Microbe Trace logo- title page




**v0.1.11 User Manual**

**April 2018**

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# Introduction

A **glossary of terms** is provided after this section for details on terms commonly used in network building and analysis. As you move through the manual, you will find that many terms or references are in blue text; clicking on these hyperlinks will take you to the relevant word in the glossary section or to a website for additional information.

MicrobeTrace is a software tool that enables rapid visualization of [networks](#Networks) and associated data. MicrobeTrace allows users to map characteristics of their data to visual on-screen characteristics (e.g., color, size, shape, etc.) of elements of the network. In addition to network visualization, MicrobeTrace also provides other analytic tools (e.g., tables, filters, geographic maps, histograms, 3D visualizations, phylogenetic tree building) to explore and contextualize nucleotide sequence and other data. These methods have been widely adopted in epidemiology, especially when responding to tuberculosis, HIV, and HCV outbreaks, but have broad applications from molecular biology to sociology.

For nucleotide sequences, a genetic network is constructed after computing genetic distances using the TN93 (Tamura-Nei, 1993) nucleotide substitution model which computes distances between two sequences based on differences in nucleotides between the sequences per site. Potential links between the individual sequences are identified using an empirically determined genetic distance cutoff. For HIV sequences, TN93 is the nucleotide substitution model used and a genetic distance of 1.5% nucleotide substitutions/site is a good initial cutoff for examining the genetic relationships in your dataset, although smaller distances such as 0.5% may improve the specificity for recent transmission. For other pathogens, our development team is in the process of testing functionality of an updated software version that allows importation of distance matrices determined using other nucleotide substitution models or hamming distances for pathogens with single nucleotide polymorphism (SNP) data.

MicrobeTrace can also generate social network diagrams using contact tracing or partner services data. All networks can be customized according to available supplemental data sources (demographic, clinical, epidemiological, etc.) and mathematical inferences like the most probable transmission pathways can be determined by using minimum-spanning methods.

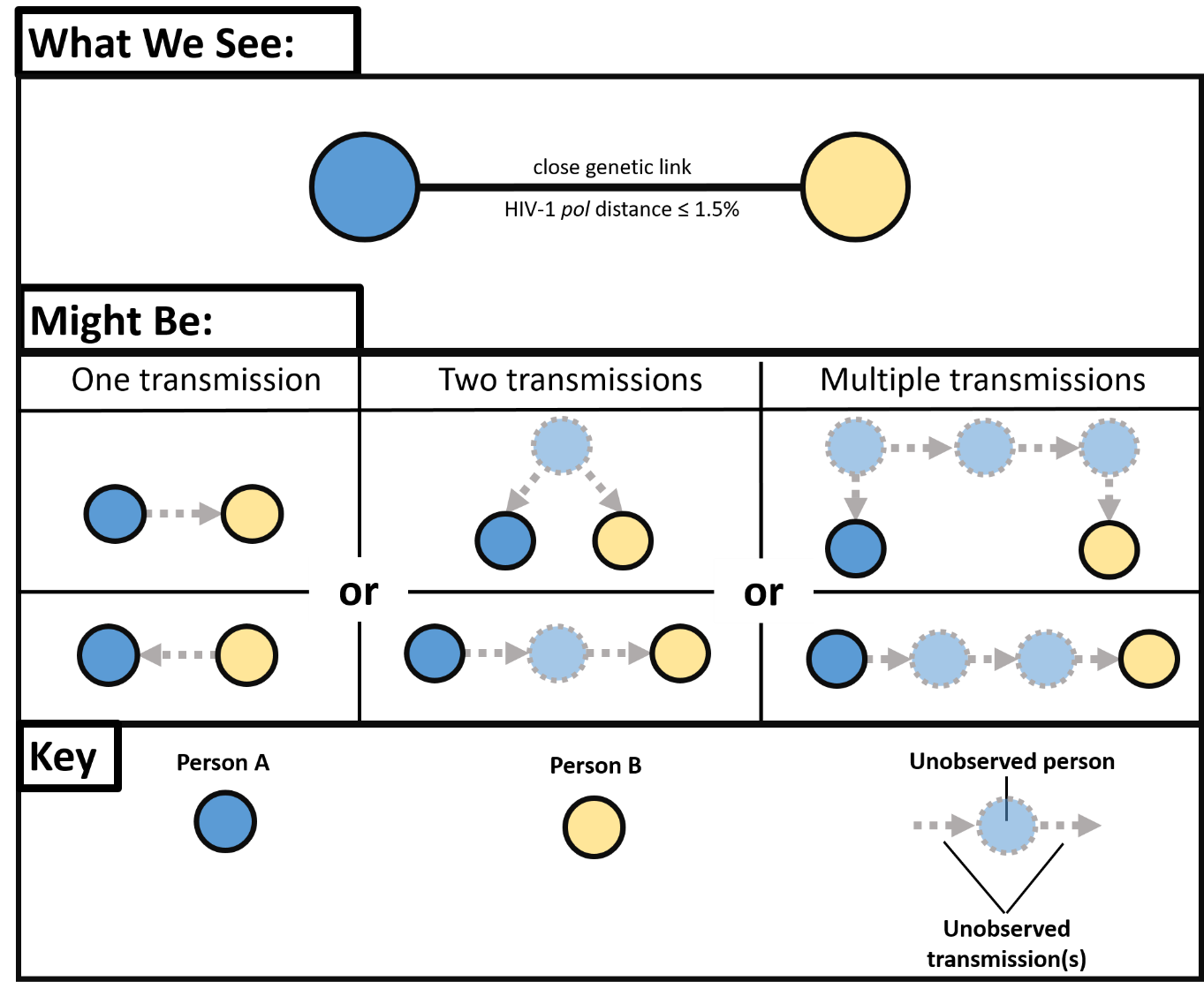
MicrobeTrace is a highly responsive, visual sequence analytics tool, which can reduce the gap between data collection and analytics and help you to discover, understand, and communicate relationships (represented as lines or [edges](#Edge)) between individuals (represented as [nodes](#Node) in the network). As a standalone desktop application, MicrobeTrace can be deployed on encrypted laptops to locations without internet access, thereby reducing both the startup cost and analysis time and effort, all while maintaining data security. Data security is of utmost importance when using sensitive data and should be given the highest consideration when using MicrobeTrace. Please follow your institution’s data security policies when using MicrobeTrace.

This user manual describes installation of MicrobeTrace, loading data for analysis, data visualization, provides troubleshooting tips, and describes how to uninstall the software if needed.

**Glossary of Terms**

***Network Terminology***

**Edge –** A link or line in a network that connects two nodes is referred to as an edge. An edge consists of the unique ID for both connected nodes that are typically labeled as “Source” and “Target”. An edge can be the close genetic relatedness between two sequences in your data, but cannot infer directionality of transmission between these two sequences.



A close genetic link between HIV-1 pol sequences (distance ≤1.5%) can represent many actual transmission scenarios that could involve at least one unobserved person. Six potential transmission scenarios are shown above.

**Edge Attribute –** A data field associated with an edge (i.e., a characteristic of the edge) that can be a categorical or numerical value. For example, one could calculate the absolute difference in ages between individuals connected by an edge.

The figure below outlines various possible transmission scenarios between Node A and Node B that make determination of directionality difficult without inclusion of additional epidemiologic information.

**Edge List –** A list in which all edges and associated information (e.g., genetic distance and/or contact type data) occur exactly once. Edge Lists are also referred to as Link Lists. For MicrobeTrace, this data is included in a CSV (comma separated values) file. CSV files can be prepared by storing the metadata in an excel file that is then saved as a CSV file. Note that reciprocal edges (**Person A 🡪 Person B** and **Person B 🡪 Person A**) are considered unique. Below is an example of an edge list.

| **Source** | **Target** | **Genetic Distance** | **Type of Contact** |
| --- | --- | --- | --- |
| Person A | Person B | 0.004 | Sexual |
| Person B | Person A | 0.004 | Social |

**Metadata –** Data that provides information about other data. Metadata can exist for both edges and/or nodes. For example, the record entry date of a new case or the type of high-risk contact associated with a link. For MicrobeTrace, this data is included in a CSV (comma separated values) file. CSV files can be prepared by storing the metadata in an excel file that is then saved as a CSV file. **Node –** A discrete object in a network that typically represents a person (as in a contact tracing network) or a nucleotide sequence (as in a genetic distance network).

**Node Attribute –** A data field associated with a node (i.e., a characteristic of the node) that can be a categorical or numerical value.

**Node List –** A list in which each node and its associated information (e.g., demographic and behavioral details) occurs exactly once. For MicrobeTrace this data is included in a CSV file. CSV files can be prepared by storing the metadata in an excel file that is then saved as a CSV file. Below is an example of a node list.

| **ID** | **Gender** | **Age (years)** | **Race/Ethnicity** |
| --- | --- | --- | --- |
| Person A | Male | 26 | White |
| Person B | Female | 23 | Black |

**Networks –** For the purposes of this software and manual, there are social or contact networks and genetic distance networks. Social and contact networks are determined from behavioral data collected by partner services during the investigation. Genetic networks are inferred from the microbial nucleotide sequences of the pathogen being studied. Both network types should be included in the analysis for optimal epidemiological understanding of the transmission network, which is a network that combines data from both the social/contact tracing and genetic networks.

**Source –** The node where an edge begins. For example, if **Person A** names **Person B**, then the source is **Person A**. Please note that in this context, source does not imply the source of transmission.

**Target –** The node where an edge ends. For example, if **Person A** names **Person B**, then the target is **Person B**. Please note that in this context, target does not imply the target of transmission.

***Genetic Analysis***

**Cluster –** A cluster is defined as a group of nodes in which each node can be reached either directly or indirectly from any other node. If no path or edge exists between two nodes, then they are considered to be in different clusters or they are singletons. ***It is important to note that the identification of a cluster will change depending on your chosen genetic distance threshold or the addition of contact tracing data.***

**Dyad** - A cluster containing only two nodes.

**FASTA File –** A text-based file format for representing a nucleotide sequence that consists of the standard [IUPAC single letter characters for a nucleotide or amino acid](https://www.bioinformatics.org/sms/iupac.html). FASTA files can have the file name extensions .FASTA, .FA, .FAS or even saved as a text file (.TXT). The FASTA file extensions do not need to be uppercase. The first line in a FASTA file starts with a “>” (greater than sign without the apostrophes) and includes the code or text used for the name of the sequence, specimen or person. The next line in the FASTA file contains the actual nucleotide sequence using the one-letter IUPAC code. **If you are uploading a sequence file as well as a corresponding node list with demographic data (CSV file),** **IDs used for sequences in the FASTA file must match exactly those in the CSV file and must also be unique.** A multiple sequence FASTA file would contain multiple iterations of unique sequence names and their corresponding sequences. Blank lines do not have to separate the first and subsequent sequences in the multiple sequence FASTA file. Here’s an example of the contents of a multiple sequence FASTA file containing three different short sequences.

>Sequence ID 1

ATCGATCGATCGATCGATCG

>Sequence ID 2

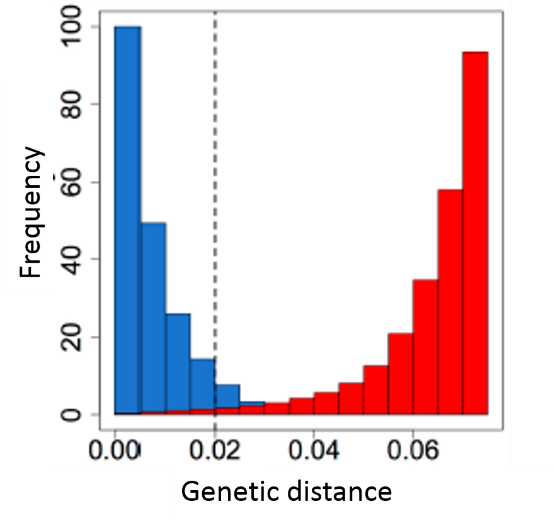
ATCGATCGATCGGGGGGGG

>Sequence ID 3

ATCGATCGATCGCCCGGGTT

**Genetic Distance Threshold –** When analyzing nucleotide sequences, the genetic distances between all possible pairs of sequences are determined using a nucleotide substitution model (MicrobeTrace uses the TN93 model). Therefore, a genetic distance threshold or cutoff for the analysis must be selected to determine potential transmission linkage. For HIV transmission, a 1.5% genetic distance threshold corresponding to 0.015 nucleotide substitutions/per site is used as a starting point to link closely related viruses. For comparison, a distance of 1.0% between two HIV *pol* sequences represents about 10 years of viral evolution within an individual mono-infected with HIV-1 subtype B. However, users will need to select an applicable threshold depending on the situation (e.g., recent vs. distant evolutionary past) and specific pathogen under investigation. The potential cutoff for your analysis can be determined by identifying a threshold that best differentiates a bimodal distribution of the genetic distances that are typically present in your sequences.

The figure below is an example of a frequency distribution of genetic distances with the blue boxes representing genetic distances from known pathogen transmission cases, and the red boxes representing genetic distances from cases without evidence of pathogen transmission. For this data set, a genetic distance of 0.02 nucleotide substitutions/site would likely best differentiate the genetic distances from viruses/pathogens associated with and without transmission (see dashed line in figure). A lower threshold will result in fewer identified linkages, but with increased specificity for recent transmission. For example, for HIV transmission, using a threshold of 0.5% (0.005 nucleotide substitutions/per site) would identify viruses that were likely transmitted very recently (i.e., within about the last 3.3 years).



**Singleton**: An isolated node. For example, a node that does not link to any other nodes in the network.

**SNP –** Single nucleotide polymorphism. This is a single nucleotide difference between two sequences that occurs at a specific position in the genome, oftentimes referred to as a genetic mutation. SNPs are more frequently used with bacterial pathogens.

***Network Visualization Parameters***

**Charge –** Nodes repel each other in the network visualization to maintain separation so all nodes are visible. To change the degree to which the nodes repel one another, this setting can be modified as desired.

**Friction –** The rate at which a node can move across the network view on your computer screen. High friction means nodes won’t move much.

**Gravity –** Nodes are drawn to the center of the network view in your computer screen in proportion to a gravitational constant. Low gravity means nodes will float toward the edges and high gravity will ensure that they are tightly clustered on-screen.

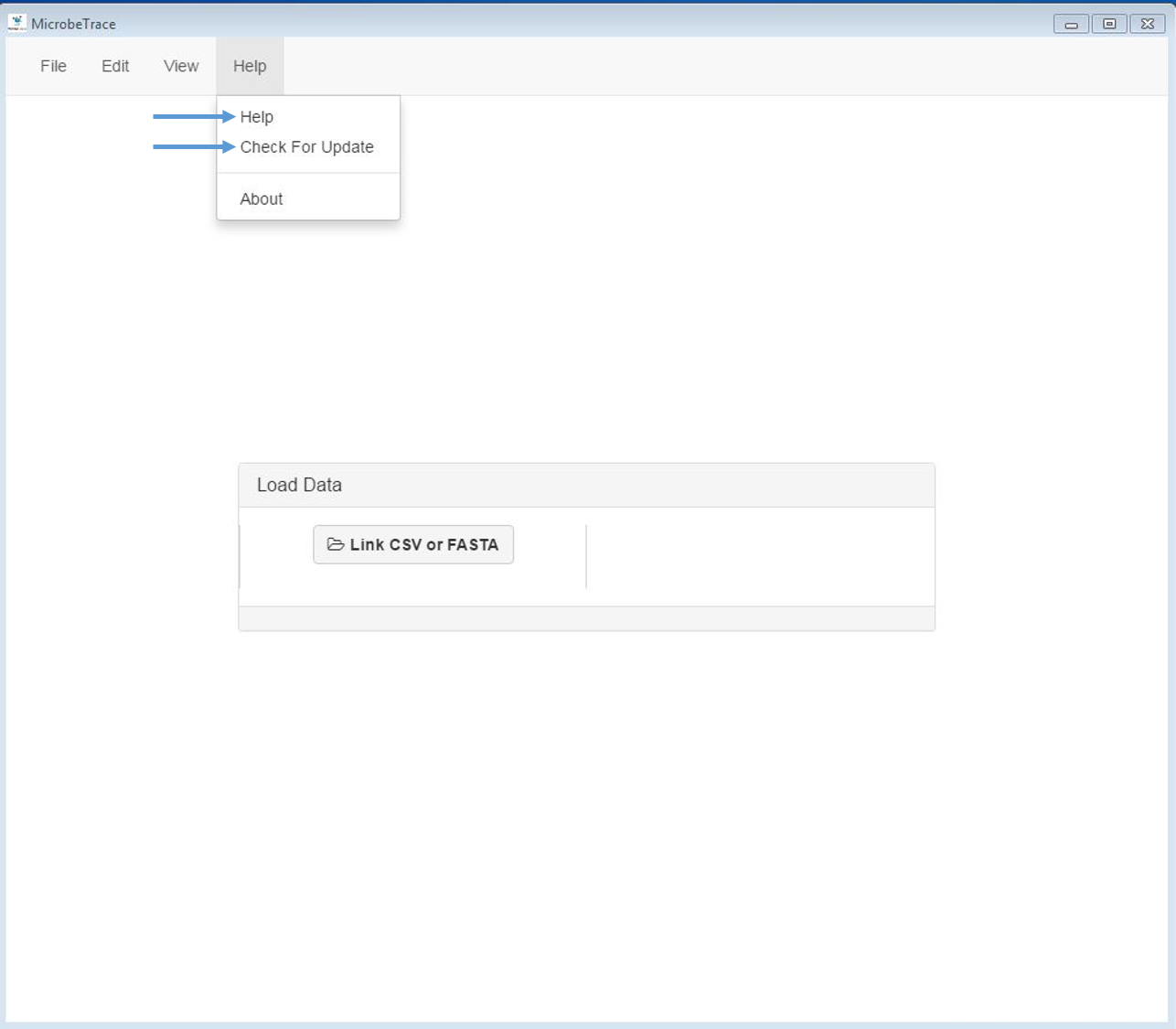
**Tool tip –** a software visualization tool that displays information when the mouse pointer hovers over an object.

**MicrobeTrace Users**

State and local public health workers investigating active microbial transmission clusters and researchers (academic and government) conducting transmission network analysis will find MicrobeTrace especially useful. Although the software was originally designed for HIV and contact tracing transmission analysis, we have added functionality for additional pathogens.

**MicrobeTrace Help and Updates**

MicrobeTrace help is always available by selecting the “Help” link on the right of the menu options as shown in Fig. 1. Help consists of very useful information and step-by-step procedures to assist you in using the tool. Please note that you can also check for updates from this same drop-down menu to make sure you are working with the latest version (Fig. 1).



**Fig. 2.** Accessing help for MicrobeTrace and checking for updates

**System Requirements**

MicrobeTrace works on both Windows and Mac platforms.

**Windows Operating Requirements:**

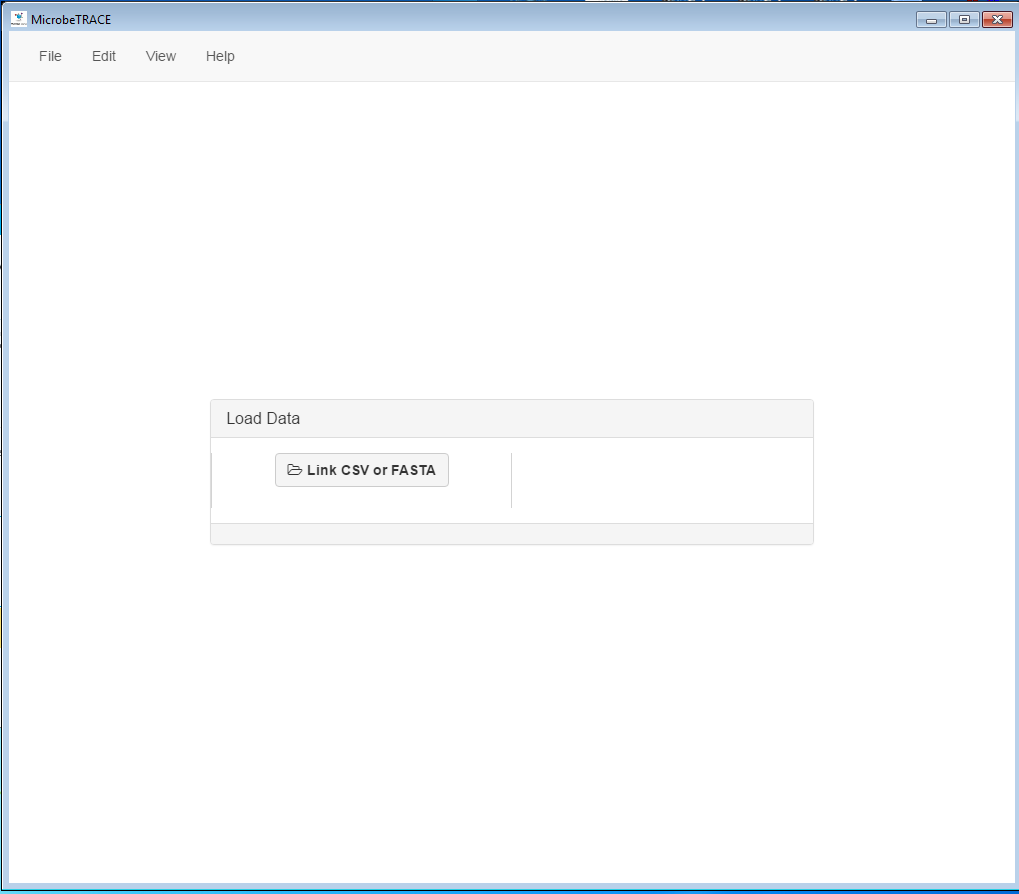
* Windows 7 or newer
* An Intel Pentium 4 or newer processor
* 512 MB of RAM
* Both x86 and amd64 (x64) binaries are provided for Windows.

**Mac Operating Requirements:**

* A 64-bit Intel processor
* 512 MB of RAM
* Only 64-bit binaries are provided for OS X, and the minimum OS X version supported is OS X 10.9.

**Installing MicrobeTrace**

First, download the appropriate program file from the MicrobeTrace [GitHub Page](https://github.com/CDCgov/MicrobeTRACE/releases/latest). There are separate versions available for different PC platforms (at present, we support Windows and Mac OS X). The Windows file is an executable installation file (.EXE). The Mac OS X version is a mountable disk image (.DMG) file. Double-clicking on the installation icon will: (1) install MicrobeTrace, (2) place a shortcut on the desktop and start menu, and (3) launch MicrobeTrace. Once installed, the home screen for MicrobeTrace (Fig. 2) displays the following fields for selecting and loading files.



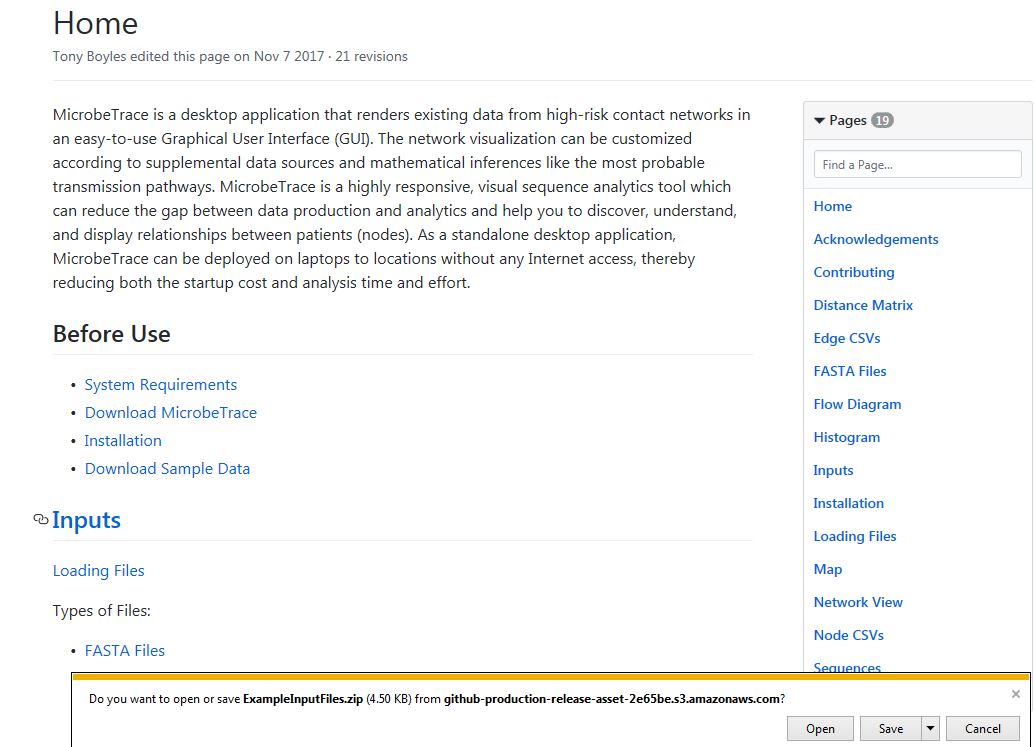
**Fig. 3.** MicrobeTrace home screen for selecting and loading data files

Example data files used to familiarize yourself with MicrobeTrace can be downloaded by selecting:

**Download Sample Data**

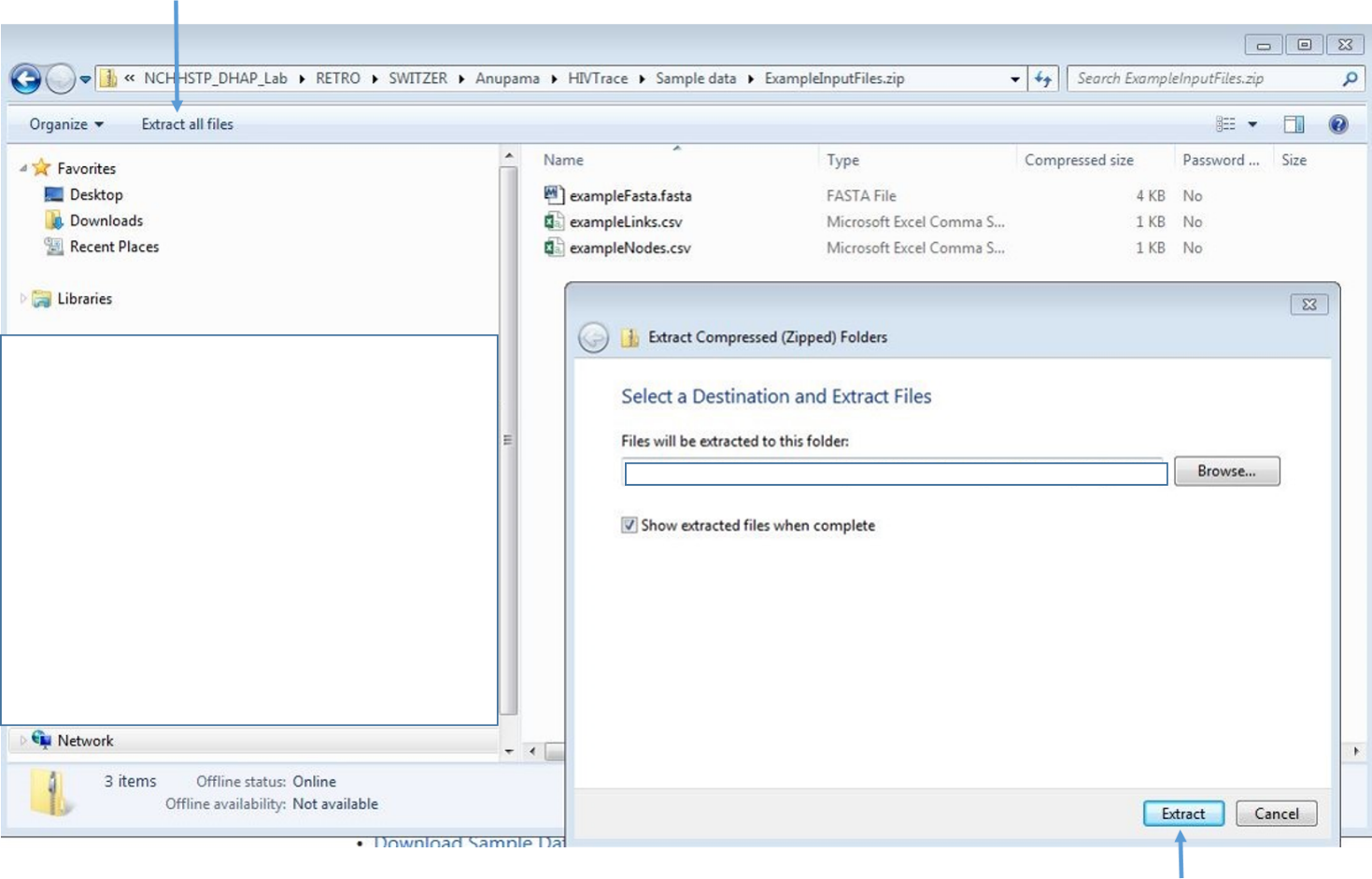
Click [here](https://github.com/CDCgov/MicrobeTRACE/wiki/_edit) to download example files

When you click on Download Sample Data on the website, you are prompted to save or open the file. Select **Save As** and save the zipped file to the location of your choice. (Fig.3)



**Fig. 3**.a. Downloading example files from MicrobeTrace website

Open the file location on your computer and you will see three zipped files. When you click on **Extract all Files**, a dialog box opens up; click on **Extract**.



