Gene Ontology Tools: A Comparative Study

Muhammad Shahzad[1,*], Kamran Ahsan[2], Adnan Nadeem[2] and Muhammad Sarim[2]

[1]*College of Computing & Information Sciences, PAF - Karachi Institute of Economics & Technology, Korangi Creek, Karachi 75190, Pakistan*

[2]*Department of Computer Science, Federal Urdu University of Arts, Science and Technology, MSC Block, Block No. 9, Gulshan-e-Iqbal, Main University Road, Karachi 75300, Pakistan*

Abstract: Gene ontology (GO) is a key initiative of bioinformatics to provide uniform description of gene product in different public databases. The GO project has initiated 3 (three) structured and organized controlled vocabularies (i.e. ontology) that describes gene products in term of cellular components, biological processes and functions in an independent of species. This resource saves lots of time and effort in finding information of any particular gene from different databases. Since 1998, many tools have been developed which at one end relieves the researchers to search particulars about gene products but at other end makes difficult to opt most appropriate tool for any certain investigation in gene ontology. Here we present the state-of-the-art web based GO tools currently used for biological ontologies. We adopt comparison methodology in conjunction with visualization capabilities and sources of annotation data. This paper considers three GO tools for the said purpose. In Visualization capabilities; Indented List, Node-link & tree and Zoomable capabilities of selected tools have been analyzed. In the data sources section; currently available sources for data annotation have been discussed. This review will facilitate potential users of the GO tools to select an appropriate tool for their need.

Keywords: Gene Ontology, Knowledge Representation, Visualization, Data Annotation.

INTRODUCTION

Ontologies are being used to describe the terms and relations for providing common terminology over a domain. Specially whenever there is a need for representing knowledge then ontology would be the way for illustration in any particular topic area. It also helps to established unified vocabulary for the communication between people, organization and computer. As a common interface, it provides a language for communication independent of internal details of the domain. Ontologies can also be used for establishing content for the source of information explicitly and provide indexing to information repository [1].

In the science of bioinformatics, with the arrival of the genomes, the knowledge of biological data stored in electronic databases is increasing exponentially. Due to hundreds of online public biological databases, same knowledge is available in difference resources but with different terms and specifications. This creates difficulties for biologist to find particular knowledge. For example, the gene itself is being used in the biological community. In some databases, the gene is defined as 'the coding section of DNA'; whereas in other database, it is defined as 'fragment of DNA fragment which has capability to translated and transcribed into a

protein' and 'region of DNA for the interest of biological investigation with a name and which carries phenotype or genetic trait' in a third [2]. These wide variations in expression and terms of same biological data in different databases waste a lot of effort for locating available information. Therefore, since last few years, many bio-ontology tools have been developed for the elimination of said problem and comparative studies have been performed. In this research, we explore and compare visualization capabilities, data sources and knowledge representation languages of three Gene Ontology (GO) tools, i.e. CGAP, RAVIGO and AmiGO Browser.

This paper includes three sections to explain modern GO tools. First, we have highlighted three latest tools for bio-ontologies. that are currently available. Secondly, selected tools are compared with respect to visualizing capabilities and data annotation sources. Final section of the manuscript contains overview of related work.

SOME MODERN GENE ONTOLOGY TOOLS

1. Cancer Genome Anatomy Project (CGAP)

Cancer Genome Anatomy Project (CGAP) was started in 1996 [3]. The aim of CGAP project is to seek or to conclude the profiles of gene expression for cancer, pre-cancer and cells, eventually help to provide an improve methods for treatment, detection and diagnosis for the patients [4]. This project (CGAP)

