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(54) **Title:** METHOD, SYSTEM AND SOFTWARE ARRANGEMENT FOR COMPARATIVE ANALYSIS AND PHYLOGENY WITH WHOLE-GENOME OPTICAL MAPS

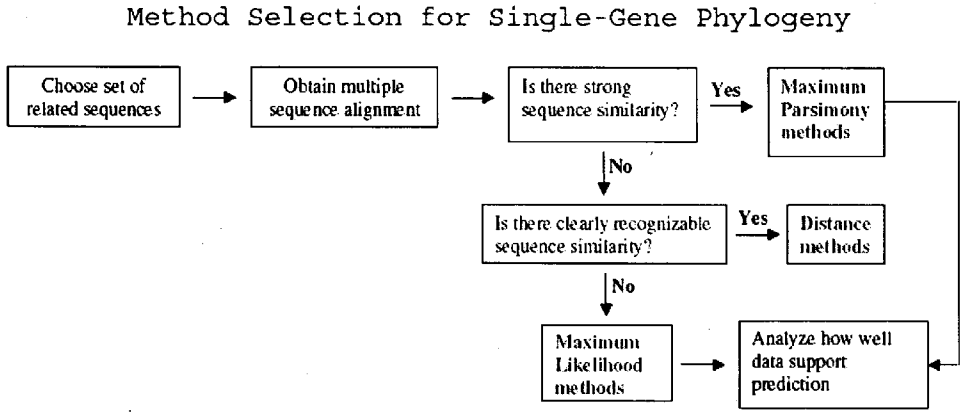

Figure 1. Procedure of selecting an appropriate method to infer phylogeny given single-gene sequences.

(57) **Abstract:** The present invention provides a method for organizing genomic information from multiple organisms. In one embodiment of the invention, phylogenetic trees can be constructed for the organisms. The method of the present invention is termed CAPO, Comparative Analysis and Phylogeny with Optical-Maps. Optical maps of organisms are obtained and phylogeny between the organisms is determined by optical map comparison and bipartite graph matching between the organisms, as, for example, computed by a stable marriage algorithm.

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METHOD, SYSTEM AND SOFTWARE ARRANGEMENT FOR COMPARATIVE ANALYSIS AND PHYLOGENY WITH WHOLE-GENOME OPTICAL MAPS

FIELD OF THE INVENTION

[0001] The present invention relates generally to methods, systems and software arrangements for characterizing whole genomes of several species and strains by comparing and organizing their genomes in a searchable database.

BACKGROUND

[0002] A phylogenetic tree represents the evolutionary history among organisms. Constructing phylogenetic trees is a crucial step for biologists to find out how today's extant species are related to one another in terms of common ancestors. Numerous computer tools have been developed to construct such trees

[0003] Given DNA sequences of various taxa, the standard technique in evolutionary analysis is to first perform a multiple sequence alignment (on DNA sequences or protein sequences). From the resultant distance matrix, a phylogenetic tree is built describing the relationship of the various taxa with respect to one another. These distance-based methods compress sequence information into a single number and the two sequences with shortest distance are considered as closely related taxa. However, the high cost of sequencing techniques and the biological diversity among the genomes, make it impossible to study phylogeny using detailed sequences of many strains of large-number of related species.

[0004] Standard methods for constructing phylogenetic trees, known to persons having ordinary skills in the art, include Unweighted Pair Group Method using Arithmetic Average (P. Sneath and R. Sokal. *The principles and practice of numerical classification*. Numerical Taxonomy, W. H. Freeman, San Francisco, 1973, incorporated herein by reference), Neighbor Joining (N. Saitou and M. Nei. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, 4:406-425, 1987, incorporated herein by reference), Fitch Margoliash (W. Fitch and E. Margoliash. The construction of phylogenetic trees – a generally applicable method utilizing estimates of the mutation distance obtained from cytochrome c sequences. *Science*, 155:279-284, 1967, incorporated herein by reference), Maximum Parsimony (J. Felsenstein. A likelihood approach to character weighting and what it tells us about parsimony and compatibility. *Biological Journal of Linnean Society*, 16:183-196, 1981, incorporated herein by reference), and Maximum Likelihood (J. Felsenstein. Evolutionary trees from DNA sequences: A maximum likelihood

approach. *Journal of Molecular Evolution*, 17:368-376, 1981, incorporated herein by reference).

[0005] The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method is a sequential clustering algorithm. It works by constructing distance matrix, amalgamating two Operational Taxonomy Units (OTUs) at each stage and creating a new internal node in the tree at the same time. Whenever two nodes are merged into a new node, it recalculates the distances between the new nodes and other nodes, repeating the process until all OTUs are grouped in a single cluster. It produces a rooted tree containing all the OTUs at the leaves of the tree. It is suitable for constructing phylogenetic tree of taxa with a relatively constant rate of evolution. It has several advantages: The algorithm is simple and fast. Its main disadvantages are: (1) It implicitly assumes the existence of an ultrametric tree: the total branch lengths from the root to any leaf are all equal. In other words, there is an assumed "molecular clock," which ticks at a constant pace, and all the observed species are at an equal number of ticks from the root; the same evolution rate is assumed to apply to all branches, which is often not the case. (2) It assumes a stringent additive property.

[0006] The Neighbor Joining (NJ) method is a heuristic greedy algorithm. It begins with distance matrix and a star-like tree. At each stage two closest neighbors are joined into a new node, which becomes the root of the new tree. The branch lengths from the two nodes to the new node are calculated. The two nodes are replaced by the new node in the distance matrix, thus reducing the number of OTUs by 1. In the process, it updates the distance matrix and performs the node merging process again. The process repeats until there are two OTUs left and they are joined into a root node. Unlike UPGMA, which chooses the neighbors with minimum distance, NJ chooses the neighbors that minimize the sum of branch lengths at each stage. It has several advantages: (1) It is fast and well suited for data sets of substantial size and also for the postprocessing step of bootstrap analysis. (2) It is especially suitable when the rate of evolution of the separate lineages under consideration varies. Its main disadvantages are: (1) It depends heavily on the evolutionary model applied. (2) Like UPGMA, it assumes a stringent additive property.

[0007] Both UPGMA and NJ employ distance matrix to reflect evolutionary relationship, compressing sequence information into a single number, and thus cannot reflect the changes of character states of sequences. UPGMA and NJ are relatively fast, so they are suitable for analyzing large data set that is not very strongly similar. In general, NJ gives better result than UPGMA.

[0008] The Fitch Margoliash (FM) method assumes that the expected error is proportional to the square root of the observed distances. It compares the two most closely related taxa to the average of all the other taxa. It then moves through the tree sequentially to calculate the distances between decreasingly related taxa until all the distances are found. Its advantages include the following: It does not assume a constant rate of evolution and therefore can produce varied branch lengths from a common ancestor. Its main disadvantage is that it requires longer computational execution time than UPGMA and NJ.

[0009] The Maximum Parsimony (MP) method is built upon the principle that simple hypotheses are more preferable than complicated ones. Consequently, the construction of the tree using this method requires the smallest number of evolutionary changes among the OTUs in order to explain the phylogeny of the species under study. This method compares different parsimonious trees and chooses the tree that has the least number of evolutionary steps (substitutions of nucleotides in the context of DNA sequence). MP is a character-based Maximum Parsimony algorithm. It starts with multiple alignment and construct all possible topologies. Based on evolutionary changes, it scores each of these topologies and chooses a tree with the fewest evolutionary changes as the final tree. An evolutionary change is the transformation from one character state to another. Character states can be DNA bases, the loss or gain of a restricted site, and the absence or presence of morphological features. Its advantages are enumerated as follows: (1) It allows the use of all known evolutionary information in tree building. (2) It produces numerous unrooted, "most parsimonious trees." Some of its disadvantages are listed below: (1) It requires long computation time, although faster than maximum likelihood. (2) It yields little information about branch length. (3) It usually performs well with closely related sequences, but often performs badly with very distantly related sequences.

[0010] The Maximum Likelihood (ML) method evaluates the topologies of different trees and chooses the best tree among all as measured with respect to a specified model. Such a model may be based on the evolutionary process that can account for the conversion of one sequence into another. It evaluates a hypothesis about evolutionary history in terms of the probability that the proposed model and the hypothesized history would give rise to the observed data set. The parameter considered in the topology is the branch length. It starts with a multiple alignment and lists all possible topologies of each data partition. It then calculates probability of all possible topologies for each data partition and combines data partitions. It identifies tree with the highest overall probability at all partitions as most likely

phylogeny. Its advantages include the following: (1) It is more accurate than other methods. It is often used to test an existing tree. (2) All the sequence information is used. (3) Sampling errors have least effect on the method. Its main disadvantage is that it is extremely slow, and thus impractical for analyzing large data set.

