

Esmail Ebrahimie, Mario Fruzangohar, Seyyed Hani Moussavi-Nik, and Morgan Newman  
**Gene Ontology-based analysis of zebrafish' omics data using the web tool  
Comparative Gene Ontology**  
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ZEBRAFISH  
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DOI: 10.1089/zeb.2016.1290*Original Article***AU1c** Gene Ontology-Based Analysis of Zebrafish Omics Data  
**AU2c** Using the Web Tool Comparative Gene Ontology**AU3c** Esmaeil Ebrahimie,<sup>1-3,\*</sup> Mario Fruzangohar,<sup>4,\*</sup> Seyyed Hani Moussavi-Nik,<sup>1</sup> and Morgan Newman<sup>1</sup>**Abstract**

Gene Ontology (GO) analysis is a powerful tool in systems biology, which uses a defined nomenclature to annotate genes/proteins within three categories: “Molecular Function,” “Biological Process,” and “Cellular Component.” GO analysis can assist in revealing functional mechanisms underlying observed patterns in transcriptomic, genomic, and proteomic data. The already extensive and increasing use of zebrafish for modeling genetic and other diseases highlights the need to develop a GO analytical tool for this organism. The web tool Comparative GO was originally developed for GO analysis of bacterial data in 2013 ([www.comparativego.com](http://www.comparativego.com)). We have now upgraded and elaborated this web tool for analysis of zebrafish genetic data using GOs and annotations from the Gene Ontology Consortium.

**AU5c** **Keywords:** genomics, bioinformatics, Gene Ontology

**AU6c** Gene Ontology (GO) analysis is a powerful tool in systems biology, which uses a defined nomenclature to annotate genes/proteins within three categories: “Molecular Function,” “Biological Process,” and “Cellular Component.”<sup>1-3</sup> GO analysis can assist in revealing functional mechanisms underlying observed patterns in transcriptomic, genomic, and proteomic data. Some genes/proteins such as transcription factors can play prominent roles in systems biology despite their low levels of expression. Therefore, a recent trend in analysis of omics data has been a consideration of the possible role and class of a gene/protein in addition to its level of expression using approaches such as network analysis and promoter profiling.<sup>4,5</sup> This strategy can be described as quality-based gene selection (compared to quantity-based gene selection). GO analysis provides the opportunity for function-based and quality-based identification of important genes/proteins in omics studies. Many GO tools have been developed in recent years,<sup>3,6,7</sup> but none of them is designed specifically for use with zebrafish omics data. In addition, to detect statistically significantly altered GOs, common GO tools only compare the enrichment of GO

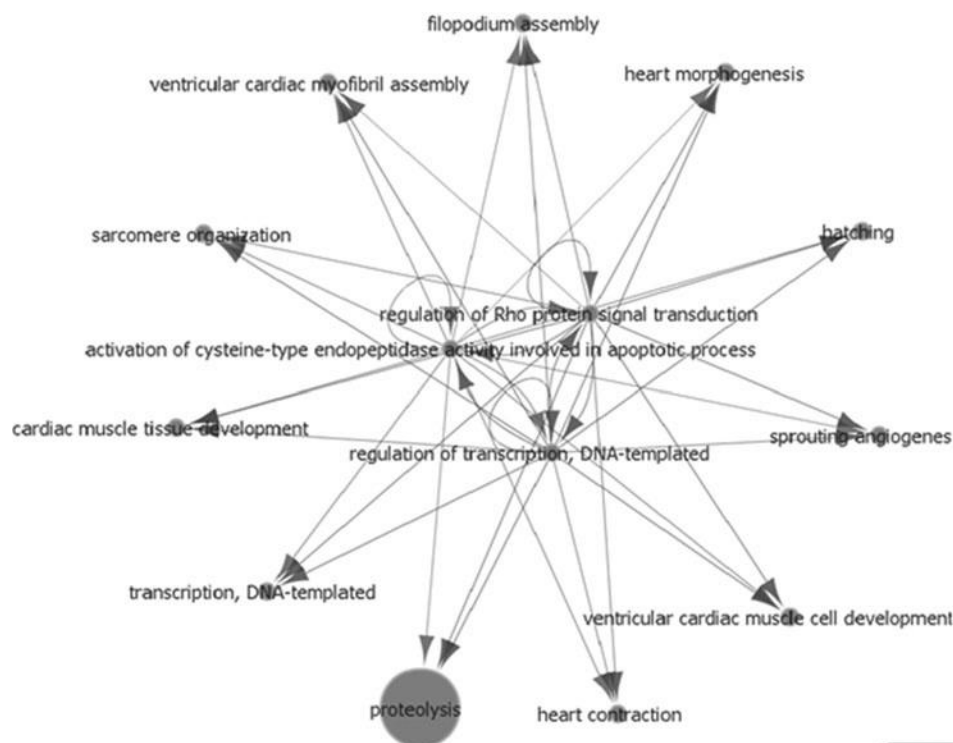
terms for a particular sample (e.g., a set of genes/proteins with significantly altered expression) versus their enrichment over the entire genome (as a reference set). These tools are not able to compare GOs between two or more samples directly.<sup>1</sup>

