Phylogenetics as motivation for pathogen sequencing, and viral genome assembly e.g. with `shiver`

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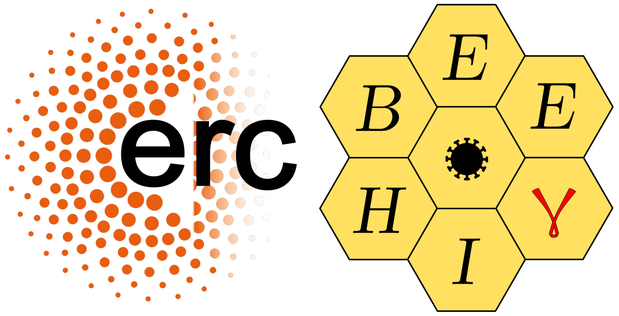


Infectious diseases: motivation

HIV:

- 35,000,000 deaths so far
- In 2017:
  - 36,900,000 people living with HIV (no cure)
  - 1,800,000 new infections
  - 940,000 deaths

Tuberculosis in 2017: 10,000,000 cases, 1,600,000 died.

Malaria in 2016: 216,000,000 cases, 445,000 deaths, disproportionately children under 5.

Viral hepatitis in 2015: >328,000,000 chronic infections, 1,340,000 deaths

$O(10^7)$ deaths anually.
Make an $O(10^{-6})$ improvement: 10 lives saved each year.

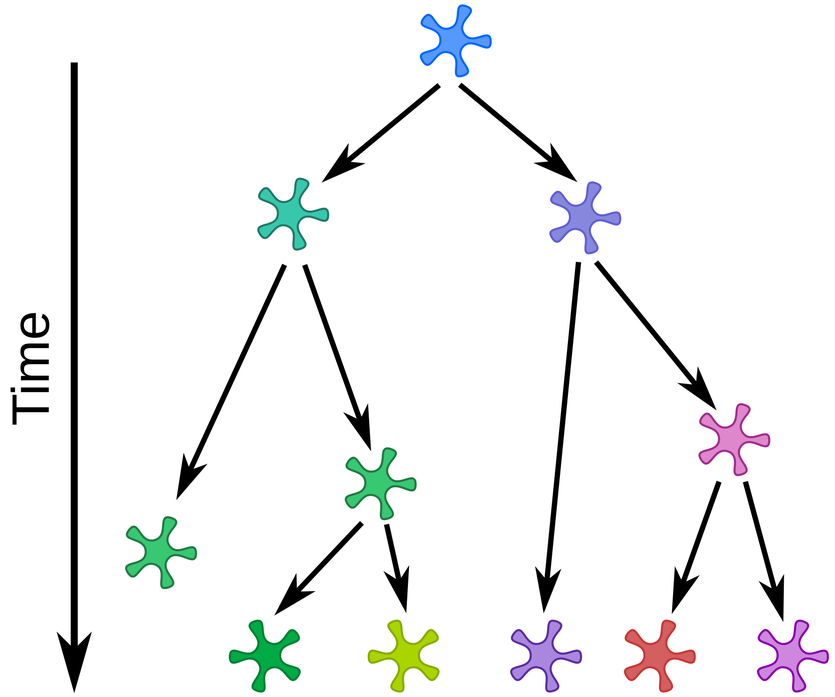
Source: WHO

Problem: how can we more effectively hinder the spread of an infectious disease?

A solution: learn more about the disease & its spread by analysing pathogen sequence data.

Genetic changes (substitution) accumulate over time

Substitution: replacement of a nucleotide (e.g. a $\rightarrow$ t)



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See also excellent webinar on understanding pathogen phylogenies by Christophe Fraser
https://www.dropbox.com/s/mia686o4oy1mhoj/181109%20Christophe%20Fraser.mp4?dl=0

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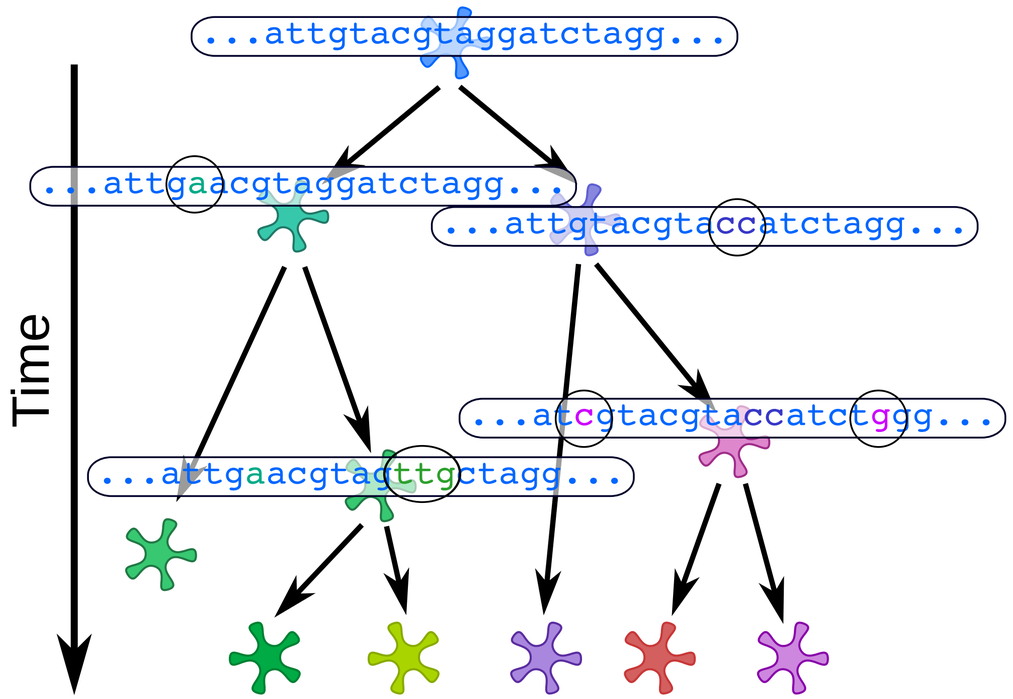

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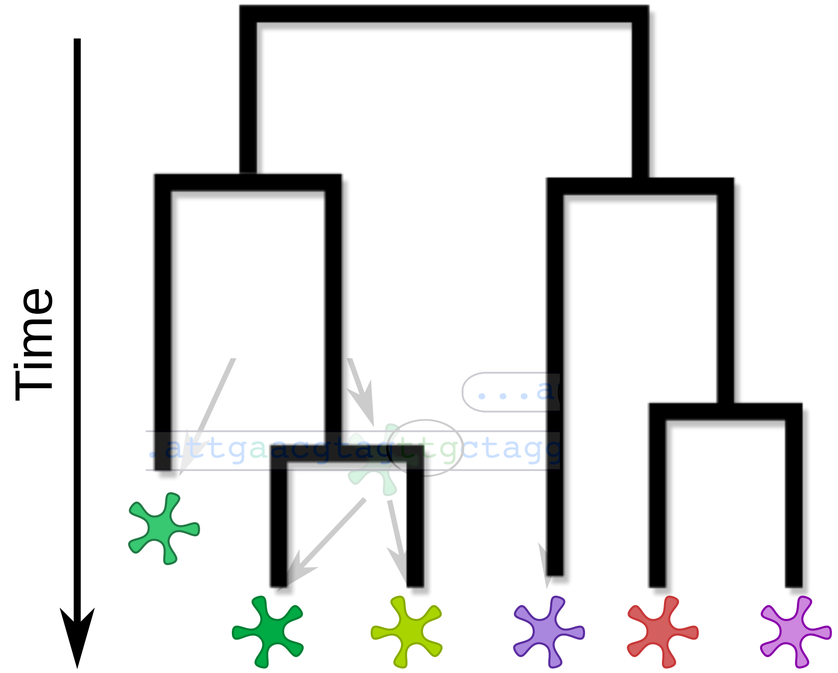
Genetic changes (substitution) accumulate over time