SUMMARY OF THE INVENTION

[0011] The present invention provides a method for organizing genomic information from multiple organisms. In one embodiment of the invention, phylogenetic trees can be constructed for the organisms. The method of the present invention is termed CAPO, Comparative Analysis and Phylogeny with Optical-Maps. This method can be used to determine phylogeny among optical maps of multiple strains or genomes. The low cost and high speed of an Optical Mapping technique provides an elegant solution to the problem posed by the high cost procedures involved in sequence generation and comparison.

[0012] In one aspect, the invention provides a method for comparative genomic analysis, the method includes comparing optical maps obtained from one or more organisms in order to obtain at least one pair-wise similarity value; and determining relatedness of the organisms based on said pair-wise similarity value. In a related embodiment, the method further includes constructing a phylogenetic tree based on the relatedness of the organisms. Exemplary organisms include a microorganism, a bacterium, a virus, and a fungus.

[0013] Another aspect of the invention provides a method for identifying an unknown organism, the method includes comparing an optical map from an unknown organism to a plurality of optical maps from a phylogenetic tree of known organisms; obtaining a pair-wise similarity value for one or more comparisons between the unknown organism and the known organism in the phylogenetic tree; and identifying the unknown organism based on the pair-wise similarity values. In a related embodiment, the method further includes, prior to the comparing step, preparing an optical map from the unknown organism. In another related embodiment, the method further includes, prior to the comparing step, constructing a phylogenetic tree of known organisms.

[0014] Another aspect of the invention provides a method for constructing a phylogenetic tree, the method includes obtaining pair-wise distances among organisms by comparing at least one pair of optical maps from the organisms in order to generate a pair-wise similarity matrix; and constructing a phylogenetic tree based on the pair-wise similarity matrix. In a

related embodiment, the method further includes, prior to said obtaining step, preparing optical maps of each organism.

[0015] Some of the steps of the methods can be accomplished by a computer utilizing various algorithms. Software instructions to perform embodiments of the invention may be stored on a computer readable medium such as a compact disc (CD), a diskette, a tape, a file, or any other computer readable storage device.

[0016] To begin the organization of genomic information, whole-genome physical maps or sequences of multiple organisms are obtained. These maps can either be partially or fully assembled. In one suitable embodiment the physical maps are optical maps. Suitable optical maps include, but are not limited to, restriction enzyme optical maps and probe hybridization optical maps. Once these maps are obtained, the maps of any two organisms are compared.

[0017] In one embodiment this comparison is done by using pair-wise map similarity values found by comparing the optical maps of organisms. The distance between the two optical maps (labeled mapA and map B) is found by taking: (alignedL_A + alignedL_B)/(L_A + L_B), where aliginedL_A is the length (in units of base pairs, bps) of aligned restriction fragments of mapA, and L_A is the total length (also in bps) of restriction fragments of mapA.

[0018] After the percentage similarity values are computed, these values are fed into a statistical package available in the language "R" and analyzed with a clustering method, which can be the nearest neighbor, furthest neighbor, or UPGMA

[0019] In another embodiment, the distance between the two optical maps is computed by a heuristic mer-based algorithm for pair-wise optical map comparison. After choosing a mer size k, the algorithm is used to generate all k-mers in an optical map for both forward and backward orientations. A k-mer is an optical map segment of length k fragments. For each genome, some k-mers occur much more, or less, frequently than chance predicts (to within a some sizing tolerance), and the distribution of k-mer frequencies comprises a type of "species signatures". The difference between k-mer distributions and profiles for two species increases as evolutionary distance increases, thus comparing k-mer profiles can be used to infer phylogenetic relationships.

[0020] To compare two optical maps i and j, the algorithm examines all common k-mers between them to count the number of common k-mers as c_{ij}, and computes the pair-wise map similarity s_{ij}, where s_{ij}=(s_i+s_j - 2c_{ij}) /(s_i+s_j), where s_i and s_j are the sizes (all measured in terms of the numbers of restriction fragments) of the two optical maps. s_{ij} = 0 if i = j. In one embodiment the common mers are computed by accounting for the sizing error. Given two

k-mers, $k_1 = (f_1, f_2, ..., f_k)$ in map 1 and $k_2 = (g_1, g_2, ..., g_k)$ in map 2 (f's and g's are both measured in units of base pairs, bps), it considers k_1 and k_2 as a pair of common k-mers if and only if the following condition is true:

$$\frac{F_i \cap G_i}{F_i \cup G_i} \geq \rho, \text{ for all } 1 \leq i \leq k.$$

[0021] where F_i is interval $(f_i - \sigma_{fi}, f_i + \sigma_{fi})$, σ_{fi} is the standard deviation for fragment f_i; G_i is defined similarly. Threshold ρ is a cutoff determining the least overlap degree between two common intervals, deemed necessary to interpret them as equal modulo statistical noise.

[0022] After the pair-wise distances among the organisms are found, a plurality of disjoint pairs of near neighbors among the organisms or their putative ancestors is obtained. In one embodiment a single pair of nearest neighbors is determined by searching all pair-wise possibilities. In another embodiment, multiple pairs of nearest neighbors are determined by using a stable marriage algorithm.

[0023] Once the nearest neighbors are determined, the plurality of pairs of neighbors are joined pair-wise to create a set of putative ancestral genomes. The determination of the plurality of disjoint pairs of near neighbors, and the pair-wise joining of such neighbors are repeated until no pair remains. These iterative steps organize the physical maps in a phylogenetic tree.

[0024] Another aspect of the invention provides a method for determining similarity among organisms, the method including, comparing optical maps from the organisms to determine relatedness of the organisms.

[0025] Other aspects of the invention will become apparent by consideration of the detailed description and accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1 is a chart showing the procedure of selecting an appropriate method to infer phylogeny given single-gene sequences.

[0027] Figure 2 shows an example of building a bipartite graph given a distance matrix. A) A distance matrix M of four items (A, B, C, D). B) The corresponding bipartite graph.

[0028] Figure 3 shows a first-degree polynomial fit for restriction fragment sizing error. (a) L vs. StdDev(L), cc=0.7428; (b) $\sqrt{L}$ vs. StdDev(L), cc=0.7562; (c) $1/\sqrt{L}$ vs. StdDev(L)/L, cc=0.8290.

[0029] Figure 4 shows Data Set I: 11 *Escherichia coli* Strains.

[0030] Figure 5 shows view maps in Data set I using MapViewer. A pair-wise alignment between *Escherichia coli* O157:H7 str. Sakai and *Escherichia coli* O157:H7 EDL933 is shown.

[0031] Figure 6 is a table showing data Set II: 28 *Enterobacteriaceae* Taxa.

[0032] Figure 7 shows view maps in Data set II using MapViewer

[0033] Figure 8 shows a Phylogenetic tree for data set I and II ($k=2$, $\rho=0.9$)

[0034] Figure 9 shows a Phylogenetic tree for data set I and II ($k=3$, $\rho=0.8$)

[0035] Figure 10 shows a Phylogenetic tree for data set I and II ($k=4$, $\rho=0.7$)

[0036] Figure 11 shows a number of clusters in the iterations of the experiments of data set I and II using CAPO SM-UPGMA/SM-NJ.

[0037] Figure 12 shows Phylogenetic trees constructed by CAPO for data set I and II using default setting and single merge mode.

[0038] Before any embodiments of the invention are explained in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description or illustrated in the following drawings. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, it is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having" and variations thereof herein is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

DETAILED DESCRIPTION OF THE INVENTION

[0039] A phylogenetic tree represents the evolutionary history among organisms. Some methods have been proposed and implemented for the construction of phylogenetic trees. They can be classified into two groups, the phenetic method (distance matrix method, P. Sneath and R. Sokal. *The principles and practice of numerical classification*. Numerical Taxonomy, W. H. Freeman, San Francisco, 1973, incorporated herein by reference) and the cladistic methods (maximum parsimony and maximum likelihood, J. Felsenstein. A likelihood approach to character weighting and what it tells us about parsimony and compatibility. *Biological Journal of Linnean Society*, 16:183-196, 1981, incorporated herein by reference). Popular programs of constructing phylogenetic trees include PHYLIP (Available at evolution.genetics.washington.edu/phylip.html; phylogenetic inference package

- J Felsenstein) and PAUP (Available at paup.csit.fsu.edu; phylogenetic analysis using parsimony – Sinauer Assoc.).

[0040] The phenetic methods use various measures of overall similarity for the ranking of species. They can use any number or type of characters, but the data has to be converted into a numerical value. The organisms are compared to each other for all of the characters and then the similarities are calculated. After this, the organisms are clustered based on the similarities. Such methods place a greater emphasis on the relationships among data sets than the paths they have taken to arrive at their current states. They do not necessarily reflect evolutionary relations.

