Phylogenetics Between and Within: Seeing Transmissions and Dual Infections in HIV Deep Sequence Data

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Mathematical and Computation Evolutionary Biology, June 2016: Forecasting Evolution

Notes on the talks:
ChrisWymant on Twitter

The BEEHIVE Study: Bridging the Epidemiology and Evolution of HIV in Europe

- Whole viral genome data from >4000 patients.
- Seroconverters: 1 year window between – and + tests, or clinical evidence
- ≥ 1 viral load measurement 6-24 months after + test, pre-ART
- Wealth of clinical data (including later response to ART)

Objectives:

1°: the viral-molecular basis of virulence

2°: molecular epidemiology

3°: dual infections and minority variants

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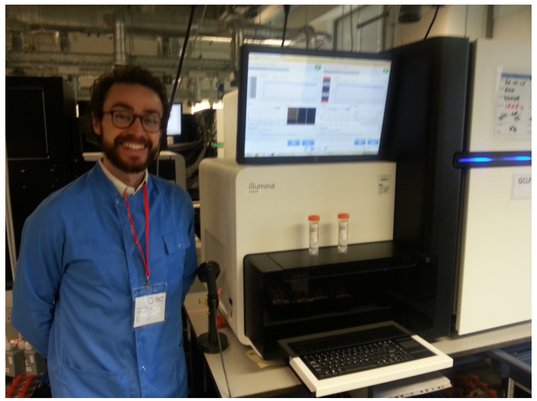
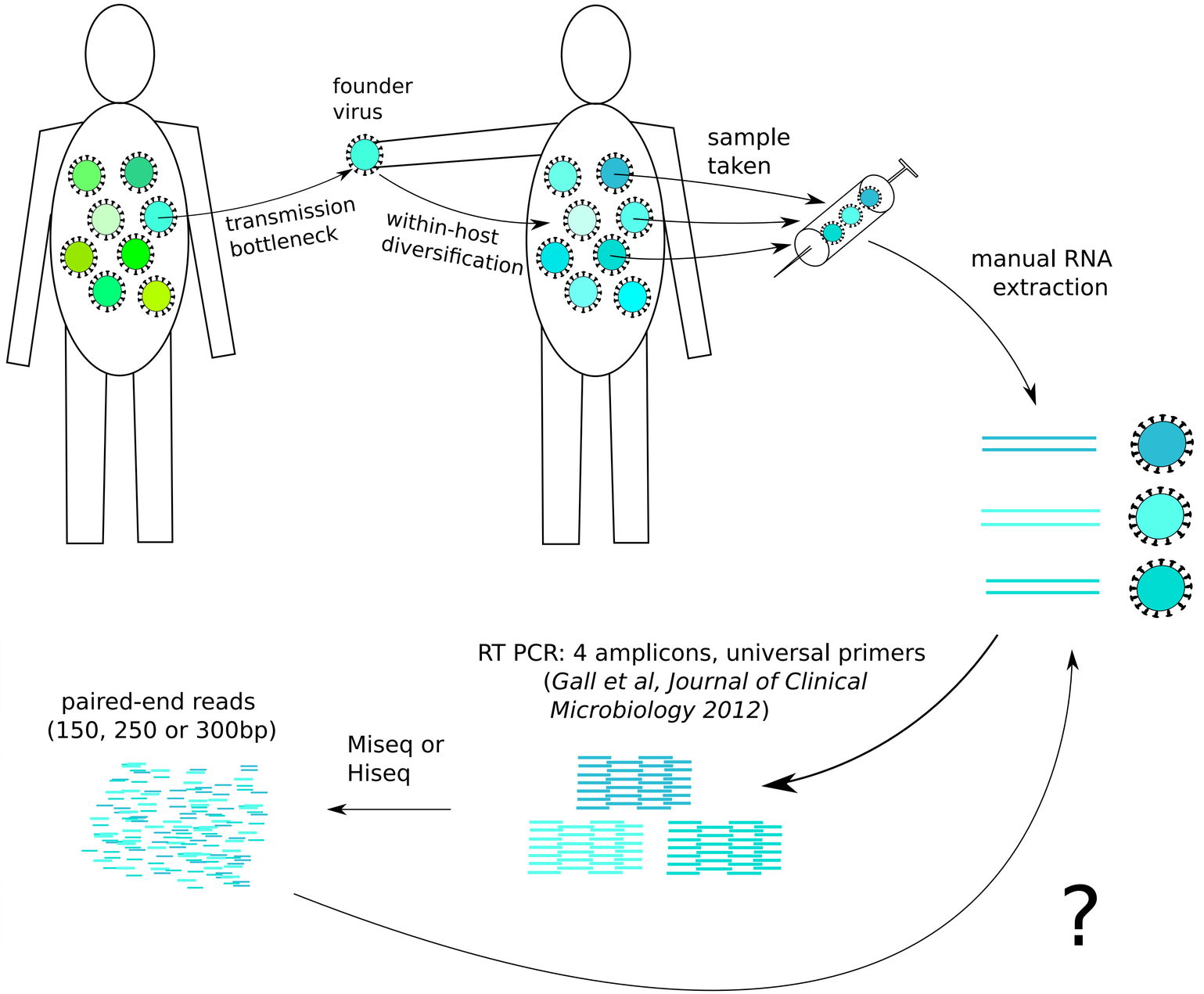
Oliver Pybus
Gil McVean
. . .and thanks to
Tiziano Gallo Cassarino
Nick Croucher
Katrina Lythgoe
Olli Ratmann

erc

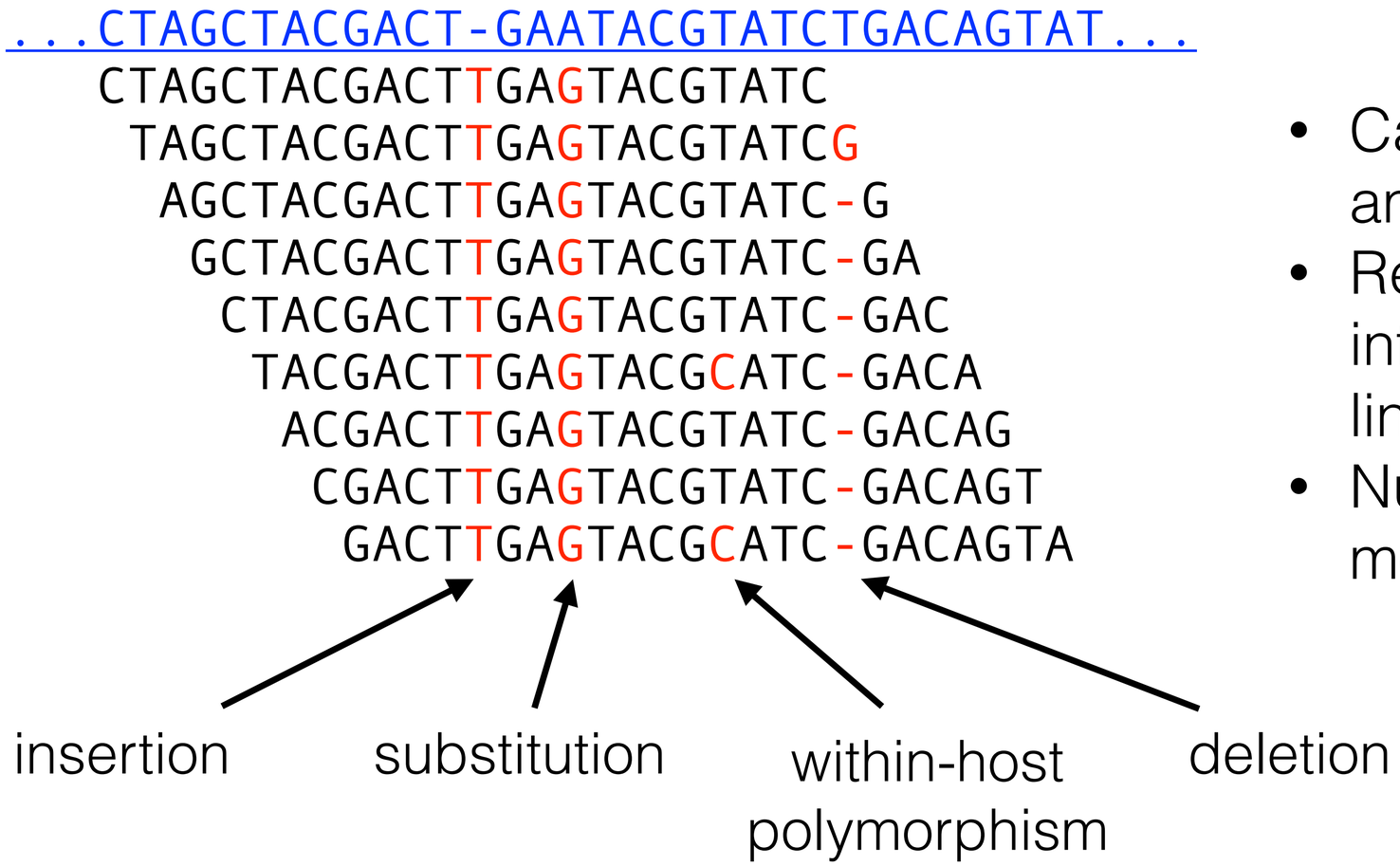
First: reconstructing the viral genotype

(Next: putting it in context.)



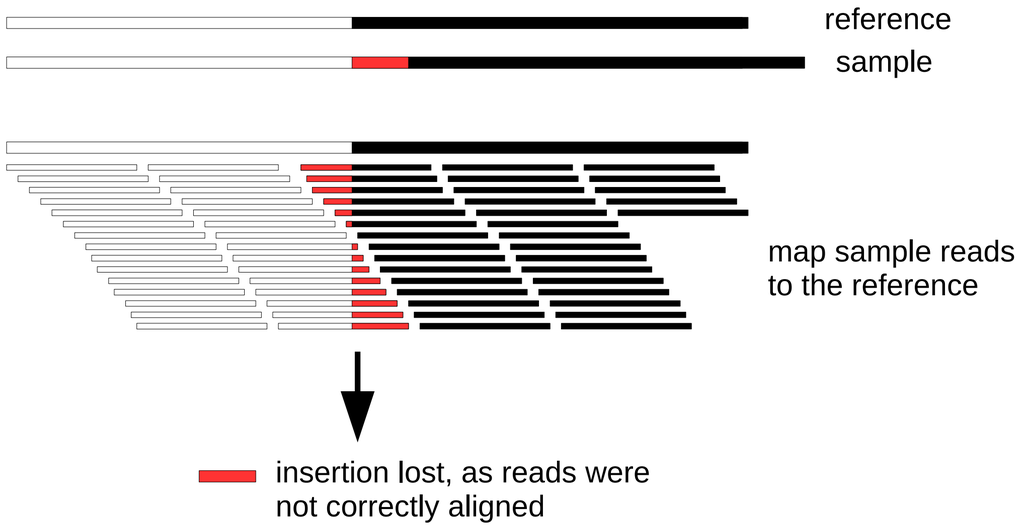

founder
virus
transmission
bottleneck
within-host
diversification
sample
taken
manual RNA
extraction
RT PCR: 4 amplicons, universal primers
(*Gall et al, Journal of Clinical Microbiology 2012*)
Miseq or
Hiseq
paired-end reads
(150, 250 or 300bp)
?

Map Reads to a Known Reference…



- Call the most common base amongst the reads at each position
- Retains within-host diversity information (base frequencies and linkage)
- Number of reads gives some measure of confidence

…but Mapping Induces Bias



The more different a read is from its reference, the more likely it is to be aligned incorrectly or not at all.

↓

Systematic bias to make new sequences resemble known examples. An extremely high mutation rate makes this an acute problem for HIV.

Real data example: what the mapper should have done:

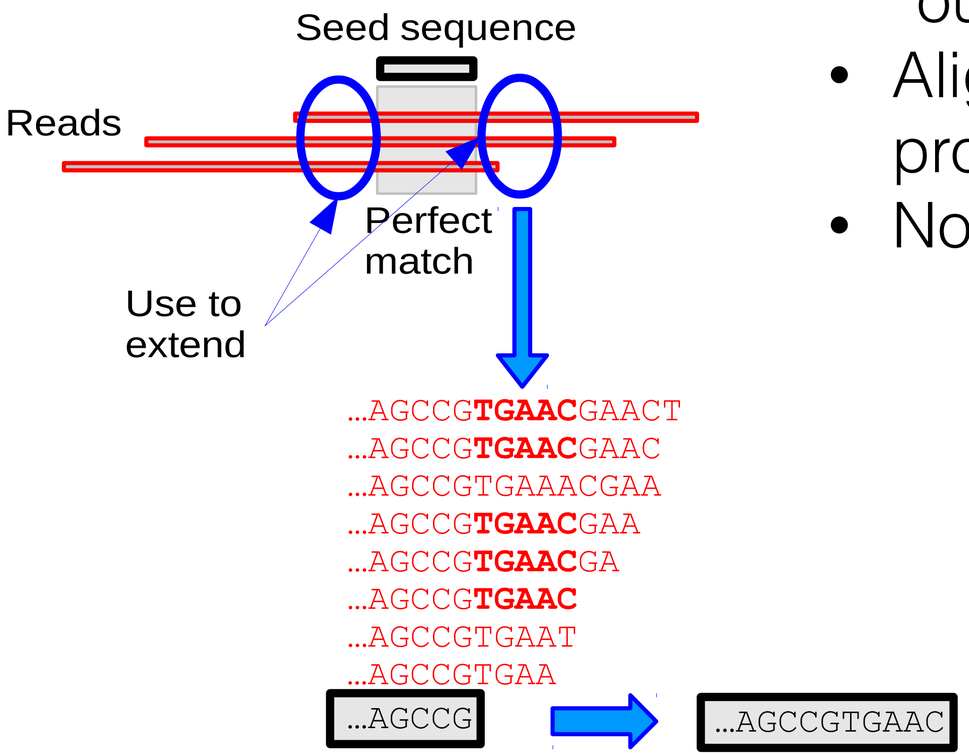
```
reference AAGTGTTAGTGTGGAAGTTTGACAGCAGCCTAGCACTTCATCACAGGGCCCGAGAGCAACATCCGGAGTTTTACAAAGACTGCTGACATC--------------------GAGTTTCCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAAGGCGTGGCCTGGGCGGG
read 1    AAGTGTTAATGTGGAAGTTTGACAGCCGCCTAGCATTCCATCACGTAGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCCTACAAAGACTGCTGACATCGAGCTTCTGCAAAGGGACTTTCCGCTGGGGACTTTCCAGG
read 2                      GACAGCCGCCTAGCATTCCATCACGTAGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCCTACAAAGACTGCTGACATCGAGCTTCTGCAAAGGGACTTTCCGCTGGGGACTTTCCAGGGGAGGCGTGGCCTGGGCGGG
```

