afkSNP:
assembly-free K-mer based SNP comparison of bacterial WGS samples

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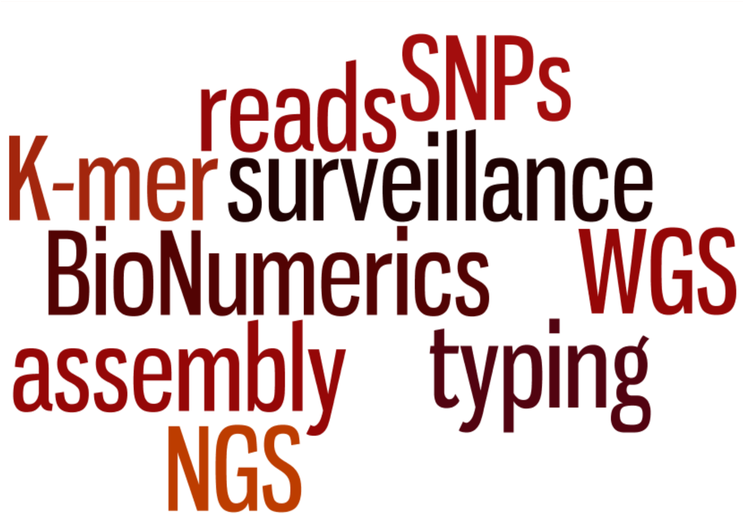

INTRODUCTION

The democratization of whole genome sequencing (WGS) creates new possibilities for molecular surveillance of bacterial pathogens. However, easy to use bioinformatics tools to analyze these samples are often lacking:

- On a technical level: routine labs often have difficult access to the bioinformatics resources required to analyze (confidential) whole-genome sequencing data.
- On a biological level: for numerous micro-organisms there are no closely related reference sequences available, compromising the resolution of reference sequence-based approaches.

OBJECTIVES

- *Reference-free*, *assembly-free* and *fast* method to compare WGS samples based on single-nucleotide polymorphisms (SNPs).
- Tools, *integrated in the software suite BioNumerics®*, enabling fast and reliable strain comparison, with subsequent phylogenetic, statistical and data mining analyses.

METHODOLOGY

- For every base, use its flanking regions to define the location.
- For each sample, calculate the complete list of pairs of words of length k. To limit sequencing errors, we filter on
  - base quality scores,
  - overall and strand-specific coverage
  - middle base ambiguity.
- Identical repeat regions yield the same set of “locations”, middle base ambiguity filtering removes all dubious “locations”.
- For each pair of samples, the word lists are compared by counting the number of words that have the same start and end, but have a different middle base. This yields a pairwise distance matrix that can be used for further analysis.

