AUTO-TUNE: FINDING AN OPTIMAL DISTANCE THRESHOLD FOR INFERRING HIV TRANSMISSION CLUSTERS

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ABSTRACT

Choosing an appropriate distance threshold is an important part of inferring a transmission network to determine the relative growth of clusters within a localized epidemic. This distance threshold determines how close two consensus sequences must be in order for a link to be created between them in the network. Using a distance threshold that is too high can result in a network with many unnecessary links, making it difficult to interpret and analyze. On the other hand, using a distance threshold that is too low can result in a network with too few links, which may not capture key insights into rapidly growing clusters among patients with shared attributes that could benefit from public health intervention measures.

Here, we present a heuristic scoring approach for tuning a distance threshold by associating each tested threshold against the maximal number of clusters created across all thresholds and the difference between the ratio (R_{12}) of the largest cluster in the network to the second largest cluster at each iteration. The number of clusters is normalized between $[0, 1]$ then gated via a Gompertz function transform. Meanwhile, the distribution of all R_{12} ratios are converted to Z scores, and normalized relative to the largest positive Z score across all candidate distances. The priority score is the sum of aforementioned two components.

Published research using the HIV-TRACE software package frequently use the default threshold of 1.5% for HIV pol gene sequences. We apply our scoring heuristic to outbreaks with different characteristics, such as regional or temporal variability, and demonstrate the utility of using the scoring mechanism's suggested distance threshold to identify clusters exhibiting risk factors that would have otherwise been more difficult to identify. For example, while we found that a 1.5% distance threshold is typical for US-like epidemics, recent outbreaks like the CRF07_BC subtype among men who have sex with men (MSM) in China has been found to have a lower optimal threshold of 0.5% to better capture the transition from injected drug use (IDU) to MSM as the primary risk factor. Alternatively, in communities surrounding Lake Victoria, where there has been sustained transmission for several years, we found that a larger distance threshold is suitable to

28 capture a more risk factor diverse populace with sparse sampling over a longer period of time.
29 Such identification may allow for more informed intervention action by respective public health
30 officials.

31 **Keywords: molecular epidemiology, HIV, network, transmission cluster, surveillance**

1 INTRODUCTION

32 Choosing an appropriate distance threshold is an important part of using a transmission network to track
33 the spread of a contagious disease. This distance threshold determines how close two individuals must be
34 in order for a link to be created between them in the network.

35   Using a distance threshold that is too small can result in a network with many unnecessary links, making
36 it difficult to interpret and analyze. On the other hand, using a distance threshold that is too large can result
37 in a network with too few links, making it difficult to accurately track the spread of the disease.

38   To ensure that the transmission network is useful and informative, it is important to carefully consider
39 the appropriate distance threshold. This may vary depending on the specific disease and the context in
40 which it is spreading. For example, a highly contagious respiratory illness may require a smaller distance
41 threshold than a less contagious illness that is primarily spread through direct contact.

42   In general, the goal is to strike a balance between having enough links to accurately track the spread of
43 the disease, while not having so many links that the network becomes difficult to interpret. This can be
44 achieved through careful analysis and consideration of the specific disease and context.

45   Overall, choosing an appropriate distance threshold is an important step in using a transmission network
46 to track the spread of a contagious disease. It can help ensure that the network is useful and informative,
47 and can ultimately aid in efforts to control and prevent the spread of the disease.

2 METHODS

2.1 Scoring Heuristic Procedure

49   Network threshold selection procedure proceeds as follows:

50 1. For each candidate threshold d_L, in increasing order, ranging from the smallest genetic distance in
51   the dataset, up to either the largest distance or a predetermined maximal threshold, we compute two
52   network statistics: R_{12}, the ratio of the largest cluster to the second largest cluster, and C – the number
53   of clusters in the network.

54 2. A priority score is assigned to each d_L. This score measures two properties of the threshold: Does
55   R_{12} jump at d_L? How far is the number of clusters C at d_L from the maximal number of clusters over
56   all threshold values? Let there be N overall d_L candidate values, and assume we are examining the
57   ith candidate, d_L^i with $W < i \leq N - W$ (W is a positive integer defined below).

