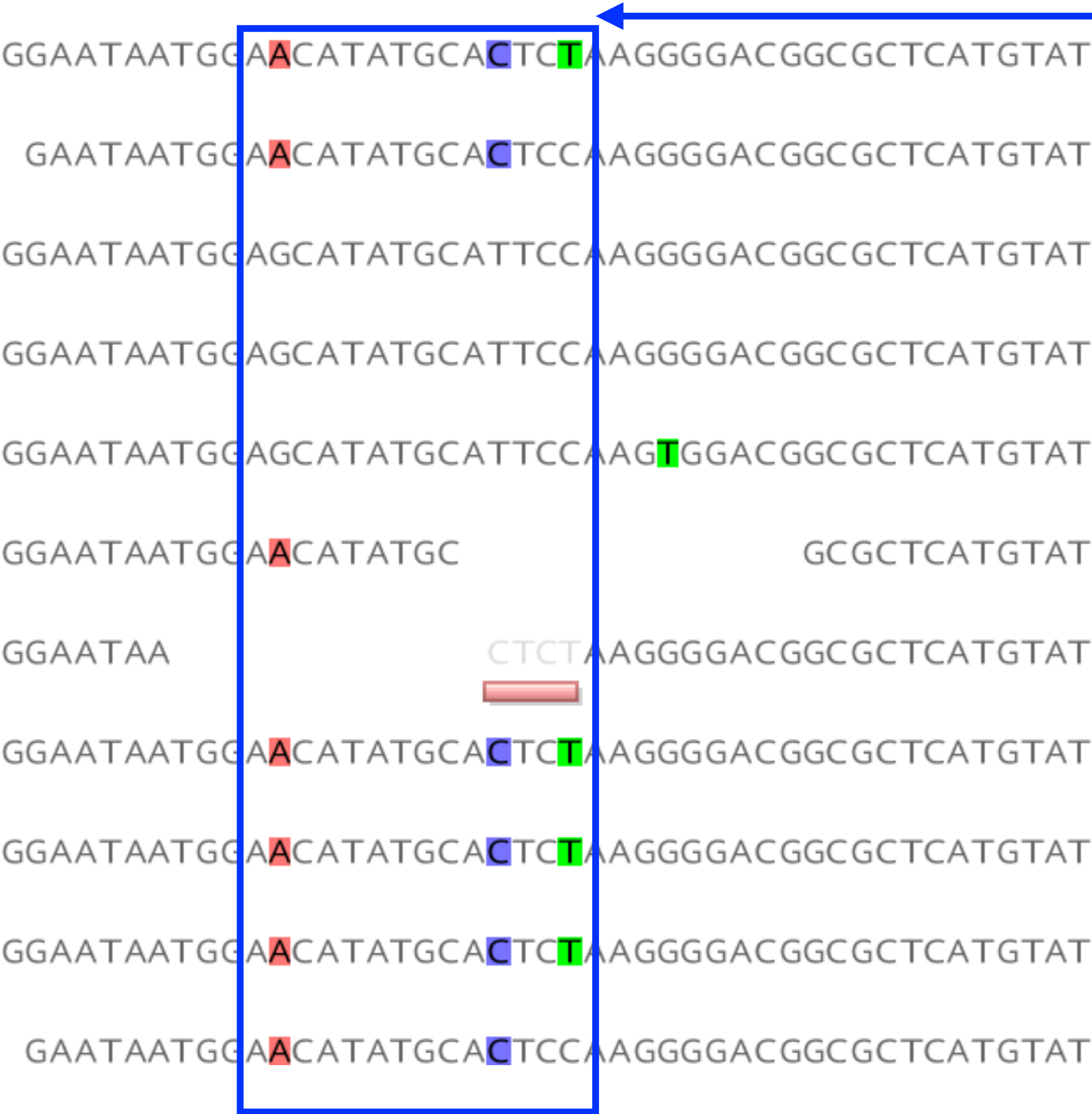
GAATAATGGAACATATGCACCTCCAAGGGGACGGCGCTCATGTAT

These bases were soft-trimmed  
(not aligned), but they support variant  
basecalls

Different mapping software could well  
produce different results.



