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SPOCS: software for predicting and visualizing orthology/paralogy relationships among genomes

Darren S. Curtis[1,†], Aaron R. Phillips[1,†], Stephen J. Callister[2], Sean Conlan[3] and Lee Ann McCue[4,*]

[1]Computational & Statistical Analytics Division, Pacific Northwest National Laboratory, [2]Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA 99352, USA, [3]Genetics and Molecular Biology Branch, National Human Genome Research Institute, NIH, Bethesda, MD 20892, USA and [4]Computational Sciences & Mathematics Division, Pacific Northwest National Laboratory, Richland, WA 99352, USA

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ABSTRACT

Summary: At the rate that prokaryotic genomes can now be generated, comparative genomics studies require a flexible method for quickly and accurately predicting orthologs among the rapidly changing set of genomes available. SPOCS implements a graph-based ortholog prediction method to generate a simple tab-delimited table of orthologs and in addition, html files that provide a visualization of the predicted ortholog/paralog relationships to which gene/protein expression metadata may be overlaid.

Availability and Implementation: A SPOCS web application is freely available at http://cbb.pnnl.gov/portal/tools/spocs.html. Source code for Linux systems is also freely available under an open source license at http://cbb.pnnl.gov/portal/software/spocs.html; the Boost C++ libraries and BLAST are required.

Contact: leeann.mccue@pnnl.gov

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1 INTRODUCTION

The prediction of orthologs and paralogs is a fundamental step in many comparative genomics studies. For example, orthology and paralogy relationships are used to identify the core and auxiliary genes among related organisms, assign protein function annotation to genes in a new genome and predict gene expansion events, such as those that give rise to protein families. Though the concepts of orthology and paralogy were defined many years ago, the advent of whole genome sequencing and the wide availability of these genomic data has been the catalyst for the development of numerous ortholog prediction programs [see Kristensen *et al.* (2011) for a recent review]. In large parts, these programs rely on the identification of homologous genes/proteins, and frequently employ BLAST (Basic Local Alignment Search Tool; Altschul *et al.*, 1997) to identify these.

Many methods have focused on eukaryotic genomes, in which widespread gene duplication and domain rearrangement have made predicting orthologs particularly challenging (Sjölander *et al.*, 2011). In prokaryotes, however, the challenge lies in keeping up with the rate of genomic data generation. As a bacterial genome can now be sequenced in a day or two, and many are often left in 'high quality draft' stage, comparative genomics studies on these data require the flexibility to quickly and accurately predict orthologs on-the-fly, when the genome data are updated frequently.

We have previously employed a graph-based ortholog/paralog prediction method (Callister *et al.*, 2008; Konstantinidis *et al.*, 2009), that combines the pairwise (two species) predictions from InParanoid (Remm *et al.*, 2001), using custom Perl scripts to produce orthologs for groups of species. Here, we present a robust software tool called SPOCS (Species Paralogy and Orthology Clique Solver) that implements this approach, providing flexible and readily extensible ortholog predictions for groups of species defined by the user.

2 APPROACH

For every pair of species (i.e. pair of protein fasta files provided by the user), we use the ortholog/paralog definitions as described by Remm *et al.* (2001) and combine the pairwise predictions using a graph-based method to identify complete or near-complete cliques. SPOCS will generate a simple tab-delimited table of orthologs, and in addition, html files that provide a visualization of the predicted orthology/paralogy relationships. The html files link back to the protein sequence data and gene/protein expression metadata may be overlaid onto the visualization.

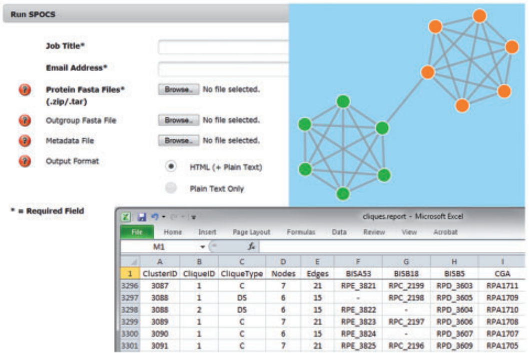
2.1 Implementation

SPOCS is written in C++ using the Boost libraries (http://www.boost.org/). For the identification of orthologs and paralogs between pairs of species, the InParanoid Perl code (Remm *et al.*, 2001) was redesigned and implemented in C++. We used the MaxCliqueDyn algorithm (Konc and Janezic, 2007) to identify ortholog cliques, and the D3 Javascript library (http://d3js.org/) to generate SVG images of the ortholog graphs.

SPOCS is available both as source code and in a web application. For local installation on Unix/Linux machines, instructions are provided in a readme file within the downloadable source tar file. To run SPOCS locally, the BLAST executables must be in the user PATH. At the command line, SPOCS requires only a list of fasta formatted protein sequence files, a scratch output directory in which SPOCS will store intermediate files and a results directory for the final output files.

