HIV-TRACE (Transmission Cluster Engine): a tool for large scale molecular epidemiology of HIV-1 and other rapidly evolving pathogens.

Sergei L. Kosakovsky Pond,[1] Steven Weaver,[1], Andrew J. Leigh Brown[2], and Joel O. Wertheim[3,*]

[1]Institute for Genomics and Evolutionary Medicine, Temple University, Philadelphia, PA, USA,

[2]Institute of Evolutionary Biology, University of Edinburgh, United Kingdom.

[3]Department of Medicine, University of California, San Diego, CA, USA.

***Corresponding author:** E-mail: jwertheim@ucsd.edu

Associate Editor: TBD

Abstract

In modern applications of molecular epidemiology, genetic sequence data are routinely used to identify clusters of transmission in rapidly evolving pathogens, most notably HIV-1. Traditional 'shoe-leather' epidemiology infers transmission clusters by tracing chains of partners sharing epidemiological connections (e.g., sexual contact). Here, we present a computational tool for identifying a molecular transmission analog of such clusters: `HIV-TRACE` (TRAnsmission Cluster Engine). `HIV-TRACE` implements an approach inspired by traditional epidemiology, by identifying chains of partners whose viral genetic relatedness imply direct or indirect epidemiological connections. Molecular transmission clusters are constructed using codon-aware pairwise alignment to a reference sequence followed by pairwise genetic distance estimation among all sequences. This approach is computationally tractable and is capable of identifying HIV-1 transmission clusters in large surveillance databases comprising tens or hundreds of thousands of sequences in near real time, i.e., on the order of minutes to hours. `HIV-TRACE` is available at `www.hivtrace.org` and from `github.com/veg/hivtrace`, along with the accompanying result visualization module from `github.com/veg/hivtrace-viz`. Importantly, the approach underlying `HIV-TRACE` is not limited to the study of HIV-1 and can be applied to study outbreaks and epidemics of other rapidly evolving pathogens.

Key words: molecular epidemiology; HIV; network; transmission cluster; surveillance

Letter

Introduction

Research into fundamental questions of epidemiology and public health, such as "Who infected whom?" (Romero-Severson *et al.*, 2016; Volz and Frost, 2013), "How does pathogen X spread through a population"? (Dennis *et al.*, 2014), and "Is a particular prevention or treatment effective at slowing or stopping the spread of disease?" (Little *et al.*, 2014) has greatly benefited from large-scale analyses of molecular sequences obtained during surveillance or through routine diagnostics. For rapidly

evolving pathogens, such as HIV-1 or hepatitis C virus, viral isolates from different hosts will typically not be genetically identical, and analyses of these genetic differences via phylogenetic, phylodynamic, or other evolutionary methods have proven tremendously powerful. Phylogenetic analyses have been used in criminal cases involving deliberate HIV-1 transmission (Scaduto *et al.*, 2010), to understand the introduction of HIV-1 into regions and countries (Gilbert *et al.*, 2007), and to define recent clusters of transmission cases (Peters *et al.*, 2016). Recent work in the field of phylodynamics has established a template on how to use sequence data to inform inference of epidemiological transmission parameters, e.g., R_0 or transmission rates between different risk groups (Frost and Volz, 2013; Volz and Frost, 2014). The fundamental insight shared by all these methods is that genetic similarity, or relatedness, between pathogen sequences can be used to identify strains that are connected in an epidemiologically meaningful way: as potential source-recipient pairs (Campbell *et al.*, 2011) or members of the a distinct transmission cluster (Campbell *et al.*, 2017; Wertheim *et al.*, 2017a).

Real time or near real time surveillance of pathogen transmission is an area of great interest to local, national, and global public health agencies (Division of HIV/AIDS Prevention, 2017). Real time surveillance seeks to quickly analyze newly obtained pathogen genetic sequences in the context of large, preexisting reference samples and to deliver actionable inference results: "A new rapidly growing HIV-1 transmission cluster has been identified", or "An unusual pattern of transmission between people with different risk factors has been detected", or "An HIV-1 transmission prevention is effectively reducing population level incidence".

Defining molecular transmission clusters is a challenging problem, and currently there is no consensus in the field of molecular epidemiology of what should or should not constitute a transmission cluster or whether certain definitions are more germane to particular research questions or public health interventions (Grabowski and Redd, 2014; Hassan *et al.*, 2017; Novitsky *et al.*, 2017; Wertheim *et al.*, 2014).

Here, we present the algorithmic, software implementation, and operational usage details for `HIV-TRACE`, a platform that has been used extensively for rapid inference of transmission networks from **large** sets of pathogen genetic sequences to identify potential transmission links and to describe putative transmission clusters. An early version of `HIV-TRACE` was used to analyze nearly 100,000 HIV-1 sequences sampled worldwide, and this analysis revealed that there was a surprising amount of global (country-to-country) connectivity in this network (Wertheim *et al.*, 2014). Since then, `HIV-TRACE` has been used to investigate transmission patterns among risk groups (Oster *et al.*, 2015; Whiteside *et al.*, 2015), characterize transmission fitness of HIV

Table 1. Key parameters controlling `HIV-TRACE`

Parameter	Meaning	Phase
`-r`, `--reference`	Reference sequence for mapping	Alignment
`-m`, `--minoverlap`	Sequences must have at least this many aligned characters	Distance estimation
`-a`, `--ambiguities`	Sets policy for handling ambiguous nucleotides	Distance estimation
`-g`, `--fraction`	Sets the maximum fraction of resolvable ambiguous nucleotides	Distance estimation
`-s`, `--strip_drams`	Mask HIV-1 drug resistance associated sites	Distance estimation
`-t`, `--threshold`	Distance threshold for drawing a network link	Network construction
`-u`, `--curate`	Sets policy for handling potential contaminants	Network construction

drug-resistance associated mutations (Wertheim *et al.*, 2017b), and to identify rapidly growing transmission clusters (Campbell *et al.*, 2017; Monterosso *et al.*, 2017).

