Genome analysis

GGRaSP: a R-package for selecting representative genomes using Gaussian mixture models

Thomas H. Clarke1,*, Lauren M. Brinkac1,2, Granger Sutton1 and Derrick E. Fouts1

1J. Craig Venter Institute, Rockville, MD 20850, USA and 2Department of Biotechnology and Food Technology, Durban University of Technology, Durban 4000, South Africa

*To whom correspondence should be addressed.

Associate Editor: John Hancock

Received on October 3, 2017; revised on March 27, 2018; editorial decision on April 11, 2018; accepted on April 12, 2018

Abstract

Motivation: The vast number of available sequenced bacterial genomes occasionally exceeds the facilities of comparative genomic methods or is dominated by a single outbreak strain, and thus a diverse and representative subset is required. Generation of the reduced subset currently requires a priori supervised clustering and sequence-only selection of medoid genomic sequences, independent of any additional genome metrics or strain attributes.

Results: The Gaussian Genome Representative Selector with Prioritization (GGRaSP) R-package described below generates a reduced subset of genomes that prioritizes maintaining genomes of interest to the user as well as minimizing the loss of genetic variation. The package also allows for unsupervised clustering by modeling the genomic relationships using a Gaussian mixture model to select an appropriate cluster threshold. We demonstrate the capabilities of GGRaSP by generating a reduced list of 315 genomes from a genomic dataset of 4600 Escherichia coli genomes, prioritizing selection by type strain and by genome completeness.

Availability and implementation: GGRaSP is available at https://github.com/JCVenterInstitute/ggrasp/.

Contact: tclarke@jcvi.org

Supplementary information: Supplementary data are available at Bioinformatics online.

1 Introduction

Next-generation sequencing technologies have resulted in a large number of publicly available microbial genome sequences. The number of genomes available for comparative genomic analysis can exceed what can be feasibly visualized or analyzed (Chan et al., 2015; Chavda et al., 2016; Zaslavsky et al., 2016). Additionally, sequencing of clonal or nearly clonal bacterial pathogens involved in disease outbreaks (e.g. Acinetobacter baumannii, Escherichia coli and Klebsiella pneumoniae) can skew the analyses; therefore, a reduction in genome redundancy to maximize diversity is necessary (Chan et al., 2015). One common method to reduce sequence redundancy while minimizing information loss is to cluster genomes by their nucleotide distance metrics and from each cluster select one genome, often a medoid (the genomes with the minimal combined distance to the other genomes in the cluster) (Chan et al., 2015; Moreno-Hagelsieb et al., 2013), as a representative. However, these methods require the user to a priori specify either the number of clusters or a distance cutoff, and they do not allow the user to use the highest quality (i.e. most complete) representative genome for each cluster. Likewise, no dedicated program exists for loading and selecting these genomes.

2 Materials and methods

Here, we introduce GGRaSP (Gaussian Genome Representative Selector with Prioritization), a R-package and associated executable...
R script program that generates a list of prioritized representative genomes from either supervised or unsupervised clustering of related genomes. GGRaSP supports three forms of input to describe the relationship between the genomes: (i) a phylogeny in Newick format; (ii) a distance or similarity matrix; or (iii) an aligned multiple FASTA file. GGRaSP uses hierarchical clustering in the hclust R function or the APE R package to create phylogenies from (ii) and (iii) (Paradis et al., 2004). By default, GGRaSP prioritizes medoids as representative genomes in order to minimize the loss of information, but this can result in removal of genomes that contain regions of interests (e.g. plasmids, antibiotic resistance islands, pathogenicity islands and prophage), have a more complete assembly, or are from a given project. Users can therefore specify criteria of genomes for selection as representatives by generating a text file containing tiered ranks of the genomes.

GGRaSP can cluster genomes using supervised methods, including specifying the number of clusters or the cluster cut-off distance, but it also allows for unsupervised clustering by using Gaussian mixture models (GMMs) to identify a cut-off value that separates the most closely related genomes from the more diverse genomes. GMMs of sequence distances have previously been used to model the evolutionary relationship between multiple genomes in metagenomes (e.g. Alneberg et al., 2014; Ji et al., 2017), and to model homologs descending from distinct ancient large-scale duplications in various eukaryotic organisms (e.g. Cui et al., 2006; Schwager et al., 2017). The GMM model could be biased or limited by collections of genomes which contain a single branch of highly related genomes (for which GGRaSP will select a cutoff that will only cluster that single branch) or a set of genomes that can be best modeled by a single Gaussian peak (in which case GGRaSP cannot find a cutoff).

In GGRaSP, GMMs are calculated using expectation maximization via mixtools or bgmm (Benaglia et al., 2009; Biecek et al., 2012). Multiple Gaussian distributions are tested incrementally until the addition of a distribution is not significant by the Likelihood Ratio test or exceeds the user defined limit. After the GMM is cleaned by removing overlapping and low count distributions, the inflection point between the first two distributions is used as the cut-off to generate the clusters (see dotted vertical line, Fig. 1). The default pipeline behavior is described earlier, but many of the parameters for the GMM-based threshold calculation are user-modifiable for the cases where the GMM varies from the default model.