[0041] The cladistic method is based on the notion that members of a group share a common evolutionary history and are more closely related to members of the same group than to any other organisms. This method emphasizes the need for large data sets but differs from phenetics in that it does not give equal weight to all characters. Cladists are generally more interested in evolutionary pathways than in relationships. FIG. 1 shows how to select an appropriate method to infer phylogeny given single-gene sequences.

[0042] Standard methods for constructing phylogenetic trees, known to persons having ordinary skills in the art, include Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Neighbor Joining (NJ), Fitch Margoliash (FM), Maximum Parsimony (MP), and Maximum Likelihood (ML) methods, and can be combined with certain basic methods related to optical mapping to infer phylogeny using optical-map comparison.

[0043] In one embodiment of the present invention, a phylogenetic tree is crafted by using pair-wise map similarity values found by comparing the optical maps of organisms. To calculate the pair-wise map similarity value, a SOMA map aligner is used to find all the local alignments between the two strains above a certain score threshold. Given two optical-maps mapA and mapB, the percentage similarity is found by taking: $(\text{alginedL}_A + \text{alginedL}_B)/(L_A + L_B)$, where alginedL_A is the length of aligned restriction fragments of mapA, and L_A is the total length of restriction fragments of mapA.

[0044] After the percentage similarity values are computed, these values are fed into a statistical package available in the language "R" and analyzed with a clustering method, which can be the nearest neighbor, furthest neighbor, or UPGMA. As an example, a pair-wise alignment was performed between *Escherichia coli* O157:H7 str. Sakai and *Escherichia coli* O157:H7 EDL933 using SOMA map aligner with its default settings, shown in Figure 5.

[0045] In another embodiment of the present invention, the distance between the two optical maps is computed by a heuristic mer-based algorithm for pair-wise optical map comparison is used to determine phylogeny among optical maps of multiple strains or genomes.

Optical mapping

[0046] Optical mapping is a single-molecule technique for production of ordered restriction maps from a single DNA molecule (Samad *et al.*, *Genome Res.* 5:1-4, 1995). During this method, individual fluorescently labeled DNA molecules are elongated in a flow of agarose between a coverslip and a microscope slide (in the first-generation method) or fixed onto polylysine-treated glass surfaces (in a second-generation method). *Id.* The added endonuclease cuts the DNA at specific points, and the fragments are imaged. *Id.* Restriction maps can be constructed based on the number of fragments resulting from the digest. *Id.* Generally, the final map is an average of fragment sizes derived from similar molecules. *Id.*

[0047] Optical mapping and related methods are described in co-pending U.S. patent application serial number 12/120,586, co-pending U.S. patent application serial number 12/120,592, U.S. Pat. No. 5,405,519, U.S. Pat. No. 5,599,664, U.S. Pat. No. 6,150,089, U.S. Pat. No. 6,147,198, U.S. Pat. No. 5,720,928, U.S. Pat. No. 6,174,671, U.S. Pat. No. 6,294,136, U.S. Pat. No. 6,340,567, U.S. Pat. No. 6,448,012, U.S. Pat. No. 6,509,158, U.S. Pat. No. 6,610,256, and U.S. Pat. No. 6,713,263, each of which is incorporated by reference herein. Optical Maps are constructed as described in Reslewic et al., Appl Environ Microbiol. 2005 Sep; 71 (9):5511-22, incorporated by reference herein. Briefly, individual chromosomal fragments from test organisms are immobilized on derivatized glass by virtue of electrostatic interactions between the negatively-charged DNA and the positively-charged surface, digested with one or more restriction endonuclease, stained with an intercalating dye such as YOYO-1 (Invitrogen) and positioned onto an automated fluorescent microscope for image analysis. Since the chromosomal fragments are immobilized, the restriction fragments produced by digestion with the restriction endonuclease remain attached to the glass and can be visualized by fluorescence microscopy, after staining with the intercalating dye. The size of each restriction fragment in a chromosomal DNA molecule is measured using image analysis software and identical restriction fragment patterns in different molecules are used to assemble ordered restriction maps covering the entire chromosome.

[0048] A current issue with optical map comparison can be understood from the following discussion: An optical map can be viewed as an ordered sequence of "restriction sites," or equivalently, "restriction fragment lengths." A vector of decimal numbers, $H_k = (h_1, h_2, ..., h_m)$, is used to represent a single map k, where h_i with index $0 < i \leq m$ is the length of the i-th restriction fragment. The size of an optical map k is defined as $s_k = \Sigma h_i$, $h_i \varepsilon H_k$. The input to the heuristic mer-based algorithm is an N by M matrix $O = (o_{ij})$, where each row corresponds to an optical map of a strain or a genome. Each column corresponds to a position in that map. N is the total number of maps, and M is the number of restriction fragments in the longest map in that input. Because sequences of different strains or genomes vary in length, the final optical maps usually do not have the same number of restriction fragments. By using the present heuristic mer-based algorithm method, the optical maps are forced to have M fragments by appending zeros to the end of shorter map vectors. Suitably, all the restriction maps in the input must be digested by the same set of restriction endonucleases to make the map comparison meaningful in genome evolution study.

[0049] The heuristic mer-based algorithm is based on pair-wise optical map comparison and bipartite graph matching, combined with standard distance methods of phylogeny tree construction. It consists of two major phases. First, pair-wise optical map comparison is performed to generate a pair-wise similarity matrix $S = (s_{ij})$, where s_{ij} is the map similarity between the i-th and j-th map in the input matrix O. S is used as input to the second phase of CAPO, which determines phylogeny among input strains or genomes. The output is in the Phylip format, used by many phylogenetic analysis packages. This format consists of a series of nested parentheses describing the branching order with the sequence names. Users can display the phylogeny tree using the NJPLOT program distributed with the ClustalX package (The latest version of the ClustalX program is available at ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). The details of the two algorithms implemented in CAPO are explained in the following sections.

Pair-wise Optical Map Comparison

[0050] In phase one of constructing a phylogenetic tree, a heuristic mer-based algorithm for pair-wise optical map comparison is used. A 'mer' (or more elaborately "restriction-fragment-mer") in an optical map is an ordered sequence of restriction fragment lengths. A 'k-mer' is a mer with k fragment lengths. Mathematically, a k-mer comprises k decimal numbers, and their positions reflect the sequence order of the corresponding restriction

fragments. After choosing a mer size k, all k-mers in an optical map for both forward and backward orientations are generated. Each k-mer is indexed by its position in the optical map. To compare two optical maps i and j, all common k-mers between them are examined as follows: the number of common k-mers are counted as c_{ij}, and the pair-wise map similarity s_{ij} is computed by using the formula s_{ij}==(s_i+s_j - 2c_{ij}) /(s_i+s_j), where s_i and s_j are the sizes of the two optical maps. s_{ij} = 0 if i = j. The computed pair-wise similarity matrix S is used as input to the next phase of inferring phylogeny.

[0051] Common mers are searched in a manner allowing for sizing errors. For example, given two k-mers, $k_1 = (f_1, f_2, ..., f_k)$ in map 1 and $k_2 = (g_1, g_2, ..., g_k)$ in map 2, k_1 and k_2 are considered as a pair of common k-mers if and only if the following condition is true:

$$\frac{F_i \cap G_i}{F_i \cup G_i} \geq \rho, \text{ for all } 1 \leq i \leq k. \quad \text{(I)}$$

[0052] where F_i is interval (f_i - σ_{fi}, f_i + σ_{fi}), σ_{fi} is the standard deviation for fragment f_i; G_i is defined similarly. Threshold ρ is a cutoff determining the least overlap degree between two common intervals. The standard deviation of a restriction fragment is estimated via observations of experiment data. Details are given in a later section.

Inferring Phylogeny

[0053] Given a matrix of distances among a set of taxa, both the UPGMA and NJ methods are widely used in phylogenetic analysis to show how similar or dissimilar they are. The UPGMA method assumes equal rates of evolution, so that branch tips come out equal. The NJ method allows for unequal rates of evolution, so that branch lengths are proportional to amount of change. The present method combines the standard stable marriage (SM) algorithm for bipartite graph matching problem with either the UPGMA or the NJ method for inferring phylogeny.