*Address correspondence to this author at the College of Computing & Information Sciences, PAF - Karachi Institute of Economics & Technology, Korangi Creek, Karachi 75190, Pakistan; Tel: (9221) 35091114-7; Cell: +92-321-2197371; Fax: (9221) 35091118; E-mail: mshahzad@pafkiet.edu.pk, mohammadshahzad@hotmail.com
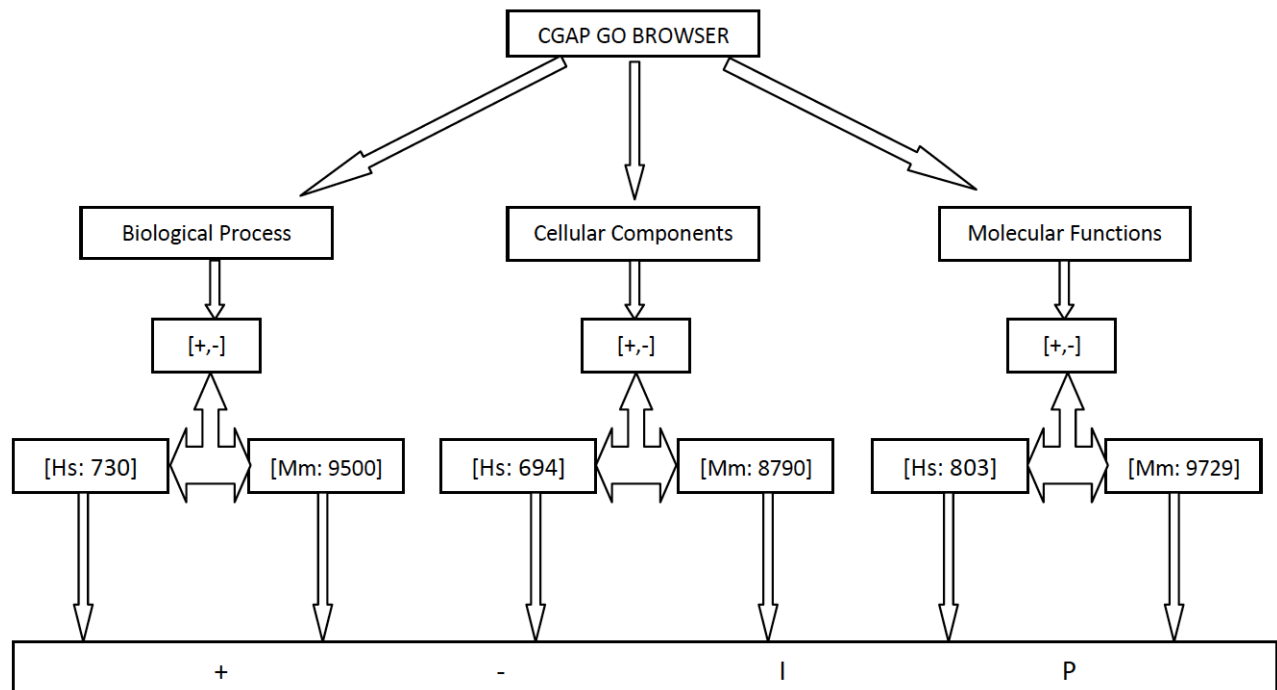
Figure 1: Classification of CGAP GOBrowser.

focuses on (a) constructing and annotating gene expression catalogues for the period of cancer development, (b) finding polymorphicgenes, and (c) constructing the resources for the characterization of molecules in cancer-related chromosomal aberrations [5]. Under the umbrella of this project, multiple tools for different investigation have been developed. Among these tools, CGAP has also developed GO browser to find GO hierarchal vocabulary of known human and mouse gene [6]. Using CGAP, we can find the details of the hierarchal structure of GO vocabulary from general descriptive terms to highly specific terms. Classifications of vocabulary with respect to biological roles of genes are: molecular function, biological process and cellular components. CGAP GO browser has further distributions of these biological roles into humans and mouse abbreviated as Hs and Mm respectively. Figure **1** shows the classification of CGAP GO browser [6].

Whereas Figure **2** shows the details symbols like +, -, I, P that used in CGAP GO browser [6]. The symbol [+, -] are non terminals term where '+' means it has descendent which is currently not displayed and '-' symbol means it has descendent and currently displayed. Symbol [I, P] are terminals term and both terms have no descendent. These symbols are further associated with either gray or blue shade which depicts the relationship of different types e.g. When symbol [+, -] are shaded with gray color, then it demonstrate is

"a kind of" its parents relationship and these symbols are in blue color shade, then it describes is "a part of" its parents relationship. Whereas symbol 'I' is a terminal term only displayed in gray color and it is "a kind of" its parents relationship. And symbol 'P' is also a terminal term and it is only shaded in blue color which means it is "a part of" its parent relationship. Figure **2** shows the summary of symbols.

2. REVIGO

REVIGO is web server based solutions that precisely define the Gene ontology terms. With clustering algorithms in REVIGO, it finds terms representative subset on the basis of similarities in semantics. It concludes the list of GO terms by eliminating the term which are redundant and displaying the GO terms in interactive graphs, tree maps, tag clouds and scatter plots [7]. On the bases of semantic similarity, it collapses GO terms and results are being showed it together with different colors according to p-value (or other metric) assigned to each GO term. The clustering-like algorithm used for finding semantic similarities depends on the previously defined measures in [8]. Following is the algorithm showing all necessary steps for clustering similar GO terms [9].