GGRaSP can output multiple supporting files as is described in detail on the R help pages including: tab-delimited files with information on the clusters; ggplot2-based images showing the GMM, the initial or the final phylogenies (Wickham, 2009) with colorspace to determine the hues of GMM and phylogeny shading (Ihaka et al., 2016); the Newick files for any phylogeny used in GGRaSP; and the iTOL-formatted text files showing the clusters on the phylogenies

(Letunic and Bork, 2016). A Rscript version of GGRaSP to run on a command line to facilitate high-throughput analyses is also provided.

3 Usage scenario

To demonstrate the usefulness of GGRaSP, we downloaded 4600 Escherichia genomes from NCBI RefSeq on 2/2/2017 using the downloader script in the LOCUST package (Brinkac et al., 2017). A whole genome-based Average Nucleotide Identity (gANI) matrix was calculated with Mash (Ondov et al., 2016). The genomes were ranked, in order by: whether it was a type strain; whether it was circular; and whether it was complete. The remaining genomes were ranked by the number of contigs and genes according to the LOCUST downloader output. The similarity matrix and the ranking file were input to GGRaSP, which computed a cut-off of 1.09% identity after modeling 9 Gaussian distributions (10 before cleanup), leading to a selection of 315 representative genomes in 98 min and 2s (Fig. 1, Supplementary Fig. S1). When using to a priori cutoff of 96.5% gANI cutoff suggested for species (Varghese et al., 2015), only nine clusters were generated with the largest cluster containing 98.9% of the genomes. Ranking the genomes as described earlier increased the completeness of retained genomes compared to selecting the representatives from an unranked set number of complete genomes (from 6.7 to 25.4%) and mean N50 (from 205 to 556 kb). All input and output files for these runs and the a priori cutoffs are available on the GitHub repository.

4 Conclusion

As the number of sequenced genomes available for comparative genomic analysis continues to expand, the need to generate robust representative genomic subsets will increase. Building off the statistical, bioinformatic, and graphical capabilities of R, GGRaSP and the accompanying Rscript provides a single and customizable platform to run multiple analyses to generate a subset of representative genomes. The user can specify clustering parameters and levels of importance for ranking the genomes, thus allowing for both generalizable high-throughput and more dataset specific use.

Acknowledgements

The authors wish to thank Jason Inman, Pratap Venepally, and Chris Greco for their assistance in providing feedback on GGRaSP.

Funding

This work was supported in whole or part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services under Award Number [U19AI110819].

Conflict of Interest: none declared.

References

Alneberg, J. et al. (2014) Binning metagenomic contigs by coverage and composition. Nat. Methods, 11, 1144–1146.
Benaglia, T. et al. (2009) mixtools: an R package for analyzing mixture models. J. Stat. Softw., 32, 1–29.
Biecek, P. et al. (2012) The R package bgmm: mixture modeling with uncertain knowledge. J. Stat. Softw., 47, 1–31.
Brinkac, L. M. et al. (2017) LOCUST: a custom sequence locus typer for classifying microbial isolates. Bioinformatics, 33, 1725–1726.
Chan, A.P. et al. (2015) A novel method of consensus pan-chromosome assembly and large-scale comparative analysis reveal the highly flexible pan-genome of *Acinetobacter baumannii*. *Genome Biol.*, 16, 143.

Chavda, K.D. et al. (2016) Comprehensive genome analysis of carbapenemase-producing *Enterobacter* spp.: new insights into phylogeny, population structure, and resistance mechanisms. *mBio*, 7, e02093-16–e02016.

Cui, L. (2006) Widespread genome duplications throughout the history of flowering plants. *Genome Res.*, 16, 738–749.

Ihaka, R. et al. (2016) colorspace: Color Space Manipulation. R package version 1.3-2, https://CRAN.R-project.org/package=colorspace.

Ji, P. et al. (2017) MetaSort untangles metagenome assembly by reducing microbial community complexity. *Nat. Commun.*, 8, 14306. ncomms14306.

Letunic, I. and Bork, P. (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res.*, 44, W242–W245.

Moreno-Hagelsieb, G. et al. (2013) Phylogenomic clustering for selecting non-redundant genomes for comparative genomics. *Bioinformatics*, 29, 947–949.

Ondov, B.D. et al. (2016) Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biol.*, 17, 132.

Paradis, E. et al. (2004) APE: analyses of Phylogenetics and Evolution in R language. *Bioinformatics*, 20, 289–290.

Schwager, E. et al. (2017) The house spider genome reveals an ancient whole-genome duplication during arachnid evolution. *BMC Biol.*, 15, 62.

Varghese, N.J. et al. (2015) Microbial species delineation using whole genome sequences. *Nucleic Acids Res.*, 43, 6761–6771.

Wickham, H. (2009) *ggplot2 - Elegant Graphics for Data Analysis*. Springer-Verlag, New York.

Zaslavsky, L. et al. (2016) Clustering analysis of proteins from microbial genomes at multiple levels of resolution. *BMC Bioinformatics*, 17, 276.