Detecting mixed *Mycobacterium tuberculosis* infection and differences in drug susceptibility with WGS data

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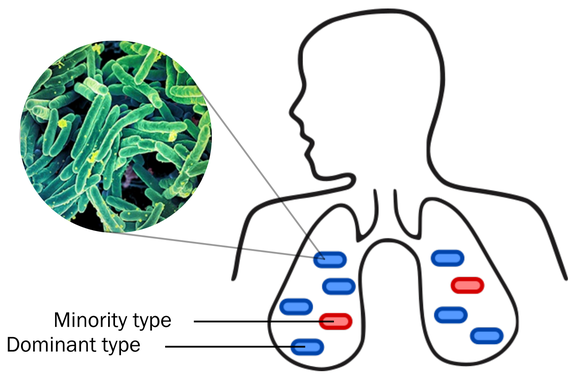
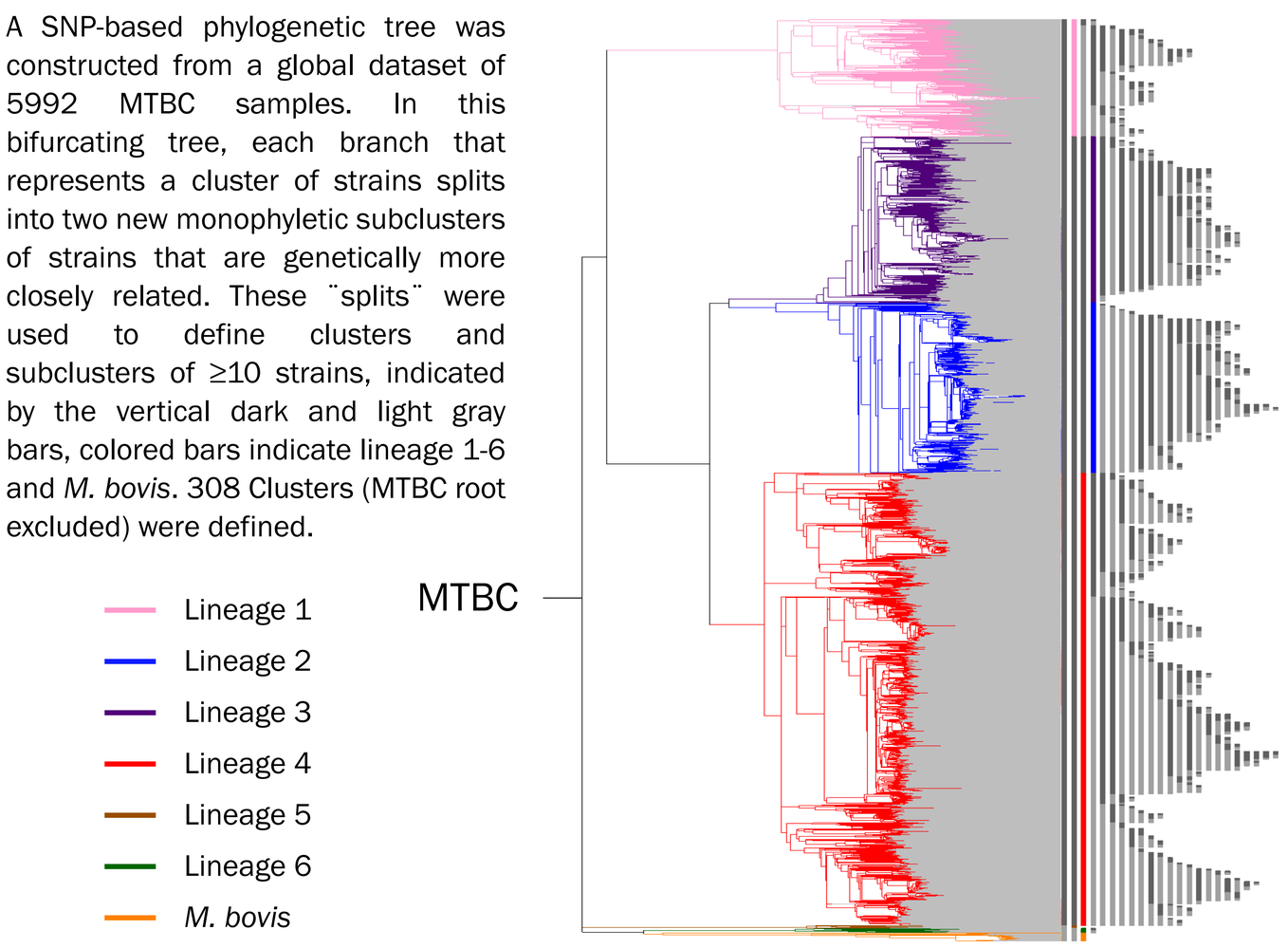
Mycobacterium tuberculosis is a clonal, bacterial pathogen that causes the pulmonary disease tuberculosis (TB) and infects and kills millions of people [1]. The study of genetic diversity within the *M. tuberculosis* complex (MTBC) is complicated by mixed TB infections, which happens when a person is infected with more than one distinct strain type of MTBC. This often results in poor diagnosis and treatment of patients as the bacterial subpopulation may have undetected differences in drug susceptibility [2].