**Fig. 3.b.** Extracting files from zipped folders

You can use these unzipped example files to explore MicrobeTrace.

**Creating and importing files**

MicrobeTrace accepts the following file formats:

* Nucleotide sequences in the [FASTA file](#FASTAFile) format.
* Standard comma- or space-separated files (.CSV files) which is an available file type option when saving a spreadsheet/workbook in Microsoft Excel. These CSV files can be [edge lists](#EdgeList) or [node lists](#NodeList) (files with sequence/patient IDs with corresponding data such as age, sex, risk-type, method of transmission, diagnosis date, sequence subtypes, etc.). Node list files are not required to build networks, but can provide valuable context to the network visualization.

***\*IMPORTANT\*: IDs used for sequences in the FASTA file must match exactly those in the CSV file and must also be unique.*** For best practices, duplicate IDs should not be used in a FASTA file. If duplicate IDs are detected, the sequences with identical IDs are automatically modified with an underscore and a consecutive number (e.g., PersonA\_1, PersonA\_2) to make them unique. New unique IDs will propagate to all data visualization layers.

**Possible File Input Combinations**

As shown in Fig. 4, a combination of data files can be input into MicrobeTrace depending on the specific analysis or network visualization desired.

**DNA Only**

User only has sequence data and would like to (1) run MicrobeTrace and (2) visualize the genetic network.

**Edge List Only**

User has a pre-existing network file from a previous session or other source and would like to visualize the genetic or contact tracing network.

Edge List (.CSV)

**Edge List + Node List**

User has a pre-existing network file from a previous session or other source. User also has additional information in tabular format and would like to (1) run MicrobeTrace and (2) interactively visualize the genetic or contact tracing network.

Edge List (.CSV)

Node Attribute Table (.CSV)

**DNA + Node List**

User has both sequence data and additional information linked to the nucleotide sequences in tabular format and would like to (1) run MicrobeTrace and (2) interactively visualize the network.

Node Attribute Table (.CSV)

**Fig. 4**. Possible file combinations to create new networks or to visualize previously created networks

The speed of network generation by MicrobeTrace will depend on the number of data files and amount and type of data. The table below gives you an estimate of time taken on average to process genetic data. This includes calculation of distance matrices and network computation.

|  |  |
| --- | --- |
| Number of  Sequences | Duration  Estimates |
| 50 | 5s – 15s |
| 100 | 15s – 30s |
| 150 | 15s – 1min |
| 200 | 30s – 2min |
| 250 | 1min – 4min |
| 350 | 2min – 6min |
| 500 | 6min – 20min |
| 750 | 15min – 40min |
| 1000 | 30min – 60min |

**Data Files used with MicrobeTrace**

Data input into MicrobeTrace has two primary network components: a [node](#Node) and an [edge](#Edge). A node can represent many things, but in the context of partner services (e.g., contact tracing); they typically represent either an infected person or their high-risk partners. In a genetic distance network, nodes represent the pathogen sequences that appear in your [FASTA](#FASTAFile) file. In a contact-tracing network, an edge represents an epidemiologic link between two people. In a genetic distance network, an edge represents the genetic relationship between two pathogens. Edges can be directed or undirected. Directed edges are represented by arrows between nodes. ***\*PLEASE NOTE\*: We STRONGLY advise that directed edges be turned off unless directionality has been supported with strong confidence using additional epidemiologic information (see*** [***Directionality***](#Directionality) ***for more information).***

**Additional Node Attributes**

Additional attributes associated with nodes (e.g., age, gender, infection status, etc.), which are typically stored in an Excel spreadsheet or database, can be visualized in MicrobeTrace by using a [node list](#NodeList).

While [node attributes](#NodeAttribute) are not required to visualize networks, they are a vital component of characterizing and exploring the network. To associate the node attributes to a network, each node ID in the node list CSV must match exactly to its corresponding ID in the provided edge list. All available [metadata](#Metadata) that might help with the network analysis should be appended as additional columns that follow the node ID column in the node list CSV file.

The node list file contains the data associated with the nucleotide sequences in the FASTA file. In order to link the metadata to the respective nucleotide sequences in the two files, entries in the first column of the node CSV file (IDs) must match the IDs of their respective nucleotide sequences. IDs in the FASTA file appear as the text after the “>” and before any space in each sequence. Alternatively, sequence names may be stored in the CSV file in any column with the column header named “ID”. If more than one “ID” column exists, the leftmost one in the file will be used.

**\*PLEASE NOTE\*: *In a CSV file, rows with identical node names cause repeating rows to be dropped. You MUST ensure that ID names are unique.***

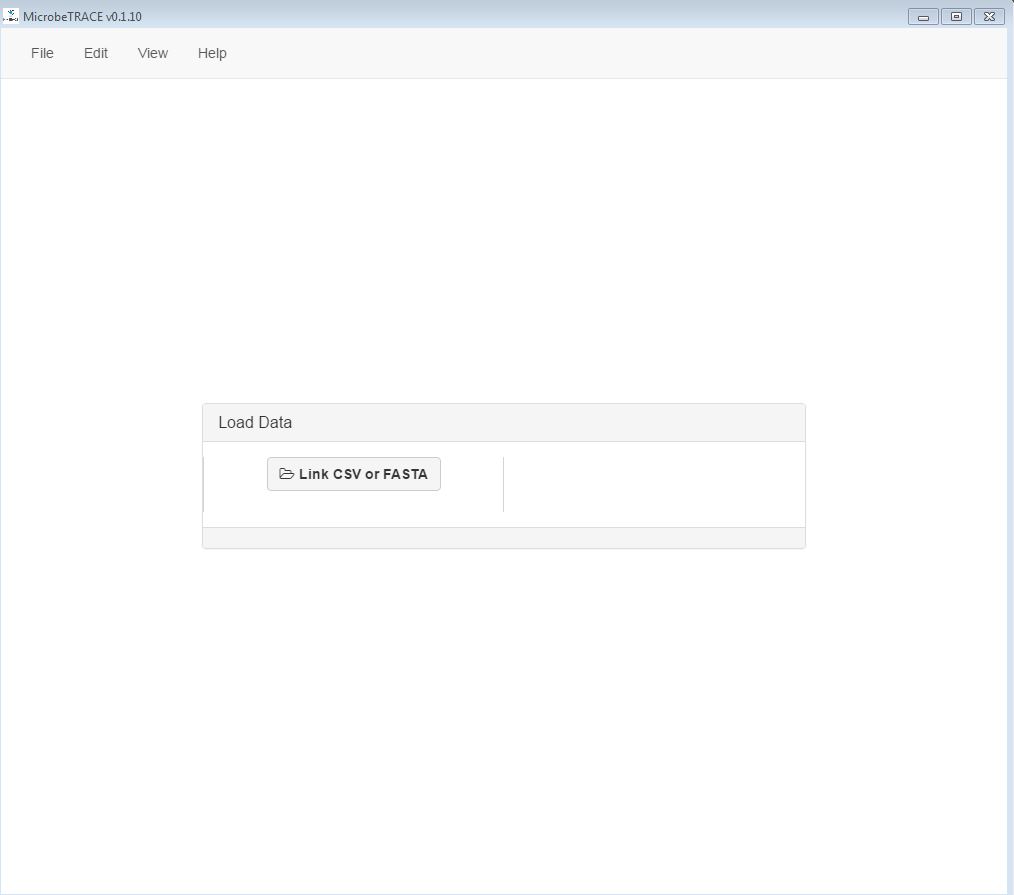
As an alternative input to a FASTA file, a list of edges can be provided which indicate connections between nodes defined in the node CSV file. This is called an “edge CSV” file and is typical of person-to-person linkages determined during contact tracing. In MicrobeTrace, this is also called a Link file or Link List or Link CSV file. Networks from edge CSV or link files are called contact tracing or social networks. Here is an example of data in an edge file.

| **Source ID** | **Target ID** | **Edge Attribute** |
| --- | --- | --- |
| John | Jacob | High-risk contact |
| John | Mary | High-risk contact |

Additional edge properties (or data) can be visualized by adding data columns to the [edge list](#EdgeList). Edge properties can be any characteristic that further define relationships between two nodes. It is important to note that edge properties should reference both nodes that are connected by an edge. Some examples of edge properties include genetic distance between two pathogens, the type of high-risk contact that occurred between two people, or the age difference between two people.

**Importing files into MicrobeTrace**:

Double-click the MicrobeTrace desktop icon to open the program. The system displays the MicrobeTrace **Load Data** view (Fig. 5).



**Fig. 5.** Loading data, sequences (FASTA) or Link/Edge files (.csv) into MicrobeTrace

**Loading a FASTA file**

***Step 1.*** Select the **Link CSV or FASTA** button. The system displays a standard windows explorer page for navigating to the desired file.

***Step 2.*** Navigate to the example FASTA file and double-click on the file to add it to the analysis, or select the file and choose **Open**. This loads the FASTA file into MicrobeTrace.

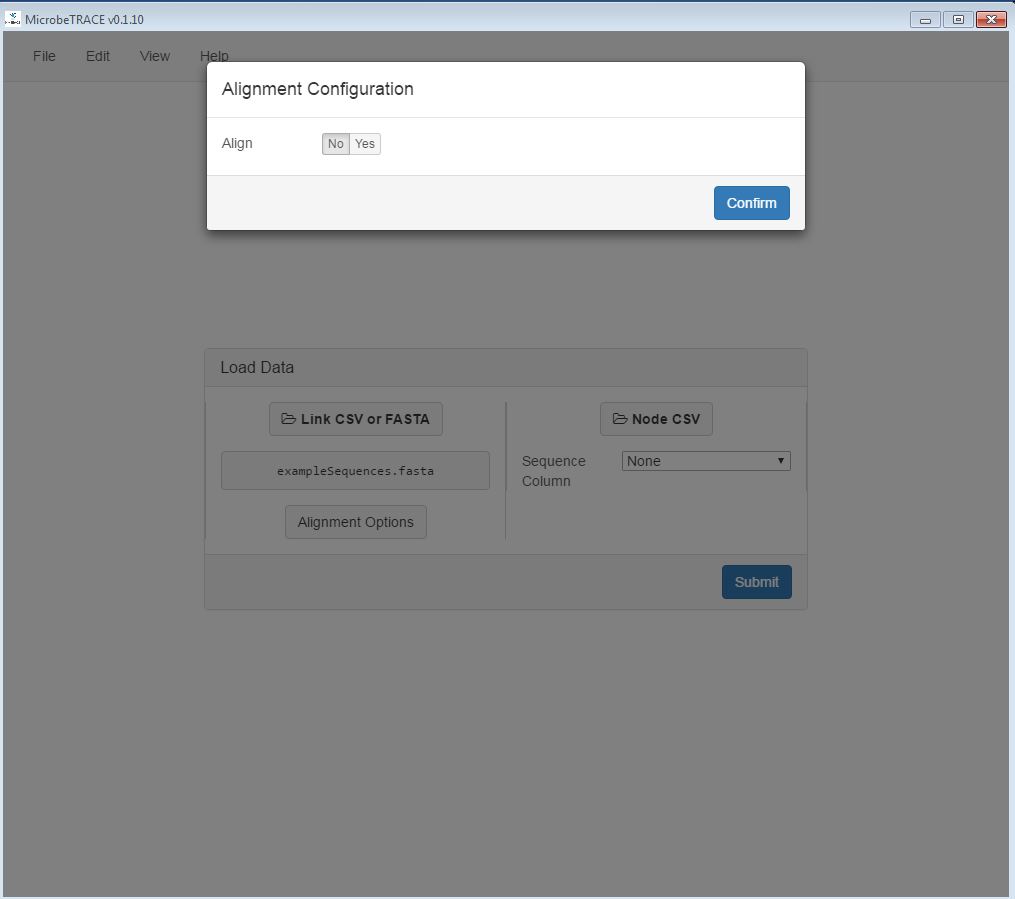


**Fig. 6**. Loading a FASTA file and choosing alignment options

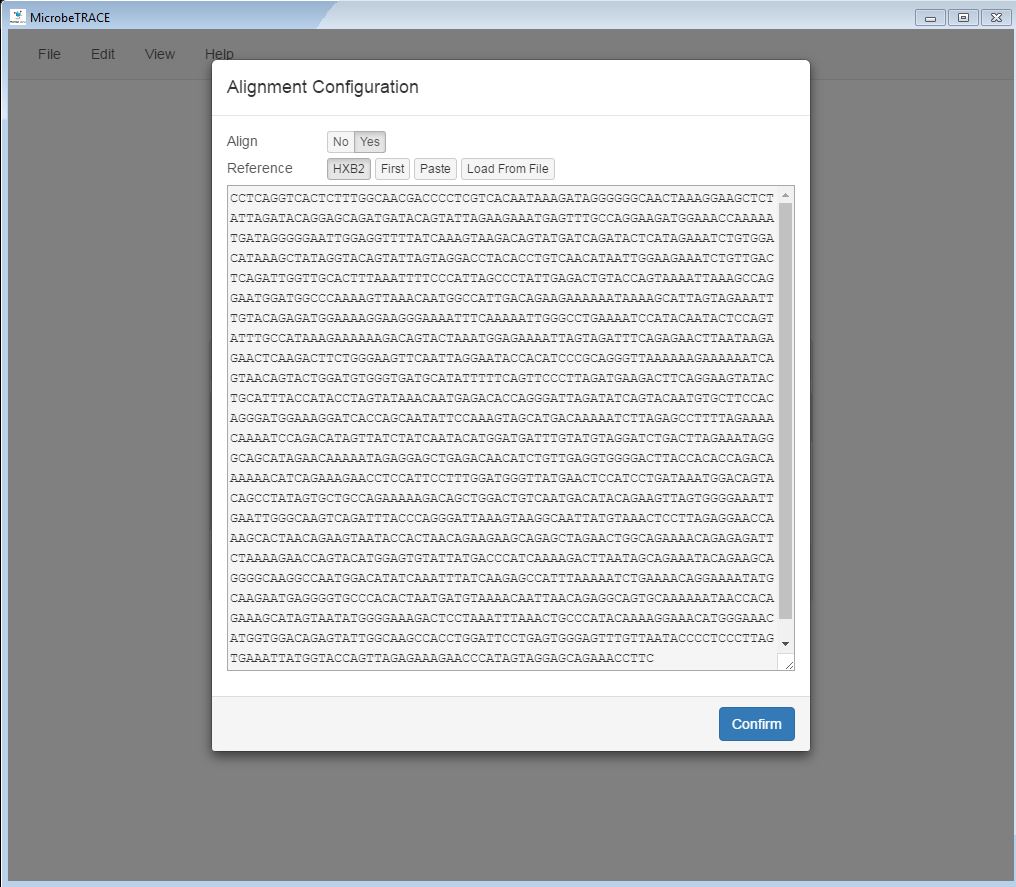
***Step 3.*** The **Alignment Options** button gives you the choice of aligning your sequences against a reference sequence. ***\*IMPORTANT NOTE\*: The default option is that your sequences will NOT be aligned. Please ensure that your sequences are aligned before network analysis, either in MicrobeTrace (steps below), or using an alignment software of your choice. Do not realign alignments using the aligner in MicrobeTrace.***

If you are using HIV-1 polymerase (*pol*) sequences, select the **Alignment Options** button.

Select **Yes** (Fig. 7).



**Fig. 7.** Alignment configuration

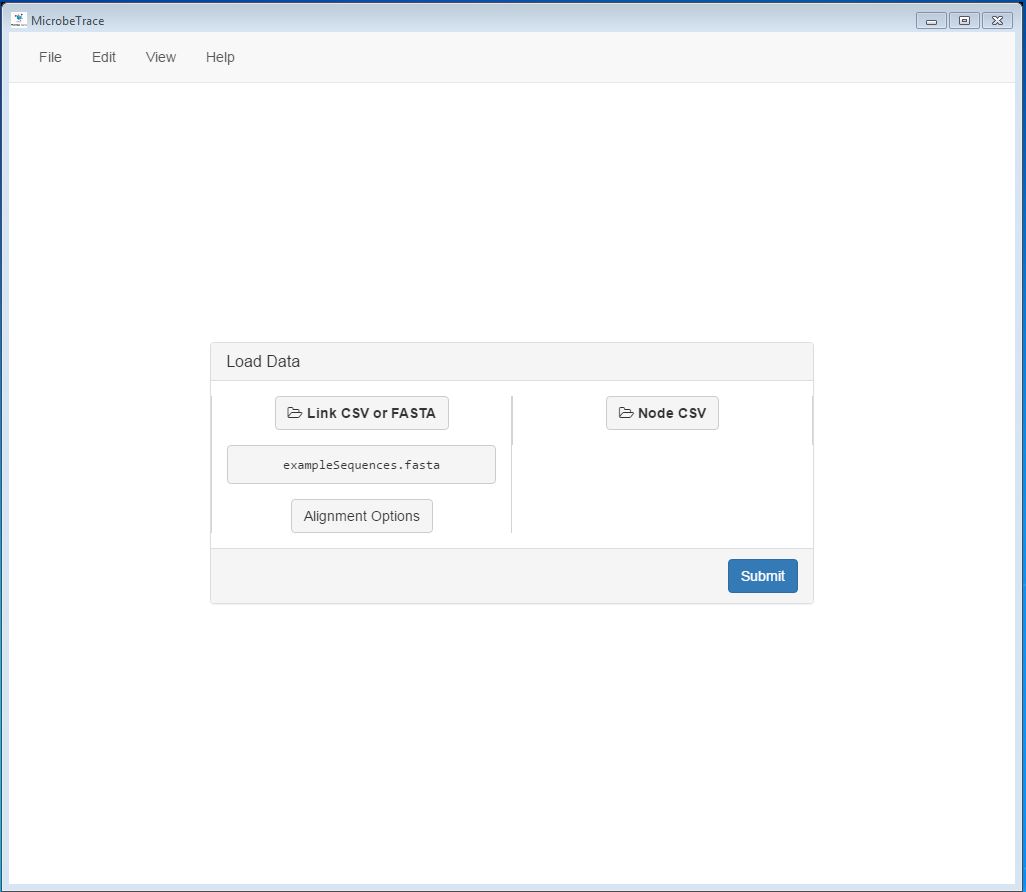


**Fig. 8.** Alignment options for HIV polymerase (*pol*) sequences in FASTA file. See descriptions below

When you select **Yes**, the program prompts you to select one of four options

1. The default setting is to align your sequences to the HIV-1 HXB2 reference (HIV-1\_HXB2 GenBank accession number K03455). You will see the reference sequence in the window. Select the **Confirm** button to accept this option.
2. If you prefer for the program to align your sequences to the first sequence in your FASTA file, select the **First** button next to **Reference**, then click **Confirm**.
3. If you prefer to paste a reference sequence, select the **Paste** button, and paste your sequence in the window, then click **Confirm**. The program will use this as a reference for the alignment.
4. If you prefer to load a specific reference sequence file, select the **Load From File** button and load your file from the appropriate location on your computer, then click **Confirm**.

Once you click Confirm, you will see the screen below (Fig. 9):



**Fig. 9.** Display after choosing alignment options

***Step 4.*** If you do not need to load a node CSV file, and would like to visualize only the genetic distance network, select **Submit** now. If you would like to load demographic/epi data in a node file, proceed to *Step 5* (optional).

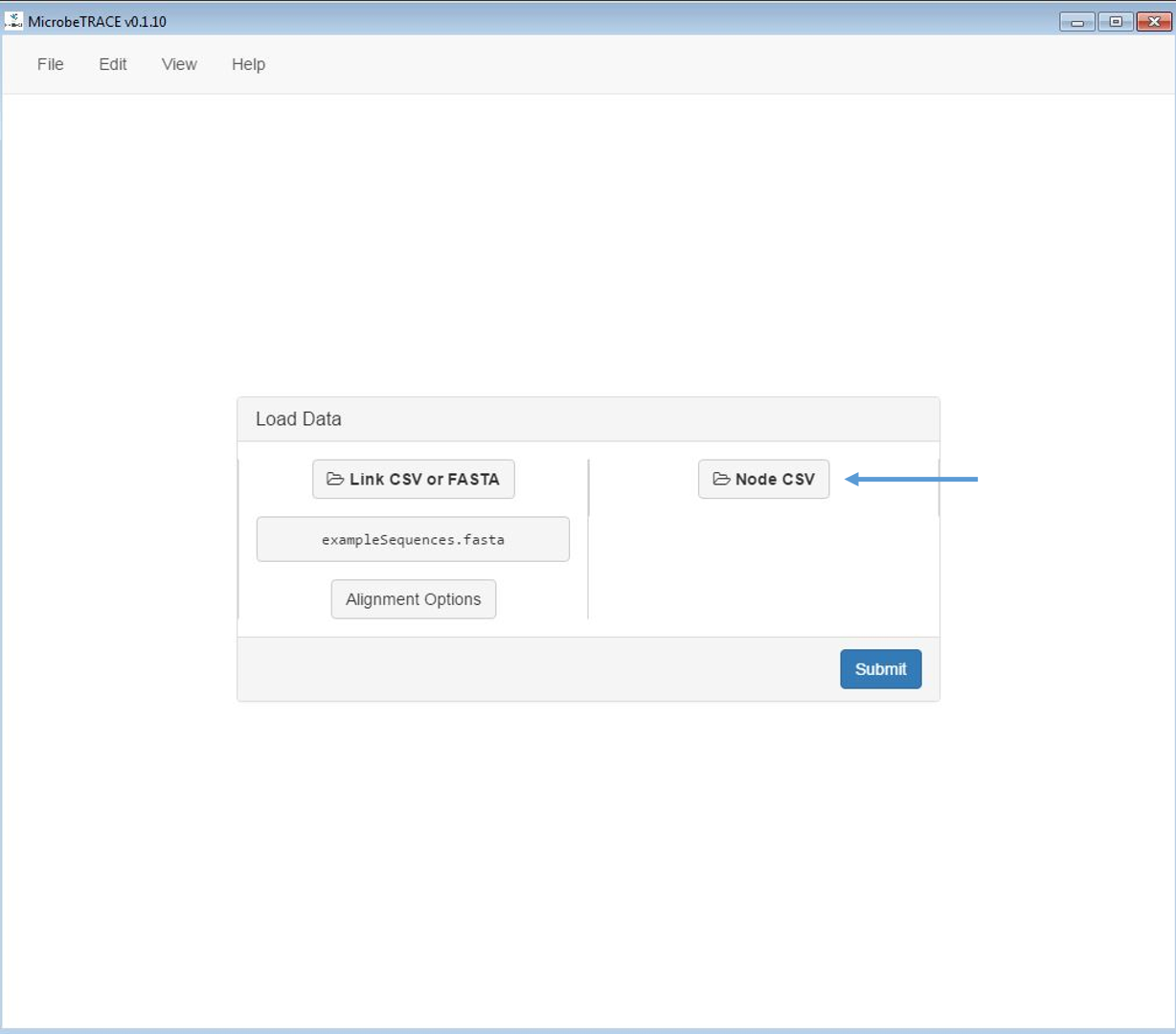
***\*IMPORTANT NOTE\*:******We STRONGLY recommend that you use pre-aligned sequences for non-HIV pathogens because MicrobeTrace is primarily not an alignment program for other pathogens.*** MicrobeTrace is currently configured for determining genetic distances between only HIV-1 *pol* sequences in the FASTA file with a reference HIV *pol* sequence (HIV\_HXB2 GenBank accession number K03455) in the embedded alignment algorithm. For other pathogens, we recommend loading a **pre-aligned** nucleotide sequence file as the FASTA file input needed for MicrobeTrace and completely skipping the alignment options step, and go with the default settings (no alignment).

