

Protocol

Use of the BioGRID Database for Analysis of Yeast Protein and Genetic Interactions

Rose Oughtred,¹ Andrew Chatr-aryamontri,² Bobby-Joe Breitkreutz,³ Christie S. Chang,¹ Jennifer M. Rust,¹ Chandra L. Theesfeld,¹ Sven Heinicke,¹ Ashton Breitkreutz,³ Daici Chen,² Jodi Hirschman,¹ Nadine Kolas,³ Michael S. Livstone,¹ Julie Nixon,⁴ Lara O'Donnell,³ Lindsay Ramage,⁴ Andrew Winter,⁴ Teresa Regulý,³ Adnane Sellam,² Chris Stark,³ Lorrie Boucher,³ Kara Dolinski,^{1,5} and Mike Tyers^{2,3,4,5}

¹Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, New Jersey 08544; ²Institute for Research in Immunology and Cancer, Université de Montréal, Montréal, Québec H3C 3J7, Canada; ³Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario M5G 1X5, Canada; ⁴School of Biological Sciences, University of Edinburgh, Edinburgh, EH9 3JR, United Kingdom

The BioGRID database is an extensive repository of curated genetic and protein interactions for the budding yeast *Saccharomyces cerevisiae*, the fission yeast *Schizosaccharomyces pombe*, and the yeast *Candida albicans* SC5314, as well as for several other model organisms and humans. This protocol describes how to use the BioGRID website to query genetic or protein interactions for any gene of interest, how to visualize the associated interactions using an embedded interactive network viewer, and how to download data files for either selected interactions or the entire BioGRID interaction data set.

Computer (Internet-connected with web browser)

BioGRID is compatible with all modern web browsers including Firefox, Chrome, Safari, and Internet Explorer, but is not compatible with Internet Explorer 6 and earlier versions.

METHOD

*BioGRID (Chatr-Aryamontri et al. 2013) is a member of the International Molecular Exchange (IMEx) consortium (<http://www.imexconsortium.org/>) (Orchard et al. 2012), which is a network of public databases that provide curated protein interaction data in a standardized Proteomics Standards Initiative Molecular Interaction (PSI-MI) format (<http://www.psdev.info/>). BioGRID is also a source of protein and genetic interaction data for the following model organism databases: *Saccharomyces* Genome Database (SGD) (<http://www.yeastgenome.org/>) (Cherry et al. 2012), FlyBase (<http://flybase.org/>) (Marygold et al. 2013), PomBase (<http://www.pombase.org/>) (Wood et al. 2012), TAIR (<http://www.arabidopsis.org/>) (Lamesch et al. 2012), and WormBase (<http://www.wormbase.org/>) (Yook et al. 2012).*

⁵Correspondence: md.tyers@umontreal.ca; kara@genomics.princeton.edu

Search Interactions for a Gene or Protein of Interest

This series of steps describes how to search BioGRID for interactions that involve a gene or protein of interest and to view additional details about interactions and associated posttranslational modifications.

1. Use any modern web browser, except Internet Explorer 6, to open the following URL: <http://www.thebiogrid.org>.
2. Locate the BioGRID search form in the upper right corner (Fig. 1), and search “By gene” or “By publication.” For example, to search for the gene *SWE1*, type “SWE1” into the search bar, select “*Saccharomyces cerevisiae*” from the organism pull-down menu, and then press the “SUBMIT GENE SEARCH” button.

The two search options (“By gene” and “By publication”) are available as vertical tabs on the right-hand side of the search form (Fig. 1). When searching for a specific protein or gene, the search can be restricted to an organism of interest, or performed on all 57 organisms simultaneously. Any of the following common types of search terms will be recognized by the gene search field:

- Gene symbols (e.g., *SWE1*), gene accession numbers from NCBI (e.g., 853252), or RefSeq (e.g., NM_001181620);
- Protein identifiers from UniProtKB (e.g., P32944) or NCBI RefSeq (e.g., NP_012348); and
- Systematic identifiers from the corresponding model organism database (e.g., YJL187C from the SGD).

