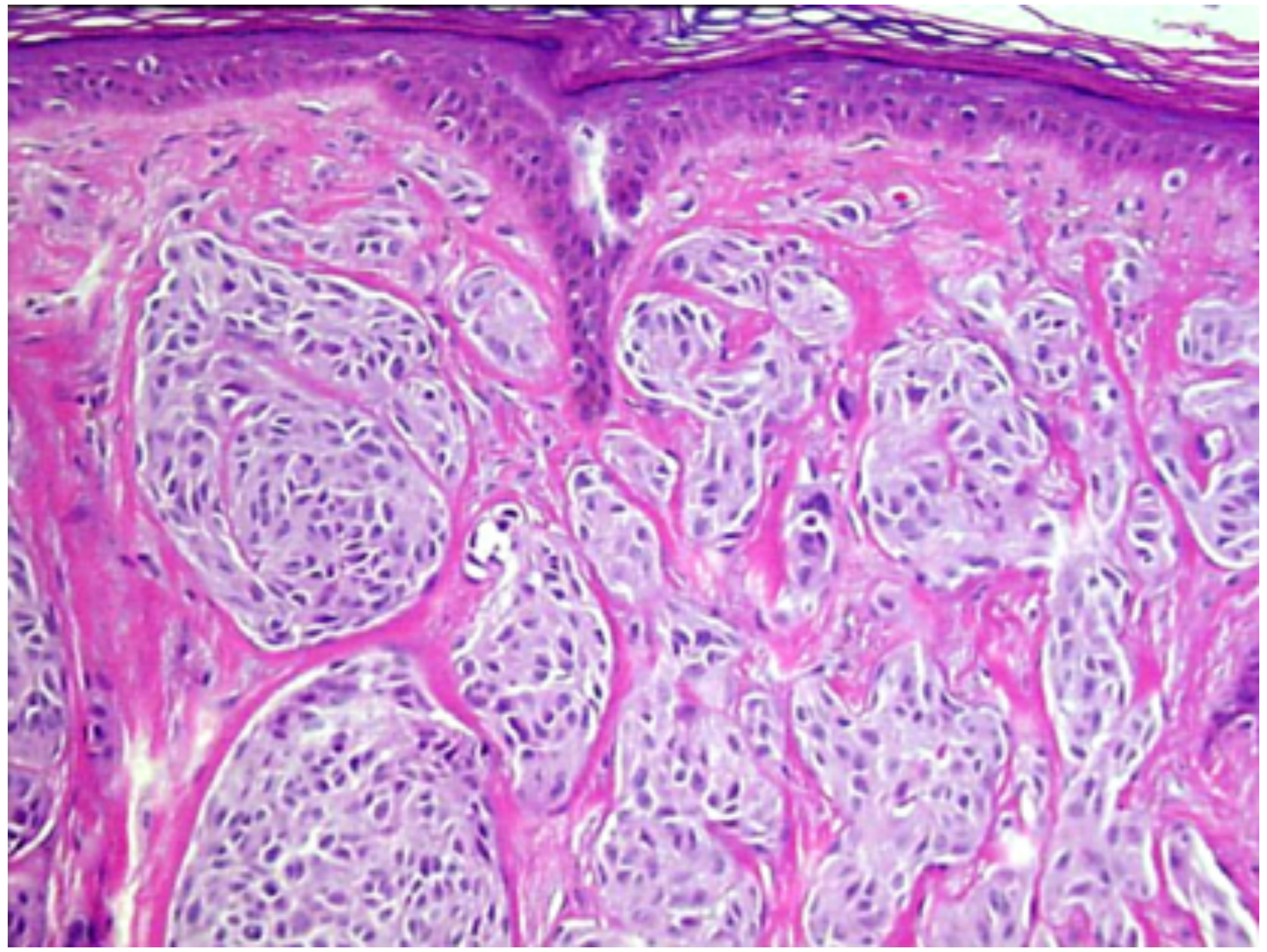
Intrahost and pooled variation

Mark Stenglein, GDW 2017

GDW
Genomics of Disease in Wildlife

Will mainly focus on intrahost virus populations, but other populations can be studied using similar methods and are similar in principle

Rare somatic variants in cancer
(cancer subclones)



Population genomics using
pools of individuals (Pool-Seq)

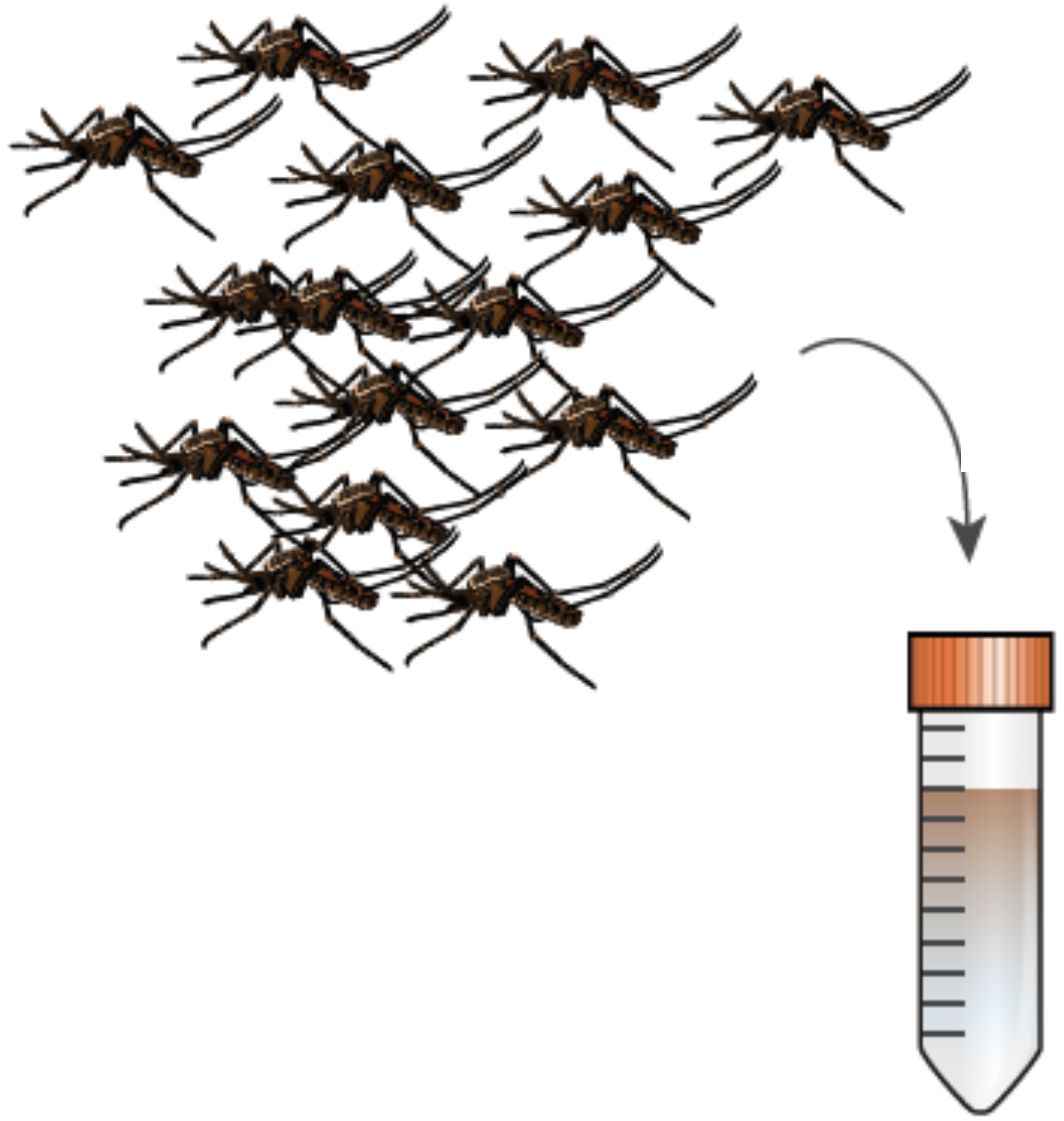
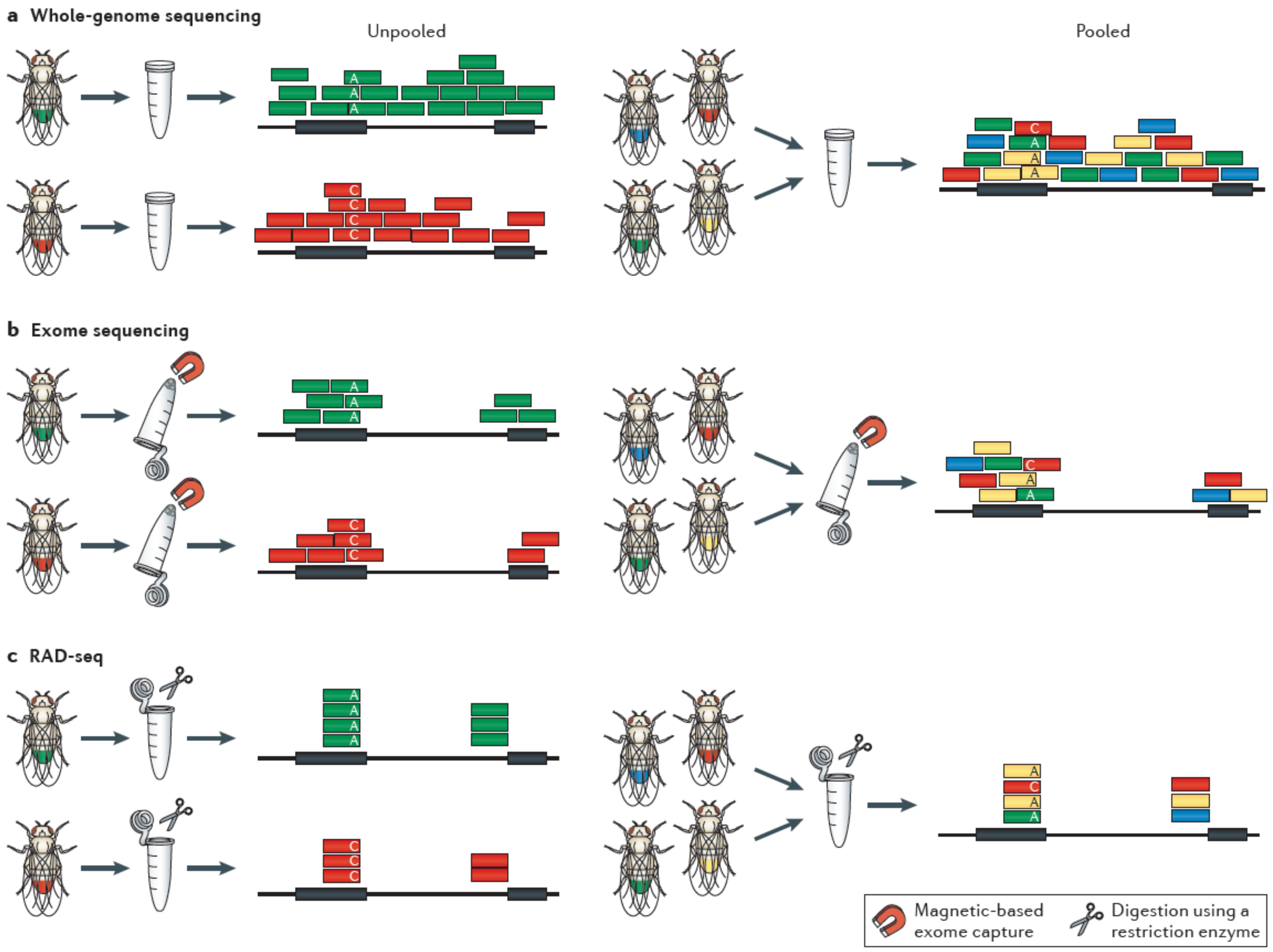


image: Magro et al (2006) Modern Path.

