genoPlotR: comparative gene and genome visualization in R
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1 INTRODUCTION
Comparison of genes and genomes has increasingly been used to infer patterns and processes in evolution and to relate phenotypic differences to genomic changes. With the recent advent of modern high-throughput sequencing technologies, the need for methods and visualization tools in comparative genomics has vastly increased. As of 2010, more than 1000 bacterial and archaeal genomes are available in public databases, making the number of possible comparisons almost infinite. Several programs such as the Artemis Comparison Tool (ACT; Carver et al., 2005), UCSC Genome Browser (Rhead et al., 2010), Mauve (Darling et al., 2004), M-CAT (Teeling et al., 2006) and Murasaki (Sakakibara et al., 2009) have extensive visualization functions. However, these tools lack the ability (i) to directly add annotations to the publication quality graphics they produce or (ii) to fully automate the production of comparative figures. GenomeGraphs (Durinck et al., 2009) is a R package, which allows the visualization of one genomic region with related datasets such as microarray data. It can display several annotations for the same region, but it cannot show several regions in a single plot.

The R package genoPlotR is an attempt to fill in those gaps by providing a flexible, automatable tool. It allows the user to graphically represent the comparison between several segments or subsegments of genomes in a linear fashion. It reads data stored in commonly used formats (EMBL, Genbank, BLAST and Mauve outputs) or in user-created tabular files and allows comparisons of one or several subsegments of a genome. A tree can be added to show the phylogenetic relationships between the segments, as can also scales and annotations to each subsegment. The use of R (R Development Core Team, 2009) and its grid package enables the use of its graphical power and flexibility to manipulate data and to integrate gene and genome maps into more complex graphics. The results can be saved either in high-quality raster or vector formats for further editing.

2 INPUT DATA
genoPlotR uses two main objects: dna_seg, which represent segments of DNA containing genes or other features, and comparison, which represent the relationships between two dna_seg. genoPlotR reads dna_seg objects from the widely used Genbank and EMBL formats (described here: http://www.ncbi.nlm.nih.gov/entrez/; Fig. 1A), or from tabular formats, either as protein table files (NCBI) or user-generated. Genbank or EMBL files can be generated by the user or downloaded from nucleotide databases, e.g. from NCBI’s Nucleotide Entrez (http://www.ncbi.nlm.nih.gov/sites/entrez). By default, only CDS features are read, but any set of features can be specified for reading. genoPlotR reads comparison objects using the tabular output of, e.g. BLAST (Altschul et al., 1990) or from user-generated tabular files (Fig. 1A). Hit tables in text format produced by stand-alone or online BLAST programs are suitable for input in genoPlotR. For example, from the NCBI BLAST web page, sequence alignments of genes and genomes can be produced with the option ‘align two or more sequences’. The hit table can then be downloaded. The backbone file produced by Mauve, a multiple-genome alignment tool (Darling et al., 2004), can be transformed into both dna_seg and comparison objects (Fig. 1B).

Both dna_seg and comparison objects can be filtered either by using arguments to the reading functions, or by using R functions, for example, to remove short genes or low-similarity comparisons. Objects can be modified to specify color, size or appearance (arrows, blocks, lines, etc.) for each element of the DNA segments and of the comparisons. Inten-containing genes in EMBL and Genbank files are automatically recognized (Fig. 1D).

In addition, a phylogenetic tree in Newick format can be parsed as a phylog object, using the package ape (Dray et al., 2007; Fig. 1A). Finally, annotation objects can be designed to add a legend to the DNA segments.

3 VISUALIZATION
After being read and modified, objects can be passed to the main graphical function plot_gene_map.

The user can define, for each segment, several subsegments that will be represented in the plot. These subsegments can be represented in reverse orientation (e.g. see Fig. 1B, first subsegment on the top DNA segment). The placement of each subsegment on the plot is either automatically determined by minimizing the area of the

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Fig. 1. Examples of gene and genome comparisons with genoPlotR. The code used to generate this figure is available at http://genoplotr.r-forge.r-project.org/screenshots.php. (A) Example of minimal code to represent a three-way comparison in genoPlotR. (B) Comparison of four Bartonella genomes using the backbone output of Mauve. The features are colored according to their order along the second segment (BG), following a rainbow palette. (C) BLAST comparison of genes in several subsegments of the same four Bartonella genomes. Note the scale of the first subsegment on the top row, which is in reverse orientation. (D) Comparison of a 220 kb segment of the Y chromosome in Homo sapiens and Pan troglodytes. In (C and D), the E-value of the BLAST alignment is represented with shades of blue and red. All datasets used here are present in the package.

4 CONCLUSIONS

By using the graphical power and flexibility of R, the package genoPlotR generates reproducible maps of genes and genomes that can be used to generate publication-ready figures, starting from a wide range of formats. Since all the instructions for drawing the figures are contained in R code, it is highly flexible, and thus it is straightforward to automate the process of drawing very similar figures for different datasets. The use of a scripting language makes it particularly suitable for integration into annotation and comparative genomics pipelines.

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REFERENCES