Whole genome sequencing (WGS) yields a great number of single nucleotide polymorphisms (SNPs) and offers an increased resolution to distinguish distinct strains of MTBC [3]. Here, I present a tool that maps sample reads against 21 bp cluster-specific SNP markers to detect a possible mixed infection and estimate the frequencies of present subpopulations.

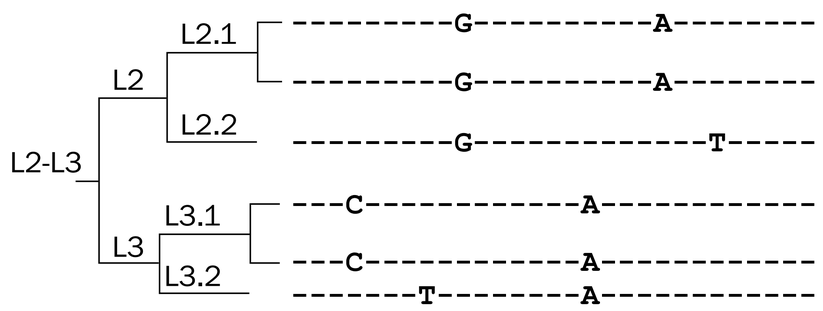
MTBC SNP-based phylogenetic tree with hierarchical clusters

A SNP-based phylogenetic tree was constructed from a global dataset of 5992 MTBC samples. In this bifurcating tree, each branch that represents a cluster of strains splits into two new monophyletic subclusters of strains that are genetically more closely related. These ¨splits¨ were used to define clusters and subclusters of ≥10 strains, indicated by the vertical dark and light gray bars, colored bars indicate lineage 1-6 and *M. bovis*. 308 Clusters (MTBC root excluded) were defined.



Global SNP association

Global SNP association was done for each hierarchical cluster to get cluster-specific SNPs. Based on the number of samples that harbor a specific SNP, a SNP was associated to a cluster when the true positive rate, true negative rate, positive predictive value, and negative predictive value were >0.95.

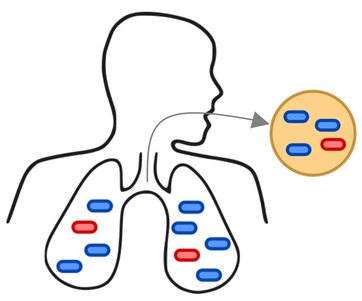


Unique cluster-specific SNP

All SNPs	13633
SNPs not within 10 bp of another SNP	13337
Generate 21 bp SNP markers (incl. complementary markers)	14861
Unique SNP markers	14823

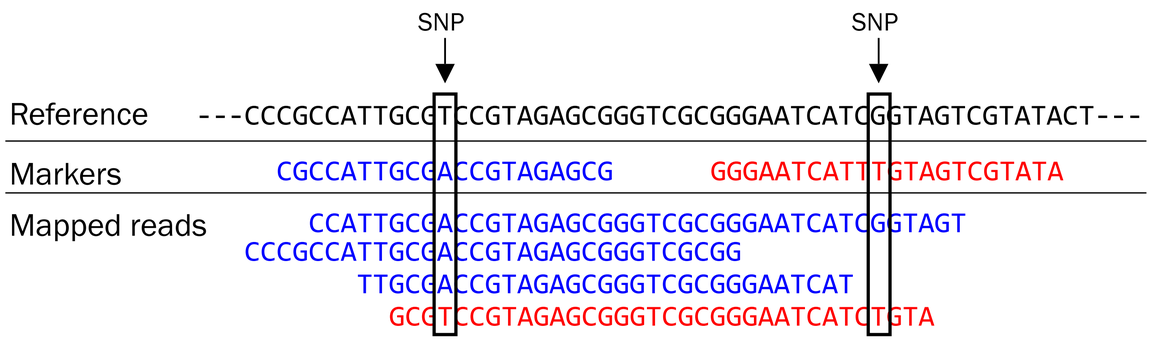
The known MTBC lineages 1 to 6 have between 355-614 SNP markers.