When searching by publication, it is possible to search for a PubMed identifier or by keywords found in the title or abstract. Additional instructions are provided in the “Advanced Search” and “Search Tips” links (Fig. 1).

3. View the search results (i.e., the Result Summary page [Fig. 2A], which provides a summary of curated interactions for the gene(s) or protein(s) of interest).

FIGURE 1. Search in BioGRID for interactions of a gene or protein of interest. The BioGRID home page is shown with available search options in the upper right corner (arrow). In the top menu, links are also provided to the help document, online tools, BioGRID statistics, and download options. In this gene search example, “SWE1” is entered as the search term and “*Saccharomyces cerevisiae*” is selected as the organism.

- i. Locate at the top left of the page a brief description of the gene/protein; links to relevant external resources such as SGD, Entrez Gene, and UniProtKB; and an option to download associated interactions.
- ii. Locate the curated interaction statistics for the gene or protein of interest in the upper right “Stats & Options” panel.

The total number and kind of interactions are listed and are color-coded throughout the page in green for genetic interactions and in yellow for protein interactions. The interactions may also be filtered via the “Search Filters” option according to whether they are derived from low- versus high-throughput experimental studies. A link to a graphical viewer, which allows for visualization of the gene- and/or protein-specific interaction network, is also available and is described in more detail in Steps 7 and 8.

- iii. Further down on the page, locate the full interactors table that displays the list of unique interactors (see Fig. 2A, “Interactors View”). View the total number of independent evidence codes for genetic and protein interactions on the far right of the interactors table. Click on the “details” link to obtain information about each of the listed interaction partners.

This action reveals a detailed list of the different experimental evidence codes associated with each interaction, including the context of the interactor as either a bait or hit and whether the experimental evidence is from a low- or high-throughput study. The different types of interaction data curated by BioGRID are described in the “Experimental Evidence Codes” help document (http://wiki.thebiogrid.org/doku.php/experimental_systems). Pointing to specific icons in the right-hand “Notes” column reveals further applicable details. These include the following:

- Tag icon: phenotypes curated by BioGRID using the Ascomycete Phenotype Ontology (APO) developed by SGD (Engel et al. 2010);
- Globe icon: posttranslational modifications associated with the *in vitro* “Biochemical Activity” experiment type, most commonly phosphorylation or ubiquitination;
- Histogram icon: quantitative scores published in the original low-throughput study or high-throughput data set, when available; and
- Tablet icon: additional notes provided by BioGRID curators to explain experimental details or significance scores.

4. Use the “Switch View” bar at the top of the full interactors table to navigate from the “Interactors” view to an “Interactions Table” (as shown for *SWE1* in Fig. 2A). Click on the column headers to sort by interactor, role (bait/hit), organism, experimental evidence code, data set publication, throughput (high/low), or interaction scores provided in the original publications.
5. Click on the “PTM Sites” button on the “Switch View” bar to access the Posttranslational Modification (PTM) viewer (Fig. 2B).

- i. View the phosphorylation sites in the protein sequence (in red; e.g., Fig. 2B, arrow b). Click on a modified residue in the protein sequence to display links to publications with supporting evidence.
- ii. Locate the table immediately below the protein sequence, which lists the individual residues and their phosphorylation sites (Fig. 2B, arrow c) along with the interacting proteins and their relationship (e.g., kinase or phosphatase), as applicable.

Additional notes associated with each phosphosite are provided in the right hand “Notes” column under a tablet icon.

- iii. Scroll down to the separate “Relationship Details” table (Fig. 2B, arrow d), which provides information about the enzymes involved in the PTMs, identifying whether each protein listed in the left column phosphorylates or dephosphorylates the query protein of interest.

