AGP: A Multimethods Web Server for Alignment-Free Genome Phylogeny

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Abstract

Phylogenetic analysis based on alignment method meets huge challenges when dealing with whole-genome sequences, for example, recombination, shuffling, and rearrangement of sequences. Thus, various alignment-free methods for phylogeny construction have been proposed. However, most of these methods have not been implemented as tools or web servers. Researchers cannot use these methods easily with their data sets. To facilitate the usage of various alignment-free methods, we implemented most of the popular alignment-free methods and constructed a user-friendly web server for alignment-free genome phylogeny (AGP). AGP integrated the phylogenetic tree construction, visualization, and comparison functions together. Both AGP and all source code of the methods are available at http://www.herbbol.org:8000/agp (last accessed February 26, 2013). AGP will facilitate research in the field of whole-genome phylogeny and comparison.

Key words: alignment-free method, genome phylogeny, tree comparison.

Introduction

Phylogenetic analysis reveals the evolutionary derivation of species. A phylogenetic tree is traditionally inferred from multiple sequence alignment of conservative proteins or genes. Alignment methods and tools have been widely used for the construction of phylogenetic trees. However, with the development of various genome sequencing projects, alignment methods meet huge challenges when dealing with whole-genome sequences. Alignment methods cannot evaluate the recombination, shuffling, and rearrangement events of the whole genomes, and whole-genome multiple alignments are computationally intensive. These obstacles for alignment-based phylogenetic reconstruction motivated several alignment-free methods in recent years. Two main categories of alignment-free methods have been proposed including methods based on word frequency and methods that do not require resolving the sequence with fixed length word.

The first category includes feature frequency profile (FFP), composition vector (CV), return time distribution (RTD), and frequency chaos game representation (FCGR). FFP assembles the frequency information for all the possible words of fixed length k (k-mers) into a CV (Qi et al. 2004; Gao and Qi 2007; Xu and Hao 2009; Yu, Liang, et al. 2010). Chaos game representation (CGR) was proposed as a scale-independent representation for genomic sequences (Jeffrey 1990). Each CGR is a unique fingerprint of the underlying sequence. However, the CGRs are not directly comparable. If the CGRs are divided by grid lines, each grid square denotes the occurrence of one pattern of k-mers in the sequence (Deschavanne et al. 1999; Almeida et al. 2001). These frequencies can be represented as FCGRs. FCGRs are numerical matrices and can be used to infer phylogenetic trees (Wang et al. 2005; Hatje and Kollmar 2012). RTD was defined as the time required for the reappearance of particular k-mers. Two statistical parameters (μ and σ) of each RTD were used to derive a feature vector (Kolekar et al. 2011, 2012). Methods that are not based on k-mers are rather heterogeneous, for example, average common subsequence (ACS), graph-based methods, the Kр estimator, and methods based on information correlation or compress. ACS calculates the pairwise genome sequence distances using average common substring at every site of each sequence (Cohen and Chor 2012). The Kr estimator designed by Haubold et al. is closely related to the ACS, which calculates the number of substitutions per site between two unaligned DNA sequences using the shortest absent substring (Domazet-Loso and Haubold 2009; Haubold et al. 2009).

Graph-based methods have been used for graphical representations of DNA sequences (Gates 1985; Nandy et al. 2006). Graph-based methods were used for graphical representations of DNA sequences (Gates 1985; Nandy et al. 2006). The features from graphical representations of DNA sequences have been developed to capture the essence of the base composition and distribution of the sequences in a quantitative manner. Deng et al. (2011) and Huang et al. (2011) used natural vector and a 10-dimensional statistical
vector to characterize the two-dimensional (2D) graphical DNA curve, they were called two-dimensional natural vector (2DNV) and two-dimensional statistical vector (2DSV), respectively. Yu, Chu, et al. (2010) converted the 2D genome space to an N-dimensional moment vector (N equals to the length of genome, and this method was called 2DMV) and used the first n components of the vectors to construct phylogenetic tree for 34 lentiviruses based on whole-genome sequences. Methods based on information correlation emphasized the base correlation property of whole-genome sequences. Methods based on information correlation were proposed using Lempel–Ziv and Kolmogorov complexity (Li et al. 2001). Lempel–Ziv complexity uses the ultimate lower bound of all measures of information, it is a theoretical limit and cannot be computed in the general case (Li et al. 2001).