58   a. The R_{12} jump is computed by looking at the normalized ratio of the mean R_{12} values computed
59   over the leading window $d_L^{i+1} \ldots d_L^{i+W}$ and the trailing window $d_L^{i-W} \ldots d_L^{i-1}$. The width of the
60   window, W, is defined as $(([\frac{N}{100}], 3), 30)$. The distribution of ratios is converted to Z scores, and
61   normalized relative to the largest positive Z score across all candidate distances, yielding the jump
62   component of the score.

63   b. The number of clusters, C_i at threshold d_L^i is first normalized to $[0, 1]$ through $\frac{C_{max} - C_i}{C_{max} - C_{min}}$ and next

64   gated via a Gompertz function transform $1 - e^{-e^{-25x+3}}$ This function provides an ad hoc means

65   for penalizing having too few clusters relative to the maximum over all ranges. For example, a

66   threshold that yields 95% of the maximal number of clusters receives a score of 0.996, while a

67   threshold that yields 85% - a score of 0.376.

68   c. The priority score for d_L^i is the sum of the two components defined in (a) and (b).

69 3. The threshold with the highest priority score will be selected as the suggested automatic distance

70   threshold, if the score is high enough (1.9 or more), and either of the two conditions hold.

71   a. No other thresholds have priority scores of 1.9 or higher

72   b. If other thresholds have priority scores of 1.9 or higher, then the range of thresholds represented by

73   these options is small (no more than $log(N)$ times the mean step between successive d_L^i).

74 4. If no single threshold can be selected in step 3, then the one with the highest priority score is suggested,

75   and an inspection of the plot like the one on the analyze page is recommended to ensure that the

76   threshold is sensible.

2.2 Assortativity

78   Degree-weighted homophily (DWH) is a measure of similarity between nodes in a network based on
79 their attributes (such as demographic characteristics or behaviors) and their degree (i.e., the number of
80 connections they have to other nodes in the network). It is used to quantify the extent to which nodes
81 with similar attributes tend to be connected to each other more frequently than would be expected by
82 chance. DWH is calculated as the ratio of the observed number of connections between nodes with similar
83 attributes to the expected number of connections between such nodes, based on their degree.

84   In mathematical terms, it is defined as:

$$DWH = \frac{W_M + W_C - 2W_X}{\frac{d_{in}}{nodes_{in}^2} + \frac{d_{out}}{nodes_{out}^2}} \tag{1}$$

85   Where

86   • W_M : Weight of in-group connections

87   • W_C : Weight of out-group connections

88   • W_X : Weight of cross-group connections

89   • d_{in} : In-group degree

90   • d_{out} : Out-group degree

91   • $nodes_{in}$: number of in-group nodes

92   • $nodes_{out}$: number of out-group nodes

93   DWH ranges from -1 to 1. A DWH value of 0 indicates that there is no more homophily than expected
94 with chance, while a value of 1 indicates that there is perfect homophily (e.g. Birds always link to birds,
95 and only birds). A value of -1 is achieved for perfectly disassortative networks (e.g. Bird never linking
96 with another bird).

97   DWH is used in social network analysis and in the study of how different attributes are related to the
98 formation of connections between individuals. It is used as a way to measure the similarity of attributes

99 between individuals in a network. Additionally, randomization is performed by shuffling attribute labels
100 among nodes, then performing DWH computation. This is useful in creating a null distribution of DWH
101 scores under random mixing. A panmictic range is reported by shuffling attributes multiple times and
102 reporting the minimum and maximum score.

103 **2.3 Implementation**

104   The software implementation involves a step-by-step process that utilizes the HIV-TRACE suite of
105 packages. It starts with calculating pairwise distances wth the tn93 tool and a supplied multiple sequence
106 alignment. This generated pairwise distances are supplied to the hivnetworkcsv script while providing the
107 -A keyword argument. A brief outline of the software's implementation are as follows

108 1. Calculate pairwise distances: The user first calculates the pairwise distances using the tn93 fast pair-
109   wise distance calculator, providing the necessary threshold value and the input FASTA file. The
110   command for this step is

```
1  tn93 -t 0.030 pol.fasta > pairwise_distances.15.tn93.csv
```

114   Please note that the threshold should include the maximal range one is intending to test.

115 2. Compute distance threshold scores: The hivnetworkcsv script is then executed with the required input
116   file, format, and autotune option to generate a tab-separated output file, as shown below

```
1  hivnetworkcsv -i pairwise_distances.15.tn93.csv -f plain -A 0 > autotune_report.tsv
```

120 3. Visualize the report: Users can upload the generated autotune_report.tsv file to
121   http://autotune.datamonkey.org/analyze for visualization and further analysis of the
122   data. This web-based platform provides an interactive environment to explore scores and other metrics
123   across the range of tested outputs.