Step1: Start

Step2: For all given GO term in one Ontology, calculate a matrix of pairwise semantic similarities
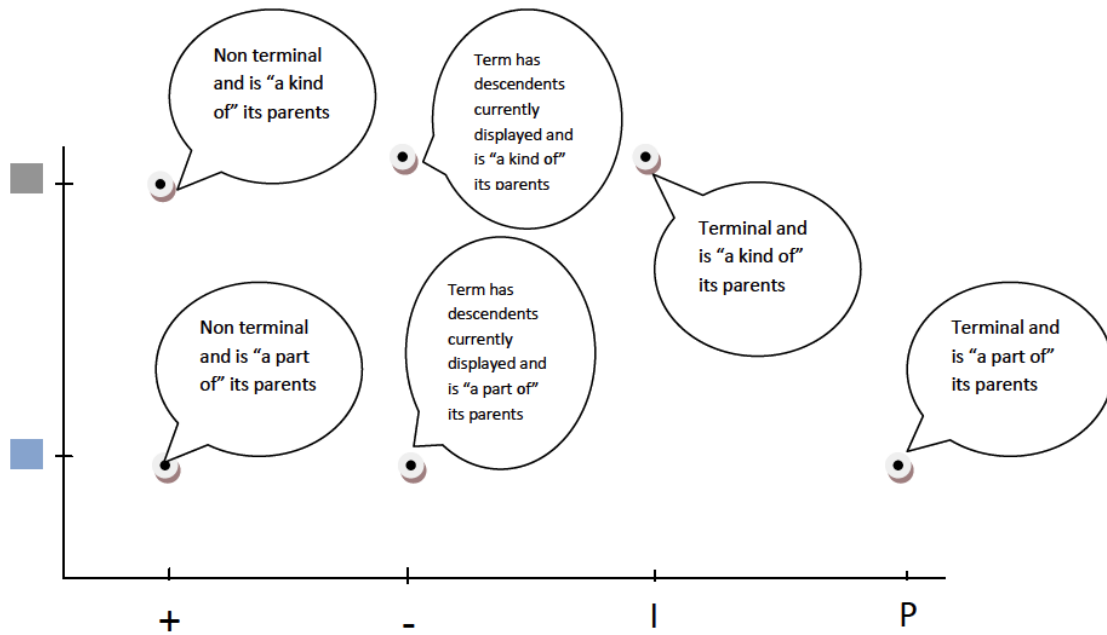
Figure 2: Description of the relationships with different symbols and colors.

Step3: Find the most similar pair of GO terms, t_i and t_j

Step4: IF t_i AND t_j less similar than the user-supplied cutoff C THEN

      Finish

      ELSE

Remove either t_i OR t_j depending on several criteria listed in the order of priority

IF one term has only very abroad interpretation (frequency >5%) THEN

      a): Reject very general term

b): GOTO Step 3

    ELSE IF One term has a less significant *p*-value THEN

      a): Reject less significant term

      b): GOTO Step 3

    ELSE IF t_i AND t_j are in parent-child relationship THEN

      a): Reject child term

      b): GOTO Step 3

    ELSE

    a): Reject t_i OR t_j at random

      b): GOTO Step 3

Step 5: Exit

The formation of the cluster or the group of highly similar GO terms depends on *p*-values and user specified cut-off C value. REVIGO also offers four types of pre-defined cut-off C values (i.e. 0.9, 0.7, 0.5 and 0.4) [9].

In REVIGO, there are four different ways for visualizing results after clustering procedure, which are: scatter plots, a graph based visualization, tree maps and tag clouds. Following are all four visualization procedures of GO: 0010447 1e-14 and GO: 0000027 1e-297.

2.1. Scatterplots

Scatter diagrams is used to examine the relationship possibilities amongst two variables which relate to the same "event" [10]. The data displayed in scatterplots is a collection of points, each point have the value of one variable determining the position on the horizontal axis and the value of the other variable determining the position on the vertical axis [11]. There are different kinds of correlation that we can investigate between the two variables with a certain confidence interval [12].