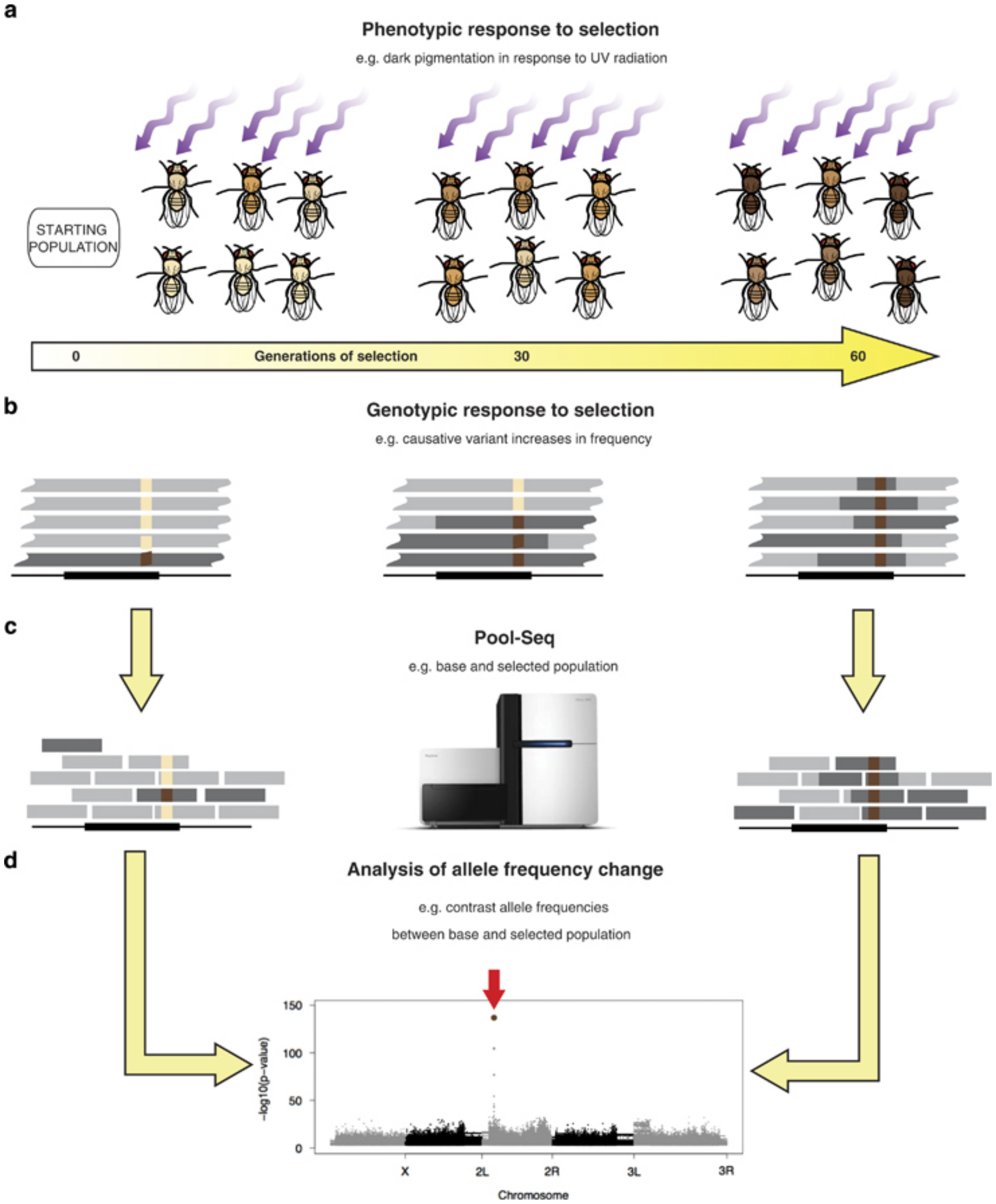
Sequencing pools of individuals — mining genome-wide polymorphism data without big funding

Christian Schlötterer[1], Raymond Tobler[1,2], Robert Kofler[1] and Viola Nolte[1]

Nat Rev Genetics 2014

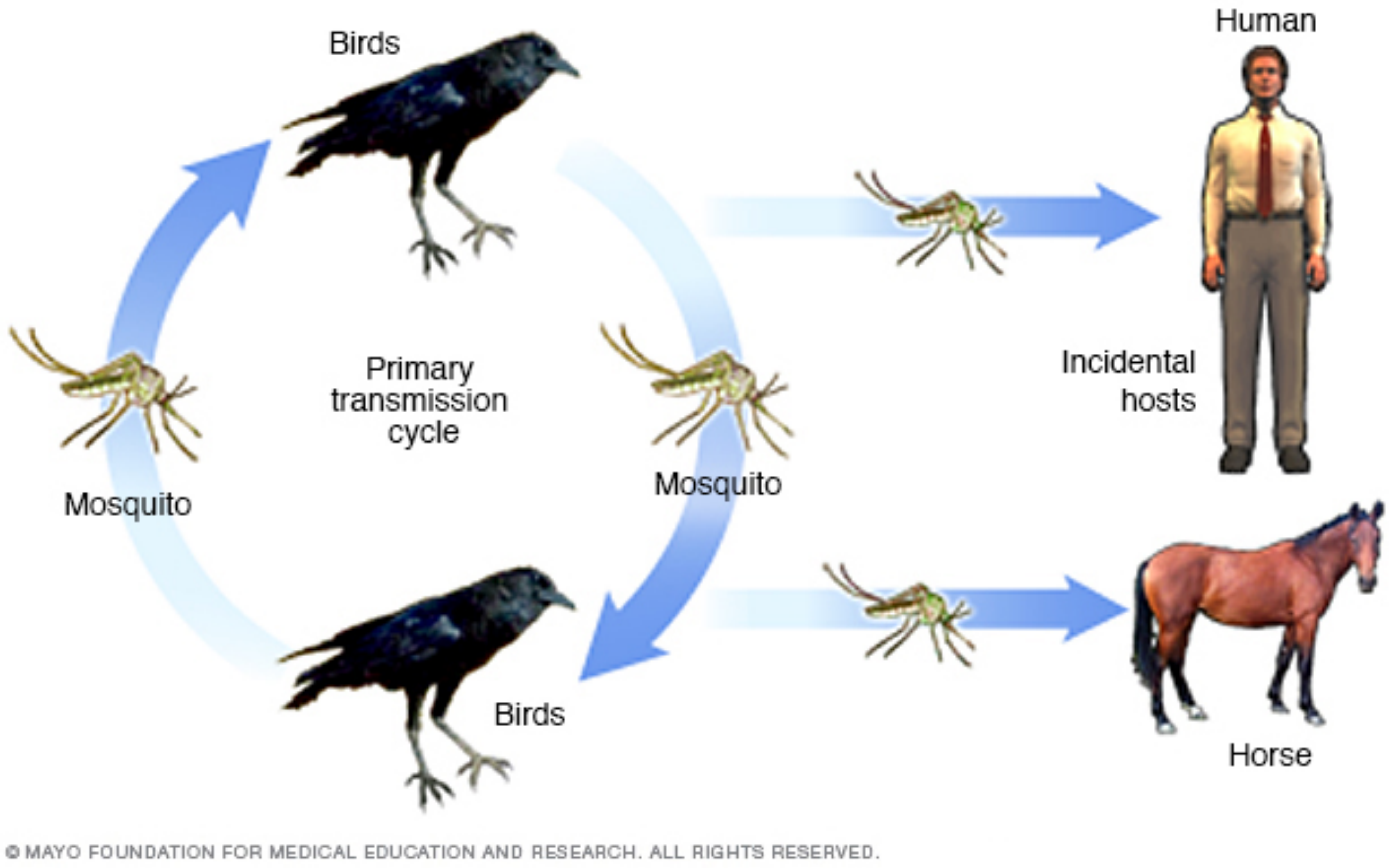


“Evolve & Resequence”



Schlötterer et al Heredity 2015

Why study intrahost viral variation?



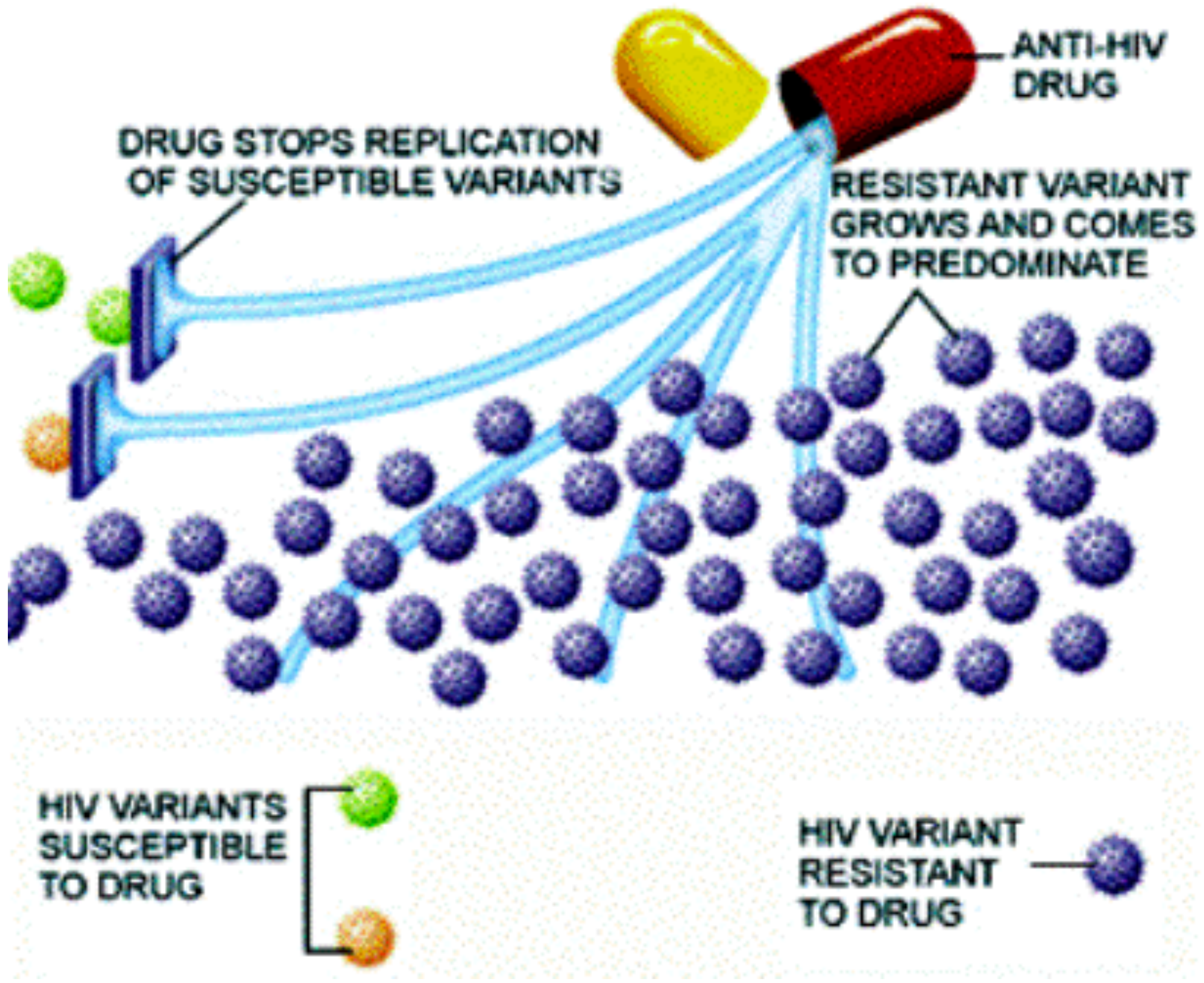
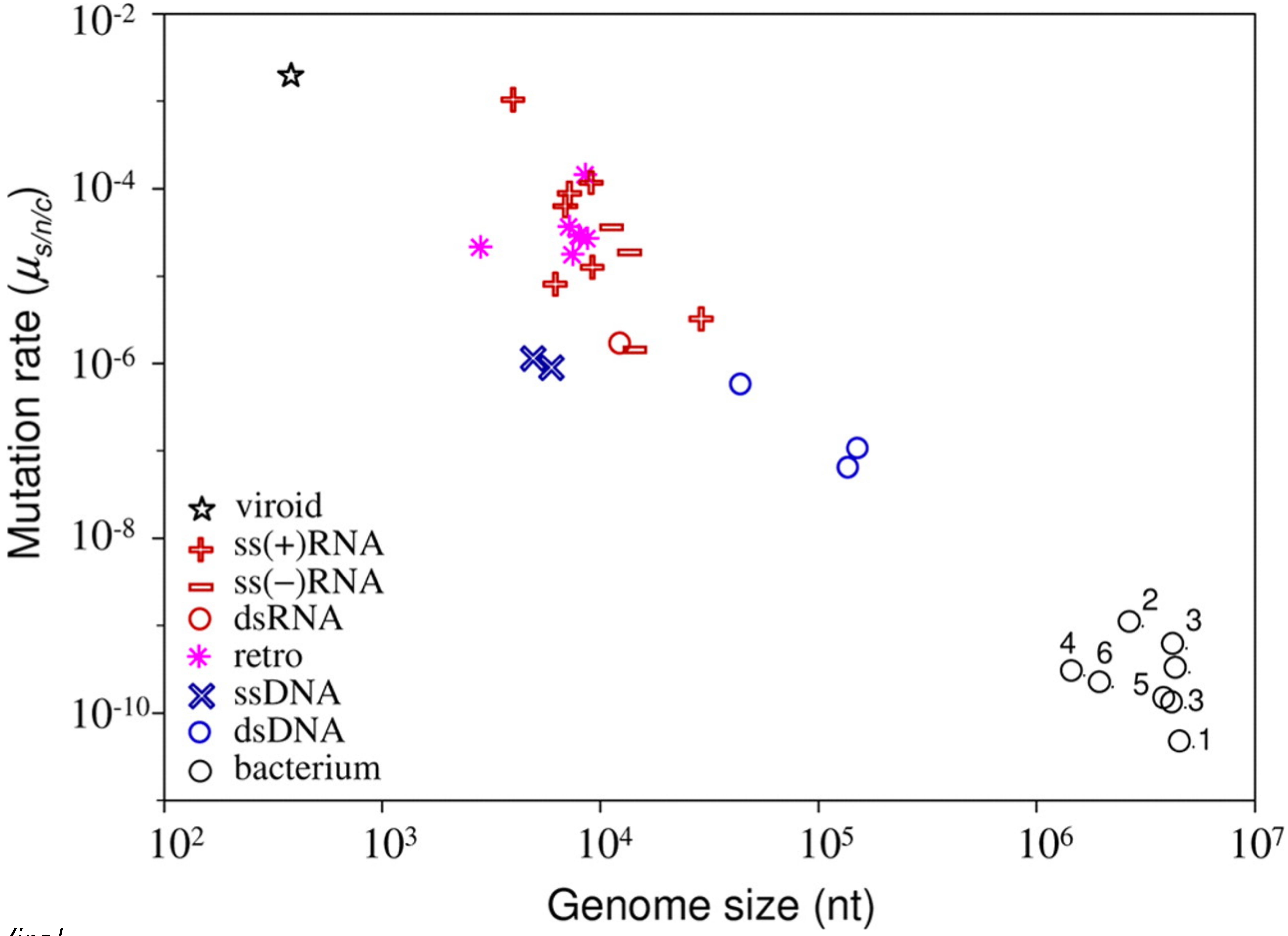


