Supplemental Materials for

Seamless, rapid and accurate analyses of outbreak genomic data, using Split *K*-mer Analysis

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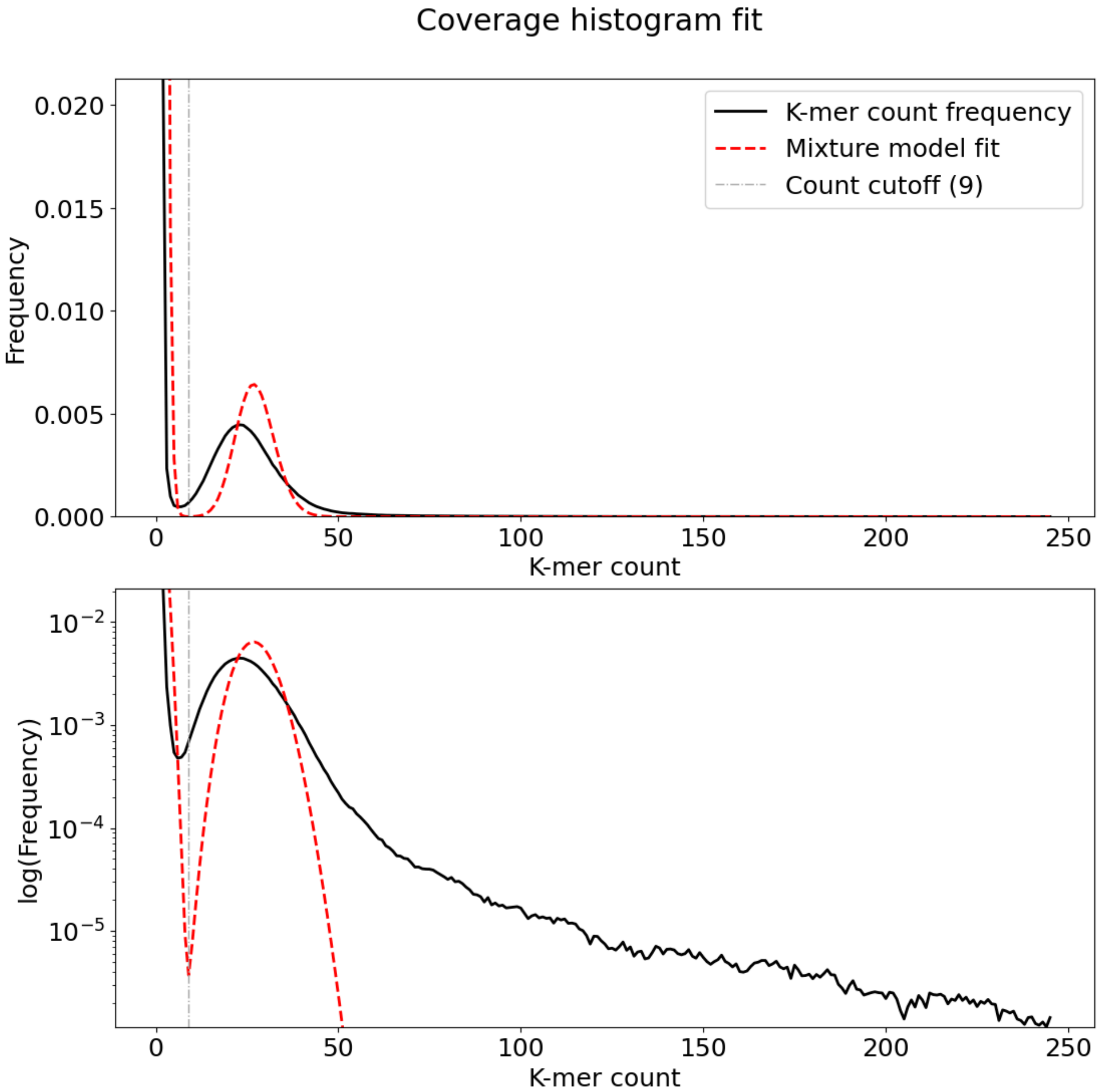
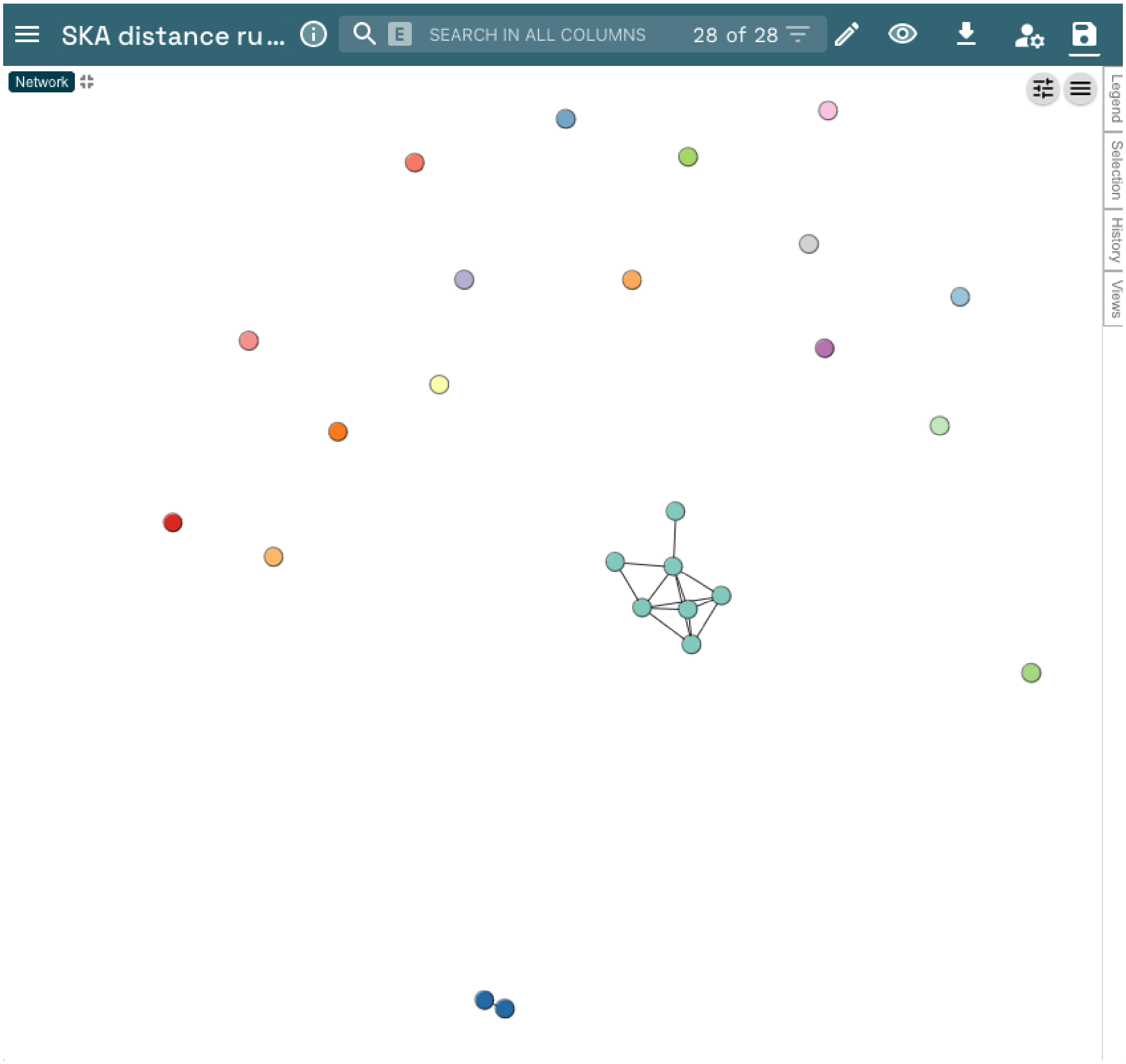
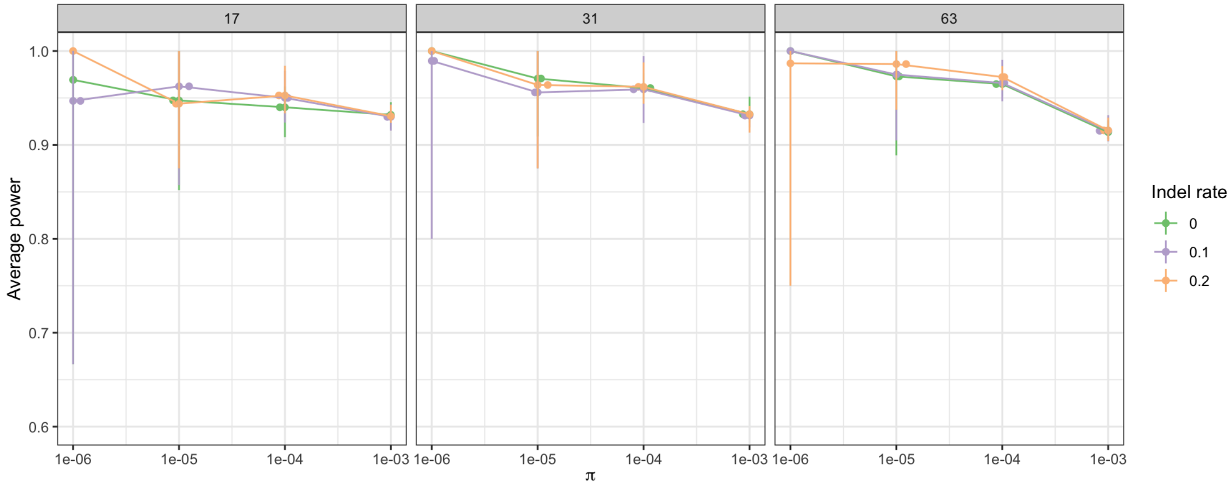
Supplemental figures



