Bioinformatics Tools for Proteomics Data Interpretation

16

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Abstract

Biological systems function via intricate cellular processes and networks in which RNAs, metabolites, proteins and other cellular compounds have a precise role and are exquisitely regulated (Kumar and Mann, FEBS Lett 583(11):1703–1712, 2009). The development of high-throughput technologies, such as the Next Generation DNA Sequencing (NGS) and DNA microarrays for sequencing genomes or metagenomes, have triggered a dramatic increase in the last few years in the amount of information stored in the GenBank and UniProt Knowledgebase (UniProtKB). GenBank release 210, reported in October 2015, contains 202,237,081,559 nucleotides corresponding to 188,372,017 sequences, whilst there are only 1,222,635,267,498 nucleotides corresponding to 309,198,943 sequences from Whole Genome Shotgun (WGS) projects. In the case of UniProKB/Swiss-Prot, release 2015_12 (December 9, 2015) contains 196,219,159 amino acids that correspond to 550,116 entries. Meanwhile, UniProtKB/TrEMBL (release 2015_12 of December 9 2015) contains 1,838,851,8871 amino acids corresponding to 555,270,679 entries. Proteomics has also improved our knowledge of proteins that are being expressed in cells at a certain time of the cell cycle. It has also allowed the identification of molecules forming part of multiprotein complexes and an increasing number of posttranslational modifications (PTMs) that are present in proteins, as well as the variants of proteins expressed.

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Proteomics data interpretation • Interactome mapping • Gene Ontology • STRING • MINT • IntAct • HPRD • BioGRID • PIPs • MPIDB • TAIR • PANTHER • DAVID • KEGG • IPA

Biological systems function via intricate cellular processes and networks in which RNAs, metabolites, proteins and other cellular compounds have a precise role and are exquisitely regulated [1]. The development of high-throughput technologies, such as the Next Generation DNA Sequencing (NGS) and DNA microarrays for sequencing genomes or metagenomes, have triggered a dramatic increase in the last few years in the amount of information stored in the GenBank and UniProt Knowledgebase (UniProtKB). GenBank release 210, reported in October 2015, contains 202,237,081,559 nucleotides corresponding to 188,372,017 sequences, whilst there are only 1,222,635,267,498 nucleotides corresponding to 309,198,943 sequences from Whole Genome Shotgun (WGS) projects. In the case of UniProKB/Swiss-Prot, release 2015_12 (December 9, 2015) contains 196,219,159 amino acids that correspond to 550,116 entries. Meanwhile, UniProtKB/TrEMBL (release 2015_12 of December 9 2015) contains 1,838,851,8871 amino acids corresponding to 555,270,679 entries. Proteomics has also improved our knowledge of proteins that are being expressed in cells at a certain time of the cell cycle. It has also allowed the identification of molecules forming part of multiprotein complexes and an increasing number of post-translational modifications (PTMs) that are present in proteins, as well as the variants of proteins expressed.

Considering that human cells contain between 20,000 and 30,000 protein-encoding genes and possibility that there could be approximately four alternative splice variants for each gene [2], the total number of proteins that could be expressed at a certain time would range between 80,000 and 120,000. Moreover, guessing four PTMs in each protein, then, the total number of proteins in a cell would range between 320,000 and 480,000. However, when we consider the more than 400 different PTMs that have been found [3] the number of proteins in a cell would easily grow to more than one million.

Proteins do not function alone; they usually carry their function by interacting with one or more partners. The main goal of the protein-protein interaction map is to catalogue interactions and to define the interactome. These interactions are currently determined using a vast array of technologies, including yeast two hybrid systems, tag-fusion proteins for the identification of interacting proteins, co-immunoprecipitation, chemical crosslinking, phage display, FRET (Fluorescence Resonance Energy Transfer), SPR (Surface Plasmon Resonance), tandem affinity purification, protein microarrays, protein domains, etc. Many of these techniques, if not all, use mass spectrometry and non-redundant gene and protein databases as the main tools for the identification of peptides and proteins. Many of the cellular protein-protein interaction networks have been catalogued and a number of interactome databases have been established. There are several protein-protein interaction databases freely available via World Wide Web that can be used to determine the putative functions of a protein based on its direct or indirect interactions. Protein-protein interaction maps in these databases are, in general, based on the information published, mostly in PubMed. In this section, we describe some of the most important databases available, including STRING, MINT, IntAct, HPRD, BioGRID, PIPs, MPIDB and TAIR. Furthermore, additional tools such as

Gene Ontology, PANTHER, DAVID, KEGG, and IPA, among others, have been developed to facilitate data mapping into these databases. We are certain that these tools will be useful in understanding the intricate interactions and functions of proteins in cells.

16.1 Gene Ontology

Many proteins are conserved through evolution and consequently share the same functions. However, the systems of nomenclature for genes and proteins stay divergent despite repeated evaluation of gene similarities by experts [4]. In order to tackle this challenge, the Gene Ontology (GO) consortium was created. The aim of the GO project is to provide a structured vocabulary to define specific biological domains that describe gene products in different organisms [5]. GO project began in 1998 as a collaborative effort between three organism databases: FlyBase (*Drosophila*), the Mouse Genome Informatics (MIG) project and the *Saccharomyces* Genome Database (SGD). The GO Consortium has been continuously growing due to the deposition of several animal, microbial and plant genome databases [6], as well as the recent addition of ontology areas, such as cell cycle and cilia-related terms, as well as multicellular organism processes [7]. By using these ontologies, it is possible to graph structures that comprise cellular components, molecular functions, biological processes, and the relationships between them in a species-independent manner [7]. In other words, GO is divided in two modules, the ontologies, called GO ontology, which includes defined terms and their relationships, and the GO annotations, which covers gene products and defined terms [8]. The GO annotation is generated either by a curator or automatically through predictive methods (95 % by this method).

The gene ontology relationships are developed like a tree, depicting a hierarchy from more general terms to more specific ones. Terms are linked by three possible relationships: "is_a", "part_of", and "positively regulates/negatively regulates". The "is_a" is a simple relationship between a class and a subclass. The "part_of" relationship is more complex than the former. C is part of D means that whenever C is present, it always belongs to D; for instance, an organelle (C) is always part of a cell (D), but not all cells have the same organelles. In the GO website (http://geneontology.org), a variety of browsers provide visualization and query capabilities for GO. For example, the AMIGO browser provides a web interface for searching and displaying ontologies, term definitions and associated annotated gene products for diverse organism databases [6]. The GO Online SQL (Structured Query Language) Environment (GOOSE) for AmiGO 2, allows users to freely enter SQL queries in the GO database. On the other hand, the PANTHER Classification System, that is further described next, provides enrichment analysis tools for GO.

16.2 PANTHER

PANTHER (Protein ANalysis THrough Evolutionary Relationships) is a classification system that combines ontology, gene function, pathways and statistical tools. This classification system can analyze sequencing, gene expression, and proteomics data [9]. PANTHER is a large database of gene families developed as a resource for family and subfamily classification of proteins [10]. PANTHER has two main components: PANTHER library (PANTHER/LIB) and PANTHER index (PANTHER/X). PANTHER library is a collection of protein families and subfamilies represented as phylogenetic trees assembled using Hidden Markov statistical models (HMMs) and a multiple sequence alignment algorithm (MSA) (Fig. 16.1a) [9–12]. PANTHER index is a set of ontological abbreviated terms that describe the function of proteins in biological processes or molecular functions [10–12]. In addition,

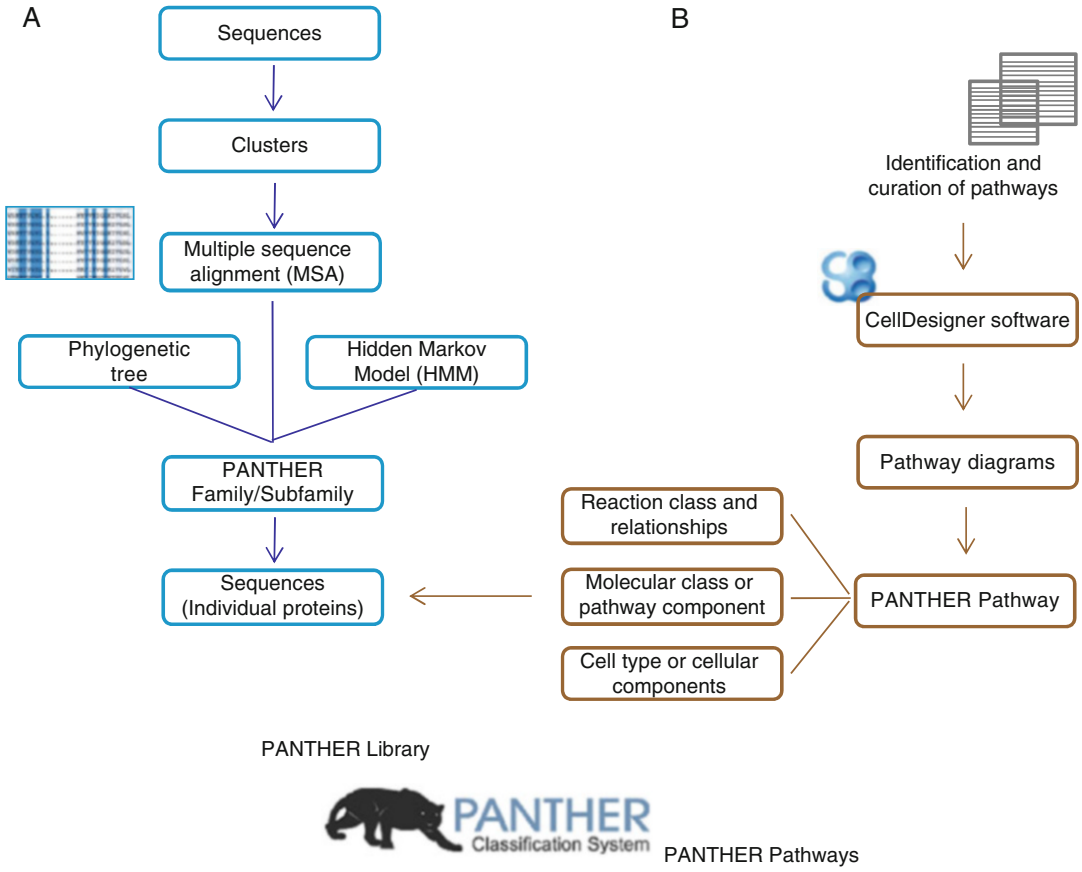


Fig. 16.1 PANTHER data overview. PANTHER has two main modules: (**a**) PANTHER Library which is a collection of families and subfamilies of proteins. This library is constructed from a selection of sequences built into clusters. These clusters are then used to generate multiple sequence alignments (MSA), phylogenetic trees, and statistical HMMs. (**b**) PANTHER Pathways are built using literature databases related to pathway components or a particular molecular class. Then, pathways are drawn and curated by expert curators using the CellDesigner software. Pathways are built based on molecular class or pathway component, reaction class and relationships, and cell type or cellular components. The pathway component is a link between various PANTHER modules

PANTHER has a Pathway module, in which the pathways are represented as a diagram generated with CellDesigner software (Fig. 16.1b) [13]. This module uses a defined vocabulary to describe pathways and their components, including pathway class and components, molecular class, reaction class, reaction relationships, cell type, and cellular components [14, 15]. PANTHER pathways are related to protein sequences in the PANTHER/LIB and, therefore, are also connected with families/subfamilies and HMM analysis (Fig. 16.1) [9, 10, 12]. Pathways are created and annotated by expert curators, according to evidence found in the literature. Moreover, pathways can be curated with the Pathway curation software (http://curation.pantherdb.org/) [14, 15]. Some of the pathways included in the PANTHER database are Cell cycle, DNA replication, General transcription regulation, Glycolysis, Tricarboxylic acid cycle, among others (http://www.pantherdb.org/pathway/

pathwayList.jsp). The PANTHER database contains the following information:

1. Genes (104 genomes; 1,424,953 total genes; 1,026,421 genes in PANTHER families with phylogenetic trees, MSA and HMMs)
2. Families (11,928 families and 83,190 subfamilies)
3. Pathways (177 pathways, 3092 pathway components, 2447 sequences related to pathways, and 2447 references captured for the pathways)
4. Ontologies (550 terms in PANTHER GO slim, 257 terms corresponding to biological process, 70 cellular components, and 223 molecular functions; 243 terms of protein class; 41,603 terms used in GO database annotations, including 9942 molecular functions, 27,852 biological processes, and 3809 cellular component terms (http://www.pantherdb.org/data).

The main window in PANTHER is composed of two main toolbars. The first one contains different links to individual topics (Fig. 16.2, items *1–5*), as well as an option for registration, login and contact (Fig. 16.2, items *6–8*). The second toolbar contains different options for data analysis, including gene list analysis, browse, sequence search, cSNP scoring, and keyword

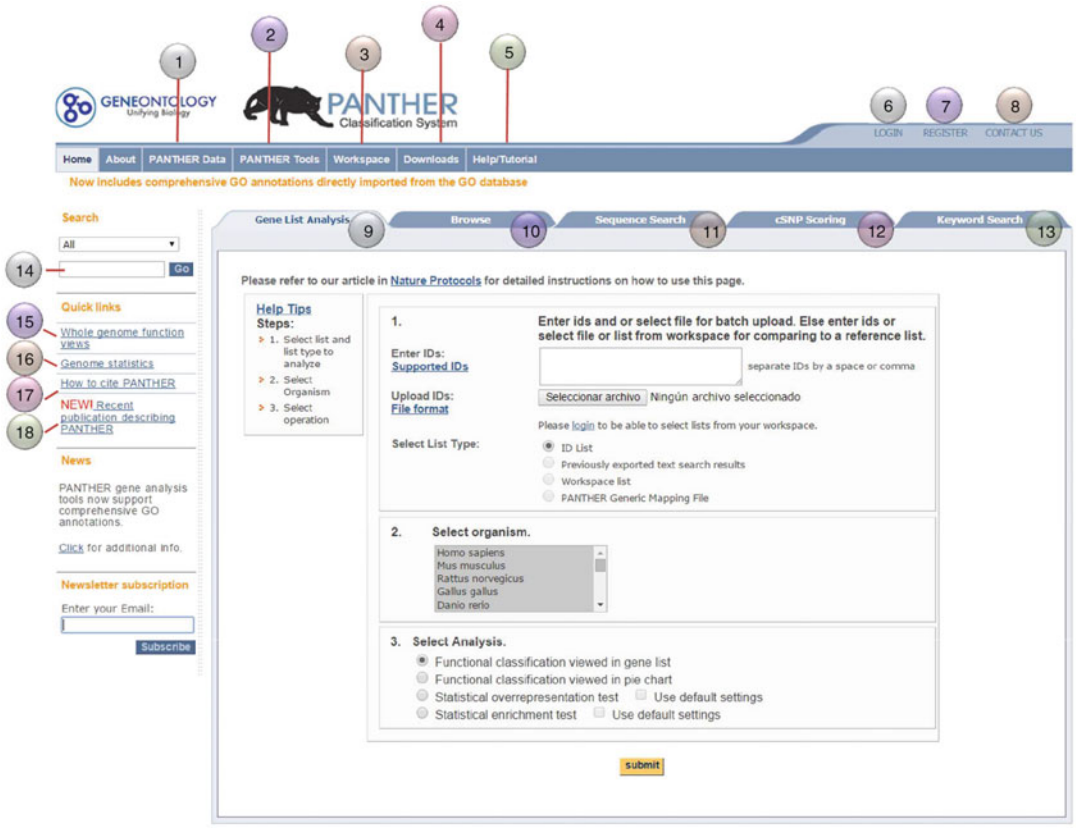


Fig. 16.2 PANTHER Classification System website. The main window in PANTHER contains two main toolbars. The first toolbar on top has links to different options incldluing: (*1*) PANTHER data, (*2*) PANTHER tools, (*3*) workspace, (*4*) downloads and (5) help/tutorial, and a section for (*6*) registration, (*7*) login, and (*8*) contact. The second toolbar, right under the first one, is for data analysis: (*9*) Gene list analysis, (*10*) browse, (*11*) sequence search, (*12*) cSNP scoping, and (*13*) keyword search. PANTHER also includes: (*14*) Quick keyword search, (*15*) whole genome function views, (*16*) genome statistics, (*17*) publications, and (*18*) recent publications describing PANTHER [16]

search (Fig. 16.2, items *9–13*). In addition, PANTHER has a panel for keyword search and quick links (Fig. 16.2, items *14–18*) [16]. In the analysis of list of genes or proteins, different functional classification views can be obtained, including gene list, bar or pie charts. Also, genes or proteins can be statistically analyzed through an enrichment test or a statistical overrepresentation test [17]. The PANTHER Ontology Browser also called PANTHER Prowler, browses and retrieves results (e.g. molecular functions, biological process, cellular component, protein class, pathway, and species) for input data related to ontology terms, such as genes and families [11, 17]. The PANTHER HMM sequence-scoring (sequence search) tool, can be used to search and compare protein sequences with the HMMs of PANTHER library. The top hit HMM can be observed in the results page, which also contains a statistical value for significance [17]. The Evolutionary Analysis of Coding SNPS (cSNP scoring) tool estimates the probability of a specific amino-acid change [17]. The keyword search tool can be used to obtain a variety of information, such as genes, families, pathways, and ontology terms for the protein of interest. However, we will focus on the generation of graphs for proteins classified in different categories.

16.3 PANTHER Gene List Analysis

To perform a gene list analysis using the PANTHER website (http://pantherdb.org), go to the toolbar gene list analysis (Fig. 16.3) and enter the

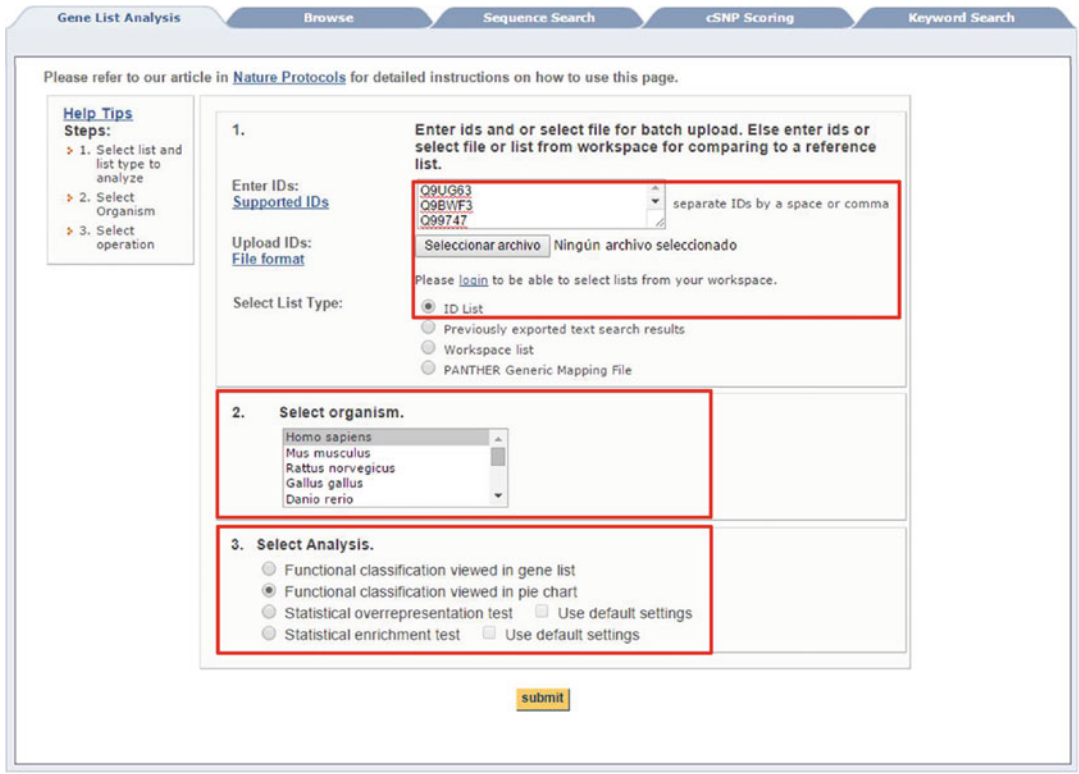


Fig. 16.3 Procedure to perform gene list analysis in PANTHER. The *red* section denotes the three primordial steps: (1) Enter the IDs of proteins to be analyzed, (2) select the organism, and (3) select the type of analysis to be performed

IDs of the genes or proteins in your list (Ensembl, Ensembl_PRO, Ensembl_TRS, Gene ID, Gene symbol, GI, HGNC, IPI, UniGene, UniProtKB ID) into the window, separating IDs by a space or comma. IDs can also be uploaded as a txt file. Then select the list type for query data (i.e. ID List, Previously exported gene list, Workspace list or PANTHER Generic Mapping File) and the organism of interest for analysis. In our example, we selected "ID list" and "*Homo sapiens*". Afterward, choose the type of analysis you like to perform. For example, we selected the "functional classification" viewed as a pie chart. Finally, click on the submit key (Fig. 16.3). In the results webpage, genes can be classified according to Molecular Function, Biological Process, Cellular Component, Protein Class, and Pathway (Fig. 16.4a). The chart obtained for a certain process can change for other processes. In addition, pie charts can be changed to bar charts and vice versa (Fig. 16.4b). The list of genes obtained in each ontological classification can be exported as a txt file. Classification categories may also contain different subcategories. When the cursor is located over a category in a chart, a message containing the following information will be displayed: Category name and its corresponding identifier, number of genes included from your list, the corresponding percentage of gene hits against the total number of identified genes, and the percentage of gene hits against the total number function hits (Fig. 16.4a). When a subcategory is selected, the corresponding gene list will be displayed (Fig. 16.5). As an example, we classified a list of overexpressed proteins in common between Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cells lines, which were recently described by Calderón-González et al. [18]. These proteins were categorized into Molecular functions and Cellular components (Fig. 16.4). In the first category, the most representative processes were: Binding and Catalytic activity with 25 and 21 genes, respectively (Figs. 16.4a and 16.5a). For Cellular component classification, categories with the higher number of genes were: Cell part (14 genes) and Macromolecular complex (10 genes) (Fig. 16.4b).