Although many alignment-free methods have been proposed, only the CV and Kr methods have been implemented as web servers, source code for FFP, and Lempel–Ziv complexity can be obtained from authors who proposed the methods. Thus, we implemented 12 popular alignment-free methods and constructed a user-friendly web platform for alignment-free genome phylogeny (AGP) research. AGP also implemented methods for phylogenetic tree visualization and comparison. AGP will facilitate the phylogenetic researches in the field of whole-genome phylogeny and comparison.

New Approaches

AGP implemented 12 alignment-free methods for the construction of phylogeny trees using whole genomes and four methods for phylogenetic tree comparison. AGP constructed the first user-friendly multimethods web server for the phylogeny analysis using alignment-free methods and whole genomes. AGP integrated functions including phylogenetic tree construction, visualization, and comparison, which is a comprehensive multimethods platform for the phylogenetic analysis of whole genomes.

Results and Discussion

There are total six pages in AGP. The home page is shown in figure 1. “METHODS” and “TREECOMPARE” pages perform the web server functions, including phylogenetic tree construction, visualization, and comparison.

Tree Construction and Visualization

“METHODS” page gives a simple introduction of all alignment-free methods and lists every method with a hyperlink, which will lead you to its input page. Detailed information about the method and input data is described on the input page. For all methods, the input genome file must be in multi-FASTA format. For methods based on k-mers (e.g., FFP, CV, FCGR, and RTD), you need to supply the k value, which indicates the fixed length of word. The k value has influence on the results of sequence comparisons, which is determined by the length of genome sequences. For BBC, IC-PIC, and 2DMV, you also need to set the k parameter, which indicates the max distance between bases and the number of components of the moment vector used in the analysis. The reference range of each k value is also supplied to users on the input page of the method. For FCGR, RTD, 2DNV, 2DSV, 2DMV, IC-PIC, and BBC, you could select one of the 10 distance methods to calculate the distance matrix among genome sequences. To compare with the traditional alignment-based method, AGP also provided functions for the phylogeny construction based on whole-genome alignment, which was implemented using MUMmer (Kurtz et al. 2004).

When all input data have been successfully submitted, the web server will return you back the computing results. The results of phylogeny analysis contain distance matrices, phylogenetic trees, and tree maps. Two kinds of distance matrices were provided in Phylip and Nexus formats. Phylogenetic trees were formatted into standard Newick and Nexus files, which can be used for editing and viewing in other tools directly (e.g., MegAlign, TreeView). Circular and rectangular tree maps were rendered into five types of figures including TIFF, GIF, JPG, PS, and PNG (Felsenstein 1989; Page 1996; Tamura et al. 2011). All results can be viewed and downloaded online. Result page for the phylogenetic analysis of 155 complete chloroplast genomes using FFP is shown in figure 2.

Tree Comparison

When you obtain phylogenetic trees using different methods with various parameters, you can compare the differences between these trees. You need to put these trees in a plain text file. Each tree starts with the symbol ">" and the name of the tree at a new line; the following lines describe the structure of the tree. Then you can submit the file to "TREECOMPARE" page. The web server will return back the comparison results in an all-by-all distance matrix. Optionally, you can choose the distance method used for the comparison. These four distance methods were implemented for tree comparison in AGP, including RobinsonFoulds, Symmetric, FalsePositivesAndNegatives, and Euclidean.

AGP implemented 12 alignment-free methods and 10 distance methods for the construction of phylogenetic trees. The phylogenetic trees constructed were outputted as standard Newick and Nexus files and visualized as circular and traditional rectangular tree maps. To compare with the traditional alignment-based method, AGP also implemented functions for the phylogeny construction based on whole-genome alignment. Furthermore, AGP implemented four methods for the comparison of phylogenetic trees constructed in the analysis. All results can be viewed and downloaded online. AGP is the first multimethods platform for alignment-free phylogeny analysis, and it will help researchers
Welcome to Alignment-free Genome Phylogeny (AGP)

AGP program

With the development of various genome sequencing projects, alignment methods meet huge challenges when dealing with whole genome sequences. Alignment methods cannot evaluate the recombination, shuffling, and rearrangement events of the whole genome, and whole genome multiple alignments are too computationally intensive to perform. These obstacles for alignment-based phylogenetic reconstruction motivated several alignment-free methods. We collected and implemented most of the popular alignment-free methods, constructed the web server of AGP. AGP implemented 12 alignment-free methods and phylogenetic tree construction, visualization and comparison functions.

What AGP do

Phylogeny analysis based on alignment-free methods: users can construct phylogenetic trees easily using different methods and parameters.