Another issue is linking or ‘phasing’ variants (haplotype reconstruction)



3 haplotypes evident here

**G T C** [reference sequence]

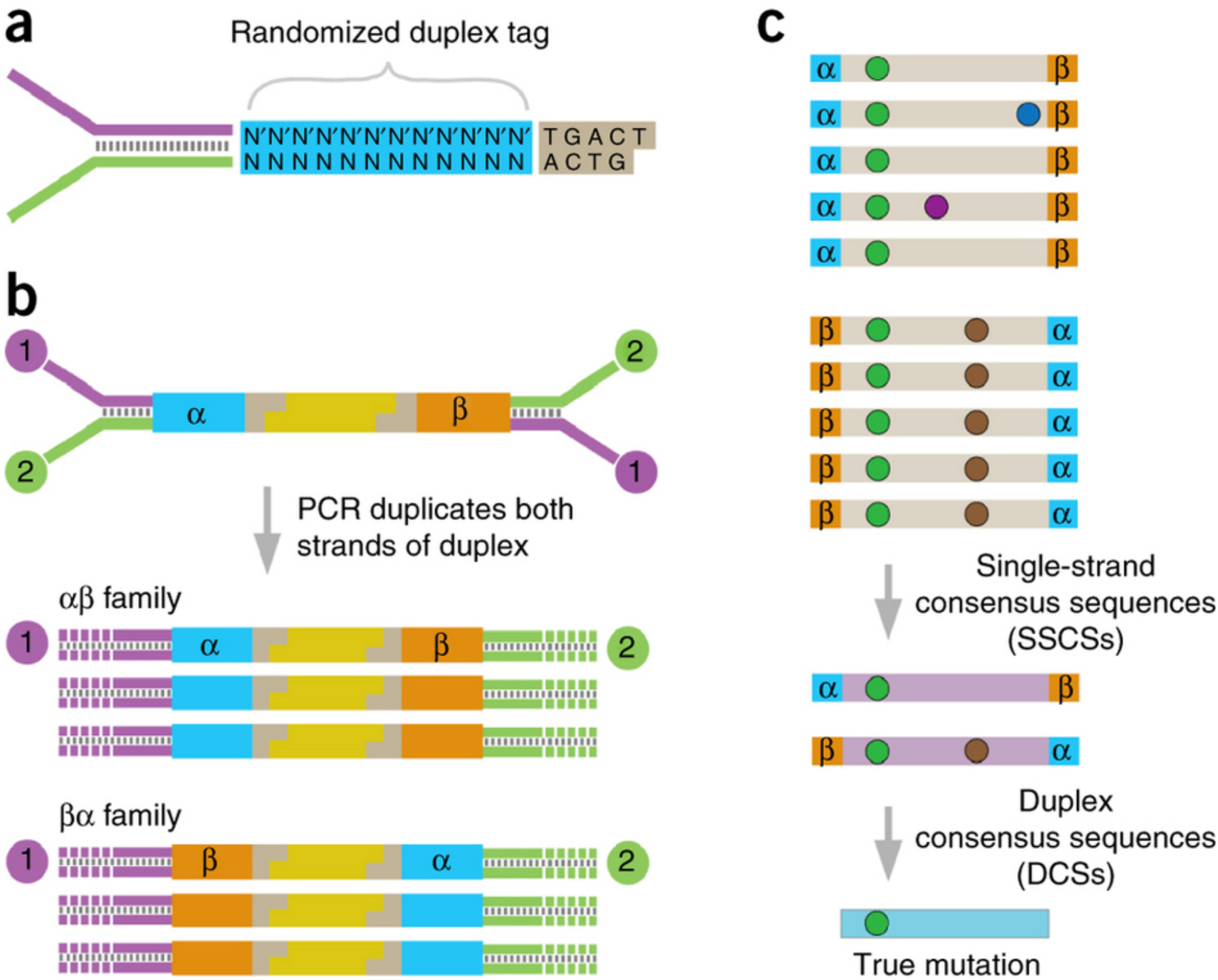
**A C C** [2 mutations]

**A C T** [3 mutations]

Much harder to link distant variants using short read data

# Clever methods have been developed to get beyond the limit of detection due to sequencing errors

Some protocols take advantage of PCR duplicates to sequence the same original molecule multiple times