16.4 DAVID

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) was developed in 2003 to address the emerging challenges posed by the post-genomic era [19]. DAVID, as well as other tools for the analysis of large gene lists, is based on the principle of gene enrichment that are functionally related to an altered gene/protein (generated by high throughput technologies). These enriched genes might potentially cooperate within a determined group and/or biological process [20]. DAVID is composed of the DAVID knowledgebase and five annotation tools:

1. DAVID Functional Annotation
2. DAVID Gene Functional Classification
3. DAVID Gene ID Conversion
4. DAVID Gene Name Viewer
5. NIAID Pathogen Annotation Browser.

The DAVID Knowledgebase is constructed around the "DAVID Gene Concept", which include tens of millions of gene/protein identifiers from several major public databases. This data concentration eliminates annotation redundancy among different resources and allows the organization of gene identifiers into more than 40 functional classification categories, e.g. Ontology (more than 40 million records), Protein-protein interactions (more than four millions), Disease gene associations (9000), Pathways (above 50,000), Functional categories (more than 6.9 millions), etc. [21].

DAVID Gene Functional Classification: This tool is useful for the exploration of large lists of genes into more feasible modules ordered according to their functional relationships. These functionally organized modules are very useful in processing large amounts of information, switching from a gene centric analysis to a module-centric analysis [21].

DAVID Functional Annotation Tool Suite: The Functional Annotation Tool Suite displays three ways for combining results: Functional Annotation Clustering, Functional Annotation Chart and Functional Annotation Table. The

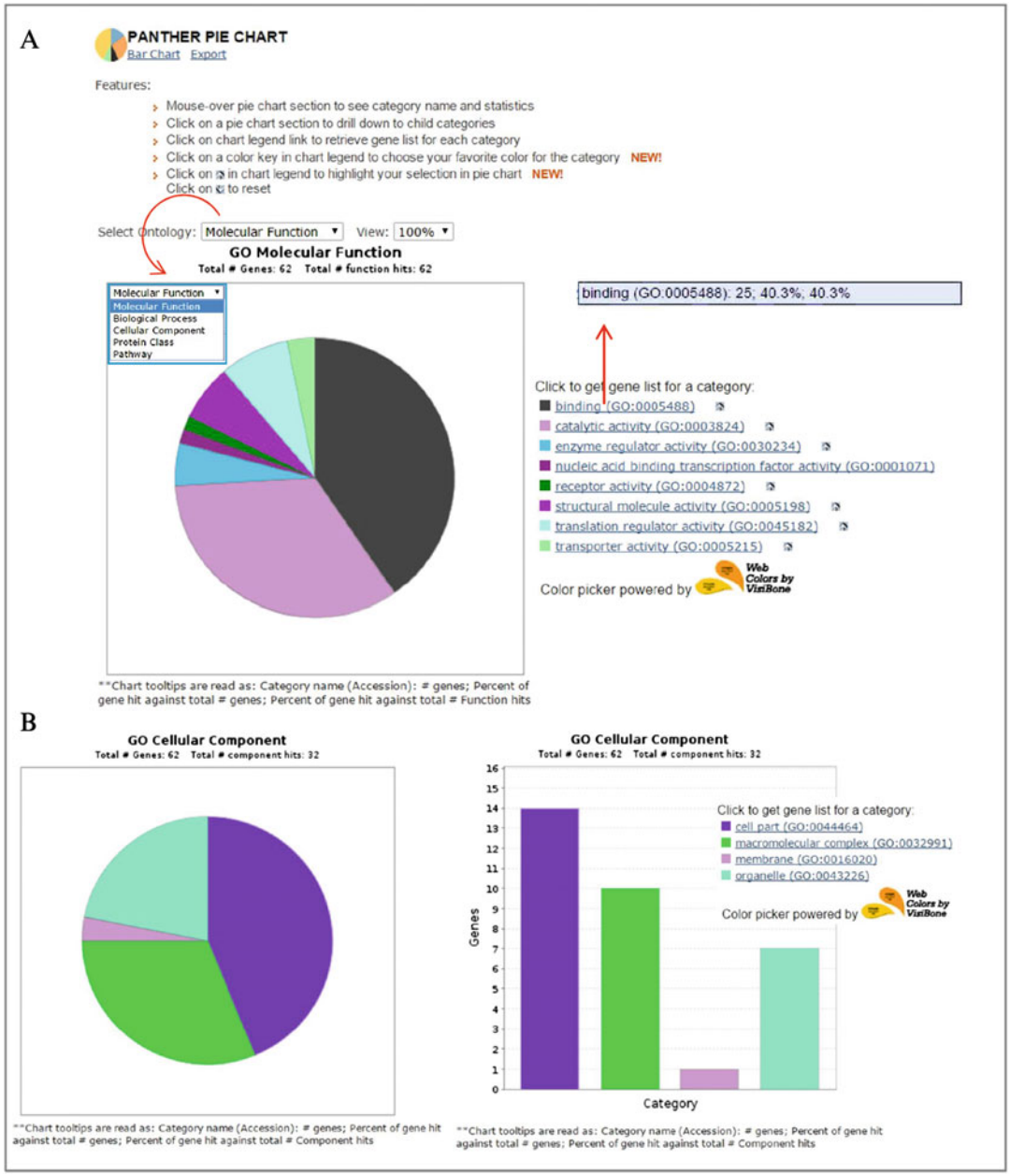


Fig. 16.4 Functional classification of proteins up-regulated in both Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cells lines. The proteins were classified into (**a**) Biological Processes and (**b**) Cellular Components. Figure shows the change of pie chart to a bar graphic as well

Functional Annotation Clustering tool allows the user to group genes depending on the degree of their functional association. It is performed with a novel algorithm that measures relationships among annotation terms. This process is useful to eliminate the redundant relationships that exist in many-genes-to-many-terms cases (i.e. when one gene is associated with many different redundant terms and one term is associated with many genes) [21]. Additional features of this

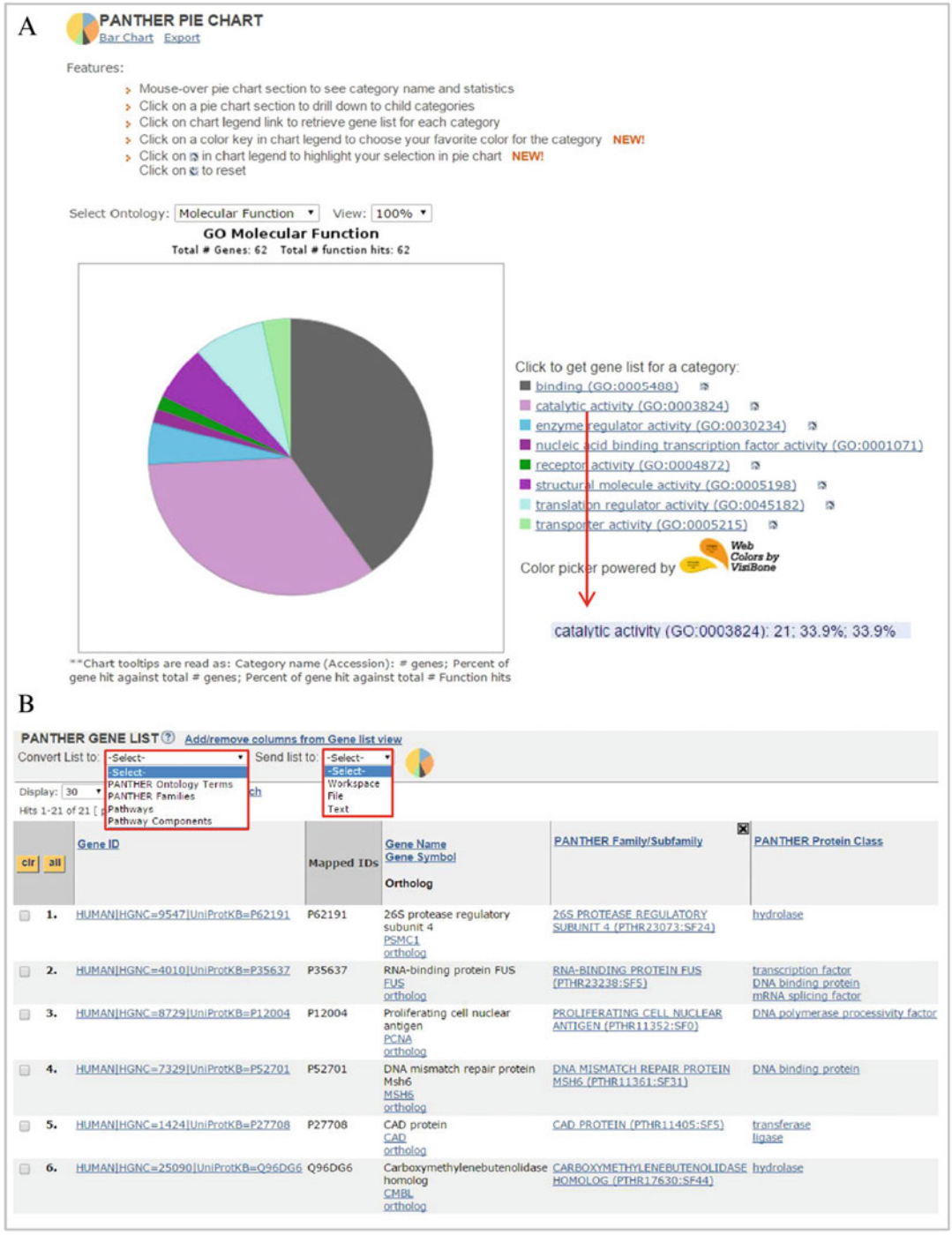


Fig. 16.5 Classification of Biological Processes for proteins up-regulated in both Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cells lines (**a**) Biological processes pie chart displaying different categories of processes, e.g. Metabolic Processes. (**b**) List of genes involved in the selected Metabolic Processes

clustering tool is the ability to rank the importance of annotation groups with an enrichment score (EASE scores) that uses the geometric mean of all the enrichment p-values of each annotation term in the group; the annotation clustering tool provides a link to a 2-D viewer for related gene-term relationships, allowing a fast way to focus on the genes that have common annotation terms [22]. On the other hand, The Functional Annotation Chart tool can be used to get the typical gene-GO term enrichment analysis (similar to other tools) to identify the most relevant (overrepresented) biological terms associated with a given gene list. However, DAVID offers extended annotation coverage in comparison to other enrichment analysis tools. The enhanced annotation coverage includes not only the GO terms but more than 40 annotation categories, such as protein-protein interactions, protein functional domains, disease associations, bio-pathways, sequence features, gene tissue expression, etc. This tool is helpful to identify enriched annotation terms associated with the gene list of interest in a linear tabular text format. Similar to the Annotation Clustering Tool, the Functional Annotation Chart also provides links to further explore the list of interacting proteins, link gene-disease associations and visualize genes on BioCarta and KEGG pathway maps [21]. Finally, the Functional Annotation Table tool is a query engine for DAVID Knowledgebase without statistical probes. It delivers annotation information in a table format for every gene from the users' gene list. This is a particularly useful tool when users want to have a closer look of some specific interesting genes and explore its annotation information.

DAVID's Gene ID Conversion tool allows conversion of user's input gene or gene product identifiers from any type to another in a more comprehensive and high throughput manner with a uniquely enhanced ID-ID mapping database leveraging heterogeneous annotations [23].

DAVID's Gene Name Viewer is another tool useful to quickly attach meaning to a list of gene IDs, translating them into their corresponding gene names. Thus, before proceeding to an in-depth analysis, researchers can quickly have an overview of gene names to gain insight into their biological system and have *a priori* general idea of interesting processes that might be involved.

DAVID's NIAID Pathogen Browser: The National Institute of Allergy and Infectious Diseases (NIAID) has defined three categories of priority pathogens, A, B and C. These pathogens are important for biodefense purposes and have become attractive study subjects because of the increasing research funding available to study them. The DAVID NIAID Pathogen Browser is provided as a support tool for researchers that would like to explore the biology of the priority pathogens types. For example, one may choose the word "anthrax" and type the key word "toxin", the result is a list of genes from *Bacillus anthracis* that matches to the typed key word. This tool may assist researchers in understanding the biology of a priority pathogen if the gene list retrieved from the DAVID NIAID Pathogen Browser is further analyzed by one of DAVID's Bioinformatics Resources [21].

Analysis of gene lists: To carry out an optimal gene list analysis, the list should; (1) have enough number of genes/proteins ranging from hundreds to thousands (e.g. 100–2000), (2) only include genes with statistical significance that show a notable up or down regulation, (3) show reproducibility between experimental replicas [22].

DAVID bioinformatics resources website is organized in two main toolbars (Fig. 16.6). There are different links, like Start Analysis, Shortcut to DAVID Tools, Technical Center, among others on top. On the left side, there are other shortcuts to DAVID Tools that also offers a brief explanation for each tool. Recently added DAVID NIAID Pathogen Annotation Browser tool can be found on the top menu in shortcut to DAVID Tools.

It is straightforward to upload a gene list for DAVID bioinformatics analysis (Fig. 16.7a). Firstly, go to https://david.ncifcrf.gov/gene2gene.jsp and select Start analysis. On the left side choose upload in the list manager, then: (1) Copy/paste the gene lists to be analyzed into box A; a text file or a gene IDs list can also be

Fig. 16.6 DAVID Bioinformatic Resources Website. This website has two main toolbars. The toolbar on the top has links to: (*1*) Start Analysis, (*2*) Shortcut to DAVID Tools, (*3*) Technical Center, (*4*) Downloads and APIs, (*5*) Terms of Service, (*6*) Why David, and (*7*) About Us. And the toolbar on the left side (*8*) has links to Tools that offer a brief explanation for each of DAVID's tool. Additionally, in (*2*) we can find the recently added tool NIAID Pathogen Annotation Browser (*9*)

uploaded in box B, (2) Choose the corresponding gene identifier type for your input gene IDs; alternatively use the ID conversion tool to seek (or convert) the correct gene identifier, (3) Select the type of list you are submitting, either gene list or gene background. The general guideline is to set up a pool of genes as population background. This usually includes all the genes that could be possibly detected (e.g. all the probes included in a particular DNA microarray). Since most of the studies are done in a genome-wide scale, there is no need to set a background (default background is the entire genome), (4) Submit the List. The different analysis suites are displayed (Fig. 16.7b) that will be applied to the submitted gene list shown on the left (highlighted in the Gene List Manager) (Fig. 16.7b). By clicking Start Analysis, users can go back at any time to upload another gene list or to access any analytical tool suite of interest.

In this section, a couple of examples are presented to showcase a few of the tools from David's toolbox that are most widely used using gene lists corresponding to proteins down regulated in both Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cell lines studied by Calderón-González et al. [18]. Selecting Functional Annotation Tool (Fig. 16.7b), results in Annotation Summary Results, which displays the number and percentage of genes (from the submitted gene list) involved in different GO categories

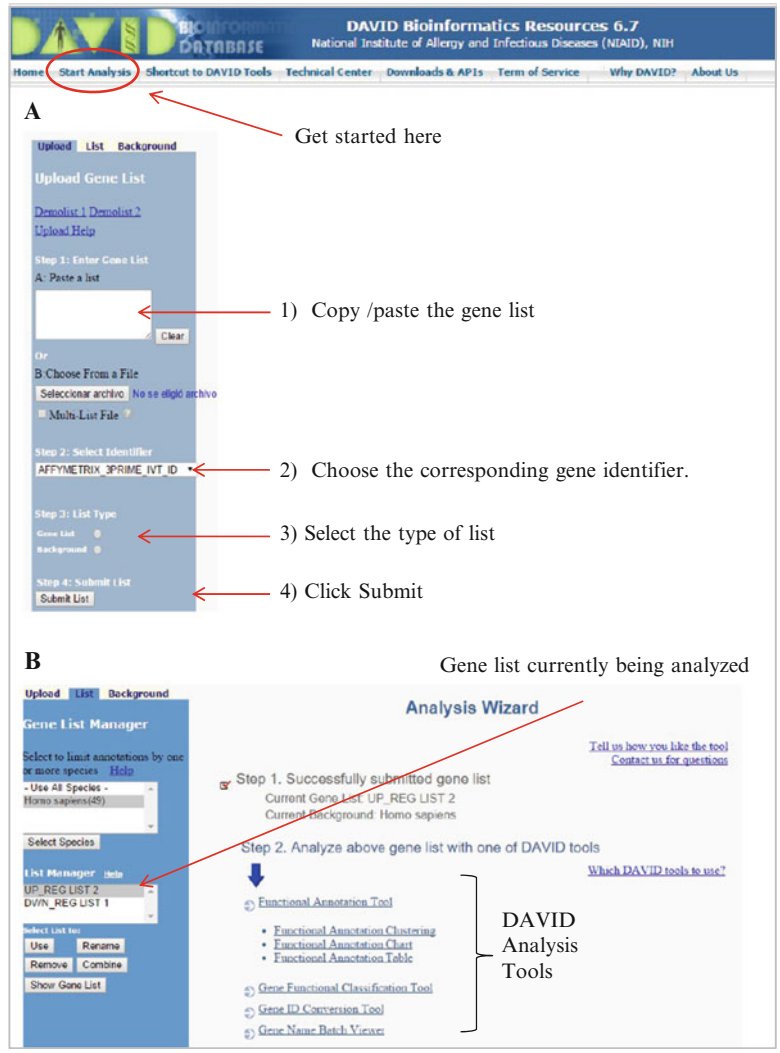


Fig. 16.7 Uploading data into David's gene list manager. (**a**) On the left side; (*1*) Upload a gene list, (*2*) Choose the corresponding gene identifier, (*3*) Select the type of list, either gene list or gene background, (*4*) Submit the gene list. (**b**) Once the user has submitted the gene list, the Analysis Wizard shows the shortcuts for the different DAVID Analysis tools

(Fig. 16.8). In each category, users can click on Chart to obtain an individual chart report for the selected category. Users can choose a number of categories for further analysis in the Combined Annotation Tools (Fig. 16.8). A table divided in several annotation clusters will be obtained by clicking on Annotation Clustering Tool. Every annotation cluster is formed by a group of terms from functionally related genes. Taken all together, the chance to identify a biological significance increases (Fig. 16.9). The degree of similarity between annotations is measured by Kappa statistics. This tool also provides a link to generate a 2D-view map that allows a fast way to associate genes that have common annotation terms.