***Step 5 (Optional).* Loading** [**Node Data**](#NodeList)

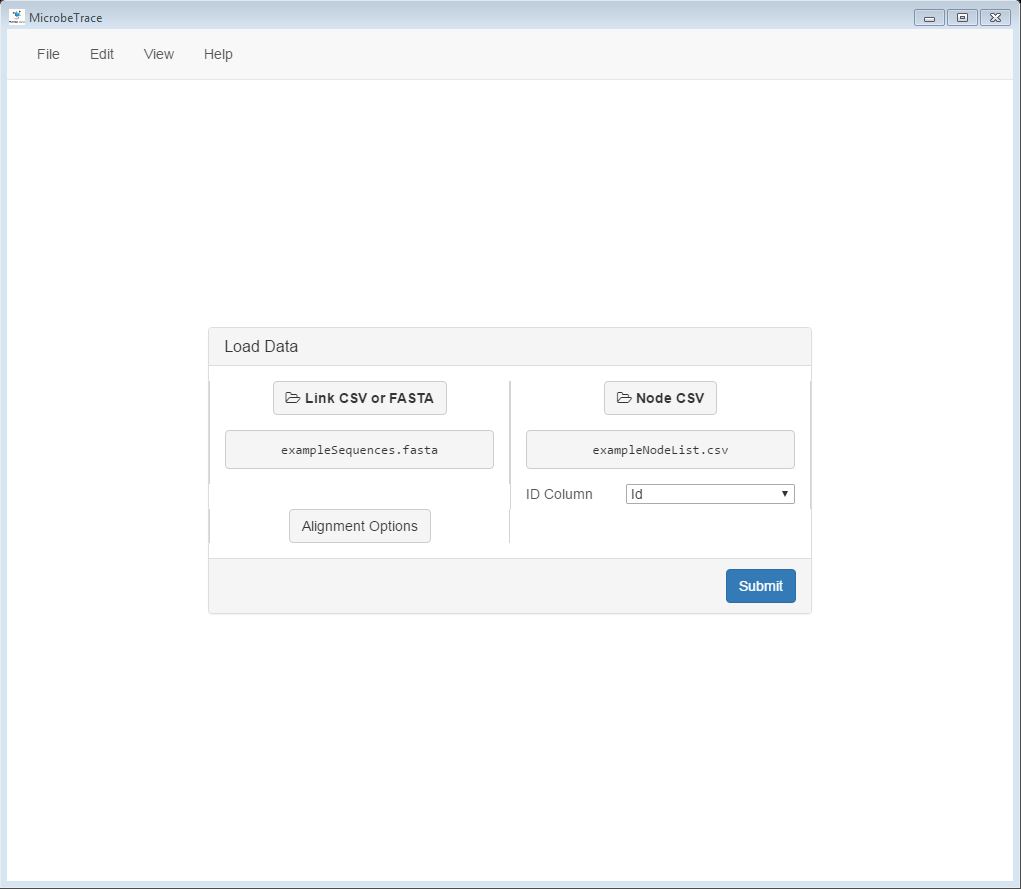
If you have additional data associated with the nodes in the network, that data can also be imported into MicrobeTrace (Fig. 10). This data must be prepared in the CSV file format, and contain an ID column with values that match the source or target columns of the Edge CSV file and/or the sequence IDs in the FASTA file. If more than one ID column exists, the left-most (first) column in that file will be used.

**To load a node file:**

***Step 1*.** Select the **Node CSV** button to load the file.



**Fig. 10.** Loading a node CSV file

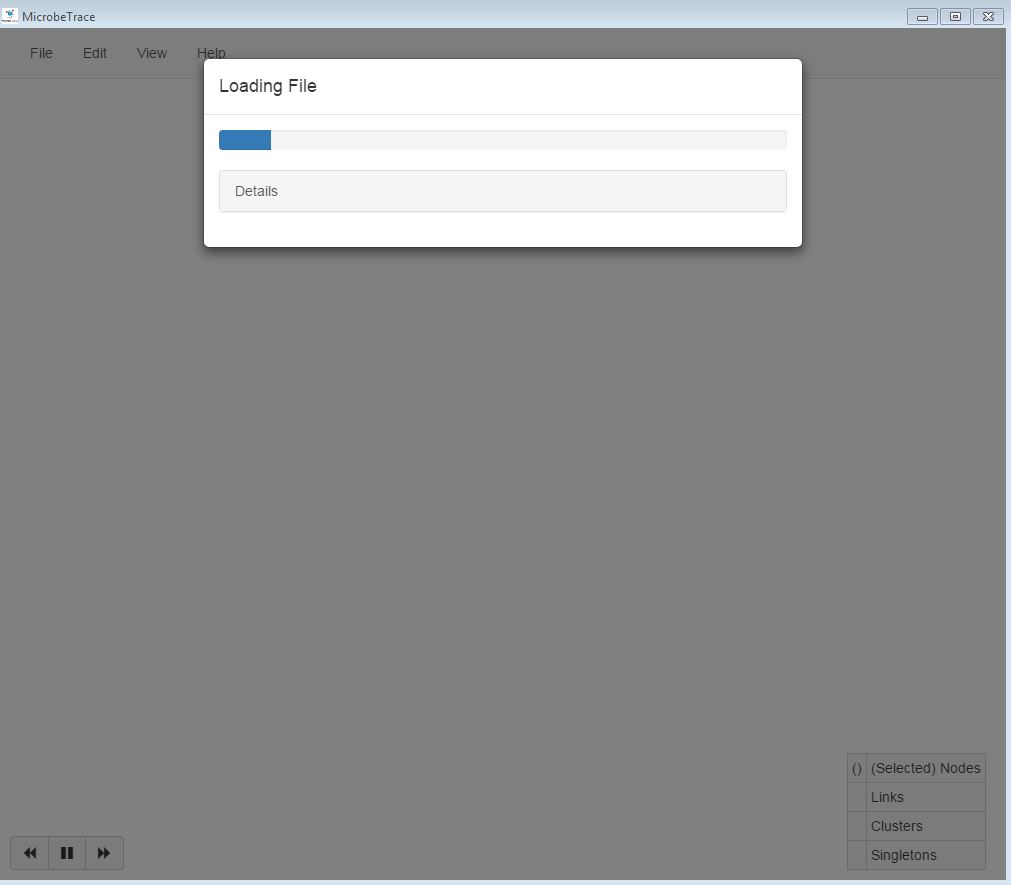


**Fig. 11.** Screen with both types of files loaded - ready for analysis

In the **ID Column** drop-down menu, the default selection for node names is ID; however, if your node file has a different column heading for IDs that you wish to use, you may select that one.

***\*IMPORTANT NOTE\*: Rows in a node list with identical node IDs cause previous rows with the same ID to be overwritten. Please ensure that node IDs are unique.***

Select **Submit** to start the analysis. A status bar will appear to show the progress of the analysis as the files are loaded and the genetic and/or social network is inferred (Fig. 12).

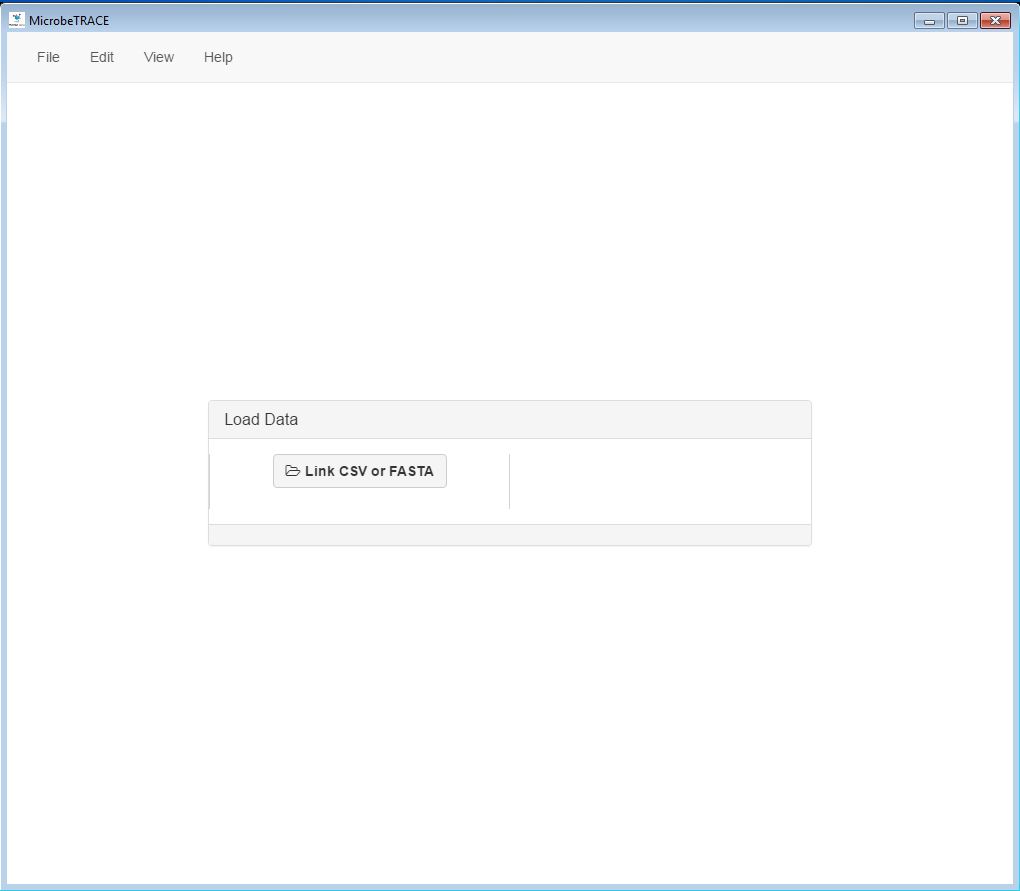


**Fig. 12.** Status bar to show progress of file loading

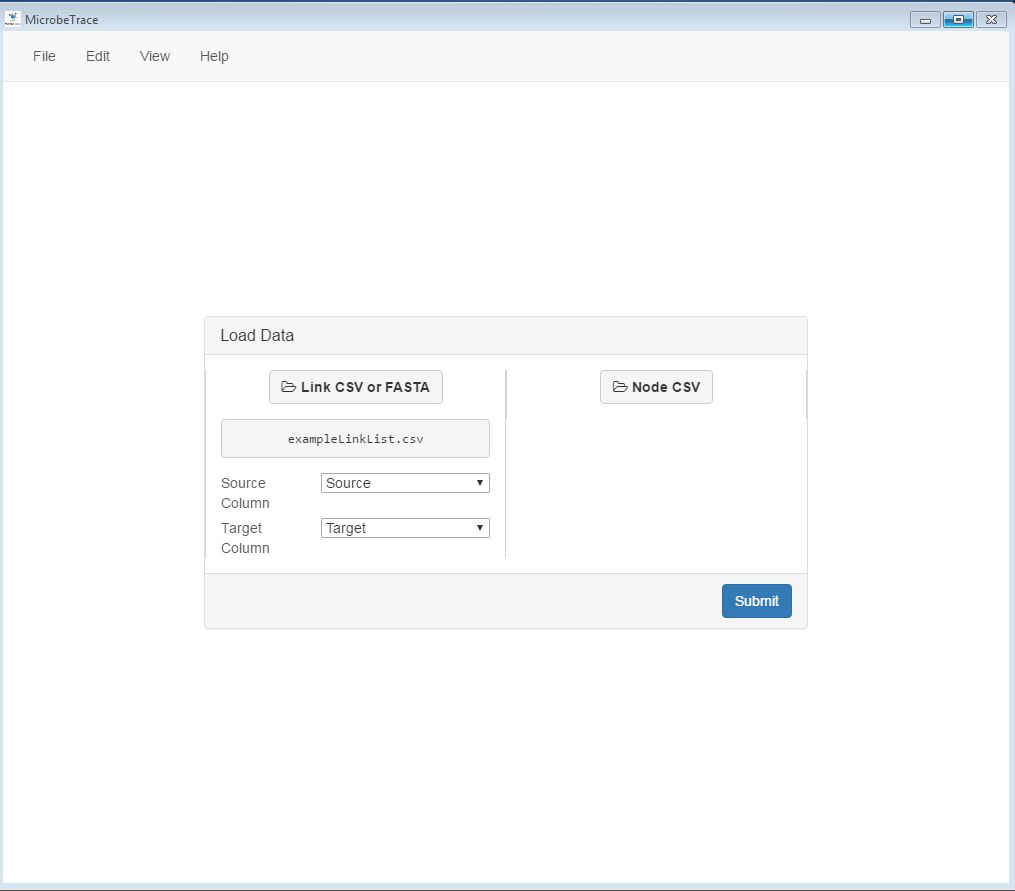
Once the file loading is complete, the software displays a network diagram and a summary statistics table at the bottom-right corner of the page (Fig. 15). This table displays the number of nodes, the number of links (edges), [clusters](#Cluster) and [singletons](#Singleton).

**Loading an** [**edge list**](#EdgeList) **instead of a FASTA file**

If you have already prepared an edge list (also referred to as a [Link CSV file](#EdgeList)), then you can load that file directly into MicrobeTrace (Fig. 13). The Link CSV file must contain a [source](#Source) (person with the infection) and [target](#Target) (recipient of the infection or contact of the infected person) column. Any additional edge properties can be included as additional columns in the edge list file.

***Step 1.*** Select the **Link CSV or FASTA** button, navigate to your CSV file and double-click on the file, or select the file and then select **Open**. 

**Fig. 13.** Loading a link file (edge list) instead of a FASTA file



**Fig. 14.** Screen after loading link file

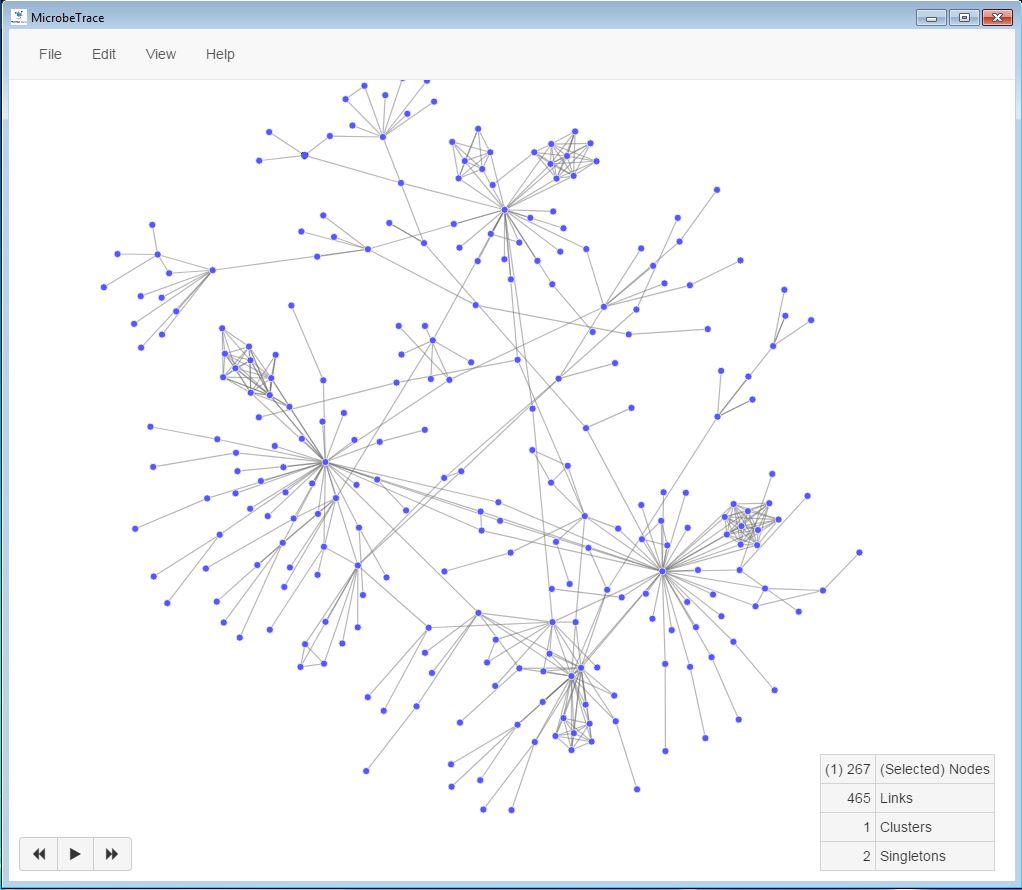
Once file is loaded, you can either click **Submit** to run the analysis (Fig. 14), or you may add a node file if you have demographic and epi data. To do this, please refer to the preceding section on loading optional node data.

***\*IMPORTANT NOTE\*: If you are done with the files you are working with and wish to load a new file/files, please make sure you close MicrobeTrace and re-launch it. If you load files by selecting New from the File menu without re-launching, the program retains information from the previously loaded file.***

***Please re-launch the program when you are done with one set of loaded files.***

**Network View**

The default data visualization and exploration method is the Network View (Fig. 15). You can select a number of different data visualizations from the **View** menu to display the data as a table, a flow (also called alluvial) diagram, a histogram, a heat map of the pairwise genetic distance matrix, an alignment of the nucleotide sequences, a phylogenetic tree of the genetic relationships of the nucleotide sequences, or a geographic map showing the location of the nodes. Some of these viewing options are specific to file type. For example, heat maps or phylogenetic trees require nucleotide sequence data. The additional data visualization views are described in the following sections.



**Fig. 15.** Network View

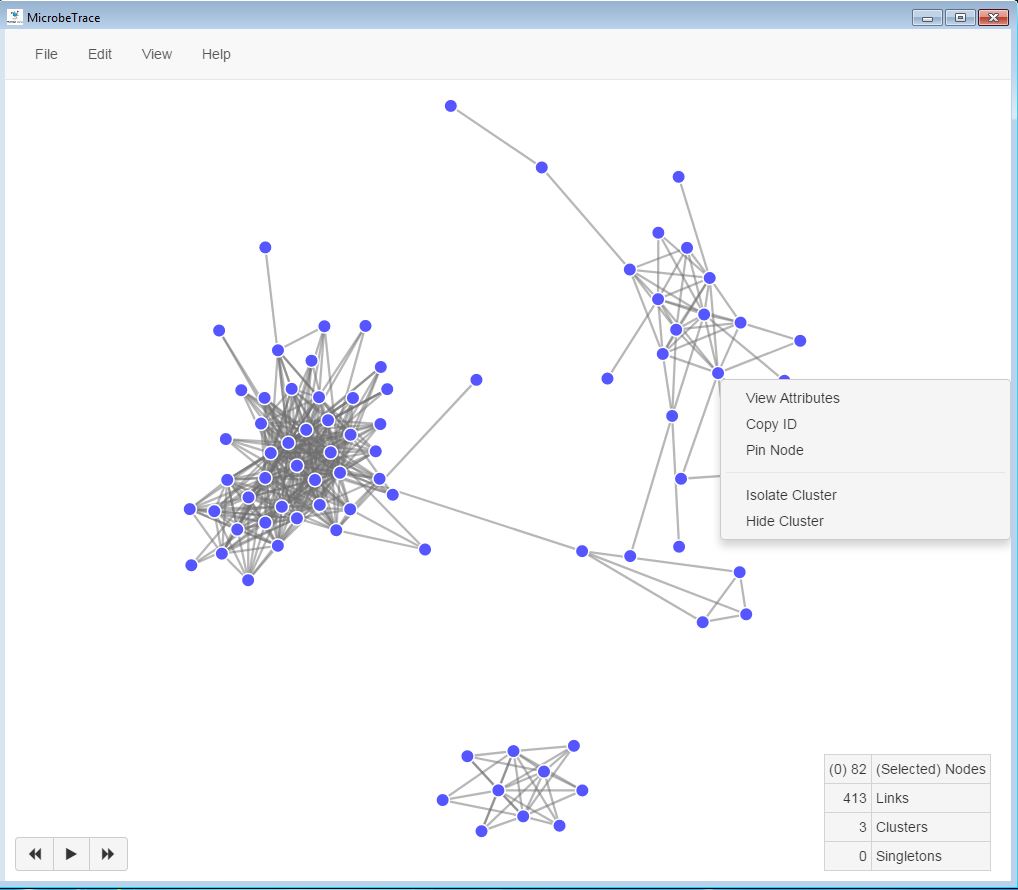
Note that the rendering of the network is updated each time you drag a node, so you will see the nodes moving around before settling into static position. You will see this rendering each time you change any of the network settings (see below for options in settings). The play/pause button at the bottom of the network display window allows you to pause this motion and/or increase and decrease the speed of the network rendering (Fig. 15).

In the Network View, you can:

* pan around by clicking on nodes and dragging or zooming in or out (use the roller on your mouse to zoom in or out)

select or de-select individual nodes (Use Ctrl+Click to select individual nodes; use Shift+Click to select multiple nodes). You can select multiple nodes and see them in a different view, which can be especially useful in Table View. This node selection feature allows you to view all the characteristics of only the selected nodes. You may want to look at the epidemiologic data for just the nodes in a cluster or any that you find interesting in the Network View.

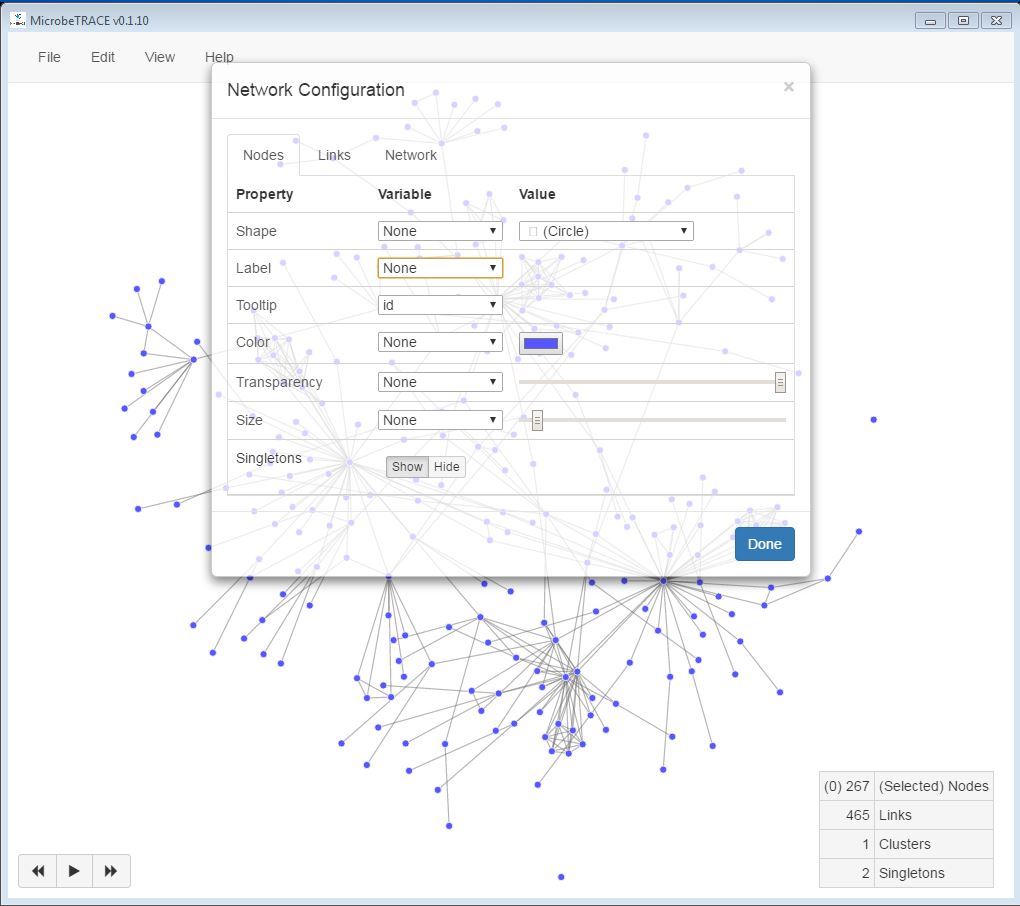
* right-click on a node to see various options (Fig. 16)
  + **View attributes:**  allows you to view node properties
  + **Copy ID:** Copy the node ID
  + **Pin a node:** Pinning a node allows you to drag it out for better visualization of that node in a cluster and to explore the cluster.
  + **Isolate Cluster:** Displays only that cluster. Once the node is isolated, if you right click on a node, you will see **De-isolate Cluster** as an option; click this option to return to the original network.
  + **Hide Cluster:** Hides a specific cluster of nodes linked by edges.
  + **Adjust parameters:** nodes may be annotated with data present in any column in the Edge CSV file (see “Network configuration” below)



**Fig. 16**. Viewing and exploring node options by right-clicking on node

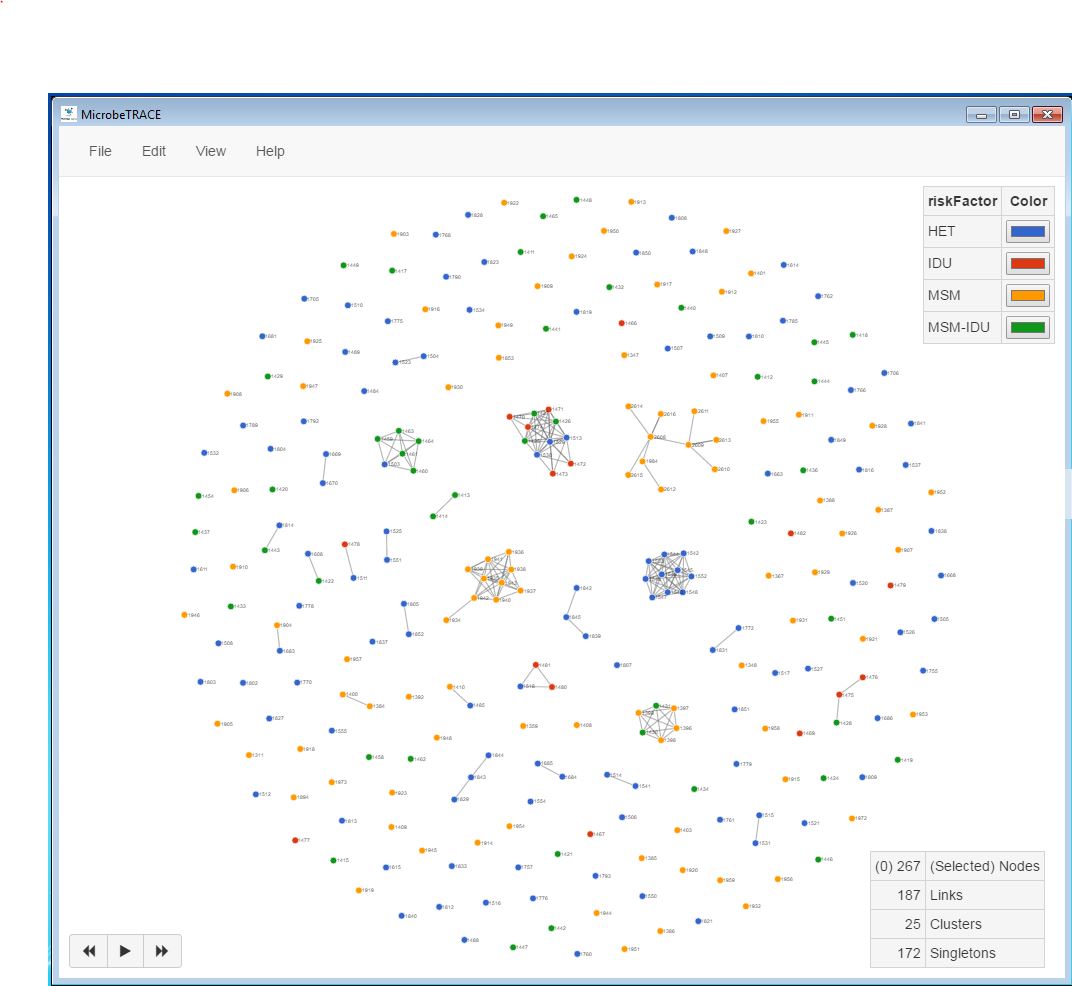
**Network configuration**

You can modify multiple visual characteristics of the displayed network. Select **Edit**🡪**Settings** from the main drop-down menu to display the Network Configuration window (Fig. 17). In the Network Configuration window, you can choose from three tabs: “**Nodes**”, “**Links**”, or “**Network**” to adjust the settings of the various network components. Using your mouse, hover over each property name to see what it represents.