[0054] Usually a phylogeny tree is constructed in stepwise manner. Every time two most similar sequences are clustered together, they are combined into a new node, representing their least common ancestor. The clustering process continues until there is only one node left. Therefore, given n taxa, traditional distance-based methods need O(n) iterations to construct a phylogenetic tree. In normal cases, the present method is capable of constructing a phylogenetic tree in log(n) iterations, though its worst-case number of iterations is comparable to traditional distance-based methods. It works as follows:

[0055] Initialization: Define T to be the set of leaf nodes, one for each given optical map. If the UPGMA method is used, the distance matrix $D=(d_{ij})=(s_{ij})$, where s_{ij} is the map similarity obtained from phase one. If the NJ method is used, $u_i=\Sigma_{j=1}^{n} s_{ij}/(n-2)$ for each node i in T, where n is the total number of nodes in T. The distance matrix D is recomputed to be $D=(d_{ij})=(s_{ij}-u_i-u_j)$.

[0056] Iteration: Build a bipartite graph. Partition D along diagonal line into two parts: the upper triangular part UT and the lower triangular part LT. Pairs in UT form the left column in the bipartite graph, and pairs in LT form the right column. Each node i has a preference list of nodes, ranked by d_{ij}.

[0057] Apply the stable marriage algorithm and produce a set X of stable pairs (B. Sun, J. Schwartz, O. Gill, and B. Mishra. Combat: Search rapidly for highly similar protein-coding sequences using bipartite graph matching. In *Computational Science - ICCS 2006: 6th International Conf.*, pages 654-661, Reading, UK., 2006, incorporated herein by reference). Such a 'stable pair' is a pair of nodes connected by the stable marriage algorithm and is be clustered into a new internal node if this pair passes the following cleaning step.

[0058] Clean the set X: sort stable pairs in decreasing order of d_{ij} and keep only the first m pairs in X that are disjoint. Note that two pairs (a, b) and (c, d) are disjoint with each other if and only if no two nodes in different pairs are the same.

[0059] Connect nodes and update the distance matrix D in a loop until X is empty. In each loop execute the following operations: I) extract the first pair (i, j) in X; II) join them with a new internal node v_{ij}. The node v_{ij} has its cluster size $n_{ij} = n_i + n_j$ (initially, $n_i = 1$).}; III) compute the distances between node v_{ij} and the remaining nodes k; IV) delete d_{ij} in D and add the new distances to D; V) connect nodes i and j in T with v_{ij}.

[0060] Termination: When only two nodes i and j remain unconnected in T, connect them to the root node of the tree T.

[0061] An example of building a bipartite graph given a distance matrix is shown in Figure 2. Each node has a preference list (gray boxes) ordered by distances. Left panel contains pairs in the upper triangular part of M; right panel contains pairs in the lower triangular part of M. For example, the first row in the left panel means "item A prefers to pair with C, B, D, in the decreasing order of preferences."

Correction of Sizing Errors

[0062] Optical maps of different strains of the same species would vary due to single nucleotide differences (SNPs), small insertions and deletions (RFLPs) as well as many genomic rearrangement events that leave their footprints on restriction site patterns. Further variations are introduced by the noises in the experimental process. These can be due to: sizing errors, partial digestion, short missing restriction fragments, false cuts, ambiguities in the orientation, optical chimerisms, and so on (T. Anantharaman, B. Mishra, and D. Schwartz. Genomics via optical mapping II: Ordered restriction maps. *Journal of Computational Biology*, 4(2):91-118, 1997; B. Mishra. Optical mapping. *Encyclopedia of the Human Genome, Nature Publishing Group, Macmillan Publishers Limited, London, UK*, 4:448-453, 2003, incorporated by reference). These error factors introduced by the experimental process are classified into three types –sizing errors, digestion errors, and orientation errors.

[0063] The sizing error statistics is estimated from observations of experiments done by OpGen, Inc. and NYU Bioinformatics Group. These observations (including fragment lengths and standard deviations) are what are reported in the output from the GENTIG (T. Anantharaman, B. Mishra, and D. Schwartz. Genomics via optical mapping III: Contiging genomic DNA and variations; B. Mishra. Optical mapping. *Encyclopedia of the Human Genome, Nature Publishing Group, Macmillan Publishers Limited, London, UK*, 4:448-453, 2003, incorporated herein by reference) software that OpGen and other practitioners of optical mapping have used to produces optical maps. A first-degree polynomial fit for the three pairs of variables: $L \sim StdDev(L)$, $\sqrt{(L)} \sim StdDev(L)$, and $1/\sqrt{(L)} \sim StdDev(L)/L$ is shown in Figure 3, where linear correlation coefficient is referred to as cc. No apparent linear relation is observed between any pair of them since none of these pairs have linear correlation coefficient close enough to one (e.g., > 0.95). These results indicate that it may not be appropriate to estimate standard deviations using any of these 'linear relations.' Therefore data interpolation is used instead to estimate standard deviations StdDev(L) for a restriction fragment whose length is L. This data interpolation step is performed in the following way: given a fragment length L, find L_l and L_r from the error plot shown in Figure below (a) where L_l and L_r are the closest left neighbor and right neighbor of L, respectively ($L_l < L < L_r$); compute StdDev(L) using $StdDev(L) = (StdDev(L_l) + StdDev(L_r)) / 2$.

[0064] The invention having now been described, it is further illustrated by the following examples and claims, which are illustrative and are not meant to be further limiting. Those skilled in the art will recognize or be able to ascertain using no more than routine

experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are within the scope of the present invention and claims.

[0065] The contents of all references and citations, including issued patents, published patent applications, and journal articles cited throughout this application, are hereby incorporated by reference in their entireties for all purposes.

EXAMPLES

[0066] Creation of Data Set I

[0067] Eleven optical maps constructed commercially by OpGen (Website of OpGen Inc. is http://www.opgen.com/) for varying *E. coli* strains. Information describing this data set is listed in Fig. 4. All the organisms described in data set I are *E. coli* bacteria, and are identified by their individual strain names. Sequence data is not available for most but four of these *E. coli* strains, including *Escherichia coli* CFT073, *Escherichia coli* K12, *Escherichia coli* O157:H7 str. Sakai, and *Escherichia coli* O157:H7 EDL933.

[0068] The following procedure was used to produce this data: i) purified chromosomal DNA is deposited onto an optical mapping surface using a microfluidic device; ii) the DNA is encased in a thin layer of acrylamide and incubated with the restriction enzyme BamHI (it cleaves at every site containing the 6 bp long sequence GGATCC) in a humidified chamber at 37°C for 60 ~ 120 mins; iii) the digested DNA is labeled with fluorescent YOYO-1 and the individual molecules are imaged with fluorescence microscopy; iv) digital images are collected by an automated image-acquisition system and image files are processed to create single-molecule optical maps; v) individual molecule restriction maps are overlapped by using GENTIG (GENomic conTIG) map-assembly software.

[0069] Briefly, GENTIG works by comparing single-molecule restriction maps and estimating the probability that these two molecules arose from overlapping genomic locations, where the probability is computed conditional to the likelihood of possible experimental errors resulting from incomplete digestion, spurious cuts, and sizing errors. Through repeated overlapping of molecules, the assembler reconstructs the ordered restriction map of the genome. This technique has been previously applied to map many other bacterial genomes.

[0070] A commercially available interface for viewing optical-maps, called MapViewer (available from OpGen, Inc.) is then used. MapViewer allows users to visualize optical-maps, to move maps around, pull up sequence information when available, and change the

orientation of the maps. Figure 5 shows the optical maps for data set I using MapViewer. A pair-wise alignment between *Escherichia coli* O157:H7 str. Sakai and *Escherichia coli* O157:H7 EDL933 is shown. Regions that match exactly once are colored green, and regions that match to more than one location are colored red.

[0071] Creation of Data Set II

[0072] Twenty-eight genomic sequences of Enterobacteriaceae taxa are downloaded from the NCBI database, and then cleaved "in silico" with the restriction enzyme BamHI. Their optical maps were constructed using the SilicoMap software provided by OpGen; The SilicoMap tool is built upon the BioPerl toolkit which is able to perform an *in silico* restriction digest, after which, it is straightforward to find the lengths of each of the resulting fragments and create the map. Information describing this data set is listed in Figure 6. Figure 7 shows the optical maps for data set I using MapViewer.

[0073] Analysis of Data Sets

[0074] Experimental results are provided in this section using CAPO on both real optical mapping data of eleven *E. coli* strains and simulated optical mapping data of twenty-eight entire genomes of *Enterobacteriaceae* taxa. All of the tests were run on a 2.4-GHz Pentium IV machine with 3GB of RAM.

[0075] *Parameter Settings*

[0076] Users have choices for two parameters in CAPO: k (mersize) and ρ (cutoff value involved in determining whether two restriction fragment lengths are 'equal' considering sizing errors). The effect of parameter settings in CAPO is tested in the following experiments using the two data sets: k=2, ρ =0.9 (see Figure 6), k=3, ρ =0.8 (see Figure 7) k=4, ρ =0.7 (see Figure 8). To adequately tolerate sizing errors it was found reasonable to use smaller cutoff value of ρ if a larger mer-size is chosen. Shown in Figure 8 – Figure 10, the 'best' results (whose phylogenetic trees are most biologically meaningful) are produced using k=3, ρ =0.8. k=3, ρ =0.8 was, therefore, subsequently used as the default parameter setting.