From this very specific gene list, we observed an enriched group of genes involved in mitochondrial function. Noteworthy, the high correlation of this result in comparison with other tools previously explored. Since the submitted gene list corresponds to down-regulated genes in a proteomic approach, this result suggests that MCF7, T47D and MDA-MB231 breast cancer

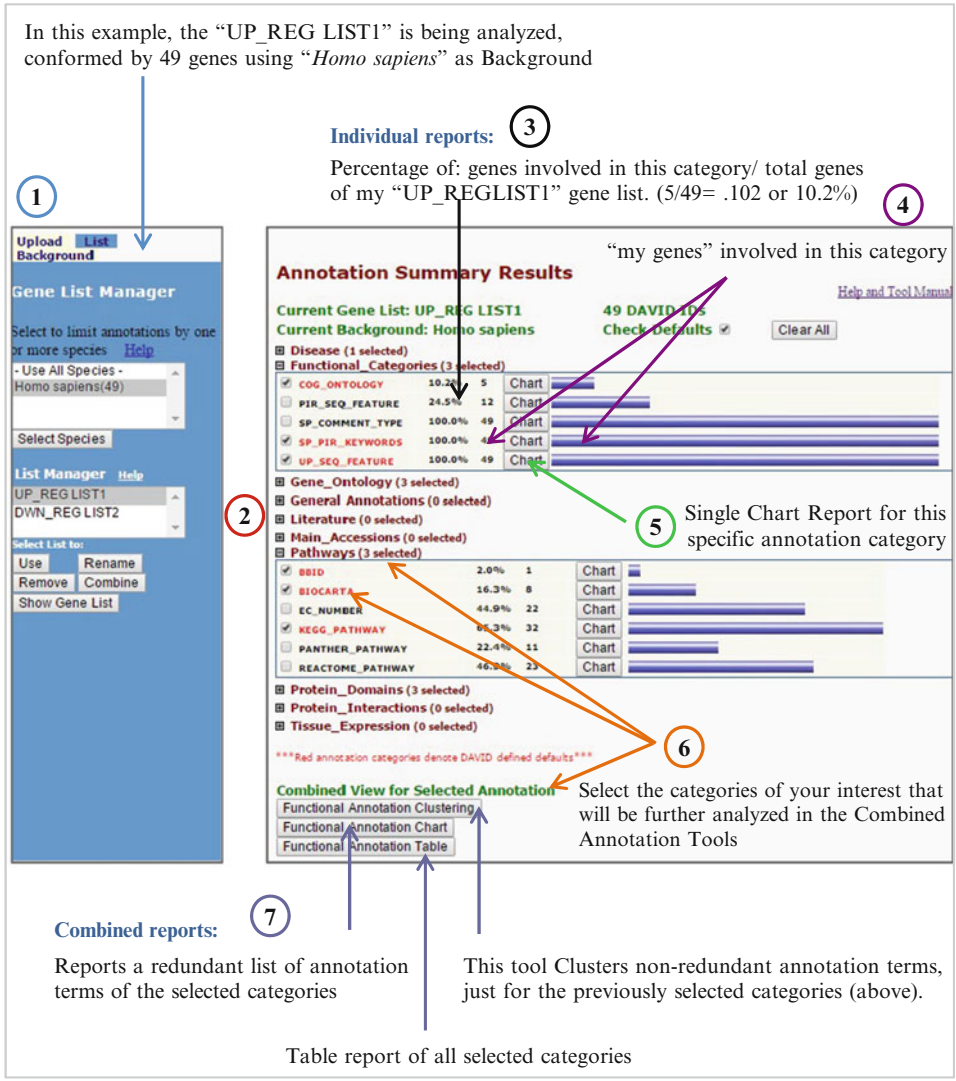


Fig. 16.8 Functional Annotation Tool Suite. (*1*) Gene List Manager showing the list that is being analyzed. (*2*) Annotation Summary results displaying different categories: (*3*) the number and (*4*) percentage of genes involved. (*5*) Clicking on this box will generate a chart report of functional categories. (*6*) The user can choose the number of categories to be considered for further analysis in the Combined Annotation Tools (*7*) by checking the check boxes next to each category

cell lines have an impaired mitochondrial function in comparison to the MCF10A control cell line.

For instance, NADH-coenzyme Q reductase, 3,2 trans-enoyl-Coenzyme A isomerase, cytochrome c oxidase, and malate dehydrogenase are some of the encoding genes that had a high EASE SCORE and are involved in the mitochondrial inner membrane function.

16.5 KEGG

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database resource designed for understanding and interpreting biological systems using high-throughput data [24–26]. KEGG is composed of 17 databases organized into four categories:

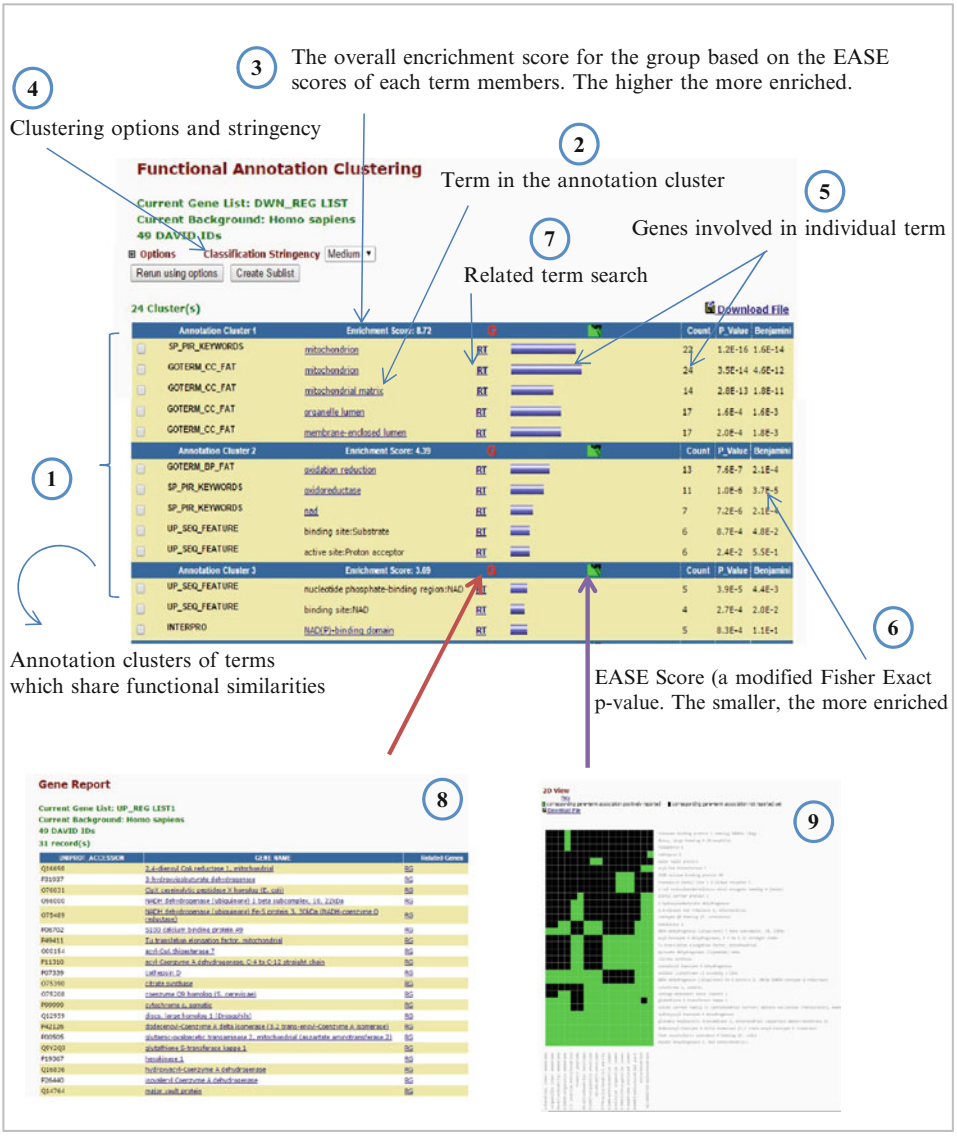


Fig. 16.9 An example of the Functional Annotation Clustering Tool. This image shows the results obtained by searching the "DWN_REG LIST". The search results show three clusters (*1*) each categorized further according to different terms (*2*). The clusters are ranked according to their enrichment score (*3*) and stringency involved in each term are shown for each clu well as the EASE score (*6*) and the related term The links to obtain the gene list in each annota (*8*) and a 2D-Map View (*9*) are provided

1. Systems information: KEGG PATHWAY (pathway maps), KEGG BRITE (functional hierarchies and table files) and KEGG MODULE (Pathway, structural complex, functional set and signature modules). These databases are manually created using published literature
2. Genomic information: KEGG ORTHOLOGY (orthology (KO) groups), KEGG GENOME (complete genomes), KEGG GENES (gene catalogs) KEGG SSDB (sequence similarity database for genes), DGENES (draft genomes) and MGENES (metagenomes). The information about genes and genomes is

obtained from different databases, such as RefSeq (prokaryotes, eukaryotes, plasmids and viruses), GenBank (prokaryotes), and PubMed (addendum: collection of manually created protein sequences entry)
3. Chemical information, also called KEGG LIGAND: KEGG COMPOUND (metabolites and other small molecules), KEGG GLYCAN (glycans), KEGG REACTION (biochemical reactions), KEGG RPAIR (reactant pairs), KEGG RCLASS (reaction class), and KEGG ENZYME (enzyme nomenclature)
4. Health information commonly called KEGG MEDICUS: KEGG DISEASE (human diseases), KEGG DRUG (drugs), KEGG DGROUP (drug groups), KEGG ENVIRON (crude drugs and health related substances), JAPIC (drug labels in Japan) and DailyMed (links to drug labels in USA) [26].

The annotation system in KEGG is based on the correlation between functional information and orthologous groups (KEGG Orthology or KO) through the assignment of KO identifiers (K number). This information is stored in the KO database and is independent of the KEGG GENE database that contains completely sequenced genomes [26]. The KO system is essential for connecting the genomic information with systemic functional information resulting in the conversion of genes to K numbers, leading to an automatic reconstruction of KEGG PATHWAYS and other networks [26, 27]. Currently, KEGG has more than 4000 complete genomes annotated with the KO system [26].

KEGG has several analysis tools:

1. KEGG Mapper which is the interface used for KEGG Mapping. This is composed of KEGG BRITE, MODULE, and PATHWAY mapping tools, which map genes, proteins, small molecules, etc. (also called objects) into all brite functional hierarchies, modules and pathways maps, respectively [28]
2. KEGG Atlas is a graphical interface to navigate the global integrated maps in KEGG. Maps available are Metabolism (Biosynthesis of amino acids, Biosynthesis of secondary metabolites, Carbon metabolism, Degradation of aromatic compounds, Fatty acid metabolism, Microbial metabolism in diverse environments, and 2-Oxocarboxylic acid metabolism) and Cancer pathway [29]
3. BlastKOALA: KOALA is defined as KEGG Orthology And Links Annotation. BlastKOALA is used for the annotation of completely sequenced genomes. This tool utilizes the Pangenomes database
4. GhostKOALA: this tool is designed by the metagenome annotation and it uses the Pangenomes and Viruses databases [26, 27], (5) BLAST/FASTA performs searches of similar sequences
5. SIMCOMP searches for similar chemical structures

Pathway Maps Analysis To map proteins of interest into Pathways, go to the KEGG website (http://www.genome.jp/kegg/) and on the Data-oriented entry points, click on the KEGG PATHWAY key (Fig. 16.10). In the Pathway Mapping menu, select the mapping tool of interest: Search Pathway, Search&Color Pathway or Color Pathway. As an example, the up and down-regulated proteins found common between Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cells lines from Calderón-González et al. were analyzed with the Search&Color Pathway tool [18]. - Up-regulated proteins were colored in red, whilst down-regulated polypeptides were presented in green (Fig. 16.11). To perform this analysis, an organism must be selected first by clicking on the org key, after which a new window is displayed to find the three to four KEGG organism code. Type the desired organism in the window and then click on select. In this example, *H. sapiens* has the hsa code. The next step is to introduce IDs in UniProtKB format, followed by the word red or green as mentioned before. Other compatible ID formats are KEGG-Identifiers, NCBI-GeneID and NCBI-ProteinID. Alternatively, a file containing IDs can be uploaded. To perform

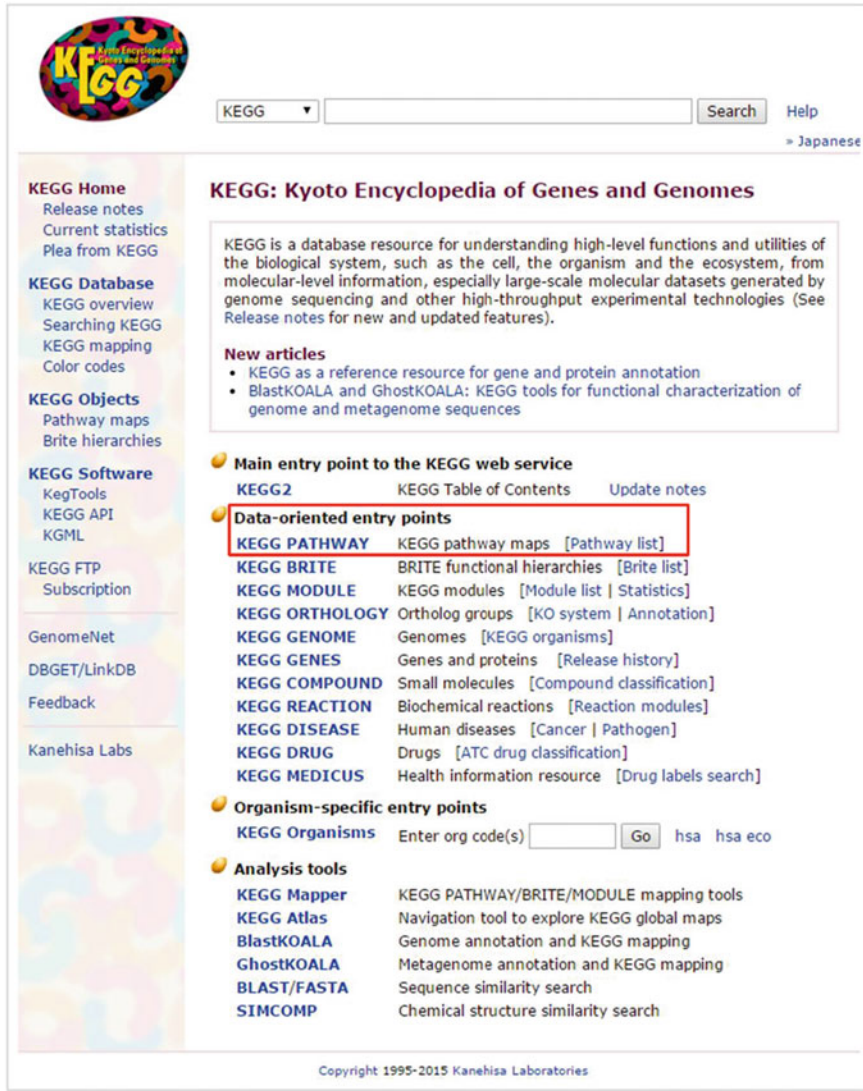


Fig. 16.10 KEGG website. This image shows the different links provided in KEGG's website, including KEGG Home, KEGG Database, KEGG Objects, KEGG Software, among others. The website also provides several tools for the data analysis including KEGG Mapper, KEGG Atlas, BlastKOALA, Ghost KOALA, BLAST/FASTA, SIMCOMP. KEGG Pathway modules are highlighted in a *red box*

the search, the following options were selected; (1) to include aliases and (2) to display objects not found in the search (Fig. 16.12a). The result window shows a list of pathways where proteins were mapped, as well as a list of protein IDs that were not found (Fig. 16.12a). A list of proteins found in each pathway, including their UniProtKB IDs and KEGG *H. sapiens* database codes is also displayed (Fig. 16.12b). Clicking a particular UniProtKB ID will display the information for the selected ID (Fig. 16.13a). On the other hand, if the code of the *H. sapiens* organism in KEGG is selected, a new window containing KEGG information about that protein, including Gene name, Disease, KEGG Orthology, Structure, Motifs in the protein, and Pathways, among other information will be displayed (Fig. 16.13b). Finally, when a certain pathway is selected, an

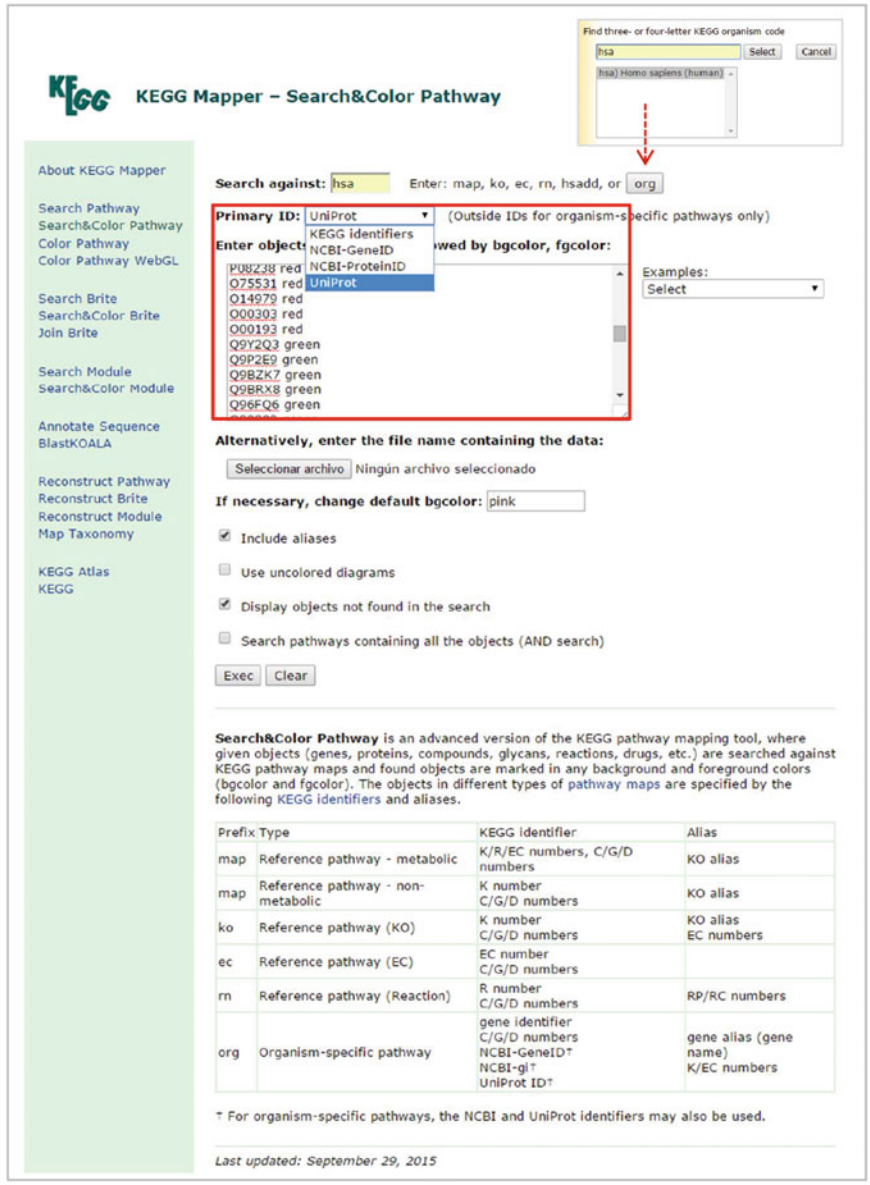


Fig. 16.11 KEGG pathway mapping tool. This image shows the general procedure for mapping proteins in Search & Color Pathway module. The format of IDs as well as the organism need to be selected. Protein accession numbers are followed with the word *red* or *green* to highlight up- or downregulated proteins, respectively

image is generated where up- or down-regulated proteins are highlighted in red or green respectively (Fig. 16.14). In the case of the breast cancer cell line, most quantified proteins mapped to metabolic processes, with 22 polypeptides [5 - up-regulated (↑) and 17 down-regulated (↓)]: ↓3HIDH, ↑ SAHH3, ↓ IVD (Amino acid metabolism), ↑ CMBL (Hydrolase), ↓ CISY (Carbon metabolism, 2-Oxocarboxylic acid metabolism, biosynthesis of amino acids, carbohydrate metabolism), ↓ AL1A3 (Carbohydrate metabolism, amino acid metabolism, metabolism of other amino acids, xenobiotics biodegradation and metabolism, chemical carcinogenesis),