**Fig. 17.** Available settings for changing the network configuration

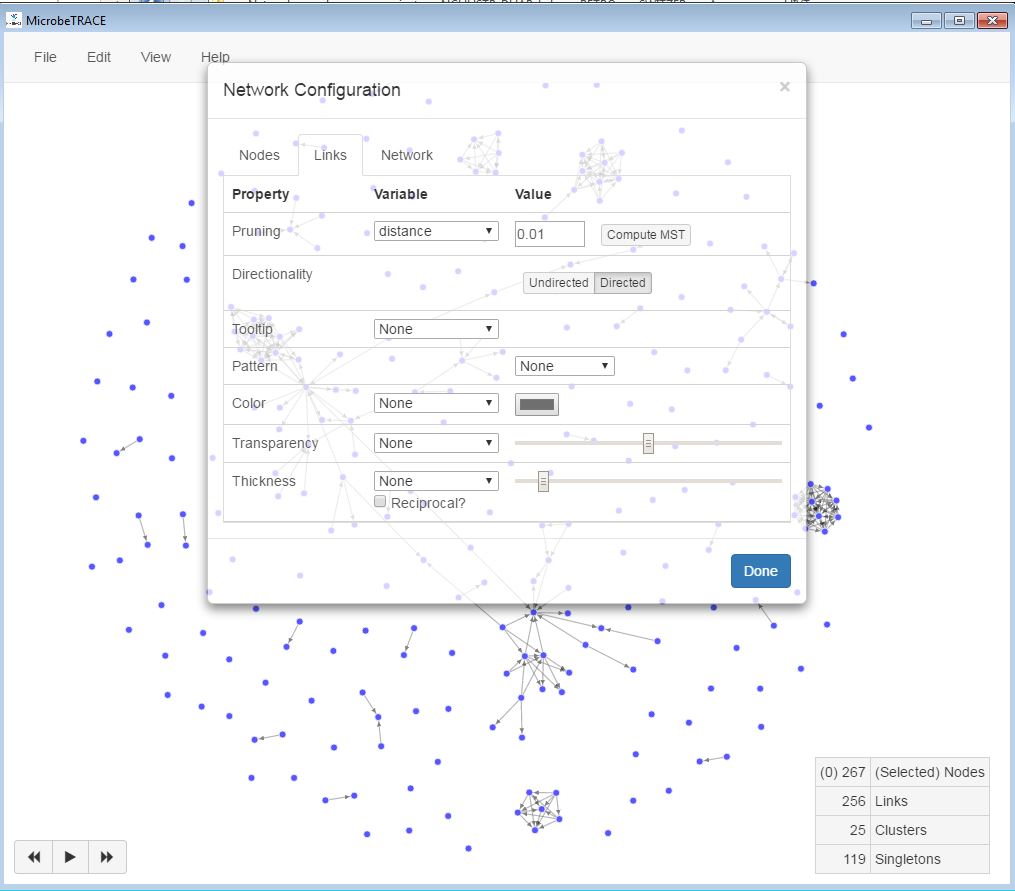
**Nodes**: The default symbol for a node is a circle but node shapes can be changed using the drop-down menu (Fig. 17). In addition to changing shape, color, size, label, etc. of the nodes using the drop-down menus, you can choose to show or hide [singletons](#Singleton) (a node not attached to another node) using the available buttons. The default view displays all nodes, including singletons. For example, select the “**ID**” variable from the **Label** drop-down menu, “risk factor” from the **Color** drop-down menu, and then select **Done.** You will see the ID labels for the nodes on the network and color-mapping options will be available in a text box in the top-right corner of the window (Fig. 18).



**Fig. 18.** Example of node settings with nodes labeled with ID and colored by risk factor

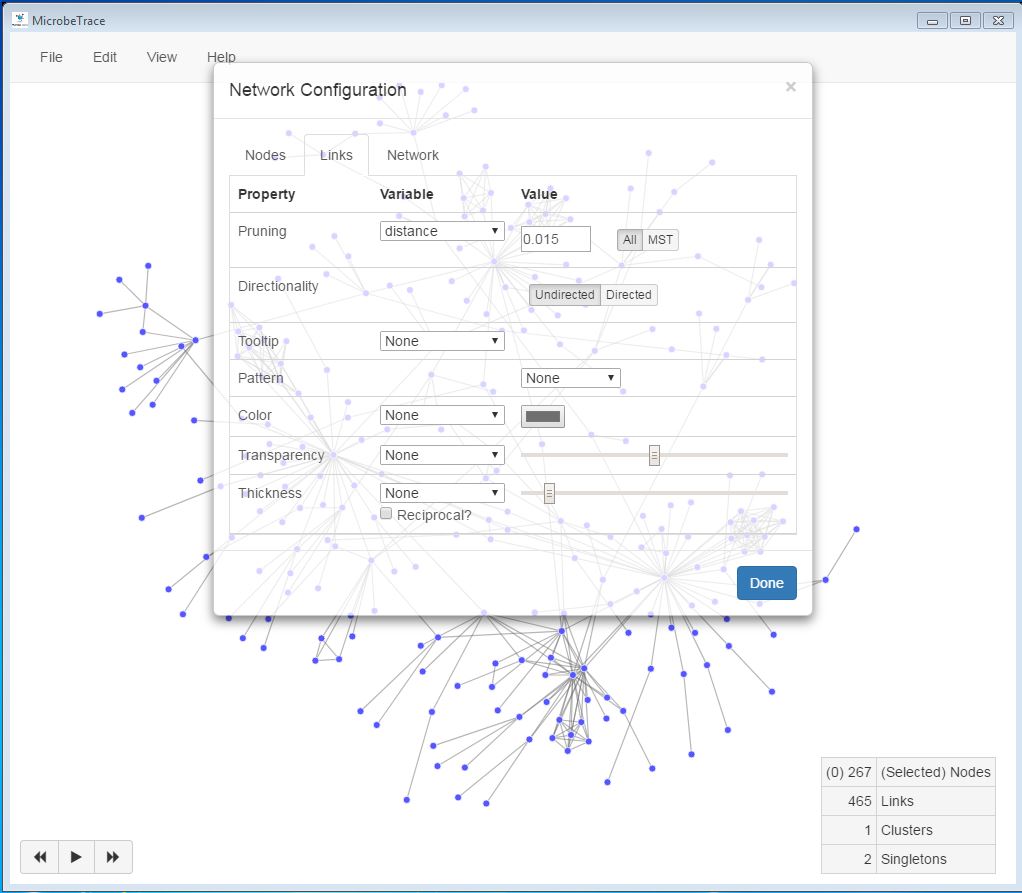
**Links:** Genetic links or edges are typically generated using a defined nucleotide distance cut-off. For HIV-1 *pol* sequences, the default is 0.015 nucleotide substitutions/site (genetic distance of 1.5%), which represents ~15 years of subtype B viral evolution within a monoinfected individual. Using the “Links” pull-down menu, you can select a different [genetic distance threshold](#GeneticDistanceThreshold) to prune the network of links and determine how this affects the network structure (e.g., a higher cutoff will link more nodes and a lower cutoff will prune links from the network) (Fig. 19). You can also choose other variables to explore the network, including the number of single nucleotide polymorphisms ([SNPs](#SNP)) if that information was included in your data.

Oftentimes, genetic distance networks are dense with many links between sequences, especially if little evolutionary time has elapsed between the sampled sequences. You can choose to view only the shortest genetic distances (links) between sequences by selecting **Compute MST** (MST = Minimum Spanning Tree; Fig. 19).



**Fig. 19.** Link settings to change pruning distance and/or compute minimum spanning tree (MST)

Once computed, you will see the screen below (Fig. 20) where you can choose to view all links (Select **All**) or just the subset of closest links (Select **MST**). The MST option will show only the closest genetic neighbor of each sequence.



**Fig. 20**. Selecting one of two viewing options for an MST tree; either all links, or just the MST links (nearest genetic neighbors)

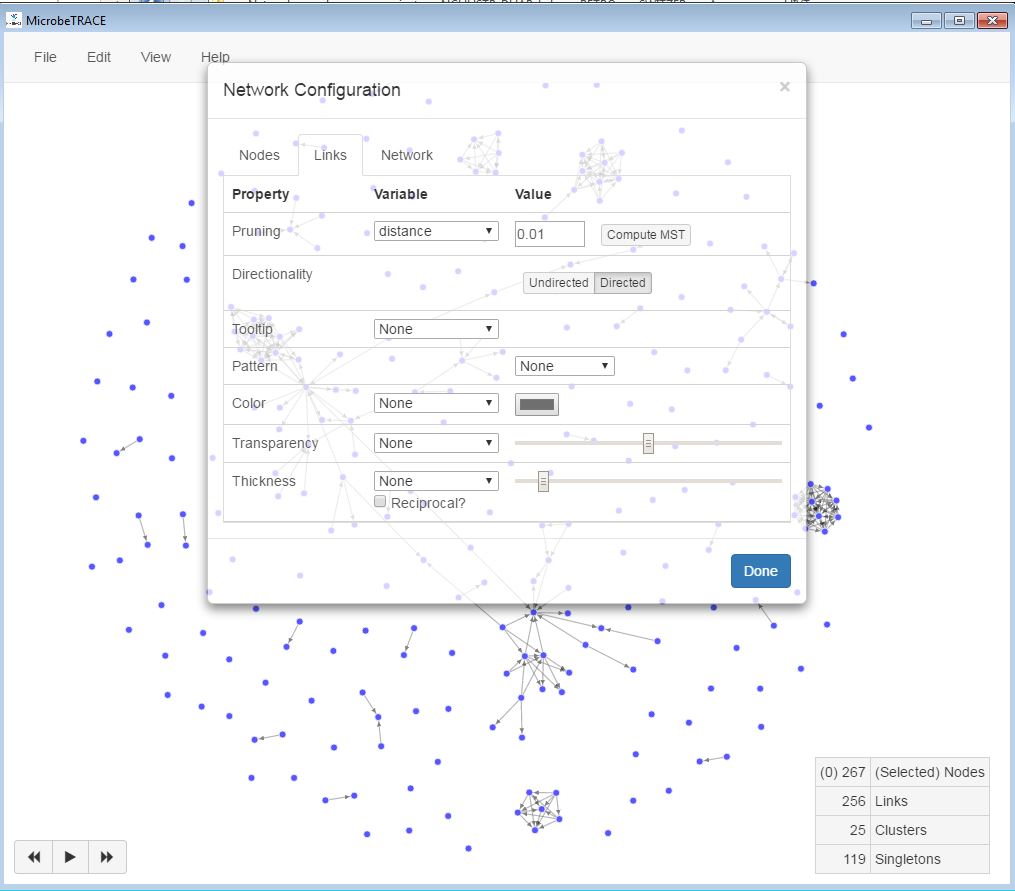
**\**Note\**:** ***It may be helpful to use the Histogram view to first identify the optimal genetic distance threshold for your data. Oftentimes, this is the threshold that best segregates a bi-modal distribution of the genetic distances (see Histogram View section below).***

**Enabling directionality in the network** (PLEASE READ IMPORTANT CAVEATS IN THE GLOSSARY ABOUT INFERRING TRANMISSION DIRECTIONALITY)

Directionality is only valid for edge lists which contain contact tracing data, and not for files containing only sequence data.

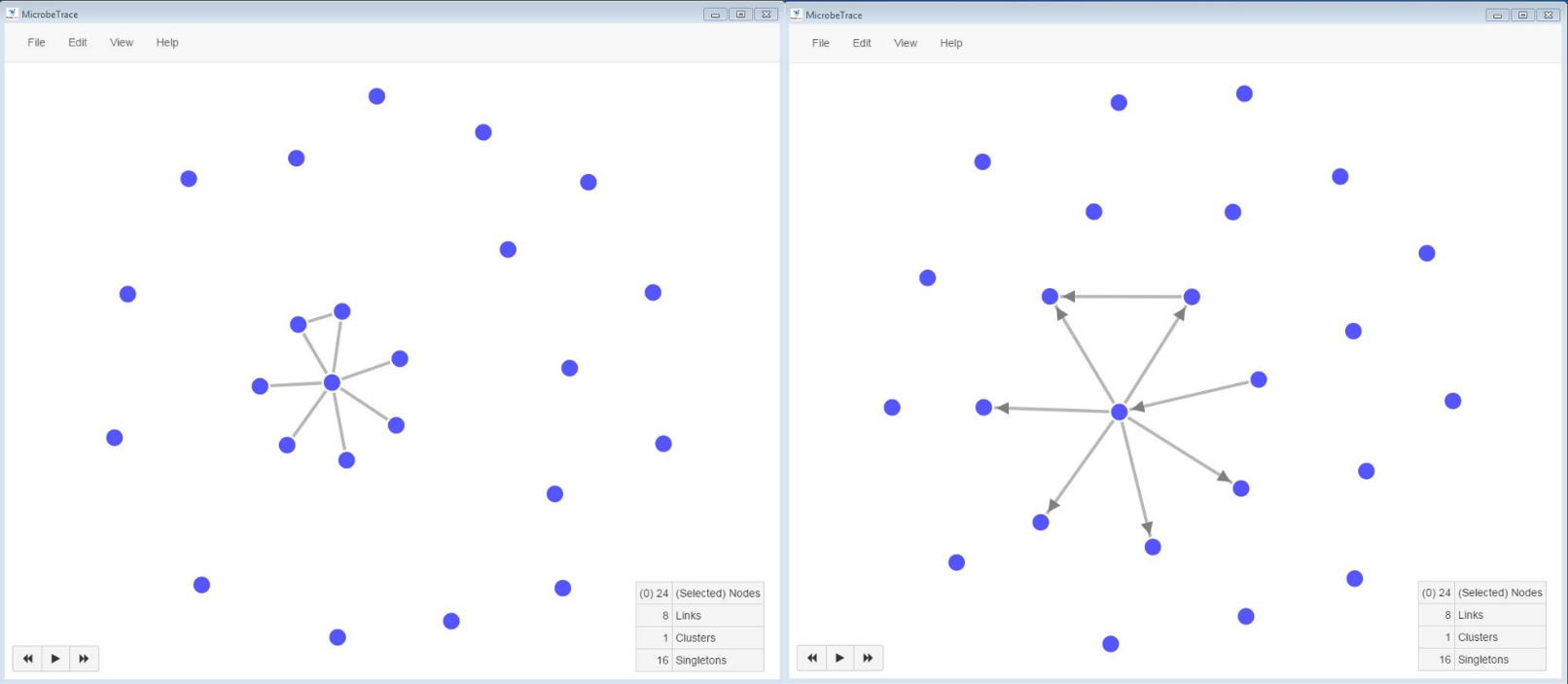
The default Link setting for HIV-1 analyses is that links are undirected. If you upload an edge list containing contact tracing information and wish to enable arrows, this can be enabled by selecting “Directed” next to the “Directionality” setting. The direction of the arrow corresponds to the order they appear in the edge list. Before enabling this feature, please [see notes above about limitations of using directionality](#Directionality) in HIV or other pathogen transmission.

For example, if you upload and edge list and select **Directed** (see Fig. 21), the lines representing edges/links will be changed to arrows (see Fig.21)



**Fig. 21.** Link settings to select directionality between links for an edge list containing both genetic information as well as contact tracing data.

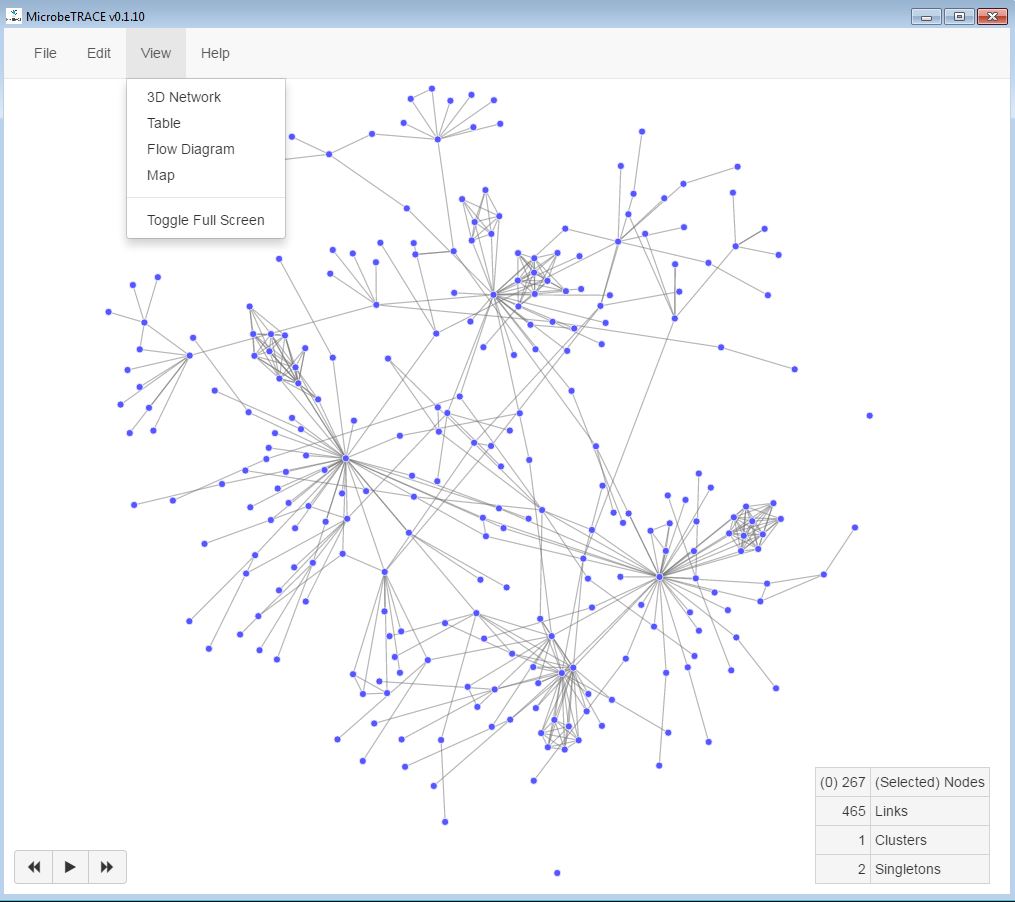
**Network:** Use this option to show/hide network statistics or to change the [friction](#Friction), [charge](#Charge) and [gravity](#Gravity) of the network, which are parameters that determine how densely packed the nodes are in the network. Learn more about these properties in the glossary by clicking the highlighted links for these terms in the previous sentence. You can also adjust the length of the link between nodes. For example, you could increase the length of the links (drag the slider bar on the **Length** option) if your clusters are too tight. This option allows the cluster structure to become more open (less dense) so the cluster nodes and edges are more easily viewed (Fig. 22).



**Fig. 22**. Left panel. Network with undirected links. Right panel. Same network as in left panel, but now shows directionality. Also, note that the cluster structure has opened up by increasing the link length using network settings. The example file used here is an edge list from contact tracing data, not viral genetic data.

**3D Network View**

You can generate a 3D version of the Network View by selecting **3D Network** from the drop-down **View** menu (Fig. 23).



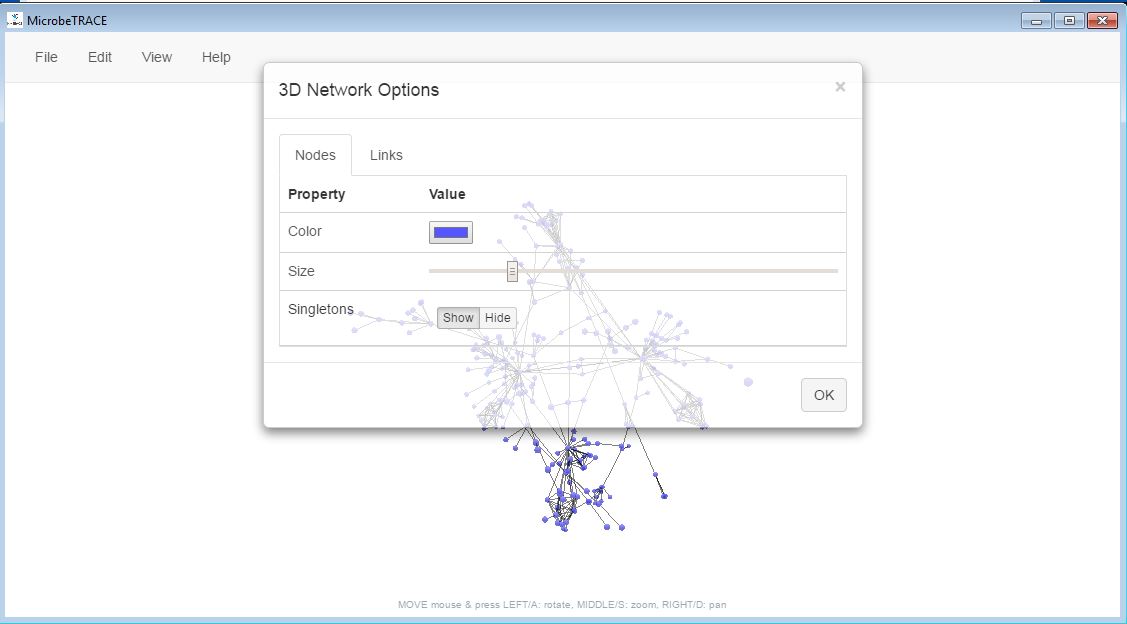
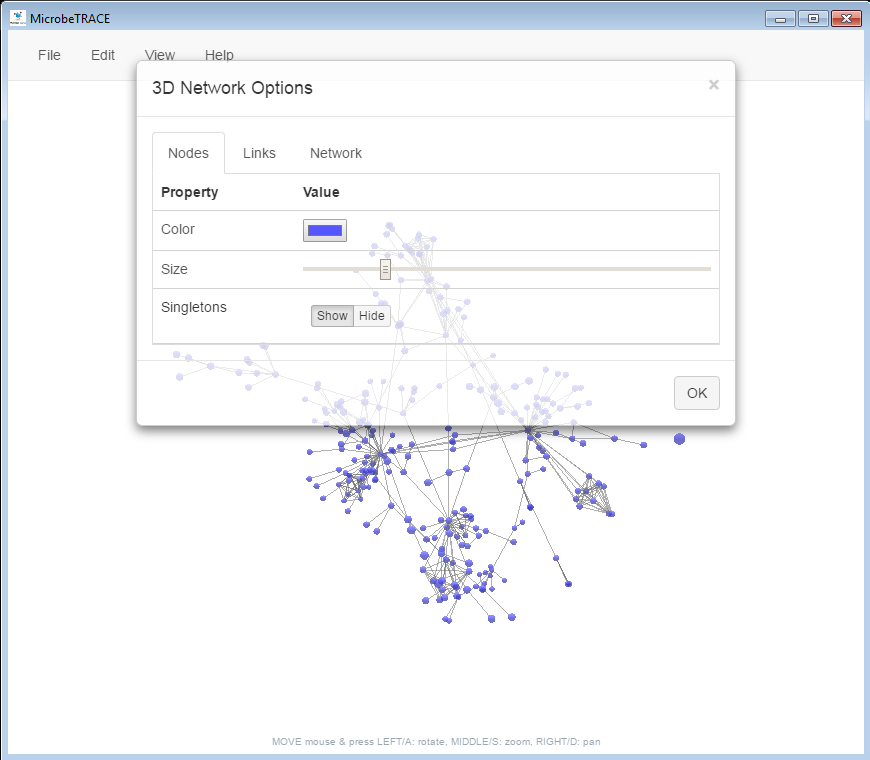
**Fig. 23**. Selecting the 3D Network View

The network will be rendered in 3D which can sometimes be useful for further exploration (Fig. 24). In the 3D view, you can zoom in or out by using the mouse wheel. Although you cannot reposition nodes in the 3D view, you can rotate the network to find the position that gives you the best visualization of clusters and links.