124 4. Run HIV-TRACE: Once AUTO-TUNEd threshold(s) are settled upon after review, the user runs the
125   HIV-TRACE command with the appropriate input FASTA file, distance threshold, and other required
126   arguments. The output is saved as a JSON file. An example command is

```
1  hivtrace -i ./INPUT.FASTA -a resolve -r HXB2_prrt -t < autotune_threshold > -m 500 -g .05
   ↪> hivtrace.results.json
```

131 2.3.1 Optional : Compute Assortatviity Metrics

132 5. Annotate results: The hivnetworkannotate script is used to annotate the results obtained from the HIV-
133   TRACE step with attributes. The script takes the JSON results file, node attributes file, schema file,
134   and a resolve flag as input.

```
1  hivnetworkannotate -n hivtrace.results.json -a node_attributes.json -g schema.json -r
```

138   For more information, users can refer to the hivnetworkannotate documentation.

139 6. Analyze the results with DWH: After the results file has been annotated, the user can proceed to the
140   assortativity page, http://autotune.datamonkey.org/assortativity, for further analysis of the output.
141   AUTO-TUNE is readily accessible on GitHub as part of the hivclustering repository
142   (https://github.com/veg/hivclustering). It is integrated into the command-line interface of the soft-
143   ware as the -A or –auto-profile argument. hivclustering is a key component of the HIV-TRACE suite
144   of tools, a resource for the inference, analysis and visualization of HIV transmission networks.

145   The Degree Weighted Homophily (DWH) calculation tool, an integral component of the assor-
146 tativity step, is developed using TypeScript, a statically typed superset of JavaScript that ensures
147 robustness and scalability. In an effort to promote accessibility and ease of integration, the DWH tool
148 is packaged and distributed through the Node Package Manager (NPM), enabling researchers and
149 developers to conveniently incorporate this advanced analytical tool into their own projects and work-
150 flows. DWH can be used in-browser or as a command line tool, allowing researchers and developers
151 to employ the tool in an interactive command-line interface or integrate it into larger software appli-
152 cations, thus catering to a diverse array of technical needs and preferences. Instructions for usage and
153 installation is found on Github (https://github.com/veg/dwh).

154   The described workflow offers a systematic approach to analyze potential distance thresholds for one's
155 data with AUTO-TUNE, from calculating pairwise distances to visualizing and annotating results.

2.4   Visualization

157   Visualizations of AUTO-TUNE results are accessible at http://autotune.datamonkey.org/analyze. It is a
158 dynamic and interactive web-based platform that offers visualization and analysis of results generated by
159 AUTO-TUNE. The website provides a comprehensive view of the data by generating various plots across
160 candidate distance thresholds. These include a score plot, allowing users to identify trends and anomalies
161 across the full range of thresholds. Additionally, it generates a graph showing the number of clusters
162 across candidate thresholds, one of the components that contribute to the score. The site also includes
163 an R1/R2 plot that displays the ratio of the largest cluster to the second largest cluster across candidate
164 thresholds, which is the other metric that contributes to the scoring heuristic.

165   An assortativity tool is available at http://autotune.datamonkey.org/assortativity, and is an advanced an-
166 alytical tool engineered to facilitate the calculation of Degree Weighted Homophily (DWH) values. It
167 utilizes the DWH NPM package to generate a tabular representation of DWH values corresponding to
168 each value for a selected attribute annotation, providing an exhaustive examination of the interrelation-
169 ships for the field. A notable feature is the computation of the panmictic range, which involves a label
170 permutation test to generate the null distribution of DWH values. This feature establishes a comparative
171 baseline that aids in determining the significance of homophily versus what would be expected by chance.
172 Lastly, the site also provides a plot of the fraction of pairwise connections, normalized by degree, for
173 each value pertinent to the selected field. This visual depiction facilitates an intuitive comprehension of
174 the distribution and interconnections within the dataset.