Substitution: replacement of a nucleotide (e.g. a $\rightarrow$ t)



Substitution patterns reflect the evolutionary history

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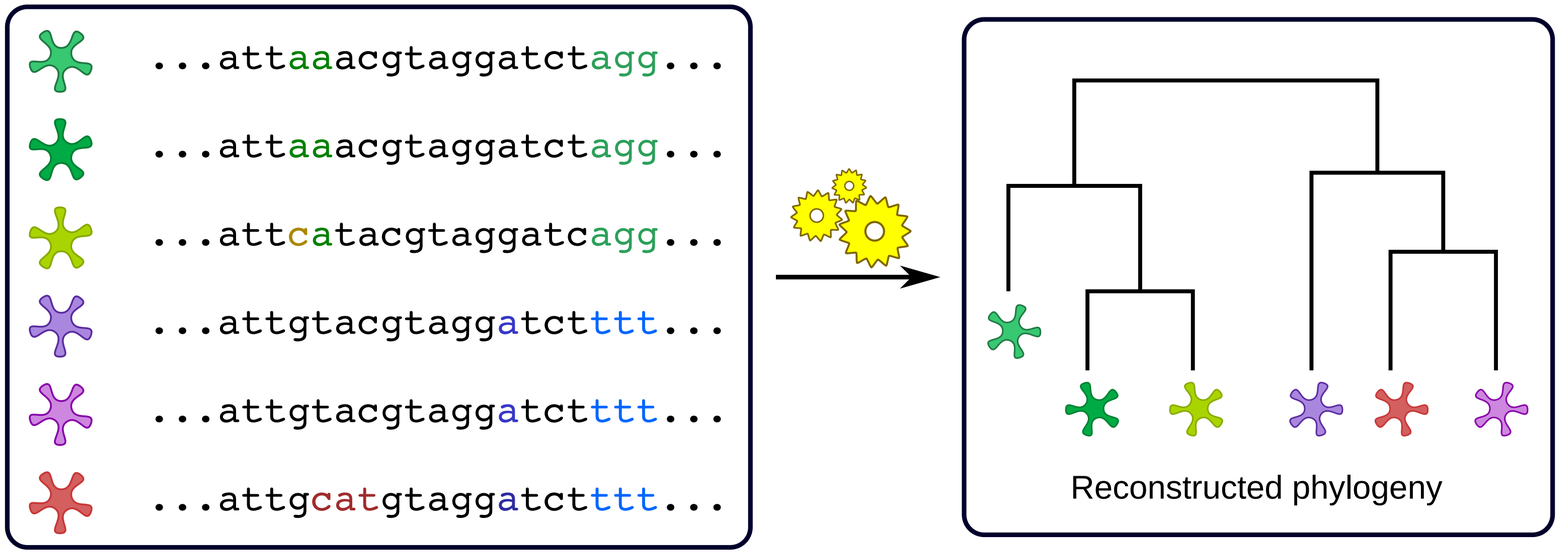
Using substitution patterns to reconstruct the evolutionary history



Phylogenetics aim to reconstruct evolutionary trees (*phylogenies*) from genetic sequence data.

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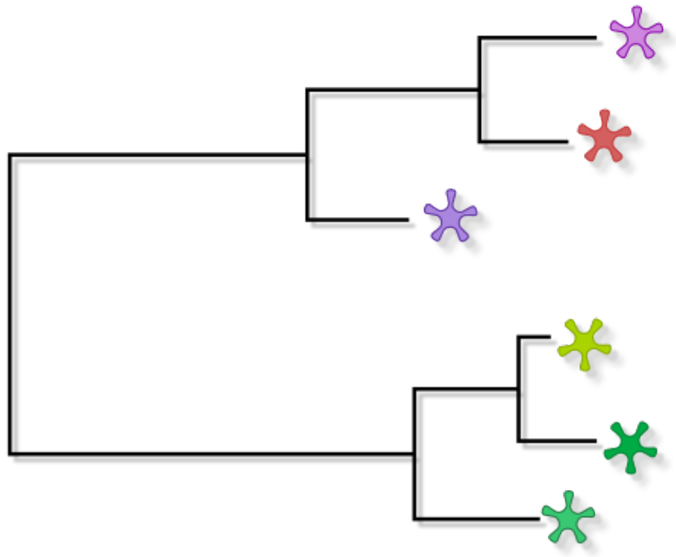
Using trees to represent the evolutionary history



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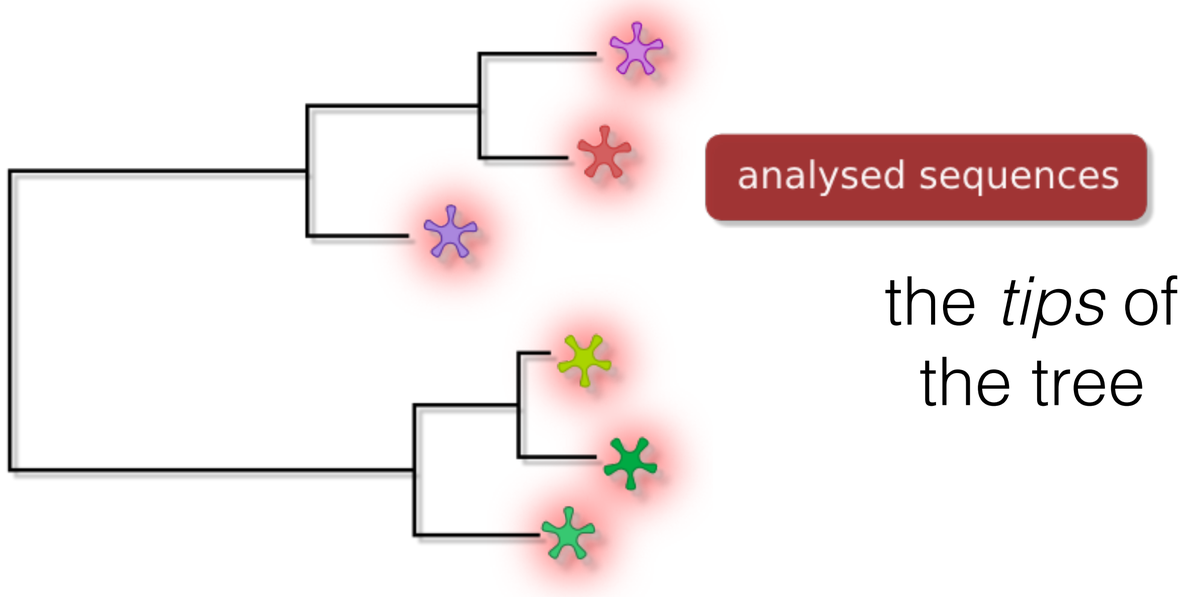
Using trees to represent the evolutionary history