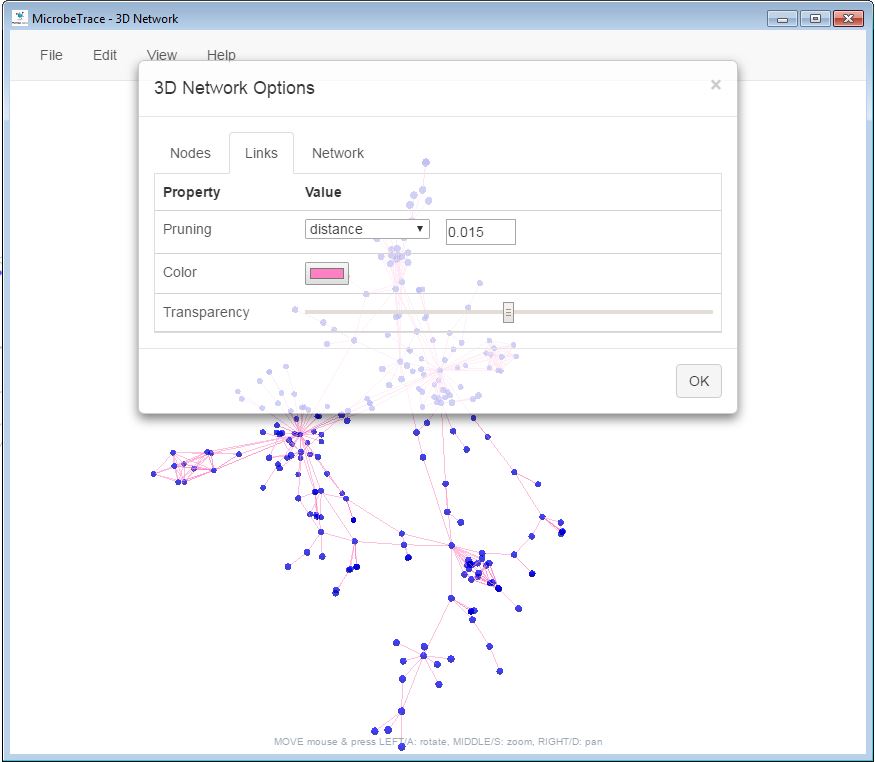
**Fig. 24.** 3D Network View. **A.** standard network view. **B.** 3D rendering of the same network in A. View was also after zooming in to show the 3D aspect of nodes as well as rotation of the network.

As with the Network View, you can change the node and link settings in the 3D view (Fig. 25). Select **Edit> Settings** for options. You can change the color and size of nodes and show or hide singletons.



**Fig. 25**. Setting node properties in the 3D network view

You can also select the **Links** tab to change the edge settings in the 3D view (Fig. 26). You can change the genetic distance cut-off value by specifying it in the textbox next to the **Pruning** option. You can also change the color of links and increase or decrease transparency using the transparency slider. Clicking on the **Network** tab lets you change the background color.

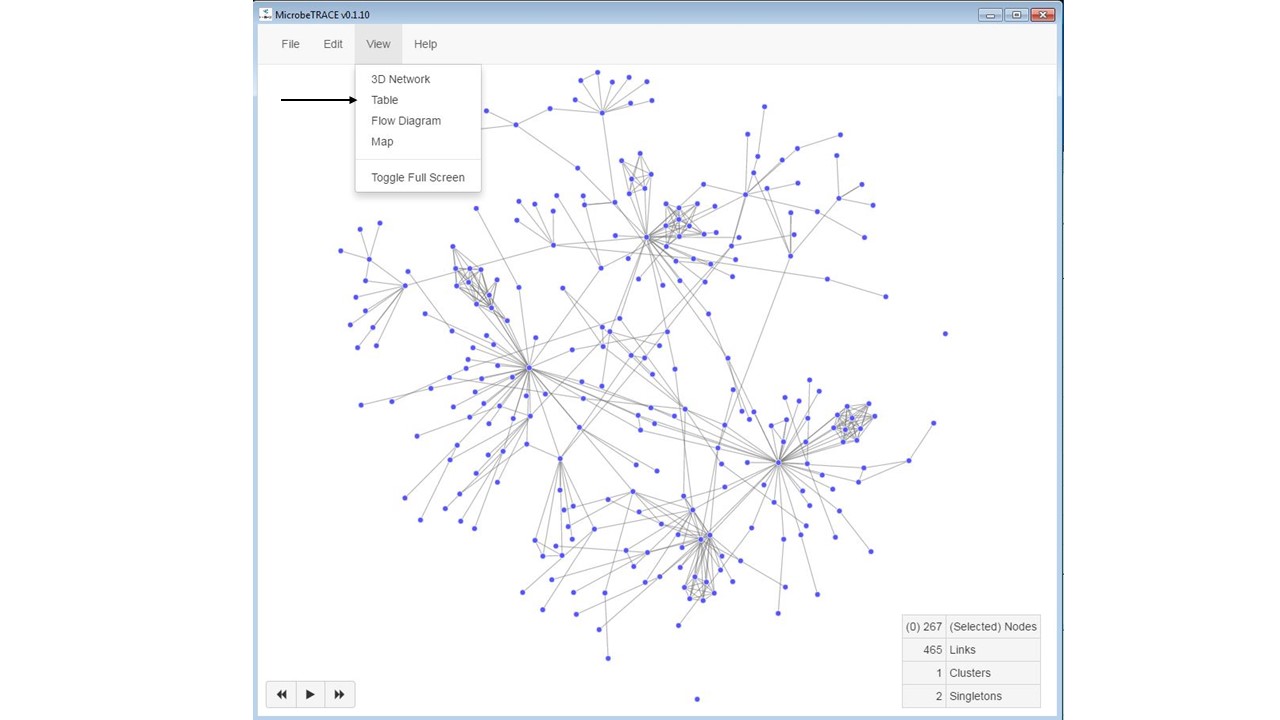


**Fig. 26.** 3D network options – setting link and network propertie**s**

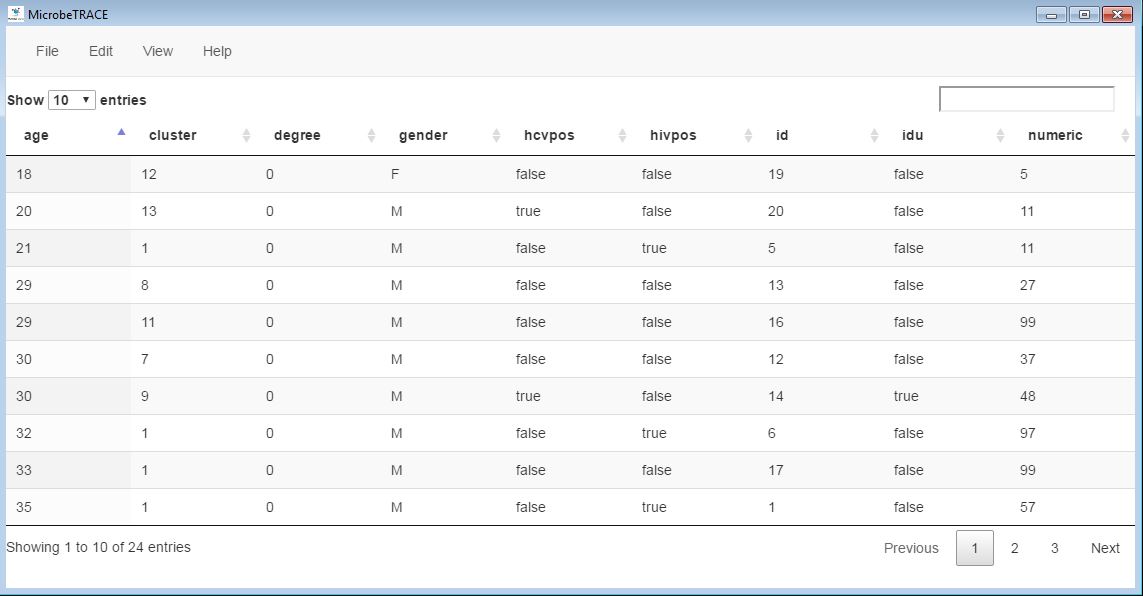
**Table View**

Table view enables you to see the data associated with the nodes in the form of a table. The Table View is similar to that of an Excel worksheet. A search box allows you to find information for nodes of interest in the table data (see below for more information).

Select **Table** under **View** (Fig. 27). The system displays the table in a new window (Fig. 28).



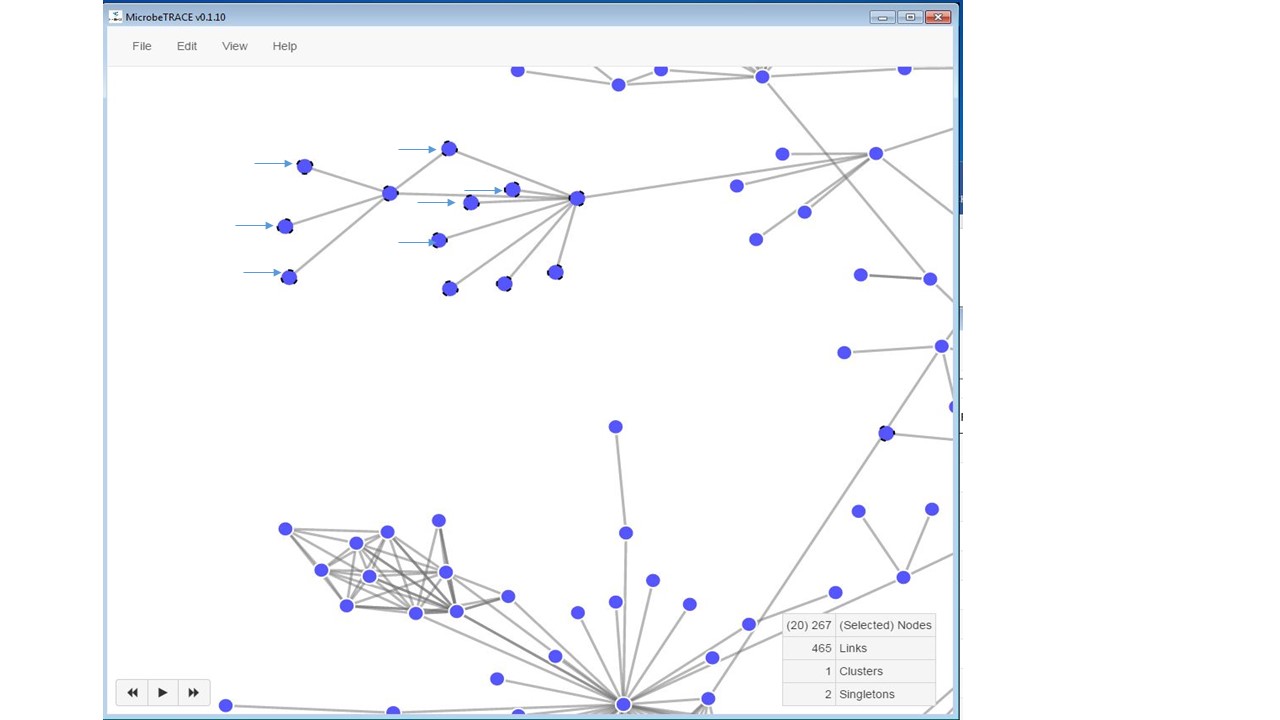
**Fig. 27.** Selecting the Table View



**Fig. 28.** Table View display

If you choose to view data for specific nodes in the Table format, then you can select as many nodes as you want to include in the Table view by holding down the Shift key to select the nodes (see Fig. 29 below to see how selected nodes are displayed). Now go back to the Table View window.

Clicking on the column headers in the table will sort all rows according to data in that column. Currently selected nodes in the network display will be sorted to the top of the table. Selecting rows in the table will highlight the corresponding nodes in the network and also the linked geographic data in the Map View (but only if the geographic data is included in your [node list](#NodeList) data file). Searches can be performed on all textual entries in the table. You can search for a specific node using an ID or other identifiers, select it in the table, and then see the corresponding highlighted node in the Network View to see links and cluster or network positions. Please note that if you chose to hide singletons in the Network View, then while you are in the Table view you select a node that happens to be a singleton, the singleton will not show up in the Network View.



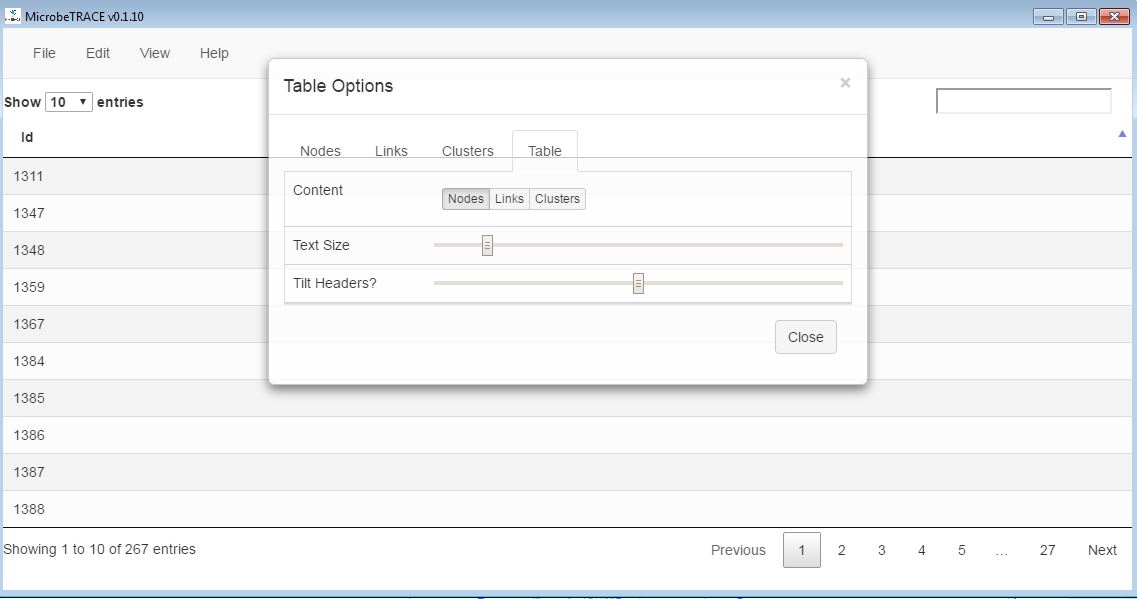
**Fig. 29.** Selected nodes are marked with an animated dashed black border (see arrows). These selections are then automatically moved to the top of the table in Table View

**Table Options**

You can change table settings by selecting **Edit> Settings>Table Options**

**Table tab:**

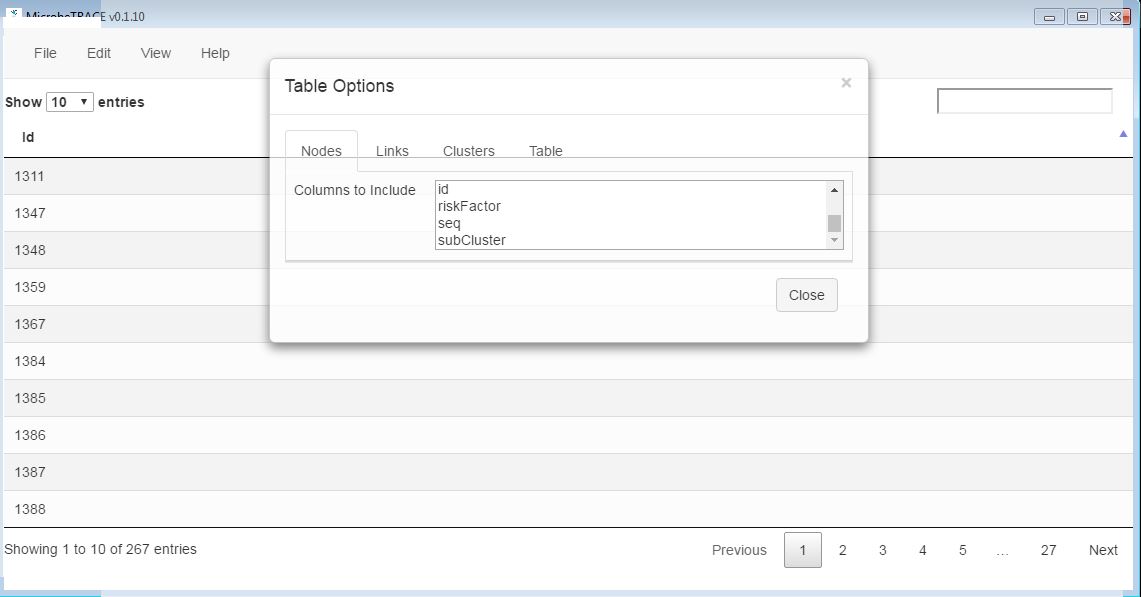
The default setting displays node data in the table columns. You can use the buttons to switch between viewing the link or cluster data in the table (Fig. 30). You can also adjust text sizes and tilt column headers by using the provided sliding scale options. Tilting the column headers may be useful if the header names are long.



**Fig. 30.** Table options - Table tab

**Nodes tab:**

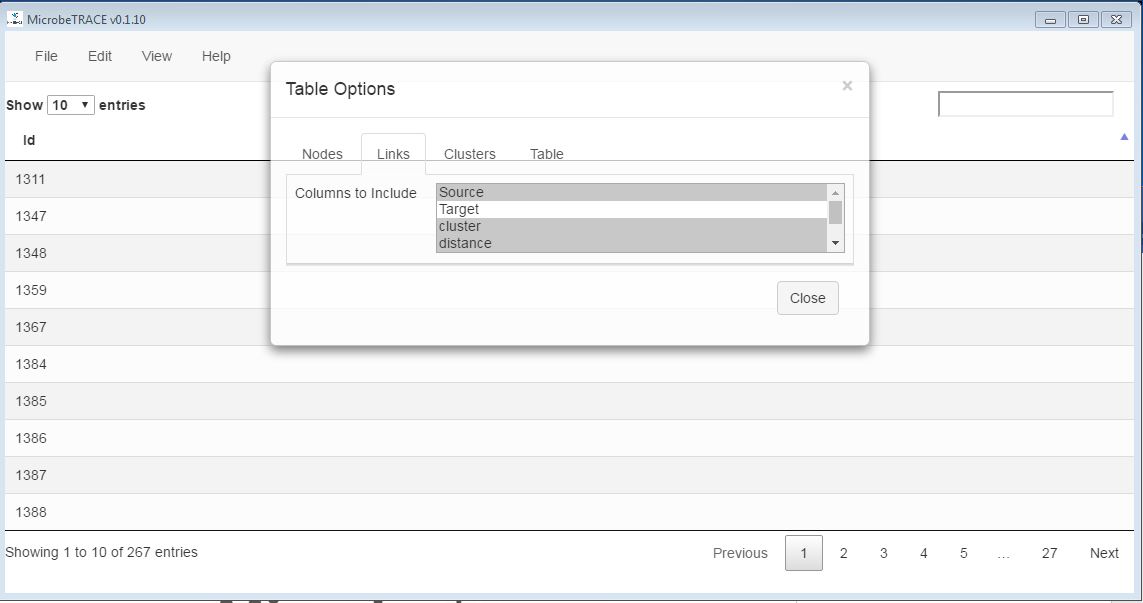
You can change which data are displayed in the table columns by selecting the **Nodes** tab and then selecting which columns of data to include by using the drop-down menu (Fig. 31).



**Fig. 31.** Table options - node tab; selecting node data to be displayedin the table

**Links tab:**

Similarly, you can change which link data to include in the table by selecting **Links** and then selecting those data columns to include in the table by using the drop-down menu (Fig. 32).

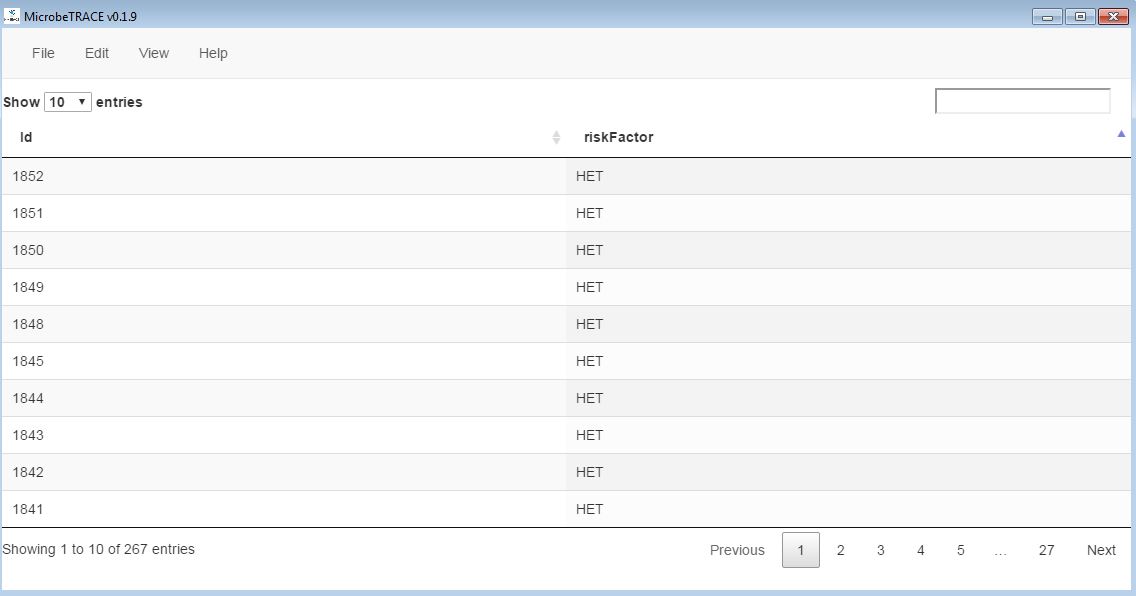
****

**Fig. 32.** Table options - Links tab; selecting link data to be displayed in the table

**Cluster tab:**

As with node and link data, you can change which cluster data to include in the table by selecting **Clusters** and then selecting those data columns to include in the table by using the drop-down menu.

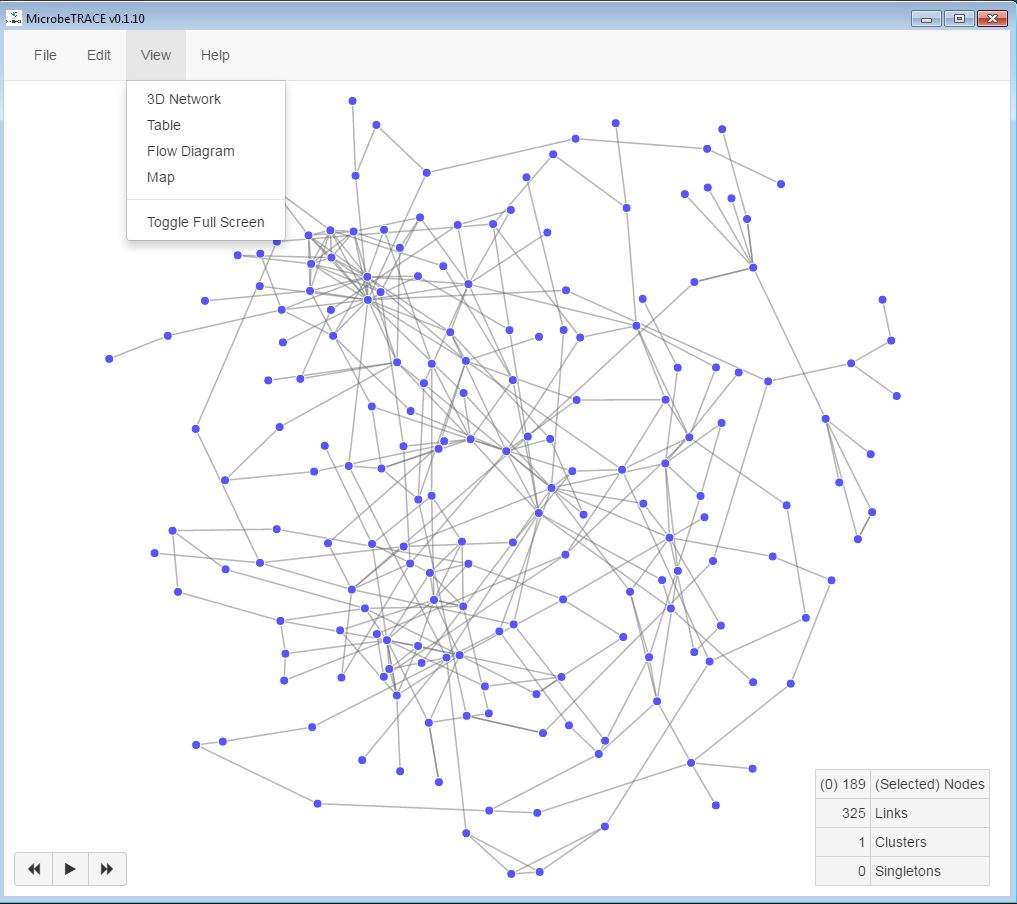
After selecting the desired table options, select **Close** or click the **X** at the top right corner of the window to close it. The table will be displayed according to the selected options. In Fig. 33 below, the variable ID and risk factor are the only two node data columns chosen to be displayed in the table.



**Fig. 33.** Table options - table with only two node properties selected for inclusion in the Table View

**Flow Diagram View**

The Flow Diagram View allows the data to be visualized in the form of a flow diagram (specifically, an [alluvial](https://en.wikipedia.org/wiki/Alluvial_diagram) or [Sankey diagram](https://en.wikipedia.org/wiki/Sankey_diagram)) and allows for a comparison of variables in the data set. The flow diagrams can be generated from any data, as they rely exclusively on node characteristics. Select **Flow Diagram** from the **View** menu (Fig. 34). The software displays the flow diagram in a new window (Fig. 35).



**Fig. 34.** Selecting Flow Diagram View



Desired variable from node list

Stream field

Stream field

**Fig. 35. Flow Diagram View -** comparison of risk factor information across zip codes (see drop-down menu options selected above the graphs)

The blocks in the flow diagram represent the relative prevalence of the selected variables and the stream fields (curved lines of variable thickness) between the blocks represent associations between selected variables.