Phylogenetic tree comparison: users can compare the trees obtained by different alignment-free methods using four distance methods.

Fig. 1. The home page of the AGP platform.
perform whole-genome phylogeny analysis and compare the results of various methods.

**Methods and Implementation**

AGP implemented 12 alignment-free methods including FFP, CV, FCGR, RTD, ACS, Kr, 2DNV, 2DSV, 2DMV, IC-PIC, BBC, and Lempel–Ziv. FCGR, RTD, 2DNV, 2DSV, 2DMV, IC-PIC, and BBC converted genome sequences into numerical multidimensional vectors and then used 10 kinds of methods to compute the distance matrix among the vectors, including Euclidean, Braycurtis, Canberra, Chebyshev, Cityblock, Correlation, Cosine, Minkowski, Seuclidean, and Squeuclidean. FFP and CV represented genome sequence as a 4^k-dimension frequency vector of k-mers and calculated the distance matrix using the distance formula published in the articles (Qi et al. 2004; Sims et al. 2009). ACS, Kr, and Lempel–Ziv did not convert genome sequences into vectors, they computed pairwise genome distances directly. When we got the distance matrix among genomes analyzed, we used neighbor-joining method to construct the phylogenetic trees (Saitou and Nei 1987). AGP implemented four methods for the comparison of phylogeny trees obtained, including the following:

*Robinson-Foulds:* This method returns the Robinsons–Foulds distance between two trees, the sum of the square of differences in branch lengths for equivalent splits between two trees (Robinson and Foulds 1981).

*Symmetric:* The symmetric distance between two trees is the sum of the number of splits found in one of the trees but not the other.
FalsePositivesAndNegatives: This method returns a tuple pair, with the first element is the number of splits in the first tree but not found in the second tree compared, whereas the second element is the number of splits in the second tree, which are not in the first tree.

Euclidean: This method returns the "branch length distance" of Felsenstein (2004), the sum of absolute differences in branch lengths for equivalent splits between two trees.

We programmed methods CV, FCGR, RTD, ACS, 2DNV, 2DSV, 2DMV, IC-PIC, and BBC according to the algorithms published with the methods. All methods were implemented using the Python language. Phylogenetic tree visualization and comparison functions were implemented based on a Python environment for tree exploration (ETE) and DendroPy Python Packages (Huerta-Cepas et al. 2010; Sukumaran and Holder 2010). The web server was implemented based on the web2py framework (http://www.web2py.com/, last accessed February 26, 2013).

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References

Almeida JS, Carrico JA, Maretzek A, Noble PA, Fletcher M. 2001. Analysis of genomic sequences by whole genome representation. Bioinformatics 17:429–437.

Cohen E, Chor B. 2012. Detecting phylogenetic signals in eukaryotic whole genome sequences. J Comput Biol. 19:945–956.

Deng M, Yu C, Liang Q, He RL, Yau SS. 2011. A novel method of characterizing genetic sequences: genome space with biological distance and applications. PLoS One 6:e17293.

Deschavanne PJ, Giron A, Vilain J, Fagot G, Fertil B. 1999. Genomic signature: characterization and classification of species assessed by chaos game representation of sequences. Mol Biol Evol. 16: 1391–1399.

Domazet-Loso M, Haubold B. 2009. Efficient estimation of pairwise distances between genomes. Bioinformatics 25:3221–3227.

Felsenstein J. 1989. PHYLIP—phylogeny inference package (version 3.2). Cladistics 5:164–166.

Felsenstein J. 2004. Inferring phylogenies. Sunderland (MA): Sinauer Associates.

Gao L, Qi J. 2007. Whole genome molecular phylogeny of large dsDNA viruses using composition vector method. BMC Evol Biol. 7:41.

Gao Y, Luo L. 2011. Genome-based phylogeny of dsDNA viruses by a novel alignment-free method. Gene. 492:309–314.

Gates MAW. 1985. Simpler DNA sequence representations. Nature 316: 219.

Hatje K, Kollmar M. 2012. A phylogenetic analysis of the brassicaceae clade based on an alignment-free sequence comparison method. Front Plant Sci. 3:192.

Haubold B, Pfaffelhuber P, Domazet-Loso M, Wiehe T. 2009. Estimating mutation distances from unaligned genomes. J Comput Biol. 16: 1487–1500.

Huang G, Zhou H, Li Y, Xu L. 2011. Alignment-free comparison of genome sequences by a new numerical characterization. J Theor Biol. 281:107–112.