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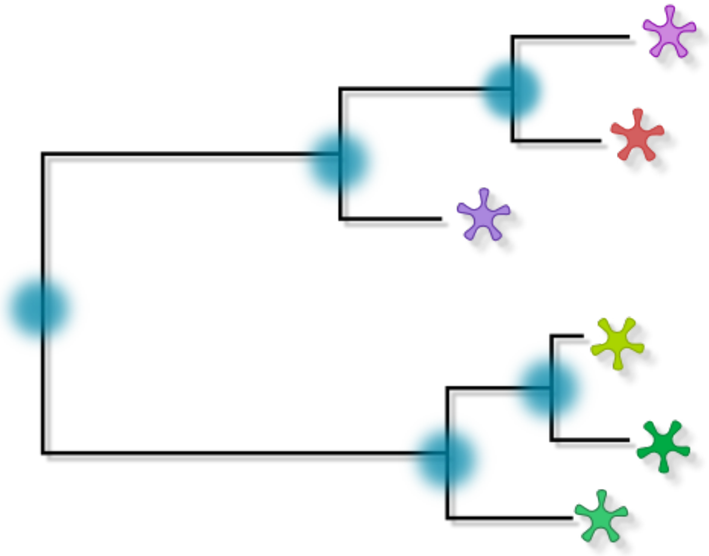
Using trees to represent the evolutionary history

Most Recent Common Ancestors (MRCA)



the *nodes* of the tree

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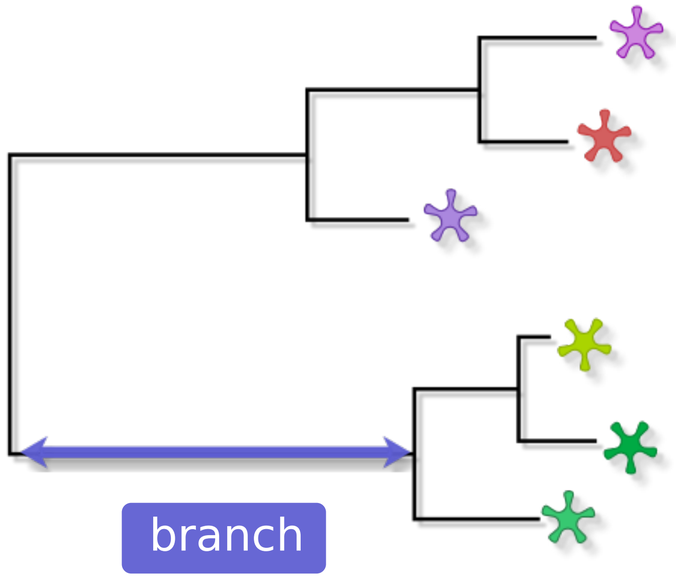
Using trees to represent the evolutionary history



length = amount of evolution

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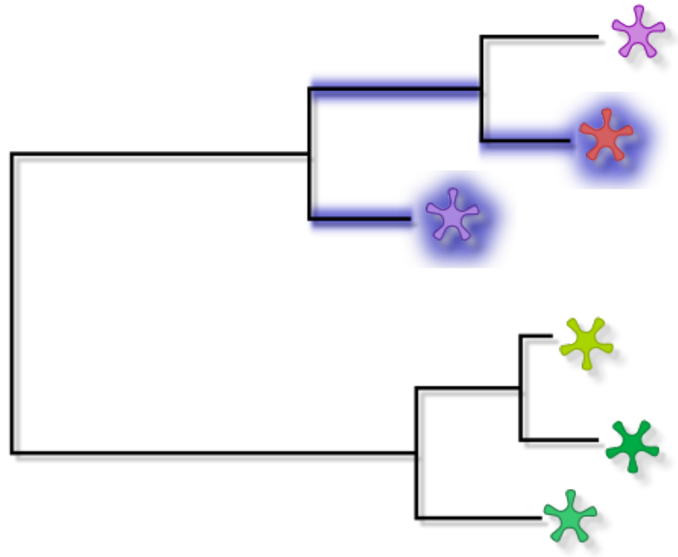
Using trees to represent the evolutionary history



distances between tips

"*patristic*" distance: sum of branch lengths

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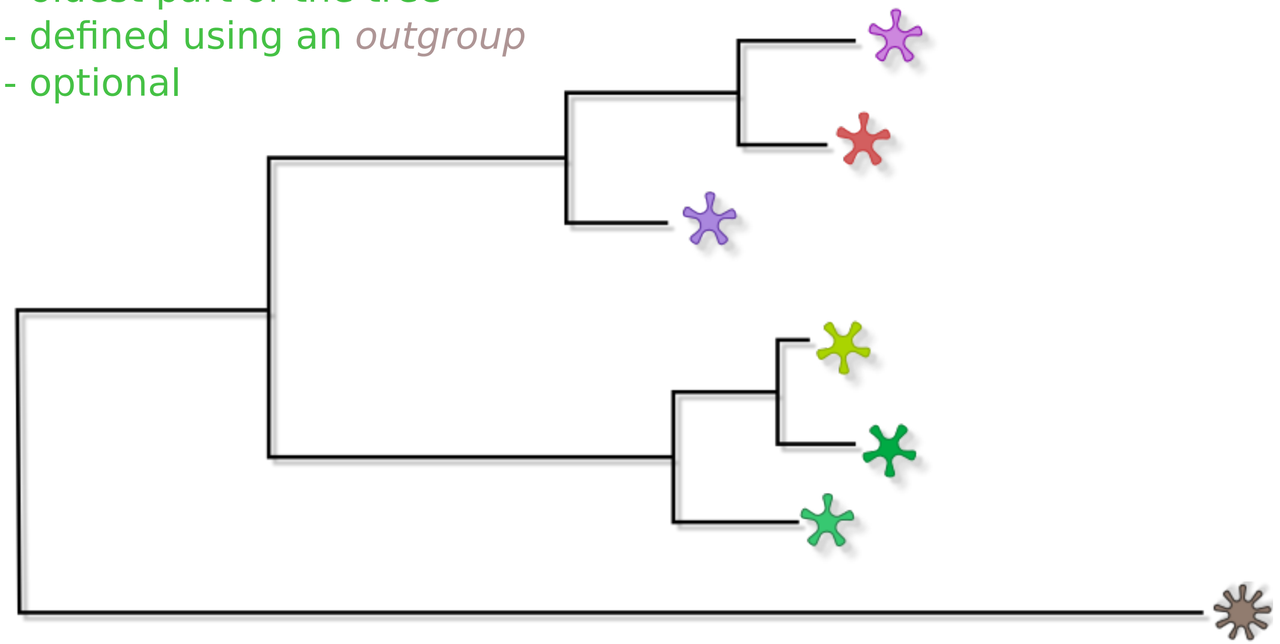
Small distance ⇒ small amount of evolution,
from which we infer proximity in a transmission network.