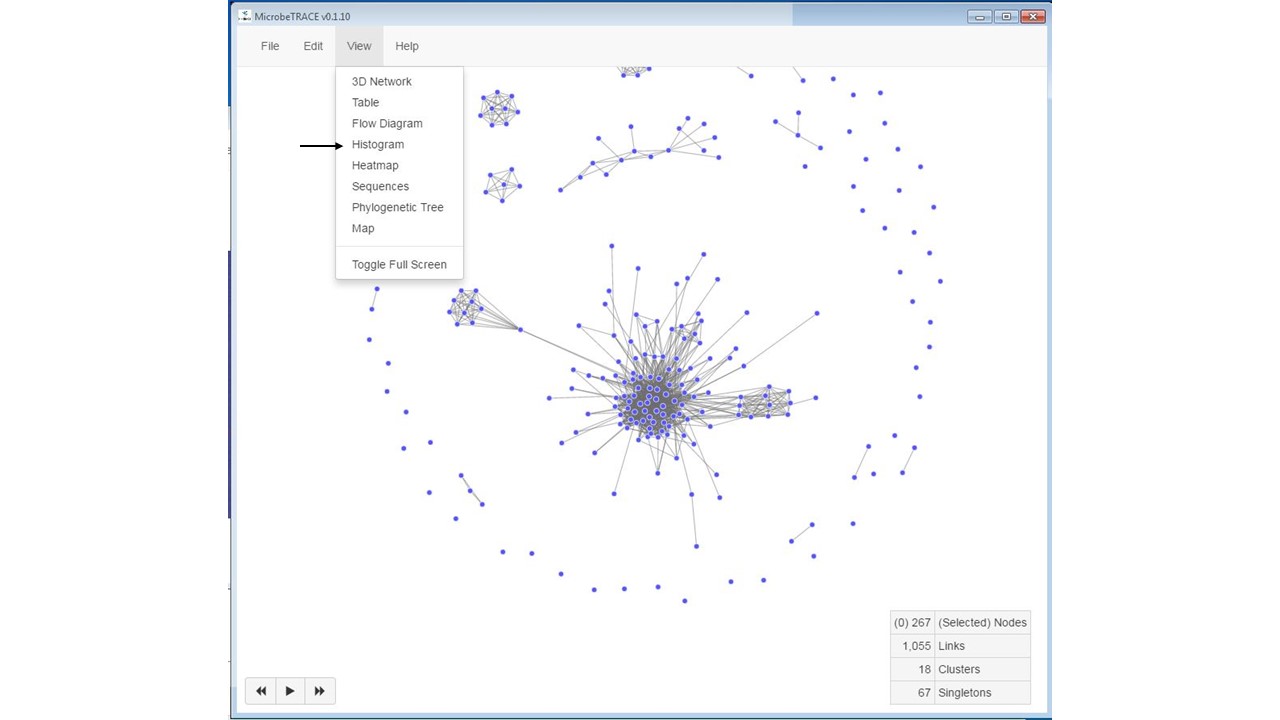
The drop-down menus on either side of the window allow you to select the two variables you want to visualize in the flow diagram. In this example, infection risk factors and zip codes were examined.

**Histogram View**

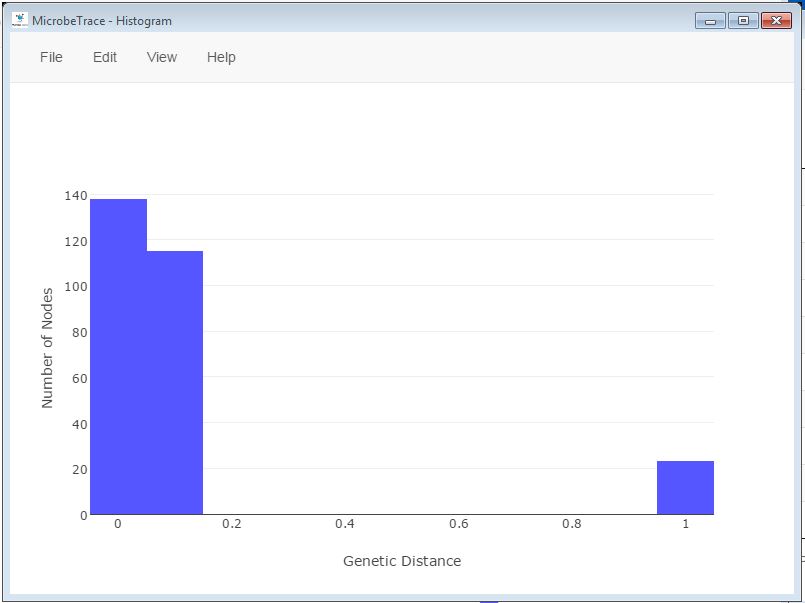
If the analysis included a FASTA file with nucleotide sequences, the genetic distance results calculated with the TN93 model can be viewed as a histogram. ***Please note that the Histogram View is not available for partner services data.***

The genetic distance histogram is a bar chart that shows the frequency with which a particular genetic distance occurs in the data set. Typically, the frequency distribution in the histogram chart appears bi-modal (two peaks). One peak will contain genetic distances of very closely related sequences and the second will contain more distantly related sequences. The genetic distance which best separates these two peaks can be used to refine the genetic distance threshold selection for your specific analysis.

Click **Histogram** under **View** (Fig. 36) to display the genetic distance histogram (Fig. 37).

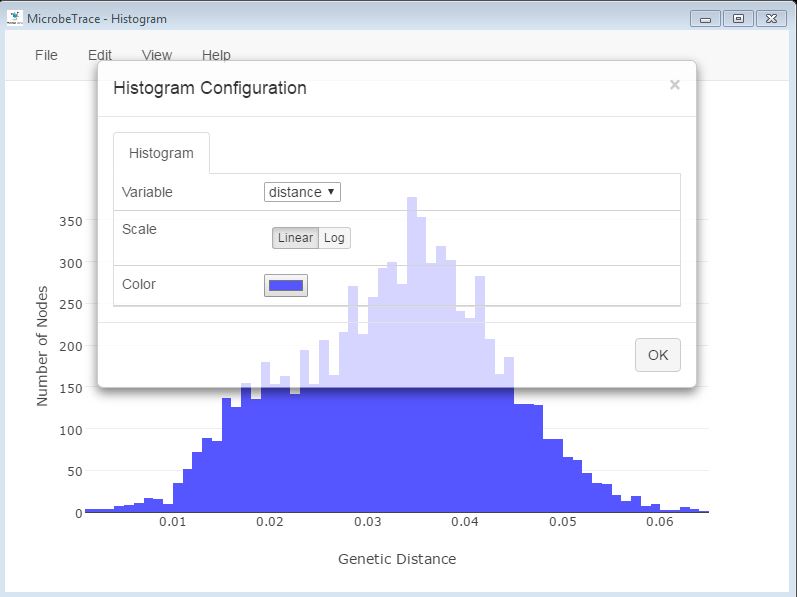


**Fig. 36.** Selecting Histogram View



**Fig. 37.** Histogram View showing the frequency of genetic distances between sequences in the given data set

The Histogram settings can be changed by selecting **Edit**> **Settings** on the menu bar. Select the relevant button to choose the type of links used for configuring the histogram (Fig. 38). The genetic distances can also be plotted in linear or log scales by toggling between linear and log scales when distance is the chosen variable to plot. The color of the histogram can be changed by selecting the color bar. The default histogram setting uses distance as the variable, displays frequency of the distances between all links and uses a linear scale for the distances. The variables to plot in the histogram can be selected from the pull-down menu. Note that values must be numeric rather than categorical.



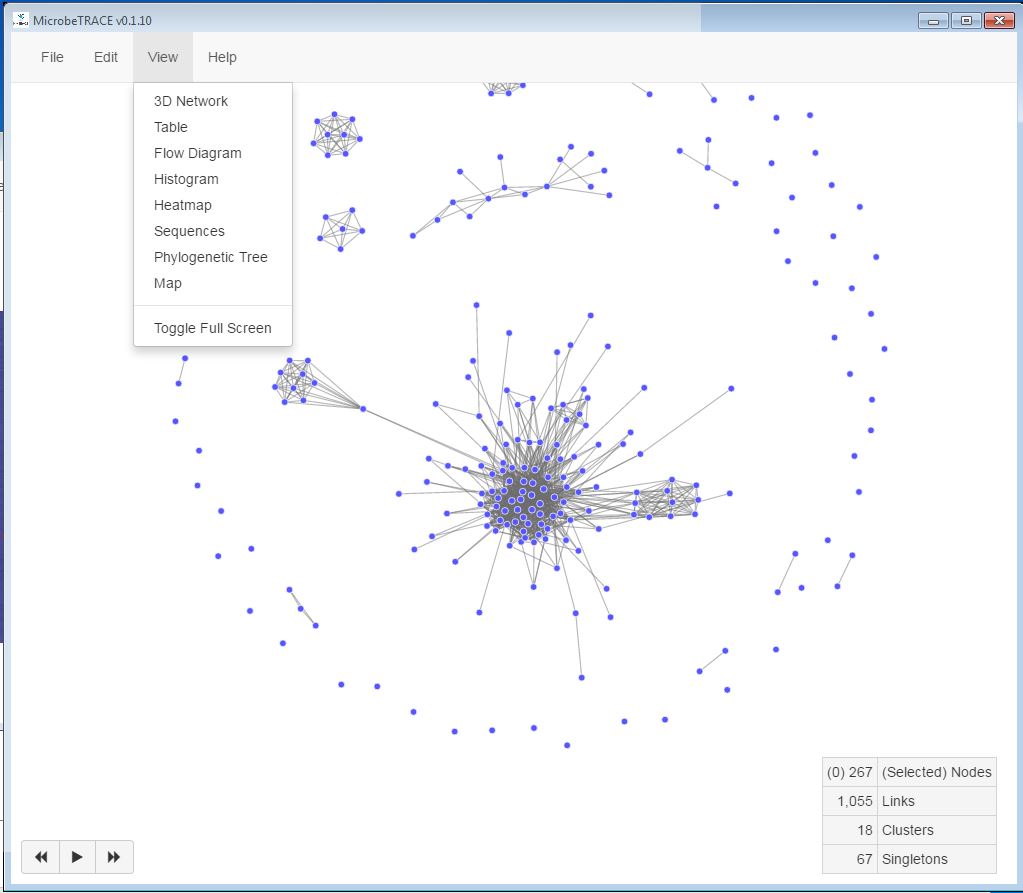
**Fig. 38.** Configuring the genetic distance histogram

The distances on the bimodal curve can be examined to determine the genetic distance cut-off to use for determining linkage of the nucleotide sequence (see [genetic distance threshold](#GeneticDistanceThreshold) in the glossary for details). If a different genetic distance cutoff value needs to be used for your specific analysis, then can go to the Network View and input this value in the genetic distance threshold under [Network Configuration](#NetworkConfig) (see Fig.19).

**Heatmap View**

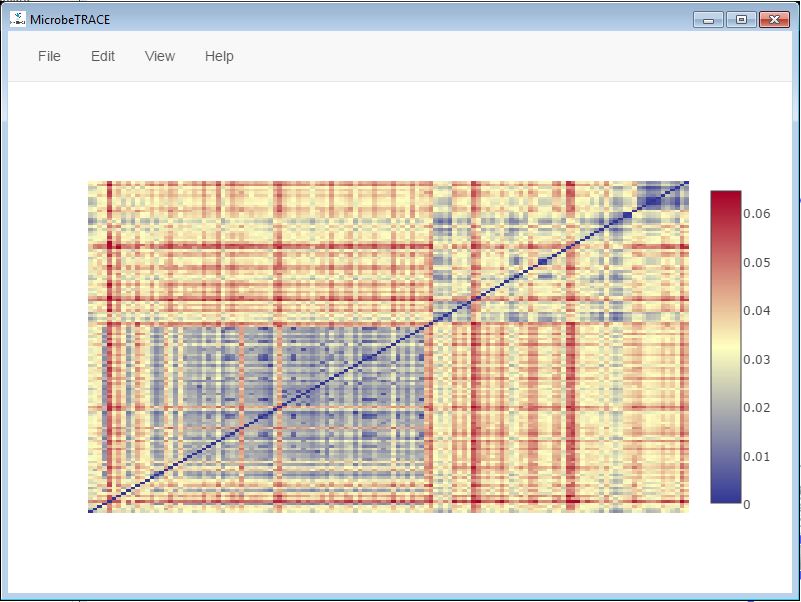
If the analysis includes a FASTA file with nucleotide sequences, MicrobeTrace offers a heatmap visualization of the calculated nucleotide genetic distance matrix.

To display the heatmap, select **View** on the Menu bar and then select **Heatmap** (Fig. 39)



**Fig. 39.** Selecting Heatmap View

This will open a new window showing the genetic distances in a heatmap matrix (Fig. 40). Note that rendering the genetic distance matrix takes a moment, so the window will appear blank at first. Once the computation is completed, the genetic distance heatmap matrix will appear.



**Fig. 40.** Heatmap View of the genetic distance matrix

Each cell in the matrix represents the genetic distance between two sequences in the dataset. The cells across the diagonal (bottom-left to top-right) represent a comparison of a sequence to itself (i.e., will have a genetic distance of zero, resulting in the visibly distinct diagonal line). The scale bar on the right of the heatmap indicates the color scheme used for the range of genetic distances in the dataset analyzed. For example, dark blue cells indicate sequences that are more closely related genetically than those in yellow or red. The actual genetic distance value for two sequences can be viewed by hovering the mouse pointer over the desired cell in the matrix. A pop-up bubble will show the IDs of the two sequences and their calculated genetic distance.

The heatmap graphic can be saved as .png or .jpg image files by selecting **File** in the Menu bar, and **Save Image**. Use the Save dialog to navigate to the desired destination on your computer and type in a filename for the image to be saved. Select **Save** to save the image file. You can also export the actual distances as a .csv file, which can then be viewed in Excel.

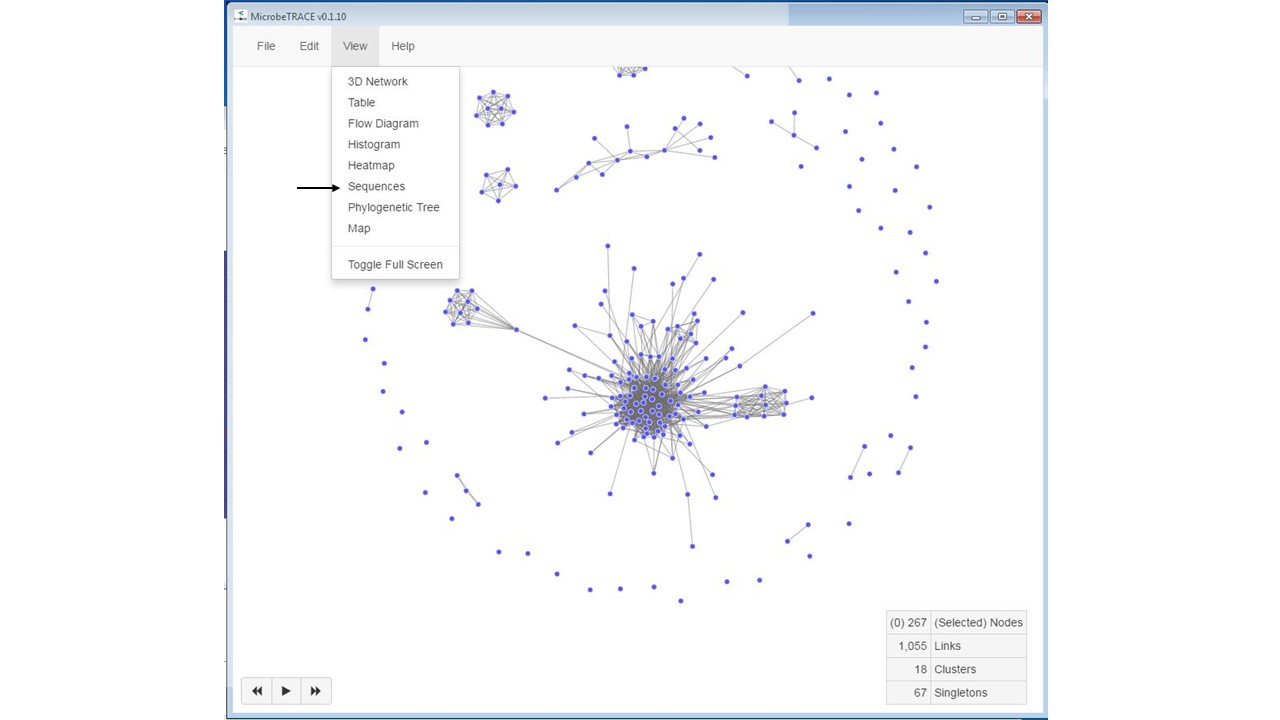
**Sequence View**

The primary function of the Sequence View is to permit a visual inspection of the quality of the final alignment to ensure that there are no unexpected gaps, insertions, etc. in the alignment. An improper alignment will greatly impact determination of genetic distances and thus also the inferred network or phylogenetic tree. This may be especially true for non-HIV sequences, since the aligner is not configured to handle sequences from other pathogens unless the appropriate reference sequence is included in the FASTA file, [as described above](#FASTAFile). Non-HIV sequences may not always properly align using the TN93 nucleotide substitution model that is commonly used for HIV. The Sequence View may have limited value if a pre-aligned FASTA file is used in the analysis unless the Sequence View is used to re-check that alignment.

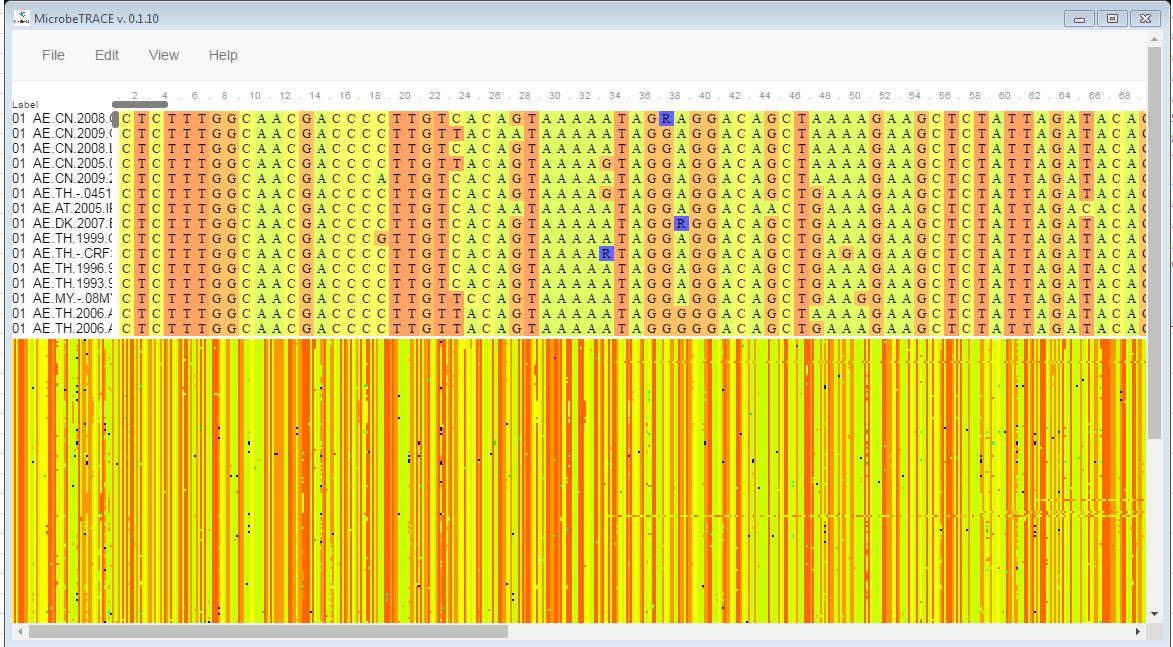
**\*IMPORTANT NOTE\*** ***We strongly recommend checking the quality of all pre-made alignments prior to using them in MicrobeTrace.*** Please note that any edits made to the alignment in the sequence viewer will not automatically be rendered in the inferred network. The edited sequence alignment file must first be saved and can then be used from the beginning of the analysis.

Select **Sequence** under the **Edit> Settings** menu (Fig. 41). The sequences are displayed as an alignment in a new window (Fig. 42). Use the scroll bars to maneuver the displayed sequence view.

***\*NOTE\*: All features available in the Sequence View are through an independent sequence analysis software embedded in MicrobeTrace called MSA Viewer (http://msa.biojs.net/).***



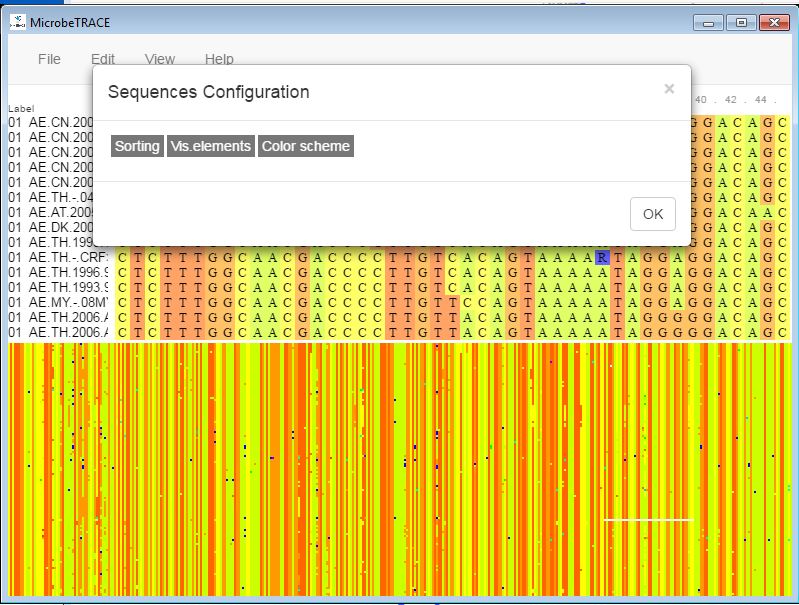
**Fig. 41.** Selecting Sequence View



**Fig. 42.** Sequence View**.** Aligned nucleotide sequences are displayed; the top half displays nucleotides while the bottom panel gives a larger overview of the quality of alignment.

**Changing the Sequence view settings**

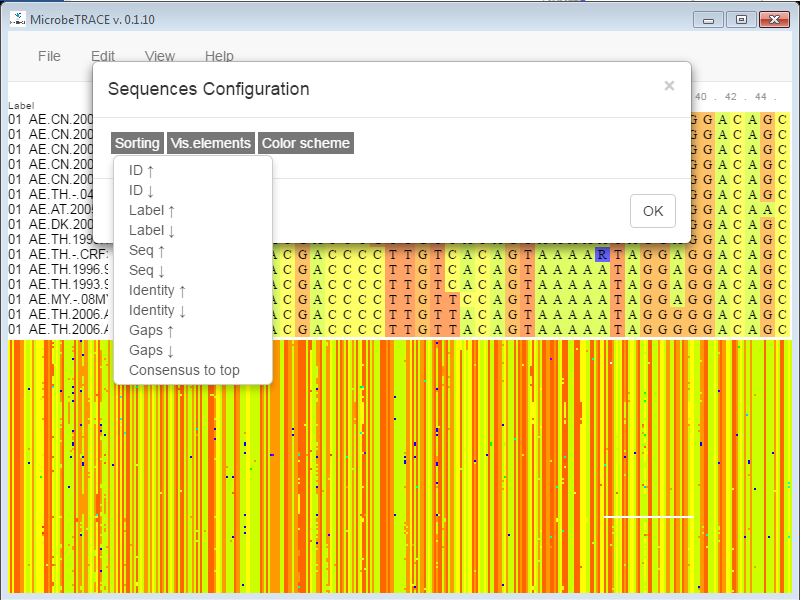
Click **Edit>Settings** to change the Sequence View settings (Fig. 43). Select the settings button in the Sequences Configuration menu to see the options available for that button.



**Fig. 43.** Sequence View configuration - available setting options

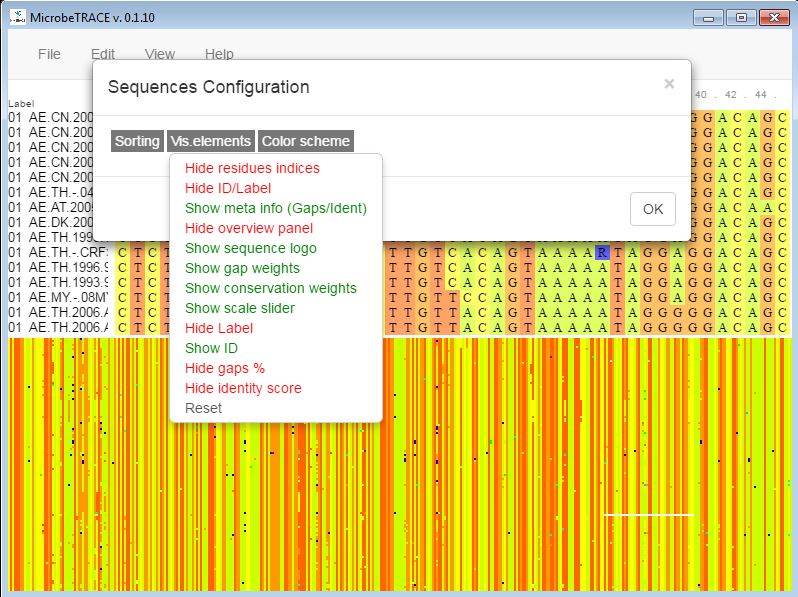
Descriptions of each Sequence View setting are as follows:

**Sorting:** Used to sort the data displayed and change the order of the sequences in the viewer using the available options (Fig. 44).



**Fig. 44.** Sorting sequences in Sequence View

**Vis. Elements**: Used to hide or show different visual elements such as labels, sliders, meta information (e.g. gaps, identity score, ID/label etc.), or to reset the sequences back to the original setting after making any changes (Fig. 45).