175   The site aims to offer a user-friendly interface for data visualization, playing an important role in in-
176 terpreting and understanding AUTO-TUNE's output data. The visualization code is available on Github
177 (https://github.com/stevenweaver/autotune-app/).

2.5   Comparisons with previously published analyses

179   In conducting our comparisons with the established clustuneR method, we procured our datasets from
180 Wolf et al. (2017) and Vrancken et al. (2017) utilizing the identical approach delineated in Chato et al.
181 (2020). These datasets, namely Middle Tennessee, Seattle, and Alberta, were processed using the work-
182 flow prescribed in Section 2.3. This enabled us to determine an optimal threshold for each dataset using
183 our proposed method, AUTO-TUNE. We further executed the command as detailed in step 4 of Section
184 2.3, deploying thresholds previously established as optimal by Chato et al. (2020).

185 To perform comparisons, we computed the average degree-weighted homophily score over a set of three-
186 year sliding windows. Specifically, the homophily among nodes was calculated for a collection of date
187 ranges as follows:

$$\bar{H} = \frac{1}{N} \sum_{i=1}^{N} H(w_i) \tag{2}$$

188 where $\bar{H}$ represents the average degree-weighted homophily score, N is the total number of sliding
189 windows, $H(w_i)$ is the homophily score for the i-th window, and the windows w_i correspond to the date
190 ranges, e.g., '2012-2015', '2013-2016', '2014-2017', etc. This methodology allowed us to compare the
191 "best thresholds" derived from our proposed AUTO-TUNE method against those defined as optimal in
192 Chato et al. (2020).

193 Second, we set out to compare the thresholds obtained in original investigations with those obtained
194 by AUTO-TUNE. To select the data sets for this analysis, we conducted a scientific literature search to
195 identify studies focused on HIV networks for public health purposes. We then filtered the studies that
196 utilized HIV-TRACE to infer genetic networks and had publicly available sequences. Thus, we attempted
197 to include studies from different countries and regions, enabling us to assess the performance of our
198 method across various epidemic contexts, risk groups, and network sizes in real-data sets that used variable
199 clustering thresholds.

200 In order to evaluate the influence of sampling density on the genetic distance threshold as determined
201 by AUTO-TUNE, we implemented a strategy of random subsampling from the original dataset sourced
202 from Rhee et al. (2019). This study was selected due to its satisfactory AUTO-TUNE score when utilized
203 in its entirety, as well as its inherent design as a Geographically-Stratified set of 716 Pol Subtype/CRF
204 (GSPS) reference sequence dataset. The dataset, which comprises 6034 samples gathered between 1959
205 and 2016, was subjected to random subsampling ten times at proportions of 25%, 50%, and 75% of the
206 original sample size. For each subsample, the optimal threshold and associated scores were determined
207 via AUTO-TUNE.

3 RESULTS

3.1 Comparison with clustuneR

209 We compared results to clustuneR, which employs the recency of sample collection or diagnosis as
210 individual-level weights in a predictive model to estimate the growth of HIV clusters. The thresholds
211 determined optimal by clustuneR were found by finding the minimum GAIC (generalized AIC) across
212 candidate distances between 0 and 0.04 in steps of 8×10^{-4}. GAIC is the difference between a null
213 model that is only influenced by cluster size, and a weighted model model that includes individual-level
214 attributes among known cases in the cluster. Using the minimum GAIC metric, it was found that 0.016
215 was the optimal threshold for Tennessee and Seattle, and 0.0104 for Northern Alberta.

216 In contrast, AUTO-TUNE does not incorporate any attribute data in its scoring heuristic. Instead, it
217 relies on clustering metrics constructed purely from pairwise distances between sequences. Using the
218 same datasets analyzed by clustuneR Chato et al. (2020), AUTO-TUNE found the thresholds with the
219 highest scores to be 0.01431 for Middle Tennessee, 0.01354 for Seattle, and 0.01099 for Northern Alberta.
220 Table 1

Performance of the inferred optimal thresholds were performed using an average degree-weighted homophily (DWH) score across 3-year collection date windows starting from the oldest collection year for each respective dataset, as that is the metadata that was consistently available across all three datasets and was the attribute of focus used by clustuneR. DWH in this case measures the affinity for nodes within the network to link with other nodes in the same collection date window. For example, samples collected between 2012-2015 linking more often with other samples within the same time window would result in a higher DWH score. It was found that, across all three datasets, using the threshold with the highest score reported by AUTO-TUNE resulted in a higher average DWH score across all three datasets.