Using trees to represent the evolutionary history

Root

- oldest part of the tree
- defined using an *outgroup*
- optional



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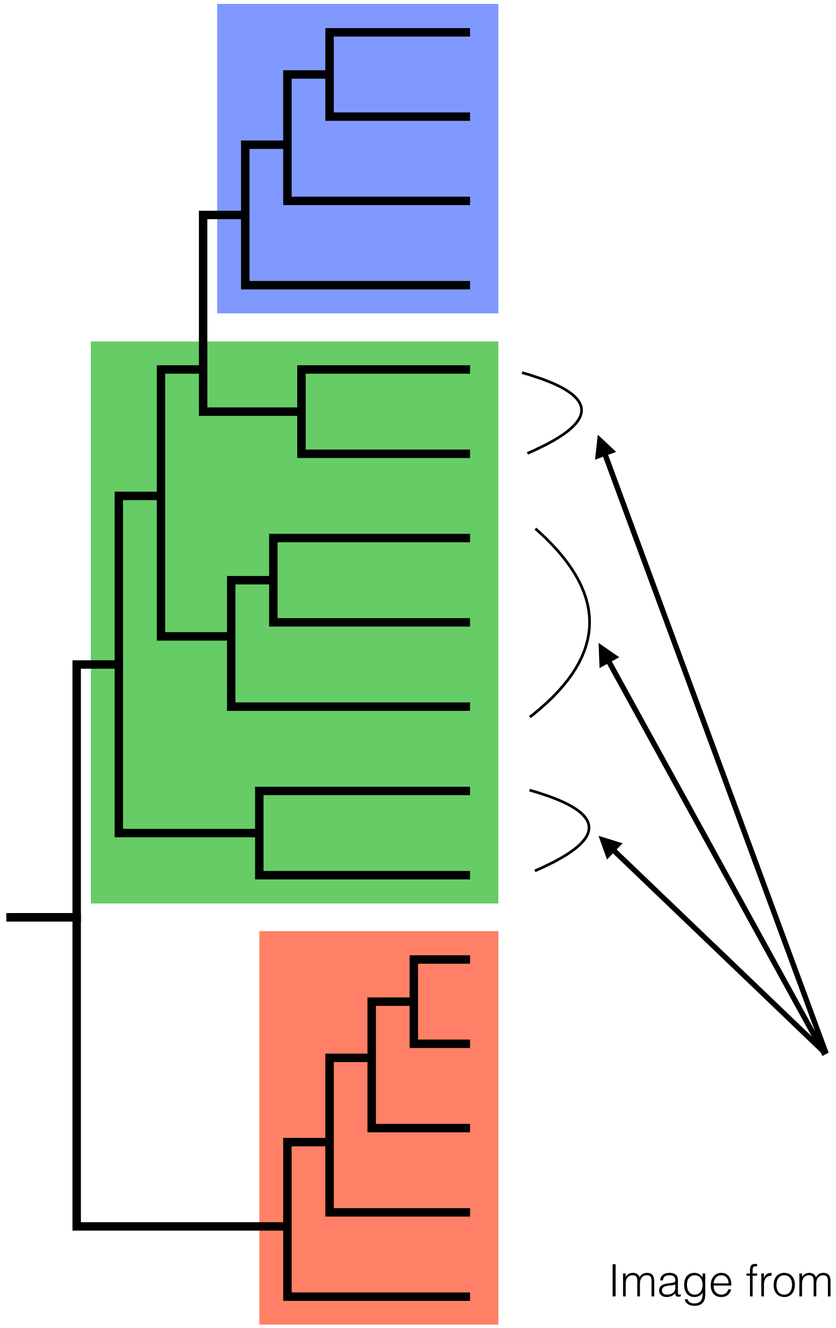


A *clade:* a group of organisms that consists of a common ancestor and all its descendants. a.k.a. a *monophyletic group*, a.k.a. a *monophylum*. The adjective is *monophyletic*. The blue and red boxes show two clades in this phylogeny (NB more clades are exist).

The group of tips in green is not monophyletic; it is called *paraphyletic*.
The set of green tips can be split into smaller groups, each of which is a clade; the minimum number needed is three, as illustrated.

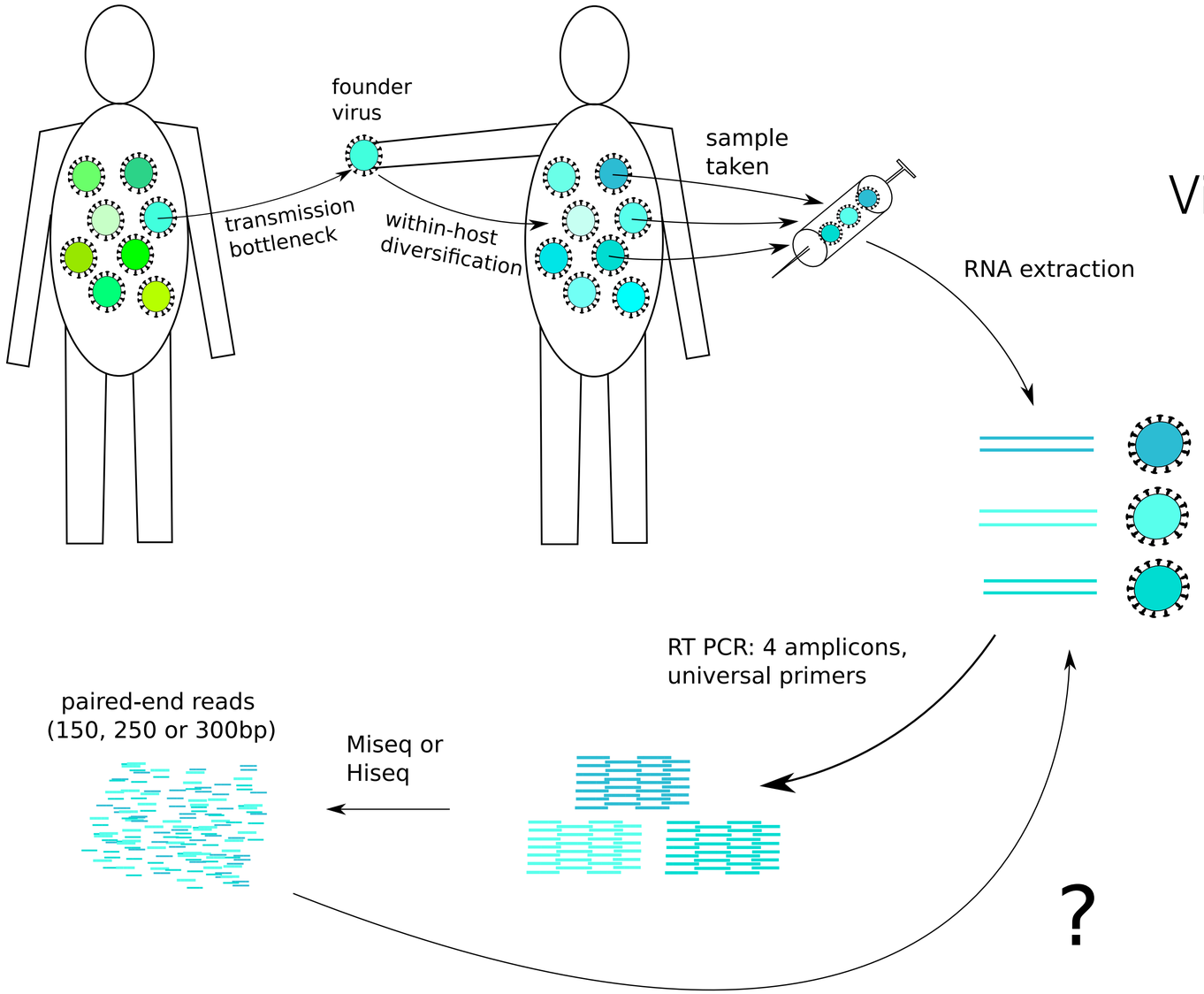
Image from https://en.wikipedia.org/wiki/Clade