What it did:

```
reference AAGTGTTAGTGTGGAAGTTTGACAGCAGCCTAGCACTTCATCACAGGGCCCGAGAGCAACATCCGGAGTTTTACAAAGACTGCTGACATCGAGTTTCCTACAAGGGACTTTCCGCTGGGGACTTTCCAGGGAAGGCGTGGCCTGGGCGGG
read 1    AAGTGTTAATGTGGAAGTTTGACAGCCGCCTAGCATTCCATCACGTAGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCCTACAAAGACTGCTGACATCGAGCTTCTGCAAAGGGACTTTCCGCTGGGGACTTTCCAGG
read 2    GACAGCCGCCTAGCATTCCATCACGTAGCCCGAGAGCTGCATCCGGAGTACTACAAAGACTGCTGACATCCTACAAAGACTGCTGACATCGAGCTTCTGCAAAGGGACTTTCCGCTGGGGACTTTCCAGGGGAGGCGTGGCCTGGGCGGG
```

reads clipped

De Novo Assembly

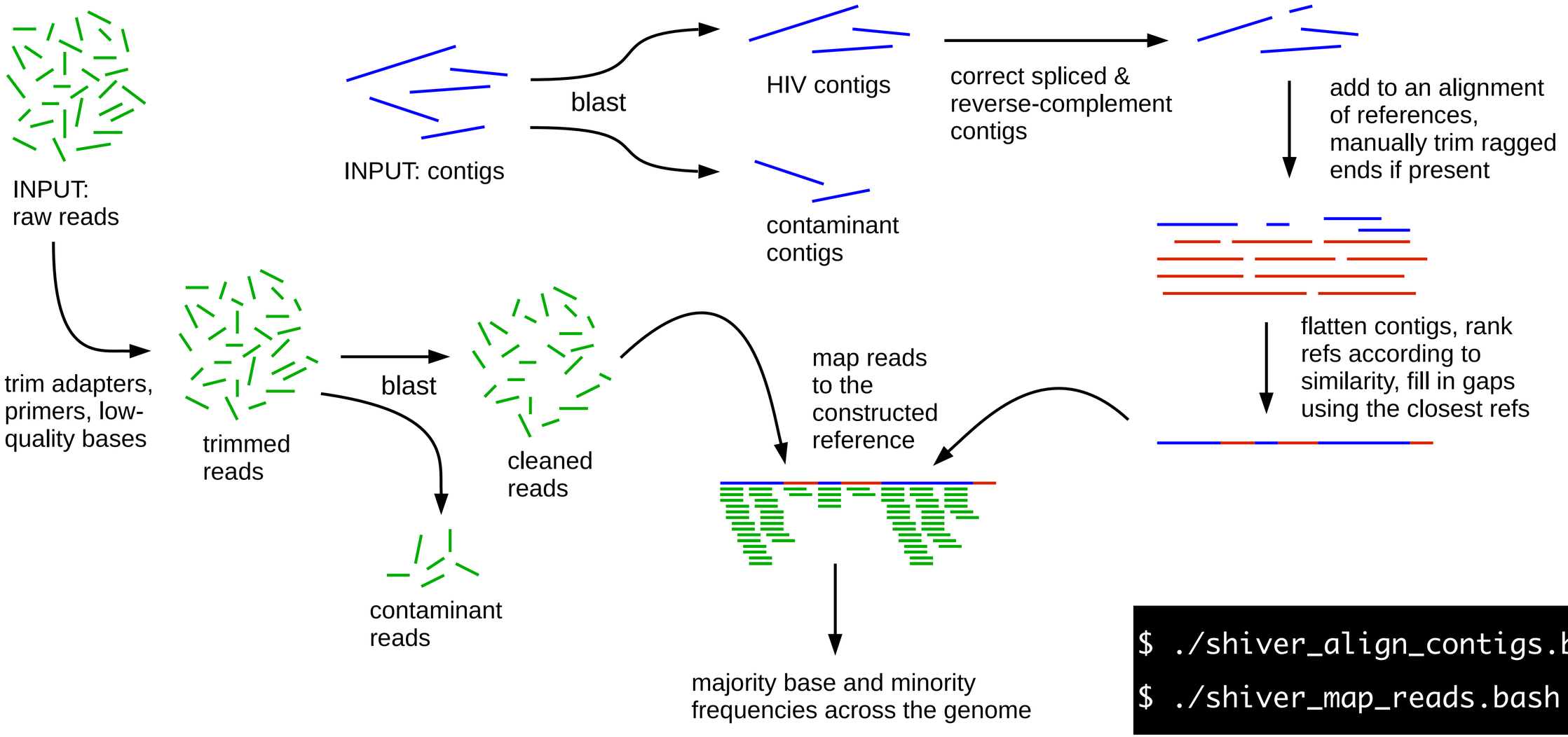


- e.g. IVA (*Hunt et al., Bioinformatics 2015*): on HIV, “outperforms all other virus de novo assemblers”.
- Align the reads to themselves, iteratively extending, producing a number of summary sequences.
- No need for a reference: no bias.

but...

- Diversity & low coverage → no contig
- Diversity & high coverage → two or more overlapping, different contigs. Which one is ‘correct’?
- No idea of base frequencies or linkage
- Occasional assembly errors: splicing separate parts of the genome, reverse complements.

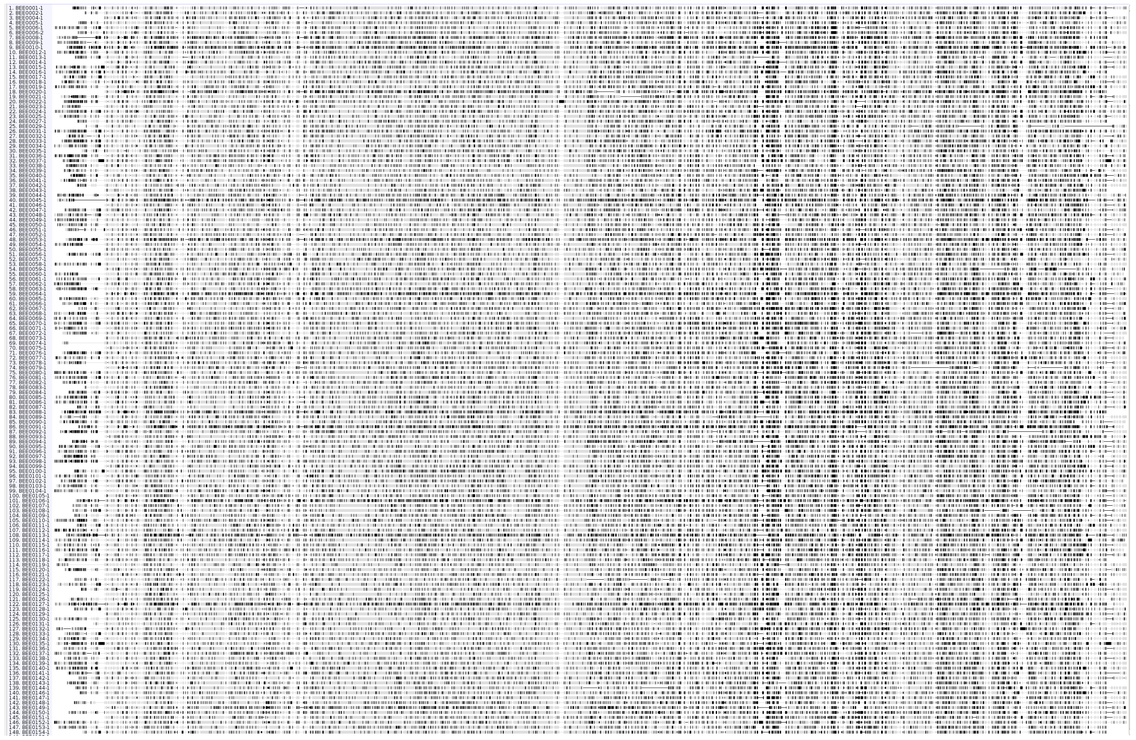
shiver - Sequences from HIV Easily Reconstructed



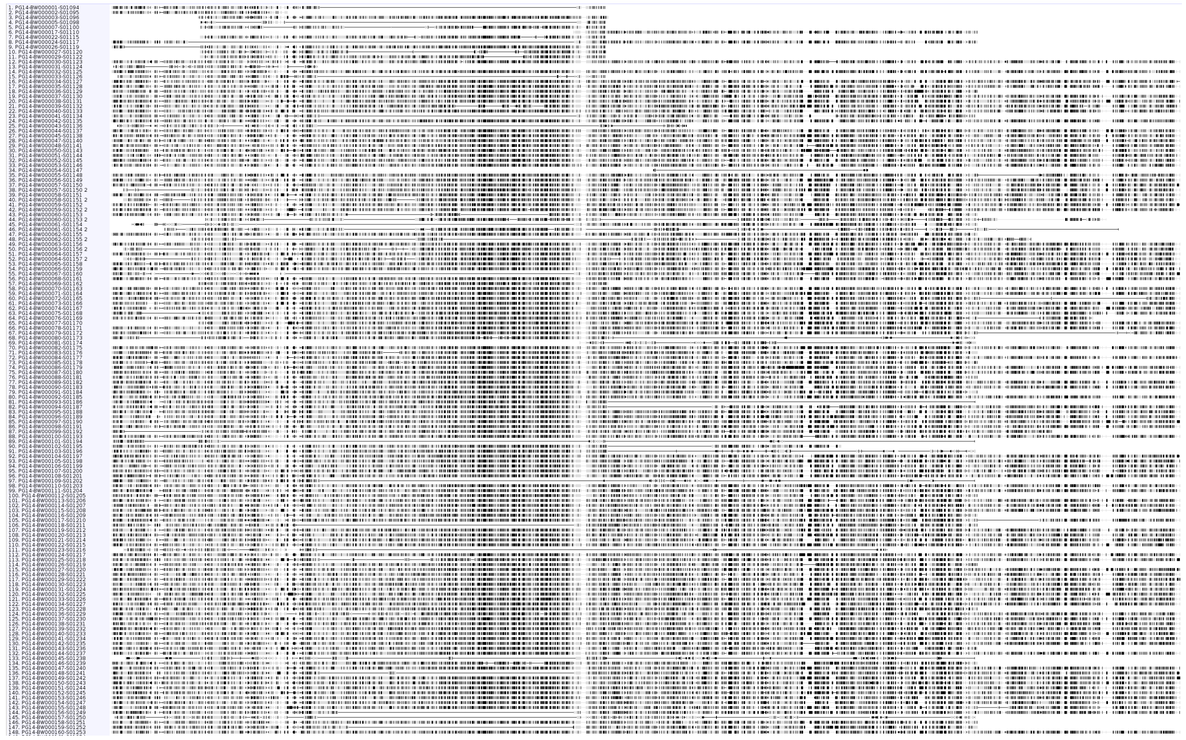

```
$ ./shiver_align_contigs.bash
$ ./shiver_map_reads.bash
```

Used to reconstruct whole* genomes for

BEEHIVE: 1700+ samples



PANGEA: 4300+ samples

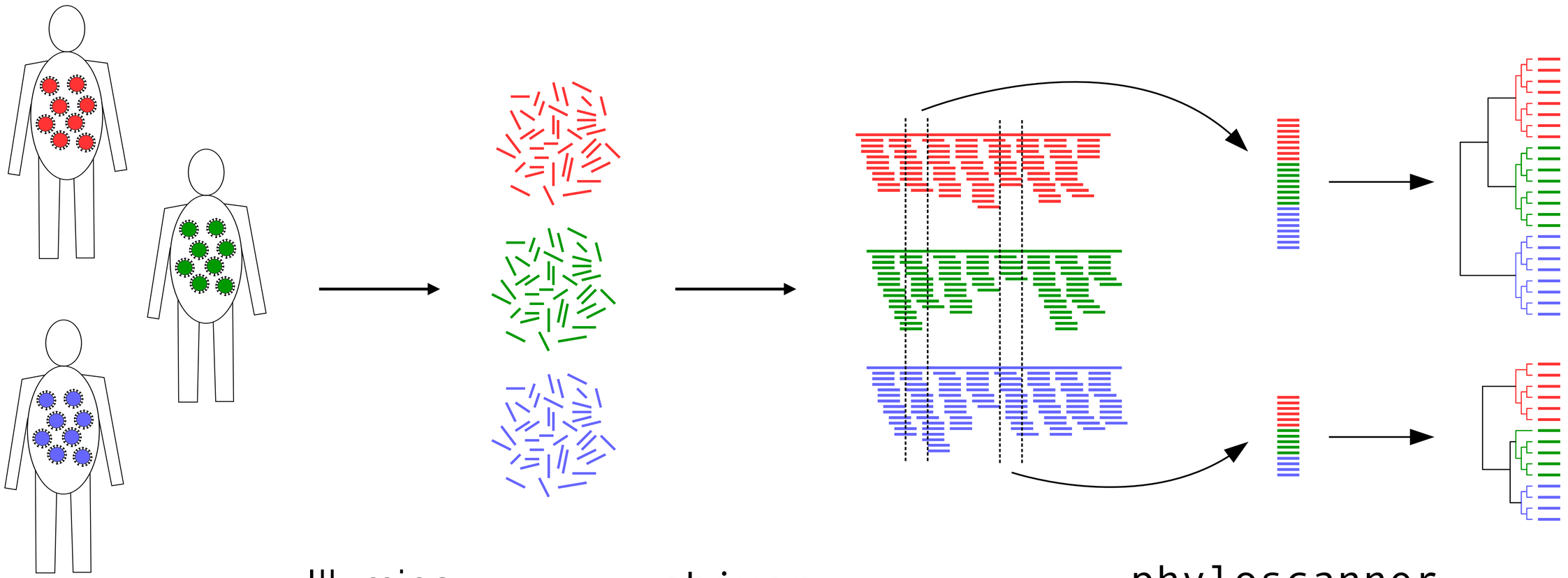


*or not, depending on the sample

A consensus sequence does not show

- **transmission** (or at least not well; important for epidemiology),
- **dual infections** (important clinically, for bioinformatics, for evolution),
- **contamination** (important to distinguish from dual infections),
- **recombination** between strains within-host.

Put within-host diversity in context: examine within- and between-host evolution at once, across the genome.