When reviewing scores across all candidate thresholds with AUTO-TUNE . none of the three datasets reached one confident score over any other. The most confident score was achieved by the Seattle dataset at 1.53325, then Tennessee with a high score of 1.25807, and lastly Canada at 1.01678. When finding peaks, a second peak in Seattle denotes that 0.01166 may also be an optimal threshold to consider, as its score, 1.52976, is only 0.003 less than the highest score. The optimal score for Tennessee and Canada are a bit more dubious, as there are multiple peaks within close scores of each other. Indeed, after applying a 0.75 minimum score threshold after visual inspection for peak ranges, standard deviation among scores were 0.0475, 0.0089, and 0.0084 for Seattle, northern Alberta, and Tennessee, respectively. This implies there may be multiple thresholds that would be considered reasonable, and downstream homophily metrics with attributes may aid in coming to a decision.

3.2 Comparison with Prior Publications Citing HIV-TRACE

Next, we curated publications citing HIV-TRACE that also had publicly accessible data associated with the study Rhee et al. (2019); Brenner et al. (2021); H et al. (2021); Liu et al. (2020); Bbosa et al. (2020); Yan et al. (2020); Dalai et al. (2018); Sivay et al. (2018). We found that a variety of different ways were used to determine distance thresholds, from precedent set by the CDC for detecting recent and rapid clusters Yan et al. (2020), because they've been used before in other studies Sivay et al. (2018), to visual inspection of number of clusters and nodes across candidate distance thresholds Liu et al. (2020). As a result, it was found that since these thresholds are determined largely qualitatively, the distance thresholds used tend to be round numbers and only somewhat tuned to their respective geographic region of research. Table 2 When utilizing AUTO-TUNE for the same datasets, more granularity in selecting thresholds can be performed in a straightforward manner. While it may seem appealing to assume that being within a 0.5% threshold of what AUTO-TUNE considers optimal is good enough, further inspection into results reveals that scores can vary widely within a very short distance. For example, when reviewing the distribution of scores for the dataset used by Dalai et al. (2018), the score is exactly 2 at 0.01848, but at thresholds tested at just 0.00002 difference, scores drop preciptously to 1.638 and 0.826 for candidates 0.01846 and 0.0185, respectively. Another example of a seemingly close threshold yet perhaps not optimal is found with Bbosa et al. (2020). While no score across candidate thresholds was found to be above 1.9, a high score was found at distance 1.707, with 1.2415. Contrast this with the threshold used, 1.5%, with a score of 0.0124.

3.3 Evaluating Performance with DWH

To evaluate the performance of an AUTO-TUNEd optimized threshold using degree-weighted homophily, we first evaluated a CRF07_BC network with data from China. 8178 HIV-1 CRF07_BC pol sequences were used from China to construct longitudinal transmission networks, each pol sequence were annotated with risk factor detailing whether the patient was heterosexual (Hetero), Person With Injected Drug Use (PWID), or Men who have Sex with Men (MSM), among other attributes. Using AUTO-TUNE,

no distance threshold receives a score above 1.9, but using the default 1.5% threshold is clearly suboptimal . Using a 1.5% threshold, the network captures 5923 nodes, of which 559 are PWID, 3371 MSM, 1993 Hetero, and received an AUTO-TUNE score of 0.029. When evaluating DWH among risk factors, MSM, Hetero, and PWID had scores of 0.211, 0.133, and 0.168, respectively.

When using AUTO-TUNE, two separate ranges appear. The highest score is obtained with distance threshold 0.76% at 1.1369. The second highest score is 1.0303 at distance threshold 0.0019. Networks at both thresholds were also evaluated with DWH based on risk factor. The network at 0.76% captured 3537 nodes, of which 236 are PWID, 2271 MSM, 1030 Hetero. When evaluating DWH among risk factors, MSM and Hetero both had slightly increased scores of 0.237 and 0.185, respectively. PWID DWH dramatically increased to 0.401. The network at 0.19% captures 1654 nodes, of which 151 are PWID, 1075 MSM, 428 Hetero. When evaluating DWH among risk factors, MSM, Hetero, and PWID had slightly increased scores over 0.76% of 0.292, 0.25, and 0.445, respectively.