Image: Mayo clinic

(RNA) Viruses typically have error rates ≈ 1 / genome size



Sanjuán et al (2010) J Virol

A typical RNA virus has a genome ~10,000 nt long and a ~1/10,000 mutation rate

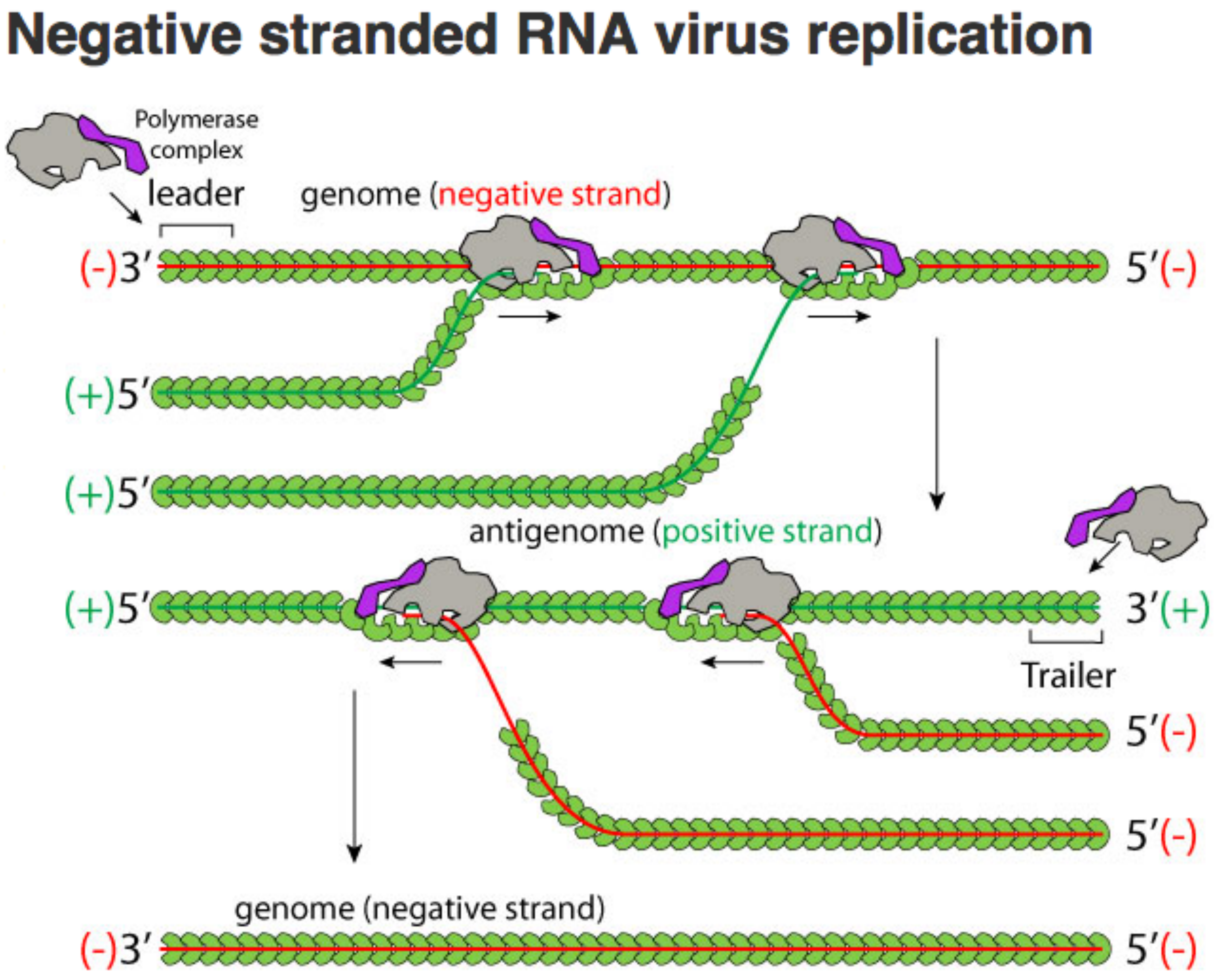
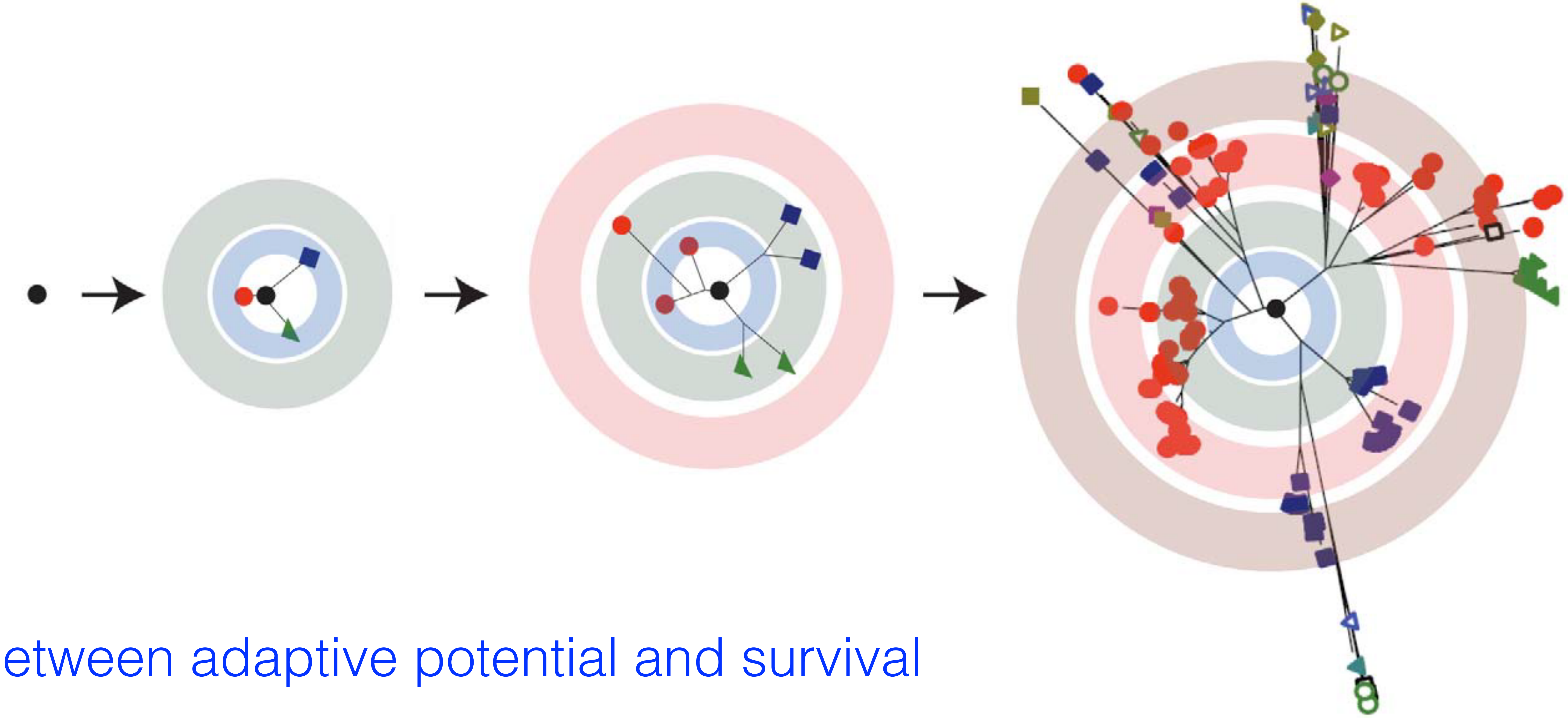


Image: viralzone.expasy.org

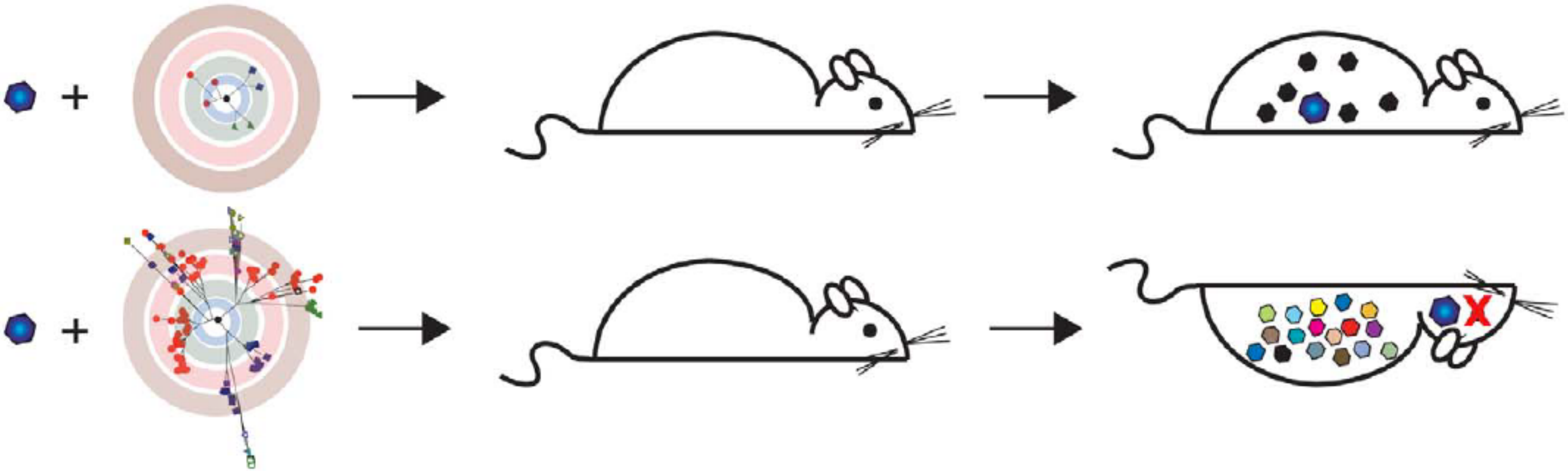
As a consequence of error-prone replication, intrahost virus populations can diversify rapidly



Balance between adaptive potential and survival

Lauring and Andino (2010) PLoS Pathogens

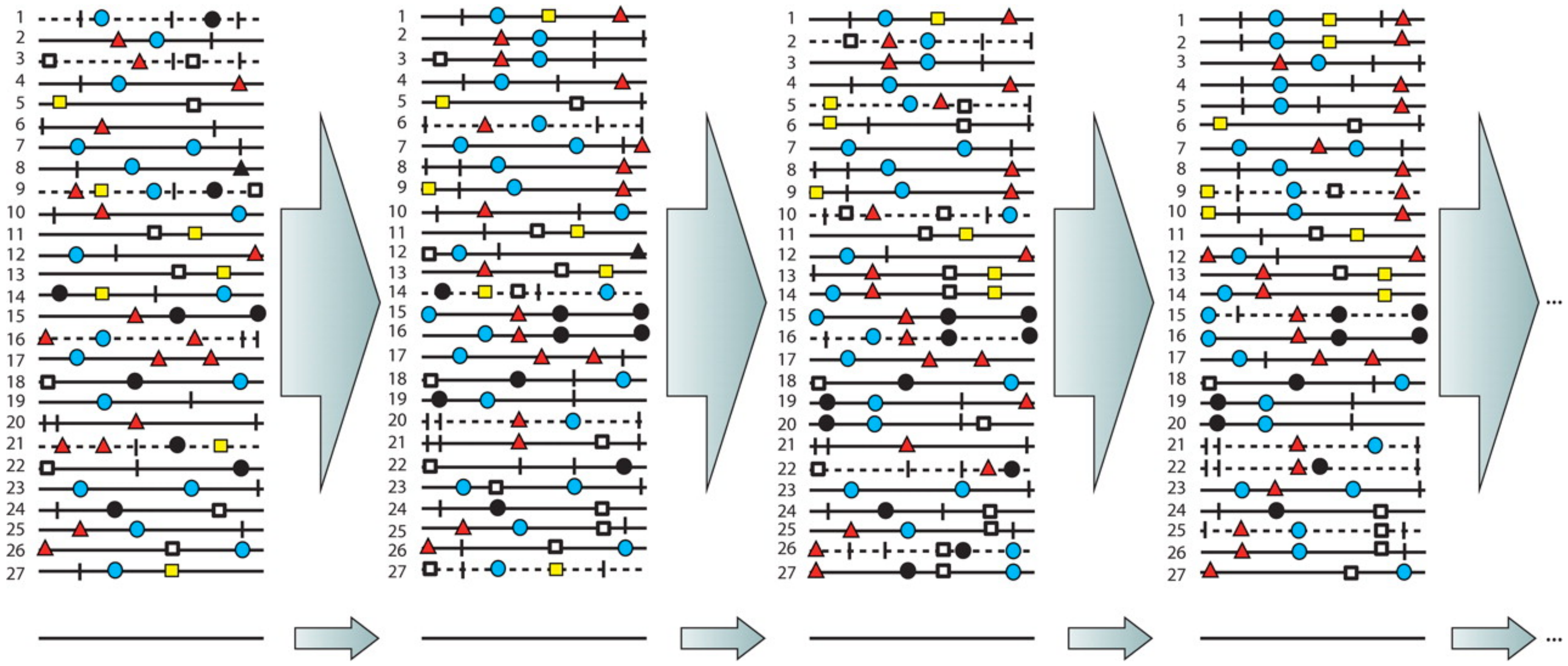
Intrahost viral population diversity can have a functional impact