[0077] *Phylogenetic Tree Evaluation*

[0078] Since there are no 'true' phylogenetic trees available for comparison with the results computed by the present method, the quality of these trees were evaluated based on optical map alignments, the taxonomy information given by the NCBI database, and tree topology overlap between the two different distance methods. Using the SOMA map aligner

developed by OpGen, it was found that the map of *Escherichia coli* K12 is very similar to that of 886, and these two strains are clustered closely by the present method with default setting (see Figure 7, A1, A2). The present method also assigns the rest of three known *E. coli* strains close evolutionary distances. Using data set II, it was observed that the present method often clustered biologically closely related taxa together (the *Buchnera aphidicola* strains, the *Candidatus Blochmannia* strains, the *E. coli* strains, the Salmonella strains, etc.), as would be desired. Lastly, phylogenetic trees produced by the present method for the same data set using different distance methods were also found to share substantial tree topology overlap.

[0079] *Cluster Sizes*

[0080] The present method (CAPO) constructs phylogenetic trees in far fewer iterations than standard distance methods. For data set I, CAPO UPGMA-flavored trees and NJ-flavored trees were constructed in 5 and 6 iterations, respectively. For data set II, CAPO UPGMA-flavored trees and NJ-flavored trees were constructed in 8 and 9 iterations, respectively. Number of remaining clusters in each iteration is shown in Figure 11.

[0081] *Impact of Single-Merge Mode and Multi-Merge Mode*

[0082] To see if there was any effect on the phylogenetic tree topology by merging more than two clusters in a single iteration. Phylogenetic trees were generated for both data sets using 'single-merge mode' (merge exactly two clusters at one iteration), as shown in Figure 12. Compared with trees produced in 'multi-merge mode' (merge multiple pairs of disjoint clusters found by the stable marriage procedure in a single iteration), as shown in Figure 9, some tree topology changes are shown, especially between Figure 12-A2 and Figure 9-A2. Because there is no reliable method for detecting the similarity level between two trees and because there is no prior knowledge about the 'true' tree topology, at this point, it remains unclear what the impact of various merging mode could be. However, almost all corresponding trees share substantial tree topology overlap, thus indicating a strong measure of consistency that can be achieved by the present method.

[0083] *Implementation and Speed*

[0084] The methods of the present invention are implemented in C++ and all experiments were performed on a Pentium IV PC with 3 GB memory. Experiments for data set I and II took ~ 4 sec. and ~ 18 sec., respectively. The computational efficiency of CAPO indicates its potential widespread usage in analyzing large genomic data sets.

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CLAIMS

1. A method for comparative genomic analysis, the method comprising:

comparing optical maps obtained from one or more organisms in order to obtain at least one pair-wise similarity value; and

determining relatedness of the organisms based on said pair-wise similarity value.

2. The method according to claim 1, further comprising constructing a phylogenetic tree based on said relatedness of the organisms.

3. The method according to claim 1, wherein the organisms are selected from the group consisting of a microorganism, a bacterium, a virus, and a fungus.

4. A method for identifying an unknown organism, the method comprising:

comparing an optical map from an unknown organism to a plurality of optical maps from a phylogenetic tree of known organisms;

obtaining a pair-wise similarity value for one or more comparisons between the unknown organism and the known organism in the phylogenetic tree; and

identifying the unknown organism based on the pair-wise similarity values.

5. The method according to claim 4, wherein prior to said comparing step, the method further comprises preparing an optical map from the unknown organism.

6. The method according to claim 5, wherein prior to said comparing step, the method further comprises constructing a phylogenetic tree of known organisms.

7. The method according to claim 4, wherein the unknown organism is selected from the group consisting of a microorganism, a bacterium, a virus, and a fungus.

8. A method for constructing a phylogenetic tree, the method comprising:

obtaining pair-wise distances among organisms by comparing at least one pair of optical maps from the organisms in order to generate a pair-wise similarity matrix; and

constructing a phylogenetic tree based on the pair-wise similarity matrix.

9. The method according to claim 8, wherein prior to said obtaining step, the method further comprises preparing optical maps of each organism.

10. The method according to claim 9, wherein the optical maps are ordered restriction enzyme optical maps.

11. The method according to claim 9, wherein the optical maps are probe-hybridized optical maps.

12. The method according to claim 8, wherein the pair-wise distances are computed by: $(\text{aligned}L_A + \text{aligned}L_B)/(L_A + L_B)$, where $\text{aligned}L_A$ is the length of aligned restriction fragments of a map of a first organism, L_A is the total length of restriction fragments of a first organism, $\text{aligned}L_B$ is the length of aligned restriction fragments of a map of a second organism, and L_B is the total length of restriction fragments of the second organism.

13. The method according to claim 8, wherein the pair-wise distances are computed by:

choosing a mer size k, and generating k-mers in the optical maps for both forward and backward orientations;

comparing two optical maps by examining common k-mers between the two optical maps and counting number of common k-mers as c_{ij},

computing the pair-wise distance as similarity s_{ij} using the formula $s_{ij}==(s_i+s_j - 2c_{ij}) /(s_i+s_j)$, where s_i is size of the first optical map and s_j is size of the second optical map.

14. The method according to claim 13, wherein the common mers are computed by accounting for the sizing error as follows:

a k-mer in the first map is $k_1 = (f_1, f_2, ..., f_k)$ and a k-mer in a second map is $k_2 = (g_1, g_2, ..., g_k)$, and the pair is considered a common k-mer if the following condition is true:

$$\frac{F_i \cap G_i}{F_i \cup G_i} \geq \rho, \text{ for all } 1 \leq i \leq k$$

where F_i is interval ($f_i - \sigma_{fi}$, $f_i + \sigma_{fi}$), σ_{fi} is the standard deviation for fragment f_i; G_i is interval ($g_i - \sigma_{gi}$, $g_i + \sigma_{gi}$), σ_{gi}; σ_{gi} is the standard deviation for fragment g_i; and threshold ρ is a cutoff determining the least overlap degree between two common intervals.

15. The method according to claim 8, wherein said constructing step comprises, (a) obtaining a plurality of disjoint pairs of near neighbors among the organisms or putative ancestors of the organisms, (b) joining pair-wise the previously computed plurality of pairs of neighbors to generate a set of putative ancestral genomes, and repeating steps (a) and (b) until no pairs remain.

16. The method according to claim 15, wherein a single disjoint pair of nearest neighbor is determined by searching all pair-wise possibilities.

17. The method according to claim 15, wherein multiple disjoint pairs of nearest neighbors are determined by using a stable marriage algorithm.

18. The method according to claim 15, wherein a single disjoint pair of nearest neighbors are joined in a single-merge mode.

19. The method according to claim 15, wherein multiple disjoint pairs of nearest neighbors are joined in a multi-merge mode.

20. A method for determining similarity among organisms, the method comprising, comparing optical maps from the organisms to determine relatedness of the organisms.

21. A computer program product for comparative genomic analysis, the computer program product being embodied in a computer readable medium and comprising computer instructions to be executed by a processor for: comparing optical maps obtained from one or more organisms in order to obtain at least one pair-wise similarity value; and determining relatedness of the organisms based on said pair-wise similarity value.

REPLACEMENT PAGE

23. A computer program product for constructing a phylogenetic tree, the computer program product being embodied in a computer readable medium and comprising computer instructions to be executed by a processor for: obtaining pair-wise distances among organisms by comparing at least one pair of optical maps from the organisms in order to generate a pair-wise similarity matrix; and constructing a phylogenetic tree based on the pair-wise similarity matrix.