We next evaluated Rhee et al. (2019) with DWH. The dataset received a clear optimal threshold of 0.01699 with an AUTO-TUNE score of 1.9998. At the default threshold of 1.5%, the AUTO-TUNE score was 0.9782. At threshold 1.5%, the network captured 1351 nodes, compared with 1592 nodes out of 6034 captured at threshold 1.699%. When evaluating DWH with country, substantial change were only found for China and Thailand, improving from no better than random linkage at threshold 1.5% with DWH -0.166 and -0.051, to 0.116 and 0.132 at threshold 1.699%, respectively. Notably, no country scored worse with the optimal AUTO-TUNE threshold, despite it being larger than the default 1.5%.

3.4 Subsampling's Effect on Optimal Thresholds and AUTO-TUNE Scores

Next, we evaluated the performance of AUTO-TUNE when subsampling a dataset. Since the Rhee et al. (2019) dataset exhibited a clear optimal peak, we used the dataset for analysis, and randomly sampled 10 times from the entire dataset at 25%, 50%, and 75% each. The original full dataset confidently determined 0.01699 (AUTO-TUNE score 1.9998).

Sampling at 25% yielded a mean top threshold of 0.021509, median at 0.019765, and standard deviation of 0.004388 . 50% yielded 0.018581 and 0.01871 mean and median, respectively with a standard deviation of 0.001629. Finally, 75% calculated mean is approximately 0.017403, with a median of approximately 0.01699. The standard deviation was 0.000924.

As the proportion increased from 25% to 50% and 75%, observable shifts were also noted in the mean, median, and standard deviation of the AUTO-TUNE scores. At 25%, the mean and median scores were 1.5585 and 1.5014 respectively, with a standard deviation of 0.3568. At 50%, both mean and median scores significantly increased to 1.8171 and 1.9191 respectively, and the standard deviation dropped to 0.2482. Upon reaching an AUTO-TUNE of 75%, the mean and median scores rose further to 1.9870 and 1.9997 respectively, while the standard deviation shrank substantially to 0.0364, indicating higher consistency in scores.

As the sample proportion increased, an upward trend was noted in average AUTO-TUNE scores. Additionally, standard deviation reduced significantly with sample proportion. This implies that as sampling becomes denser, AUTO-TUNE will become more confident in determining the optimal threshold for a particular dataset.

4 DISCUSSION

303 The results of our study suggest that AUTO-TUNE, which relies solely on clustering metrics from pair-
304 wise distances, could be an effective alternative to the clustuneR methodology. AUTO-TUNE generated
305 thresholds for all three examined datasets (Middle Tennessee, Seattle, and Northern Alberta) that outper-
306 formed clustuneR using DWH on 3-year collection date windows across all three datasets. This indicates
307 that even without incorporating attribute data, AUTO-TUNE's scoring heuristic could provide reliable
308 thresholds for HIV clusters.

309   Our evaluation of publications citing HIV-TRACE revealed the largely qualitative determination of dis-
310 tance thresholds. This approach may result in less accurate or suboptimal thresholds due to a lack of
311 systematic analysis. In contrast, AUTO-TUNE offers a more systematic and granular approach to thresh-
312 old selection, with our findings demonstrating that even minor adjustments to the distance can drastically
313 change the score. Therefore, using AUTO-TUNE could potentially improve the quality of HIV clustering
314 and transmission network studies.

315   The Degree-Weighted Homophily (DWH) evaluation showed that AUTO-TUNE could improve net-
316 work quality based on specific attributes, such as risk factor, which is an important part of HIV studies
317 and informing prevention measures. **?** For example, the use of AUTO-TUNE resulted in an increased
318 DWH among the MSM, Hetero, and PWID groups when analyzing a CRF07_BC network. Addition-
319 ally, the results from the Rhee et al. dataset also demonstrated AUTO-TUNE's ability to improve DWH
320 geographically, enhancing the network's ability to accurately reflect transmission dynamics.