Lauring and Andino (2010) PLoS Pathogens

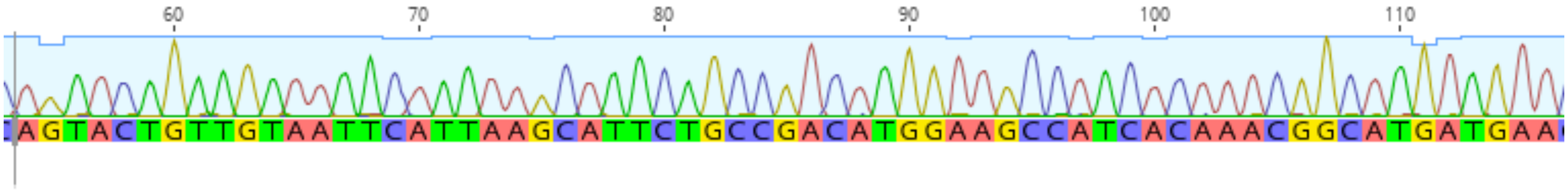
Vignuzzi et al (2006) Nature

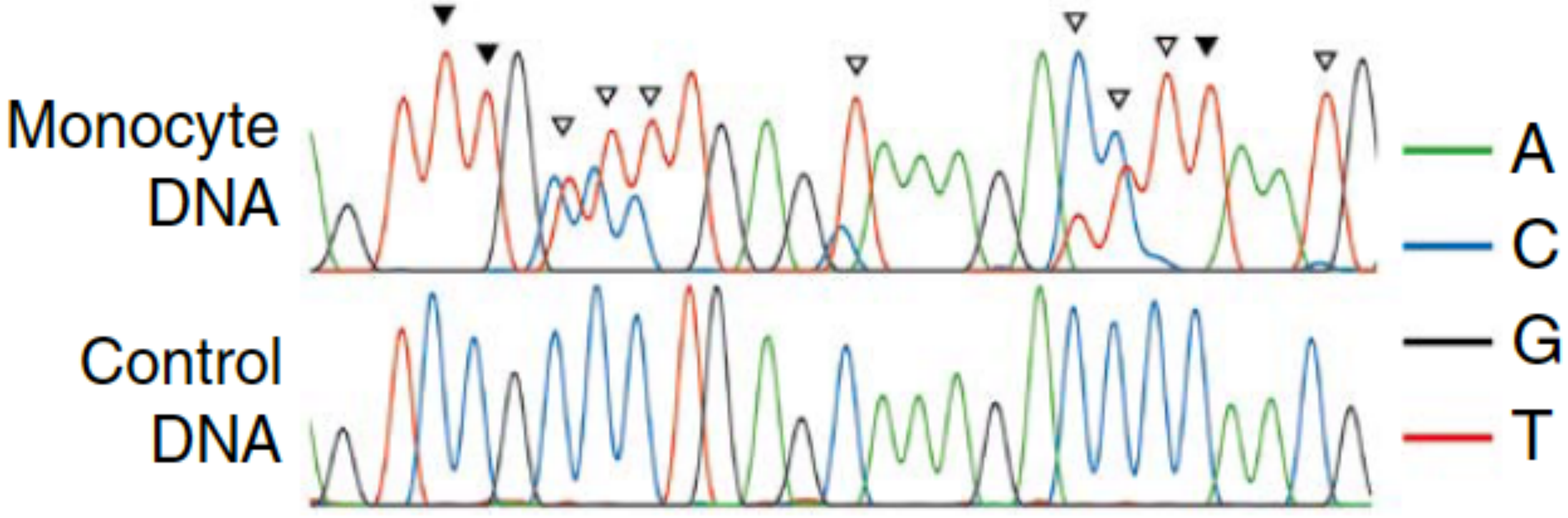
The shifting 'mutant swarm' may not change consensus sequence



Domingo et al (2012) Microbiol Mol Biol Reviews

Sanger sequencing typically produces consensus sequence



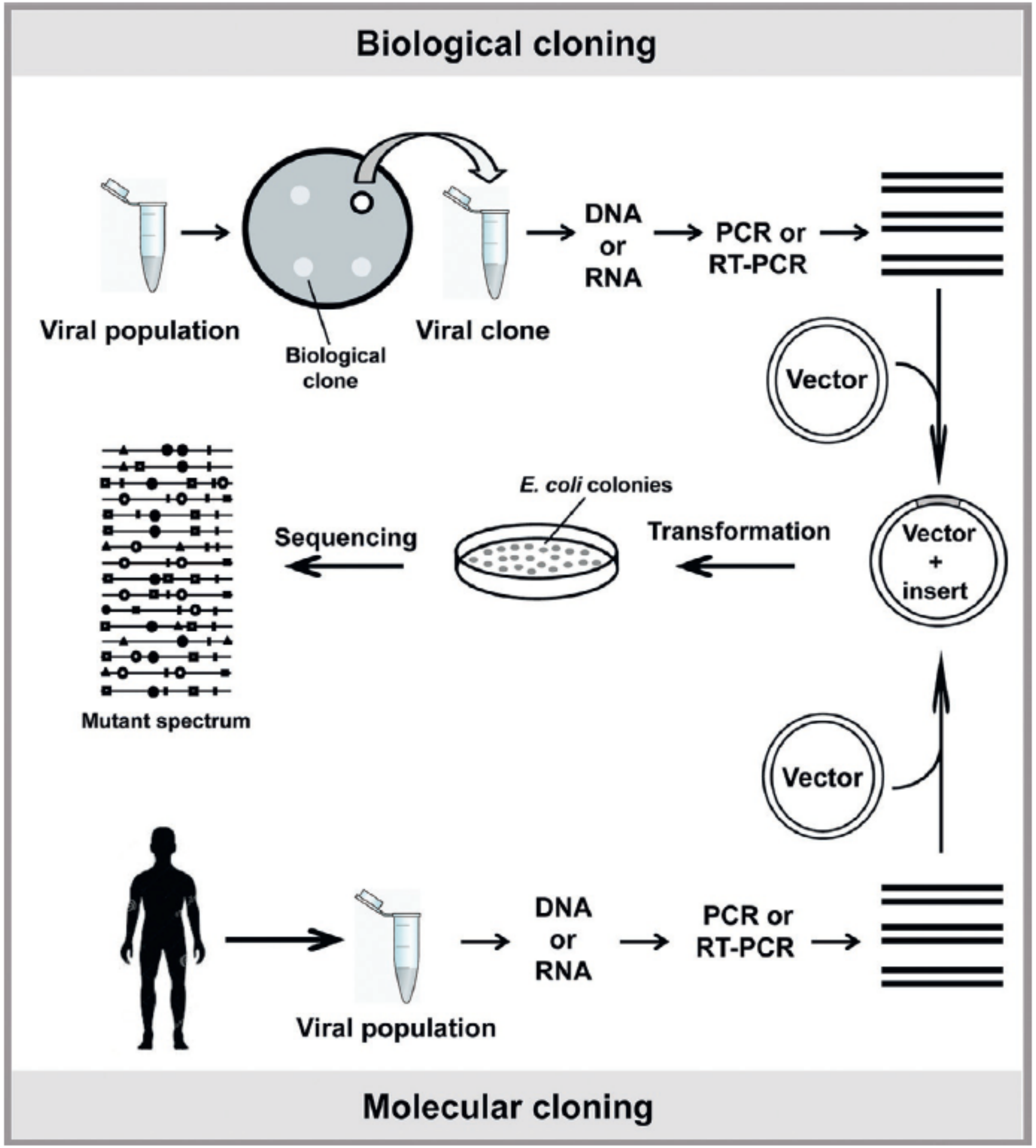


It is possible to analyze chromatograms to obtain variant frequencies

Cloning facilitated analysis of virus populations

Not easily scalable

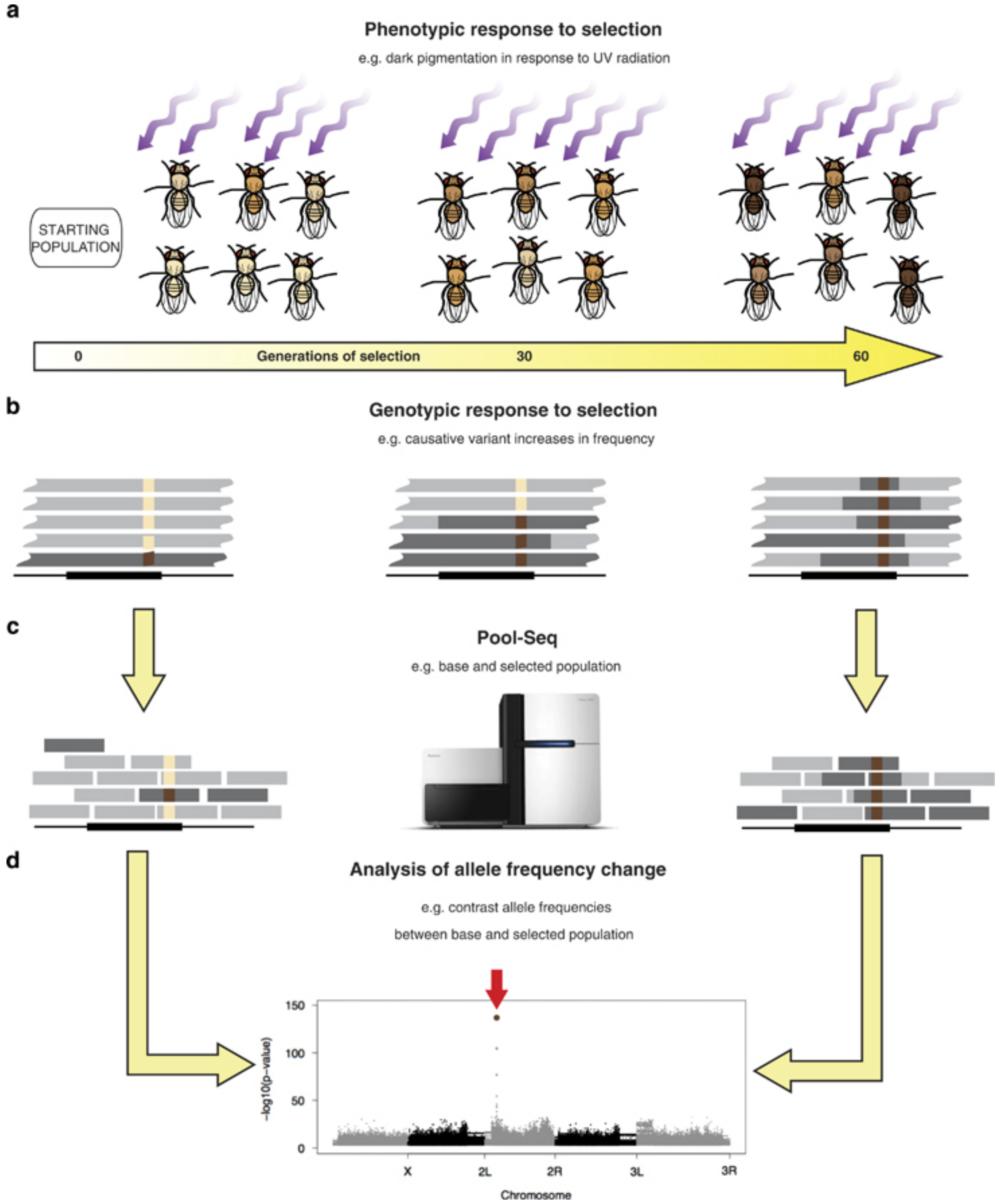
E. Domingo (2016)



NGS has emerged as powerful tool to study variants in populations

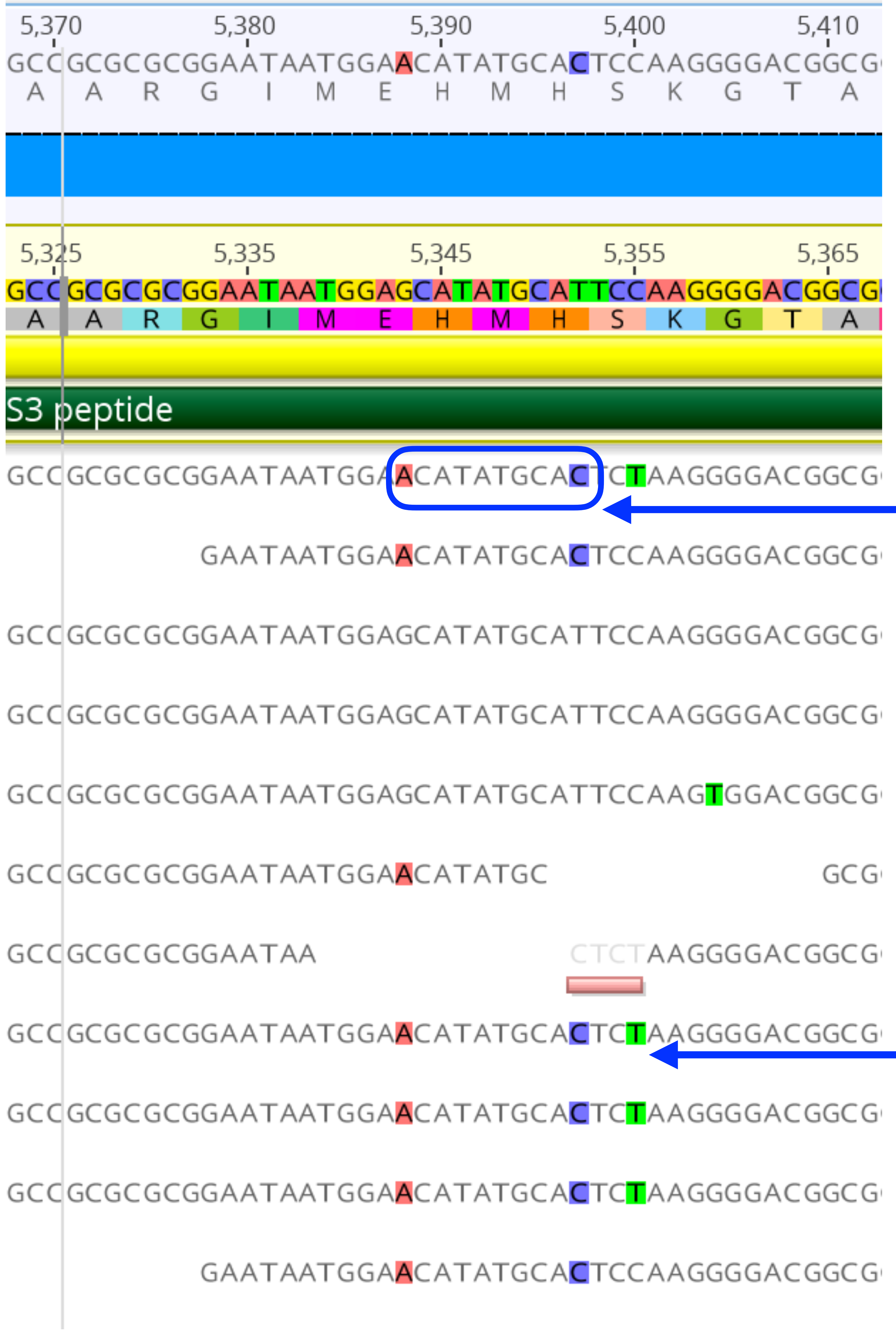
Assumes that frequencies in reads correspond to frequencies in genomes in the population