The already extensive and increasing use of zebrafish for modeling genetic and other diseases highlights the need to develop a GO analytical tool for this organism. The web tool Comparative GO was originally developed for GO analysis of bacterial data in 2013<sup>1</sup> ([www.comparativego.com](http://www.comparativego.com)) and extended for human samples recently.<sup>8</sup> We have now upgraded and elaborated this web tool for analysis of zebrafish genetic data using GOs and annotations from the Gene Ontology Consortium.<sup>9</sup> We update these GOs and annotations in our database regularly every 6 months. Database tables were designed in a PostgreSQL database system and a Java-based application is used for data extraction from source files, integrated with an R package to perform the statistical analysis and a Cytoscape plugin for visualization of GO networks. The web tool performs the following analyses: (1) GO classification of zebrafish genes/proteins in terms of “Molecular

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FIG. 1. Gene Ontology regulatory network of up-regulated genes in the zebrafish brain under hypoxia. The *gray* and *green* colors represent upregulation and down-regulation of GO interactions, respectively. See Supplementary File S1 for more information on outputs from the Comparative GO web tool. GO, Gene Ontology.



Function,” “Biological Process,” and “Cellular Component,” (2) comparison of the GO distribution of a sample of genes/proteins versus that of the genome using a hypergeometry approach (with statistical significance testing by Fisher’s exact test), (3) direct (tabular) comparison of GO enrichments between two or more samples of genes and determination of the key upregulated/downregulated GO groups based on the fold change of GO enrichment (as yet without statistical significance testing although this may be developed in future), and (4) statistics-based comparison of overall GO enrichment between two or more samples using the Wilcoxon signed rank test with continuity correction, the two-sample Kolmogorov–Smirnov test, and the chi-square test. The Comparative GO web tool also produces the following graphical outputs: (1) pie charts of GO classification of one or more samples and (2) networks visualizing GO relationships and highlighting central regulatory GO mechanisms. “GO regulatory networks” find the GOs with the highest number of interactions with the other GO groups and place them in the center of a network to build a regulatory network. The web tool includes a facility to incorporate information on gene/protein expression levels with GO enrichment data, since levels of expression can significantly affect GO enrichment.<sup>7</sup> The user has this opportunity to save the gene list in the web tool permanently to be accessed from any computer when the user logs in.

As guidance for the use of the Comparative GO tool and as an example of its functions and outputs, we have provided **SDc** Supplementary Information (Supplementary Data are available online at [www.liebertpub.com/zeb](http://www.liebertpub.com/zeb)) showing GO classification and analysis (Supplementary File S1) of up- and downregulated genes from an mRNA-level comparison of hypoxic and normoxic adult zebrafish brains (Supplementary File S2).

**SF1c**

**SF2c**

GO network analysis (Fig. 1) highlighted the central regulatory role of the GO *activation of cysteine-type endopeptidase activity involved in apoptotic process* (Gene Ontology ID: 6919, including *diabloba*), *regulation of transcription, DNA templated* (Gene Ontology ID: 6355, including *bzw1b*), and *regulation of Rho protein signal transduction* (Gene Ontology ID: 35023, including *arhgef9b*) in the zebrafish brain’s response to hypoxia. **b F1**

#### Acknowledgments

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#### Supplementary File S1

A guideline for Gene Ontology (GO)-based analysis of the adult zebrafish brain under hypoxia using the Comparative GO web tool.

#### Supplementary File S2

Differential expression analysis data of microarray data from a comparison of adult zebrafish brains under hypoxia versus normoxia.

#### Disclosure Statement

No competing financial interests exist.

**b AU7**

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