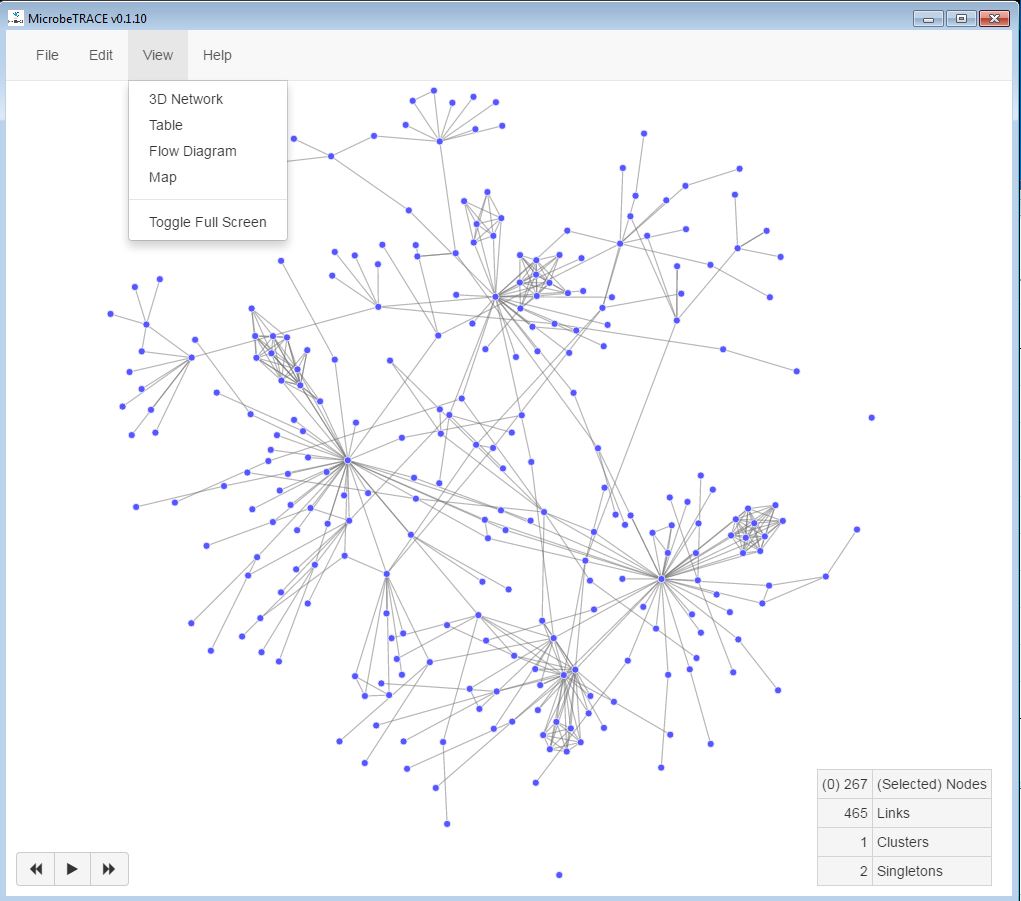
**Fig. 45.** Sequence View - selecting elements to display

**Color scheme:** Select one of the pre-loaded color schemes, or opt to not use colors.

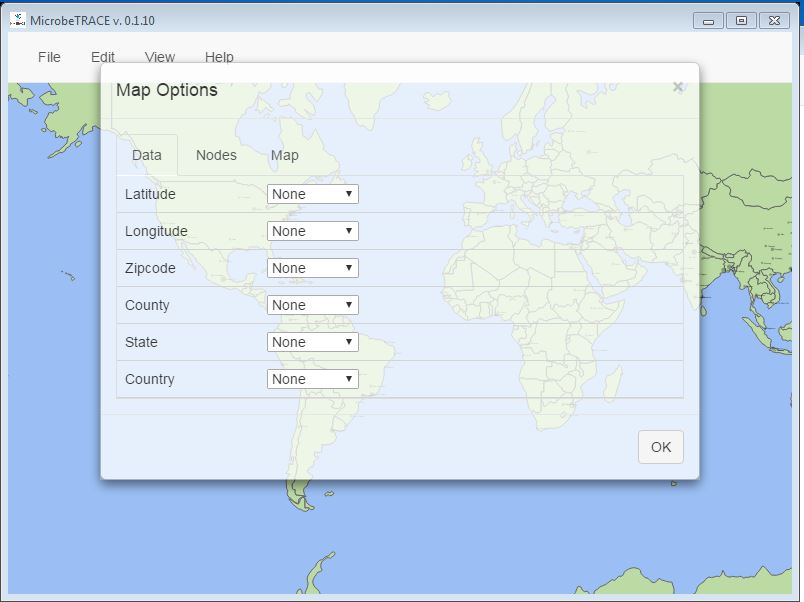
**Map View**

The nodes in the data can be displayed in a global geographic map using Map View if the latitude and longitude geo-coordinates for the nodes are included in the node file. The map allows you to zoom to the geolocation indicated in the node list. Note that latitude and longitude are ideal, but zip codes and other geopolitical demarcations (counties, cities and states) can also be rendered on the map.

Select **View** and select **Map** (Fig. 46). This brings up the Map View with the Map options window open (See Fig. 47).



**Fig. 46.** Selecting Map View



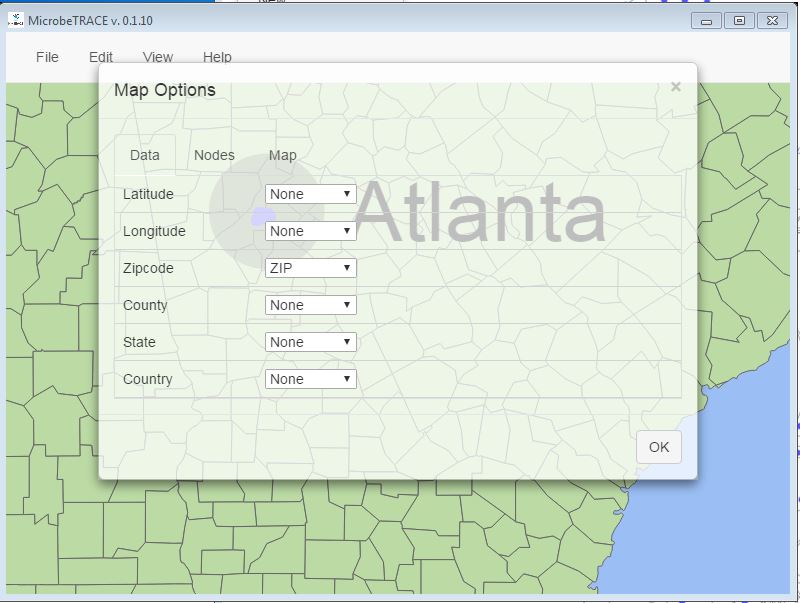
**Fig. 47.** Map options. Select the option that is best for visualization of your data based on the information stored in your node file.

**Changing map options**

The Data, Nodes and Map options can be changed by selecting each of them in turn.

**Data:**

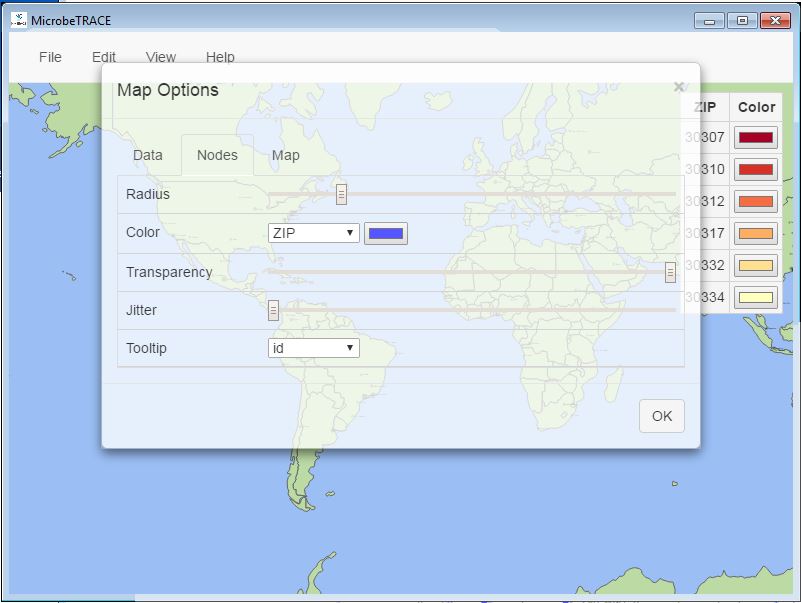
Selecting **Data** will show the pull-down menu options for this feature. In this example, we would like to visualize data by zip code. ***\*NOTE\*: The map display is hierarchical, so if your data set has all the data columns listed below, and you select multiple properties, the map displayed will default to the highest available level of geographic precision.*** Please ensure you select only the variable that works best for your dataset, and leave the others as **None**. In this example, a dataset of Atlanta area sequences, we have zip code information in the node list, and have selected zip code as the geographic parameter to use (Fig. 48).



**Fig. 48.** Map options – selecting zip code displays nodes on map

**Nodes:**

Select **Nodes** to change the appearance of nodes on the map (Fig. 49). Node size can be changed using the **Radius** slider bar. Nodes can be colored by any variable in your node file, and the transparency and jitter speed of the nodes can be changed using the respective slide bars. ***\*PLEASE NOTE: Please make sure you slide the jitter option bar towards the right if you want to see node separation.***  Use **Tooltip** (See definition here [**Tooltip**](#ToolTip)**)** to change which variables are displayed when the mouse pointer is placed (“hovered”)over a node. For example, if you choose **ID** from the Tooltip drop-down menu, the node ID will be displayed when the mouse pointer is over that node.



**Fig. 49.** Map options; changing the node color and tooltip features to be displayed.

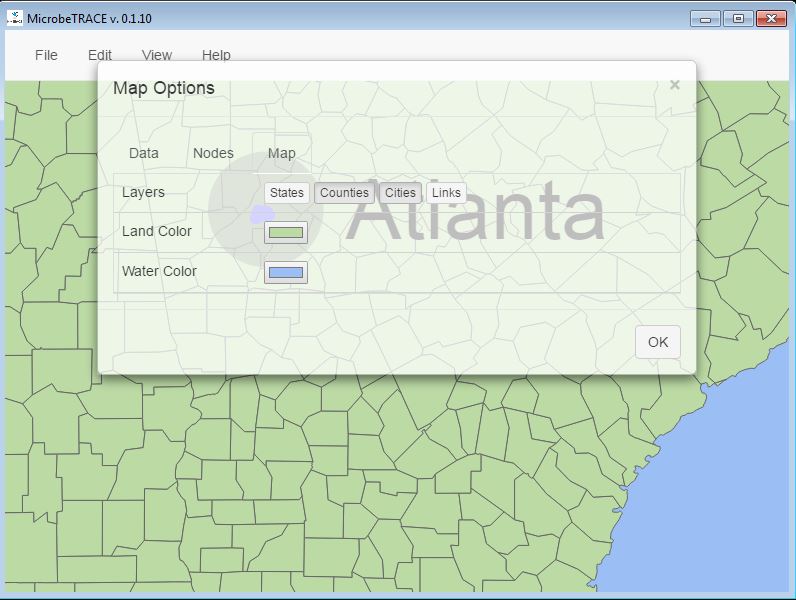
**Map:**

Select **Map** to change the land and water colors to be displayed (Fig. 50). You can also select one or more levels of map detail to be displayed, including down to the state and city level boundaries. In Fig. 38, county and city are selected. Once the map settings have been selected, chose **OK** to see the changes to the map (Fig. 51).

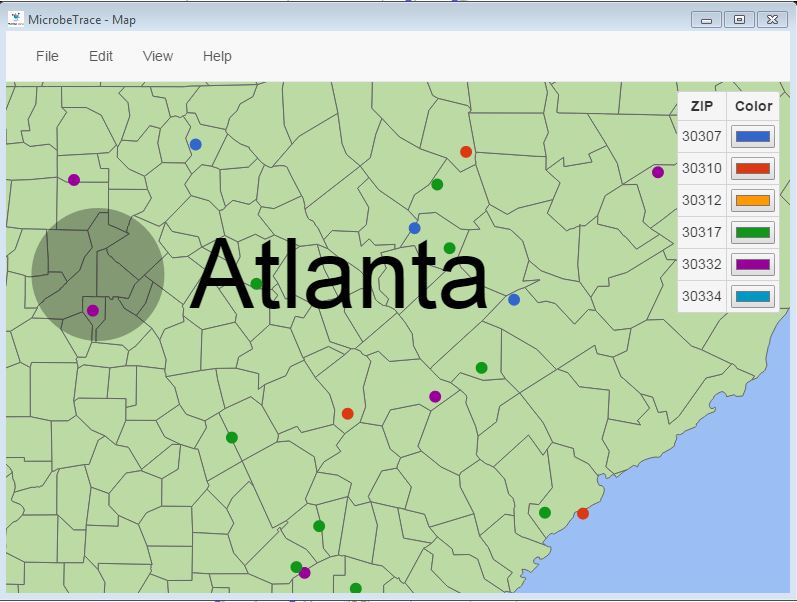
Selecting the **Links** button will highlight links (edges) in red on the map (see Fig. 52).

The software displays the node data in the map based on the various options you select. When viewing a map, the scroll bar on your mouse can be used to pan or zoom in and out. Individual or multiple nodes can be selected or de-selected by using the mouse pointer. These selections will propagate to the network and table views. This enables tracking of particular individuals between multiple visualization windows.

Map images can be exported and saved as .png or .jpg image files.



**Fig. 50.** Map options; changing the land and water colors in the map and the geographic density.



**Fig. 51.** Map View after selecting county level boundaries and visualizing the location and population size of Atlanta.



**Fig. 52.** Selecting **Links** in the **Map** tab highlights links in red. Image is zoomed in to better view links between nodes in this example.

**Phylogeny View**

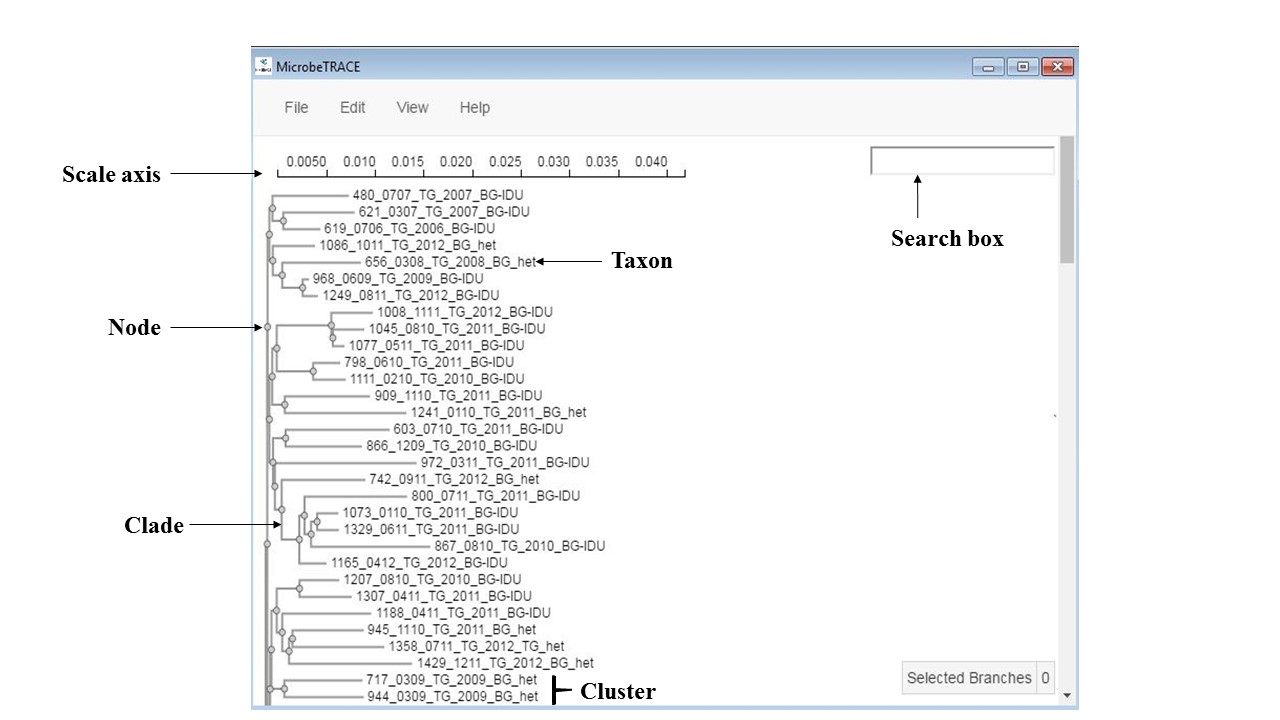
If you loaded a FASTA file with nucleotide sequences into MicrobeTrace, in the Phylogeny View you can generate a phylogenetic tree using the genetic-distance based neighbor-joining (NJ) method. Like genetic distance networks, a phylogenetic tree represents the evolutionary relationships among a set of sequences from a group of organisms. For example, phylogenetic analysis has shown that HIV-1 is composed of three main groups, M, N, O, and P. Group M is the most common group that has spread globally. Group M is further divided into multiple subtypes which represent closely related but distinct virus genotypes. Subtype B is the most common subtype in the US. The horizontal lines are called branches or tips of the tree and represent each taxon or descendant, as in a descendant in a “family tree”. The nodes on the tree represent the inferred common ancestor for one or more taxa (plural of taxon). Clusters of sequences or taxa are called clades, which represent closely genetically related sequences. A clade is a group of taxa that includes an ancestor and all descendants or taxa of that ancestor. For example, HIV-1 subtype B can be considered a clade consisting of all subtype B sequences.

The phylogenetic software embedded in MicrobeTrace is a JavaScript program called Phylotree (https://github.com/veg/phylotree.js/tree/master).

Phylogenetic trees can be rooted or unrooted. Rooted trees provide information about the order of nodes in the tree. The root of the tree is the oldest ancestral lineage of the dataset examined. Unrooted trees show the relationships of the taxa without making assumptions about ancestry. The NJ method used for Phylogeny View will infer a rooted tree.

The length of the horizontal branch is directly proportional to the amount of genetic change in your dataset. The scale bar above the tree in the Phylogeny View provides the number of nucleotide substitutions/site in the dataset for the branch lengths in the inferred tree. The vertical lines have no meaning and are used to only evenly display the taxa in the tree.

To display the phylogenetic tree, select **Phylogenetic Tree** from the **View** menu. The tree will then be displayed in a new window (Fig. 53). You can use the mouse to select different taxa in the tree. The number of selected branches is displayed at the bottom right of this window. A search box on the top right of the Phylogenetic View allows the tree to be searched by taxon (sequence) name. The taxon name is highlighted in blue. This feature is useful if you have many taxa in the tree resulting in a large tree. For large trees, use the scroll bar on the right of this window to move vertically in the tree.



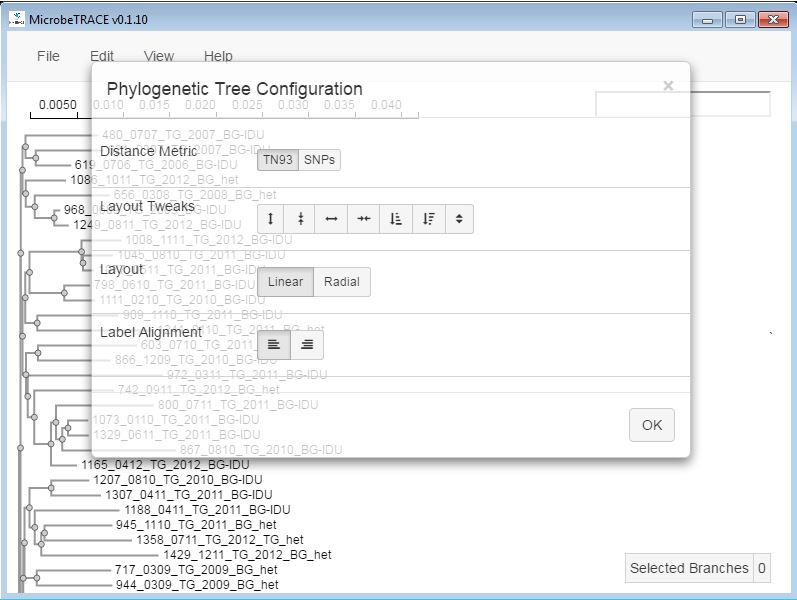
**Fig. 53**. Phylogenetic relationship of sequences in your FASTA file using the neighbor joining method

Select **Edit>Settings** for the tree configuration menu for changing various parameters for how the tree is displayed (Fig. 54).

**Distance matrix:** TN93 is the default nucleotide substitution model for the genetic distance matrix used to infer the tree. Currently, we do not offer additional nucleotide substitution models. However, SNPs can be used to infer the phylogeny if you chose to compute SNPs during the sequence alignment step.

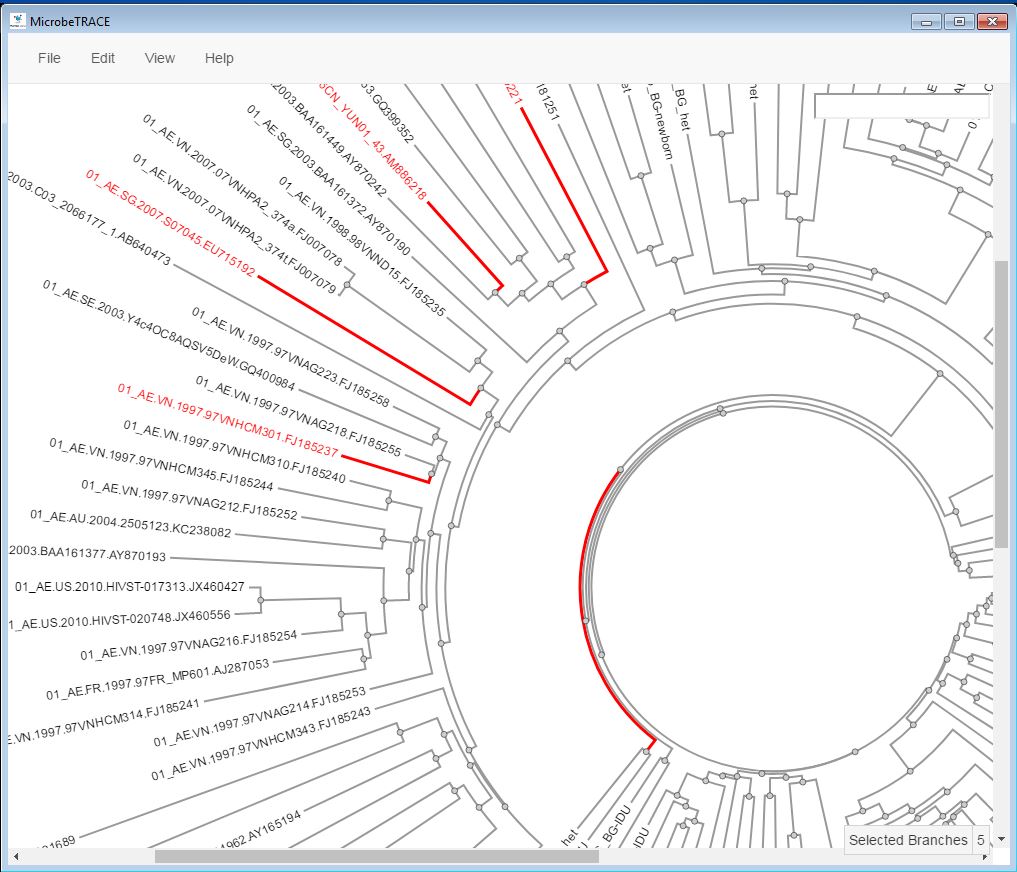
**Layout tweaks:** This option is used to change the spacing between clades so the branches and taxa are more visible if you have a large tree. You can also sort the deepest clades to the bottom or to the top of the tree. Deep clades are those closest to the root of the tree and hence are usually older lineages. You can restore original settings by clicking the furthest right button (▲▼) in **Layout Tweaks**. Hover your mouse pointer over each button to see its function.

**Label alignment:** Used to right align the taxa labels but does not alter the branch length.



**Fig. 54.** Available tree configuration options

Figure 55 shows a radial tree with increased branch spacing. Radial view is another option to display the tree layout such that the tree expands outward radially with the root in the center. In the Phylogeny View, you can select as many branches as you wish to highlight in either the radial or linear tree layout. The total number of branches you select will be indicated in the “Selected Branches” box in the bottom right of the Phylogeny View window (Fig. 55).



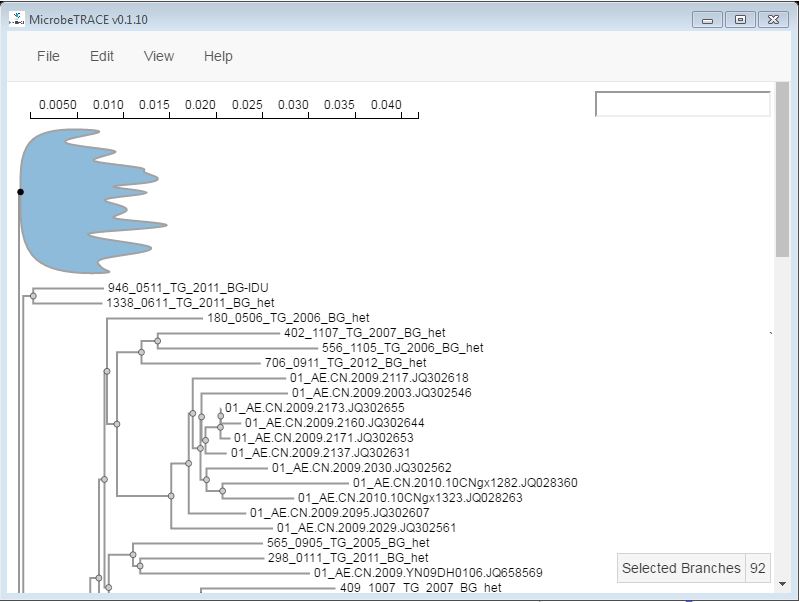
**Fig. 55**. Node and clade options showing the selection of five branches (red) on a radial tree

Selecting **re-root on any node** re-roots the tree to that node. This option is only used if you have prior information about the potential history of the taxa or if an outgroup was included in the analysis. The outgroup is used to “root” the tree. An outgroup is a set of taxa that are close but distinct from the taxa you wish to analyze. For example, HIV-1 subtype J could be used as an outgroup for an analysis of subtype B sequences. Please note that there is no direct undo option for re-rooting. If you re-root on a node and would like to go back to the original, please close the window, select **MapView** from the **View** menu, and it will once again display the original tree.