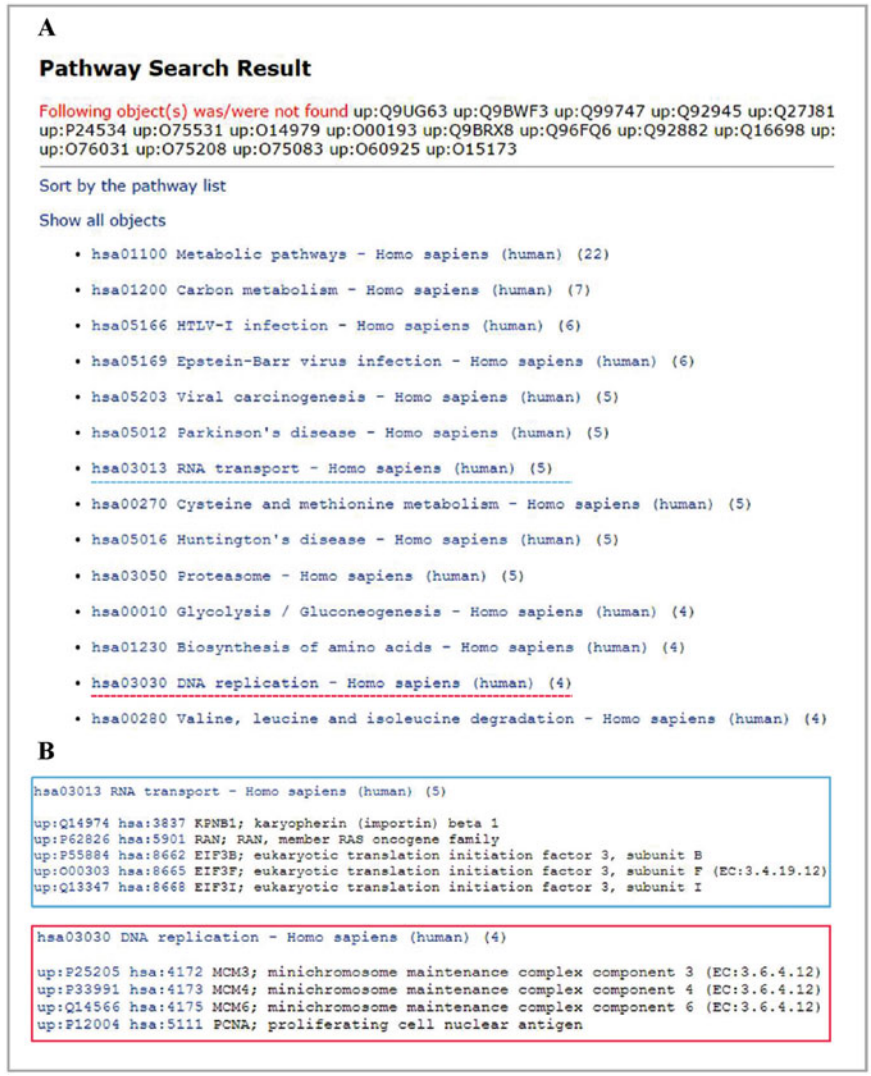


Fig. 16.12 Search & Color Pathway result. (**a**) A list of proteins that were not found are shown at the *top*. The list of different pathways is also displayed with the number of proteins involved. (**b**) Two examples of proteins involved in RNA transport and DNA replication processes

↓ AATM (Carbon metabolism, 2-Oxocarboxylic acid metabolism, biosynthesis of amino acids, amino acid metabolism, fat digestion and absorption), ↓ HCDH (Fatty acid metabolism, carbohydrate metabolism, lipid metabolism, amino acid metabolism), ↓ HXK1 (Carbon metabolism, carbohydrate metabolism, biosynthesis of other secondary metabolites, HIF-1 signaling pathway, insulin signaling pathway, carbohydrate digestion and absorption, central carbon metabolism in cancer, endocrine and metabolic diseases), ↓ ACADM (Carbon metabolism, fatty acid metabolism, carbohydrate metabolism, lipid metabolism, amino acid metabolism, metabolism of other amino acids, PPAR signaling pathway), ↑ METK2 (Biosynthesis of amino acids, amino acid metabolism), ↓ MDHM (Carbon metabolism, carbohydrate metabolism, amino acid metabolism), ↓ NDUBA, ↓ NDUS3 (Energy metabolism, neurodegenerative diseases,

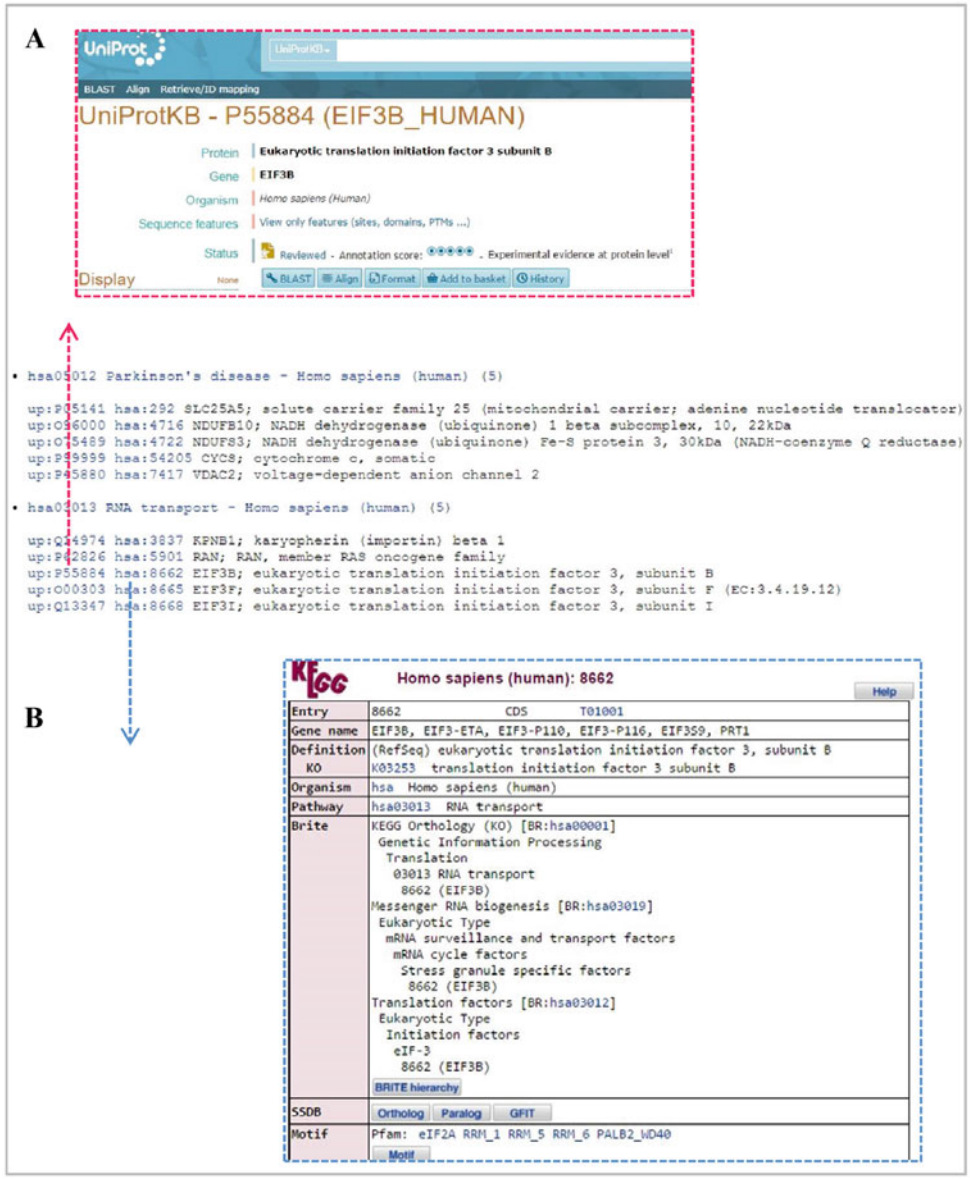


Fig. 16.13 Additional information for proteins in KEGG Database. The proteins displayed in each pathway have a link to additional information: (**a**) UniProtKB website and (**b**) KEGG database

endocrine and metabolic diseases), ↓ DHB12 (Fatty acid metabolism, lipid metabolism), ↓ ODPB (Carbon metabolism, carbohydrate metabolism, HIF-1 signaling pathway, glucagon signaling pathway, central carbon metabolism in cancer), ↑ PGAM1 (Carbon metabolism, biosynthesis of amino acids, carbohydrate metabolism, amino acid metabolism, glucagon signaling pathway, central carbon metabolism in cancer), ↓ CYC (Energy metabolism, cellular processes, pathways in cancer, neurodegenerative diseases, cardiovascular diseases, endocrine and metabolic diseases, infectious diseases), ↓ RPN1 (Glycan biosynthesis and metabolism, folding, sorting

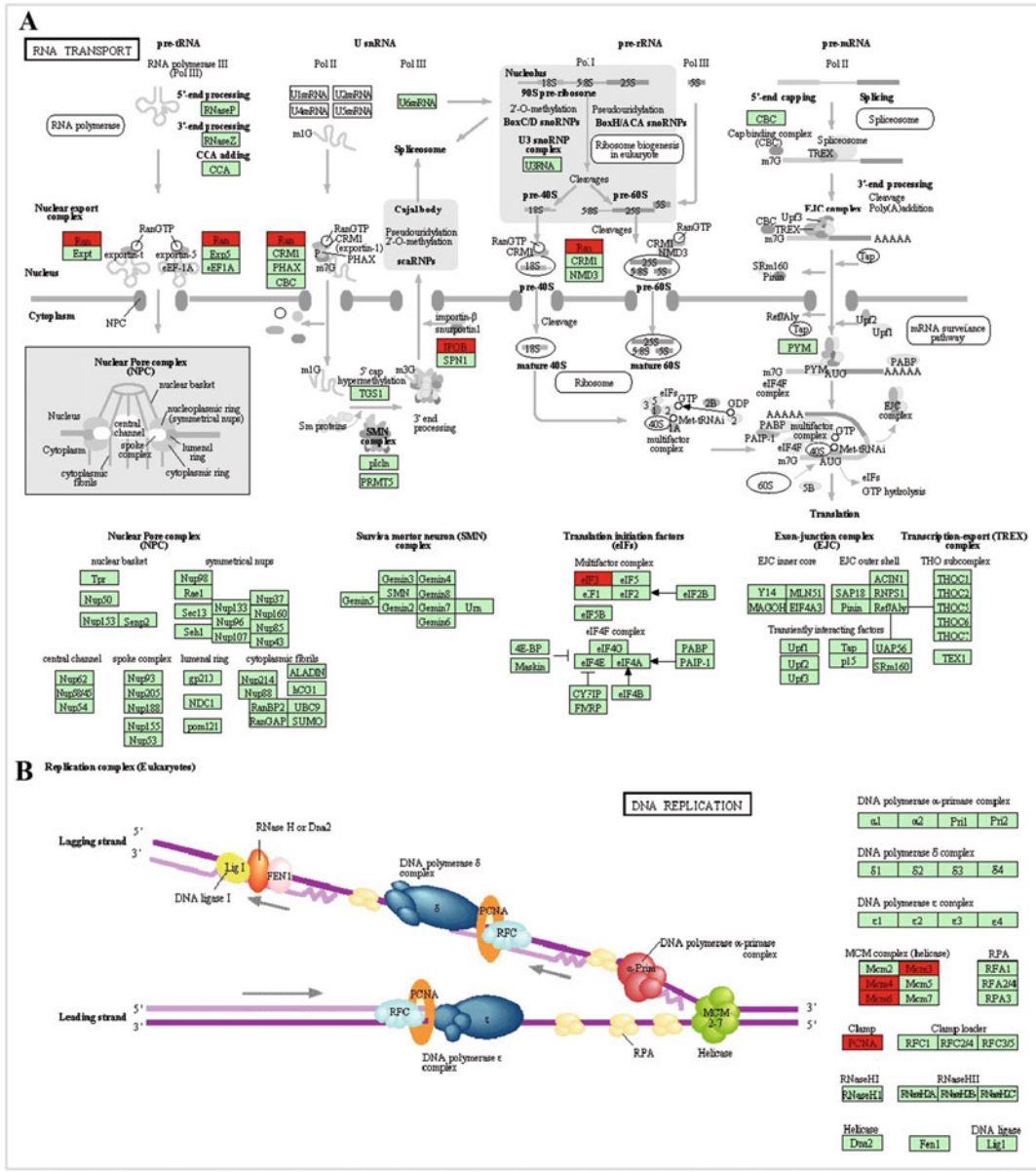


Fig. 16.14 Proteins mapped into KEGG PATHWAYS. Polypeptides found up- or down-regulated in both Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cell lines were submitted to KEGG mapping. Some of the processes found to be affected are, (**a**) RNA transport process, and (**b**) DNA replication process. Up-regulated proteins are colored in *red* and down-regulated proteins are in *green*

and degradation), ↓ NLTP (Lipid metabolism, cellular processes, PPAR signaling pathway), ↓ SPEE (Amino acid metabolism, metabolism of other amino acids), ↑ PYR1(Nucleotide metabolism, amino acid metabolism). Others mapped pathways were: RNA transport with 5 proteins ↑ IMB1, ↑ RAN, ↑ EIF3B, ↑ EIF3F, ↑ EIF3I) (Fig. 16.14a) and DNA replication with 4 polypeptides involved (↑MCM3, ↑ MCM4, ↑ MCM6, ↑ PCNA) (Fig. 16.14b).

16.6 Ingenuity Pathway Analysis (IPA)

Ingenuity Pathway Analysis (IPA, QIAGENs Redwood City, www.qiagen.com/ingenuity) is a software application platform developed for analysis, understanding, integration and interpretation of biological data [30]. Ingenuity can analyze data acquired using platforms such as microarrays, proteomics, metabolomics, etc. IPA uses the QIAGEN's Ingenuity Knowledge Base in which contents extracted from articles, biomedical literature, reviews, internally curated knowledge, and other sources are structured into Ontology terms. The information in this platform are categorized into several knowledgebases:

1. Ingenuity expert information, including Ingenuity expert findings and Ingenuity expert assist findings
2. Ingenuity supported third party information including MicroRNA-mRNA interactions (miRecords, TarBase, TargetScan)
3. Protein-Protein Interactions including BIND, cognia, DIP, Interactome studies, MINT, and MIPS
4. Additional sources: An open access database of genome-wide association results, BIOGRID, Breast cancer information core (BIC), Catalogue of somatic mutations in cancer (COSMIC), Chemical Carcinogenesis Research Information System (CCRIS), ClinicalTrials.gov, ClinVar, DrugBank, GO, GVK Biosciences, Hazardous Substances Data Bank (HSDB), HumanCyc, IntAct, miRBase, Mouse Genome Database (MGD), Obesity Gene Map Database, and Online Mendelian Inheritance in Man (OMIM).

The principal components of IPA suite are

1. Core Analyze
2. IPA-Tox
3. IPA-Biomarker
4. IPA-Metabolomics (Fig. 16.15)

Fig. 16.15 The main page of Ingenuity Pathway Analysis suit. All functions are listed via in two main tabs, Learning IPA, and shortcuts. The shortcut tab contains the dataset- and pathway options, as well as different analysis options, including Core, IPA-Tox, IPA-Biomarker and IPA-Metabolomics

Core Analyze consists of classified data sets mapped into biological processes, networks and pathways. IPA-Tox module includes data classified in the context of toxicological processes. In this tool the toxicity and safety of compounds is evaluated. IPA-Tox keeps track of the biological processes that are related to compound toxicity at various biochemical and molecular levels. IPA-Biomarker tool is used to identify and prioritize potential biomarker candidates. The selection of these putative biomarkers is based on their biological characteristics. Finally, the fourth application IPA-Metabolomics, is able to analyze metabolomics data, which are then contextualized into biological insights (metabolism and cell physiology).

IPA supports several types of identifiers including Affymetrix, Affymetrix SNP ID, Agilent, CAS registry number, CodeLink, dbSNP, Ensembl, GenBank, Entrez gene, Gene Symbol-mouse, Gene Symbol-rat and Gene symbol—Human (Hugo/HGNC), GenPept, GI number, Human Metabolome Database (HMDB), Illumina, Ingenuity, International Protein Index, KEGG, Life Technologies (Applied Biosystems), miRBase (mature), miRBase (stemloop), PubChem CID, RefSeq, UCSC hg18 and 19, UniGene and UniProtKB/Swiss-Prot accession number. The confidence reported by IPA are either experimentally determined or theoretically predicted. Some tissues and cell lines covered by IPA include tissue and primary cells from nervous and other organ systems and cell lines from breast cancer, cervical, central nervous system (CNS), colon, hepatoma, immune, kidney, leukemia, lung, lymphoma, macrophage, melanoma, myeloma, neuroblastoma, osteosarcoma, ovarian, pancreatic, prostate and teratocarcinoma model systems. Mutations covered include functional effect, inheritance mode, translation impact, unclassified mutation, zygosity and wild type.

IPA analysis core protocol: To use IPA, a license needs to be purchased but one can use a trial version for a limited period of time. To perform an analysis in IPA, first an analysis dataset need to be created (Fig. 16.16). To create an analysis dataset, go to Annotate datasets

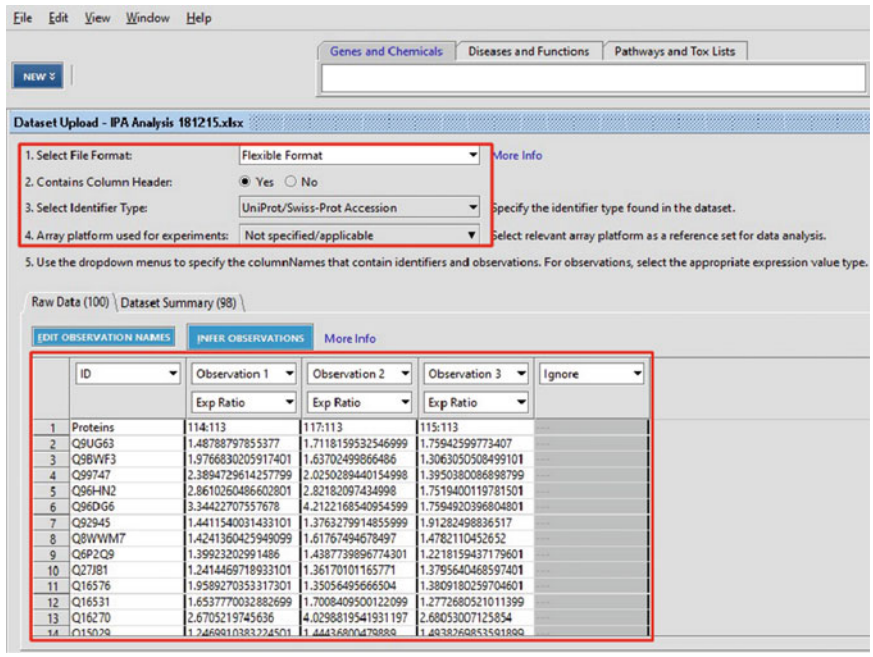


Fig. 16.16 Creation of a dataset with the IPA software. *Red* rectangles spotlight the basic steps to perform an analysis for a dataset

option in the IPA window (Fig. 16.15), select the file you wish to analyze and save the file. For illustration purposes, we analyzed proteins differentially expressed in common in Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cell lines from Calderón-González et al. [18]. It is necessary to specify the following information for the data that you wish to analyze:

1. File format: Flexible format
2. Column header: Yes
3. Identifier type: UniProt/Swiss-Prot accession
4. Array platform: In this case, it does not apply

Then the observation names must be edited, specifying the ID of proteins; in our case, the observation option 1 was selected (114:113. MCF7/MCF 10A), 2 (117:113. T47D/MCF 10A), 3 (115:113 MDA-MB-231/MCF10A), according to data number. Finally, the quantitative data format must be specified, which in our case we chose Exp Ratio (Fig. 16.16).

To carry out IPA Core analyses, we first uploaded the dataset previously created and then specified the parameters according to the goals of our study. The IPA platform gives different options to filter the data. We filtered the parameters for breast cancer disease as follows:

1. General settings: Ingenuity knowledge base (genes only). Considering direct and indirect relationships
2. Networks: 25 interaction networks with 35 molecules per interactome. Include endogenous chemicals (default parameters)
3. Data sources: All
4. Confidence: All
5. Species: Human with stringent filter
6. Tissues and cell lines: Mammary gland as organ and all breast cancer cell lines of database
7. Mutations: All.