Figure **3** is the diagram of scatterplots, in which the biggest challenge is to assign coordinates x and y to each term in the way so that semantically identical GO terms can be plotted closer to each other. The REVIGO uses a procedure with multidimensional scaling which initially sets the terms with decomposition of eigen value and pairwise distance matrix of term. This is further defined by the minimization of stress step, which iteratively improves the contract between the closer terms showed in 2 dimensional spaces and
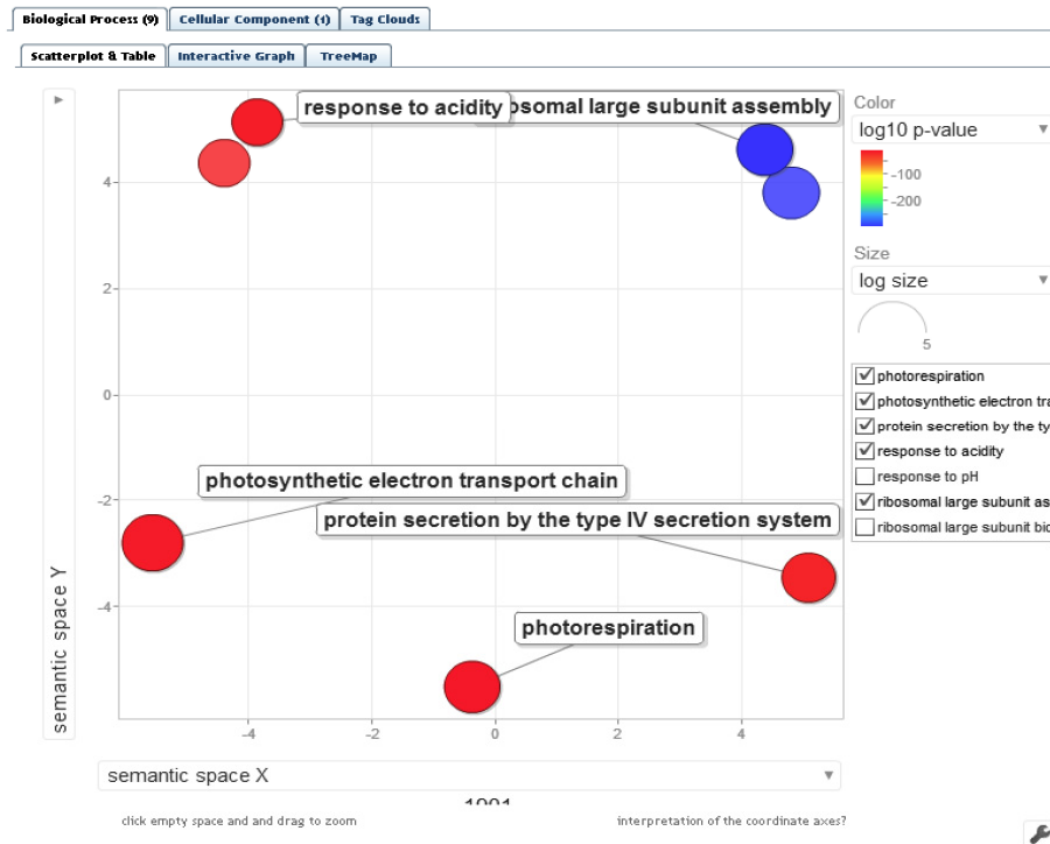
Figure 3: The Scatterplot view of REVIGO. The scatterplot shows the representation of the GO terms left after the process of reduction from the redundancy. The two dimensional space is being result from use of multi-dimensional scaling to a matrix of the GO terms' semantic similarities. User-provided p-value is showed in the form of Bubble color that indicates the legend in upper right-hand corner; size indicates the frequency of the GO term in the underlying GOA database (bubbles of more general terms are larger) [10].

semantically similar GO terms. The terms of GO and related data like p-values/enrichments, term description, uniqueness, etc can be further exported to a suitable table of text type and can be downloaded as well [9].

2.2. Interactive Graph

Graph-based visualization (Figure **4**) is also available in REVIGO. The node in graph is representing the GO terms, and 3% pairwise similarities of the strongest GO terms are denoted by edges in graph. The 3% value is threshold which is empirically derived and found an excellent balance between connected graphs. Moreover, there are no subgroups in the graph based visualization, but graphs with lot of small groups are used [9]. The nodes placement is identified by the algorithm named as 'Force Directed layout' which is implemented in Cytoscape Web [13]. Moreover for the web browser view, the graph can be translated into a XGMML file and used in the standalone program of Cytoscape [14] through JWS (Java Web Start) so that to produce high

quality solution and images with publication-quality. Both visualizations specifies the GO terms with generality with the bubble radius, in which small bubbles denotes more precise terms whereas color shading are used for showing the users' provided p-values/enrichments.

2.3. Tree Maps

Each rectangle in the Tree Map view has a single cluster for representation. These representatives further are joined together to build 'superclusters' that is loosely related terms and displayed in different colors. P-value or the GO terms' frequency is reflected by the adjustment of the size of rectangles [9].