The source code, installation instruction (via `pip3`), and documentation for `HIV-TRACE` is available at `github.com/veg/hivtrace`, and the accompanying result visualization module – at `github.com/veg/hivtrace-viz`. In addition, a public instance of the `HIV-TRACE` web-application is hosted at `www.hivtrace.org`, as a part of the Datamonkey family of services (Weaver *et al.*, 2018).

New Approaches

`HIV-TRACE` does not infer a phylogenetic tree from sequence data because phylogenetic inference is a computational bottleneck and because the phylogenies themselves are typically not directly useful for epidemiological inference. In most applications, phylogenies are converted to summary features (e.g., clades) or summary statistics (e.g., patristic distances) to identify clusters. In lieu of phylogenetic inference, `HIV-TRACE` identifies groups of putative transmission partners and assembles these partners in transmission clusters. This approach is analogous to the traditional epidemiological definition of an infectious disease transmission cluster: a group of infected people with direct or indirect epidemiological connections. In `HIV-TRACE`, genetic linkage serves as a proxy for these direct or indirect epidemiological connections, and a cluster is constructed based on these connections. This approach is fundamentally different from phylogenetic-based cluster inference (Grabowski and Redd, 2014; Wertheim *et al.*, 2014), which seeks to identify a point in evolutionary history from which all cluster members descend (i.e., a point that gives rise to a clade on a phylogeny). Importantly, several independent studies have shown that in many cases relevant to HIV-1 epidemiology, `HIV-TRACE` reports very similar sets of clusters to phylogeny-based methods (Poon,

2016; Rose *et al.*, 2017b), although whether or not clusters arise due to increased transmission rate or from increased sampling rates or recent transmission is potentially difficult to identify with this (or alternative) approaches (Le Vu *et al.*, 2017; McCloskey and Poon, 2017).

Inference procedure

`HIV-TRACE` takes in a collection of N unaligned coding viral sequences sampled from $M \leq N$ individuals (multiple sequences per individual are supported) formatted as a FASTA file, and it outputs a JSON file containing the description of the inferred transmission network as nodes (individuals) and links (potential transmission partners). When additional clinical, demographic, or other data are available, they can be included in the network as attributes. Key parameters controlling network inference are summarized in Table 1, and the schematic of program flow is depicted in Figure 1.

To demonstrate method performance, we downloaded all publicly available HIV-1 *polymerase* sequence (one sequence per patient, minimum length 500 nt) from the Los Alamos National Laboratories HIV database (`hiv.lanl.gov`), resulting in $N = M = 185{,}849$ sequences. We randomly sampled a set of 256, 1,024, 4,096, 16,384, and 65,536 sequences to plot computational time scaling. We ran each step of the pipeline 10 times (to average out computing environment stochasticity) on a 64-core (2x32 AMD Opteron 6356) system running at 2 GHz clock rate (Figure 1).

Sequence alignment. `HIV-TRACE` first aligns each of the input sequences to a single reference sequence using a codon-aware extension of the Smith-Waterman dynamic programming algorithm (Smith and Waterman, 1981), previously developed by us in the context of high throughput sequencing read mapping (e.g., Gianella *et al.* (2011)). For standard HIV-1 analyses, the HXB2 sequence (GenBank Accession number: K03455) is used as a reference sequence, although any in-frame coding sequence can be supplied as reference. Both the forward and the reverse-complement versions of each sequence are considered, and the one with the higher alignment score is retained. Codon-aware alignment leverages protein homology to align nucleotide data and is able to identify and correct relatively frequent (i.e., up to 5% of sequences in some datasets) frame-shifting insertions or deletions involving one or two nucleotides. In this case, correction means maintaining the frame relative to the reference. The resulting pairwise alignment is merged into a single multiple-sequence alignment (MSA). As the vast majority of HIV-1 sequence data arise from surveillance screening for drug resistance in a 1497 nucleotide *protease* and *reverse transcriptase* genomic region, which only rarely exhibit insertions/deletions relative to the reference

sequence, this "mapping" approach is effective and scales linearly in the number of sequences. Traditional progressive alignment methods have superlinear (e.g., up to quadratic) computational cost. In our example, computational complexity scaled linearly as expected, and the alignment of 185,849 sequences to a reference took about 20 minutes on average. Importantly, `HIV-TRACE` is also capable of handling previously aligned sequences, which may be desirable for analyses of HIV-1 *envelope* sequences or other pathogens with low evolutionary conservation, where "all-to-one" alignment is not likely to recover more distant homologies. However, genes or sequence regions that are challenging to align may be suboptimal for molecular epidemiology applications.

Estimation of genetic distances. Given a multiple sequence alignment on N sequences, `HIV-TRACE` computes all $N \times (N-1)/2$ pairwise genetic distances under the Tamura-Nei 93 (TN93) (Tamura and Nei, 1993) nucleotide substitution model, which is the most general nucleotide substitution model for which distances can be estimated directly from counts of nucleotide pairs in aligned sequences. Whereas more complex models substitution models are typically preferable in the context of phylogenetic inference, especially for more distantly related strains, (Posada and Crandall, 2001), when genetic distances are low (e.g. 0.05), all sensible nucleotide distance measures perform comparably (Wertheim and Kosakovsky Pond, 2011). A key option controlling this step in `HIV-TRACE` is how to handle ambiguous nucleotide characters that represent within-host population polymorphisms or sequencing errors (see Parameterizing genetic distance estimates). An important example of epidemiological processes that yield sequences with high fractions of ambiguous nucleotides is multiple (super- or dual-) HIV infection (Pacold *et al.*, 2010).

