

SANS serif: alignment-free, whole-genome based phylogenetic reconstruction

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Supplementary Material

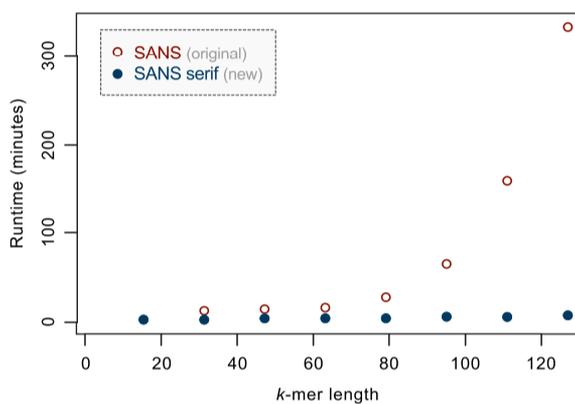


Fig. 1. Runtime (user time) of SANS evaluated for different k -mer lengths. For random subsamples of 100 assemblies from the *Salmonella* data set, the 1000 highest weighting splits were output. Values were averaged over processing three random subsamples each.

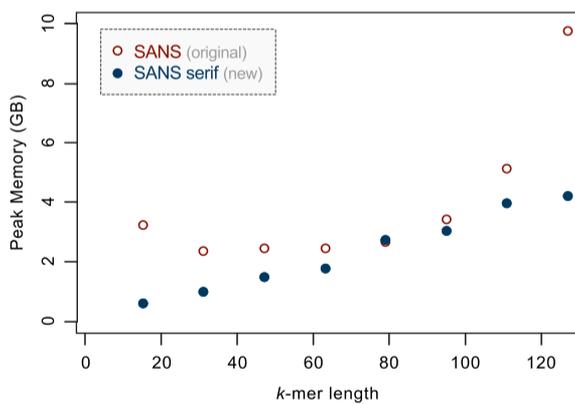


Fig. 2. Peak memory usage of SANS evaluated for different k -mer lengths. For random subsamples of 100 assemblies from the *Salmonella* data set, the 1000 highest weighting splits were output. Values were averaged over processing three random subsamples each.

Table 1. Runtime (user time) and peak memory usage of SANS serif on the complete *Salmonella enterica* data set evaluated for different parameters: processing the sequence files directly (`-input`), processing a colored de Bruijn graph generated from the sequences using Bifrost (`-graph`), processing a list of splits and applying different filters (`-filter`), and processing the sequence files considering IUPAC characters (`-iupac`). The overall weight of each split was calculated using the geometric mean without pseudocounts (`-m geom`). Values were averaged over three different runs each.

Parameter	Runtime (min)	Memory (GB)
<code>-input</code>	132.71	47.57
<code>-graph</code>	69.08	51.28
<code>-filter</code> <i>strict</i>	0.06	0.01
<i>2-tree</i>	0.06	0.01
<i>weakly</i>	467.89	0.01
<code>-iupac</code>	153.29	47.59

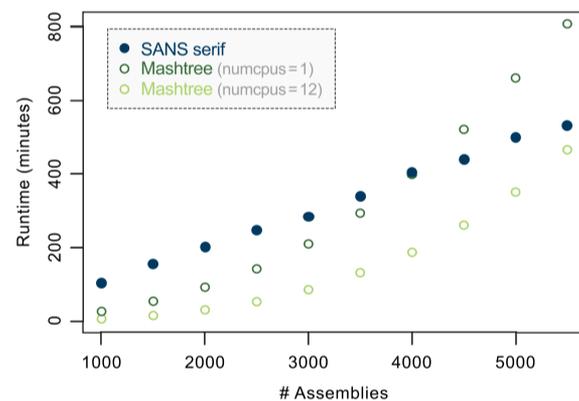


Fig. 3. Runtime (real time) comparison of SANS serif and Mashtree (Katz *et al.*, 2019) on *Salmonella* Typhimurium assemblies. For 6000 randomly selected genomes from a total of 19237 that were annotated as serovar *Typhimurium* in EnteroBase (Alikhan *et al.*, 2018) (on April 09, 2019), 5712 assemblies were available and have been downloaded. For random subsamples of n assemblies, both Mashtree and SANS serif have been run with $k=21$ (Mashtree default). For SANS serif, the $10n$ highest weighting splits were determined using the geometric mean and filtered for a tree. Mashtree has been run with default parameters as well as using multi-threading (`numcpus=12`). Values were averaged over processing two random subsamples each. The peak memory usage of SANS with 29.9GB was higher than that of Mashtree with 9.3GB for processing 5500 assemblies (data not shown). The runtime of Mashtree shows a quadratic increase (least squares regression, adjusted R-squared 0.9998), whereas SANS serif shows a linear trend (adjusted R-squared 0.9975).

Table 2. Effect of N's in the input sequences. A phylogeny with 100 leaf genomes of length ~ 96 kb and an evolutionary distance of 5 PAM to the root has been simulated with ALF (Dalquen *et al.*, 2012). (See software repository for the complete parameter setting.) DNA characters in the genomes were randomly substituted by N's at a rate of 0.1% and the sequences were processed by SANS serif before and after the substitution with k -mers containing N's being skipped (default) or N's replaced by all possible bases (parameter `-iupac`, $x=16$). For the obtained tree-filtered split sets, precision and recall (cf. Figure 1) as well as weighted precision (total weight of called splits also in the reference tree) / (total weight of all called splits) and weighted recall (total weight of reference splits also in the call set) / (total weight of all reference splits) have been computed.

	original	with 0.1% N's	
		skipped	replaced
<i>unweighted</i>			
precision	0.90	0.63	0.78
recall	0.70	0.38	0.58
<i>weighted</i>			
precision	0.98	0.95	0.98
recall	0.88	0.68	0.88

References

- Alikhan, N.F. *et al.* (2018) A genomic overview of the population structure of *Salmonella*, *PLoS Genet.*, **14**(4), 1007261.
- Dalquen, D.A. *et al.* (2012) ALF – A simulation framework for genome evolution *Mol. Biol. Evol.*, **29**(4), 1115–1123.
- Katz, L. *et al.* (2019) Mashtree: a rapid comparison of whole genome sequence files, *J. Open Source Software*, **4**(44), 1762.