At the end of the page, cutoff values are selected. We focused on up- and down-regulated proteins (Fig. 16.17). The statistical significance was determined by Fisher´s Exact Test, for which the p-value cutoff was set at 0.05. As a result of this analysis, we obtained three summary results, one for each observation. Then, we performed a Core Comparison Analysis. This analysis was performed using the following option (Core: Compare analysis). The procedure also requires

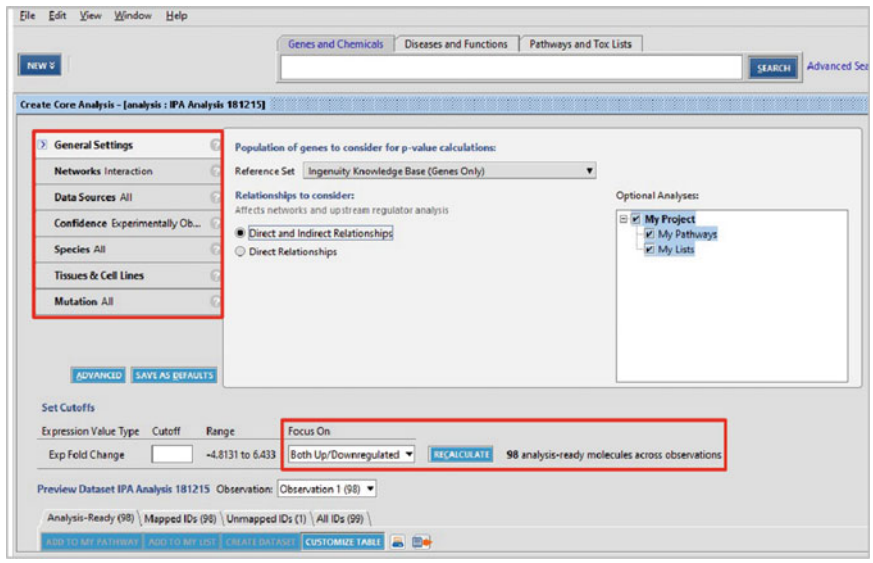


Fig. 16.17 Core parameters needed for IPA analysis. Figure shows the different parameters that need to be set to perform and delimit a Core Analysis. In this case the analysis was focus on breast cancer disease

selecting files for comparison. The summary results for all observation are reported in a single file. The Core Analysis result window shows different tool bars:

1. Canonical Pathways (Chart and HeatMap)
2. Upstream Analysis (Table and HeatMap)
3. Diseases & Functions (Chart and HeatMap)
4. Regulator effects (Table)
5. Networks (Networks for each observation or overlapping networks)
6. Molecules (Tables).

We focused our analysis on canonical pathway result obtained as a chart (Fig. 16.18a) or a HeatMap (Fig. 16.18b). In both cases, the number of up- and down-regulated proteins and their statistical probability were reported. Some of the

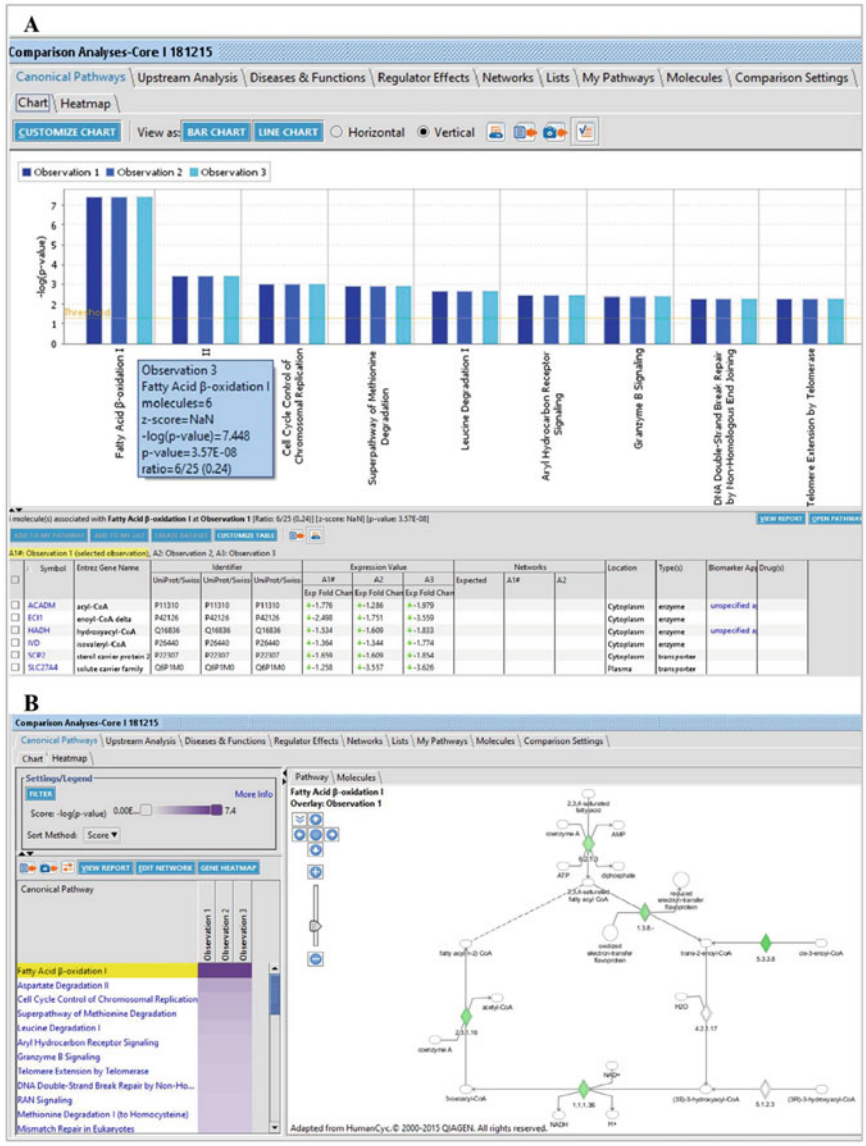


Fig. 16.18 Classification of proteins found up- or down-regulated in both Luminal A and Claudin-Low breast cancer cell lines into canonical pathways with IPA software. The result can be displayed as (**a**) Bar chart or (**b**) Heatmap

processes affected were: Fatty acid oxidation I (↓ACADM, ↓ECI1, ↓HADH, ↓IVD, ↓SCP2, ↓SLC27A4 with a p-value 3.57×10^{-8}), aspartate degradation II (↓GOT2 and ↓MDH2, p-value of 3.78×10^{-4}), cell cycle control of chromosomal replication (↑MCM3, ↑MCM4 and ↑MCM6, p-value 1.01×10^{-3}), telomere extension by telomerase (↑XRCC5 and ↑XRCC6, p-value 5.44×10^{-3}), and protein and ubiquitination pathway (HSP90AB1, ↑PSMA3, ↑PSMC1, ↑PSMD2, ↓PSMD3, and ↑PSMD7, p-value 8.65×10^{-3}).

Diseases functions are divided into two categories, Diseases and Bio Functions and Tox Functions. We only obtained the first category. We found the affected processes to be:

1. Cell-to-cell signaling and interaction: Formation of focal adhesions (↓CTNND1 and ↑STMN1, p-value 1.30×10^{-3})
2. Cellular assembly and organization: Formation of focal adhesions (↓CTNND1 and ↑STMN1, p- value 2.39×10^{-2}) and polymerization of microtubules (↑STMN1, p-value 2.39×10^{-2})
3. Cellular function and maintenance: Formation of focal adhesions (↓CTNND1 and ↑STMN1, p-value 1.30×10^{-3}) and polymerization of microtubules (↑STMN1, p-value 2.39×10^{-2})
4. Cell death and survival: Anoikis (↓CTNND1 and ↑ILK, p-value 3.99×10^{-3}) and cytotoxicity of breast cancer cell lines (↓RELA, p-value 3.17×10^{-2})
5. Drug metabolism: Synthesis and oxidation of tretinoin (↓ALDH1A3, p-value 8.02×10^{-3})
6. Cellular development: Epithelial-mesenchymal transition of breast cancer cell lines (↑ILK and ↑STMN1, p-value 4.45×10^{-2}) among other processes

The interactome data obtained in three separate experiments were processed resulting in identification of two principal networks related to: (1) Cellular development, cellular growth and proliferation, cellular movement, cell death and survival, and cancer, with a score of 19 and 14 molecules involved (↓ALDH1A3, ↓CTSD, ↓DLG1, ↓EZR, ↑FUS, ↑ILK, ↑KPNB1, ↓MVP, ↓RELA, ↓S100A8, ↑SET, ↓SLC25A5, ↑XRCC5 and ↑XRCC6) (Fig. 16.19a). (2) Cell death and survival, cellular development, DNA replication, recombination and repair, cancer and hereditary disorder obtained 12 proteins (↑ABCF2, ↑CAD, ↓CTNND1, ↓CYCS, ↑HSP90AB1, ↓LGALS3BP, ↑MAT2A, ↑MCM6, ↑MSH6, ↑NUMA1, ↑PCNA, ↑SNRPG) with a score of 15 (Fig. 16.19b). Proteins in red and green represent the up- and down- regulated proteins, respectively. Small molecules are shown in gray color to highlight their relationship with our proteins. Created Networks can be exported to IPA pathway for subcellular localization and decoration of network with organelles and backgrounds.

16.7 Biomarkers Module

To perform biomarker filtration, we used the Biomarkers module. As a first step in using the Biomarker module, we selected the analysis dataset function and choose a dataset created previously. Next we chose the following parameters:

1. Species: Human
2. Tissues and cell lines: mammary gland as organ and breast cancer cell lines
3. Molecules: All
4. Diseases: Cancer
5. Biofluids: All
6. Biomarkers: All biomarkers application (diagnosis, disease progression, efficacy, not applicable, prognosis, response to therapy, safety and unspecified application) and breast disease (breast cancer, breast carcinoma, ductal carcinoma, ductal carcinoma in situ, infiltrating ductal breast carcinoma, infiltrating lobular breast carcinoma, invasive ductal breast cancer, lobular breast cancer, mammary neoplasm, metastasic breast cancer) (Fig. 16.20a).

We then ran the analysis, saved the results, and performed a comparative analysis on our

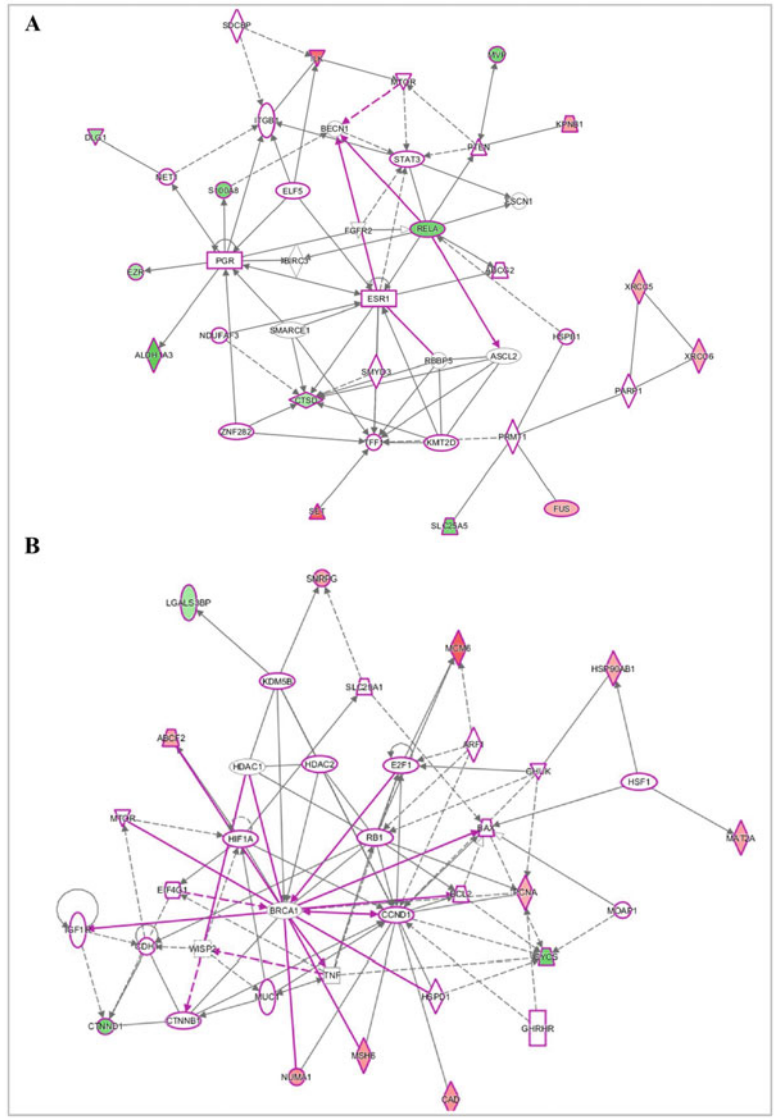


Fig. 16.19 IPA Networks of proteins found up- or down-regulated in both Luminal A and Claudin-Low breast cancer cell lines. The up- and down-regulated proteins are represented by molecules in *red* and *green* color, respectively. (**a**) Interactome related to cellular development, cellular growth and proliferation, cellular movement, cell death and survival, and cancer. (**b**) Interactome involved in cell death and survival, cellular development, DNA replication, recombination and repair, cancer and hereditary disorder

datasets. In this analysis, we had three datasets to compare (Fig. 16.20b) and only considered proteins found in all three datasets. We found four candidate biomarkers common between the luminal A and Claudin-low cells falling into different biomarker application categories: unspecified application (↑KHSRP protein found in nucleus and ↓S100A8 with cytoplasmic localization), diagnosis, efficacy (↓RELA localized in nucleus and ↑STMN1 found in cytoplasm) RELA was also found related to the drug NF-kappa B decoy (Fig. 16.21). All proteins were found in blood and all are related to cancer; however, they are not unique to this disease, as they are found in other diseases.

16.8 Protein-Protein Interactions Databases

16.8.1 STRING

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database of

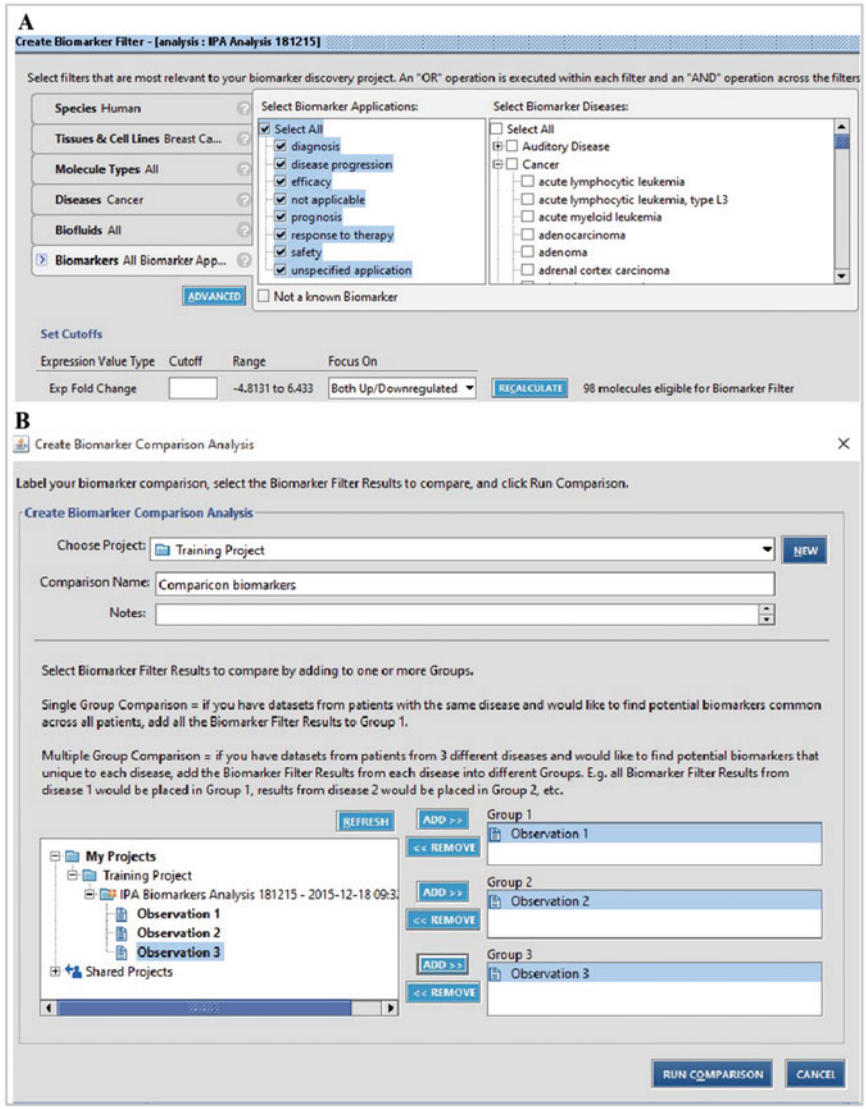


Fig. 16.20 Filter parameters for biomarker analysis in IPA software. (**a**) Creating a filter for putative biomarkers. (**b**) Comparison analysis between all observations (MCF7, T47D and MDA-MB-231)

known and predicted protein interactions [31]. This database was developed by the Center for Protein Research (CPR), The European Molecular Biology Laboratory (EMBL), The Swiss Institute of Bioinformatics (SIB), The University of Copenhagen (KU), The Technische Universität Dresden (TUD), and The Universität Zürich (UZH). STRING version 10.0 has 9,643,763 proteins from 2031 organisms. The main objective of this database is to integrate, predict and unify several protein-protein interactions [31, 32]. Associations between proteins can be physical (direct) or functional (indirect). The functional associations are defined as the interaction between two proteins that participate or contribute in the same cellular process or metabolic pathway, as well as other functional processes [32–34].

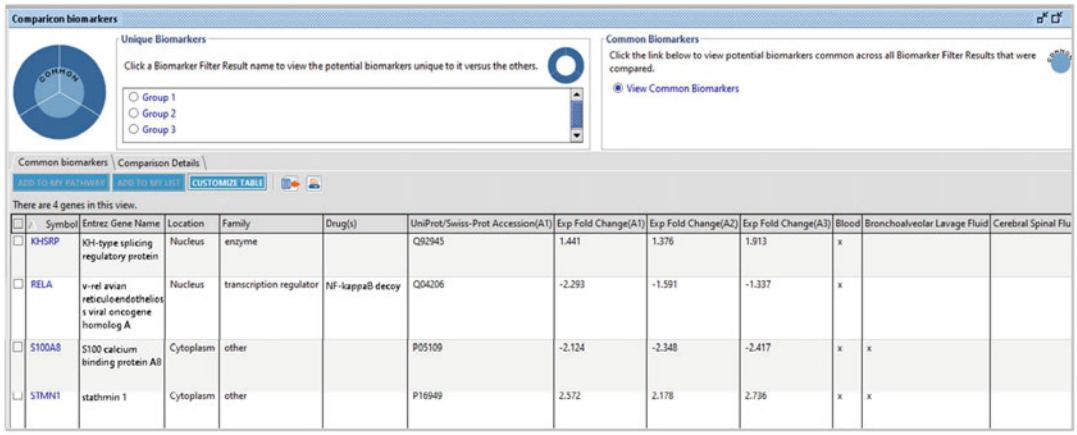


Fig. 16.21 Result of biomarker filter. Figure shows the four common biomarkers between. Luminal A and Claudin-low breast cancer cell lines

STRING database uses the following type of information to predict possible interaction:

1. Genomic data
2. High throughput experiments
3. Co-expression
4. Data extracted from literature

STRING import knowledge about protein-protein interactions from other databases such as IntAct, MINT, BioGRID, Reactome, KEGG, BIND, HPRD, DIP, NCI-Nature Pathway Interaction, GO, and EcoCyc [33]. In addition, STRING has a large collection of predicted interactions that are produced *de novo* using prediction algorithms [33, 35]. *De novo* predictions are made using genomic context such as conserved genomic neighborhood, gene fusion events, and co-occurrence of genes across the genome [34]. STRING also performs searches for genes with similar transcriptional response through a variety of conditions (co-expression) [33]. Information extracted from literature is another source used to extract protein association information from. In this case, STRING obtains information from all abstracts in PubMed database directly [36]. Finally, STRING assigns a probabilistic confidence score to all associations obtained through comparison of the association predictions against a reference database. STRING uses the KEGG database because this is manually curated [32, 37].