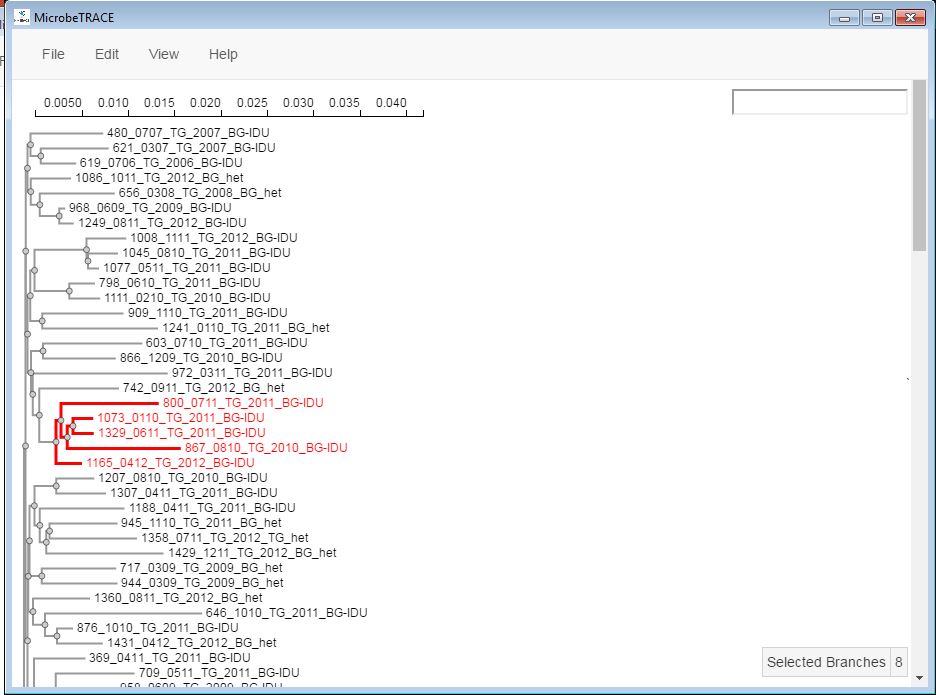
Multiple options are available for displaying and exploring the phylogenetic tree, including collapsing taxa in a clade or cluster into a compact shape (Fig. 56), highlighting a subset of taxa (Fig. 57), highlighting the terminal braches in a clade (Fig. 58), highlighting only branches internal to the node and not the terminal ones (Fig. 59), highlighting the branch that links the selected node to its predecessor (Fig. 60), highlighting branches that lead back to the root from a selected node (Fig. 61), and hides all clades originating from the selected node (hide subtree; Fig. 62).

All tree view images can be saved, and also exported as Newick files to analyze in other phylogeny or tree viewing programs (Fig. 63). The Newick file format is the standard for representing trees in a computer-readable form.

Select **File> Save image** or **File>Export Newick**



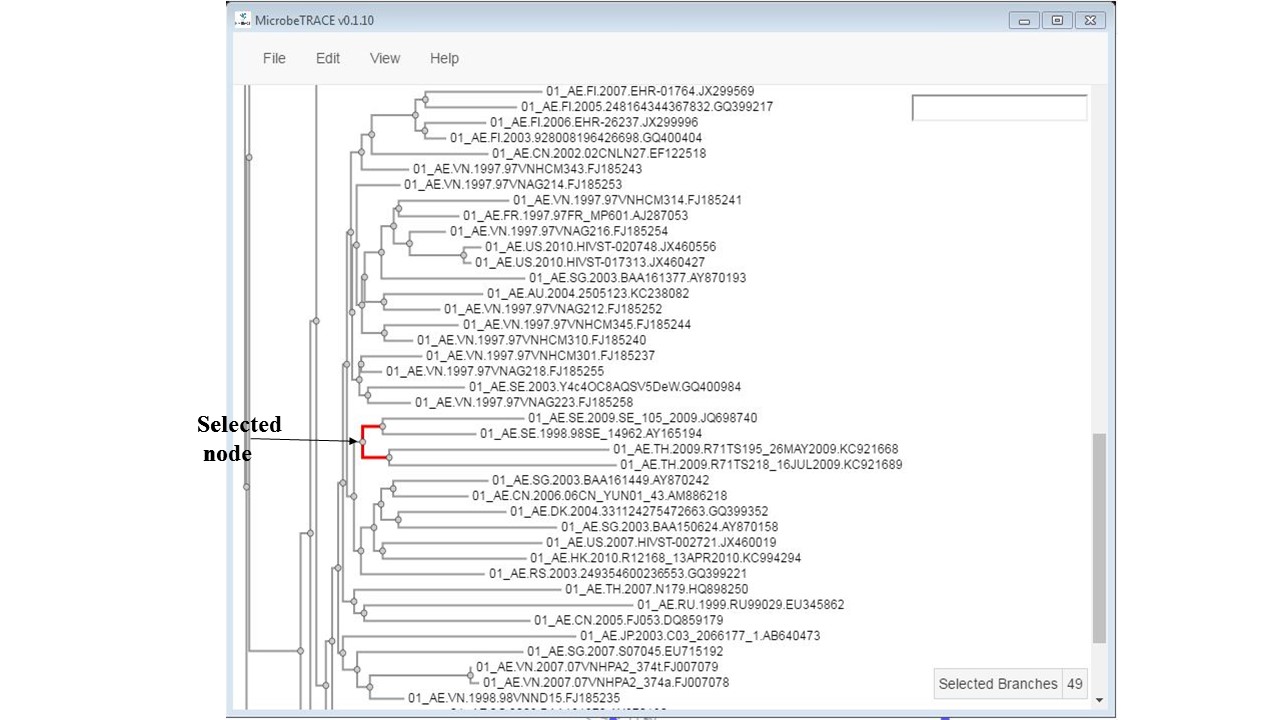
**Fig. 56.** Collapsing a clade collapses branches at that node into a compact shape to help improve tree visualization. Selecting nodes in the tree will display a window showing options for that node, including collapsing the branches descendant from that node into a single object or expanding a collapsed node back to the original layout. This feature is especially useful for large datasets where it is difficult to see the tree in its entirety.



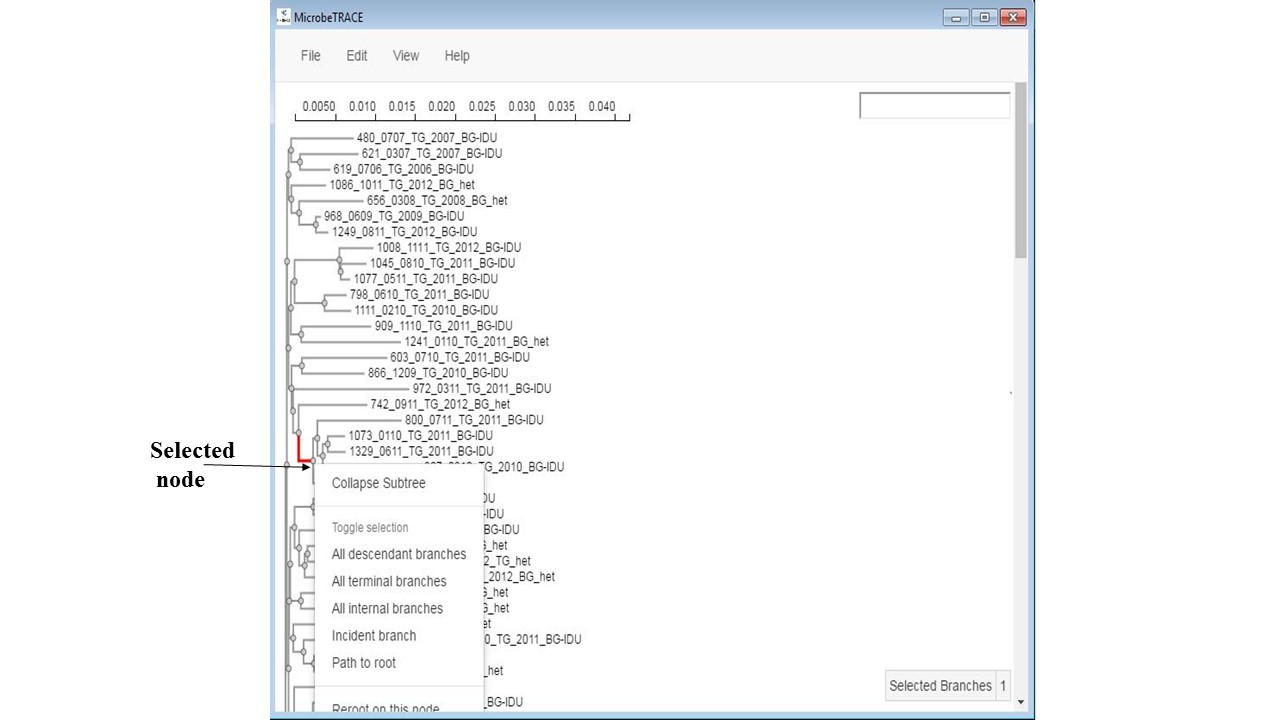
**Fig. 57.** Selection of a subset of all descendent branches



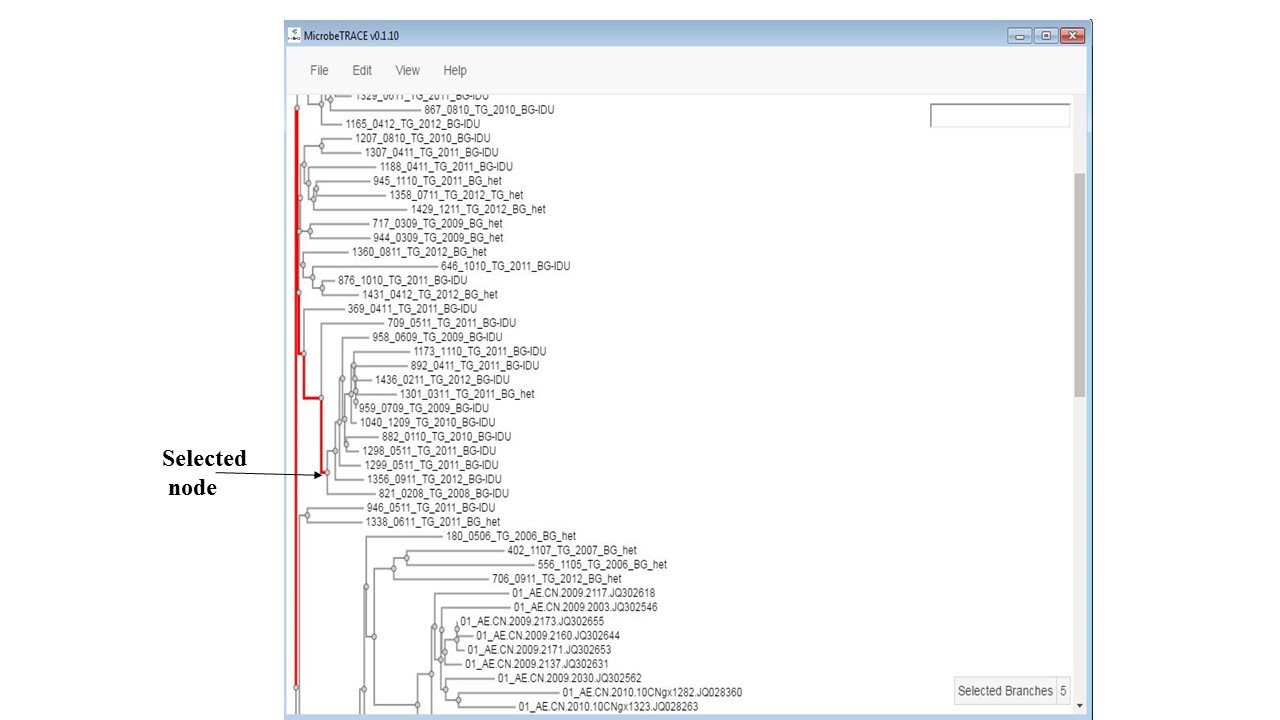
**Fig. 58.** Selection of terminal branches of a specific clade that are at the terminus or tip of the selected node



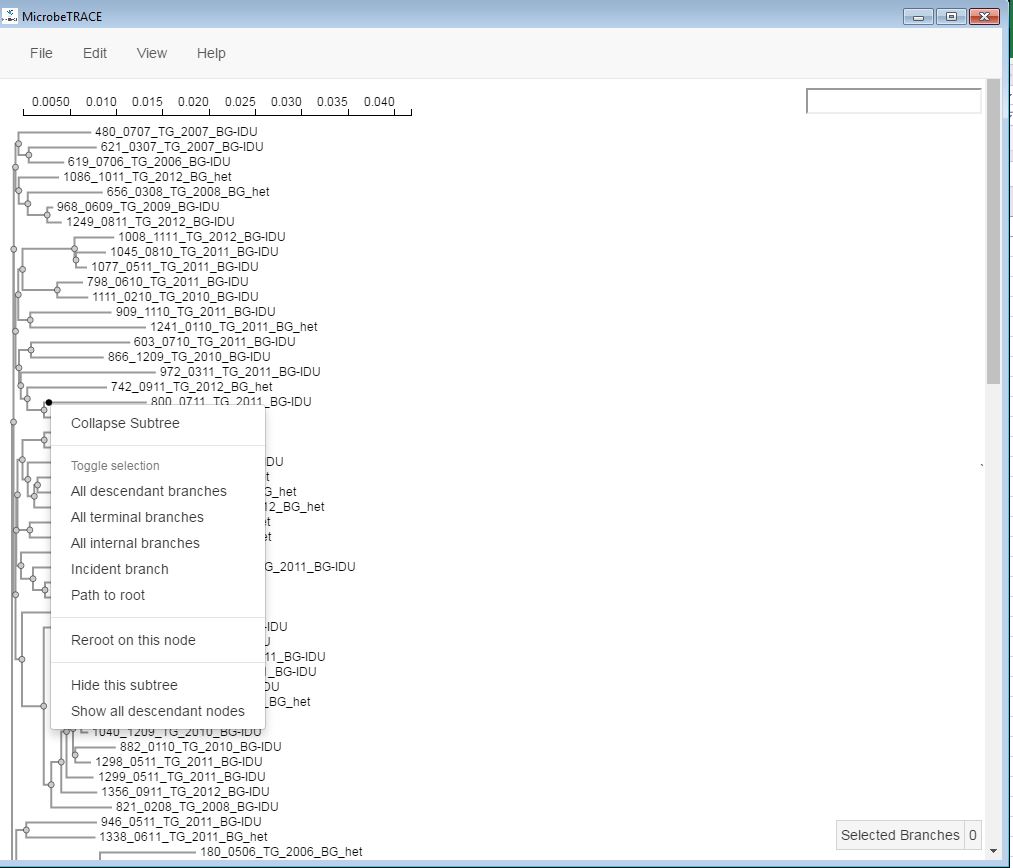
**Fig. 59.** Selecting “All internal branches” highlights only branches internal to the node and not the terminal ones.



**Fig. 60.** Selecting “Incident Branch” highlights the branch that links the selected node to its predecessor.

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**Fig. 61.** Selecting “Path to root” highlights branches that lead back to the root from a selected node.



**Fig. 62.** Selecting “Hide this Subtree” hides all clades originating from the selected node. Again, this feature is useful for large trees, and makes the tree view more manageable. The hidden subtree is indicated by a black node, and clicking on the black node allows all descendent node to be visible again.

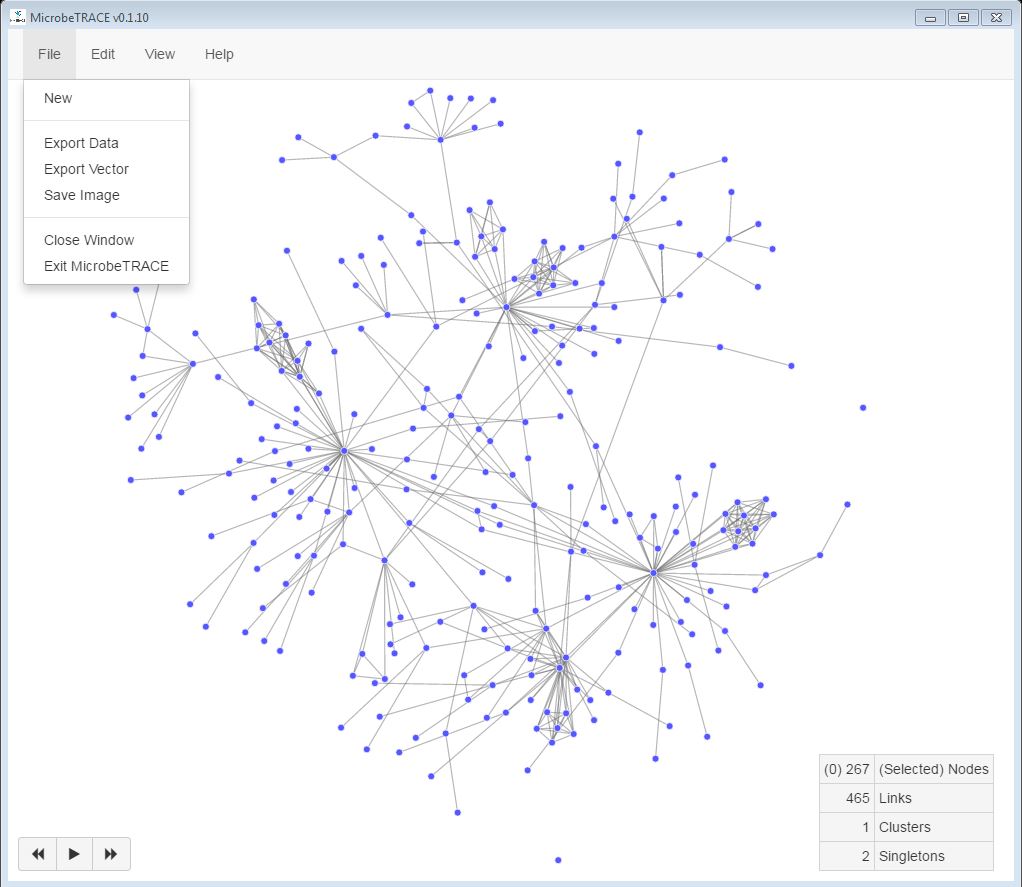
![Save tree image or export as Newick tree format for use in other programs.

](data:image/jpeg;base64,/9j/4AAQSkZJRgABAQEAYABgAAD/4RE0RXhpZgAATU0AKgAAAAgABAE7AAIAAAAjAAAISodpAAQAAAABAAAIbpydAAEAAABGAAAQ5uocAAcAAAgMAAAAPgAAAAAc6gAAAAgAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAFNoYW5rYXIsIEFudXBhbWEgKENEQy9PSUQvTkNISFNUUCkAAAAFkAMAAgAAABQAABC8kAQAAgAAABQAABDQkpEAAgAAAAM3OQAAkpIAAgAAAAM3OQAA6hwABwAACAwAAAiwAAAAABzqAAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA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**Fig. 63.** Save tree image or export as Newick tree format for use in other programs.

**File options**

From the main menu, you can access various file options in the File menu (Fig. 40).



**Fig. 64.** File options

File options include:

**Export Data:** exports your aligned sequence data as a FASTA file or MEGA file. MEGA is a software suite for analysis of DNA and protein sequence data and can be used for alignment, model selection, phylogeny etc. ([http://www.megasoftware.net/](http://www.megasoftware.net/).%20)). This option also allows a FASTA file to be created from a column in a CSV file containing nucleotide sequences.

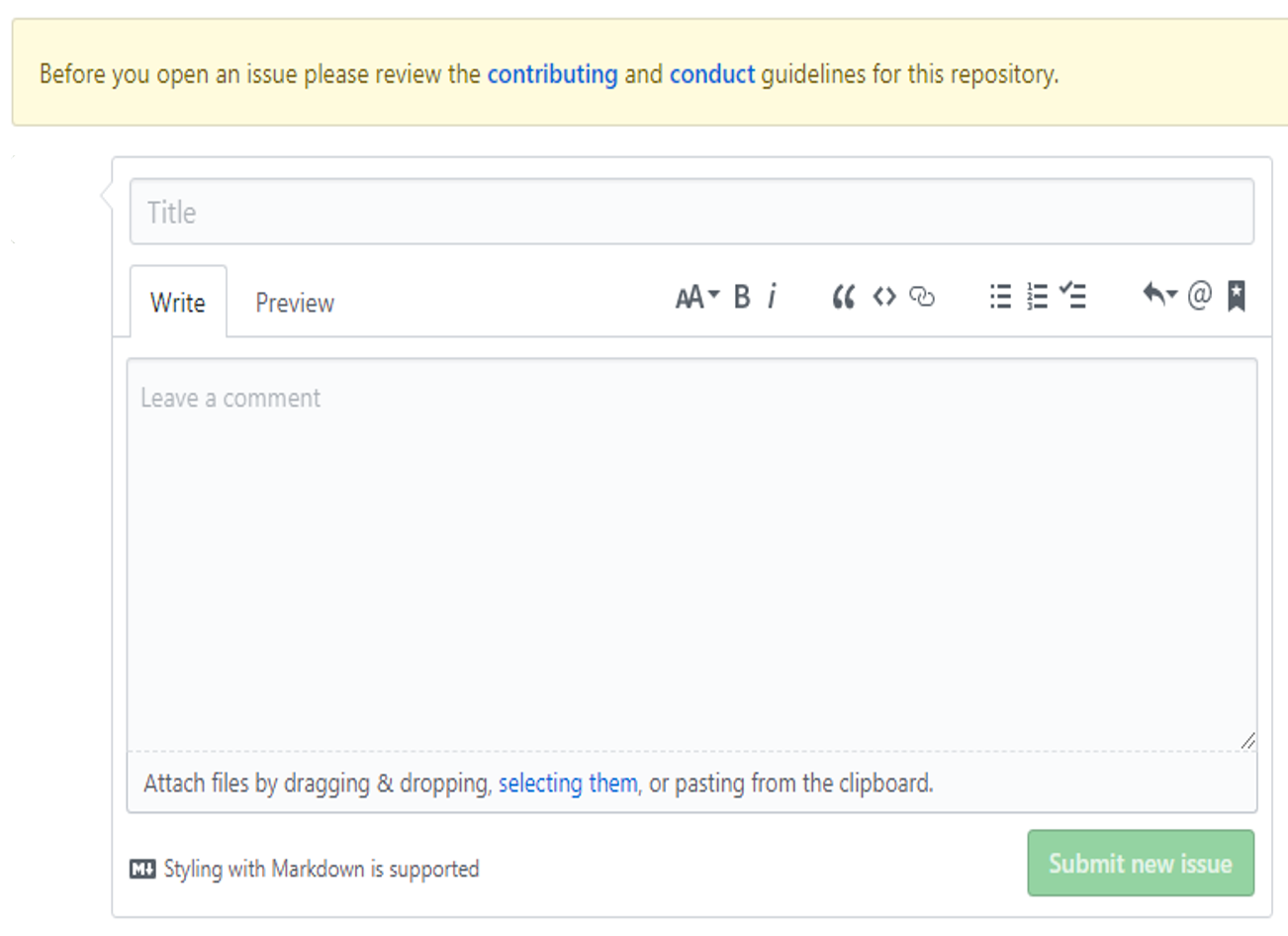
**Export Vector:** exports the network displayed as a scalable vector graphics (SVG) format image file

**Save Image:** saves the currently displayed image. This option is available in all of the Views. Images can be exported in either PNG (potable network graphic) or JPEG (joint photographic experts group) formats.

**Troubleshooting**

The MicrobeTrace GitHub site is used to track any software issues. To report any MicrobeTrace issues, use the link to go to the MicrobeTrace “new issues” website <https://github.com/CDCgov/MicrobeTRACE/issues/new>. Access to this site requires a GitHub account. If you do not have a GitHub account, then you will be prompted to create one. GitHub accounts are free and access requires only an email address and a self-generated password.

On the MicrobeTrace issues website you will be prompted with a form requesting a title and description of the issue (Fig 65).



**Fig. 65**. Reporting issues to the MicrobeTrace team

In the title, please include what View in the software program you were using. In the comment, please provide as much detail about the issue as possible, including the operating system used. You can also use this form to let us know how we can improve the software.

For example, [Issue #27](https://github.com/CDCgov/MicrobeTRACE/issues/27) is titled “The Group key table won't scroll past the bottom of the page”. While being clear about how the bug violates user expectations, this statement doesn’t describe what the “Group Key Table” is or where it can be found when encountered using the software. By contrast, [issue #125](https://github.com/CDCgov/MicrobeTRACE/issues/125) “Option to Switch Distance Matrix from TN93s to SNPs” both describes where the problem was encountered (in the Distance Matrix) and what is desired (the option to switch between the TN93 nucleotide substitution model for the analysis, and SNPs, which is available but unused).

You can also send an e-mail to request help from a CDC MicrobeTrace support representative at [\_-----@cdc.gov](mailto:nsp3@cdc.gov) (Please note: we are working on a dedicated email ID for user support)./When drafting your email, please be as thorough as possible, listing out every action you took in the program prior to encountering the problem. The more detail you can provide, the greater the likelihood that we'll be able to help resolve the issue.

**Uninstalling MicrobeTrace**

To uninstall MicrobeTrace, right-click on the MicrobeTrace icon on your desktop. In the window that opens, select **Open file location**, which will open a directory at the path

C:\Users\<Your Username>\AppData\Local\Programs\microbetrace. In this directory, you will find the file entitled **Uninstall MicrobeTrace.exe.** Double-click this file and you will be asked to confirm the uninstallation. Select **Yes** to uninstall MicrobeTrace.

**References**

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HIV-TRACE**:** <https://github.com/veg/hivtrace>

Guy Yachdav, Sebastian Wilzbach, Benedikt Rauscher, Robert Sheridan, Ian Sillitoe, James Procter, Suzanna E. Lewis, Burkhard Rost, Tatyana Goldberg; MSAViewer: interactive JavaScript visualization of multiple sequence alignments, Bioinformatics, Volume 32, Issue 22

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