Problem: to meaningfully interpret small differences between closely related viruses, we want *accurate* genomes.

Problem: to make robust statements, need *many* genomes.

Solution: high-throughput sequencing + accurate, scalable sequence processing.

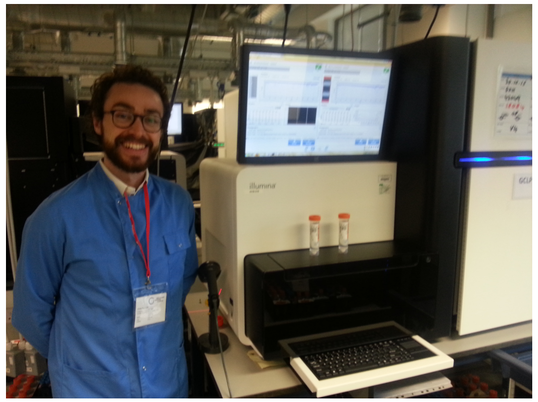
Our HIV Data*



Cornelissen *et al.*
Virus Research 2016

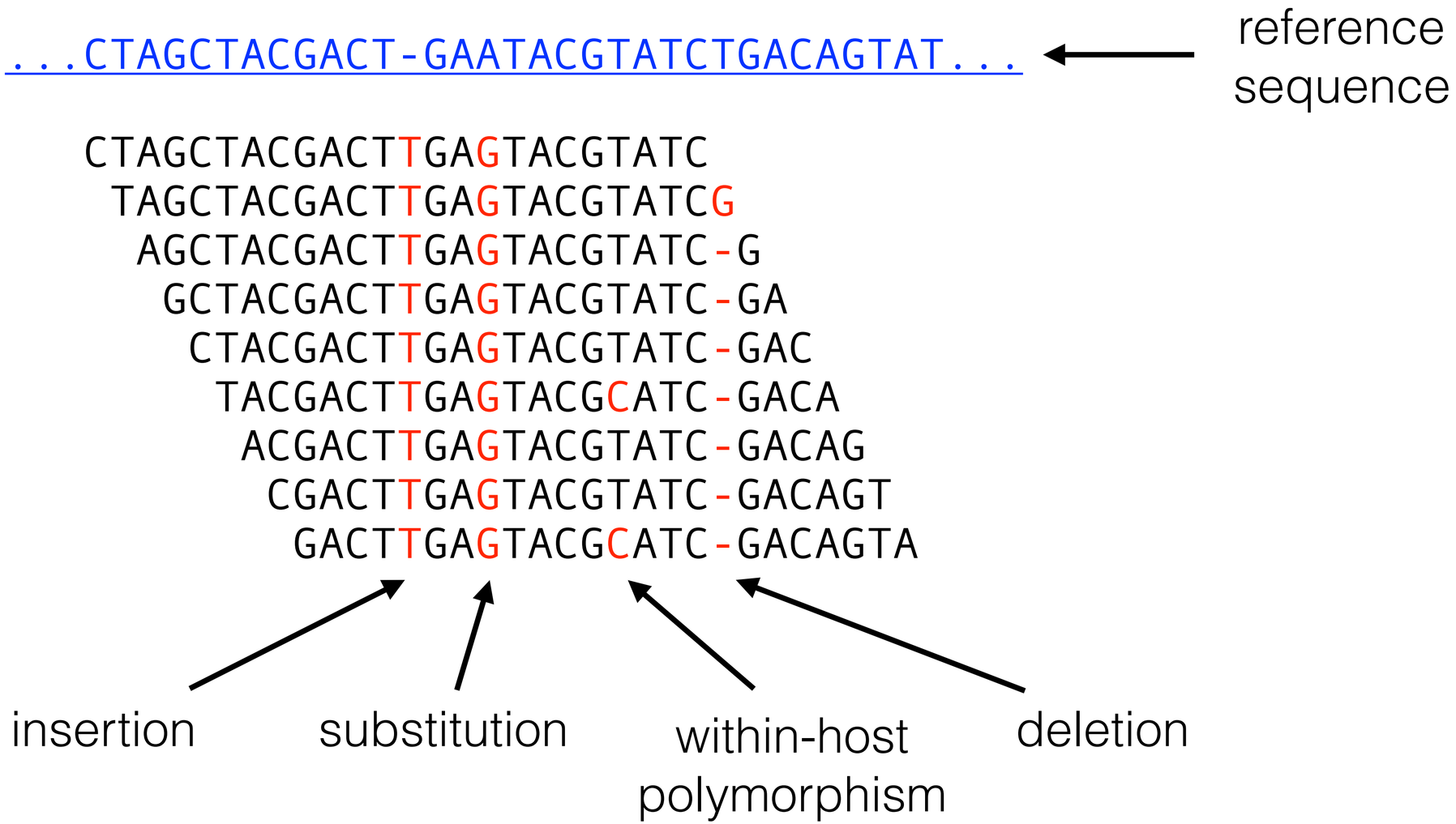
Gall *et al.*
J. Clin. Microbiol.
2012

* until now. See Bonsall, Golubchik et al. bioRxiv Aug 24th



↑ visiting, not helping

Mapping Reads to a Reference



Mapping Reads to a Reference

```
...CTAGCTACGACT-GAATACGTATCTGACAGTAT...
   ???GCTACGACTTGAGTACGTATC-GACA???
  CTAGCTACGACTTGAGTACGTATC
   TAGCTACGACTTGAGTACGTATCG
    AGCTACGACTTGAGTACGTATC-G
     GCTACGACTTGAGTACGTATC-GA
      CTACGACTTGAGTACGTATC-GAC
       TACGACTTGAGTACGCATC-GACA
        ACGACTTGAGTACGTATC-GACAG
         CGACTTGAGTACGTATC-GACAGT
          GACTTGAGTACGCATC-GACAGTA
```

consensus sequence, requiring 4X coverage (just as an example)