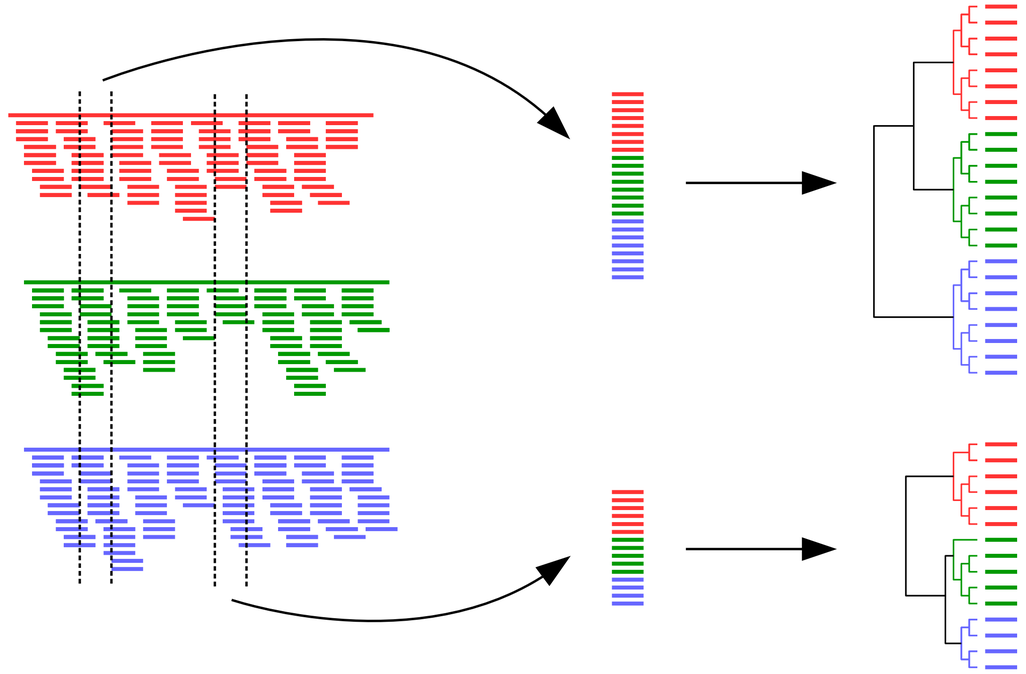
Illumina sequencing

`shiver`

(or your own pipeline)

`phyloscanner`

Easy



```
$ ./phyloscanner.py  MyBamFiles.txt  MyRefFiles.txt  --windows 1,300,200,500,...
```

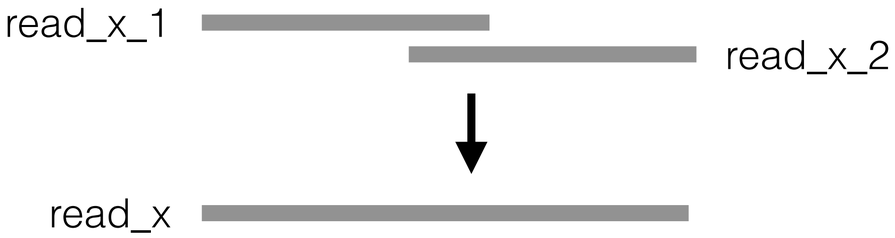
```
red.bam
green.bam
blue.bam
```

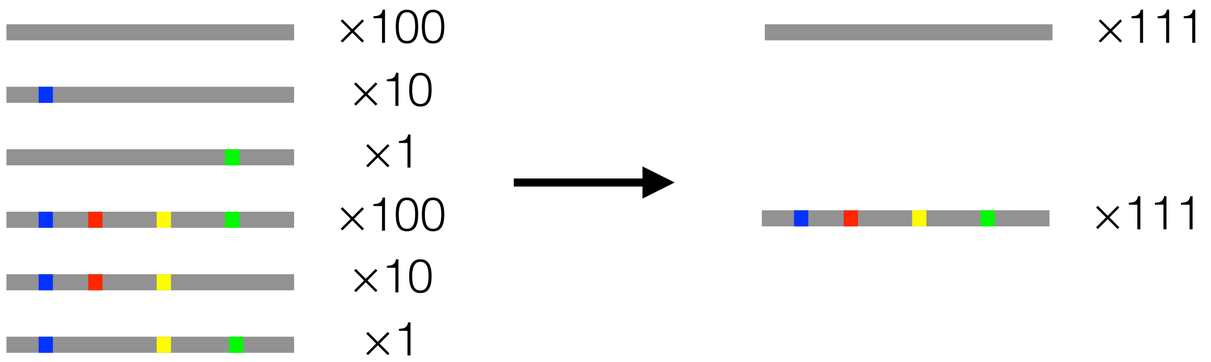
```
redRef.fasta
greenRef.fasta
blueRef.fasta
```

Genomic coordinates of the windows to be analysed; here, 1-300, 200-500, ...

Feature-packed 1

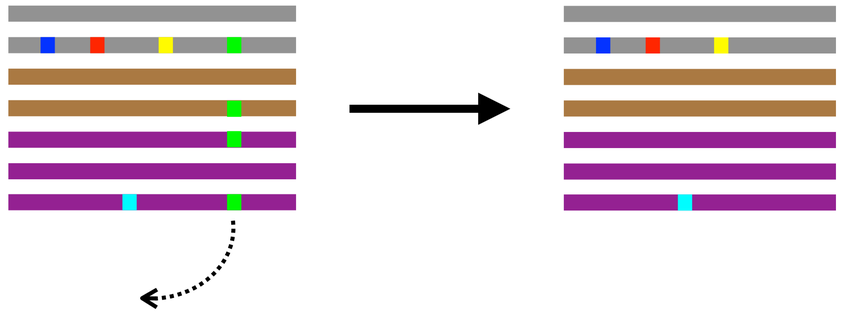
Merge overlapping paired reads into longer reads.





Similarity- and frequency-based read merging, for speed.

Excise specified positions (e.g. sites under selection).

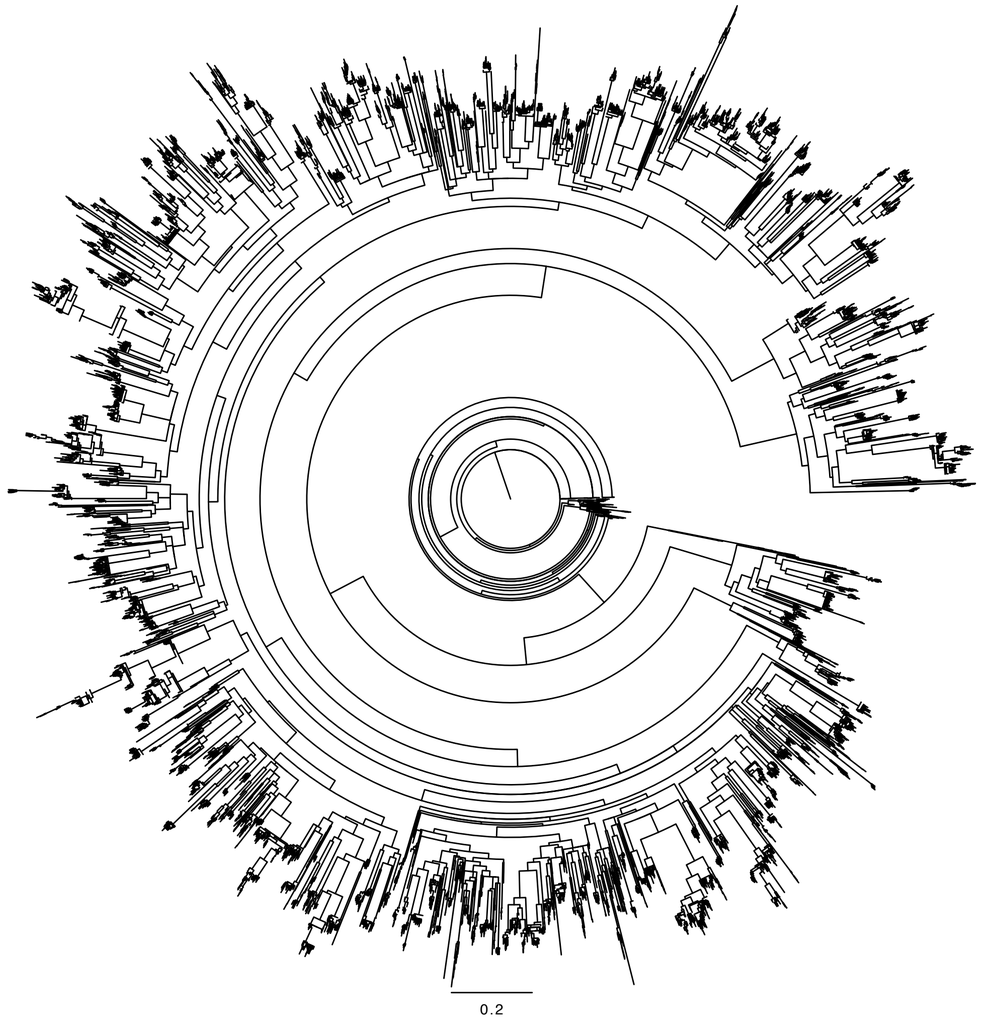


Feature-packed 2

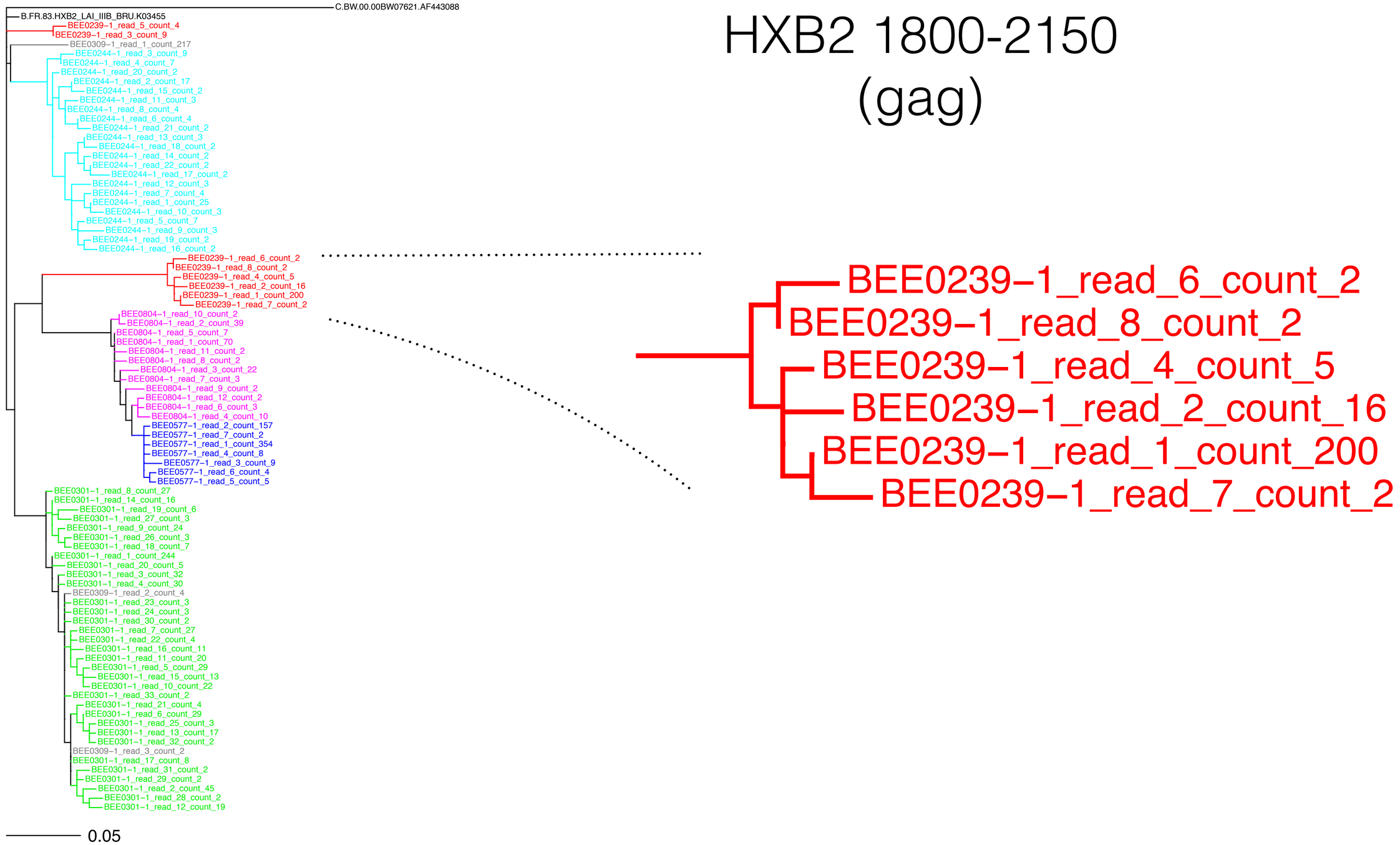
- Include known references with the reads.
- Trim and/or discard low-quality reads.
- Minimum read count.
- Check every read for duplication in any other patient to find contamination. Remove duplicates depending on counts.
- Bootstraps.

Trivially parallelisable: split the whole genome into windows, run each window as a separate job on your cluster.

<1 day for O(1000) patients with coverage O(10,000) (`RAxML` the bottleneck).



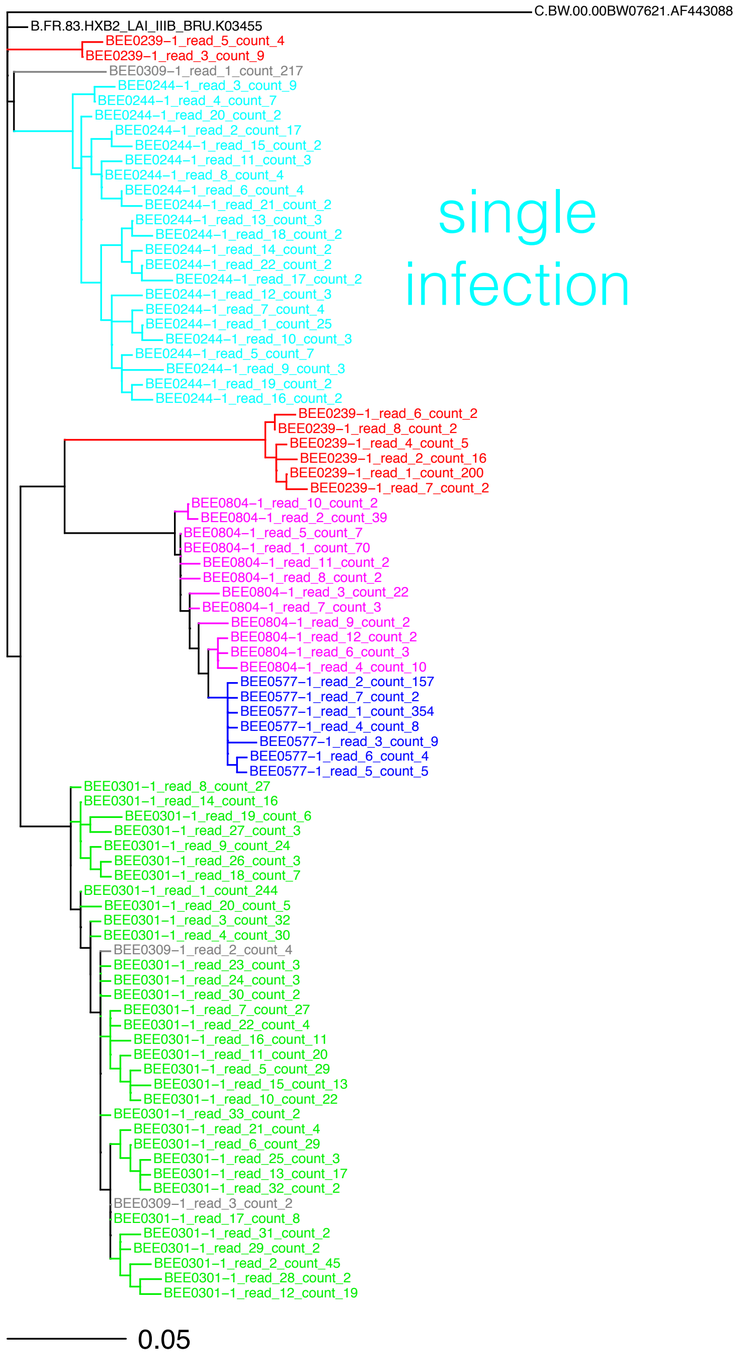
375bp at the end of the gp120 gene. 700 patients, 8574 distinct reads.
(merging threshold = 1bp, minimum read count = 2)