2.4. Tag Clouds

In the list of user supplied, all showed words in the GO terms description are overrepresented with letters having darker and larger signifies stronger over representation. Those are keywords which are underrepresented are not showed in the Tag Cloud [9].
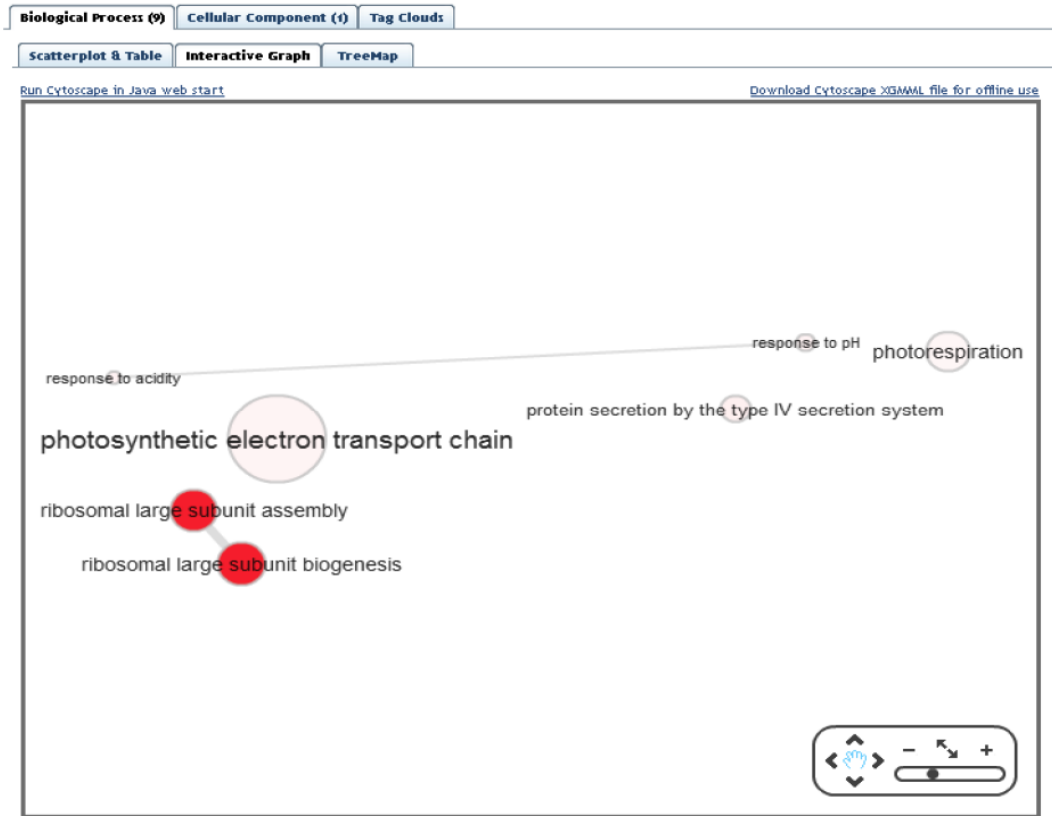
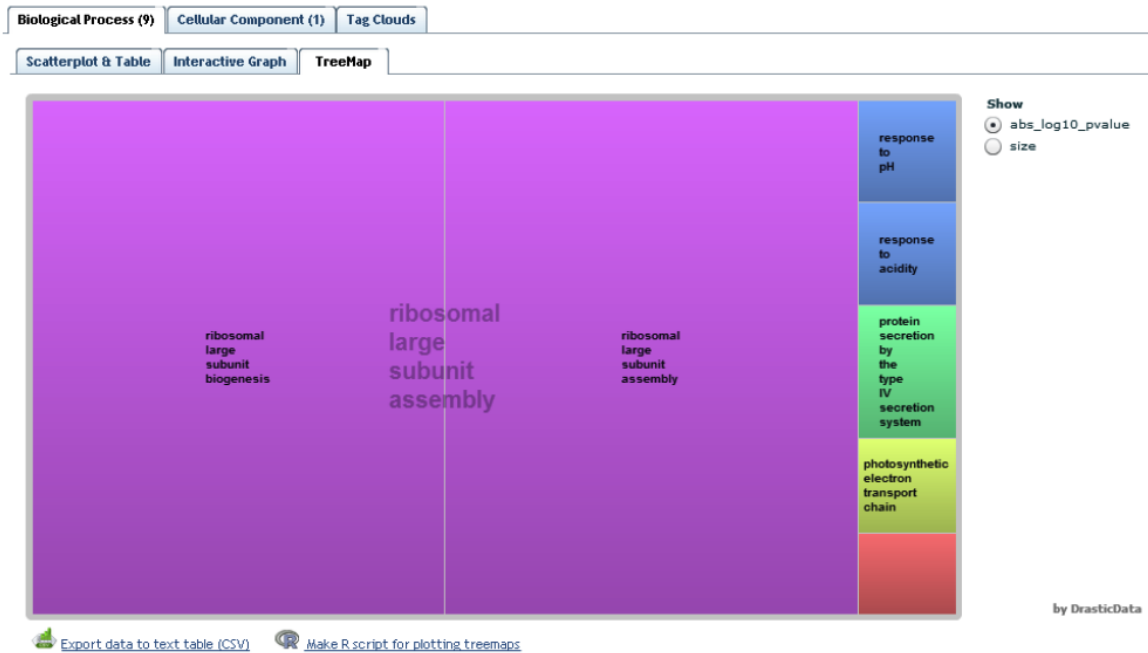**Figure 4:** view of Graph-based visualization [9].