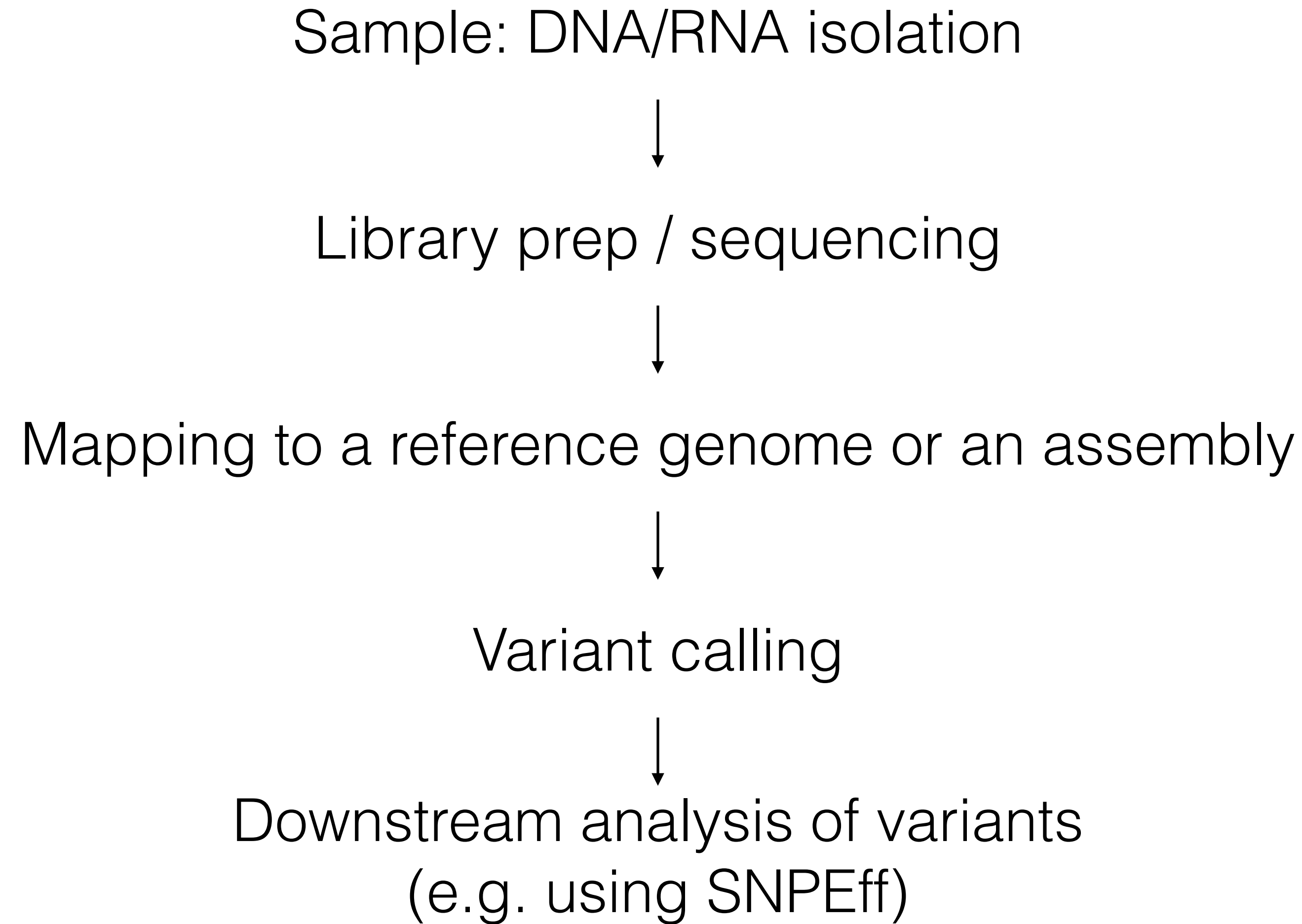




# The good news!

You don't necessarily or even often need linked variants or ultra low frequency variants to infer population genetic parameters (or otherwise answer your question of interest)

# A typical workflow for variant identification





The standard format for variant data is the vcf file (variant call format)

```
##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 NA00003
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 1/1:43:5:.,.
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 0/0:41:3
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 2/2:35:4
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 0/0:61:2
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 1/1:40:3
```

<https://github.com/samtools/hts-specs>

# Let's call some variants!

GGAATAATGGA~~A~~CATATGCAC~~C~~TC~~T~~AAGGGGACGGCGCTCATGTAT

GAATAATGGA~~A~~CATATGCAC~~C~~TCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT

GGAATAATGGAGCATATGCATTCCAAG~~T~~GGACGGCGCTCATGTAT

GGAATAATGGA~~A~~CATATGCGCGCTCATGTAT

GGAATAACTCTAAGGGGACGGCGCTCATGTAT



GGAATAATGGA~~A~~CATATGCAC~~C~~TC~~T~~AAGGGGACGGCGCTCATGTAT

GGAATAATGGA~~A~~CATATGCAC~~C~~TC~~T~~AAGGGGACGGCGCTCATGTAT

GGAATAATGGA~~A~~CATATGCAC~~C~~TC~~T~~AAGGGGACGGCGCTCATGTAT

GAATAATGGA~~A~~CATATGCAC~~C~~TCCAAGGGGACGGCGCTCATGTAT