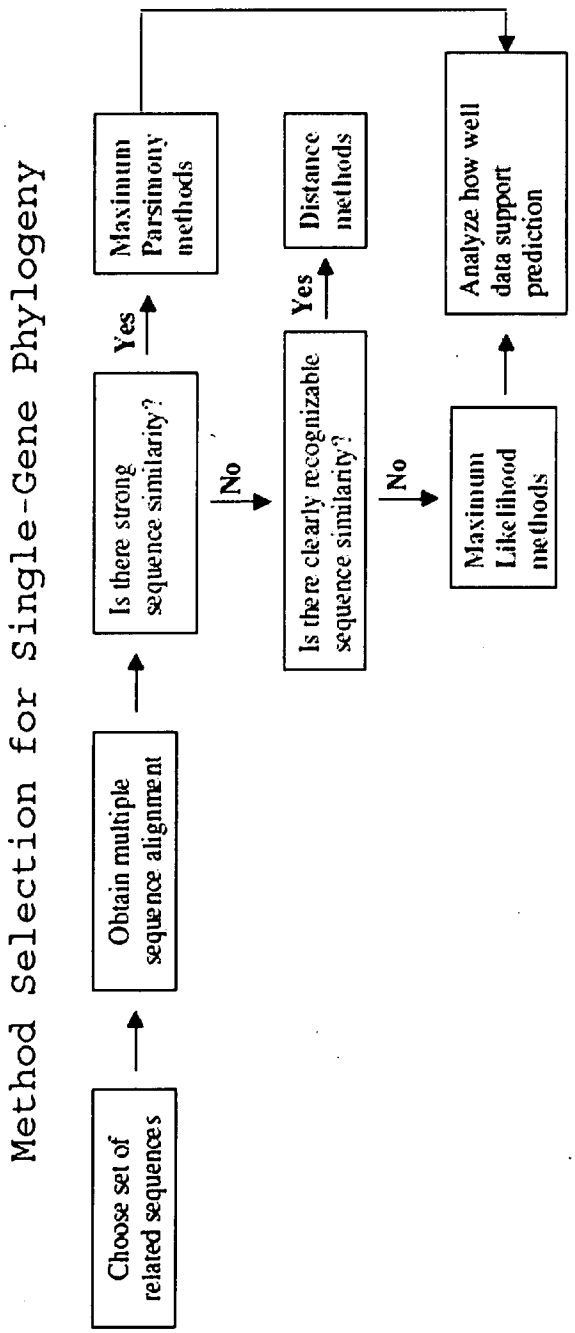


Figure 1. Procedure of selecting an appropriate method to infer phylogeny given single-gene sequences.

A) Distance Matrix for four items (A, B, C, D)

	A	B	C	D
A	0	8	7	12
B	8	0	9	14
C	7	9	0	11
D	12	14	11	0

B) Build Bipartite Graph

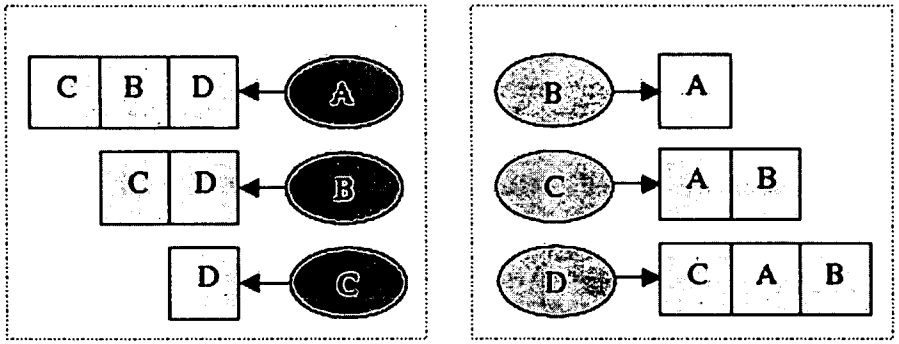


Figure 2. An example of building a bipartite graph given a distance matrix. A) A distance matrix M of four items (A, B, C, D). B) The corresponding bipartite graph.

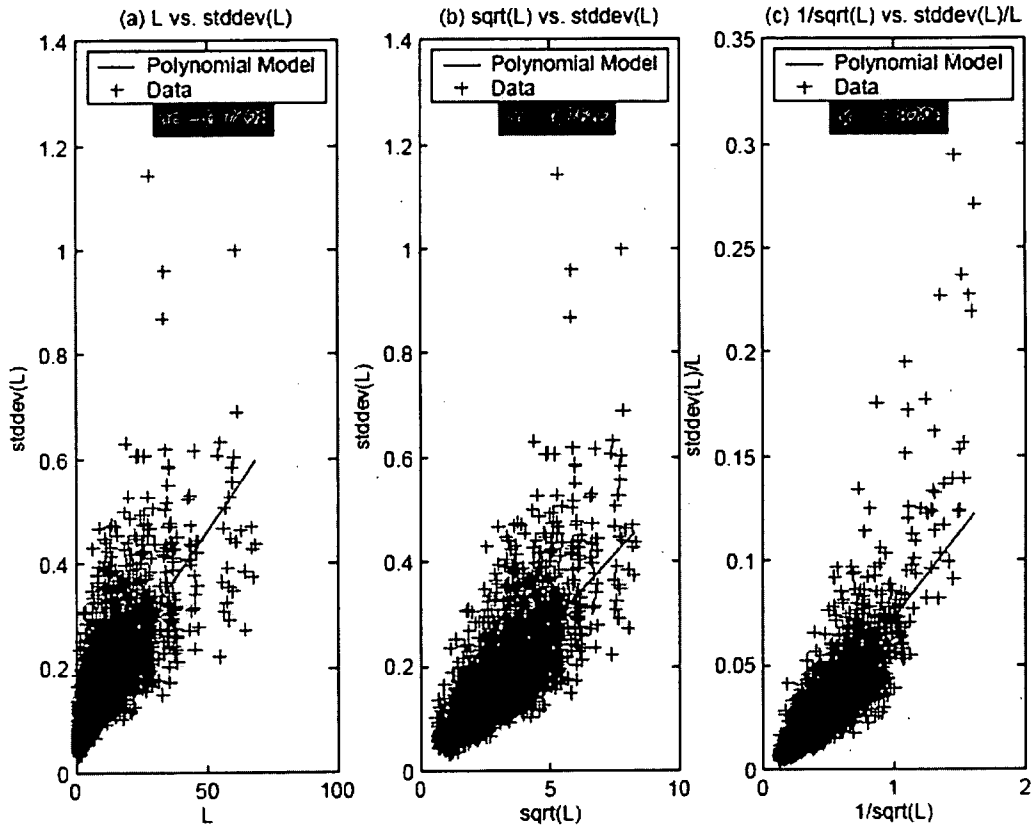


Figure 3. First-degree polynomial fit for restriction fragment sizing error. (a) L vs. stddev(L), cc=0.7428; (b) \sqrt L vs. stddev(L), cc=0.7562; (c) $1/\sqrt{L}$ vs. stddev(L)/L, cc=0.8290.

Species	Genome Refseq ID	Length(no. of nt.)
*Escherichia coli CFT*073	NC_004431	5,231,428
*Escherichia coli K*12	NC_000913	4,639,675
*Escherichia coli O*157 : *H*7 *str. Sakai*	NC_002695	5,498,450
*Escherichia coli O*157 : *H*7 *EDL*933	NC_002655	5,528,445
*EC*1231	NA	NA
400	NA	NA
536	NA	NA
*AB*1	NA	NA
*DEC*5*A*	NA	NA
503	NA	NA
886	NA	NA

Figure 4. Data Set I: 11 *Escherichia coli* Strains

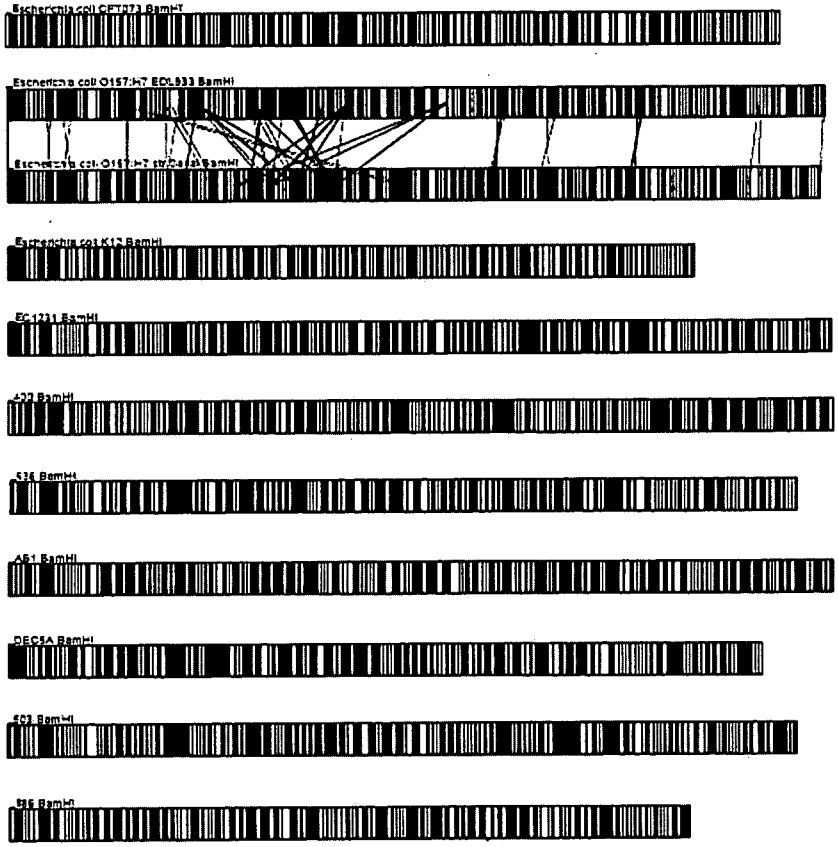


Figure 5. View maps in Data set I using MapViewer. A pair-wise alignment between *Escherichia coli* O157:H7 str. Sakai and *Escherichia coli* O157:H7 EDL933 is shown.