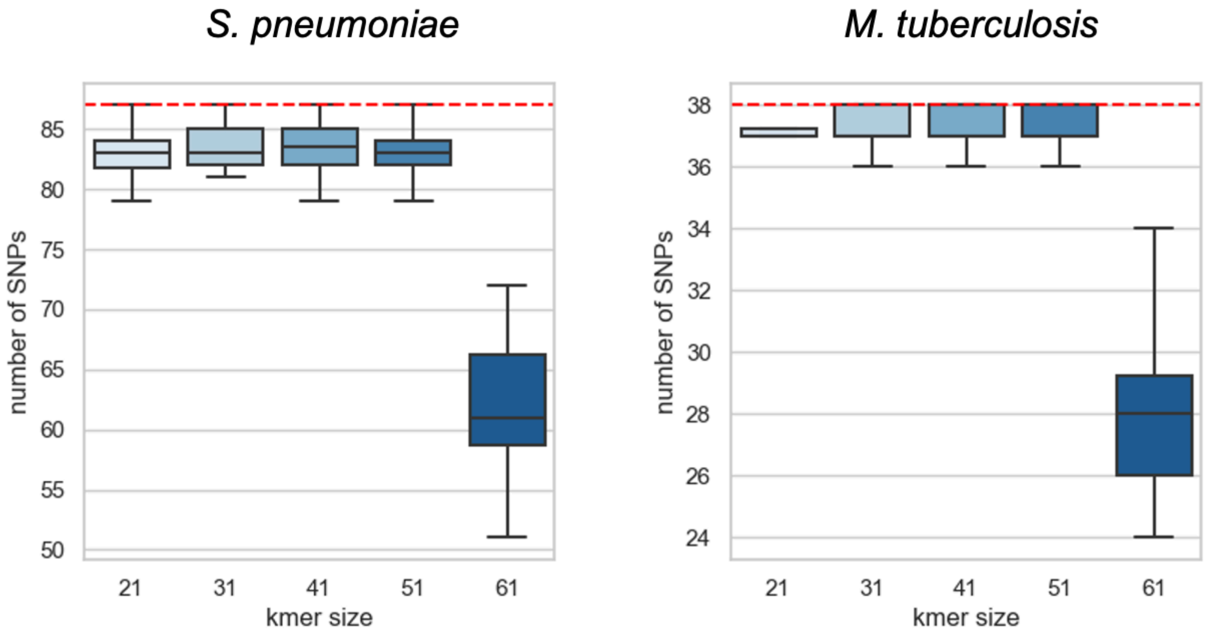
Supplemental figure 1: Example output of the coverage model *ska cov* fitted to k-mer count data from Illumina sequencing.