Generally good assumption



Schlötterer et al Heredity 2015

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



Consensus sequence

Reference sequence

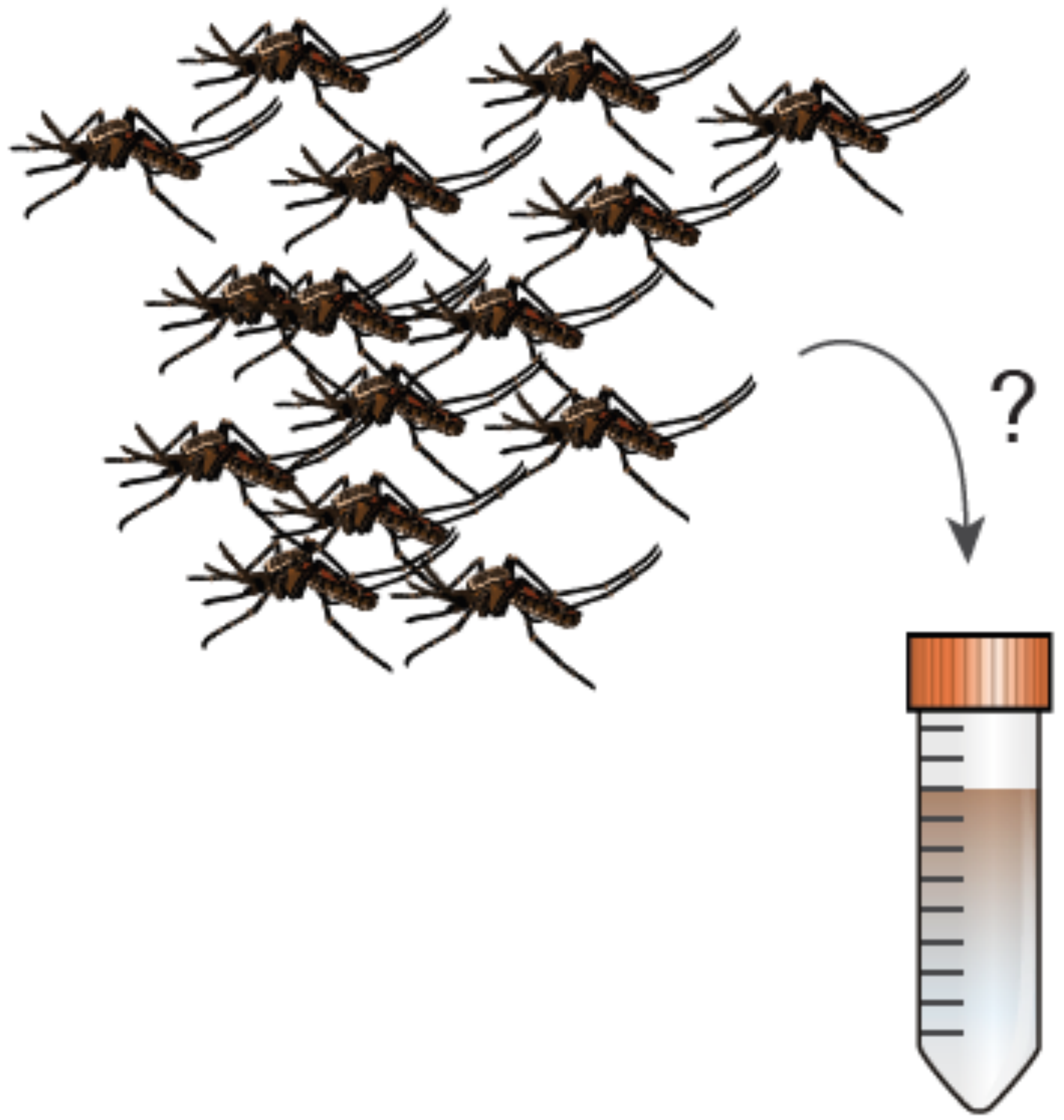
These 2 variants are at a >50% allele frequency and so are consensus changing variants

They are also both synonymous mutations

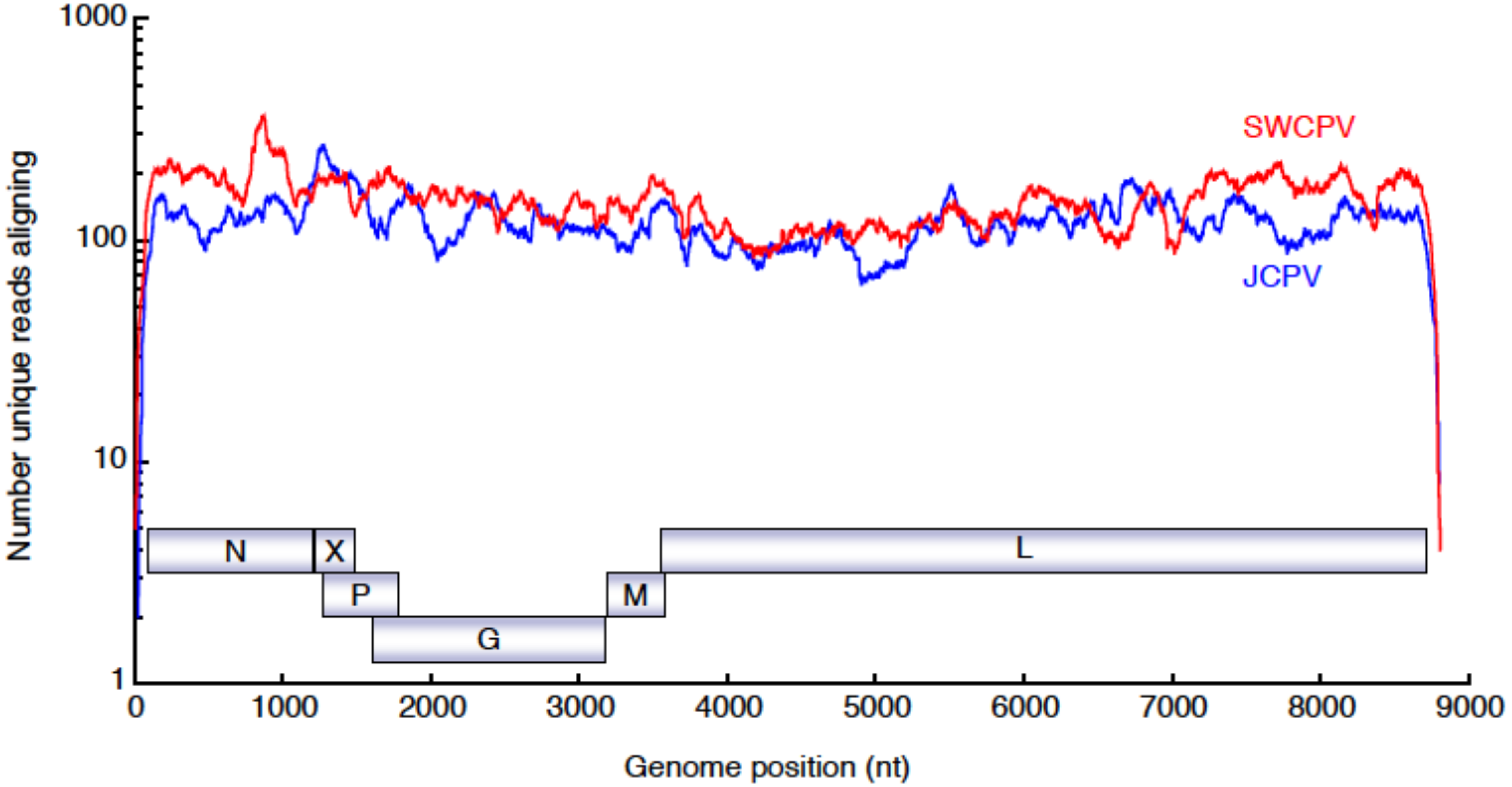
This T's allele frequency is 42% -> no consensus change