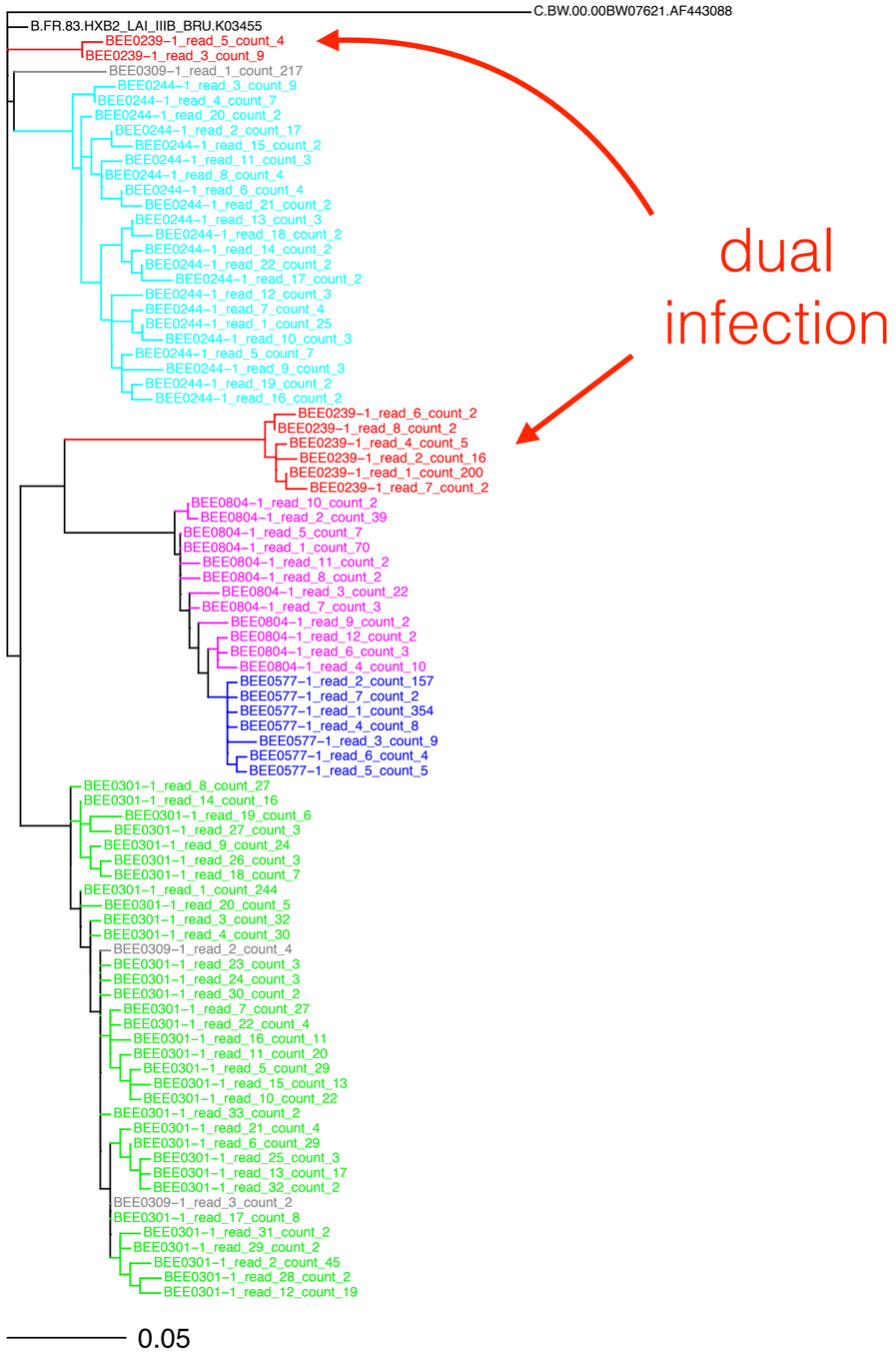
HXB2 1800-2150 (gag)

BEE0239−1_read_6_count_2
BEE0239−1_read_8_count_2
BEE0239−1_read_4_count_5
BEE0239−1_read_2_count_16
BEE0239−1_read_1_count_200
BEE0239−1_read_7_count_2

HXB2 1800-2150 (gag)



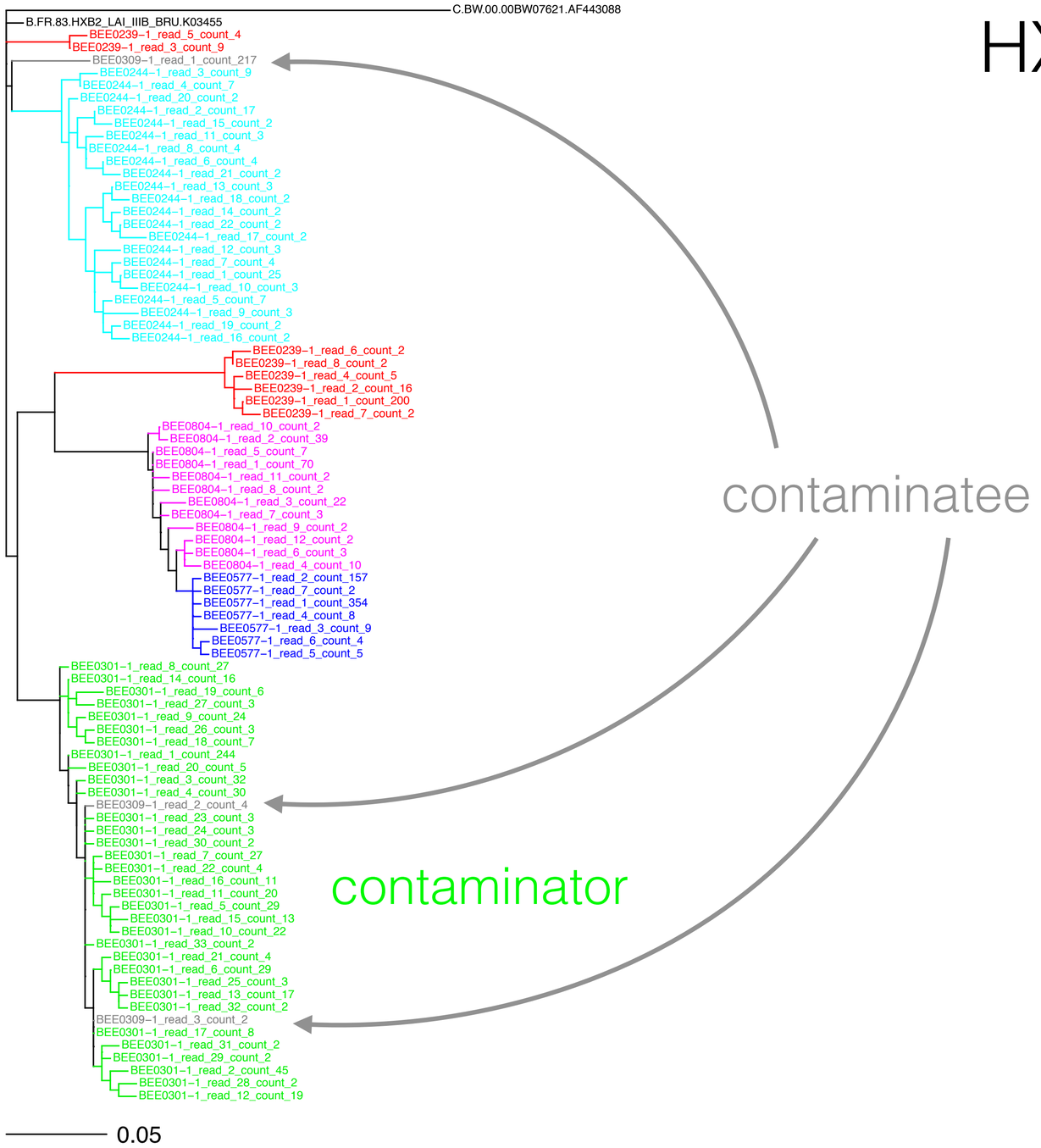
single infection



dual infection

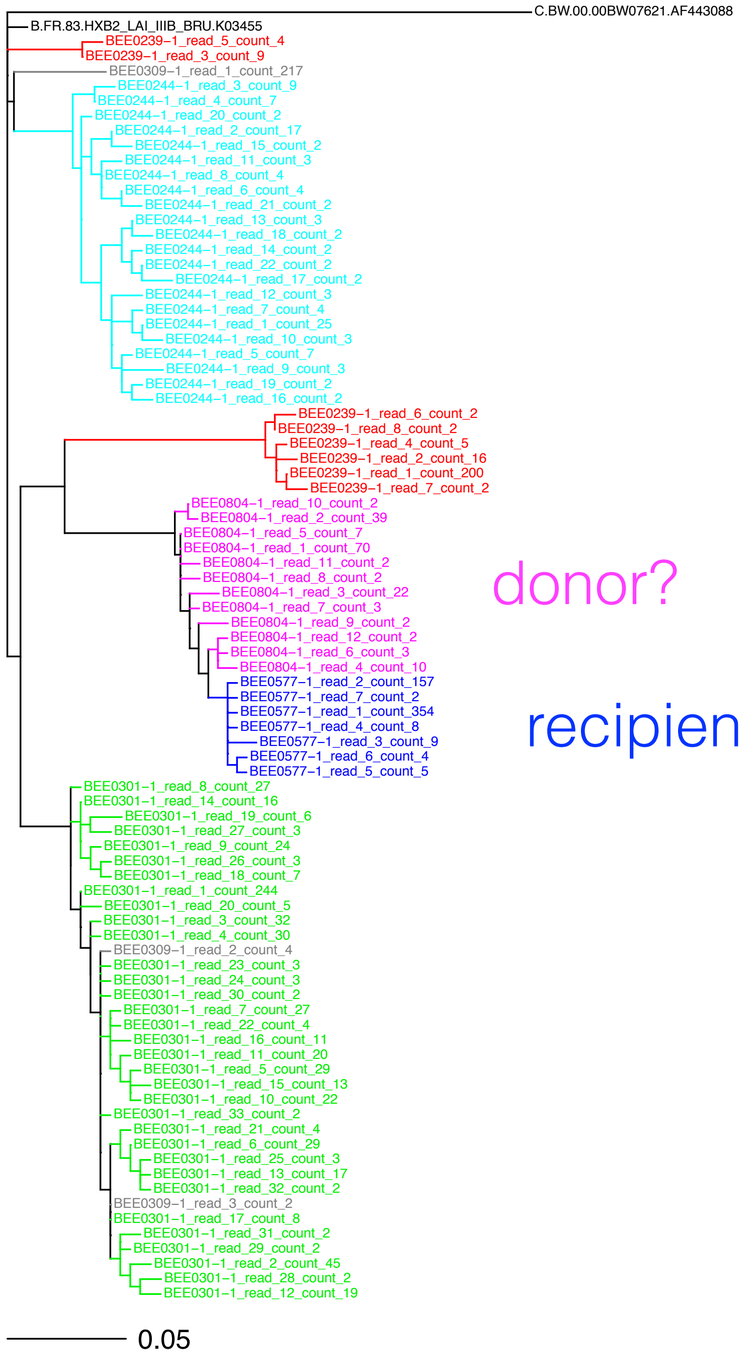
HXB2 1800-2150 (gag)

HXB2 1800-2150 (gag)



contaminatee

contaminator



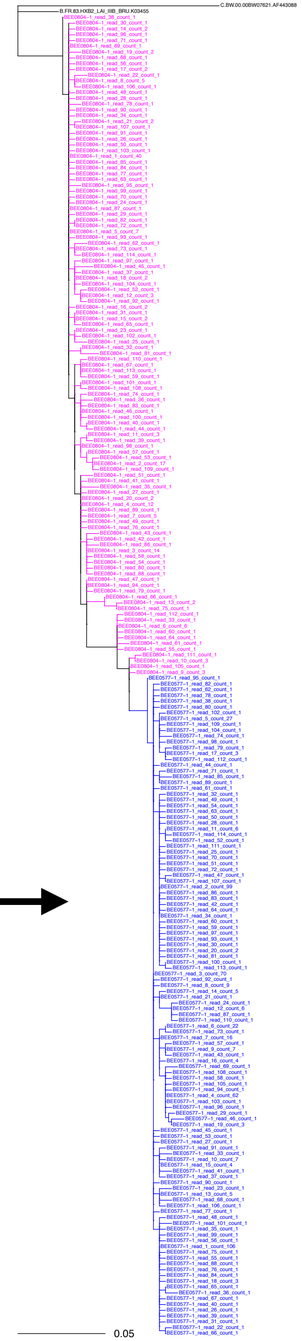
donor?

recipient?