Pairwise distances are reported to a comma separated file, and are typically limited only to those pairs that are below a user-specified threshold (e.g., 0.015 substitutions/site) to retain only pairs of sequences that have an epidemiological link. This step is computationally costly, scaling as N^2, but an efficient parallelized implementation of the tool allows rapid processing of $10^5 - 10^6$ sequences. For instance, it took approximately 32 minutes to compute all pairwise distances between 185,849 sequences. Our implementation is also memory efficient, requiring $O(NL)$ space, where L is the sequence length. For datasets of this size, traditional rapid phylogeny reconstruction techniques, such as Neighbor Joining are already infeasible, because they scale as N^3 and require the storage of the entire distance matrix (this would require approximately 256GiB of RAM for our example), which `HIV-TRACE` deliberately avoids. Because most phylogenetic methods for cluster definition require some measure of clade support

(e.g. Grabowski and Redd (2014)), it is also necessary to perform a version of bootstrapping. Our implementation compares favorably to even the fastest tree construction methods, such as FastTree 2 (Price *et al.*, 2010a) or IQ-Tree (Nguyen *et al.*, 2015), which takes at least $10x$ longer to process these sizes of data; for example, typical run times of FastTree 2 (the fastest tool to our knowledge) on $\sim$200,000 sequences is on the order of $10-20$ hours (Price *et al.*, 2010a). It is worth noting that FastTree 2 has an asymptotically better run time $O(N^{3/2}\log N)$, but it does considerably more work than needed for our application (resulting in slower run times), and uses heuristics which are not guaranteed to always find all distances below a certain threshold.

Network construction. The transmission network is inferred from the file of pairwise distances and optionally annotated with data from attribute files. Nodes within the network are all keyed on either the entire sequence name or parts thereof extracted by regular expressions. A link is drawn between two individuals if and only if the pairwise distances between any of the paired sequences from these individuals is below a user specified threshold, D. e.g. $D=0.015$. A cluster is defined as a connected component of the network. Optionally, the network can be screened for contaminants (i.e., any query sequences that link to lab strains or other user-specified contaminant sequences). Global statistics of the network, such as the number of nodes, edges, clusters, cluster sizes, and the degree distribution, are computed and reported. Lastly, the degree distribution is fit to one of four generative models of network growth: random attachment, preferential attachment, preferential attachment mixed with a component of random attachment, and power law, using the methods described by (Handcock and Jones, 2004). If the best fitting model is from a scale-free family (i.e., preferential attachment), the characteristic exponent ρ of the network is estimated and reported. This step is computationally relatively inexpensive, taking only a few seconds.

Parameterizing genetic distance estimates Selecting appropriate parameters governing genetic distance estimation is critical to `HIV-TRACE` analysis. Investigations in the U.S., the U.K., and Canada have consistently found natural breakpoints in genetic distance between putative transmission partners and 'random' cases or within-host and between-host diversity (Lewis *et al.*, 2008; Poon *et al.*, 2015; Rose *et al.*, 2017a; Smith *et al.*, 2009; Wertheim *et al.*, 2017a). In New York City, genetic distance thresholds between 0.01 and 0.02 substitutions/site were more strongly associated with probable transmission partners than traditional epidemiological connections (i.e., naming of sexual and injection drug using partners) and that a distance of 0.015 could serve

as a use proxy for epidemiological relatedness in a surveillance setting (Wertheim *et al.*, 2017a). Moreover, these genetic distance thresholds have been validated by molecular epidemiological studies in U.S. public health surveillance populations (Oster *et al.*, 2015; Wertheim *et al.*, 2016, 2017b; Whiteside *et al.*, 2015), which have reported results that are typically robust to thresholds in this range. Lower distance thresholds (e.g., 0.005 substitutions/site) may be more appropriate for distinguishing rapidly growing clusters (Division of HIV/AIDS Prevention, 2017) or populations where faster evolving (i.e., non-B subtypes) predominate. As distance thresholds increase, smaller clusters merge into larger, less informative clusters Figure 2A. At the extreme, all sequences would belong to a single cluster, which while technically correct, since all HIV-1 sequences are related through a series of transmissions, this finding is unlikely to be of interest in the context of molecular epidemiology. The same principle – that D should separate within-host or epidemiologically recent diversity from between-host diversity has been used successfully for other epidemics, genetic regions and viruses. For example Rose *et al.* (2017b) used $D = 0.053$ for HIV-1 gp41, Bartlett *et al.* (2017) selected $D = 0.03$ for the core gene of Hepatitis C virus. Regional and national epidemics HIV-1 also tend to require larger thresholds due to sparser sampling and the prevalence of chronically infected individuals (Hassan *et al.*, 2017).

Nucleotide ambiguities (e.g., `Y` indicating a mixed population of both `C` and `T` at the same genomic position) have the potential to compromise `HIV-TRACE` analysis, or phylogenetic inference in general. By default, `HIV-TRACE` will resolve (here, to 'resolve' means to choose the value of the ambiguity to match the other nucleotide if possible) the genetic distance between ambiguities (i.e., Y is 0 substitutions from both `C` and `T`). However, sequences with a high fraction of nucleotide ambiguities have the tendency to link to distantly related sequences when ambiguities are resolved, resulting in artifactual larger clusters Figure 2B. When ambiguities are properly accounted for, `HIV-TRACE` clusters tend to resemble clades on a phylogenetic tree Figure 2C. However, when distances ambiguities are resolved irrespective of ambiguity fraction, distantly related sequences are connected through these high ambiguity sequences, forming large artifactual clusters (Figure 2D and Aldous *et al.* (2012)). Therefore, `HIV-TRACE` includes a parameter (ambiguity fraction) that averages the genetic distance from ambiguities (i.e., `Y` is 0.5 substitutions from both `C` and `T`) in sequences with a higher proportion of ambiguities than the indicated ambiguity fraction. In cohorts of fewer than 1000 individuals (i.e., San Diego Primary Infection Cohort), an ambiguity fraction of 0.05 is appropriate based on empirical

network sensitivity analyses. For US surveillance data, an ambiguity fraction above 0.015 produces spurious clusters. As a consequence, sequences with high ambiguity fractions are less likely to cluster using `HIV-TRACE`.