*The BioGRID Posttranslational Modification (PTM) viewer integrates more than 20,100 phosphorylation sites on approximately 3200 yeast proteins, curated by PhosphoGRID (version 2.0; www.phosphogrid.org) from both low- and high-throughput *S. cerevisiae* studies (Sadowski et al. 2013). More than 2500 of these phosphosites are associated with specific kinases and/or phosphatases, and more than 3200 of the sites occur on 1100 proteins under specific environmental conditions. The PTM viewer has been expanded to include sites of ubiquitin modification and will include other modifications in the future.*

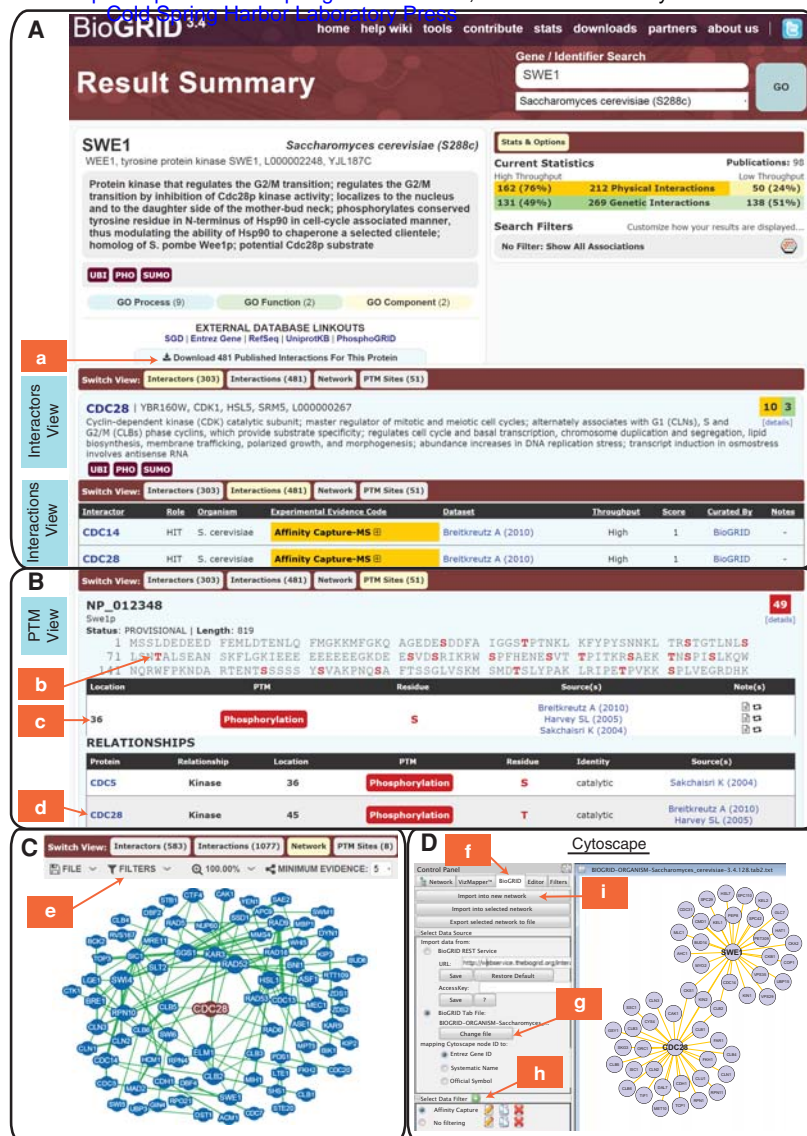


FIGURE 2. (A) Result Summary page that displays information for the query gene or protein. Results for the query gene *SWE1* are shown. The Interactors View displays unique interactors for the query gene or protein, sorted from highest to lowest number of curated interactions. The Interactions View allows results to be ordered based on interactor, role (bait/hit), organism, experimental evidence code, data set publication, throughput (high/low), or interaction scores given in the original publications. Genetic and protein interactions are color-coded in green and yellow, respectively, throughout the display. Filtering options in the *top right* "Stats & Options" box may be used to sort the table based on high- or low-throughput studies. Genetic and protein interactions for the query gene can be downloaded in all supported BioGRID download formats by clicking "Download Published Interactions for This Protein" (arrow a). (B) Integrated posttranslational modification (PTM) viewer within BioGRID. The *Swe1* protein sequence is displayed with documented phosphorylation sites shown in red (arrow b). The table below the sequence shows additional details for each phosphosite, including the corresponding enzyme and its relationship to the query protein (arrow c). The relationship table provides further details, such as whether the interactor in the *left* column phosphorylates or dephosphorylates the main query protein (arrow d). The PTM viewer initially incorporated phosphosites curated by PhosphoGRID, but has also been expanded to include other types of PTMs, such as ubiquitination sites, also as curated by BioGRID. (C) Interaction network viewer within BioGRID. A filtered view is shown for interactions of *CDC28*, which is located at the center of the viewer. Larger-diameter nodes have a greater number of connections, and thicker edges are supported by more experimental evidence. All nodes can be dragged by the user to optimize the layout. Filters are applied by choosing the "FILTERS" option in the top menu of the graphic (arrow e). In this instance, interactions have been filtered to show only genetic interactions. The MINIMUM EVIDENCE option allows edges to be shown or hidden based on the number of curated interactions for that gene/protein pair. The higher the number, the more evidence in support of that interaction. The network also shows secondary interactor interactions, with the same level of minimum evidence. Clicking on each edge in the network reveals experimental