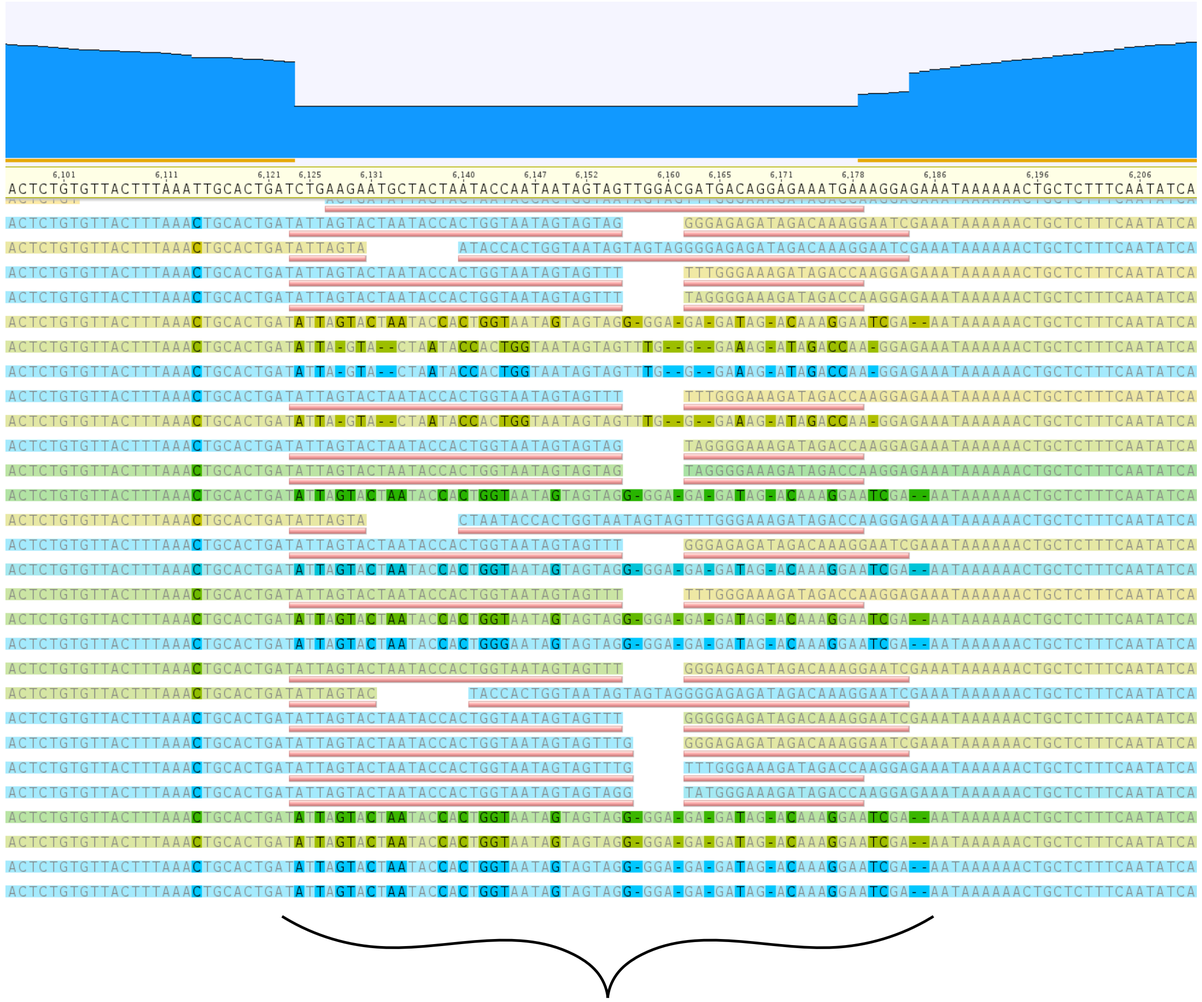
Note: Sanger sequencing, which was usually used when sequencing HIV until recently, only produces a consensus sequence. Having all these reads is very useful: stay tuned!

However, the more different a read is from its reference, the more likely it is to be aligned incorrectly or not at all. So **mapping causes biased loss of information**.

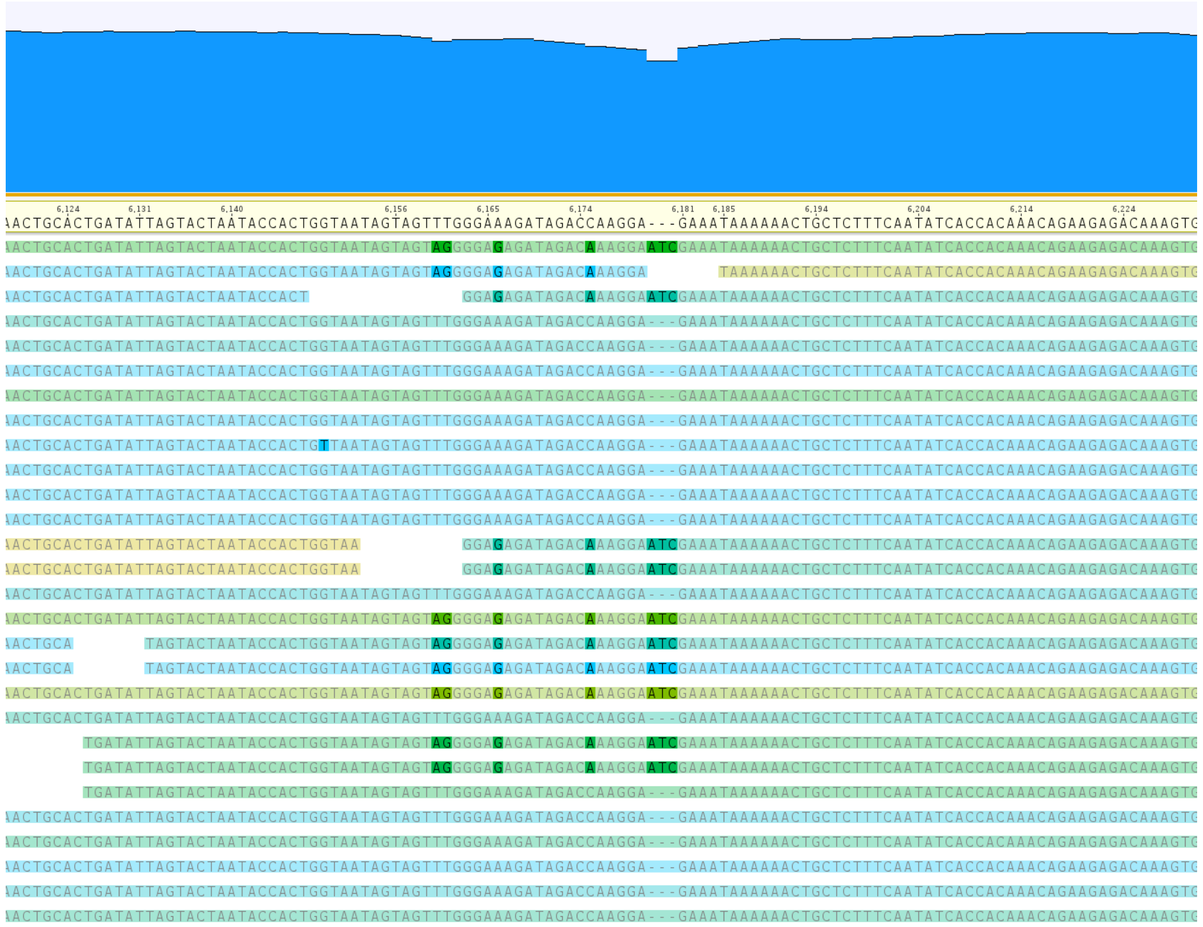
e.g. use the same reference for mapping for a group of individuals: bias their viruses to look similar to each other
→ false inference of transmission.