In `HIV-TRACE`, excluding sites containing ambiguities has a similar effect on network construction as resolving ambiguities. Many popular phylogenetic packages used for constructing HIV-1 molecular transmission networks (e.g., BEAST (Drummond *et al.*, 2012) and FastTree (Price *et al.*, 2010b) exclude sites containing ambiguities from likelihood calculations. It remains unclear how treatment of nucleotide ambiguities will affect phylogenetic inference of HIV transmission clusters (Fearnhill *et al.*, 2017).

Visualization

The `JSON` file output by `HIV-TRACE` can be explored using an interactive JavaScript application which we call `hivtrace-viz`. It is based on the open source data visualization library `d3.js`. This application runs within any modern web-browser and provides means to view the overall structure of the network, explore individual clusters, display network summary, and explore associations among attributes for connected nodes. When clinical and demographic attributes are available, they can be overlaid on the network structure as shown in Figure 3.

Software components

Alignment. `bealign` is implemented in `Python 3` as a part of `BioExt` library (`github.com/veg/BioExt`) which extends the functionality of the popular `BioPython` library (Cock *et al.*, 2009). The core alignment routine is implemented in `C` and incorporated via `Cython`. When the program is run in a multicore/multiprocessing environment, it will distribute alignment tasks across cores.

Distance calculation. `tn93` is a self-contained `C++` program (available from `github.com/veg/tn93`) which is tuned to allow $\sim 10^5 - 10^6$ distance calculations per second per core on $\sim$1000 bp long sequences. It uses `OpenMP` to distribute distance calculations across multiple CPU cores whenever possible. For example, `tn93` achieved parallelized (64 cores) throughput of $\sim 10^7$ pairwise distance calculations per second when computing distances on the LANL example dataset.

Network inference. `hivnetworkcsv` is a `Python 3` module, which is available from `github.com/veg/hivclustering`, along with the attendant documentation.

Concluding Remarks

`HIV-TRACE` is a powerful computational tool for the rapid and automated characterization of molecular transmission clusters in populations of HIV infected individuals. Its applicability for HIV research and public health surveillance and prevention activities is apparent, as first

illustrated by the unsupervised recovery of many previously characterized clusters (defined via phylogenetic analyses) in our global-scale analysis of HIV-1 databases (Wertheim *et al.*, 2014). As viral sequence sequence databases increase in size and transition to using Next Generation Sequencing (NGS) data, scalable tools like `HIV-TRACE` will be increasingly relevant.

`HIV-TRACE` can accommodate NGS data in three different ways . Firstly, NGS data can be used to generate a consensus sequence for each individual, which is then handled the same way as Sanger sequences are now. Phylogenetic approaches most commonly use this route, and `HIV-TRACE` has already been used in this context (Rose *et al.*, 2017b). Secondly NGS reads could be converted into a smaller collection of individual haplotypes; `HIV-TRACE` can directly handle multiple sequences per individual, and supports two mode of drawing links between individuals A and B: single linkage (at least one pair of sequences from A and B are closer than D substitutions per site) or complete linkage (all pairs of sequences are closer than D substitutions per site. Lastly, for NGS amplicon data that have been mapped to the reference, `HIV-TRACE` can be used to quickly compute the distribution of genetic distances between reads from individuals A and B; links can then be drawn if the distribution meets a particular condition, for example, at least $X\%$ of read pairs are closer than D substitutions per site.

In addition to extensive applications in the HIV-1 domain, `HIV-TRACE` has demonstrated utility for other pathogens including acute hepatitis C virus infection (Bartlett *et al.*, 2017; Rose *et al.*, 2017a) and norovirus (Drumright *et al.*, 2014). As any computational tool, `HIV-TRACE` has advantages and drawbacks. Speed, easy to understand clusters definitions, persistence of clusters when more sequences are added, robustness to recombination, and systematic handling of mixed bases count among the former. The latter include the difficulty in interpreting what variables drive cluster formation and growth, inability to ascertain that any particular link is a direct transmission (i.e., source attribution), and loss of information contained in the phylogenetic tree, including timing (which can be leveraged by molecular clock methods), and branching (which can be taken advantage of by phylodynamics methods). For most rigorous analyses, clusters identified by `HIV-TRACE` are further analyzed using compute-intensive molecular clock phylogenetic inference tools (e.g., BEAST; Drummond *et al.* (2012)) (Chaillon *et al.*, 2017; Wertheim *et al.*, 2016, 2017b). By using `HIV-TRACE` first to identify transmission cluster of interest, these more computationally intensive tools can be reserved for smaller, focused analyses.

Acknowledgments

This work was supported in part by grants R01 AI134384 (NIH/NIAID), R01 GM093939 (NIH/NIGMS) and U01 GM110749 (NIH/NIGMS). JOW was funded by an NIH-NIAID Career Development Award (K01AI110181) and the California HIV/AIDS Research Program (ID15-SD-052). We thank N. Lance Hepler for his work on the initial development of `HIV-TRACE`.

References

Aldous, J. L., Pond, S. K., Poon, A., Jain, S., Qin, H., Kahn, J. S., Kitahata, M., Rodriguez, B., Dennis, A. M., Boswell, S. L., Haubrich, R., and Smith, D. M. 2012. Characterizing HIV transmission networks across the united states. *Clin Infect Dis*, 55(8): 1135–43.

Bartlett, S. R., Wertheim, J. O., Bull, R. A., Matthews, G. V., Lamoury, F. M., Scheffler, K., Hellard, M., Maher, L., Dore, G. J., Lloyd, A. R., Applegate, T. L., and Grebely, J. 2017. A molecular transmission network of recent hepatitis C infection in people with and without HIV: Implications for targeted treatment strategies. *J Viral Hepat*, 24(5): 404–411.