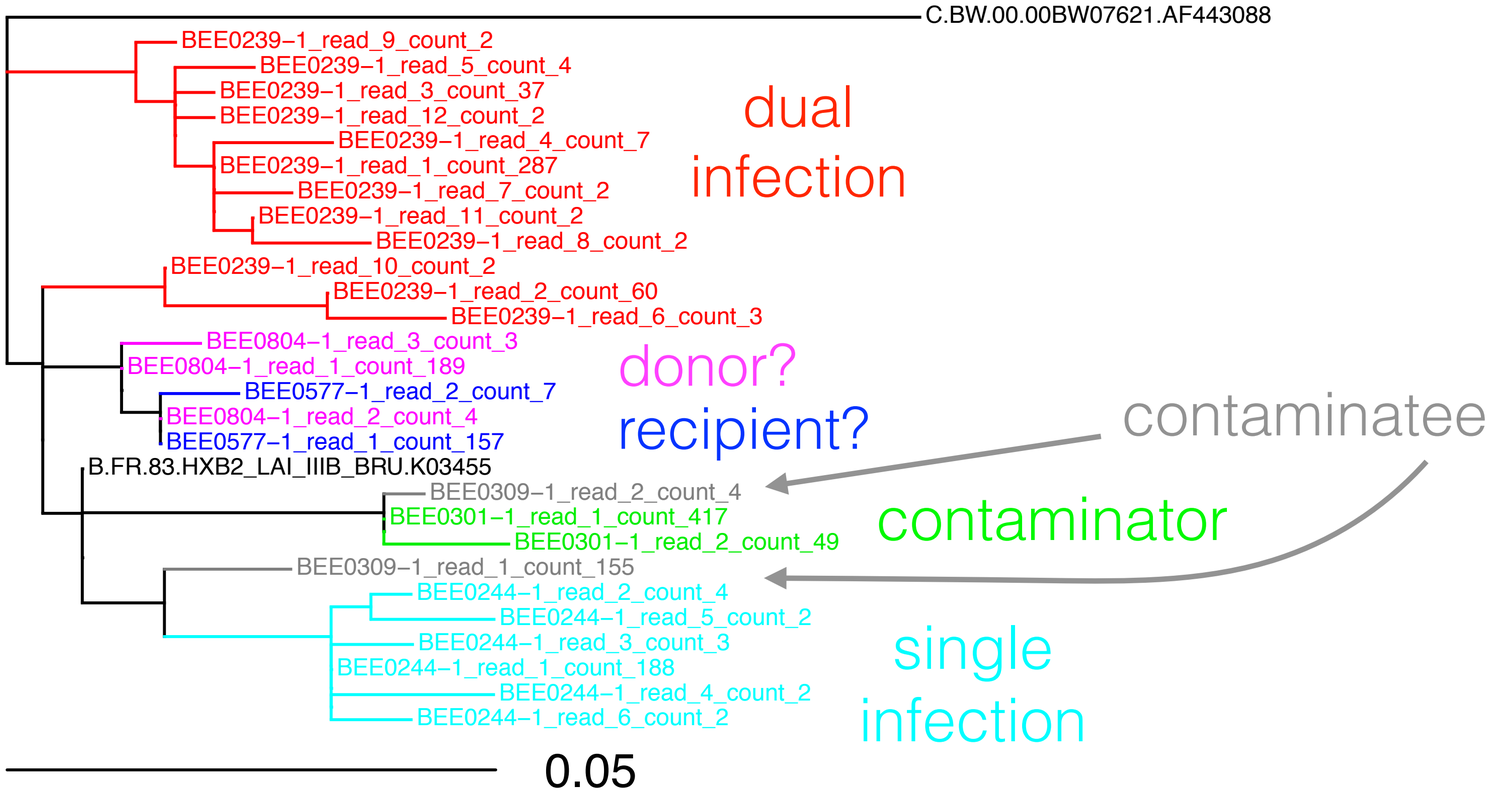
HXB2 1800-2150 (gag)

Left tree: 1bp merging, minimum read count = 2.

Re-run with just the transmission pair, no merging and no minimum read count for a clearer picture



HXB2 2800-3150 (pol)



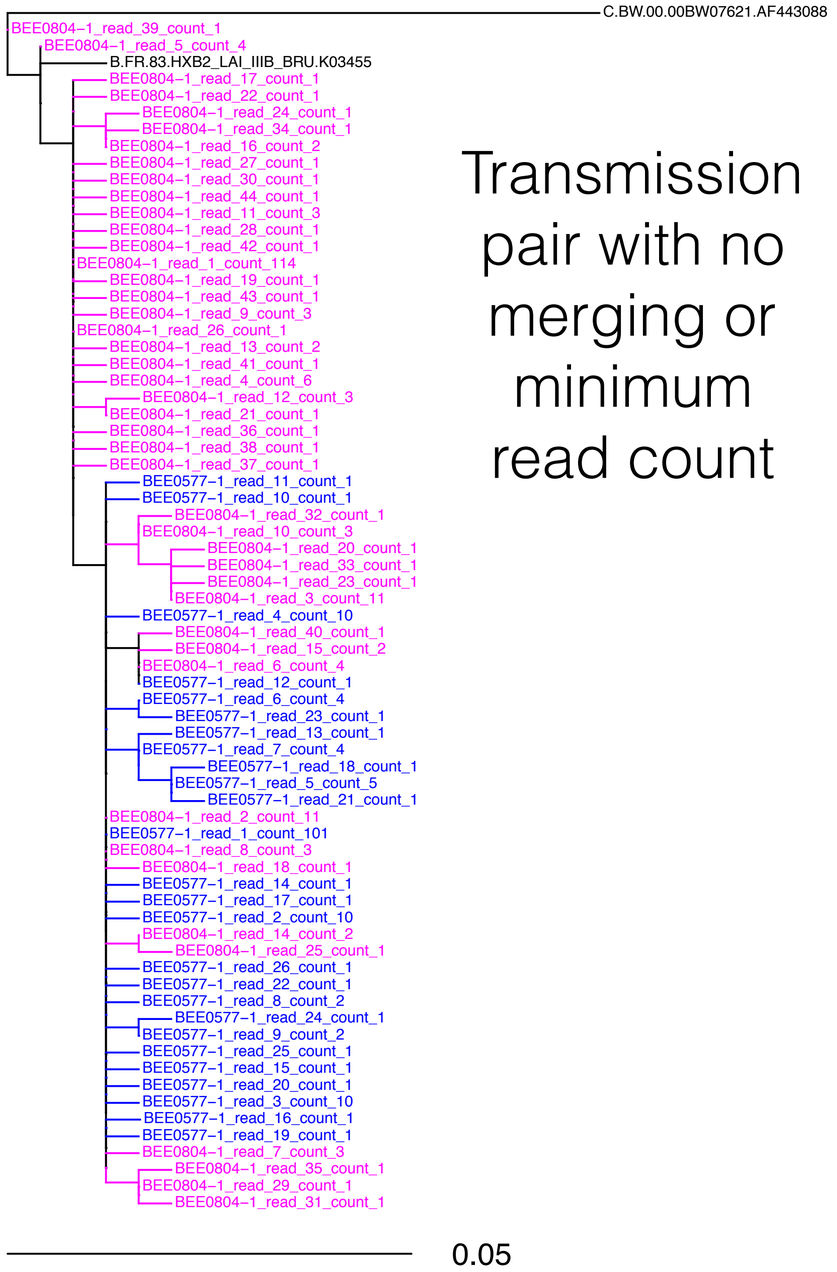
dual infection

donor? recipient?

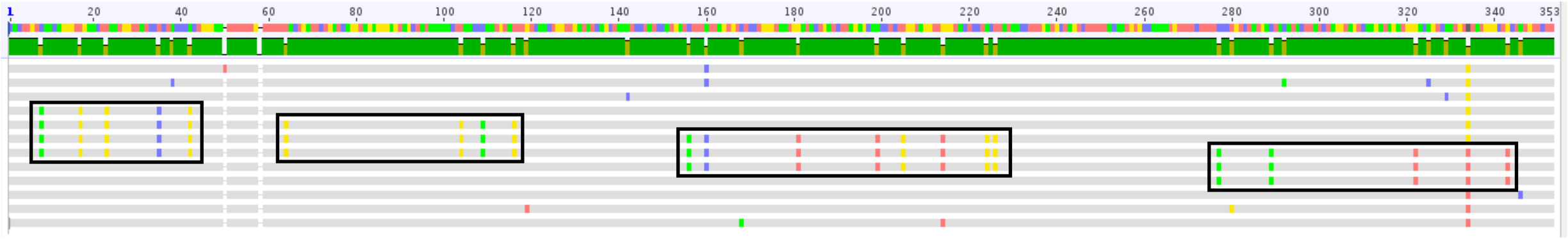
contaminatee

contaminator

single infection

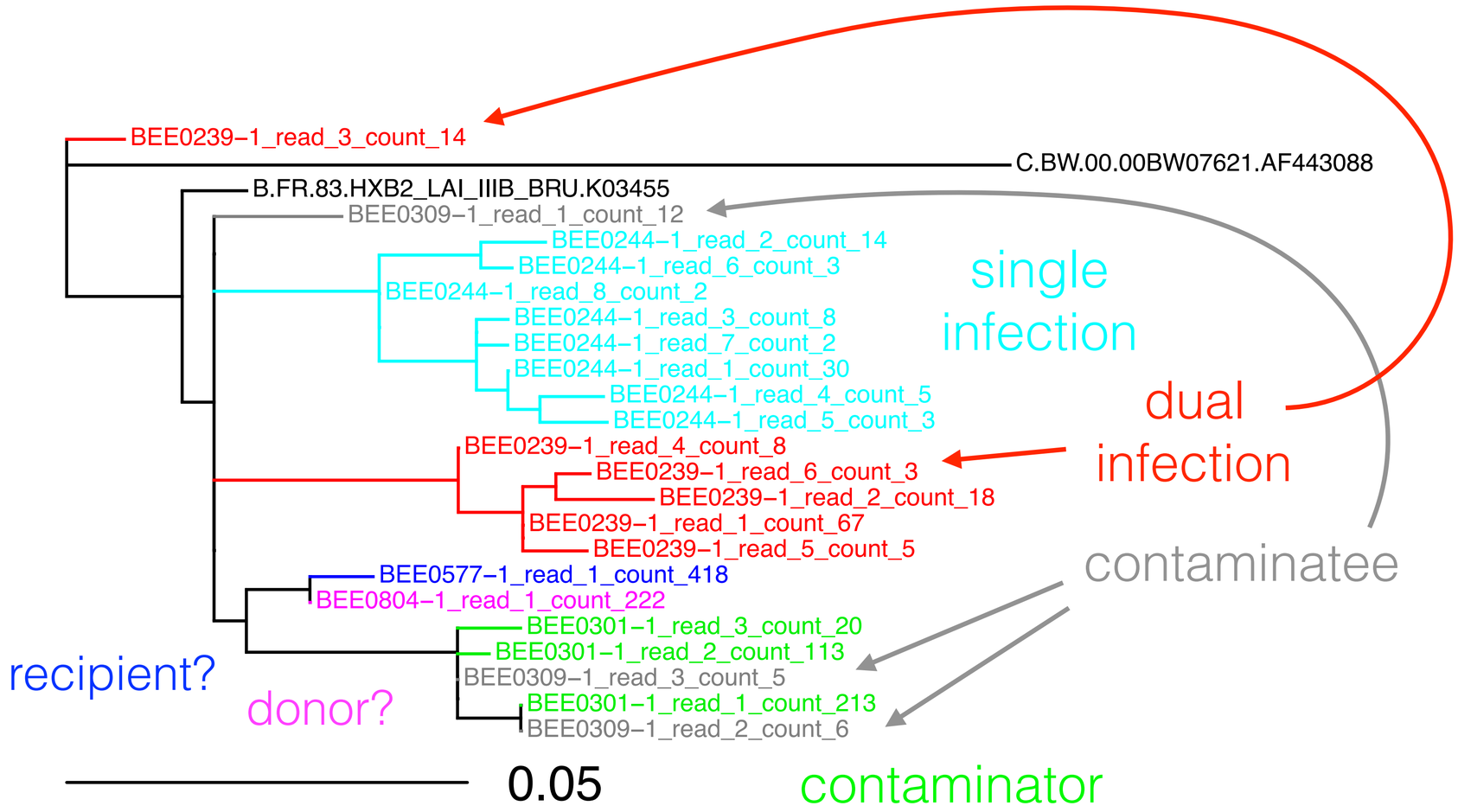


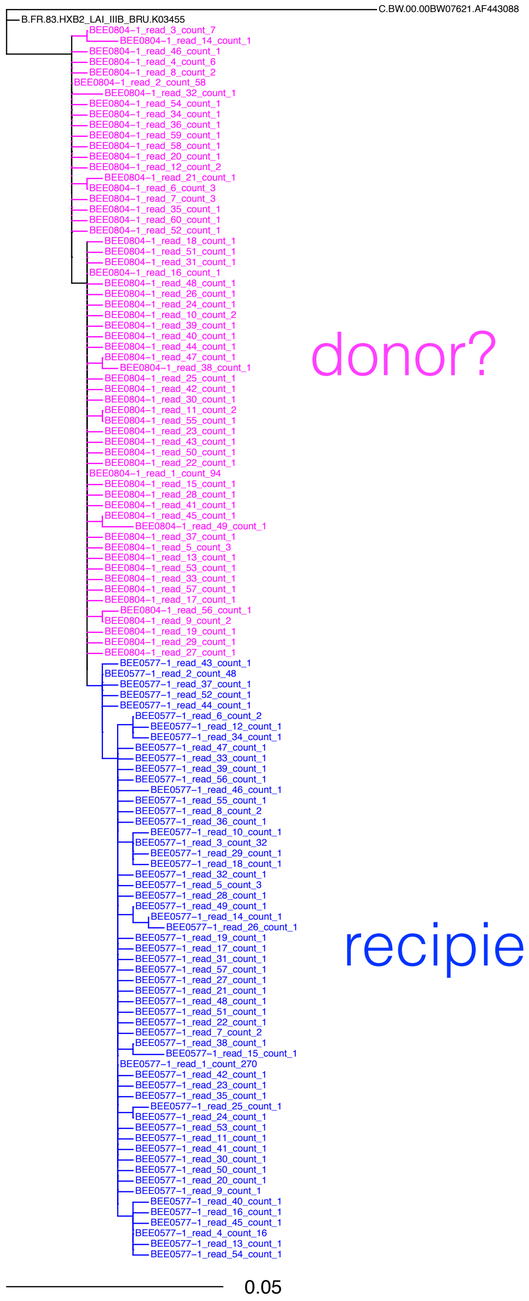
Transmission pair with no merging or minimum read count

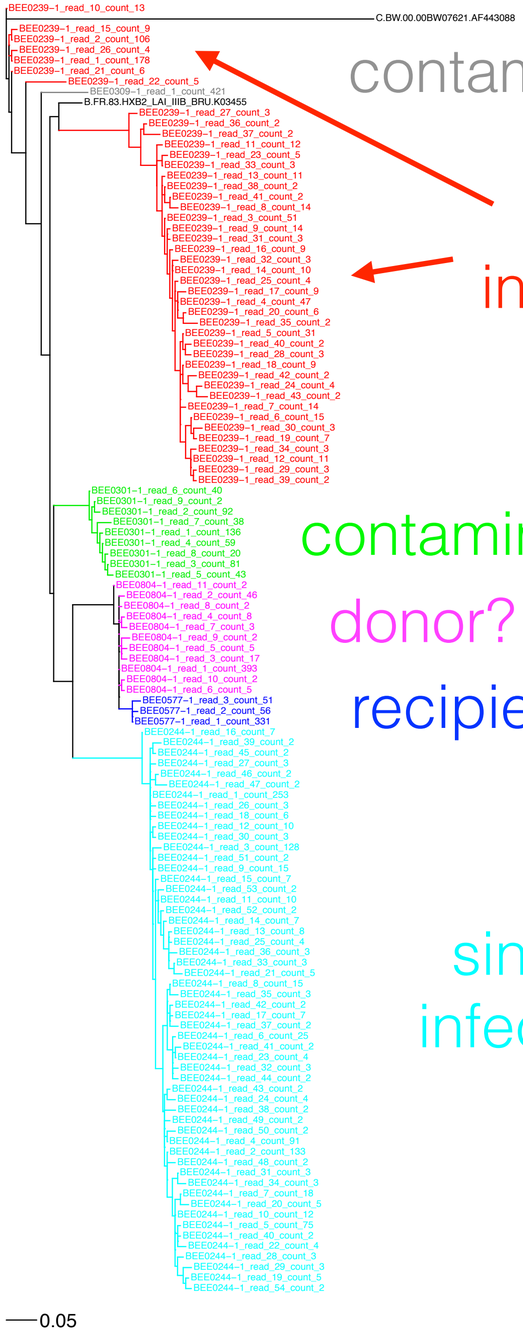


dual infection, point of recombination

HXB2 4800-5150 (pol & vif)







contaminatee (not here)

dual infection

contaminator (not here)