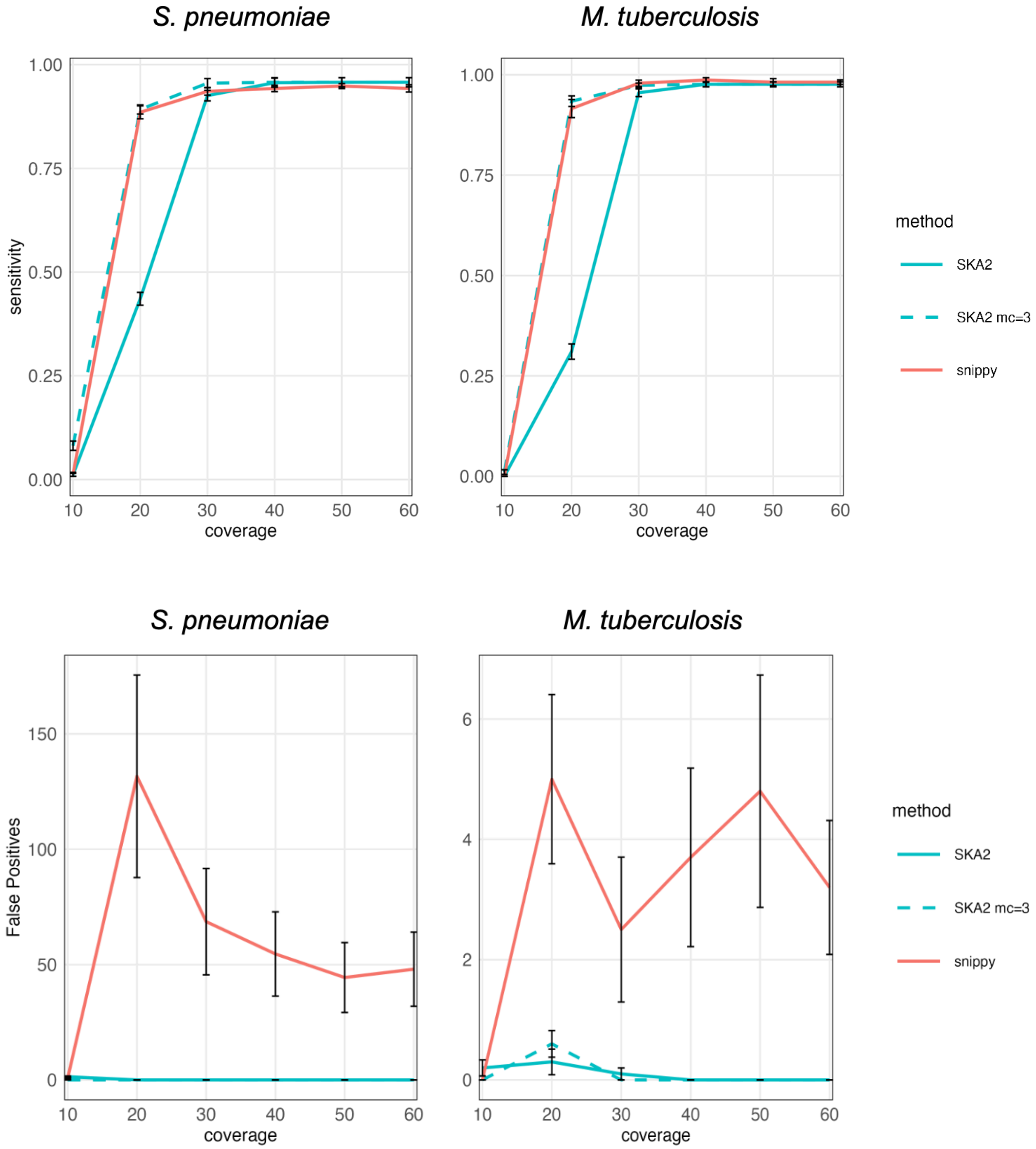
Supplemental figure 2: Example output of *ska distance*, connecting clusters below a given SNP threshold. Also available online https://microreact.org/project/icypNiESu31YhN1V8xzqX1-ska-distance-run-on-2023-jun-18-1730 and https://microreact.org/project/rNPR9KCnEWidVwvDmfbgQP-ska-distance-run-on-2023-jun-19-1119