Campbell, E. M., Jia, H., Shankar, A., Hanson, D., Luo, W., Masciotra, S., Owen, S. M., Oster, A. M., Galang, R. R., Spiller, M. W., Blosser, S. J., Chapman, E., Roseberry, J. C., Gentry, J., Pontones, P., Duwve, J., Peyrani, P., Kagan, R. M., Whitcomb, J. M., Peters, P. J., Heneine, W., Brooks, J. T., and Switzer, W. M. 2017. Detailed transmission network analysis of a large opiate-driven outbreak of HIV infection in the United States. *J Infect Dis*, (in press).

Campbell, M. S., Mullins, J. I., Hughes, J. P., Celum, C., Wong, K. G., Raugi, D. N., Sorensen, S., Stoddard, J. N., Zhao, H., Deng, W., Kahle, E., Panteleeff, D., Baeten, J. M., McCutchan, F. E., Albert, J., Leitner, T., Wald, A., Corey, L., Lingappa, J. R., and Partners in Prevention HSV/HIV Transmission Study Team 2011. Viral linkage in HIV-1 seroconverters and their partners in an HIV-1 prevention clinical trial. *PLoS One*, 6(3): e16986.

Chaillon, A., Avila-Ríos, S., Wertheim, J. O., Dennis, A., García-Morales, C., Tapia-Trejo, D., Mejía-Villatoro, C., Pascale, J. M., Porras-Cortés, G., Quant-Durán, C. J., Lorenzana, I., Meza, R. I., Palou, E. Y., Manzanero, M., Cedillos, R. A., Reyes-Terán, G., Mehta, S. R., and Mesoamerican Project Group 2017. Identification of major routes of HIV transmission throughout Mesoamerica. *Infect Genet Evol*, 54: 98–107.

Cock, P. J. A., Antao, T., Chang, J. T., Chapman, B. A., Cox, C. J., Dalke, A., Friedberg, I., Hamelryck, T., Kauff, F., Wilczynski, B., and de Hoon, M. J. L. 2009. Biopython: freely available Python tools for computational molecular biology and bioinformatics. *Bioinformatics*, 25(11): 1422–3.

Dennis, A. M., Herbeck, J. T., Brown, A. L., Kellam, P., de Oliveira, T., Pillay, D., Fraser, C., and Cohen, M. S. 2014. Phylogenetic studies of transmission dynamics in generalized HIV epidemics: an essential tool where the burden is greatest? *J Acquir Immune Defic Syndr*, 67(2): 181–95.

Division of HIV/AIDS Prevention 2017. Detecting, investigating, and responding to HIV transmission clusters. Technical report, Centers for Disease Control and Prevention.

Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol*, 29(8): 1969–73.

Drumright, L. N., Leigh Brown, A. L., and Frost, S. D. W. 2014. The global circulation of norovoris GII.3 and GII.4. In *21st International HIV Dynamics and Evolution Conference*.

Fearnhill, E., Gourlay, A., Malyuta, R., Simmons, R., Ferns, R. B., Grant, P., Nastouli, E., Karnets, I., Murphy, G., Medoeva, A., Kruglov, Y., Yurchenko, A., Porter,

K., and CASCADE Collaboration in EuroCoord 2017. A phylogenetic analysis of HIV-1 sequences in Kiev: Findings among key populations. *Clin Infect Dis*.

Frost, S. D. W. and Volz, E. M. 2013. Modelling tree shape and structure in viral phylodynamics. *Philos Trans R Soc Lond B Biol Sci*, 368(1614): 20120208.

Gianella, S., Delport, W., Pacold, M. E., Young, J. A., Choi, J. Y., Little, S. J., Richman, D. D., Kosakovsky Pond, S. L., and Smith, D. M. 2011. Detection of minority resistance during early HIV-1 infection: natural variation and spurious detection rather than transmission and evolution of multiple viral variants. *J Virol*, 85(16): 8359–67.

Gilbert, M. T. P., Rambaut, A., Wlasiuk, G., Spira, T. J., Pitchenik, A. E., and Worobey, M. 2007. The emergence of HIV/AIDS in the Americas and beyond. *Proc Natl Acad Sci U S A*, 104(47): 18566–70.

Grabowski, M. K. and Redd, A. D. 2014. Molecular tools for studying HIV transmission in sexual networks. *Curr Opin HIV AIDS*, 9(2): 126–33.

Handcock, M. S. and Jones, J. H. 2004. Likelihood-based inference for stochastic models of sexual network formation. *Theor Popul Biol*, 65(4): 413–22.

Hassan, A. S., Pybus, O. G., Sanders, E. J., Albert, J., and Esbjörnsson, J. 2017. Defining HIV-1 transmission clusters based on sequence data. *AIDS*, 31(9): 1211–1222.

Le Vu, S., Ratmann, O., Delpech, V., Brown, A. E., Gill, O. N., Tostevin, A., Fraser, C., and Volz, E. M. 2017. Comparison of cluster-based and source-attribution methods for estimating transmission risk using large HIV sequence databases. *Epidemics*.

Lewis, F., Hughes, G. J., Rambaut, A., Pozniak, A., and Leigh Brown, A. J. 2008. Episodic sexual transmission of HIV revealed by molecular phylodynamics. *PLoS Med*, 5(3): e50.

Little, S. J., Kosakovsky Pond, S. L., Anderson, C. M., Young, J. A., Wertheim, J. O., Mehta, S. R., May, S., and Smith, D. M. 2014. Using HIV networks to inform real time prevention interventions. *PLoS One*, 9(6): e98443.

McCloskey, R. M. and Poon, A. F. Y. 2017. A model-based clustering method to detect infectious disease transmission outbreaks from sequence variation. *PLoS Comput Biol*, 13(11): e1005868.

Monterosso, A., Minnerly, S., Goings, S., Morris, A., France, A. M., Dasgupta, S., Oster, A., and Fanning, M. 2017. Identifying and investigating a rapidly growing HIV transmission cluster in Texas. In *Conference on Retroviruses and Opportunistic Infections*, page 845LB.

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., and Minh, B. Q. 2015. Iq-tree: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*, 32(1): 268–74.