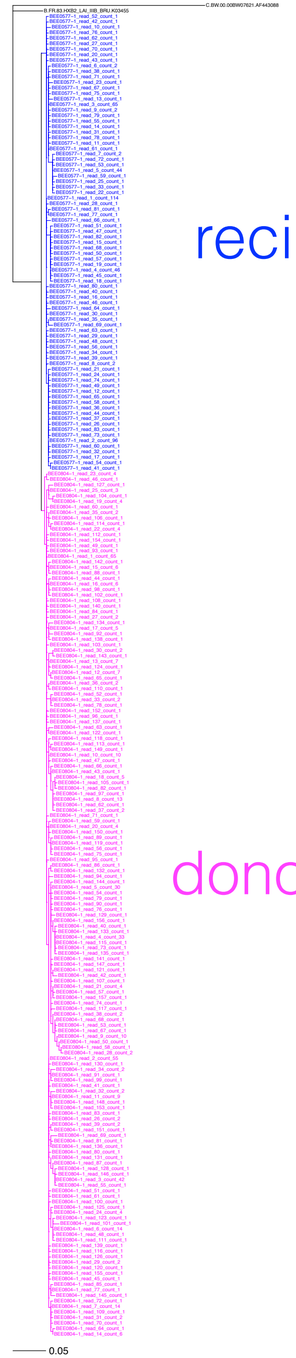
donor?

recipient?

single infection

HXB2 6050-6400 (vpu)

All trees excise the DRMs of *Johnson et al., Top. Anti. Med. 2011* and *Gatanaga et al., J. Biol. Chem. 2002* and the CTL-escape sites of *Carlson et al., J. Virology 2012*

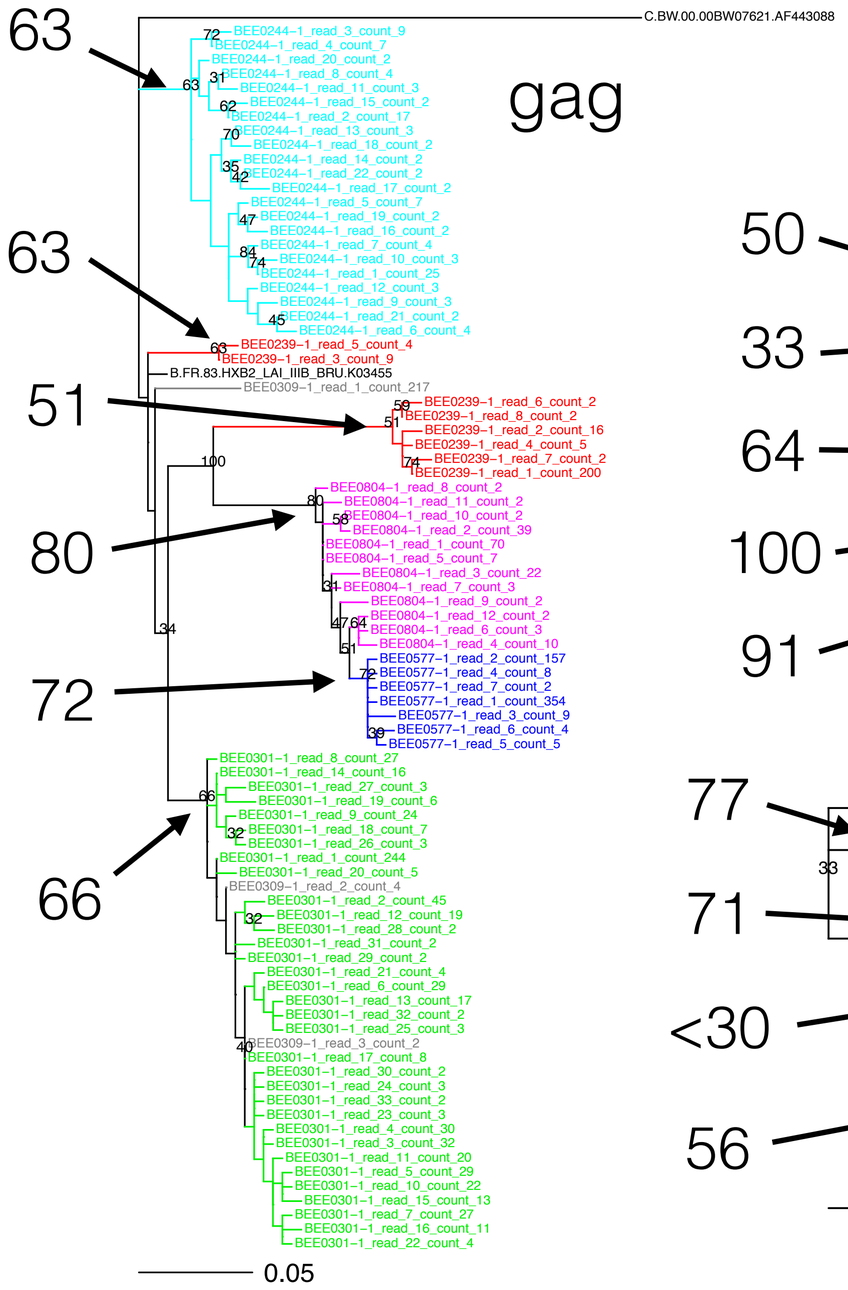


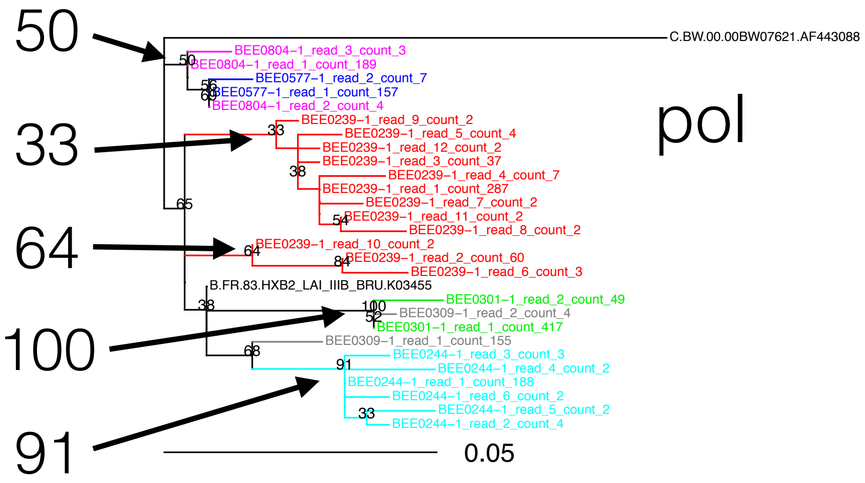
recipient?

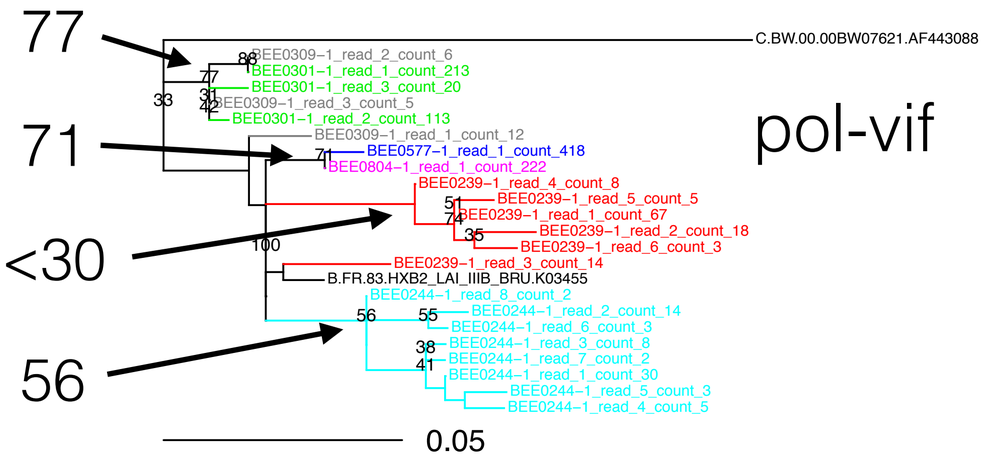
donor?

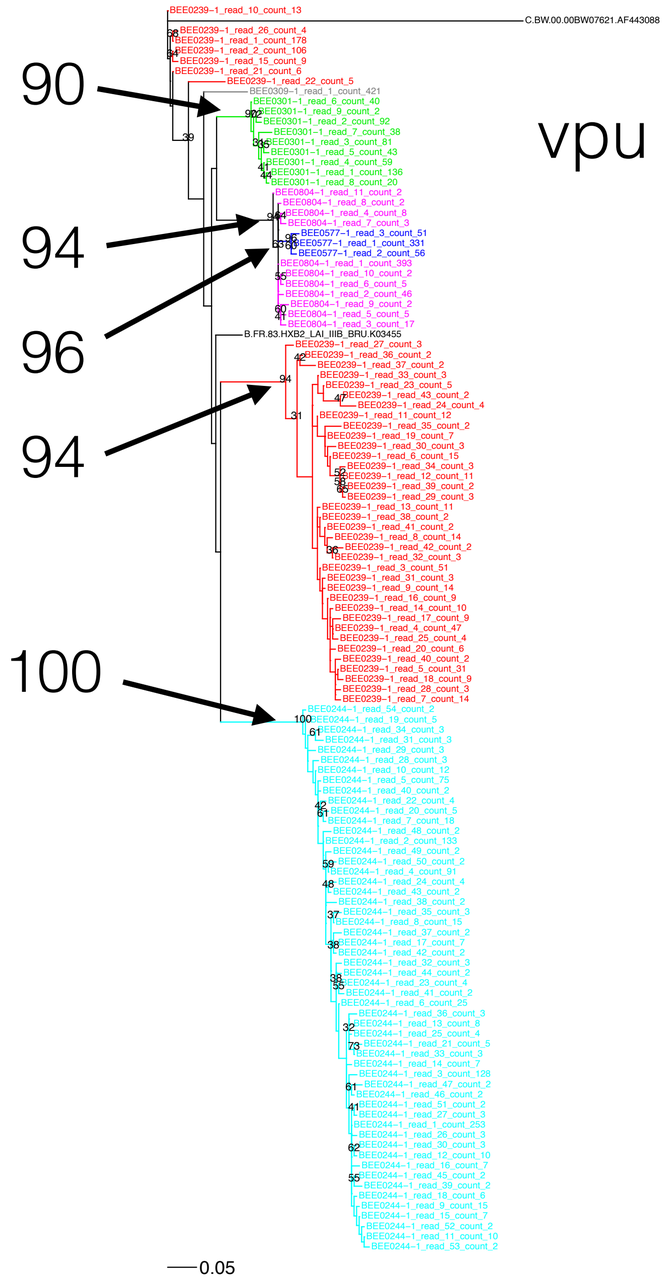
bootstraps (%)