Figure 5: Tree Maps view [9].

3. AmiGO

The open source based web application 'AmiGO' allows users to browsing query and visualizing ontologies and data related to gene product annotation [15]. AmiGO is open source software and free to download and installed. AmiGO is being maintained and developed by GO Consortium. Following is the brief functionalities of the AmiGO [16].
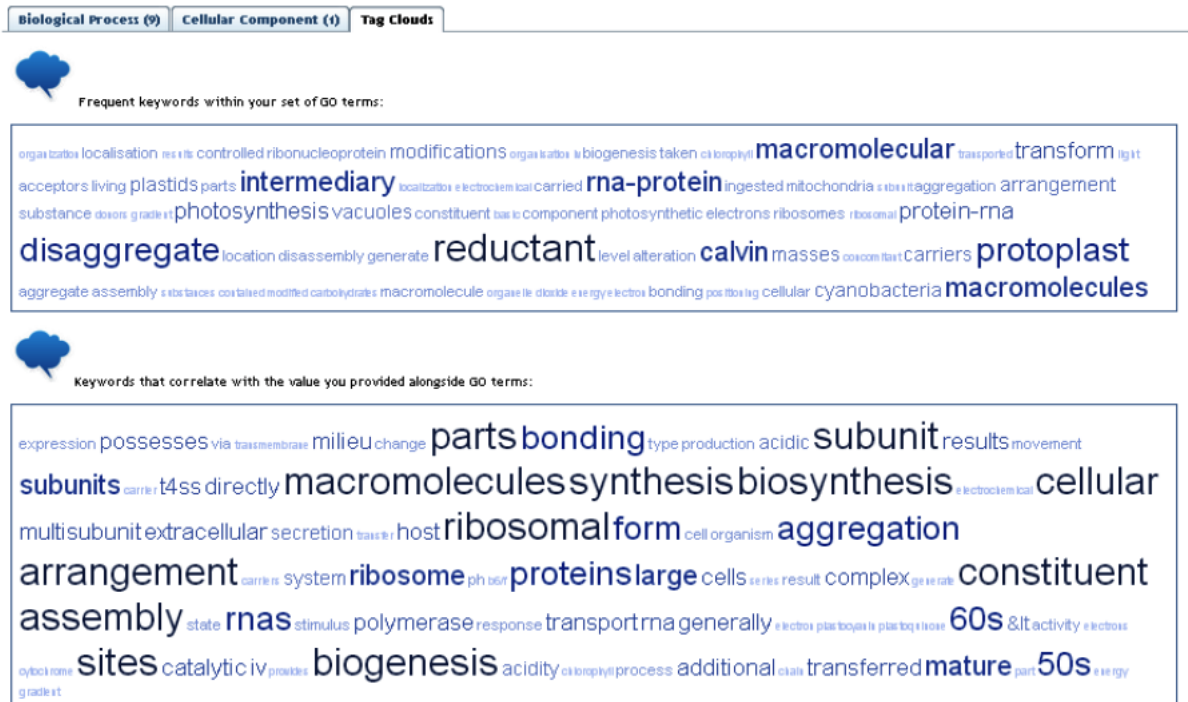
Figure 6: Tag clouds view [9].

Table 1:   Core AmiGO Functionalities

Functionalities	Details
search for GO terms [17]	view the genes or gene products they are annotated to
sequence identity BLAST search [18]	view the GO term associations for the genes or proteins returned
browse the GO ontology [19]	AmiGO presents the ontology as a tree structure; this view is available from the Browse link in the menu bar on each page, and in the Term Lineage section of the term details page.
slimmer tool [20]	can be used to map the granular annotations of the query set of genes to one or more high-level
term enrichment tool [21]	is used to discover what a set of genes may have in common by examining annotations and finding significant shared GO terms.
GOOSE [22]	is for advanced users who want to run custom SQL queries against the GO database.

3.1. Search For GO Terms

The toolbar to search for gene products or GO terms is available at the home page of AmiGO website. One can enter textin the query box and set the search type by selecting the terms or the gene or protein radio button. Protein kinase activity, DNA repair, or mitochondrion are examples of GO terms. And DMC1 or fuzzy onions is an example of genes or proteins.

Exact match checkbox is being used when someone know the name or gene product symbol orthe name or GO term ID. It returns the items which fully match the query.