Novitsky, V., Moyo, S., and Essex, M. 2017. Phylogenetic inference of hiv transmission clusters. *Infectious Diseases and Translational Medicine*, 3(2): 51–59.

Oster, A. M., Wertheim, J. O., Hernandez, A. L., Ocfemia, M. C. B., Saduvala, N., and Hall, H. I. 2015. Using molecular HIV surveillance data to understand transmission between subpopulations in the United States. *J Acquir Immune Defic Syndr*, 70(4): 444–51.

Pacold, M., Smith, D., Little, S., Cheng, P. M., Jordan, P., Ignacio, C., Richman, D., and Pond, S. K. 2010. Comparison of methods to detect HIV dual infection. *AIDS Res Hum Retroviruses*, 26(12): 1291–8.

Peters, P. J., Pontones, P., Hoover, K. W., Patel, M. R., Galang, R. R., Shields, J., Blosser, S. J., Spiller, M. W., Combs, B., Switzer, W. M., Conrad, C., Gentry, J., Khudyakov, Y., Waterhouse, D., Owen, S. M., Chapman, E., Roseberry, J. C., McCants, V., Weidle, P. J., Broz, D., Samandari, T., Mermin, J., Walthall, J., Brooks, J. T., Duwve, J. M., and Indiana HIV Outbreak Investigation Team 2016. HIV infection linked to injection use of oxymorphone in Indiana, 2014-2015. *N Engl J Med*, 375(3): 229–39.

Poon, A. F. Y. 2016. Impacts and shortcomings of genetic clustering methods for infectious disease outbreaks.

Virus Evol, 2(2): vew031.

Poon, A. F. Y., Joy, J. B., Woods, C. K., Shurgold, S., Colley, G., Brumme, C. J., Hogg, R. S., Montaner, J. S. G., and Harrigan, P. R. 2015. The impact of clinical, demographic and risk factors on rates of HIV transmission: a population-based phylogenetic analysis in British Columbia, Canada. *J Infect Dis*, 211(6): 926–35.

Posada, D. and Crandall, K. A. 2001. Selecting models of nucleotide substitution: an application to human immunodeficiency virus 1 (HIV-1). *Mol Biol Evol*, 18(6): 897–906.

Price, M. N., Dehal, P. S., and Arkin, A. P. 2010a. Fasttree 2–approximately maximum-likelihood trees for large alignments. *PLoS One*, 5(3): e9490.

Price, M. N., Dehal, P. S., and Arkin, A. P. 2010b. FastTree 2–approximately maximum-likelihood trees for large alignments. *PLoS One*, 5(3): e9490.

Romero-Severson, E. O., Bulla, I., and Leitner, T. 2016. Phylogenetically resolving epidemiologic linkage. *Proc Natl Acad Sci U S A*, 113(10): 2690–5.

Rose, R., Lamers, S. L., Massaccesi, G., Osburn, W., Ray, S. C., Thomas, D. L., Cox, A. L., and Laeyendecker, O. 2017a. Complex patterns of Hepatitis-C virus longitudinal clustering in a high-risk population. *Infect Genet Evol*, 58: 77–82.

Rose, R., Lamers, S. L., Dollar, J. J., Grabowski, M. K., Hodcroft, E. B., Ragonnet-Cronin, M., Wertheim, J. O., Redd, A. D., German, D., and Laeyendecker, O. 2017b. Identifying transmission clusters with Cluster Picker and HIV-TRACE. *AIDS Res Hum Retroviruses*, 33(3): 211–218.

Scaduto, D. I., Brown, J. M., Haaland, W. C., Zwickl, D. J., Hillis, D. M., and Metzker, M. L. 2010. Source identification in two criminal cases using phylogenetic analysis of HIV-1 DNA sequences. *Proc Natl Acad Sci U S A*, 107(50): 21242–7.

Smith, D. M., May, S. J., Tweeten, S., Drumright, L., Pacold, M. E., Kosakovsky Pond, S. L., Pesano, R. L., Lie, Y. S., Richman, D. D., Frost, S. D. W., Woelk, C. H., and Little, S. J. 2009. A public health model for the molecular surveillance of HIV transmission in San Diego, California. *AIDS*, 23(2): 225–32.

Smith, T. F. and Waterman, M. S. 1981. Identification of common molecular subsequences. *J Mol Biol*, 147(1): 195–7.

Tamura, K. and Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol*, 10(3): 512–26.

Volz, E. M. and Frost, S. D. W. 2013. Inferring the source of transmission with phylogenetic data. *PLoS Comput Biol*, 9(12): e1003397.

Volz, E. M. and Frost, S. D. W. 2014. Sampling through time and phylodynamic inference with coalescent and birth-death models. *J R Soc Interface*, 11(101): 20140945.

Weaver, S., Shank, S. D., Spielman, S. J., Li, M., Muse, S. V., and Kosakovsky Pond, S. L. 2018. Datamonkey 2.0: a modern web application for characterizing selective and other evolutionary processes. *Mol Biol Evol*.

Wertheim, J. O. and Kosakovsky Pond, S. L. 2011. Purifying selection can obscure the ancient age of viral lineages. *Mol Biol Evol*, 28(12): 3355–65.

Wertheim, J. O., Leigh Brown, A. J., Hepler, N. L., Mehta, S. R., Richman, D. D., Smith, D. M., and Kosakovsky Pond, S. L. 2014. The global transmission network of HIV-1. *J Infect Dis*, 209(2): 304–13.

Wertheim, J. O., Oster, A. M., Hernandez, A. L., Saduvala, N., Bañez Ocfemia, M. C., and Hall, H. I. 2016. The international dimension of the U.S. HIV transmission network and onward transmission of HIV recently imported into the United States. *AIDS Res Hum Retroviruses*, 32(10-11): 1046–1053.

Wertheim, J. O., Kosakovsky Pond, S. L., Forgione, L. A., Mehta, S. R., Murrell, B., Shah, S., Smith, D. M., Scheffler, K., and Torian, L. V. 2017a. Social and genetic

networks of HIV-1 transmission in New York City. *PLoS Pathog*, 13(1): e1006000.