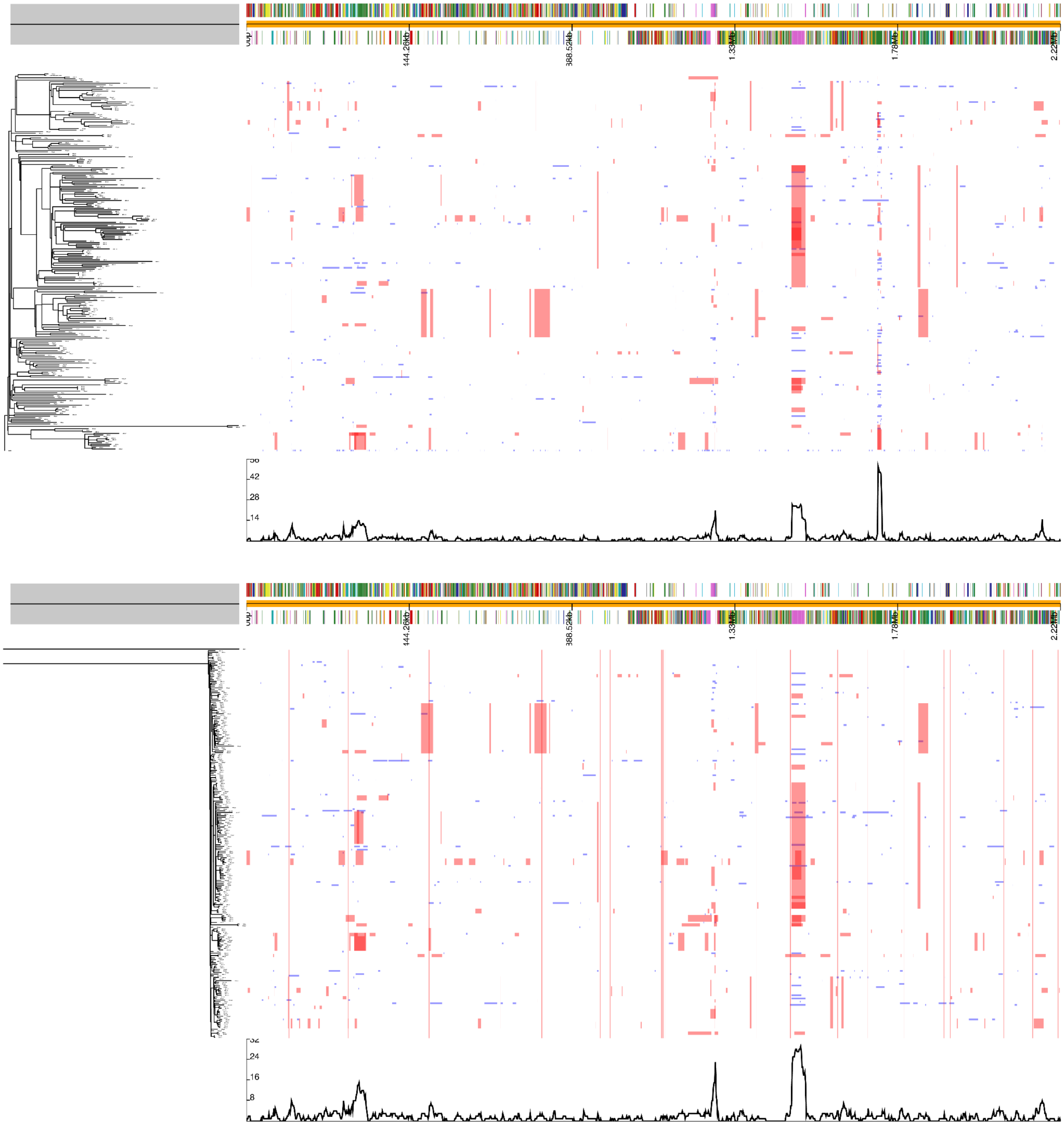
Supplemental figure 3: Effect of indels on SKA2's recall, same simulation setup as figure 2 but varying indel rate shown. Error bars are the 95% range from 20 repeats of the simulation.