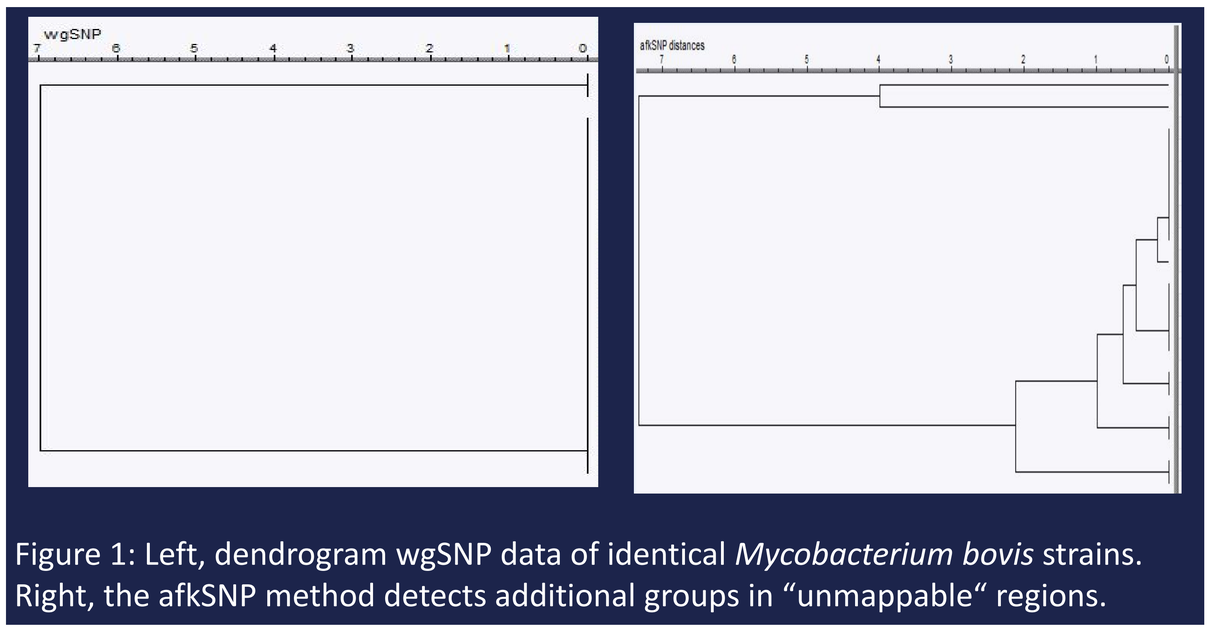

Figure 1: Left, dendrogram wgSNP data of identical *Mycobacterium bovis* strains. Right, the afkSNP method detects additional groups in “unmappable“ regions.

EXPERIMENTAL VALIDATION

- We use 22 WGS samples of identical *Mycobacterium bovis* (MBO) strains to investigate the stability and the reproducibility, and compared the results to a reference-based SNP calling procedure (wgSNP). For both methods, we found a total of 7 mutations. The afkSNP method revealed slightly more mutations in repeat regions that have been excluded from the reference-based approach due to unreliable mapping (see figure 1).
- afkSNP analysis of a set of 26 outbreak-related *Mycobacterium tuberculosis* (MTBC) samples shows the potential of the method to reveal the structure of the outbreak in a fast and objective manner. The correlation between distances calculated by afkSNP and wgSNP in this sample is 99,9% (see figure 2).

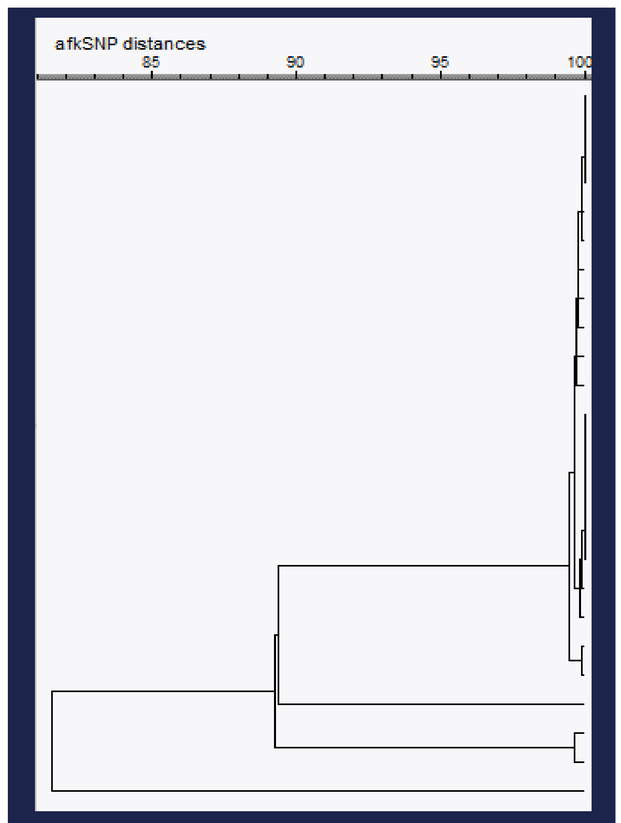

Figure 2: afkSNP dendrogram of 26 outbreak-related *Mycobacterium tuberculosis strains.* The afkSNP similarities correlate extremely well with the wgSNP data (99.9% correlation)

CONCLUSIONS

- The afkSNP method gives almost identical results as the classical SNP detection methods, but does not require a closely related reference.
- The afkSNP method compares the complete genomic content of all WGS samples, in a pairwise manner, and not just what is common with the reference sequence.
- The use of only *isolated* SNPs does restrict the analysis, but at the same time this approach avoids clusters of SNPS that have been introduced by a single evolutionary event, thus providing a less disturbed counting of evolutionary events. Therefore, the afkSNP method covers a middle ground between reference-based SNPs and whole genome multi-locus sequence typing.

ACKNOWLEDGEMENTS

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