Species	Genome Refseq ID	Length (no. of nt.)
Buchnera aphidicola str. APS(Acyrthosiphonpisum)	NC_002528	640,681
Buchnera aphidicola str. Sg(Schizaphisgraminum)	NC_004061	641,454
Buchnera aphidicola str. Bp(Baizongtapistacae)	NC_004545	615,980
Candidatus Blochmannia floridanus	NC_005061	705,557
Candidatus Blochmannia pennsylvanicus str. BPEN	NC_007292	791,654
Erwinia carotovora subsp. atroseptica SCRI1043	NC_004547	5,064,019
Escherichia coli CFT073	NC_004431	5,231,428
Escherichia coli K12	NC_000913	4,639,675
Escherichia coli O157 : H7 str. Sakai	NC_002695	5,498,450
Escherichia coli O157 : H7 EDL933	NC_002655	5,528,445
Escherichia coli UTI89	NC_007946	5,065,741
Escherichia coli W3110 DNA	AC_000091	4,646,332
Photorhabdus luminescens subsp. laumondii TTO1	NC_005126	5,688,987
Salmonella typhimurium LT2	NC_003197	4,857,432
Salmonella enterica subsp. enterica serovar Typhi Ty2	NC_004631	4,791,961
Salmonella enterica subsp. enterica serovar Typhi str. CT18	NC_003198	4,809,037
Salmonella enterica subsp. enterica serovar Paratyphi A str. ATCC 9150	NC_006511	4,585,229
Salmonella enterica subsp. enterica serovar Choleraesuis str. SC – B67	NC_006905	4,755,700
Shigella flexneri 2a str. 301	NC_004337	4,607,203
Shigella boydii Sb227	NC_007613	4,519,823
Shigella sonnei Ss046	NC_007384	4,825,265
Shigella dysenteriae Sd197	NC_007606	4,369,232
Sodalis glossinidius str. 'morsitans'	NC_007712	4,171,140
Wigglesworthia glossinidia endosymbiont of Glossinabrevipalpis	NC_004344	697,724
Yersinia pestis CO92	NC_003143	4,653,726
Yersinia pestis biovar Medievalis str. 91001	NC_005810	4,595,065
Yersinia pestis KIM	NC_004088	4,600,755
Yersinia pseudotuberculosis IP 32953	NC_006155	4,744,671

Figure 6 Data Set II: 28 *Enterobacteriaceae* Taxa

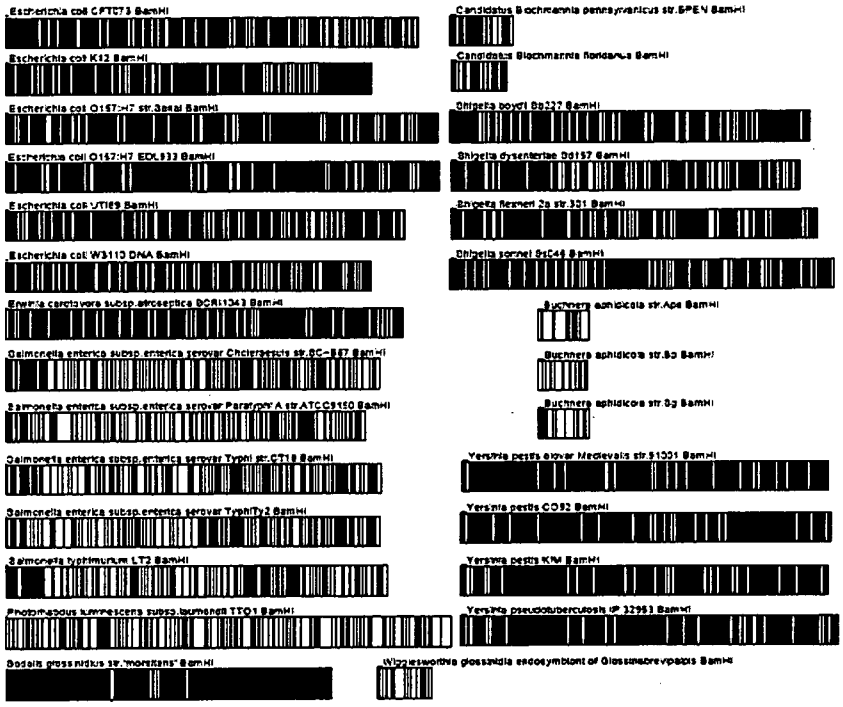
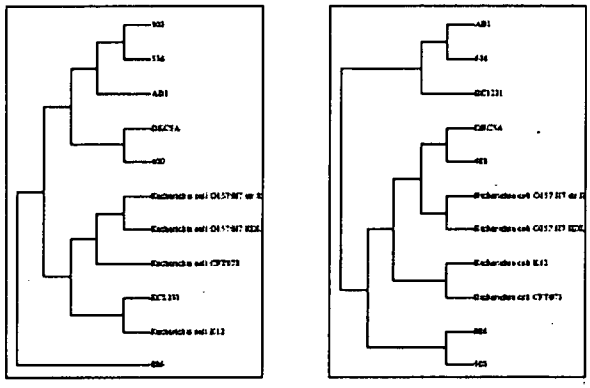
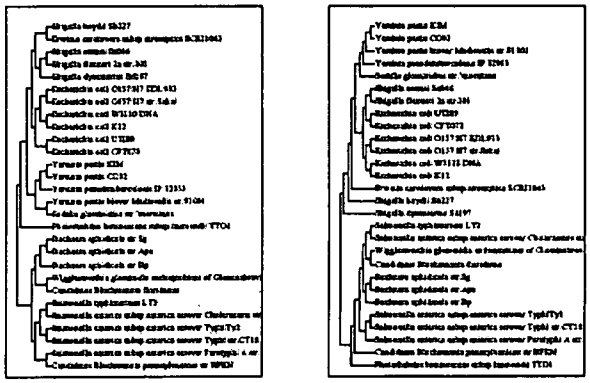


Figure 7. View maps in Data set II using MapViewer

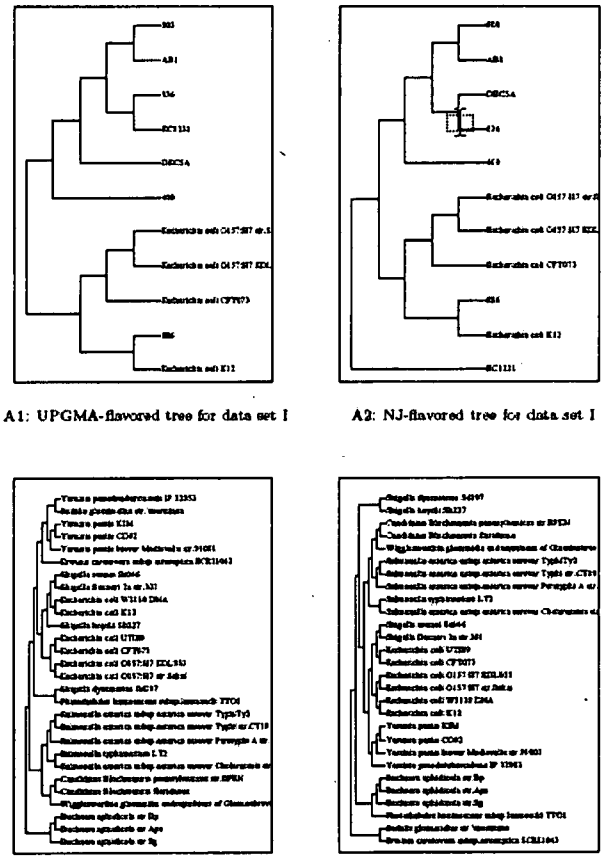


A1: UPGMA-flavored tree for data set I A2: NJ-flavored tree for data set I



B1: UPGMA-flavored tree for data set II B2: NJ-flavored tree for data set II

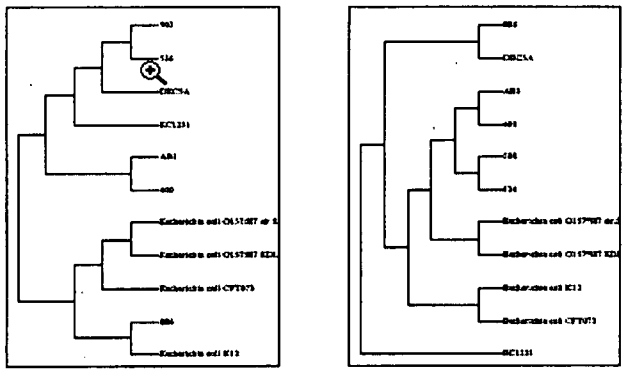
Figure 8 Phylogenetic tree for data set I and II (k=2, ρ =0.9)