e.g. use old references for mapping new samples
→ false inference of slow evolution

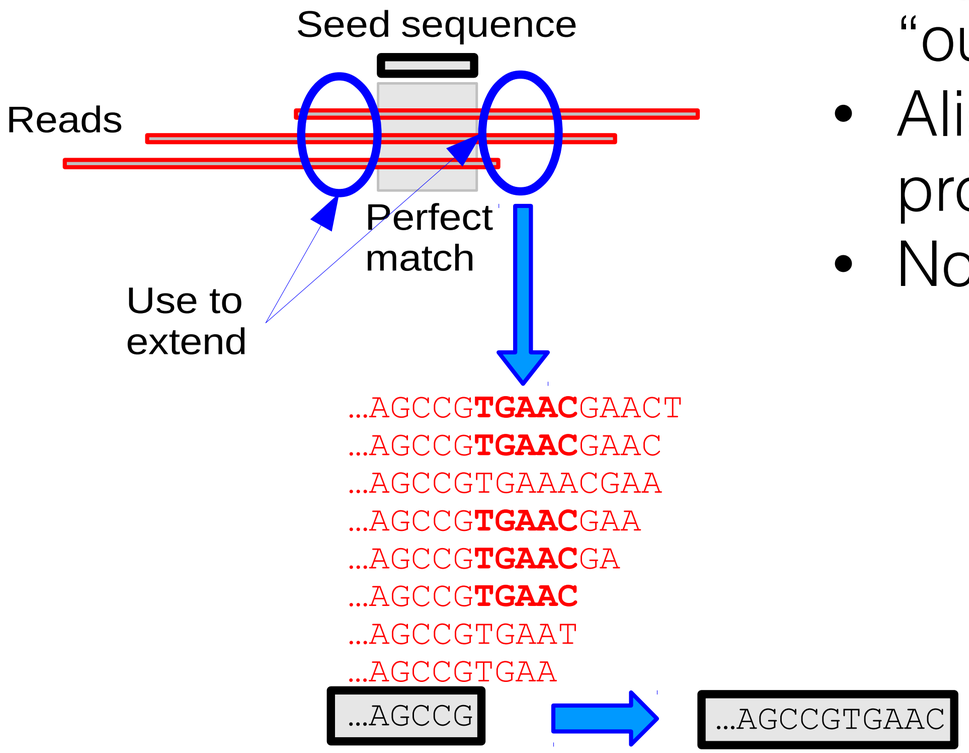
inappropriate reference ← same reads & mapping algorithm → appropriate reference



13 base miscalls, a false insertion and a false deletion



De Novo Assembly



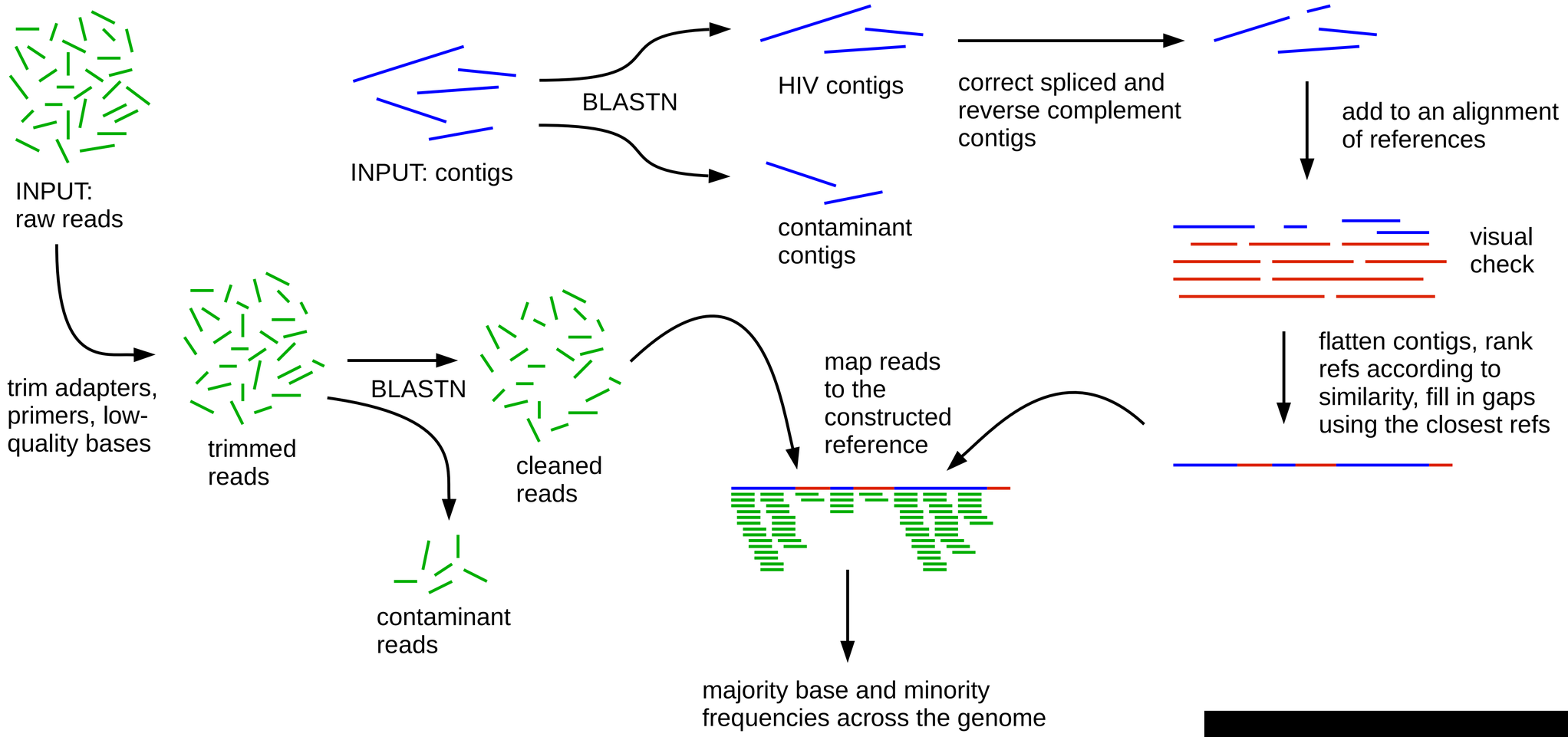
- e.g. IVA (*Hunt et al., Bioinformatics 2015*): on HIV, "outperforms all other virus de novo assemblers".
- Align the reads to themselves, iteratively extending, producing a number of summary sequences.
- No need for a reference: no bias.

but...