evidence related to the interacting pairs. The network may also be exported as a graphic file using the FILE option in the top menu. (D) Use of Cytoscape with the BiogridPlugin2.2. After copying the BiogridPlugin2.2.jar into the plugins directory of Cytoscape, select the "BioGRID" tab in the control panel (arrow f). In the "Import data from" window select the "BioGRID Tab File" and choose the tab2 file of interest (arrow g). In the "Select Data Filter" window, click on the green plus symbol (arrow h). After setting up the filter described in Step 15 in the text, click the "Import into new network" button to view the interaction network (arrow i).

6. Navigate to another gene of interest in BioGRID by clicking on any gene name link listed in one of the table views. For example, in the “PTM sites” view, click on any interacting gene listed on the left-hand side of the “Relationships” table (e.g., CDC28; Fig. 2B, arrow d) to go to the “Result Summary” page for that particular gene.

Interaction Network Visualization

An interactive network viewer is embedded within the BioGRID interface to allow users to immediately visualize interactions of interest. This new Javascript-based BioGRID visualization tool was built using the Cytoscape.js platform (Shannon et al. 2003). The viewer shows the interaction network of the gene of interest based on its direct genetic and/or protein interaction partners. In addition to BioGRID’s integrated network viewer linked from each gene page, selected interaction data may be downloaded in formats compatible with either the Cytoscape (<http://www.cytoscape.org/>) (Shannon et al. 2003; Cline et al. 2007) or Osprey (Breitkreutz et al. 2003; Stark et al. 2006) network visualization systems. These formats include the BioGRID TAB 2.0 delimited file format for Cytoscape, and an Osprey Custom Network File format for the Osprey visualization system. A BioGRID Cytoscape plugin 2.0 (<http://wiki.thebiogrid.org/doku.php/biogridplugin2>) is available to facilitate the import of BioGRID interaction data into Cytoscape to visualize interaction networks and integrate interactions with gene expression profiles (Winter et al. 2011). GeneMania (<http://www.genemania.org/>) is also compatible with BioGRID formats (Montejo et al. 2010; Zuberi et al. 2013). This next series of steps describes how to visualize a local network of interactions in the BioGRID viewer and in Cytoscape.

BioGRID Viewer

7. Click on the “Network” link in the “Switch View” bar of any gene page (e.g., CDC28).

This action opens up a network view that graphically displays the interaction network around the query gene. The query gene is represented by a node at the center of the layout, and the interacting genes are displayed as associated nodes with each line (edge) between a pair of genes representing interactions (Fig. 2C). Greater node size represents increased connectivity, and thicker edge sizes represent increased evidence supporting the interaction.

8. Display the desired information about the gene of interest as follows.

- i. Filter the network display via options provided in the top “FILTERS” menu option (Fig. 2C, arrow e).