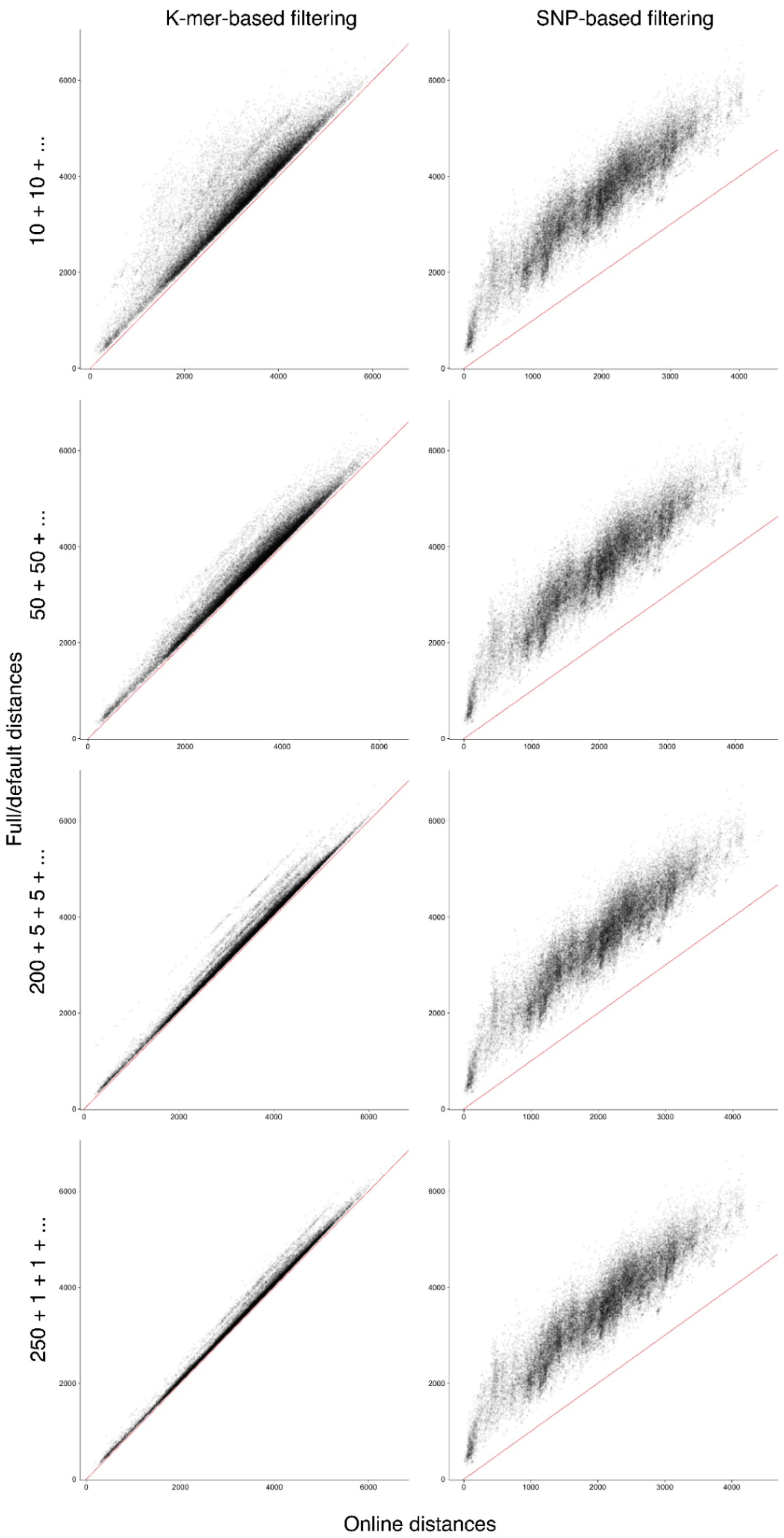
Supplemental figure 4: Number of SNP identified by SKA2 at different kmer sizes. The red dotted lines indicate the expected number of SNPs for each simulated outbreak.



Supplemental figure 5: SNP detection at low coverages. The simulated outbreak analyses were repeated at low coverage settings for outbreaks generated from the strain genomes ATCC_700669 and D39V, and H37Rv and lin_4.8 for *S. pneumoniae* and *M. tuberculosis* respectively (i.e., a total of 10 simulated outbreaks at each coverage for each species). Error bars represent the 95% confidence interval.