Submit Query option is used to start the search.

3.2. Sequence Identity BLAST Search

The server of AmiGO BLAST uses the database of GO protein sequence to search the sequences, which consists of gene products and protein sequences of genes which are annotated to a GO term and put forwarded to the Consortium of GO. BLASTP is being used to search protein queries, while DNA sequences may be searched using BLASTX.

3.3. Browse the GO Ontology

The browser of AmiGO visualizes and navigates the terms in the Gene Ontology.

Figure 7: Interface of the AmiGO term search results [17].

Figure 8: View of BLAST search in AmiGO [18].

Tree View

In the form of tree structure, the AmiGO presents ontology; on each page, there is a Browse link where this tree structure view is available. Figure **9** shows a tree view of gene ontology.

The tree view structure represents the information related to GO terms. It also holds the relationships between different terms. To represent term parentage, indentation is used. The child term also known as subclasses are being indented with respect to parent terms. Same level terms are also indented which referred to as siblings.

Each and every line the view includes the GO term name and GO ID. When we click on the ID or name

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☐ 🔲 GO:0008150 : biological_process [166899 gene products] ⬏
    ⊞ 🔲 GO:0022610 : biological adhesion [1615 gene products]
    ⊞ 🔲 GO:0065007 : biological regulation [32432 gene products]
    ⊞ 🔲 GO:0001906 : cell killing [233 gene products]
    ⊞ 🔲 GO:0009987 : cellular process [79984 gene products]
    ⊞ 🔴 GO:0048519 : negative regulation of biological process [5976 gene products]
    ☐ 🔲 GO:0043473 : pigmentation [269 gene products] ⬏
        ⊞ 🔲 GO:0033059 : cellular pigmentation [64 gene products]
        ⊞ 🔲 GO:0033060 : ocellus pigmentation [20 gene products]
        ⊞ 🔲 GO:0043476 : pigment accumulation [13 gene products]
        ⊞ 🔵 GO:0043474 : pigment metabolic process during pigmentation [0 gene products]
        ⊞ 🔲 GO:0048066 : pigmentation during development [177 gene products]
    ⊞ 🟢 GO:0048518 : positive regulation of biological process [7630 gene products]
    ⊞ 🔴 GO:0050789 : regulation of biological process [30093 gene products]
    ─
```

Figure 9: An example of part of the GO tree under biological process [19].

then the Term details page will be opened which contain more detail information regarding the GO term.

Viewing Options

To save the created tree, links box is used to display the view and save the tree. With the use of GraphViz plugin, Graphical View display the tree as a graph, and it can be downloaded by the users.

Permalink provides a stable link to the tree. The tree downloading is also being entertained as GO RDF-XML or GO flat file format.

3.4. Slimmer Tool

The another tool is Slimmer tool which provide us more broader and high level parent term which referred as GO slim term for the granular annotations of the

Figure 10: View of slimmer tool [20].

gene query set. Because of the relationship of parent:child among GO slim term and granular term, this is possible with GO. This tool may also be helpful in presenting the results of genome GO annotations. Analysis of the results of microarray expression data, or cDNA collection is using a high level view of the three ontologies.

3.5. Term Enrichment Tool

This tool serves an important role to discover the gene that has common properties. Query/Input set for gene can also be described with the shared GO terms or parents of those GO terms. The GO-Terminder perl module is used in term enrichment tool written by Shuai Weng and Gavin Sherlockl.

3.6. GO Online SQL Environment (GOOSE)

This web based tool or utility provide sits user the facility to write and execute queries (SQL) in the GO database environment without any extra utility. GOOSE utility has numerous samples of queries to help new users. It also let the users to generate results as tab-delimited text or as a web page.

A COMPARISON OF SOME MODERN TOOLS

The comparison between the selected GO tools is summarized in Table **1**. The criteria used in these tables are described in detail in the following.

1. Visualization Capabilities

Visualization of bio-ontologies has been a challenge for a long time for the bioinformatics communities. Information associated with the ontology contains not only hierarchy of concepts. In fact there are also many relations exist between these concepts in a particular domain. Moreover, each and every concept in ontological based representation has also variety of other attributes which ranges from one to thousands of types. To thoroughly observes the data and derive the knowledge out of bio-ontologies, it is a need for biologist to effectively and efficiently visualize complete ontological information. To accommodate this need,

different GO tools have been developed with advanced visualizing capabilities. With strong visualizing capabilities in GO tools, it helps researchers to perform variety of operations on the ontology simultaneously.

