K When a Score is Below 1.9

Supplementary Note 1: Figure cap-

tions

K When a Score is Below 1.9



Figure 1. (A) Box plot representing the AUTO-TUNE scores across ten random samples at 25%, 50%, and 75% of the (Rhee et al., 2019) dataset, showing a trend of increasing confidence in score estimates with denser sampling. (B) Box plot of the selected distance thresholds across the same random samples at 25%, 50%, and 75% proportions, demonstrating improved consistency in threshold selection with increased sample size. (C) Scatterplot of the chosen thresholds (Y-axis) against their corresponding AUTO-TUNE scores (X-axis) for the three subsample proportions.



Figure 2. Figure A and B present the effects of subsampling on network structure using different thresholds. Figure A illustrates the proportion of nodes subsampled that remained clustered in both the original and the subsampled networks, with an observable increase in nodes captured as the threshold transitions from 1.5

	5481.0		
Upload a multiple sequence alignment:		OR	Upload an AUTO-TUNE results file : Choose file
	Choose file		
Instructions To generate a results file t Once a pairwise distance An example workflow is as	rat is compatible with this page, use a multiple lie is created, use the himetworkcay script with follows:	sequence alignment with the tn03 fast pairs 1 the -A keyword argument to generate the t	rise distance calculator. ab-separated output compatible with this page.
./tn93 -t 0.03 pol	.fasta > pairwise_distances.tn93.csv pairwise_distances.tn93.csv -f pl	v hivnetworkcsv −i ain −A 0 ≻ autotune_report.tsv	
1.8 - 1.6 - 1.4 -			
1.2			
	ANY .		
1.0-			
1.0 - 0.8 -	요. 영원 :		
10 - 0.8 - 0.6 -			

Figure 3. The user interface of the AUTO-TUNE web application (http://autotune.datamonkey.org/analyze). The platform provides a multi-faceted view of AUTO-TUNE's analysis, including a score plot that visualizes trends across different genetic distance thresholds. It also displays graphs of the number of clusters and the R1/R2 ratio—both key metrics in AUTO-TUNE's heuristic scoring system. These interactive visualizations aid researchers in making nuanced decisions for threshold selection, especially when multiple thresholds yield similar scores.



Figure 4. Examples of AUTO-TUNE scores profiles. (A). Lowering the genetic distance threshold removes some of the edges from the network (shown in grey) and disconnects a large cluster into color-coded smaller clusters; here "None" means that the node is not connected to anything at the lower threshold. (B). Raising the genetic distance threshold adds edges to the network (shown in grey) and connectes previously separte clusters into a larger component. (C). Each circle is a cluster in the larger threshold network, and with a proportion of nodes removed when the threshold is lowered. (D). Changes to the node degree distribution (colors represent the counts of nodes with the same degree). (E). A significant enlargement of a small network at a higher threshold, with grey edges only present at the larger threshold.

K When a Score is Below 1.9



Figure 5. Examples of how changing thresholds affects inferred networks. (A). A high-scoring network Bbosa et al. (2020) has a distance threshold which achieves the number of clusters near the maximum, while also avoiding the formation of a large (weakly connected) cluster. (B). A low-scoring network Liu et al. (2020) has a misalignment between the distance for which the maximum number of clusters is found, and where the big jumps in the cluster size ratio occur. Here, AUTO-TUNE effectively optimizes the number of clusters while preventing excessive growth of the largest cluster.