STRING website is composed of two components, the first component deals with protein analysis and the second covers the platforms (Fig. 16.22). The window of results displays the networks of protein-protein associations. The resulting interactome is represented by connecting lines. Each one of these lines represents different types of evidence. Networks can be viewed in three forms:

1. Evidence view in which connections are color coded as follows, neighborhood (green), gene fusion (red), co-occurrence (blue), co-expression (black), experiments (purple), database (light blue), text mining (yellow), and homology (gray)
2. Confidence view in which the thickness of connecting lines correlates with the strength of the associations
3. Interaction view in which the type of interactions is color coded as follows; activation (brilliant green), inhibition (red), binding (blue), phenotype (brilliant blue), catalysis (purple), posttranslational modifications (lilac), reaction (black) and expression (olive green)

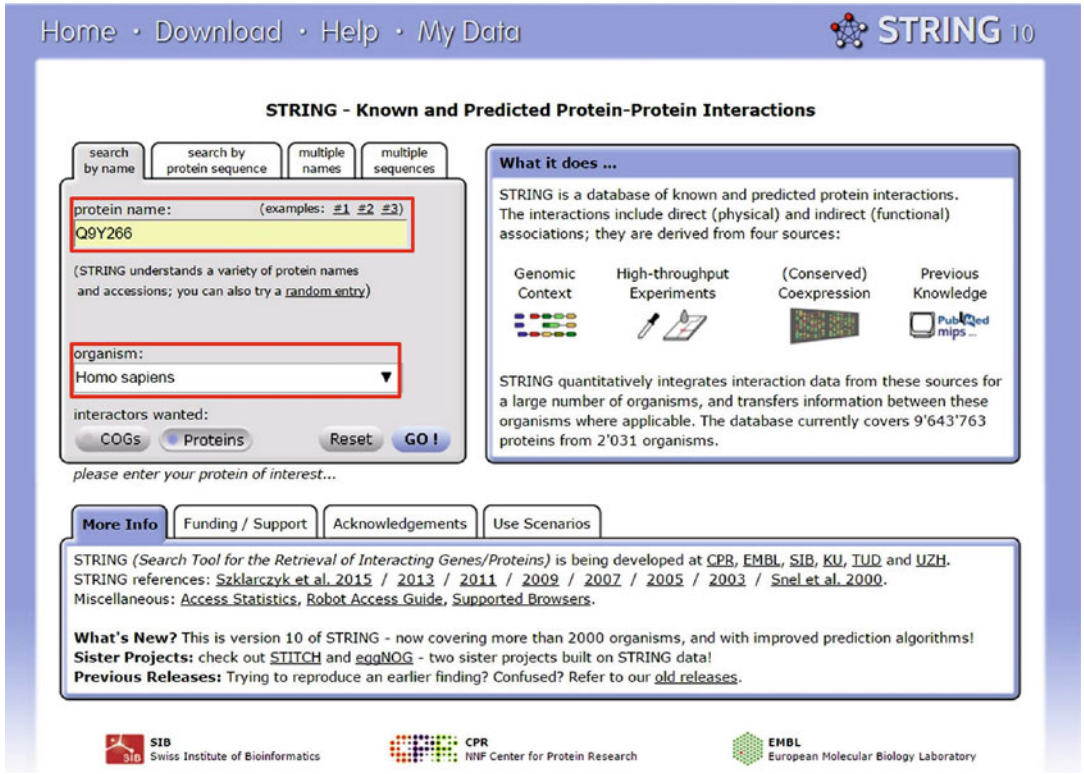


Fig. 16.22 STRING window view. The STRING webpage has different options to perform interaction analysis. The search can be done by the name of the protein or a protein sequence. The analysis can be performed for multiple proteins in the same way. In addition, the main page has various tabs with information about this platform

STRING has also an interactive view. In this option the network can by reordered by moving the proteins in the network. In advanced option, the network can be enriched into a GO Biological Processes, GO Molecular functions, GO Cellular components, KEGG Pathways, PFAM domains, INTERPRO domains, and Protein- Protein interactions. In each enrichment category, a new window is displayed containing a list of interactors, which contains different processes, the number of proteins involved as well as a p-value.

16.8.2 Protein-Protein Interaction Networks

To determine the protein-protein interaction of overexpressed NUDC protein exclusively found in Claudin-low breast cancer cell line [18], we accessed the STRING website http://string-db.org/.

To generate a network of protein interactions, a list (one or more) of protein names, accession number, or sequence, as well as the organism or species they originated from, need to be specified (Fig. 16.22). At the bottom of the result window there is a parameter box. The options in the parameter box are used to select the active prediction algorithm. The confidence score as well as the number of interactors can be adjusted as well (Fig. 16.23). The interactome can be seen according to evidence (Fig. 16.24a), confidence (Fig. 16.24b) and action (Fig. 16.24c). In each network, a score is generated according to each protein's interaction evidence. In addition, a brief description for each protein is also displayed (Fig. 16.24). NUDC protein is associated with PAFAH1B1

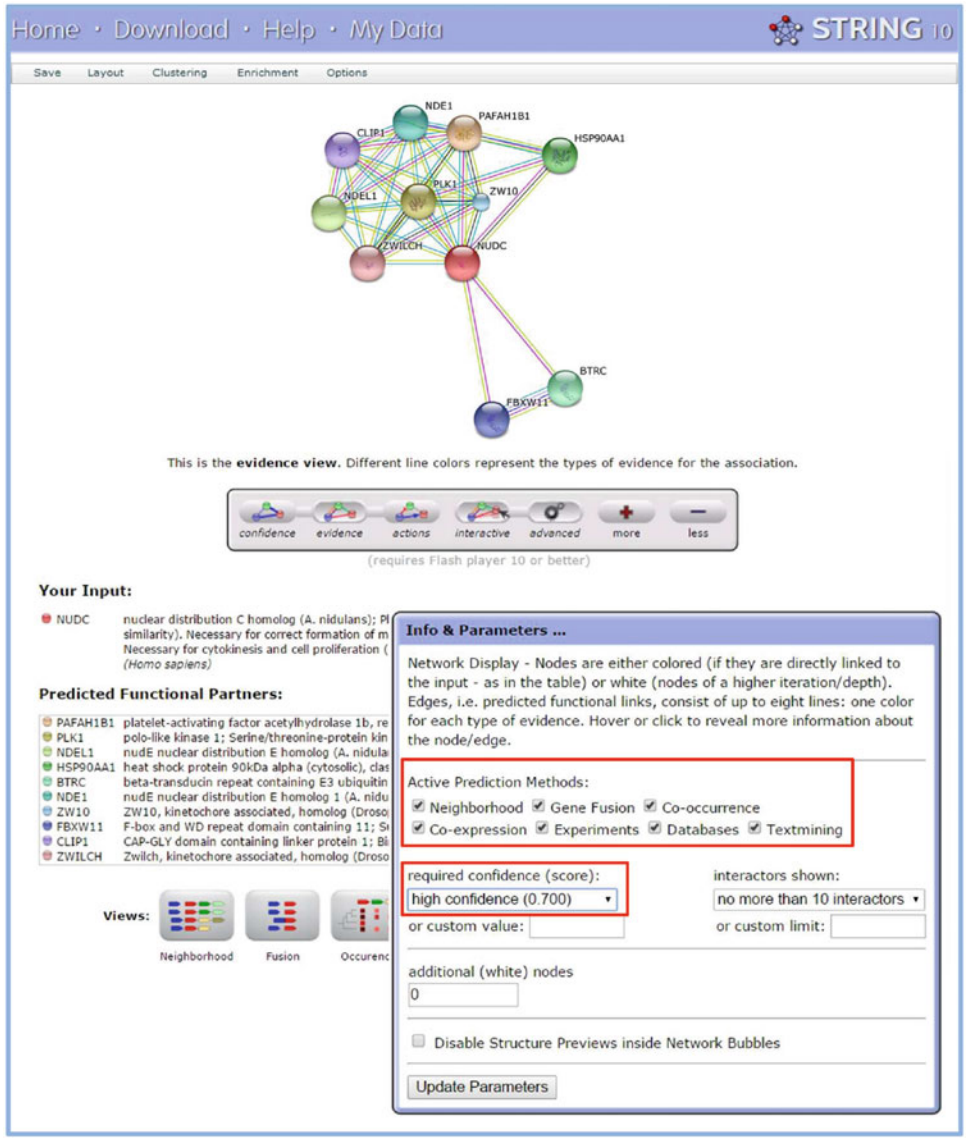


Fig. 16.23 STRING results view. A window containing different parameters is shown at the *bottom*. The active prediction methods as well as the confidence of the interactions in the network can be selected in this window

(platelet-activating factor acetylhydrolase 1b), PLK1 (polo-like kinase 1), NDEL1 (nudE nuclear distribution E homolog (A. nidulans)-like 1), HSP90AA1 (heat shock protein 90 kDa alpha), BTRC (beta-transducin repeat containing E3 ubiquitin protein ligase), NDE1 (nudE nuclear distribution E homolog 1 (A. nidulans)), ZW10 (ZW10, kinetochore associated, homolog (Drosophila), FBXW11 (F-box and WD repeat domain containing 11), CLIP1 (CAP-GLY domain containing linker protein 1) and ZWILCH (Zwilch, kinetochore associated, homolog (Drosophila)). All interactions have more than 0.90 score. In

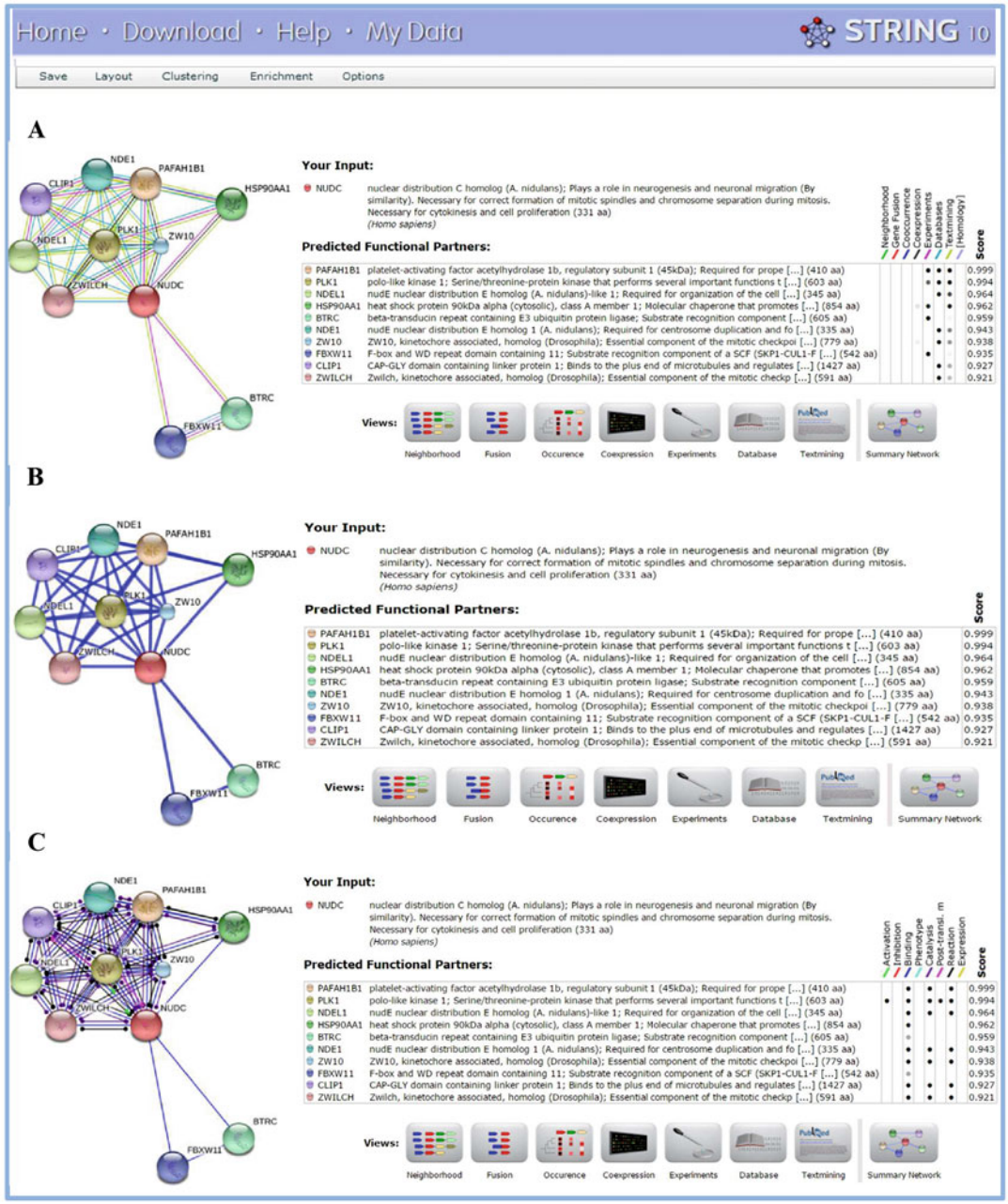


Fig. 16.24 Interaction network of NUDC protein. This polypeptide is overexpressed exclusively in Claudin-low breast cancer cell line. The interactome can be seen in three options. (**a**) Evidence view, where the color lines represent the diverse evidences of interactions: *Green*, neighborhood; *red*, gene fusion; *blue*, co-occurrence; *black*, co-expression; *purple*, experiments; *light blue*, database; *yellow*, text mining; *gray*, homology. (**b**) Confidence view where thicker lines represent stronger associations. (**c**) Interaction view, where the different modes of action are represented by different colors. *Brilliant green*, activation; *red*, inhibition; *blue*, binding; *brilliant blue*, phenotype; *purple*, catalysis, lilac, PTMs; *black*, reaction; *olive green*, expression. The three view modes provide a score of the different evidence of interaction

addition, the network was enriched into GO Biological Processes. Processes showed Enrichment with statistical significance were:

1. Mitotic prometaphase (4.940×10^{-13})
2. Mitotic anaphase (8.089×10^{-12})
3. Mitotic M phase (6.309×10^{-11})
4. M phase (6.309×10^{-11})
5. Mitotic cell cycle phase (4.300×10^{-10})
6. Cell cycle phase (4.300×10^{-10})

All processes mentioned above have at least eight proteins involved. We selected the cell cycle phase process as an example. The proteins enriched in this process are shown in color red (Fig. 16.25a). We selected the interacting proteins NUDC and ZW10 as examples to extract interaction information. ZW10 was selected because it is an essential component of the mitotic checkpoint that prevents cells from prematurely exiting mitosis. The evidence supporting the functional link between these two proteins are the following:

1. Co-expression (putative homologs are co-expressed in other species, score 0.065)
2. Association in curated database (score 0.900)
3. Co-mentioned in PubMed abstracts (score 0.285)

Also putative homologs are mentioned together in other species (score 0.192). The combined score is 0.938. There is also activity evidence, such as catalysis (score 0.900), binding (score 0.900) and reaction (score of 0.900) that support the interaction between these two proteins (Fig. 16.25b). For proteins selected in a network, STRING displays a window with information about their 3D structure, as well as links to Ensembl, GeneCards, KEGG, Nextprot and UniProt. Also, STRING can show the protein sequence and the sequence of its homologs in organisms stored in STRING. NUDC has three 3D structures obtained from Protein DataBase (PDB) (Fig. 16.25c). As mentioned above, STRING can perform network analysis for multiple proteins as well. We performed an interactome analysis for the up- and down-regulated proteins common in Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cells lines [18]. In this case, we used the highest confidence (0.900) possible to generate our interaction network. The network has several interaction nodes related to:

1. Energy metabolism
2. Translation
3. Proteasome
4. Replication and repair
5. Transcription

Red and green arrows indicate up- and down-regulated proteins, respectively (Fig. 16.26).

16.8.3 MINT

The Molecular INTeraction database or MINT is an open source protein-protein interaction database developed at the Università degli Studi di Roma Tor Vergata that has been experimentally verified [38, 39]. The webpage can be found at http://mint.bio.uniroma2.it/mint/Welcome.do (Fig. 16.27). The current version of MINT database (November 2015) contains 241,458 interactions, corresponding to 35,553 proteins and 5554 PMIDS (PubMed unique identifiers). Species included are *Drosophila melanogaster, Saccharomyces cerevisiae, Caenorhabditis elegans*, mammals and viruses, with mammal databases being the main datasets. Evidences for protein-protein interactions include association studies, co-localization, direct interactions, interactions in form of complexes, enzymatic reactions, and high throughput studies. Protein-protein interactions have been identified by a number of methods including co-immunoprecipitation with either anti-bait or anti-tag antibodies, fluorescence microscopy, peptide arrays, protein arrays, pull down experiments, SPR, tandem affinity isolation, two hybrid arrays, two hybrid pooling, and two hybrid systems, etc. Additionally, the MINT database is freely available for academic and commercial users.

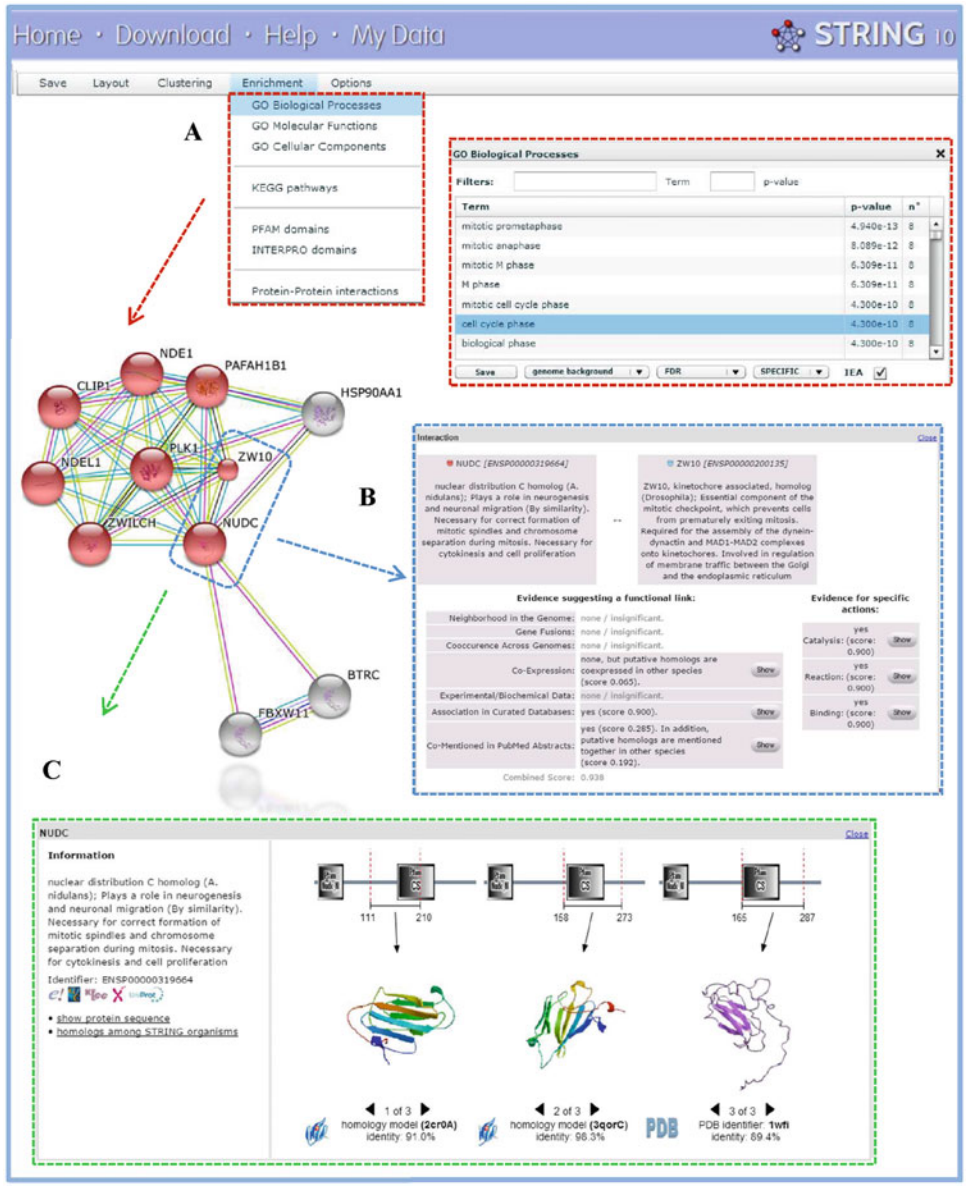


Fig. 16.25 Interaction network of NUDC overexpressed protein found exclusively in Claudin- low breast cancer cell line. STRING platform provides different information for the generated network. (**a**) Network enrichment for GO Biological Processes. The proteins in *red* which have a statistical significance (p-value) are involved in cell cycle phase. (**b**) Evidence supporting interaction between NUDC and ZW10. (**c**) 3D protein structure information

There are three additional databases available via MINT website including HomoMINT, Domino, and VirusMINT. The first one is an inferred network for human; the second is specialized in domain-peptide interactions, and the last is a protein-protein interaction database specialized on viruses.