Biological and technical limitations to the ability to detect rare variants

Pool size could limit the ability to detect rare variants

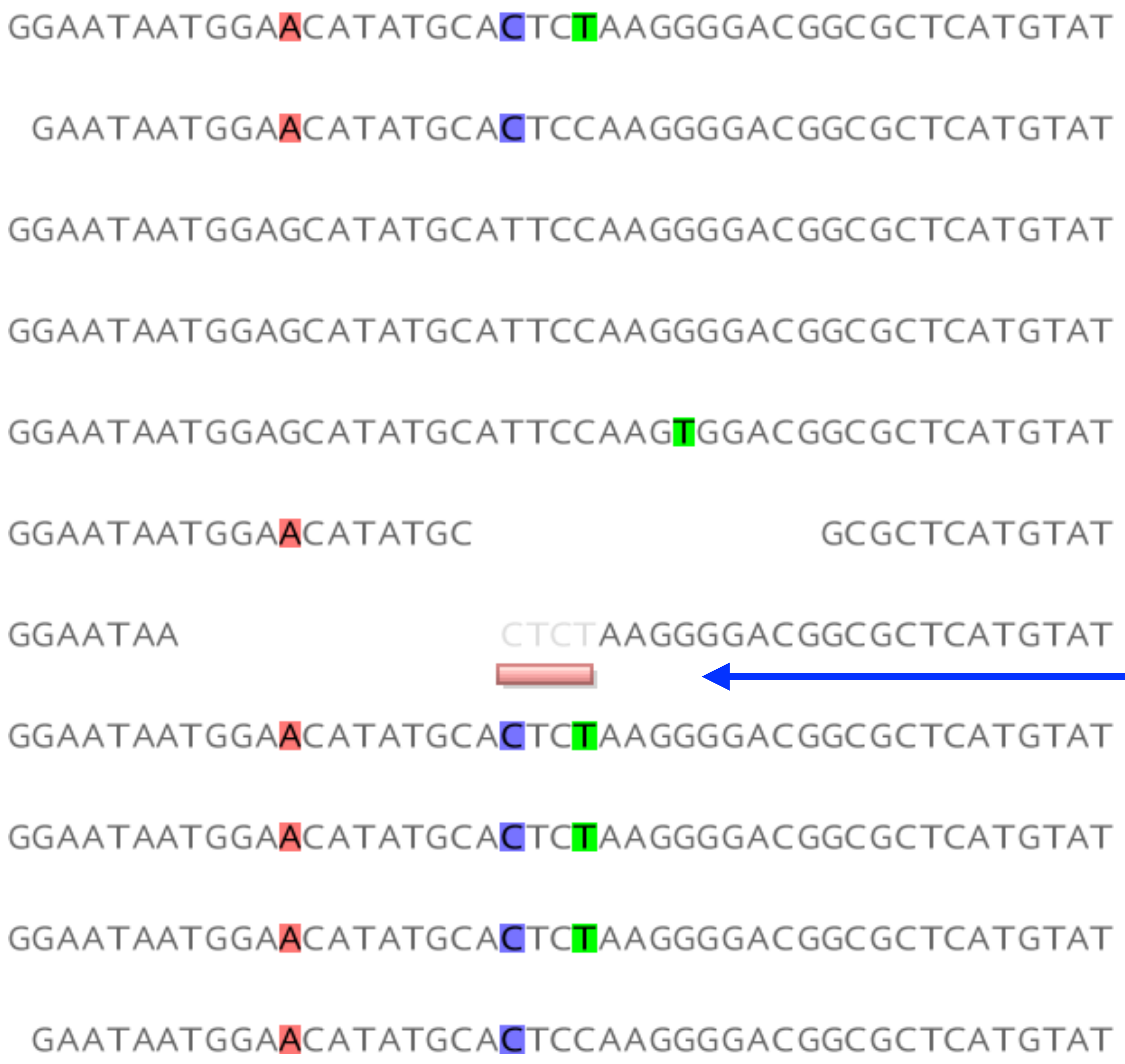


unlikely to observe variants with frequency < 1% in these datasets



Distinguishing sequencing errors from true rare variants is a central challenge

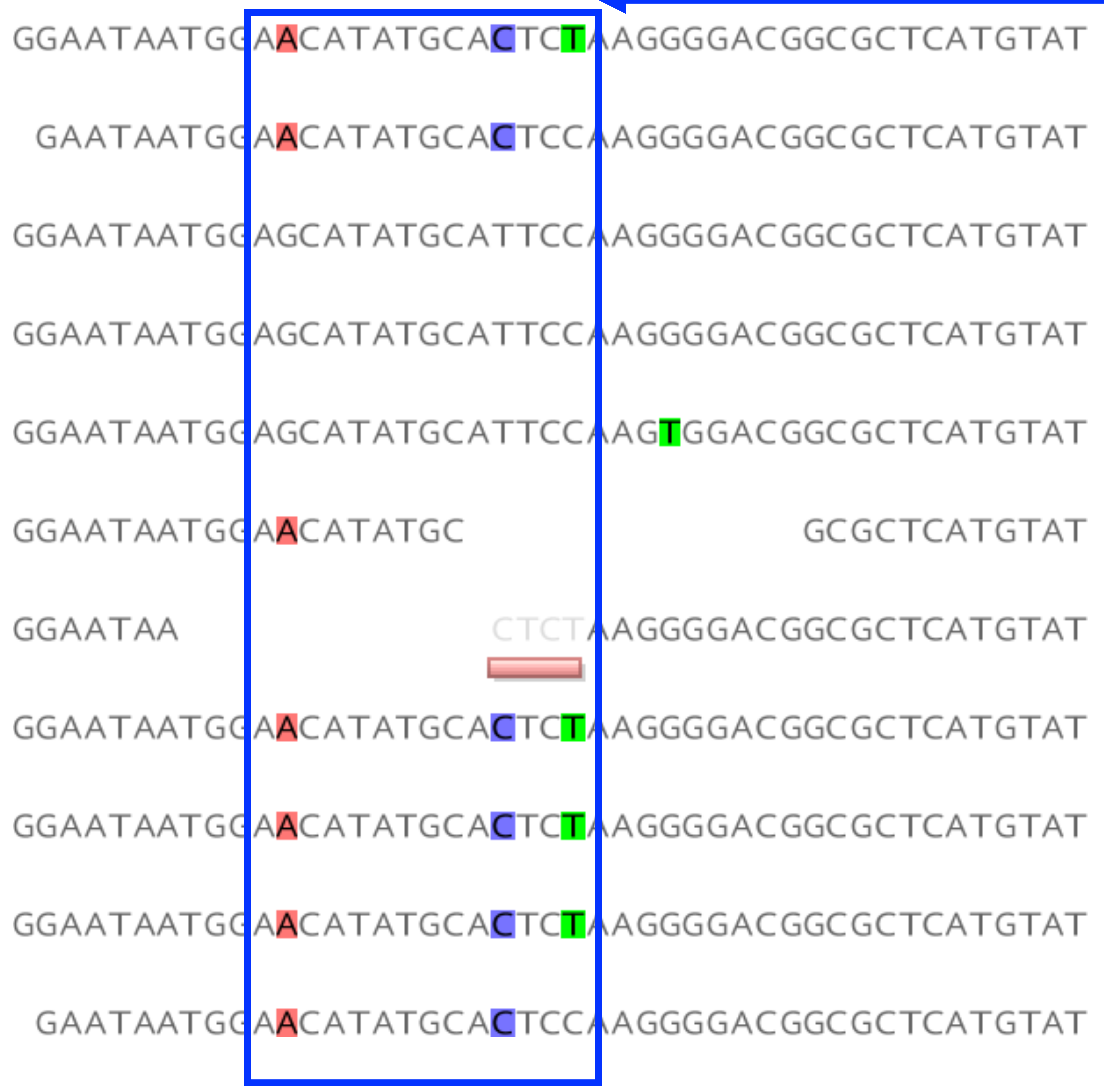
Variant calling is also sensitive to mapping



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)

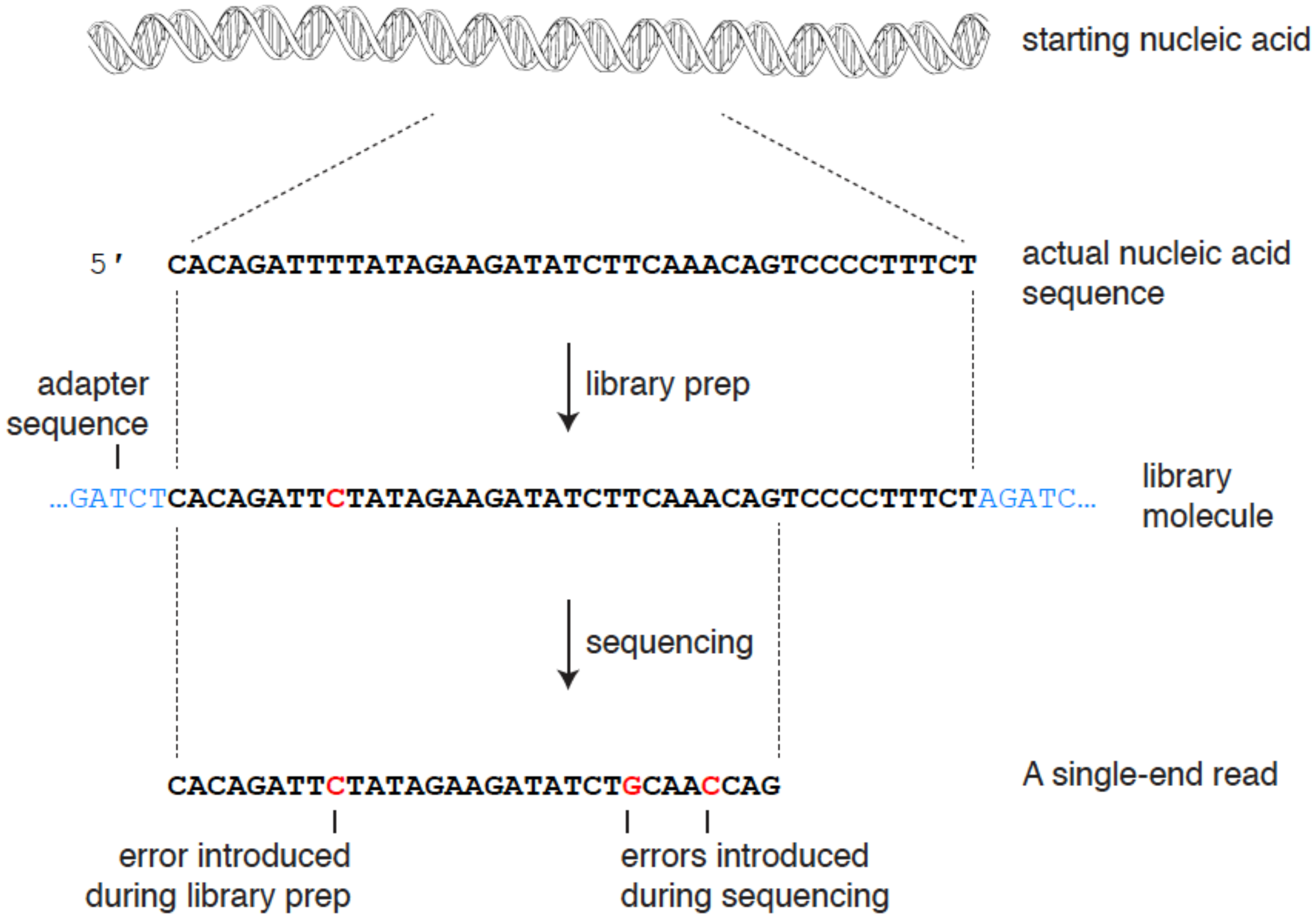


3 haplotypes evident here

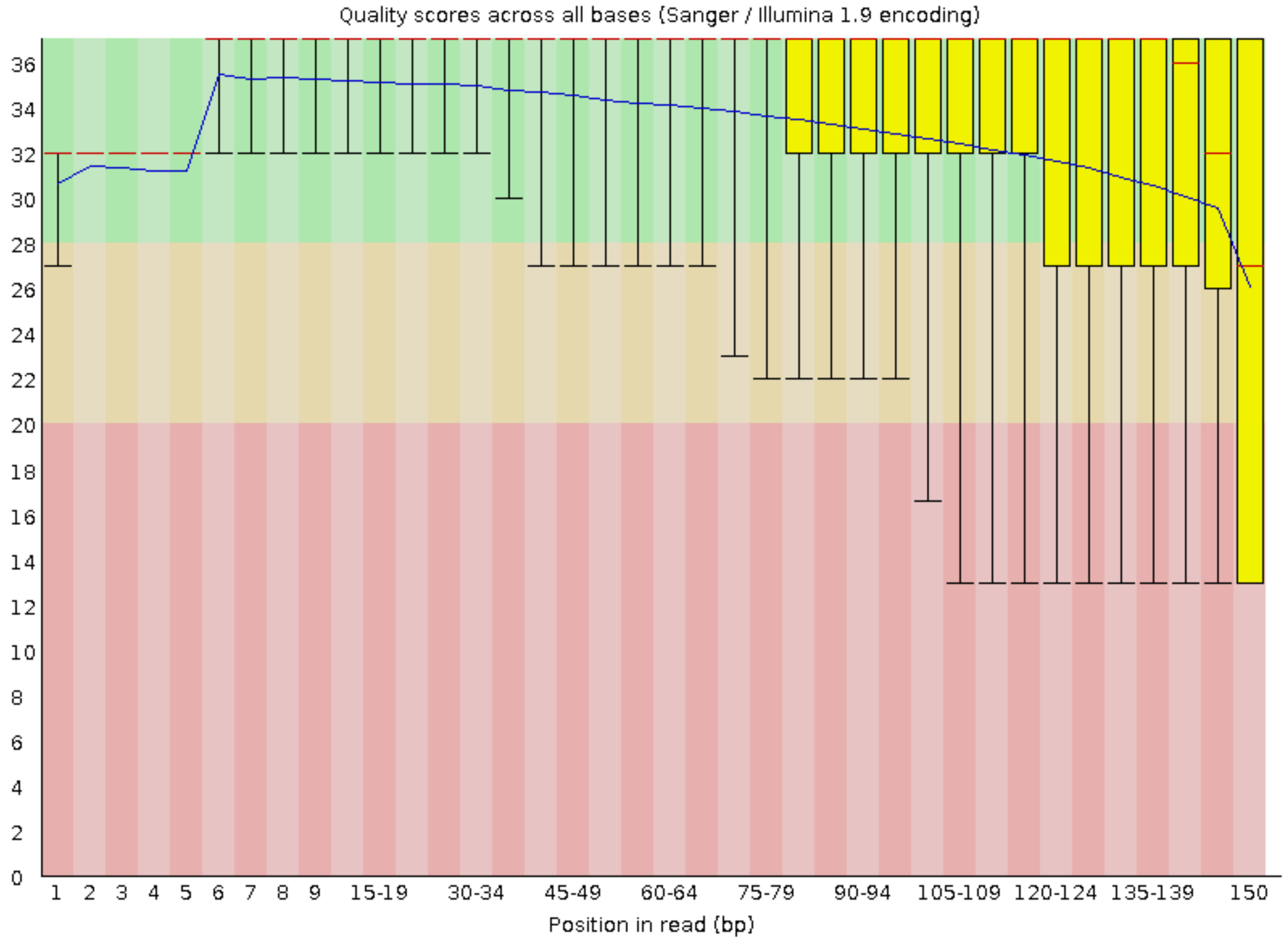
G T C [reference sequence]
A C C [2 mutations]
A C T [3 mutations]

Much harder to link distant variants using short read data

Errors in sequence reads can be introduced during library prep and during sequencing



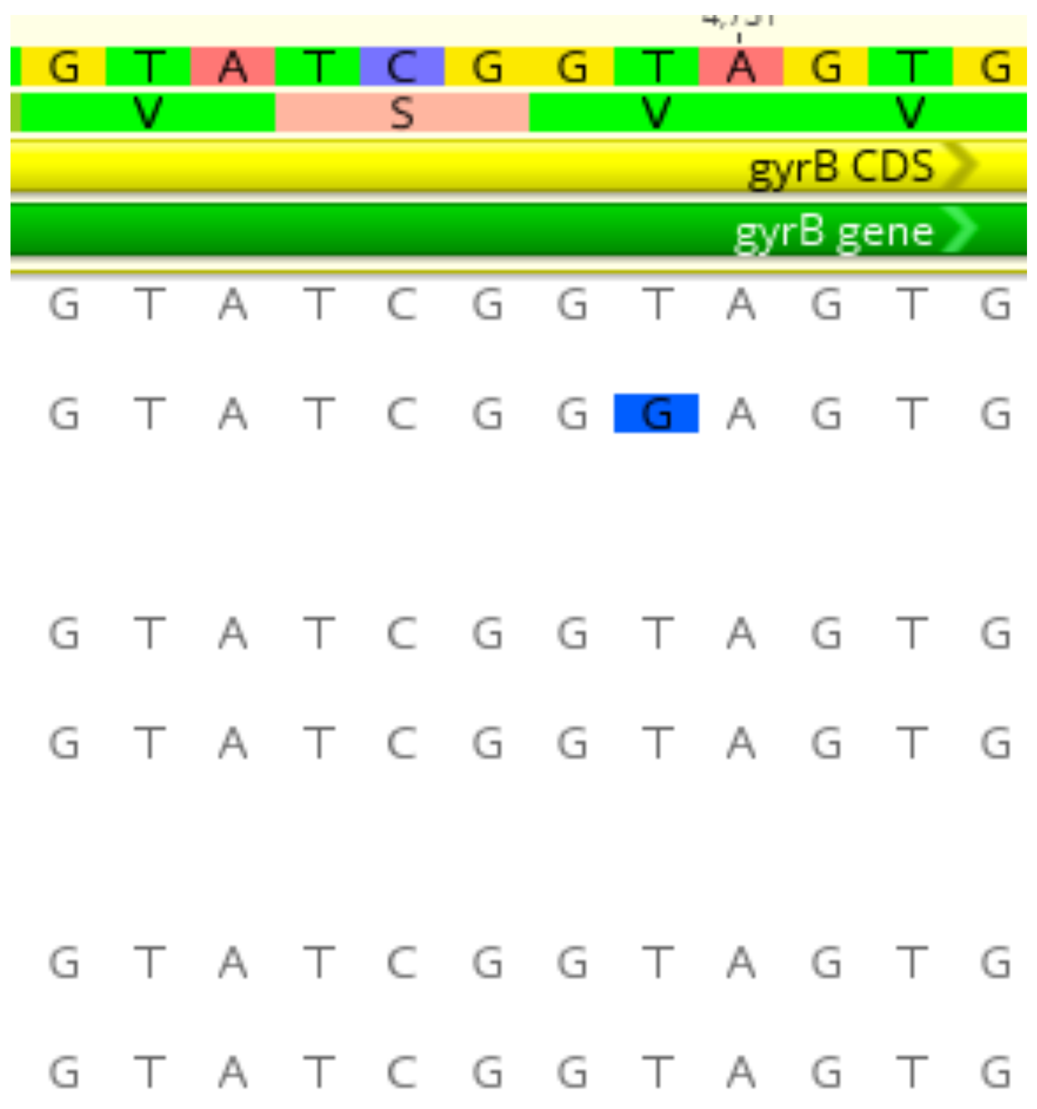
Error rates are fairly low, but they apply to huge #s of basecalls