Wertheim, J. O., Oster, A. M., Johnson, J. A., Switzer, W. M., Saduvala, N., Hernandez, A. L., Hall, H. I., and Heneine, W. 2017b. Transmission fitness of drug-resistant HIV revealed in a surveillance system transmission network. *Virus Evol*, 3(1): vex008.

Whiteside, Y. O., Song, R., Wertheim, J. O., and Oster, A. M. 2015. Molecular analysis allows inference into HIV transmission among young men who have sex with men in the united states. *AIDS*, 29(18): 2517–22.

***N** unalighned FASTA sequences*

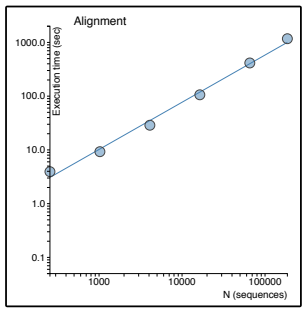
```
CCTCAGATCACTCTTTGGCAACGACCMC…
CAAATCACTCTTTGGCAACGACCTCTTG…
ATCACTCTTTGGCAGCGACCACTCGTCC…
TCACTCTTTGGCAACGACCCCTTGTCAC…
```

*Reference Sequence, e.g. **HXB2 pr_rt***

```
CCTCAGGTCACTCTTTGGCAACGACCCCTC…
```

*Map each read to reference with **`bealign`***

O(N)

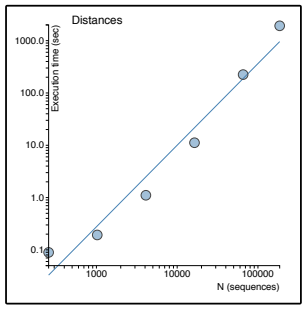


Aligned FASTA sequences

```
CCTCAGATCACTCTTTGGCAACGACCMCTCGTCACAATAAAGATAGGGGGGCAATTAAAG
---CAAATCACTCTTTGGCAACGACCTCTTGTCACAGTAAAAATAGCAGGACAGCTAAAA
------ATCACTCTTTGGCAGCGACCACTCGTCCCAATAAGGATAGGGGGGCAACTAAAG
-------TCACTCTTTGGCAACGACCCCTTGTCACAGTAAAAATAGGAGGACAGATGATA
```

*Compute pairwise distances with **`tn93`**, reporting those under 0.02*

O(N^2)

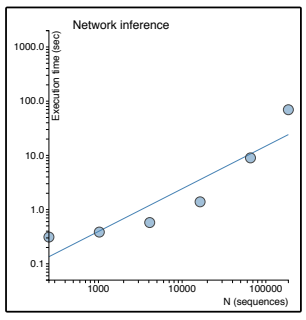


Distances between pairs of sequences, up to a threshold

ID1	ID2	Distance
B_US_KT167882_1985	B_US_GQ209649_2002	0.0152805
07_BC_CN_KT379202_2011	07_BC_CN_KR187686_2011	0.0100482
07_BC_CN_KT379202_2011	07_BC_CN_KR187866_2012	0.0110194
01_AE_CN_KX583284_2014	01_AE_CN_KU050634_2007	0.0186584
07_BC_CN_KR187686_2011	07_BC_CN_KR187866_2012	0.0124912

*Construct and describe the network using **`hivnetworkcsv`***

~O(N)



Network file (JSON), network visualization

```
…
Nodes": [
      {
         "attributes": [],
         "baseline": null,
         "cluster": 1,
         "edi": null,
         "id": "B_JP_AB868660_2010"
      },
…
```

Network Statistics	
Clusters	1396
Edges	22138
Nodes	5466
Sequences used to make links	5466
Links/node	
Mean	8.1
Median	1.0
Range	1 - 247
Interquartile range	1 - 4
Cluster sizes	
Mean	3.9
Median	2.0
Range	2 - 361
Interquartile range	2 - 3

Genetic distances among linked nodes.

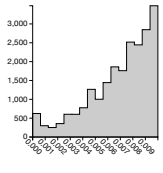


FIG. 1. A schematic of the `HIV-TRACE` workflow. For each stage, we show example input and output data, indicate computational complexity, and provide empirical run-times as functions of the number of sequences on the example HIV-1 datasets described in the text. Trend lines show linear fits in the log-log space.