Protein interaction searches in MINT database (Fig. 16.28a) can be carried out using PubMed ID, D.O.I, or author's name. Alternatively, this

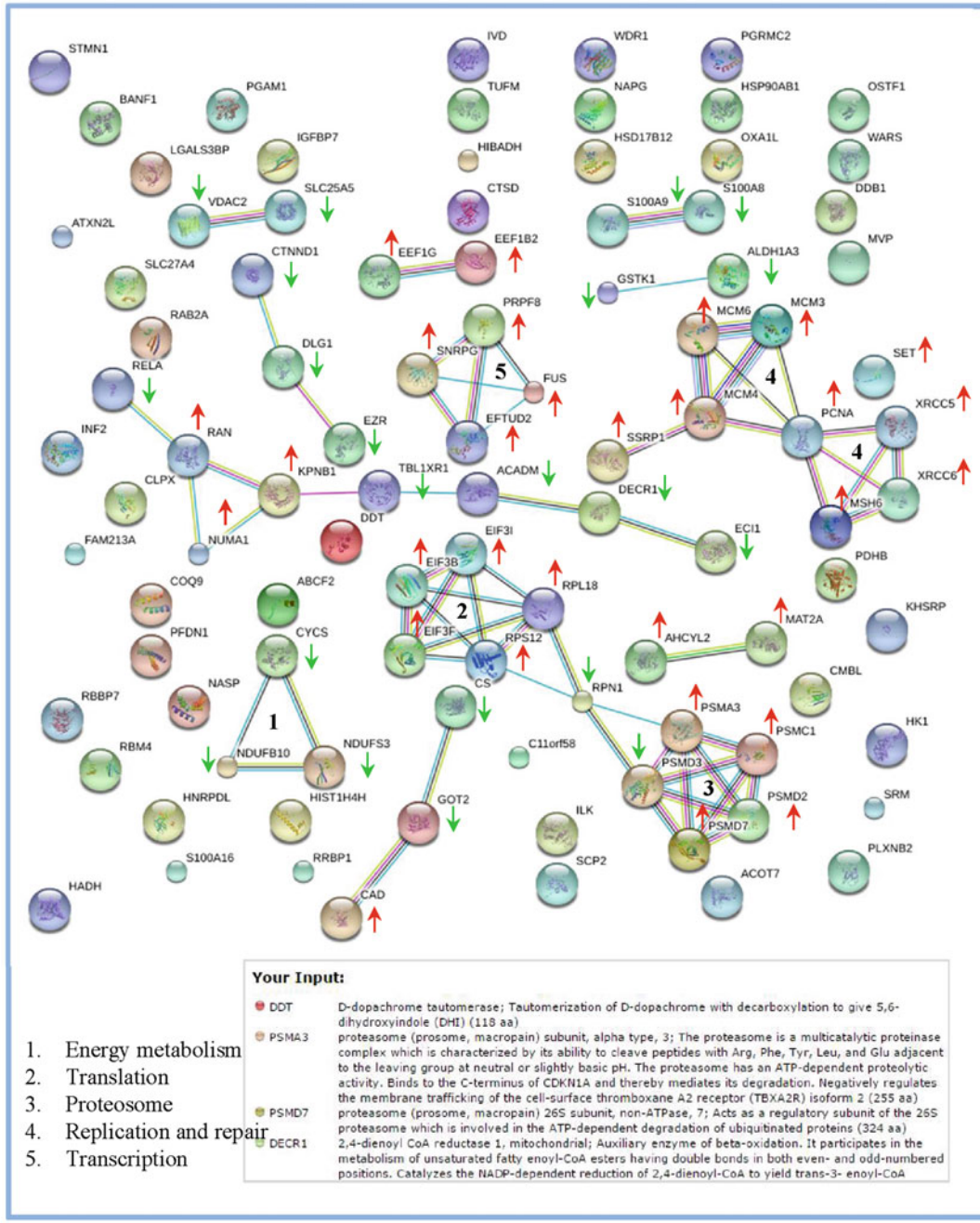


Fig. 16.26 STRING interaction network of proteins found up- or down-regulated in both Luminal A (MCF7 and T47D) and Claudin-low (MDA-MB-231) breast cancer cell lines. This list has interaction nodes related to: (*1*) Energy metabolism, (*2*) Translation, (*3*) Proteosome degradation, (*4*) Replication and repair, (*5*) Transcription. Colored lines represent different evidence of interaction: *Green*, neighborhood; *red*, gene fusion; *blue*, co-occurrence; *black*, co-expression; *purple*, experiments; *light blue*, database; *yellow*, text-mining; *gray*, homology. *Red arrows* indicate up-regulation and *green arrows* down-regulation. A box with information about some proteins is also shown

Fig. 16.27 Homepage of the Molecular INTeraction database, MINT

database can be searched against protein or gene name, protein accession number (Protein AN) or keywords. Protein accession numbers recognized by MINT search engine are FlyBase, Ensembl, Human Identified Gene Encoded Large Protein Analyzed database (HUGE), Nematode database (WormBase), OMIM, REACTOME pathway database, the *Saccharomyces* Genome Database (SGD), and Universal Protein Resource Knowledgebase (UniProtKB).

To demonstrate how MINT database works, we selected the vesicle-fusing ATPase NSF (P46459) for analysis. This protein is part of a set of proteins that were found overexpressed in several breast cancer cell lines [18]. To follow our analysis, click on the Search tab and type P46459 (Fig. 16.28, arrow 1) and then select the organism (Fig. 16.28, arrow 2) and then press the Search key (Fig. 16.28, arrow 3). Results show certain information for the queried protein including its ID, species, synonyms, domains found in query, a link to its role in diseases, its gene ontology, references covering the target protein, prediction of its modular domain interactions (ADAN), and its orthologs in MINT database (Fig. 16.28). Results also display a window containing a list of molecules interacting with the target according to MINT database, evidence for each interaction and a global score for each interaction (Fig. 16.28).

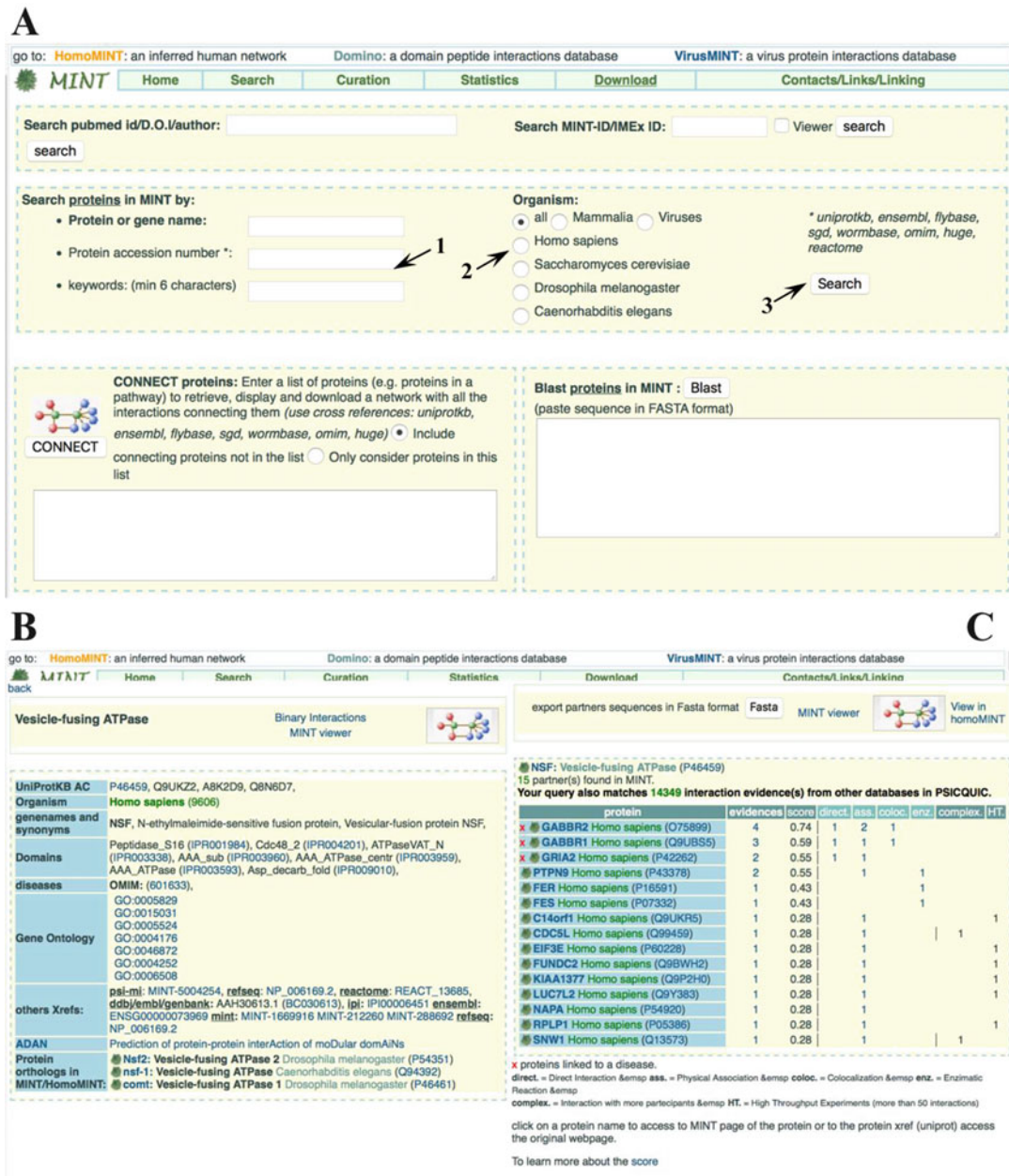


Fig. 16.28 MINT search webpage. (**a**) Search in MINT can be performed using: (*1*) Gene or protein name, Protein ID or keywords and the species of interest or the whole database, (*2*) Protein sequence in FASTA format, (*3*) a list of proteins. (**b**, **c**) Result of a query for vesicle-fusing ATPase NSF from Homo sapiens (UniProtKB/Swiss-Prot ID P46459). (**c**) List of NSF interactors are shown

Clicking on the MINT viewer will generate a list of interactions that are displayed as a function of score threshold. For each partner, a number showing evidence for interaction is shown (Fig. 16.29). As an example, we clicked on number 4 and a new window appeared showing the partner name, ID, and techniques used to determine the interaction, as well as a PubMed identifier containing this information (Fig. 16.29).

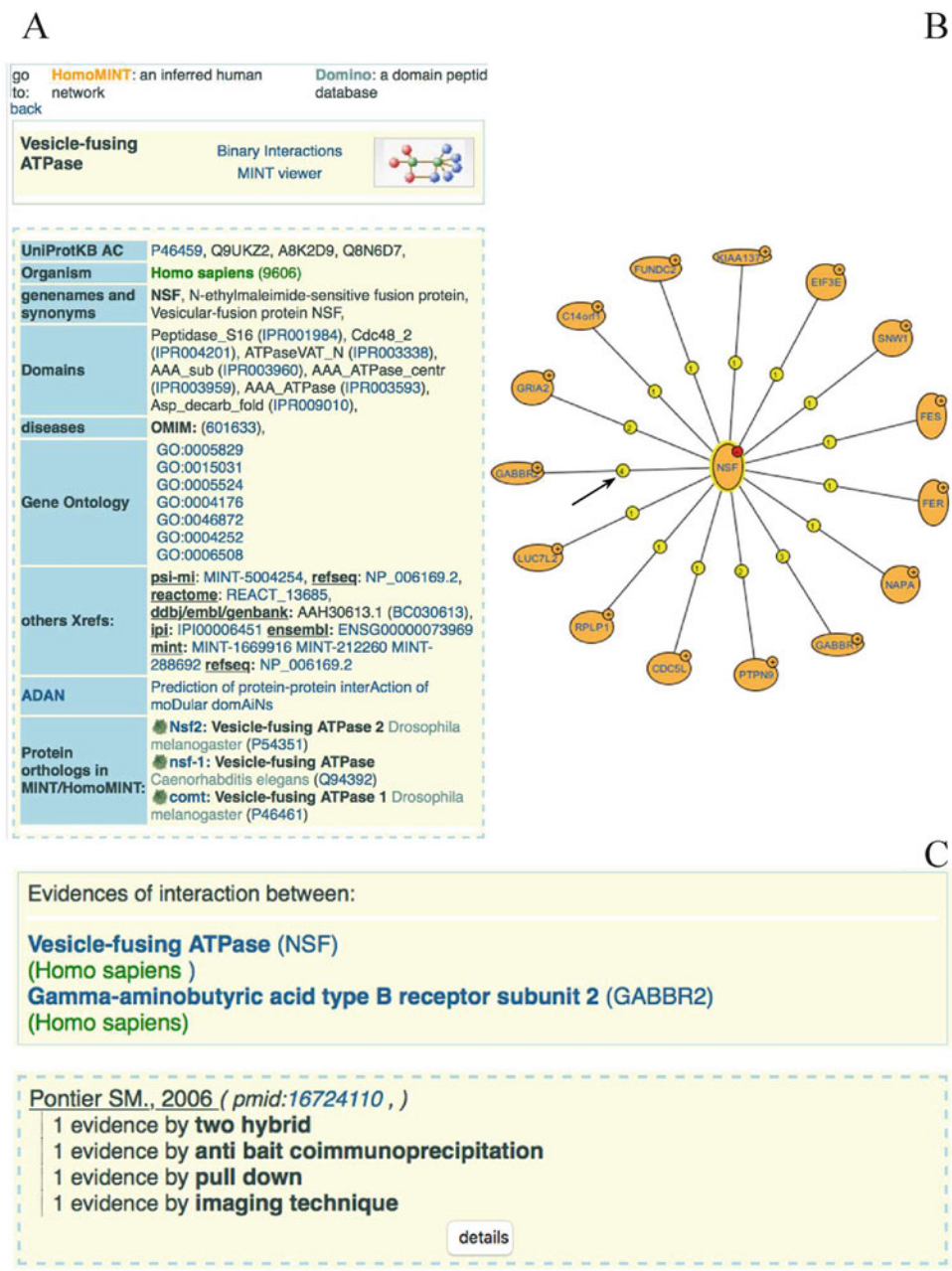


Fig. 16.29 Binary interactions of the N-ethylmaleimide-sensitive fusion protein NSF viewed in MINT database. (**a**) Basic information queried for NSF. (**b**) Binary interaction map of NSF with 15 interactors found in MINT database. (**c**) Selecting number 4 in (**b**), a new window is displayed showing the name of the corresponding interactor (GABBR2, Gamma-aminobutyric acid type B receptor subunit 2) and the experimental methods used to determine this interaction, as well as the PMID ID for the publication describing it

16.8.4 IntAct

IntAct is a database of protein-protein interactions, as well as a suite of analytical tools at The European Bioinformatics Institute (EBI), which is part of the European Molecular Biology Laboratory (EMBL) [40, 41]. All information has been curated by experts at the IntAct team.

This freely available database can be accessed through its webpage http://www.ebi.ac.uk/intact/.

As of November 26th, 2015 this database had registered 355,819 interactions, which included 89,340 interactors (proteins) described in 36,864 experiments, 13,892 PMIDs, and 564,831 binary interactions. Methods used for the determination of protein-protein interactions include tandem affinity purification, anti-tag co-immunoprecipitation, two hybrid systems, pull down experiments, two hybrid arrays, anti-bait co-immunoprecipitation, two hybrid pooling approach, and co-sedimentation, among others. The source of information mainly comes from human (42.5 %), various *S. cerevisiae* strains (22.8 %), *Mus musculus* (11.3 %), and *D. melanogaster* (8.1 %). Other species included are *Escherichia coli, C. elegans, A. thaliana, Campylobacter jejuni*, etc. MINT and IntAct databases have recently joined their individual efforts to optimize resources as the MIntAct project, thus avoiding duplication of activities [42].

IntAct model has three main components, interactions, interactors, and experiments used to determine interactions. Protein interactions are inferred using scientific publications, including binary interactions or complexes. An interactor can be defined as a biological molecule (mainly a protein) involved in a specific interaction. An interaction is not circumscribed to binary interactions only; it also includes interactions with more partners identified in the experiment performed, e.g. precipitation of multi-protein complexes. Search in IntAct database can be performed in different ways, including name of gene, protein, RNA or chemical compound, or UniProtKB, ChEBI (Chemical Entities of Biological Interest), RNA Central, PMID or IMEx (International Molecular Exchange) IDs. The principal page of IntAct (Fig. 16.30) contains links to other websites the might be of interest. These sites include MINT, UniProtKB, The Swiss Institute of Bioinformatics (SIB), The Interologous Interaction Database (I2D), The Innate Immune Response Database (Innate Database), Molecular Connections, The Extracellular Matrix Interactions Database (MatrixDB), The Modular Approach to Cellular Functions Resource (MB Info), a curated resource for functional analysis of agricultural plant and animal gene products (AgBase), and The cardiovascular Gene Annotation database at the London's Global University (UCL).

As an example of the function of IntAct, we selected the protein XRCC6 (X-ray repair cross-complementing protein 6, UniProtKB ID P12956), which was found overexpressed in both Luminal A and MDA-MB-231 breast cancer cell lines [18]. This protein is a single-stranded DNA-dependent and ATP-dependent 3′–5′ DNA helicase involved in DNA non-homologous end joining (NHEJ) required for double-strand break repair and V(D)J recombination. To reproduce our analysis, in the search window (Fig. 16.30) type XRCC6 or P12956 ID and push the search key. A new window will appear on screen with the results for your query (Fig. 16.31). There are 324 binary interaction found for XRCC6 protein up to date. These interactions are displayed as a table, where molecule A is your query or bait, and B molecules are proteins interacting with your query. For each interaction, a list of interaction methods used for the determination of such interactions is shown, their corresponding IDs, and the source database as well. When you click on the interactors tab, a new page will be shown containing a list of all interactors, showing the type of interactor, the number of interactions described, a link to access the description in UniProtKB, and a description of the interaction (Fig. 16.32). More information, including interactions described, the