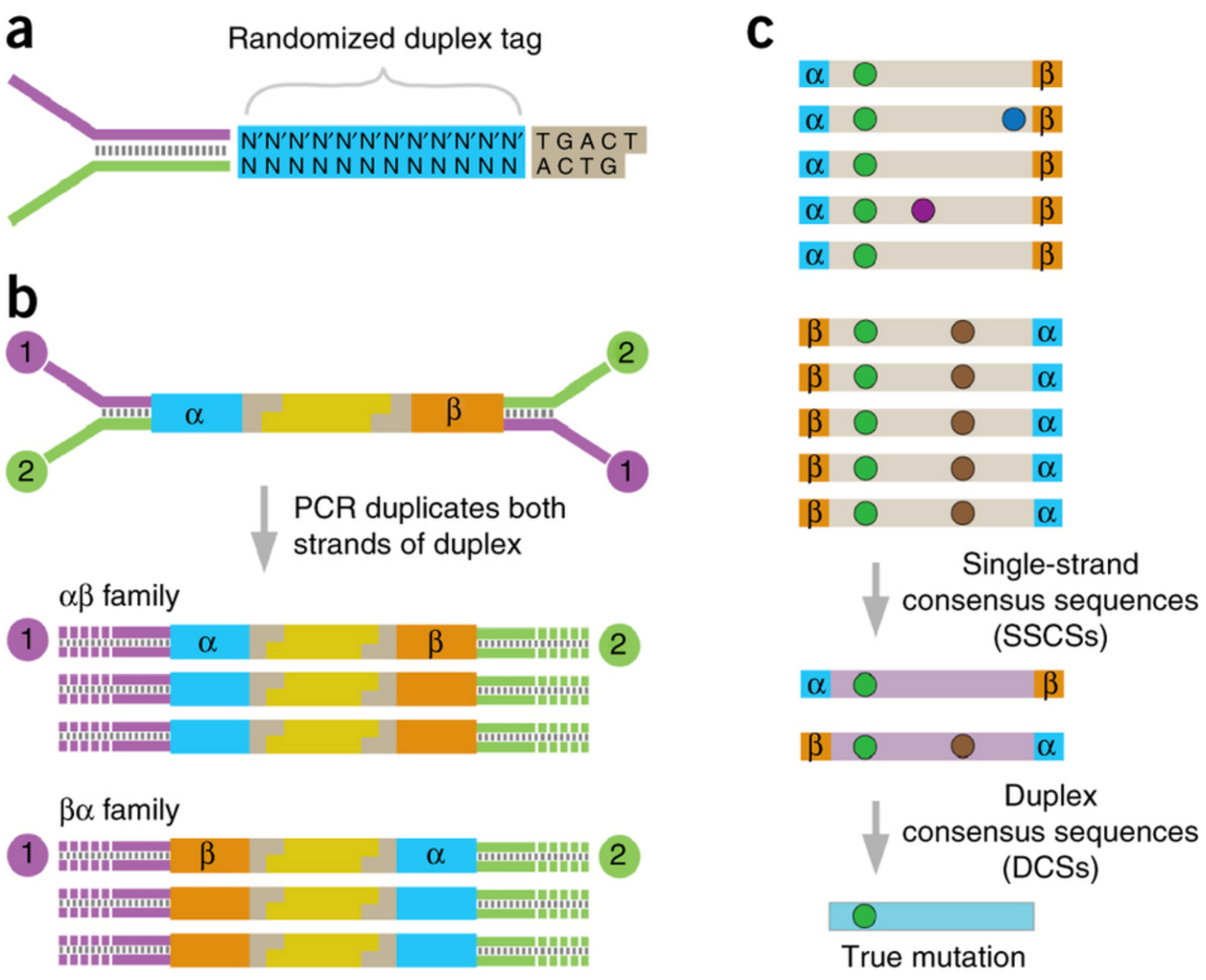
If the average error rate is 0.1%, and a sequencing run produces 100M 100 nt reads, there will be 10M incorrect basecalls in the dataset

Incorrect baseball

Incorrect basecall

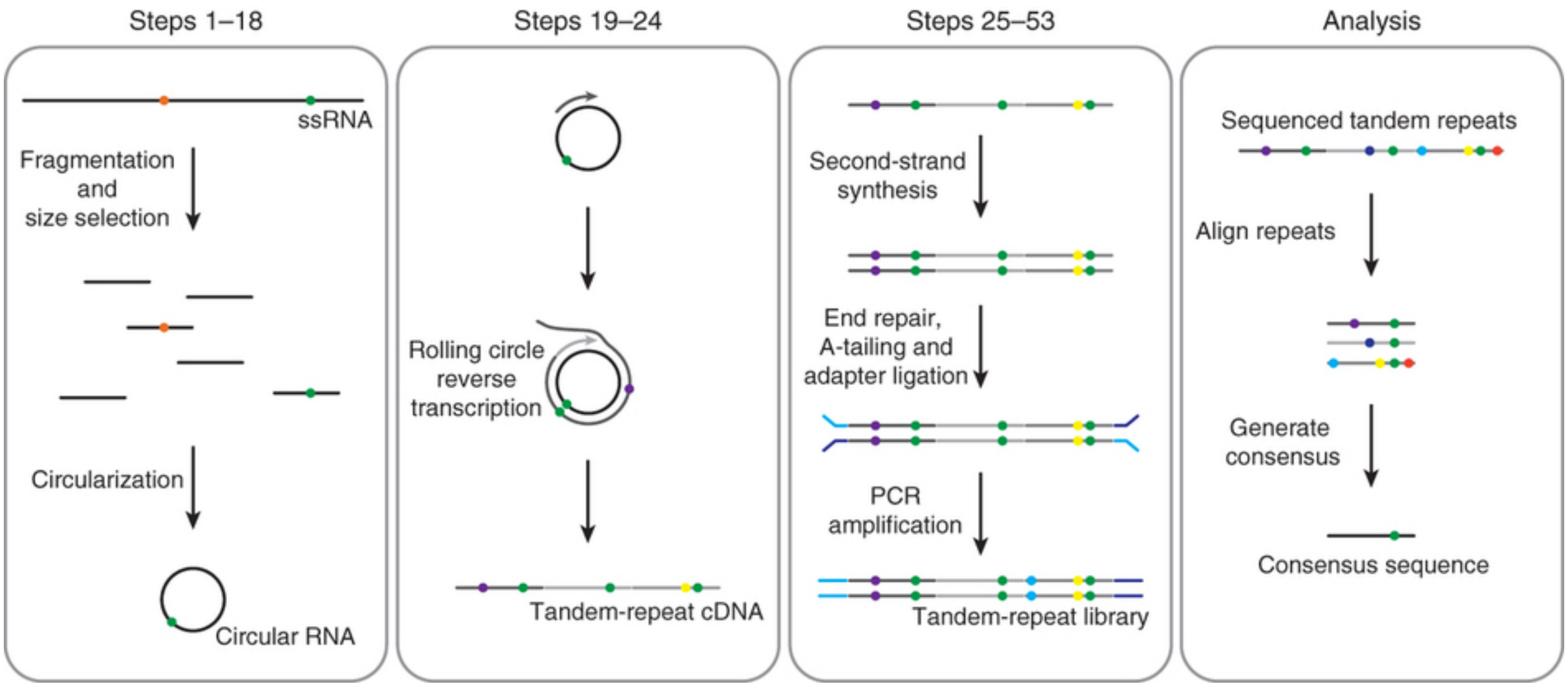


Several clever methods have been developed to get beyond the limit of detection due to sequencing errors



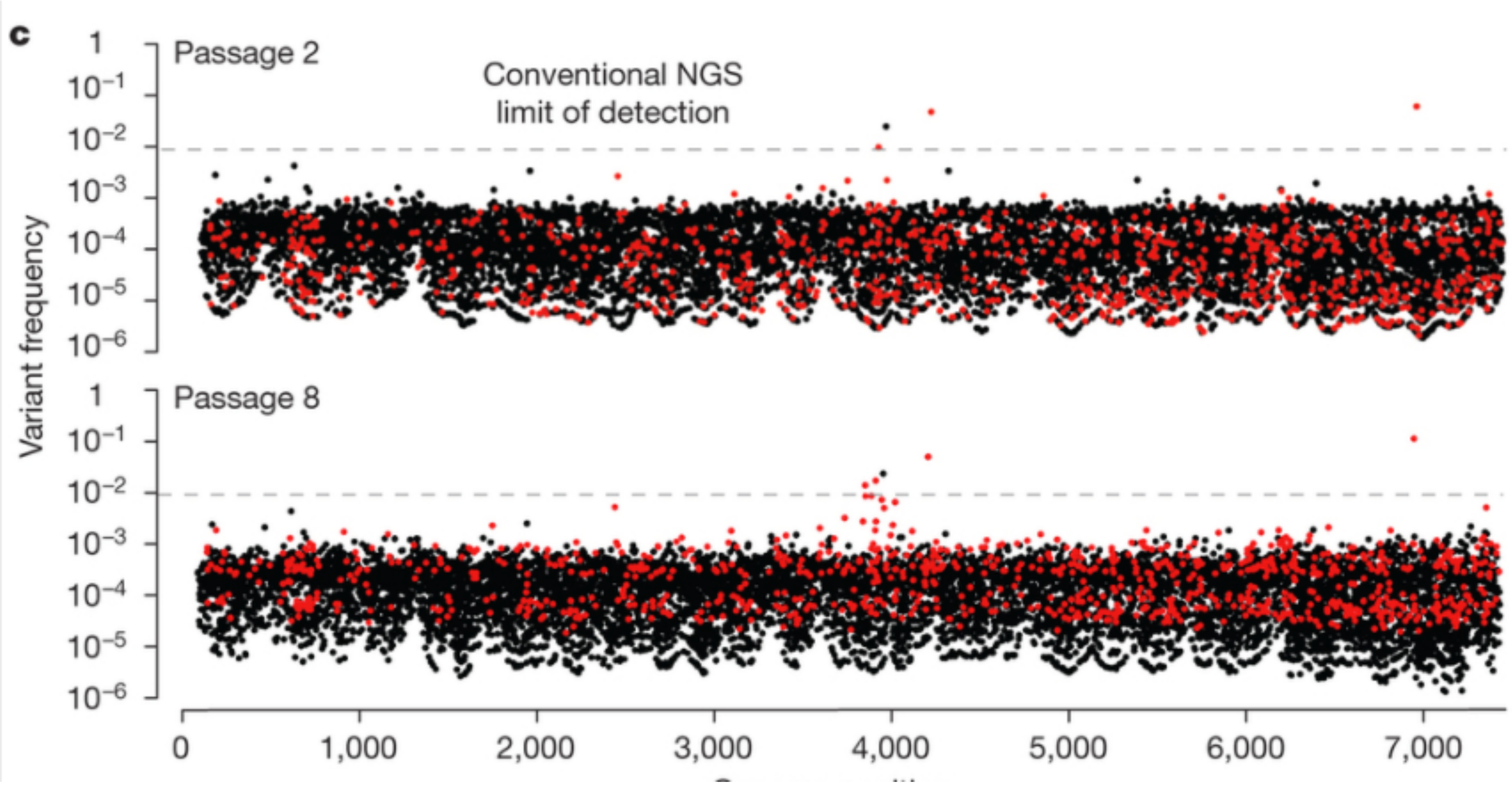
Kennedy et al (2014) Nature Protocols

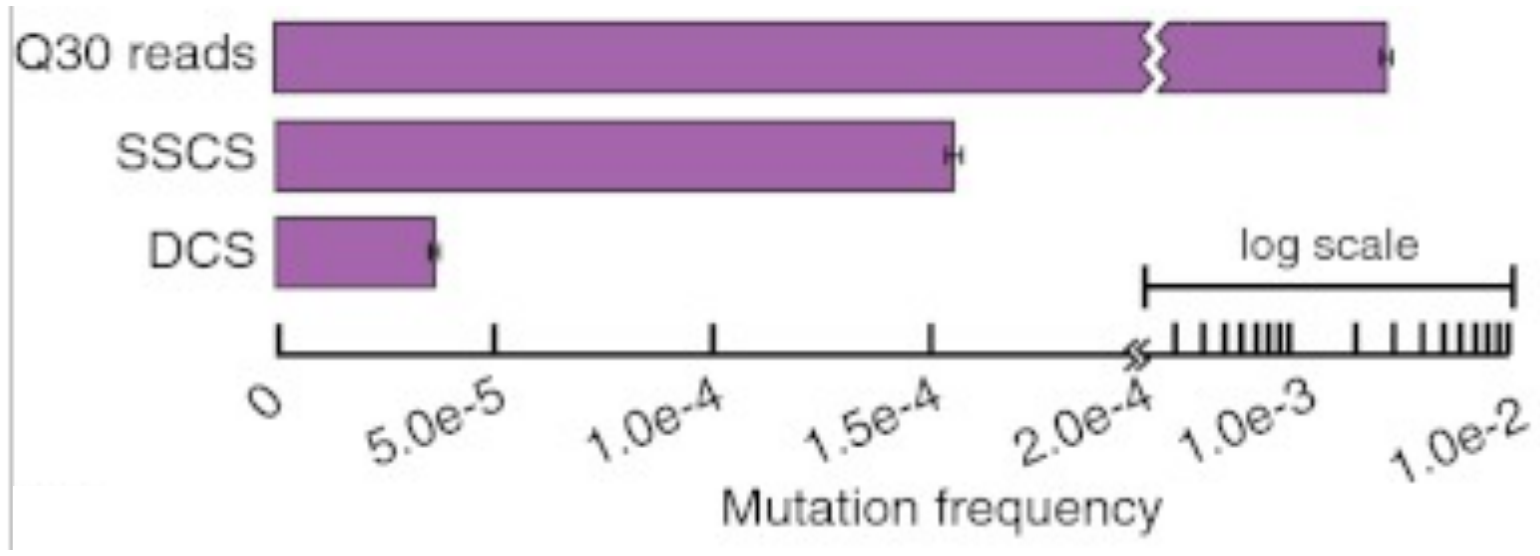
CirSeq aims to measure lower frequency variants in RNA viruses