values > 30 shown on nodes

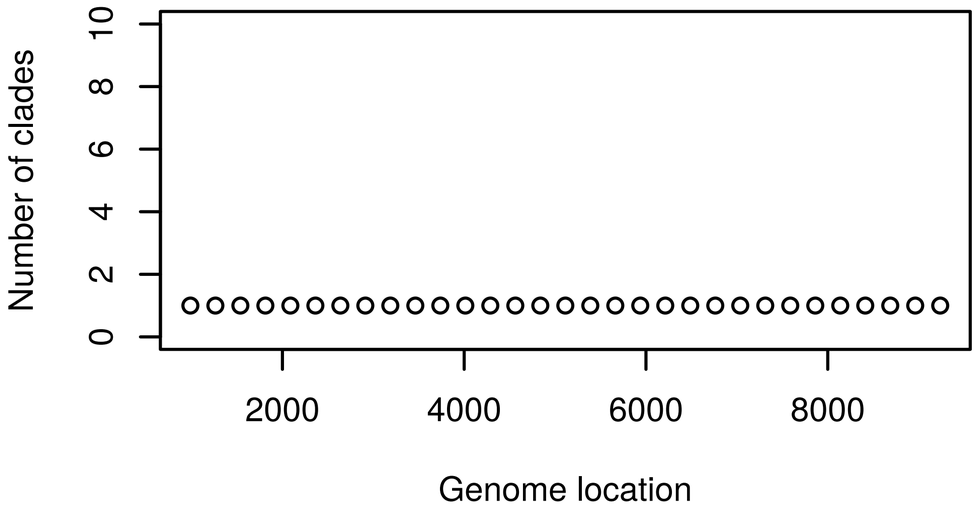






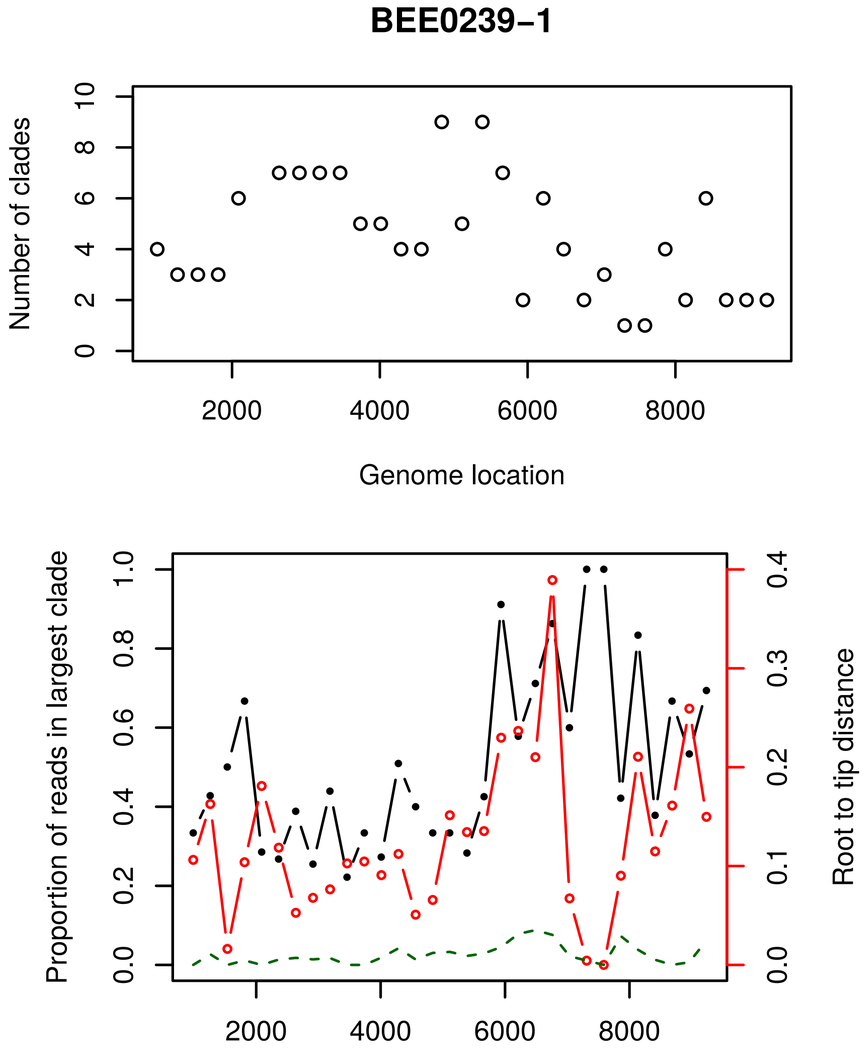


Per-patient summaries over the genome



single infection

clades = 1 throughout

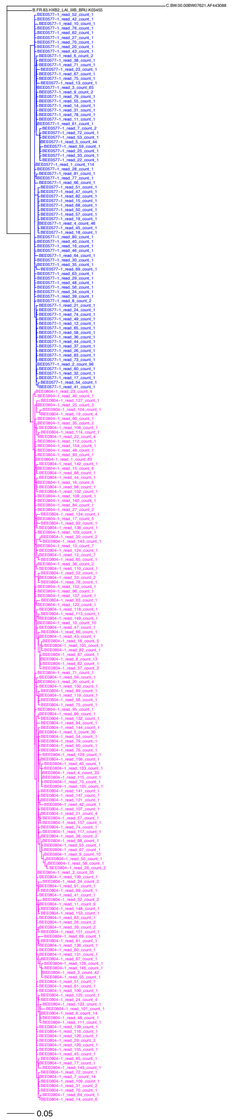


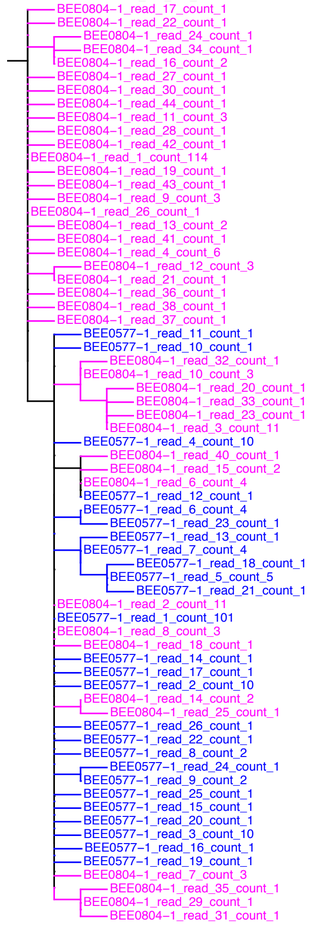
dual infection

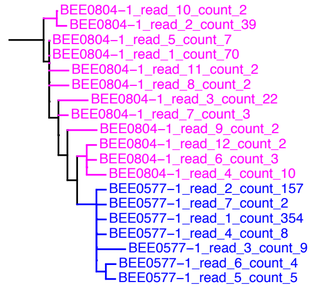
>1 clade, large root-tip, large proportion of reads outside largest clade

Two patients' reads can be...

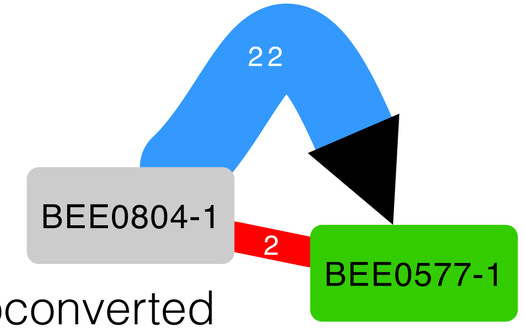
separated, entangled, or clearly directed.







Count these over all windows (31 here):



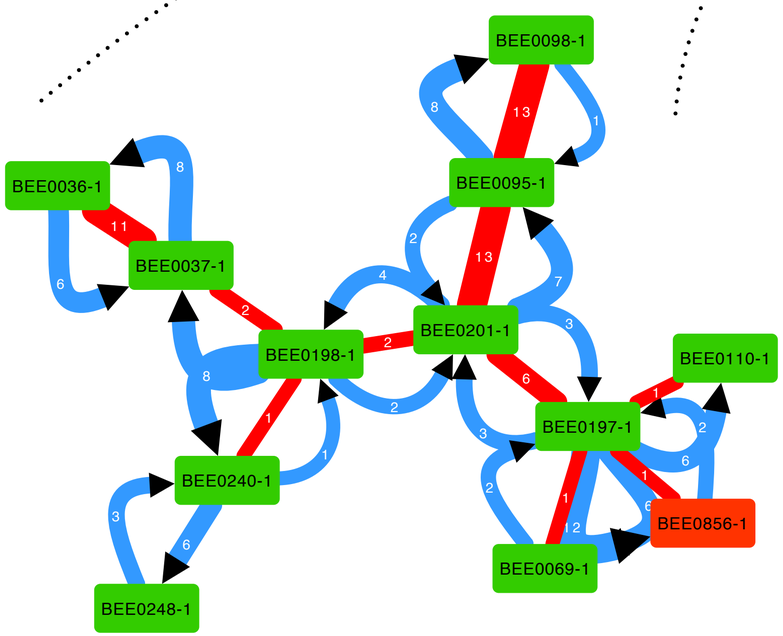
seroconverted 2007, Amsterdam

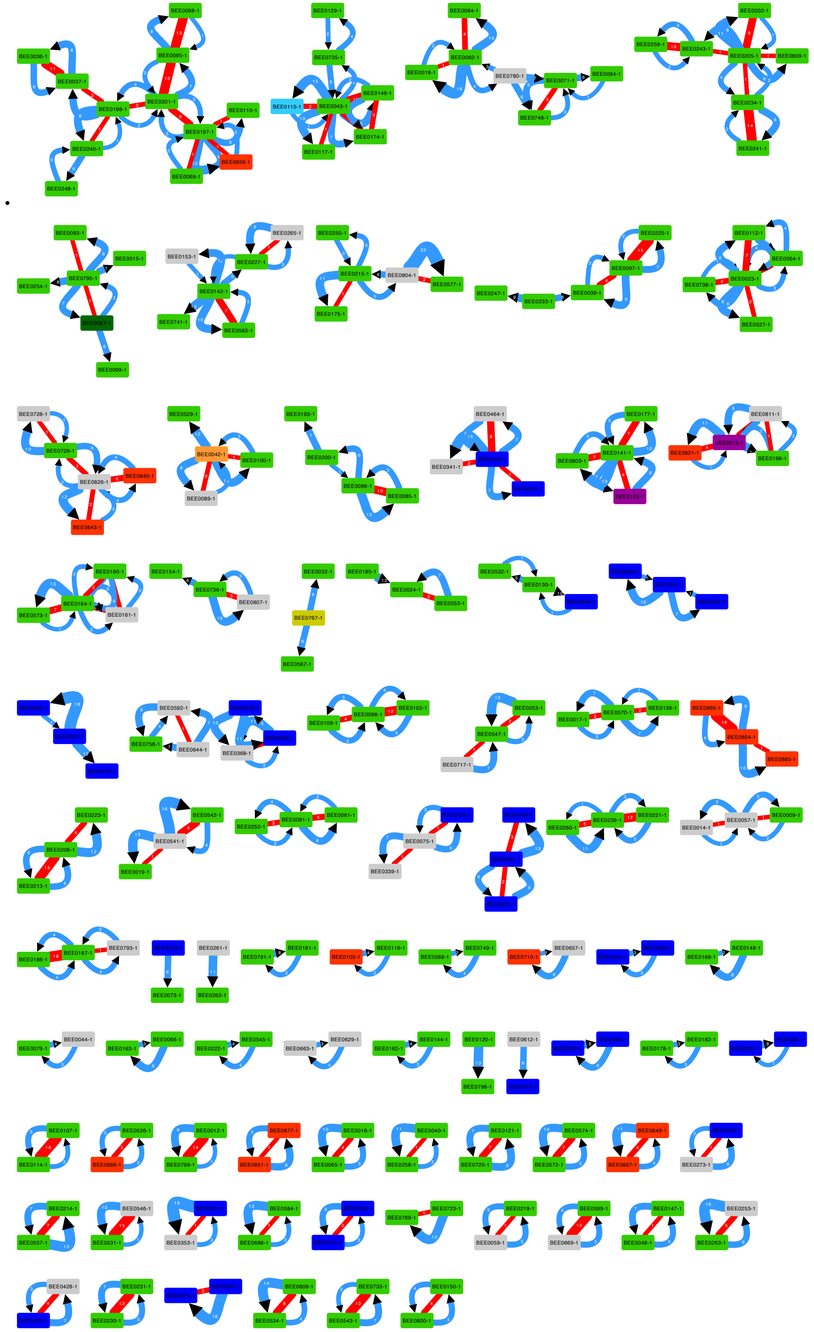
seroconverted 2010, Amsterdam

Matthew Hall

See BEAST2 package *BEASTLIER* (github: *twoseventwo*)

c.f. Ypma et al., *Genetics* 2013;
Didelot et al., *Mol. Biol. Evol.* 2014;
Hall et al. *PLoS Comp. Biol.* 2015:
Kenah et al., arxiv 2016.
Romero-Severson et al. 2016

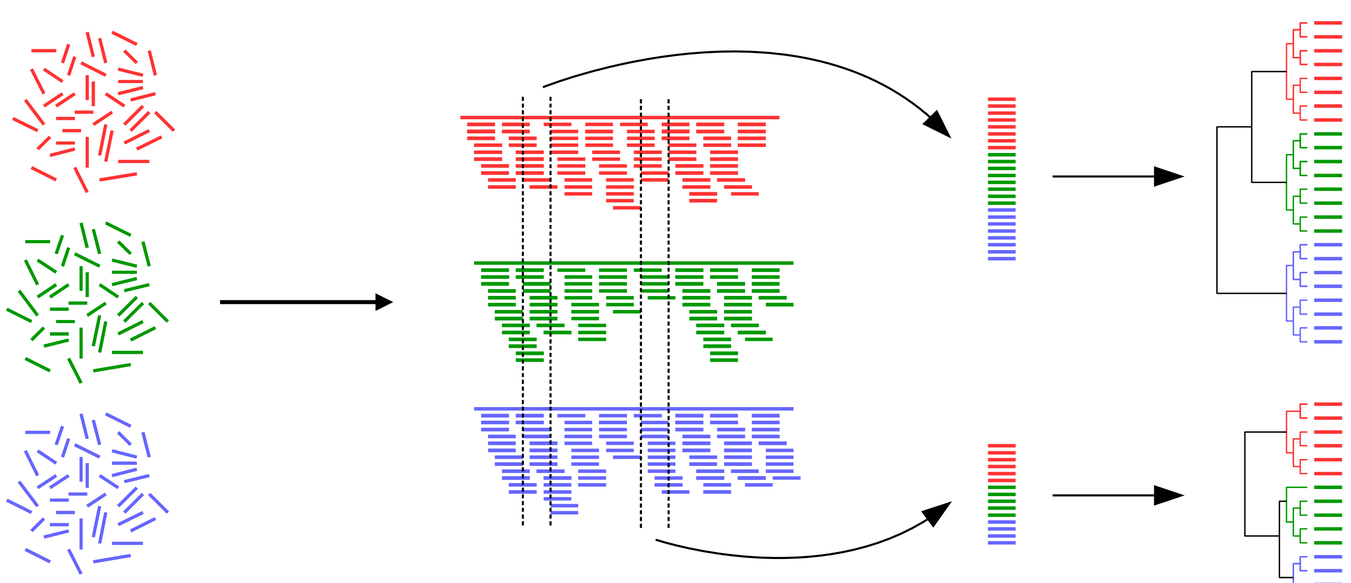




Node Fill Color	LONG_COUNTRY_INF
	Belgium
	Germany
	Morocco
	Netherlands
	Portugal
	Spain
	United Kingdom
	United States
	Unknown

Edge Stroke Color (Unselected)	interaction
	int
	trans

Summary



`shiver` `phyloscanner`

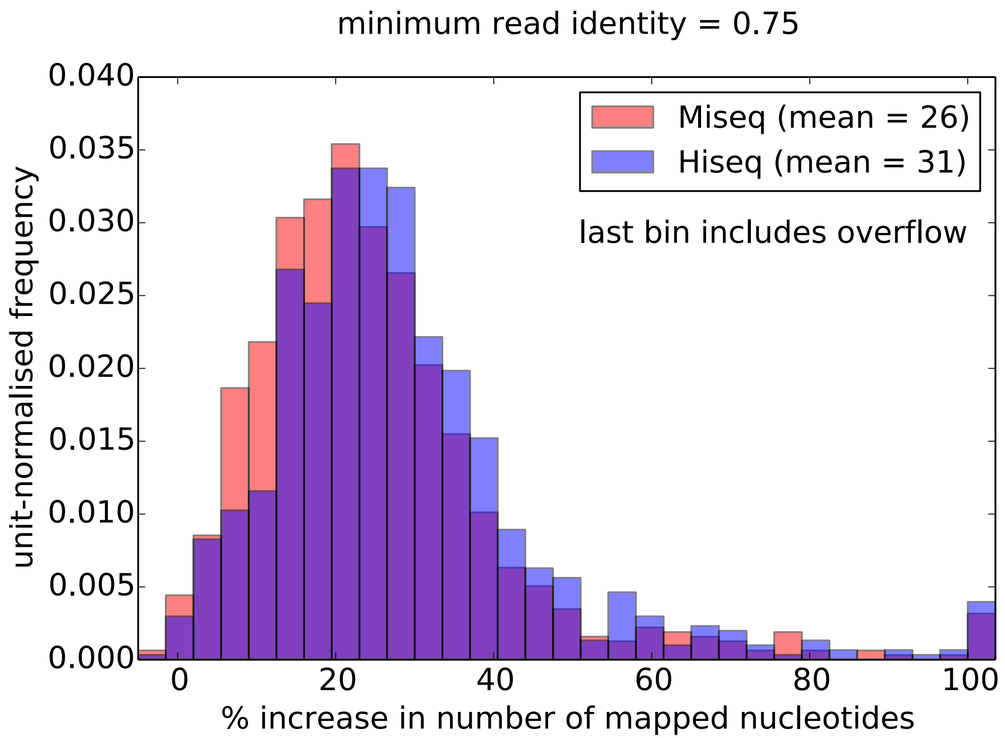
Analysis of phylogenetics between and within, along the genome, shows:

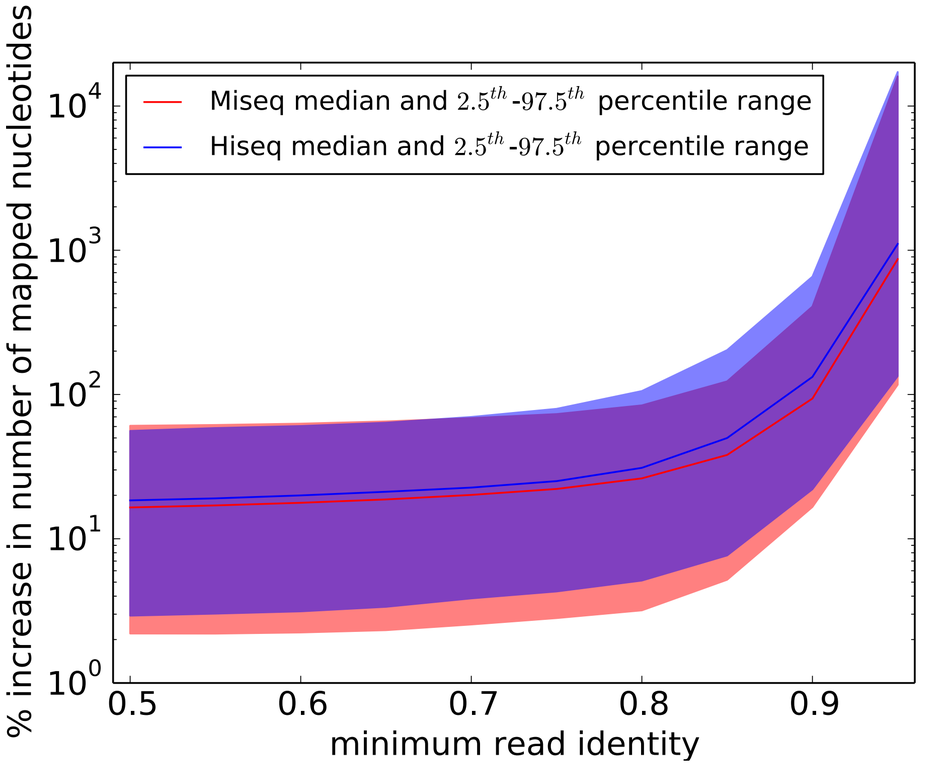
- **transmission**: one quasispecies as the descendant of another
- **dual infections**: two distinct quasispecies
- **recombination**: intermediates between two quasispecies
- **contamination**: repeated exact duplication of reads

Extra slides

Quantifying, not just arguing,
why you should use `shiver`

shiver maps more nucleotides





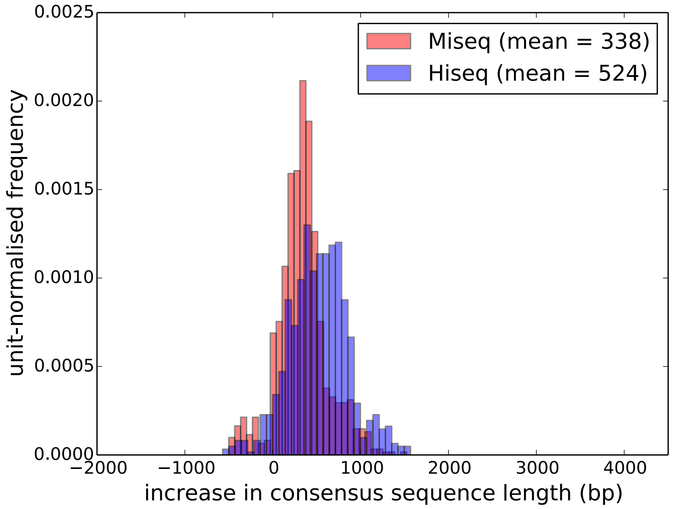
Map 904 Miseq and 864 Hiseq samples first to the closest of 160 existing references (chosen with `Kraken`, often HXB2) then to the custom reference constructed by `shiver`. Compare the number of nucleotides mapped. Repeat for different thresholds on read identity - the fraction of a read that agrees with the reference.

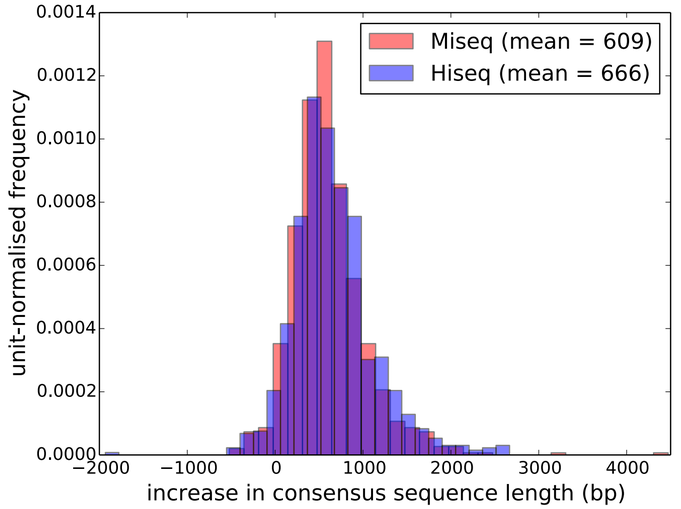
`shiver` reconstructs more of the genome

lenient on read identity (>50%)

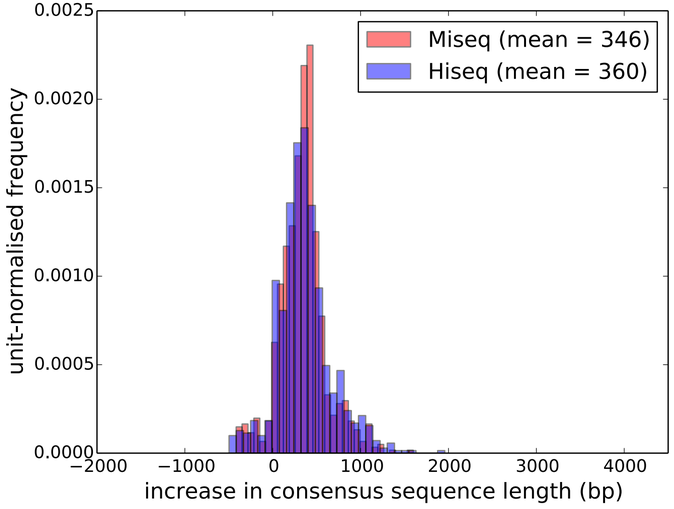
strict on read identity (>85%)

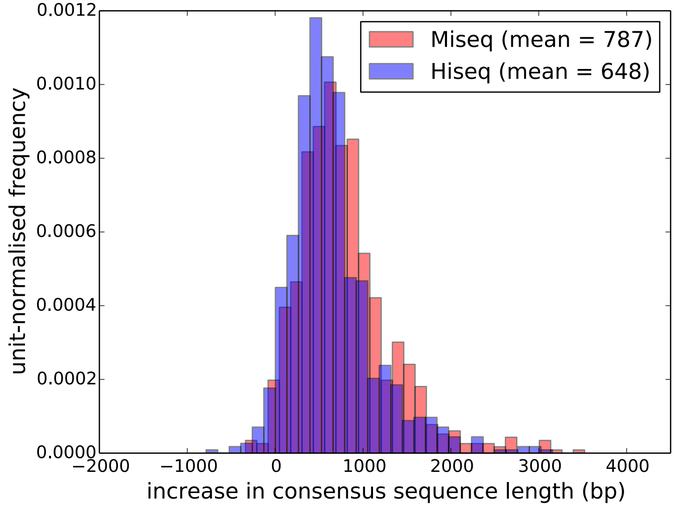
lenient on coverage (>10)



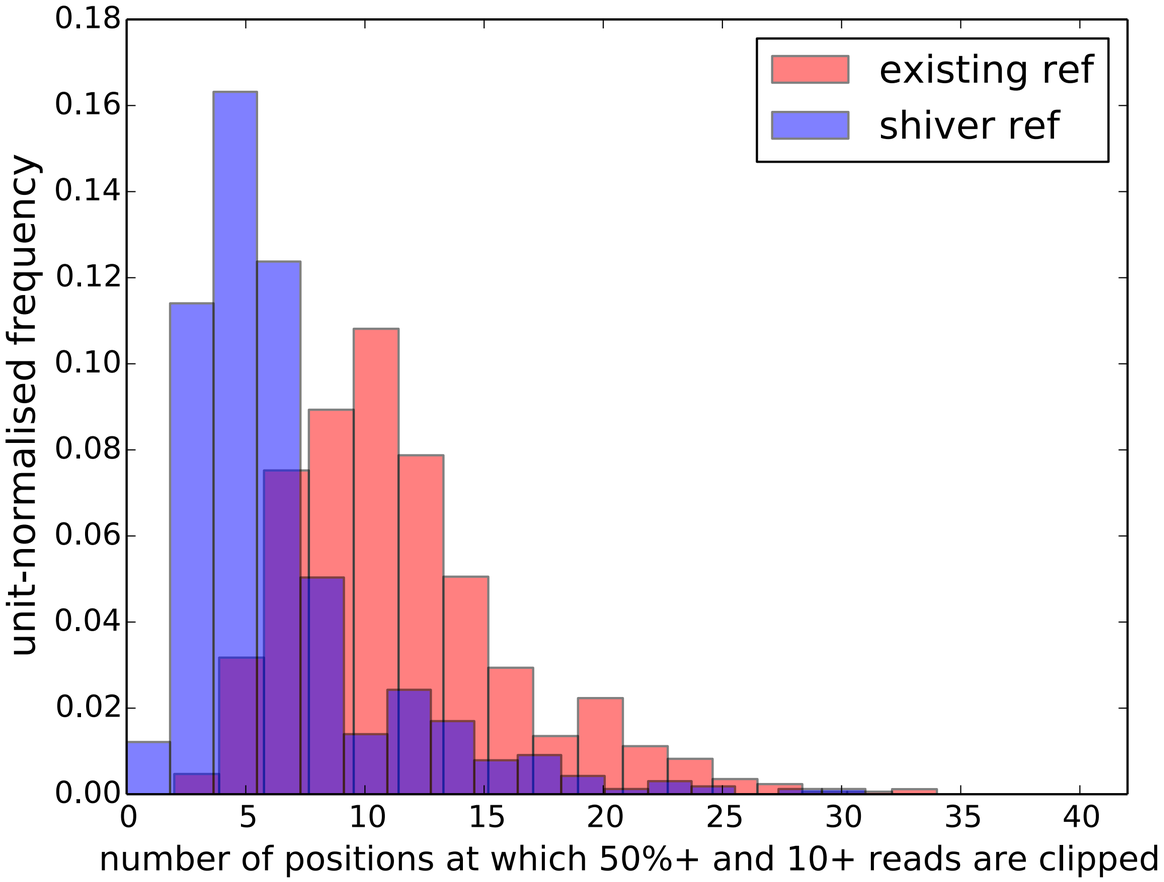


strict on coverage (>100)

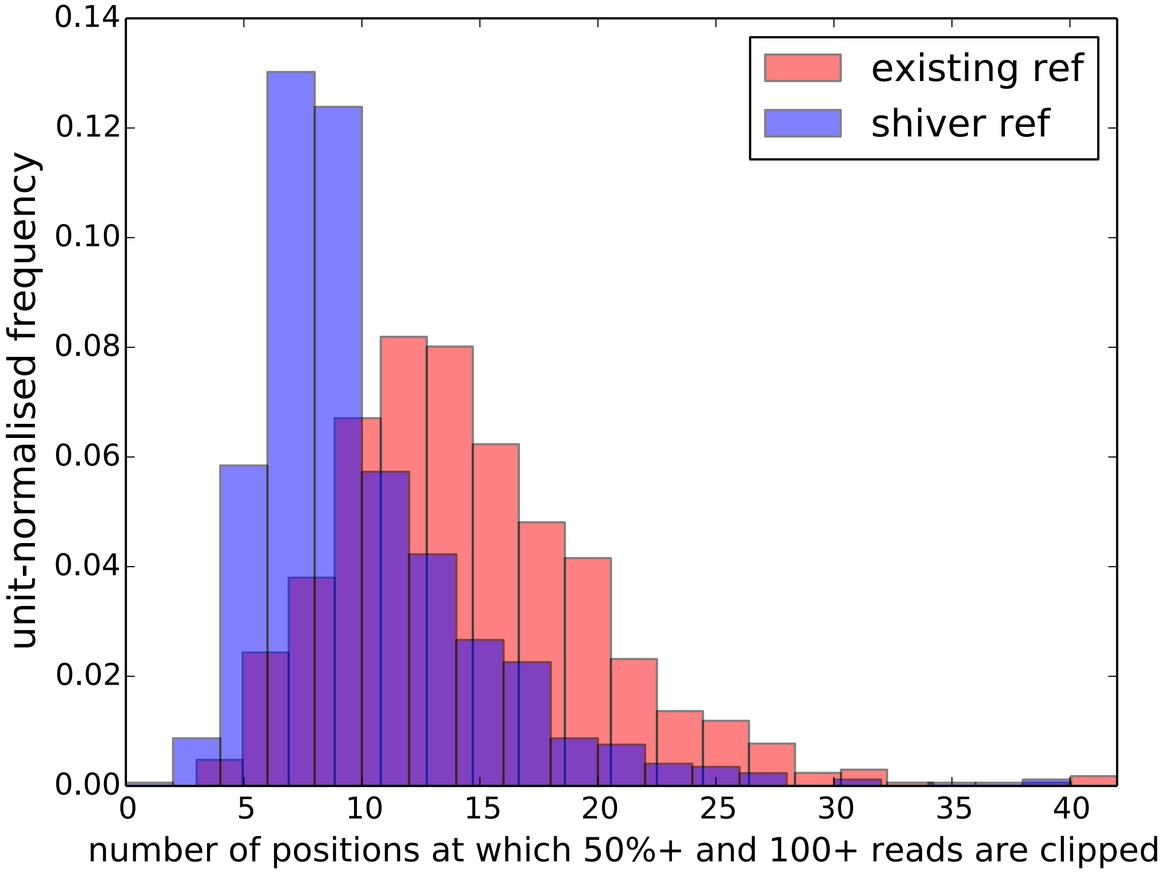




`shiver` decreases bias in the consensus



Miseq



Hiseq