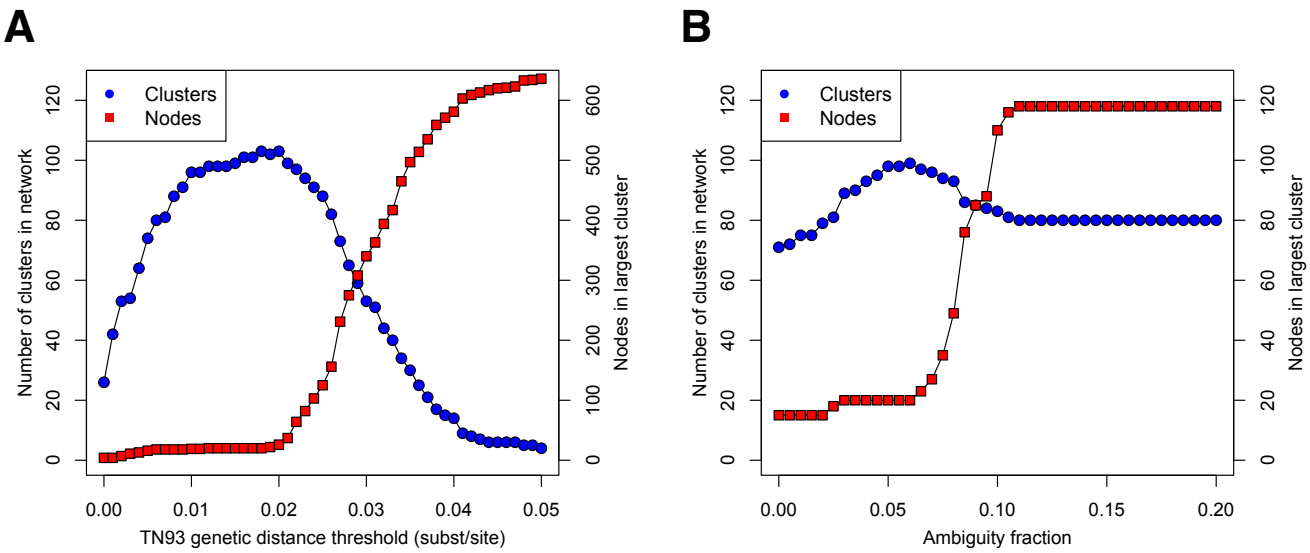


FIG. 2. Effect of genetic distance threshold and ambiguity fraction on network construction. (A) Number of clusters and size of largest cluster across increasing genetic distance thresholds. (B) Number of clusters and size of largest cluster across increasing ambiguity fractions. (C) Largest clusters ($\geq$7 nodes) from the San Diego Primary Infection Cohort, inferred with a 0.015 substitutions/site genetic distance threshold and a 0.05 ambiguity fraction on a phylogenetic tree (each cluster has its own color and is shown in bold). (D) Members of the large, artifactual cluster when ambiguity fraction is increased to 1.0 and distances from ambiguities in all sequences are resolved (shown in bold, colored in red). San Diego sequence data are from Little *et al.* (2014), and phylogeny was inferred using FastTree2 (Price *et al.*, 2010b)

.

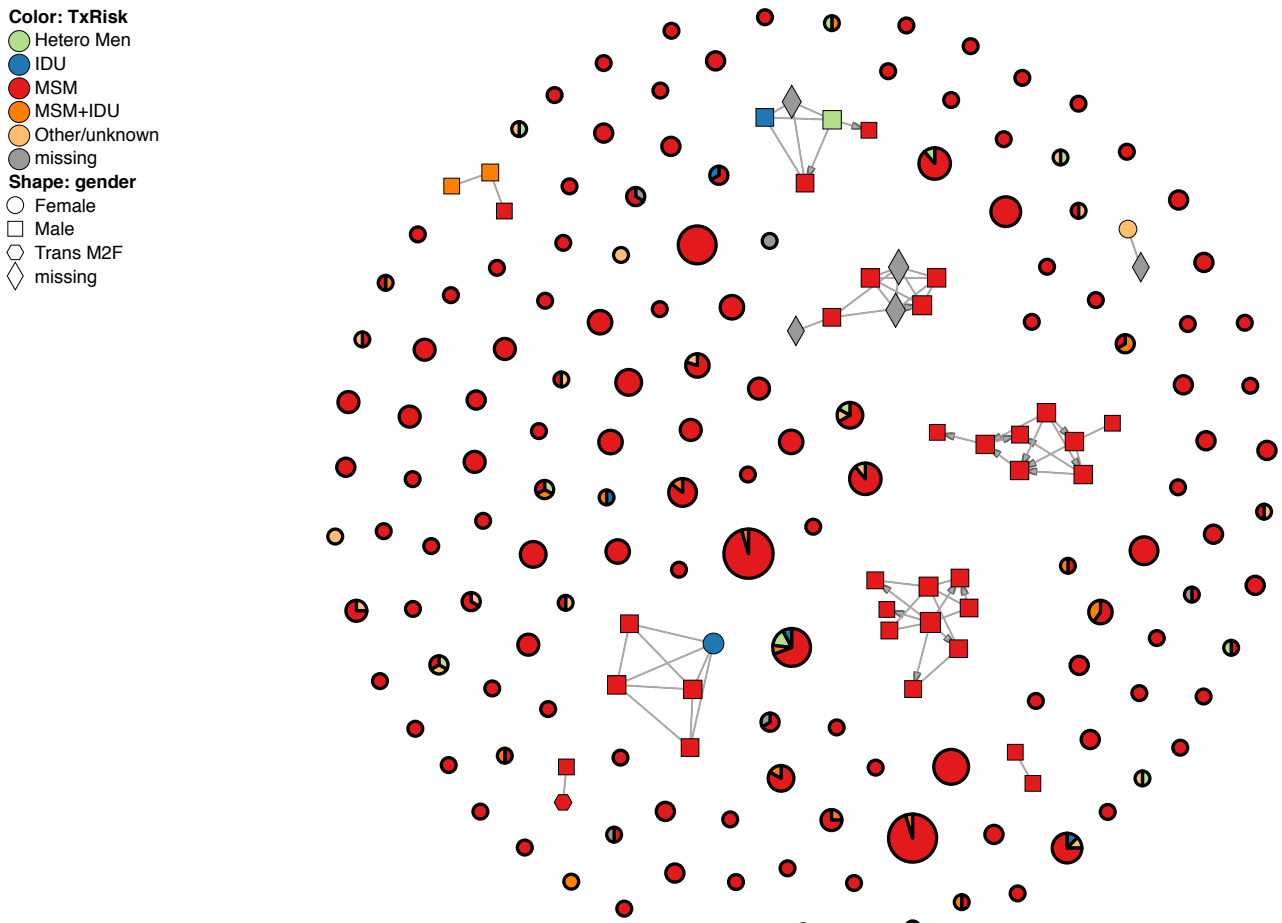


FIG. 3. Visualization of the San Diego Primary Infection Cohort cluster (Little *et al.*, 2014) using `hivtrace-viz`. Circles without connections and darker borders represent clusters, and their are is proportional to cluster size. 9 of the clusters have been expanded, showing individual nodes (individuals) and edges (putative transmission links). Nodes and clusters are colored by risk factor (this is user selectable, and is obtained from network annotation data); for clusters, the distribution of risk factors is shown as a pie chart. The shape of individual nodes indicates the gender of the corresponding individual.