*To whom correspondence should be addressed.

†The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors



Run SPOCS

Job Title*

Email Address*

Protein Fasta Files* (.zip/.tar) Browse.. No file selected.

Outgroup Fasta File Browse.. No file selected.

Metadata File Browse.. No file selected.

Output Format

● HTML (+ Plain Text)

○ Plain Text Only

* = Required Field

cliques.report - Microsoft Excel

	A	B	C	D	E	F	G	H	I
1	ClusterID	CliqueID	CliqueType	Nodes	Edges	BISA53	BISB18	BISB5	CGA
3296	3087	1	C	7	21	RPE_3821	RPC_2199	RPD_3603	RPA1711
3297	3088	1	DS	6	15	-	RPC_2198	RPD_3605	RPA1709
3298	3088	2	DS	6	15	RPE_3822	-	RPD_3604	RPA1710
3299	3089	1	C	7	21	RPE_3823	RPC_2197	RPD_3606	RPA1708
3300	3090	1	C	6	15	RPE_3824	-	RPD_3607	RPA1707
3301	3091	1	C	7	21	RPE_3825	RPC_2196	RPD_3609	RPA1705

Fig. 1. Screen shots of the SPOCS web application home page, a sample of the text output (viewed in Excel) and an html page for a Degenerate group that will resolve two cliques (orange and green)

The web application requires only the protein fasta files, a job title and an email address (Fig. 1, upper left). The user is notified by email when results are available, because jobs may require hours to run, depending on the number of species involved. The stand-alone Linux implementation and the web application generate the same outputs: a tab-delimited report file in which each line represents a predicted set of orthologs (Fig. 1, lower right), and optionally, self-contained html output with visualizations of the ortholog relationships (Fig. 1, upper right). SPOCS also offers several options to control parameters associated with utilization of an outgroup species, identification of the reciprocal BLAST hits, generation of html files for visualization, and incorporation of metadata on gene/protein expression that can be overlaid on the html images.

2.2 Application

Provided a set of *n* proteomes (fasta-formatted files of the predicted proteins from a species) and an optional proteome to serve as the outgroup, SPOCS proceeds through three main stages. First, SPOCS executes a series of BLAST (Altschul *et al.*, 1997) runs between every pair of species to identify reciprocal best hits; this is the most compute-intensive stage, requiring n^2 independent BLAST runs. When SPOCS is run locally, the BLAST results are stored in the scratch directory, allowing subsequent SPOCS runs that include some of the same *n* species to avoid performing BLAST runs if they already exist. In the second stage, SPOCS uses the BLAST results to generate an orthology/paralogy relationship graph based on merging the pairwise ortholog and in-paralog relationships [as defined by Remm *et al.*, (2001)]. Finally, SPOCS identifies cliques in each graph by breaking it into subgraphs and using the branch and bound clique-finding algorithm (Konc and Janezic, 2007) to iteratively find all cliques in each subgraph.

We classify the graphs into four subjective categories: Complete, SemiComplete, Incomplete and Degenerate. The Complete graphs include no more than one protein per species and every protein (node in the graph) is connected to every other protein by a reciprocal best hit (edge), i.e., a maximum clique. SemiComplete graphs may be reported in which no more than one protein per species is present but with less than the maximal number of edges (this is a tunable parameter for which the default is 5%). By definition, these Complete and SemiComplete cliques are the result of single unique graphs that resolve to single unique cliques. The Incomplete graphs have no more than one protein per species, and less than the maximal number of edges (more missing edges than specified for SemiComplete). The Degenerate graphs have more than one protein (node) from one or more of the species. For the Incomplete and Degenerate graphs, the clique-finding algorithm will identify as many maximum cliques as possible.

2.3 Visualization

Using the `-H` flag with the stand-alone software, or the html radio button on the web application will generate self-contained html output, which allows the user to navigate through the graph space and view gene relationships. The graph structure is rendered using a force-directed layout engine, which uses charged particles to place related genes closer together in the graph; the html output provides this graph image as well as a link to the fasta file of those proteins and a table providing their functional annotation. Generation of the html output does not appreciably affect run-time, but will dramatically increase the number of files generated. Though no upper limit has been imposed on the number of genomes that SPOCS can process, the visualization functions were designed to facilitate the analysis of targeted datasets chosen to explore specific hypotheses, and can become difficult to interpret as the number of genomes increases.

3 CONCLUSION

SPOCS provides a flexible and extensible software tool for the prediction of orthologs and paralogs among closely related genomes. In addition to a standard table of orthologs, the html output presents a visualization of the predicted orthology/paralogy relationships, provides hooks back to the protein sequence data and allows the overlay of gene/protein expression metadata.

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Conflict of Interest: none declared.

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