Pairwise interactions may be restricted to either physical interactions (yellow edges) or genetic interactions (green edges), or both may be displayed. Any interacting pairs associated with both genetic and physical evidence are linked by purple edges.

- ii. Limit the number of displayed interactions via the “MINIMUM EVIDENCE” option in the upper menu.

Selecting a number allows edges to be shown or hidden based on the number of curated interactions associated with the gene/protein pair. The higher the selected number, the more evidence is associated with that interaction. In the example given in Fig. 2C, the displayed interactions for CDC28 have been restricted to genetic interactions that are associated with at least five unique curated interactions (minimum evidence = 5). This network includes secondary interactor interactions that are also associated with the same level of minimum evidence.

- iii. Click on any edge (e.g., between CLB5 and CDC28) to view the “Association Details” between interacting genes/proteins.

This opens up a window with “Association Details” between the two interactors including relevant experimental evidence codes and publications.

Cytoscape

9. Download BioGRID interaction data in tab2 format as follows.

- i. Visit the BioGRID download page (<http://thebiogrid.org/download.php>).
- ii. Click on “Current Release,” and then click on the “BIOGRID-ORGANISM-(VERSION NUMBER).tab2.zip” to begin downloading the available interaction data, which will be broken into distinct files by organism.

- iii. Open the zip file and locate “BIOGRID-ORGANISM-Saccharomyces_cerevisiae-(VERSION NUMBER).tab2.txt”.

This file contains all of the budding yeast interactions from BioGRID and will be used in this example.

10. Download a copy of Cytoscape version 2.8.2 from the following URL: <http://www.cytoscape.org/download.html>.

Note that the BioGRID Cytoscape plugin is not currently compatible with version 3.x of Cytoscape but will be updated in the future.

11. After installing Cytoscape, download the BiogridPlugin version 2.2 located in the folder “BiogridPlugin2” within “Cytoscape Plugins” on the BioGRID download page (<http://thebiogrid.org/download.php>).

12. Copy the downloaded file, “BiogridPlugin2.2.jar,” into the plugins directory of Cytoscape, located in the folder in which Cytoscape is installed.

Typically this will be located in “C:\Program Files\Cytoscape_v2.8.2\plugins” on Windows-based computers, or “/Applications/Cytoscape_v.2.8.2/plugins” on a Mac.

13. Launch Cytoscape and select the “BioGRID” tab in the control panel (Fig. 2D, arrow f) for a list of input variables for the BioGRID plugin.

14. In the “Import data from:” window (Fig. 2D, arrow g) select the “BioGRID Tab File:” option box and change the file to the one that was downloaded in Step 9.iii.

15. Filter interaction data using the plugin. As an example, set up a filter to show only Affinity Capture–MS interactions for the budding yeast proteins CDC28 and SWE1.

- i. Bring up the filter window by clicking on the green and white plus symbol just to the right of the “Select Data Filter” window (Fig. 2D, arrow h).
- ii. Assign the filter a name: In this example use “Affinity Capture-MS Filter.”
- iii. In the “Filter by gene” window, add the protein names CDC28 and SWE1, make sure the drop down is set to “Include only,” and check the box for “and their primary interactors.”
- iv. In the “Filter by evidence code (include checked)” window, select the experimental system “Affinity Capture-MS” (Fig. 2D, arrow h). Remove any genetic evidence codes.
- v. Click “OK” to save this filter.

16. Once the filter is setup, click the “Import in new network” button (Fig. 2D, arrow i), which will produce an interaction network containing the query genes of interest and their interactors.

17. To better visualize the network, select one of the built-in layouts in Cytoscape. Navigate to the “Layout” menu at the top and select “Cytoscape Layouts → Forced-Directed Layout → (unweighted).”

Cytoscape will generate a network display with each query gene at the center of its set of interactors (Fig. 2D).

Download Options and Formats

Interaction data in BioGRID are updated on a monthly basis and may be downloaded for each query gene or protein, or by publication, in various formats.