Huerta-Cepas J, Dopazo J, Gabaldon T. 2010. ETE: a python environment for Tree exploration. BMC Bioinformatics 11:24.

Jeffrey HJ. 1990. Chaos game representation of gene structure. Nucleic Acids Res. 18:2163–2170.

Jun SR, Sims GE, Wu GA, Kim SH. 2009. Whole-proteome phylogeny of prokaryotes by feature frequency profiles: an alignment-free method with optimal feature resolution. Proc Natl Acad Sci U S A. 107:133–138.

Kolekar P, Kale M, Kulkarni-Kale U. 2012. Alignment-free distance measure based on return time distribution for sequence analysis: applications to clustering, molecular phylogeny and subtyping. Mol Phylogenet Evol. 65:10–522.

Kolekar PS, Kale M, Kulkarni-Kale U. 2011. Genotyping of Mumps viruses based on SH gene: development of a server using alignment-free and alignment-based methods. Immune Res. 7:1–7.

Kurtz S, Phillippy A, Delcher AL, Smoot M, Shumway M, Antonescu C, Salzberg SL. 2004. Versatile and open software for comparing large genomes. Genome Biol. 5R12.

Li M, Badger JH, Chen X, Kwong S, Kearney P, Zhang H. 2001. An information-based sequence distance and its application to whole mitochondrial genome phylogeny. Bioinformatics 17:149–154.

Liu ZH, Jiao D, Sun X. 2005. Classifying genomic sequences by sequence feature analysis. Genomics Proteomics Bioinform. 3(4):201–205.

Liu ZH, Meng JH, Sun X. 2008. A novel feature-based method for whole genome phylogenetic analysis without alignment: application to HEV genotyping and subtyping. Biochem Biophys Res Commun. 368:223–230.

Liu ZH, Sun X. 2008. Coronavirus phylogeny based on base-base correlation. Int J Bioinform Res Appl. 4:211–220.

Liu ZH, Zeng X, Yang D, Ren GM, Chu GY, Yuan ZR, Luo K, Xiao PG, Chen B. 2010. Identification of medicinal vines by ITS2 using complementary discrimination methods. J Ethnomedical 14:242–249.

Liu ZH, Zeng X, Yang D, Chu GY, Yuan ZR, Chen SL. 2012. Applying DNA barcodes for identification of plant species in the family Araliaceae. Gene. 499:76–80.

Nandy A, Harle MS, Basak C. 2006. Mathematical descriptors of DNA sequences: development and applications. Arch Org Chem. 9: 211–238.

Otu HH, Sayood K. 2003. A new sequence distance measure for phylogenetic tree construction. Bioinformatics 19:2122–2130.

Page RD. 1996. TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci. 12:357–358.

Qi J, Luo H, Hao B. 2004. CVtree: a phylogenetic tree reconstruction tool based on whole genomes. Nucleic Acids Res. 32:W45–W47.

Robinson DF, Foulds LR. 1981. Comparison of phylogenetic trees. Math Biosci. 53:131–147.

Saitou N, Nei M. 1987. The neighbor-joining method: a new method for characterizing genetic distances between genomes. J Mol Evol. 25:407–425.

Sims GE, Jun SR, Wu GA, Kim SH. 2009. Alignment-free genome comparison with feature frequency profiles (FFP) and optimal resolutions. Proc Natl Acad Sci U S A. 106:2677–2682.

Sims GE, Kim SH. 2011. Whole-genome phylogeny of Escherichia coli/ Shigella group by feature frequency profiles (FFPs). Proc Natl Acad Sci U S A. 108:8329–8334.

Sukumaran J, Holder MT. 2010. DendroPy: a Python library for phylogenetic computing. Bioinformatics 26:1569–1571.
Yu C, Liang Q, Yin C, He RL, Yau SS. 2010. A novel construction of genome space with biological geometry. DNA Res. 17:155–168.

Yu ZG, Chu KH, Li CP, Anh V, Zhou LQ, Wang RW. 2010. Whole-proteome phylogeny of large dsDNA viruses and parvoviruses through a composition vector method related to dynamical language model. BMC Evol Biol. 10:192.

Zeng X, Yuan ZR, Tong X, Li QS, Gao WW, Qin MJ, Liu ZH. 2012. Phylogenetic study of Oryzoideae species and related taxa of the Poaceae based on atpB-rbcL and ndhF DNA sequences. Mol Biol Rep. 39(5):5737–5744.