A1: UPGMA-flavored tree for data set I A2: NJ-flavored tree for data set I

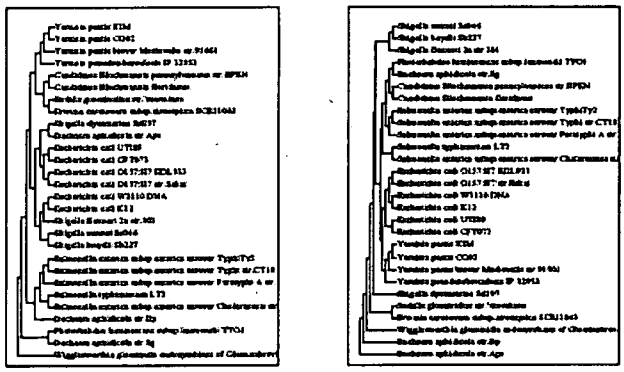
B1: UPGMA-flavored tree for data set II B2: NJ-flavored tree for data set II

Figure 9 Phylogenetic tree for data set I and II (k=3, ρ =0.8)



A1: UPGMA-flavored tree for data set I

A2: NJ-flavored tree for data set I



B1: UPGMA-flavored tree for data set II

B2: NJ-flavored tree for data set II

Figure 10 Phylogenetic tree for data set I and II (k=4, ρ =0.7)

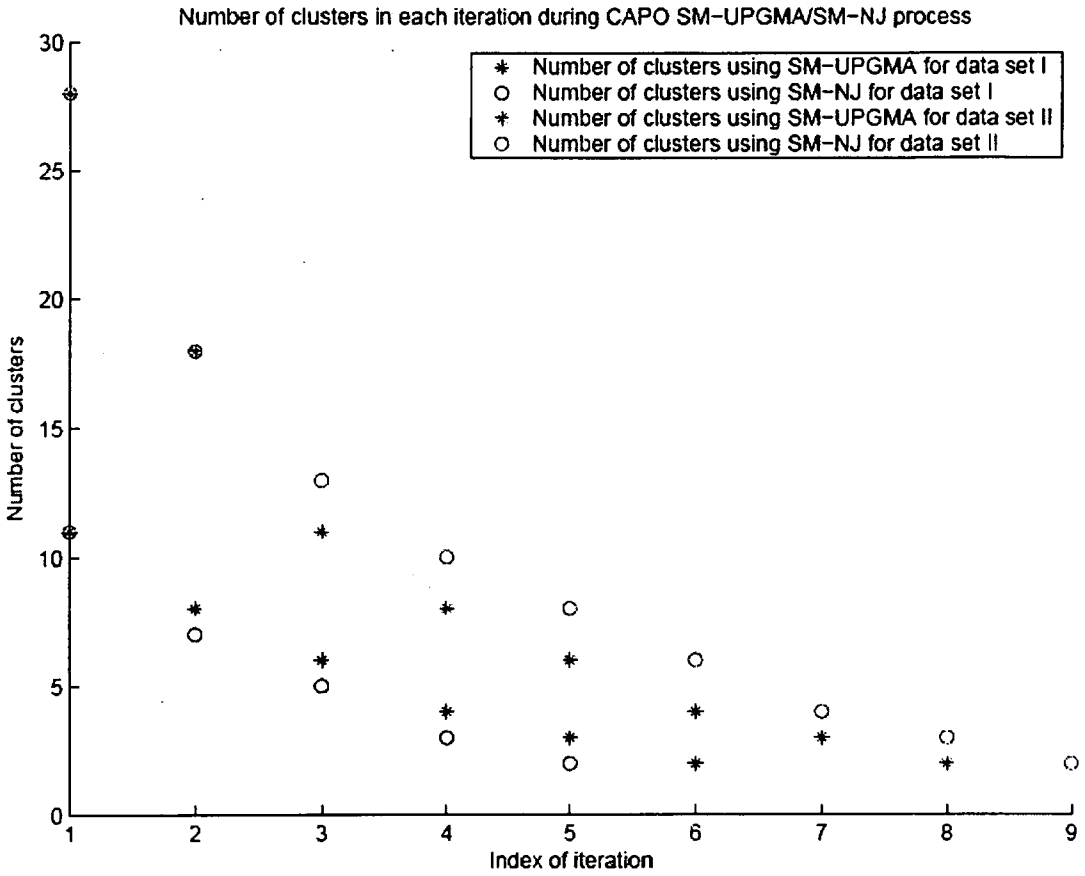
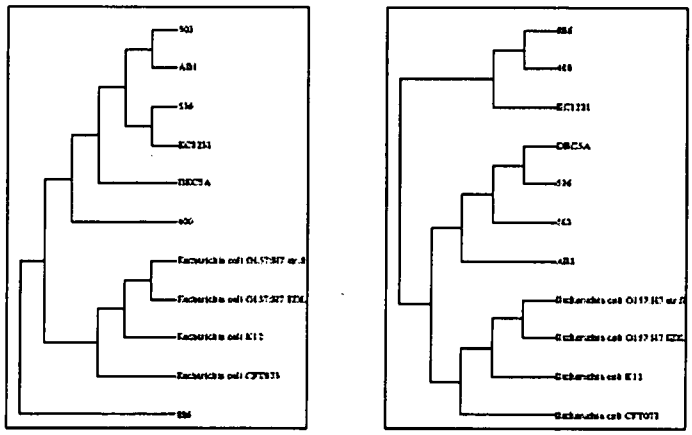
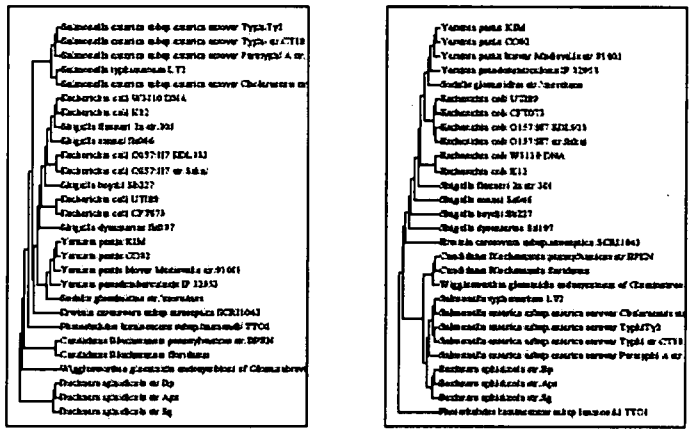


Figure 11 Number of clusters in the iterations of the experiments of data set I and II using CAPO SM-UPGMA/SM-NJ.



A1: UPGMA-flavored tree for data set I A2: NJ-flavored tree for data set I



B1: UPGMA-flavored tree for data set II B2: NJ-flavored tree for data set II

Figure 12 Phylogenetic trees constructed by CAPO for data set I and II using default setting and single merge mode.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/73282

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/48 (2008.04)
USPC - 702/20
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 702/20

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 702/19
IPC(8) - G01N 33/48 (2008.04)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, USOC, EPAB, JPAB), Google Patents and Google: optical, visualize, probe, map, pair-wise, phylogenetic, tree, algorithm, similarity, matrix, stable marriage

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUN, "Pairwise Comparison Between Genomic Sequences and Optical-maps," PhD Dissertation, Department of Computer Science, New York University, September 2006.	1-23
A	US 6,738,502 B1 (Coleman et al.) 18 May 2004 (18.05.2004) Col 9, ln 3-20, 35-38; Fig 1, 11, 14, 22, 23; col 11, ln 10-20; col 20, ln 12-28, 45-57; col 25, ln 50-61.	1-11, 21-23
A	JP 1993/128171 A (Tajima) 25 May 1993 (25.05.1993). Entire document, particularly Specifications, Claims, Drawings 10, 13.	1-11, 15-23

☐ Further documents are listed in the continuation of Box C ☐

* Special categories of cited documents
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 December 2008 (19.12.2008)	06 JAN 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571 272-4300 PCT OSP: 571-272-7774

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