Detect mixed TB infection and estimate frequencies

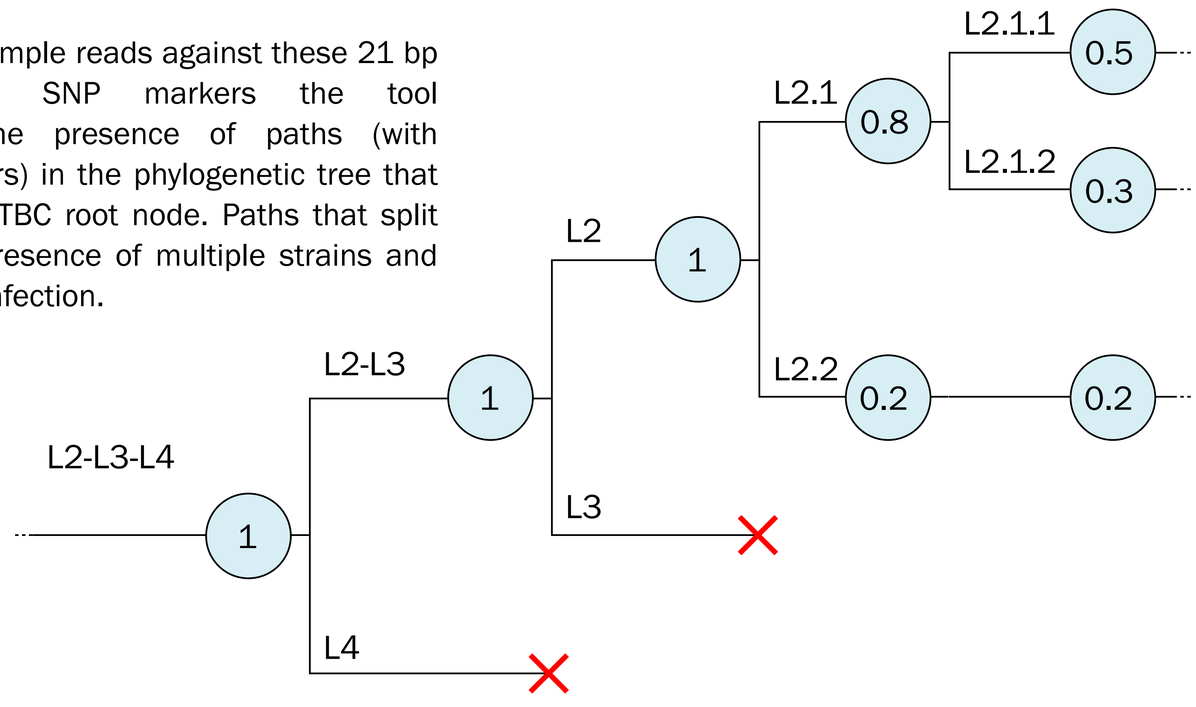


Multiple copies of a genome

Sample reads

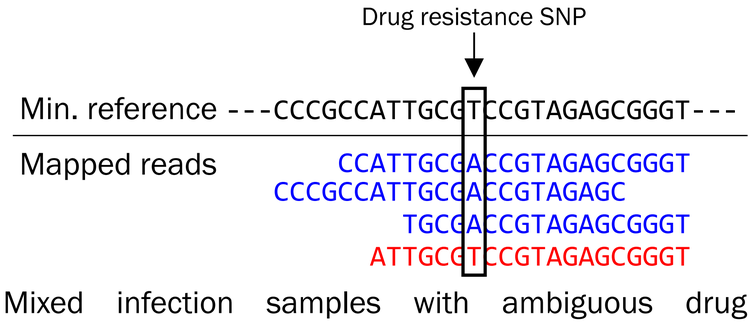


By mapping sample reads against these 21 bp cluster-specific SNP markers the tool determines the presence of paths (with present clusters) in the phylogenetic tree that start at the MTBC root node. Paths that split indicate the presence of multiple strains and thus a mixed infection.



Drug resistance detection

A minimized reference genome consisting of drug resistance genes and 1000 bp flanking regions was used to map sample reads and call variants. Detected variants are compared to a library of drug resistance mutations from Cohen et al. (2015) [4].



Mixed infection samples with ambiguous drug resistance mutations indicate drug susceptibility differences of the present subpopulations.

Prevalence of mixed TB infections in global dataset

7661 TB samples were tested, present strain(s) and frequencies could be predicted for 7495 samples of which 914 (~12%) are mixed infections.

Number of subpopulations	1	2	3	>3
Number of samples	6581	798	95	21

References

1. World Health Organization. *Global Tuberculosis Report*. World Health Organization, Geneva, Switzerland, 2014.
2. N. M. Zetola et al. Mixed *Mycobacterium tuberculosis* complex infections and false-negative results for rifampicin resistance by GeneXpert MTB/RIF are associated with poor clinical outcomes. *Journal of Clin. Microb.*, 52:2422-2429, 2014.
3. M. Coscolla and S. Gagneux. Consequences of genomic diversity in *Mycobacterium tuberculosis*. *Seminars in Immunol.*, 26:431–444, 2014.
4. K. A. Cohen et al. Evolution of extensively drug-resistant tuberculosis over four decades: Whole genome sequencing and dating analysis of *Mycobacterium tuberculosis* isolates from KwaZulu-Natal. *PloS Med*, 12(9): e1001880, 2015.