18. Obtain interactions for individual genes or publications as follows.

For a Single Query Gene

- i. Click on the “Download Published Interactions for this Protein” button on any gene page above the “Switch View” option (Fig. 2A, arrow a).
- ii. When the “BioGRID Downloads” page has opened, select the desired file format.

Documentation is available at <http://wiki.thebiogrid.org/doku.php/downloads>. The BioGRID Tab 2.0 format is the most commonly downloaded tab-delimited text format and is provided as the default option. Other formats include the IMEx-compatible PSI-MI XML or MITAB formats.

- iii. Click the “Download Interactions” button to obtain the requested file.

Based on Publication

- i. Search BioGRID using the “By Publication” option (see Step 2).

Entering any PubMed ID returns the publication of interest.

- ii. Click on the “Download Interactions For This Publication” option below the abstract.

19. Obtain the complete collection of curated interactions as follows.

- i. Click on the “Downloads” menu option at the top right of any BioGRID page to go to the main download page (<http://thebiogrid.org/download.php>).

- ii. Select the “Current Release” folder to expand a list of downloadable file formats.

A description of the different formats is available in the right hand panel of the page.

- iii. Download the interaction data based on organism type or experimental system and in the desired format.

The entire collection is available in various formats, including the BioGRID Tab 2.0 format, and the IMEx-compatible PSI-MI XML or MITAB formats.

The source code for BioGRID is also available without any restrictions (http://wiki.thebiogrid.org/doku.php/development_overview).

20. Build a specific data set based on a subset of genes, proteins, or publications as follows.

- i. Click on the “custom download generator” link toward the bottom of the main download page to open up the BioGRID Download Dataset Generator page (<http://thebiogrid.org/downloadgenerator.php>).

- ii. Select the PublicationID(s) or Gene Identifier(s) option.

The former is for PubMed IDs, and the latter is for official gene symbols, systematic names, and external database IDs. Identifiers that may be used are listed at <http://wiki.thebiogrid.org/doku.php/identifiers>. Up to five PubMed IDs or ten Gene IDs may be entered per search.

- iii. If desired, refine the search by selecting an organism of interest.

- iv. Choose an output file format.

- v. Click the “Build Download Dataset” button to obtain the specific interaction data set.

21. (Optional) Retrieve interaction data in BioGRID automatically via the BioGRID REST service.

Details regarding the REST service are documented on a wiki page (<http://wiki.thebiogrid.org/doku.php/biogridrest>) and are also published (Winter et al. 2011). The documentation includes information on how to obtain a unique access key, which is required for all queries to the web service, and how to fetch and filter interactions for a single query gene. Several examples are given along with a table listing the complete set of query parameters that may be used. A formal description of the REST service is also provided in Web Application Description Language (WADL) at <http://webservice.thebiogrid.org/application.wadl>. BioGRID is also part of the PSICQUIC project (<https://code.google.com/p/psicquic/>) (del-Toro et al. 2013), which is part of the HUPO Proteomics Standard Initiative (HUPO-PSI) to standardize automated access to molecular interaction databases by providing a standard web service and common Molecular Interactions Query Language (MIQL).

Community and User Feedback

22. Contact the BioGRID team at biogrid.admin@gmail.org as needed.

The BioGRID values feedback from the yeast research community and other users to correct curation errors, extend interaction coverage, request new features, or support and/or contribute published or prepublication interaction data. The BioGRID programming team will assist with extraction of data features, resolution of data discrepancies, and links to BioGRID pages or the REST service. The BioGRID curation team will correct any reported errors in the subsequent monthly release and assist with confidential upload of new data sets to coincide with publication date. Finally, BioGRID welcomes collaborators with an interest in contributing domain expertise or curation capacity. Further details can be found at <http://wiki.thebiogrid.org/doku.php/contribute>.

RELATED INFORMATION

For background on the BioGRID database, see Introduction: **BioGRID: A Resource for Studying Biological Interactions in Yeast** (Oughtred et al. 2015).

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