Acevedo and Andino (2014) Nature Protocols

These methods aim to decrease variant frequency limit of detection





Acevedo et al (2014) Nature

Schmitt et al (2012) PNAS

These approaches have practical limitations

That’s a lot of (poly-A) RNA

> according to the manufacturer guidelines. Then 2–5 μg of poly(A)-containing RNA was fragmented with fragmentation reagent (Ambion) for 7.5 min at 70 °C. A practical minimum for this library preparation is 1 μg to ensure that enough fragmented RNA is obtained to produce a library with sufficient complexity and handle reproducibly. Approximately 80–90-base RNA fragments

Acevedo et al (2014) Nature

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

Sample: DNA/RNA isolation

↓

Library prep / sequencing

↓

Mapping to a reference genome or an assembly

↓

Variant calling

↓

Downstream analysis of variants

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

A couple reviews to get you started

Schlötterer et al (2014) Nat Rev Gen doi:10.1038/nrg3803

Posada-Cespedes et al (2016) Virus Res doi:10.1016/j.virusres.2016.09.016

Table 2 | **To pool or not to pool?**

Scenario	Pool-seq recommended?
Small sample size (<40 individuals)	Yes, but only appropriate when carried out on genomic windows containing multiple SNPs instead of on individual SNPs
Phenotypes of individuals are or will be available	RAD-seq of individuals is probably better suited for many cases
Linkage disequilibrium is key to data analysis	RAD-seq of individuals is probably better suited for many cases
High confidence about low-frequency SNPs is needed	Not with current protocols; sequencing of individuals is preferred
Simple population genetic analyses, such as population differentiation or average heterozygosity	Yes, but when coverage is low it results in a lower confidence of the allele frequency estimate of individual SNPs
Identification of selective sweeps	Yes, but only limited information about linkage disequilibrium can be obtained
Time series with large sample sizes and many replicates	Yes
Mapping of induced mutations	Yes, identification of the causative site is possible
GWAS	Yes, provided that replicates and large pool sizes are available, but other approaches should also be considered
QTL mapping	Yes, but no effect sizes are estimated
Intraspecific polymorphism of bacterial and viral populations	Yes
Information about dominance and effect size is important	No
Cancer	Pool-seq is a natural approach to analyse the cell population

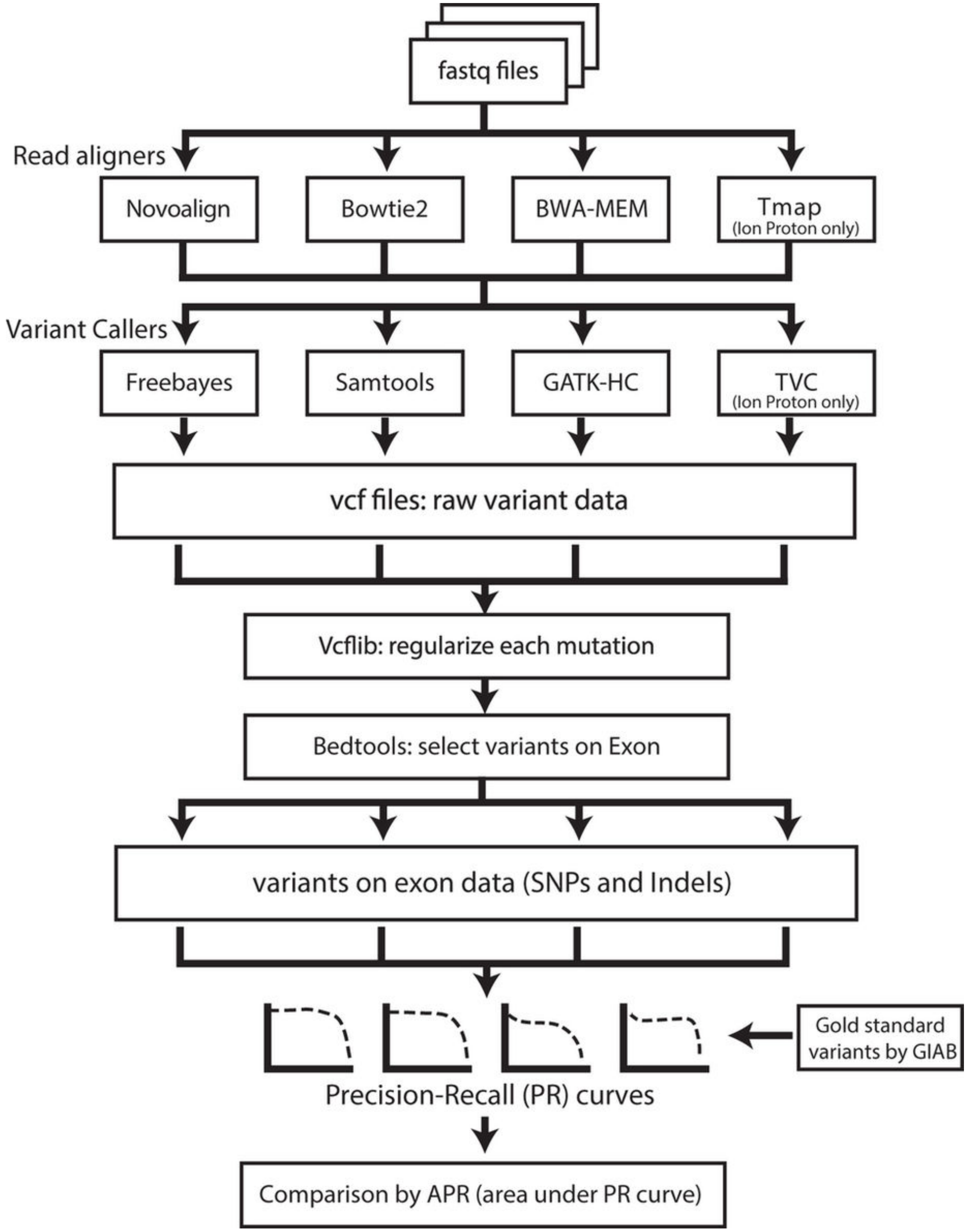
GWAS, genome-wide association study; QTL, quantitative trait locus; RAD-seq, restriction-site-associated DNA sequencing; SNP, single-nucleotide polymorphism.

These reviews summarize relevant software, pitfalls, best practices, etc.

Population genetics		
PoPoolation	Estimates variation within populations	39
PoPoolation2	Estimates differentiation between multiple populations	132
Pool-HMM	Detects selective sweeps from the allele frequency spectrum using a hidden Markov model	133
npstat	Computes a wide range of population genetic estimators; may be used in conjunction with an external SNP caller; every contig needs to be analysed separately	134
Stacks	Developed for population genomics with RAD-seq; may also be used with pooled RAD-seq data	135
Bayenv2	Estimates differentiation between populations	79
SelEstim	Detects and measures selection	136
KimTree	Infers population histories	137

Schlötterer et al (2014) Nat Rev Gen
Posada-Cespedes et al (2016) Virus Res

Several papers fairly recently compared variant calling software



Hwang et al (2015) Sci Rep
DOI: 10.1038/srep17875

Fairly good overlap from different pipelines using Illumina data

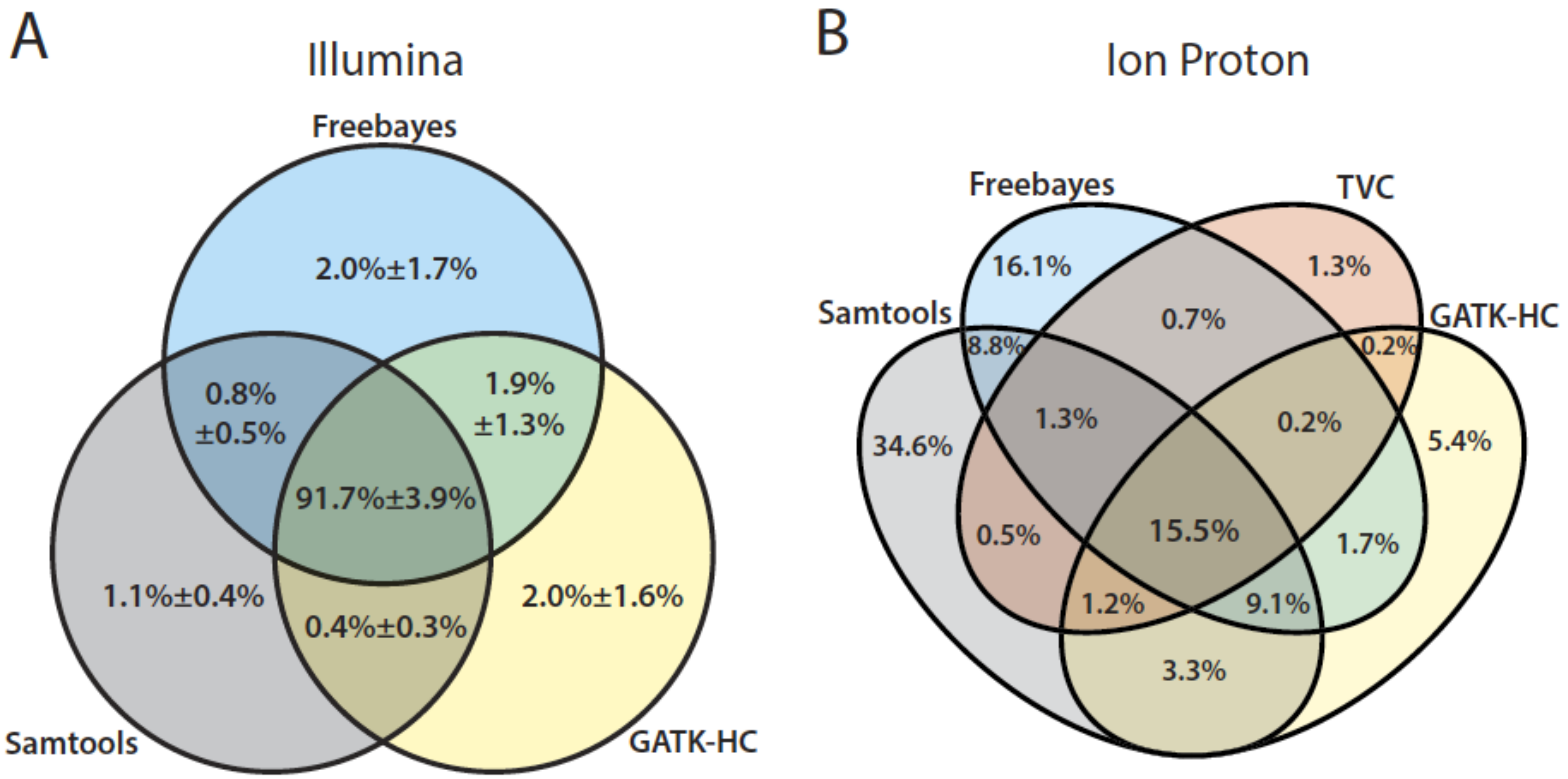


Figure 3. Venn diagrams summarizing called variants by different callers. The mean percentage with

Different s/w had different biases
Avoid using Ion Proton data for variant calling

Hwang et al (2015) Sci Rep
DOI: 10.1038/srep17875

A point we've been making repeatedly: analyze your datasets using several different software tools.

You ought to get (more or less) the same answer if an effect is real.

If you don't, that should be concerning.

Let’s call some variants, gosh darn it!!

```
GGAATAATGGAACATATGCACTCTAAGGGGACGGCGCTCATGTAT
 GAATAATGGAACATATGCACTCCAAGGGGACGGCGCTCATGTAT
GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT
GGAATAATGGAGCATATGCATTCCAAGGGGACGGCGCTCATGTAT
GGAATAATGGAGCATATGCATTCCAAGTGGACGGCGCTCATGTAT
GGAATAATGGAACATATGC              GCGCTCATGTAT
GGAATAA             CTCTAAGGGGACGGCGCTCATGTAT
GGAATAATGGAACATATGCACTCTAAGGGGACGGCGCTCATGTAT
GGAATAATGGAACATATGCACTCTAAGGGGACGGCGCTCATGTAT
GGAATAATGGAACATATGCACTCTAAGGGGACGGCGCTCATGTAT
 GAATAATGGAACATATGCACTCCAAGGGGACGGCGCTCATGTAT
```