321   Our analysis of AUTO-TUNE's performance on subsamples of a dataset revealed its sensitivity to
322 sample size. The results indicated a correlation between increased sample size and higher average AUTO-
323 TUNE scores, as well as lower score variability. This suggests that denser sampling could enhance
324 AUTO-TUNE's ability to determine the optimal threshold for a dataset. Further studies might be needed
325 to establish the minimum sample size required for reliable threshold determination.

4.1 When a Score is Below Two

5 CONCLUSION

327 AUTO-TUNE operates solely utilizing genetic sequence data to ascertain a decisive threshold. It employs
328 a scoring heuristic, which is based on the number of clusters produced by a pairwise distance threshold
329 and the ratio of the largest cluster to the second largest across a range of possible thresholds using sliding
330 windows.

331   A key advantage of this approach is its autonomy from supplementary data. When a patient tests positive
332 for HIV, data collection protocols can greatly vary, and additional data are not always available or con-
333 sistent. However, by leveraging only genetic sequence data, AUTO-TUNE eliminates the need for such
334 information.

335   Consequently, AUTO-TUNE's performance is consistently controlled, irrespective of the fluctuations
336 seen in data collection protocols after a positive HIV diagnosis. This level of adaptability demonstrates its
337 suitability for integration into various contexts related to HIV, and possibly other viral cluster detection and
338 response protocols. This versatility underscores the strong methodological foundation of AUTO-TUNE
339 and its potential utility.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

The Author Contributions section is mandatory for all articles, including articles by sole authors. If an appropriate statement is not provided on submission, a standard one will be inserted during the production process. The Author Contributions statement must describe the contributions of individual authors referred to by their initials and, in doing so, all authors agree to be accountable for the content of the work. Please see here for full authorship criteria.

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SUPPLEMENTAL DATA

Supplementary Material should be uploaded separately on submission, if there are Supplementary Figures, please include the caption in the same file as the figure. LaTeX Supplementary Material templates can be found in the Frontiers LaTeX folder.

DATA AVAILABILITY STATEMENT

Data are available at GenBank accession numbers JX160108-JX161480,JX498971-JX498972,JX498976-JX498990,JX498992-JX499018,KU190031-KU190839,KY34691-KY37792,KY883695-KY883762,KY888784-KY888875,KY921717-KY921757,MG434786-MG435347,MG435358-MG436769,MH352627-MH355541,MK2:MK25548,MN424584-MN427369,MT336755-MT336776,MT368043-MT369927.

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TABLES

6 FIGURE CAPTIONS

Table 1. clustuneR Comparison

Dataset	clustuneR		AUTO-TUNE		
	Threshold	Avg. Homophily	Threshold	Avg. Homophily	Max Score
Middle Tennessee	0.0160	0.0079	0.01431	0.0147	1.25807
Seattle	0.0160	0.0259	0.01354	0.0348	1.53325
Northern Alberta	0.0104	-0.0536	0.01099	-0.0448	1.01678

Table 2. Threshold Comparison with Prior Publications Citing HIV-TRACE

PMID	Country	Collection Date	Threshold Used	AUTO-TUNE
29975689	South Africa	2011-2015	2.5%	2.584%
30574123	USA	1997-2008	2%	1.848%
32500089	China	2008-2015	0.5%	0.675%
32693608	Uganda	2009-2016	1.5%	1.707%
33281803	China	2000-2016	0.5%/0.7%	0.676%
33901684	China	2008-2012	1.5%	1.215%
34452506	Canada	1996-2017	1.5%/2.5%	0.547%
31041344	USA	1997-2017	1.5%	0.927%

Table 3. CRF07_BC DWH and Panmictic Range at Different Thresholds

Record	Threshold 1.5%		Threshold 0.76%		Threshold 0.19%	
	DWH	Panmictic Range	DWH	Panmictic Range	DWH	Panmictic Range
MSM	0.211	[-0.105, -0.205]	0.237	[-0.120,-0.240]	0.292	[-0.146, -0.280]
Hetero	0.133	[-0.092, -0.190]	0.185	[-0.100,-0.211]	0.25	[-0.093, -0.256]
PWID	0.168	[-0.001, -0.089]	0.401	[-0.005,-0.081]	0.445	[-0.012, -0.129]

Figure 1. Hola

Figure 2. Hola 2

Figure 3. Hola 3

Figure 4. Hola 4

Figure 5. Hola 5