- Assembly failure (no contigs)
- Assembly errors (incorrect contigs)
- No minority variant information (contigs as summaries of reads)

Figure courtesy Martin Hunt

`shiver` - Sequences from HIV Easily Reconstructed



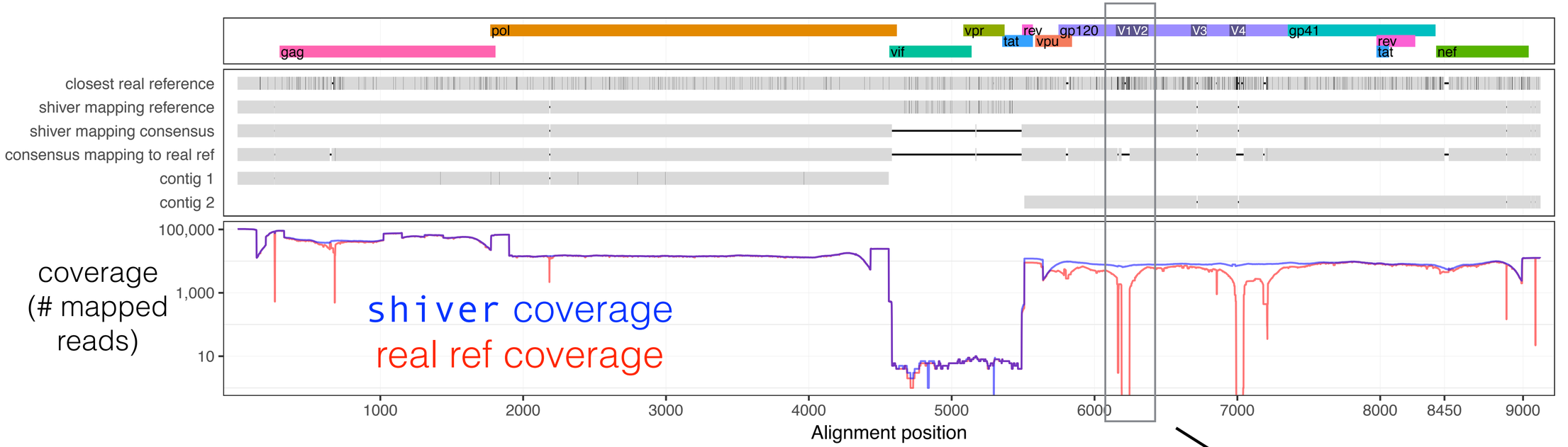
Wymant *et al.*, Virus Evolution 2018

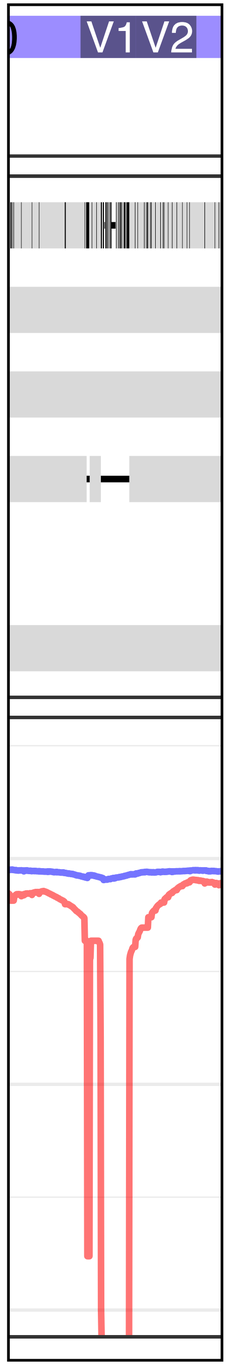
github.com/ChrisHIV/shiver

Works for other viruses too!

```
$ shiver_align_contigs.sh
$ shiver_map_reads.sh
```

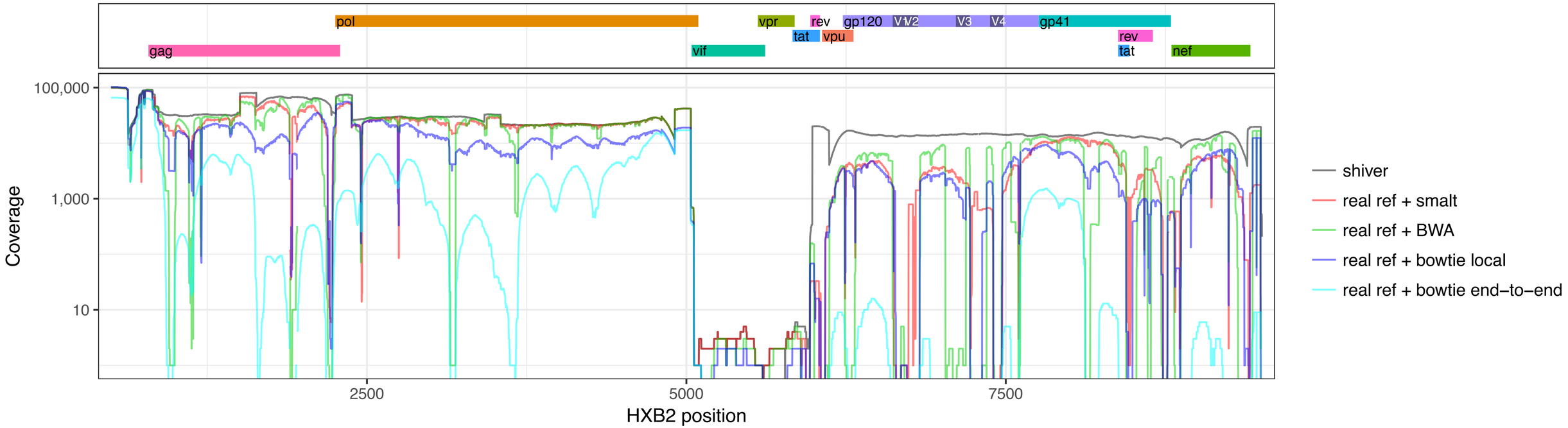
Map to `shiver`'s constructed reference,
also map to the closest of 3259 real references (from database),
compare.





Over 50 BEEHIVE samples + 65 public samples:
median number of bases called differently with higher coverage:
13; with lower coverage, 0.
Recover missing sequence, often in *envelope* (vaccine design!).

Sometimes mapping to the closest real reference really sucks.



Application of `shiver` for 3 HIV projects led by Christophe:

Investigate the viral-genetic basis of virulence

BEEHIVE: N ~ 3,500
PopART phylo: N ~ 6,000 currently, 9,000 eventually
PANGEA(2): N ~ 13,000 currently, 23,000 eventually

Help interpret the effect of a population-level test-and-treat intervention in Zambia and South Africa

Generate new insights into transmission dynamics in generalised epidemics in Africa

Tanya Golubchik