Supplemental figure 6: Gubbins analysis of PMEN1 samples. Top: original analysis using mapping and SNP calling from sequence reads against the Spn23F reference. Bottom: analysis using ska map. Visualised in phandango.



Supplemental figure 7: SNP distances between all pairs of samples in the online analysis versus the full analysis. Each row shows a different addition strategy. Each column shows a different filtering strategy.

Supplemental tables

Supplemental table 1: Genome assembly accessions used as root and reference for the simulations of outbreaks

Species	**Strain/lineage name**	**Assembly ID**
Streptococcus pneumoniae	ATCC_700669	NC_011900.1
	D39V	NZ_CP027540.1
	GPSC47	NZ_LR216060.1
	Taiwan19F-14	NC_012469.1
Mycobacterium tuberculosis	H37Rv	NC_000962.3
	4.8	NZ_CP041804.1
	3	NZ_CP041871.1
	1	NZ_AP018033.1

Supplemental table 2: List of the 288 *E.coli* genome GenBank accession numbers used in the online analysis.

GCA_010806885.1 GCA_902710505.2 GCA_014482275.1 GCA_018156485.1 GCA_009684105.1
GCA_014510545.1 GCA_012714485.1 GCA_014094315.1 GCA_013026005.1 GCA_000459175.1
GCA_012351795.1 GCA_002886425.1 GCA_016655085.1 GCA_000352825.1 GCA_003308975.1
GCA_017716315.1 GCA_012856475.1 GCA_013392695.1 GCA_002466895.1 GCA_018185135.1
GCA_014137255.1 GCA_003591475.1 GCA_014643235.1 GCA_014487175.1 GCA_000459795.1
GCA_000777995.1 GCA_012376145.2 GCA_012992085.1 GCA_902668645.1 GCA_012903805.1
GCA_017004115.1 GCA_013969625.1 GCA_009683345.1 GCA_014682415.1 GCA_018194835.1
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GCA_013176125.1 GCA_015620605.1 GCA_014327635.1 GCA_017132035.1 GCA_902161155.1
GCA_003145805.1 GCA_011318155.1 GCA_000503435.1 GCA_017946865.1 GCA_006230795.1
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GCA_003885225.1 GCA_012670565.1 GCA_904419595.1

Supplemental table 3: Fitted minimum count thresholds for simulated read data at lower coverages on *Mycobacterium tuberculosis*. The fitted threshold is the model value output by *ska cov*, the minimum threshold is the first minima in the table of counts output by *ska cov*.

Coverage	Fitted threshold	Minimum threshold
10x	4	2
20x	6	3
30x	8	4
40x	9	6

Supplemental methods

Transphylo parameters

	S. pneumoniae	*M. tuberculosis*
Neg	250/365	100/365
w.scale	1.5625	0.1
w.shape	1	10
pi	0.5	0.25
off.r	1.5	5
dateStartOutbreak	2005	2005
dateT	2007	2009
nSampled	12	30

phastSim commands

M. tuberculosis:

```
phastSim --outpath test_sim --mutationRates HKY85 0.23 0.5 0.17 0.33 0.33 0.17
--reference H37Rv.fna --treeFile mod_TransPhylo.tre --seed 0
--rootGenomeFrequencies 0
```

S. pneumoniae:

```
phastSim --outpath test_sim --mutationRates HKY85 0.23 0.5 0.17 0.33 0.33 0.17
--reference Spn_ATCC_700669.fna --treeFile mod_TransPhylo.tre --seed 0
--insertionRate CONSTANT 0.0876 --deletionRate CONSTANT 0.0876
--insertionLength DISCRETE 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1
--deletionLength DISCRETE 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1 --indels
--rootGenomeFrequencies 0
```

Commands used to run the BWA+BCFtools pipeline

```
bwa mem -t 1 -o sample.sam reference_genome.fna sample_1.fq.gz
sample_2.fq.gz
samtools view -q 20 -bS sample.sam > sample.bam
samtools sort -m 8G -o sample.sorted.bam sample.bam
samtools depth -Q 20 -q 20 -a sample.sorted.bam > sample_cov.txt
bcftools mpileup -Q 20 -f Spn_ATCC_700669.fna sample.sorted.bam -o
sample.vcf1
bcftools call -f GQ -o sample_SNPs.vcf -V indels -cv sample.vcf1
```