We compare visualization capabilities of our selected tools on the basis of Indented List, Node-Link & Tree view, Zoomable and 3D. Indented List capability presents an ontology tree view structure of Windows Explorer like. This tree view structure dictate the *is-a* inheritance relationship of the ontology taxonomy. CGAP and AmiGO have this visualization capability whereas REVIGO has not. In a tree view and Node-Link group of techniques visualize the ontologies in the manner of nodes that are interconnected, displaying the classification (taxonomy) with a left to right or top–down layout [23]. The Users can open (expand) and close (retract) the nodes and sub trees. This expansion and retraction helps to avoid show clutter. It also sets the detail of showed information. Amongst our selected tools, only AmiGO has this capability. Our third parameter under the umbrella of visualization capability is Zoomable. This category (Zoomable) display nodes with all methods at the lower level hierarchy which are nested under nodes' parents. These methods provide the users to enlarge the child nodes with zoom-in, adjusting these nodes as the level of current viewing level. AmiGO and REVIGO has this capability and CGAP has not.

2. Data Source

Many of the available GO tools use data annotation from a single public database and some GO tools use several sources. There have different pros and cons of both approaches. The data source used by CGAP is available in ASCII format which can be downloaded from the directory ftp://ftp1.nci.nih.gov/pub/CGAP. In general CGAP databases named as dpGAP/Data Matrix and Mitelman database have two kinds of data, one is about human and other is about mouse [4].

Whereas the REVIGO uses EBI GOA database [24] as a data source for its finding the semantic similarities

Table 2:   Comparison Summary

Tools	Visualization Capabilities			Data Source
	Indented List	*Node-Link & Tree View*	*Zoomable*	
CGAP	✓			dpGAP/Data Matrix
REVIGO			✓	UniProt(Default)
AmiGO	✓	✓	✓	GO Database

amongst the GO terms. But users can optionally select 11 types of different data sources which are also subsets of GOA database. If some specific organism is not available in REVIGO then it uses UniProt database as a default data source. Moreover REVIGO has also facilitated their users with the integration capability between different servers and software.

In AmiGO, three different types of data sources are being used which are categorized as Mouse Genome Database (MGD), Saccharomyces Genome Database (SGD) and FlyBase (Drosophila). Furthermore data sources from microbial, plant and animal are also becoming parts of GO data sources.

For single database advantage, that is whenever the database is used, it is for all the time up to date. Whereas the disadvantage is that, there is not a single database currently provides us a complete picture [25]. The next level analysis as discussed here may be quite prevailing i.e. additional types of data integration in a coherent way. For this reason, as compare to any single database, an integration of different types of data with a devoted database annotation from multiple sources e.g. KEGG pathways becomes is more useful. But the downside of this kind of database will require frequent updation of source database every time and in fact difficult to establish such a database. In our conclusion, as AmiGO uses more varieties of sources as compared to REVIGO and CGAP, that's why AmiGO is more powerful GO tool in the science of gene ontology tools.

COMPARISON ANALYSIS

In many GO tools, we have evaluated that there are many diversities for representing biological data in different ways using different data sources. In fact all parameters that we consider in visualization capabilities are not necessarily needed to adopt in all GO tools, but Node-link & Tree View would probably intensify the visualization aspect of CGAP. Indented List and Node-Link & Tree View factors are strongly needed in REVIGO, because it helps investigator to understand and elaborate summarization in REVIGO. In AmiGO, all our visualization considerations are there and turn out to be current ultimate standard of GO tools. Another aspect of our comparative methodology is the data source, in which if all our selected tools provide combo list to select any data source would be more efficient and effective for researcher.

Moreover are markable facet of our selected tools except AmiGO, are in general help-systems poorly.

Even though help documents and manuals are exists, our selected tools must have suitably example based help in the system. As a tool of ontological engineering fields is some what new, a wide-range of help will be appeared which is the most potential element of the tools. In CGAP and REVIGO however, there is no detail help. AmiGO is the only tool providing all type of detail help about with useful examples. Different kinds of examples are another way of supporting users in understanding GO tools. If tools come with a guided tour, then it would be quite useful for new users.

CONCLUSION

In this paper we have evaluated CGAP, REVIGO and AmiGO for their use as modern web based tools in the field of Gene Ontology. We used comparative methodology as test case. No system is preferred in all situations. All systems haves their strengths and weaknesses. The main strengths of CGAP compared to the other systems that it is used to determine the gene expression profiles of normal, pre-cancer, and cancer cells and provide improved detection, diagnosis, and treatment for the patient. REVIGO main strengths are summarization the lists of Gene Ontology terms by removing redundant terms and visualizing the remaining ones in different interactive visual effects. AmiGO has one of the main advantages over the CGAP and REVIGO is that it has a BLAST tool, tools allowing analysis of larger data sets and a user friendly interface to query the GO database directly.

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