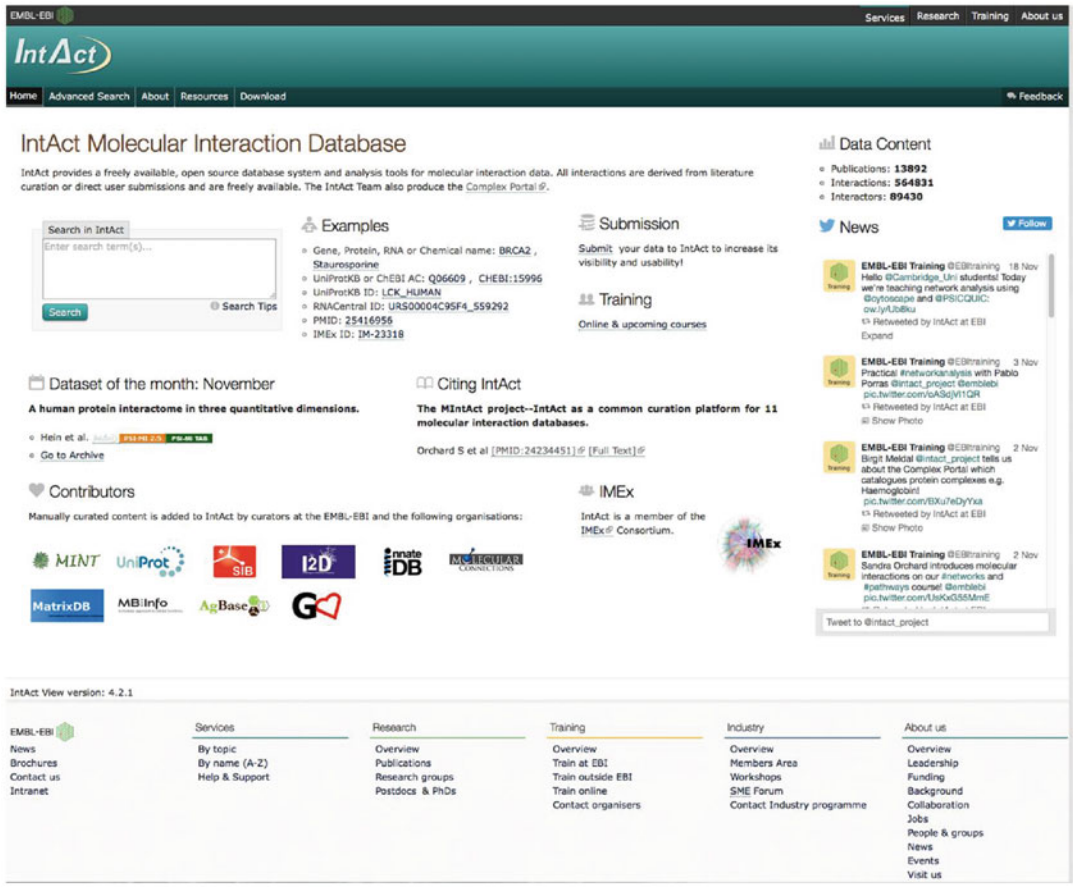


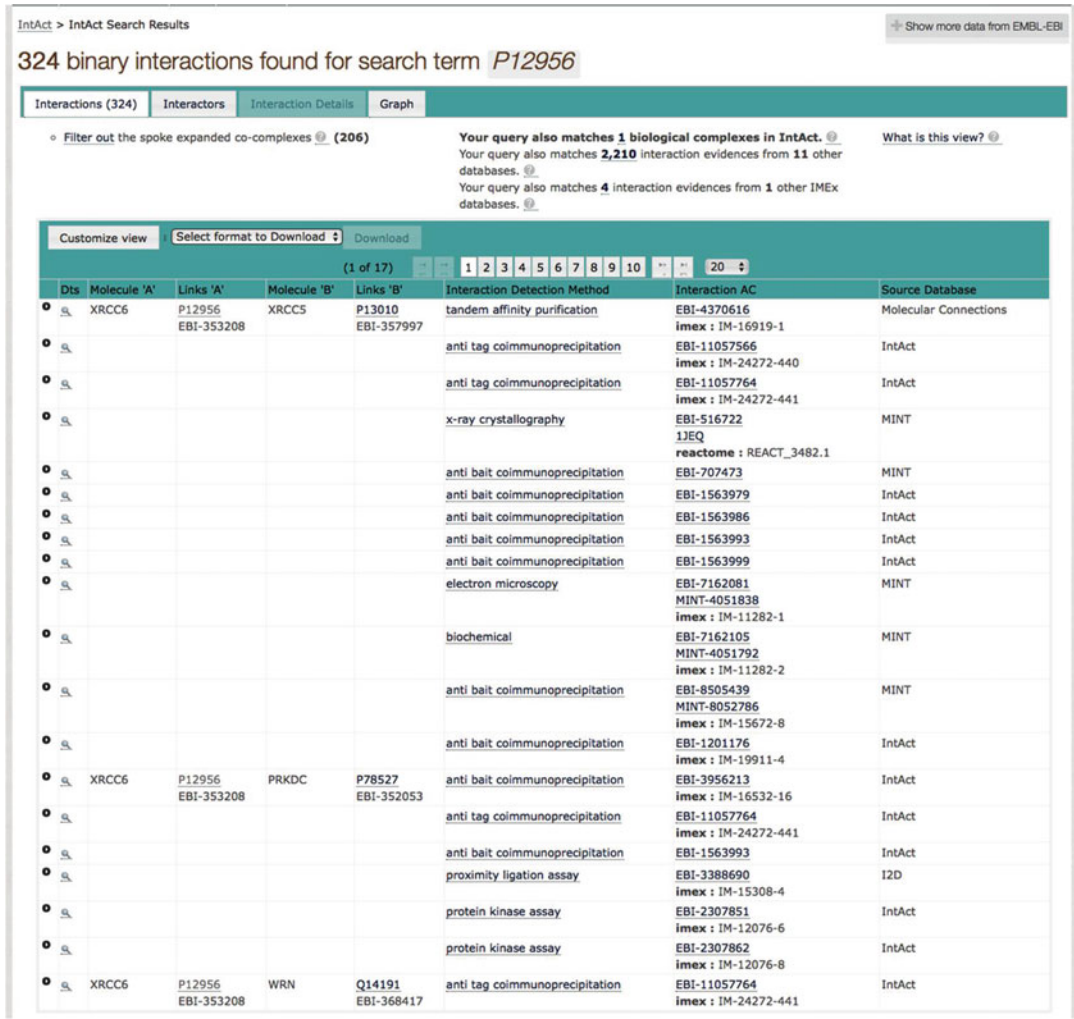
Fig. 16.30 Homepage of the IntAct Molecular Interaction Database

chromosome location in Ensembl webpage, the mRNA expression for interactor in the Expression Atlas webpage, and pathways is displayed when interactors are searched separately. The map of interactions for your query can be displayed in three layouts, force directed (Fig. 16.33), radial (Fig. 16.34) or circle (Fig. 16.35). In all cases, you can zoom in the graph with the tool window at the bottom.

Search can also be performed for a list of identifiers. The result will be more complex as all interactions for each member of your list will be shown. As an example, we only show the graph for ten proteins overexpressed in Luminal A and MDA-MB-231 breast cancer cell lines [18], where a total of 1101 binary interactions were found in database (Figs. 16.36, 16.37 and 16.38).

16.8.5 HPRD

The Human Protein Reference Database (HPRD) is a free web resource containing information of human proteins, including an information summary for each protein, their PTMs, protein-protein interactions, expression levels in tissues, mRNA and protein sequences, non-protein interactions, alternate names, participation in diseases, and domains found in proteins. All the information stored in this database is curated by a group of expert biologists from the Pandey Lab at Johns Hopkins University and the Institute of Bioinformatics in Bangalore, India [43]. The current version of HPRD is 9. It contains information for 30,047 proteins, 41,327 protein-protein interactions, 93,710 PTMs, 112,158



IntAct > IntAct Search Results

Show more data from EMBL-EBI

324 binary interactions found for search term *P12956*

Interactions (324) | Interactors | Interaction Details | Graph

Filter out the spoke expanded co-complexes (206)

Your query also matches 1 biological complexes in IntAct.
Your query also matches **2,210** interaction evidences from **11** other databases.
Your query also matches **4** interaction evidences from **1** other IMEx databases.

What is this view?

Customize view | Select format to Download | Download

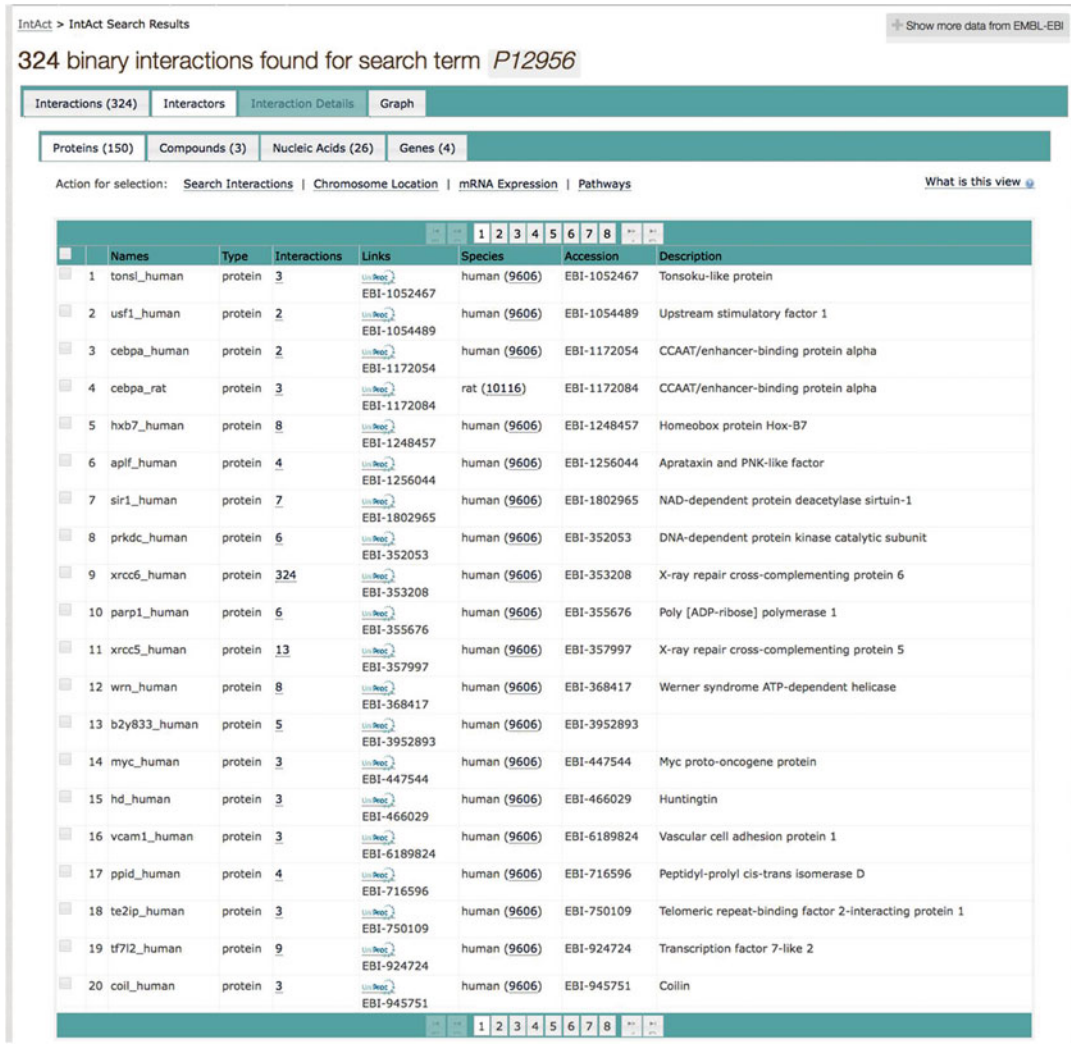
(1 of 17) 1 2 3 4 5 6 7 8 9 10 20

Dts	Molecule 'A'	Links 'A'	Molecule 'B'	Links 'B'	Interaction Detection Method	Interaction AC	Source Database
	XRCC6	P12956 EBI-353208	XRCC5	P13010 EBI-357997	tandem affinity purification	EBI-4370616 **imex :** IM-16919-1	Molecular Connections
					anti tag coimmunoprecipitation	EBI-11057566 **imex :** IM-24272-440	IntAct
					anti tag coimmunoprecipitation	EBI-11057764 **imex :** IM-24272-441	IntAct
					x-ray crystallography	EBI-516722 1JEQ **reactome :** REACT_3482.1	MINT
					anti bait coimmunoprecipitation	EBI-707473	MINT
					anti bait coimmunoprecipitation	EBI-1563979	IntAct
					anti bait coimmunoprecipitation	EBI-1563986	IntAct
					anti bait coimmunoprecipitation	EBI-1563993	IntAct
					anti bait coimmunoprecipitation	EBI-1563999	IntAct
					electron microscopy	EBI-7162081 MINT-4051838 **imex :** IM-11282-1	MINT
					biochemical	EBI-7162105 MINT-4051792 **imex :** IM-11282-2	MINT
					anti bait coimmunoprecipitation	EBI-8505439 MINT-8052786 **imex :** IM-15672-8	MINT
					anti bait coimmunoprecipitation	EBI-1201176 **imex :** IM-19911-4	IntAct
	XRCC6	P12956 EBI-353208	PRKDC	P78527 EBI-352053	anti bait coimmunoprecipitation	EBI-3956213 **imex :** IM-16532-16	IntAct
					anti tag coimmunoprecipitation	EBI-11057764 **imex :** IM-24272-441	IntAct
					anti bait coimmunoprecipitation	EBI-1563993	IntAct
					proximity ligation assay	EBI-3388690 **imex :** IM-15308-4	I2D
					protein kinase assay	EBI-2307851 **imex :** IM-12076-6	IntAct
					protein kinase assay	EBI-2307862 **imex :** IM-12076-8	IntAct
	XRCC6	P12956 EBI-353208	WRN	Q14191 EBI-368417	anti tag coimmunoprecipitation	EBI-11057764 **imex :** IM-24272-441	IntAct

Fig. 16.31 List of binary interactions found for XRCC6 (the X-ray repair cross-complementing protein 6 from *Homo sapiens*, UniProtKB/Swiss-Prot ID P12956) in IntAct database. A total of 324 interactions were found for this protein

sites of protein expression, 22,490 sites of intracellular localization, 470 domains, and 453,521 PMIDs. In addition, two other applications have been recently added, the PhosphoMotif Finder and NetPath resources, which allow the identification of phosphorylation motifs for known kinases/phosphatases and binding motifs for phospho serine/threonine or phospho tyrosine in a compendium of signaling pathways in humans [43].

To perform a search, click on the Query key, type your query and push the Search button on the upper left part on screen (Fig. 16.39, arrow). There are several options for a query, including Protein Name, Accession Number (RefSeq, GenBank, OMIM, UniProtKB and Entrez Gene Name), HPRD identifier, Gene Symbol, Chromosome locus, Molecular Class (e.g. Nuclease, Serine Proteinase, Translation Regulatory protein, Glycosylase, etc.), PTMs (e.g. ADP



IntAct > IntAct Search Results

Show more data from EMBL-EBI

324 binary interactions found for search term *P12956*

Interactions (324) | Interactors | Interaction Details | Graph

Proteins (150) | Compounds (3) | Nucleic Acids (26) | Genes (4)

Action for selection: Search Interactions | Chromosome Location | mRNA Expression | Pathways

What is this view

1 2 3 4 5 6 7 8

	Names	Type	Interactions	Links	Species	Accession	Description
1	tonsl_human	protein	3	EBI-1052467	human (9606)	EBI-1052467	Tonsoku-like protein
2	usf1_human	protein	2	EBI-1054489	human (9606)	EBI-1054489	Upstream stimulatory factor 1
3	cebpa_human	protein	2	EBI-1172054	human (9606)	EBI-1172054	CCAAT/enhancer-binding protein alpha
4	cebpa_rat	protein	3	EBI-1172084	rat (10116)	EBI-1172084	CCAAT/enhancer-binding protein alpha
5	hxb7_human	protein	8	EBI-1248457	human (9606)	EBI-1248457	Homeobox protein Hox-B7
6	aplf_human	protein	4	EBI-1256044	human (9606)	EBI-1256044	Aprataxin and PNK-like factor
7	sir1_human	protein	7	EBI-1802965	human (9606)	EBI-1802965	NAD-dependent protein deacetylase sirtuin-1
8	prkdc_human	protein	6	EBI-352053	human (9606)	EBI-352053	DNA-dependent protein kinase catalytic subunit
9	xrcc6_human	protein	324	EBI-353208	human (9606)	EBI-353208	X-ray repair cross-complementing protein 6
10	parp1_human	protein	6	EBI-355676	human (9606)	EBI-355676	Poly [ADP-ribose] polymerase 1
11	xrcc5_human	protein	13	EBI-357997	human (9606)	EBI-357997	X-ray repair cross-complementing protein 5
12	wrn_human	protein	8	EBI-368417	human (9606)	EBI-368417	Werner syndrome ATP-dependent helicase
13	b2y833_human	protein	5	EBI-3952893	human (9606)	EBI-3952893	
14	myc_human	protein	3	EBI-447544	human (9606)	EBI-447544	Myc proto-oncogene protein
15	hd_human	protein	3	EBI-466029	human (9606)	EBI-466029	Huntingtin
16	vcam1_human	protein	3	EBI-6189824	human (9606)	EBI-6189824	Vascular cell adhesion protein 1
17	ppid_human	protein	4	EBI-716596	human (9606)	EBI-716596	Peptidyl-prolyl cis-trans isomerase D
18	te2ip_human	protein	3	EBI-750109	human (9606)	EBI-750109	Telomeric repeat-binding factor 2-interacting protein 1
19	tf7l2_human	protein	9	EBI-924724	human (9606)	EBI-924724	Transcription factor 7-like 2
20	coil_human	protein	3	EBI-945751	human (9606)	EBI-945751	Coilin

1 2 3 4 5 6 7 8

Fig. 16.32 List of binary interactions found for XRCC6 (the X-ray repair cross-complementing protein 6 from Homo sapiens, UniProtKB/Swiss-Prot ID P12956) in IntAct database. There are 150 proteins, three chemical compounds (XAV939, 15-deoxy-Delta(12,14)-prostaglandin J2 and Midostaurin), 26 nucleic acid molecules, and four genes (Klk3, kallikrein-related peptidase 3 encoding gene; Tmps2, Transmembrame protease serine 2). here only a list of 20 protein interactors is shown

Ribosylation, Glycation, Nitration, Sumoylation. Ubiquitination), Cellular Component, Domain Name, Motif, Expression Site, Length of Protein sequence, Molecular Mass, and Diseases (Fig. 16.40). To present an example, we searched NUMA1. Results are shown in Fig. 16.41. Information retrieved includes the name of protein (NUMA1 corresponds to the Nuclear mitotic apparatus protein 1, isoform 1), Molecular Class (Structural protein), Molecular Function (Structural molecule activity), and Biological Process (Cell growth and/or maintenance). Seven additional tabs are provided, which are Summary, Sequence, Interactions, External Links, Alternate Names, Diseases, PTMs, and Substrates. The General tab contains the

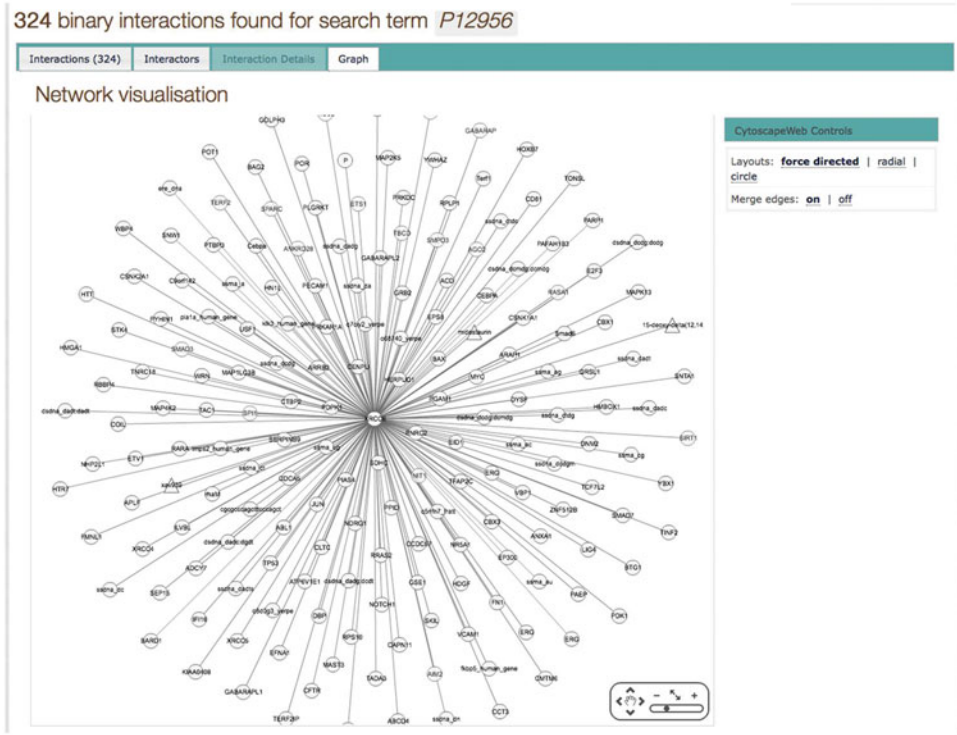


Fig. 16.33 Force-directed layout of the interaction map found for XRCC6 in IntAct database. XRCC6 protein is at the center of the map

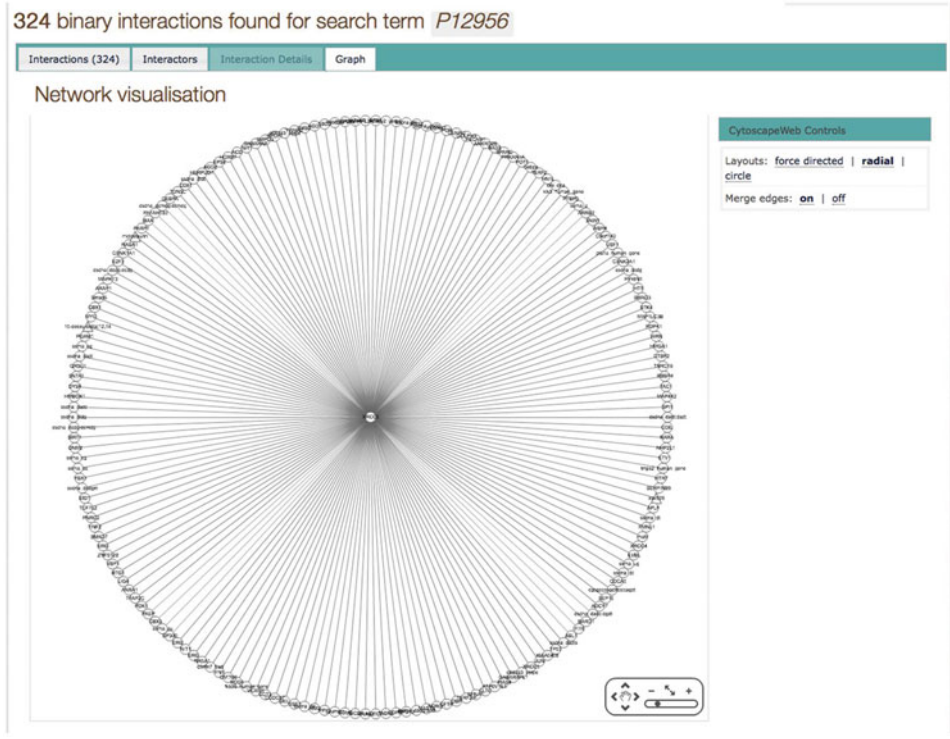


Fig. 16.34 Radial layout of the interaction map found for XRCC6 in IntAct database. XRCC6 protein query is at the center of the map

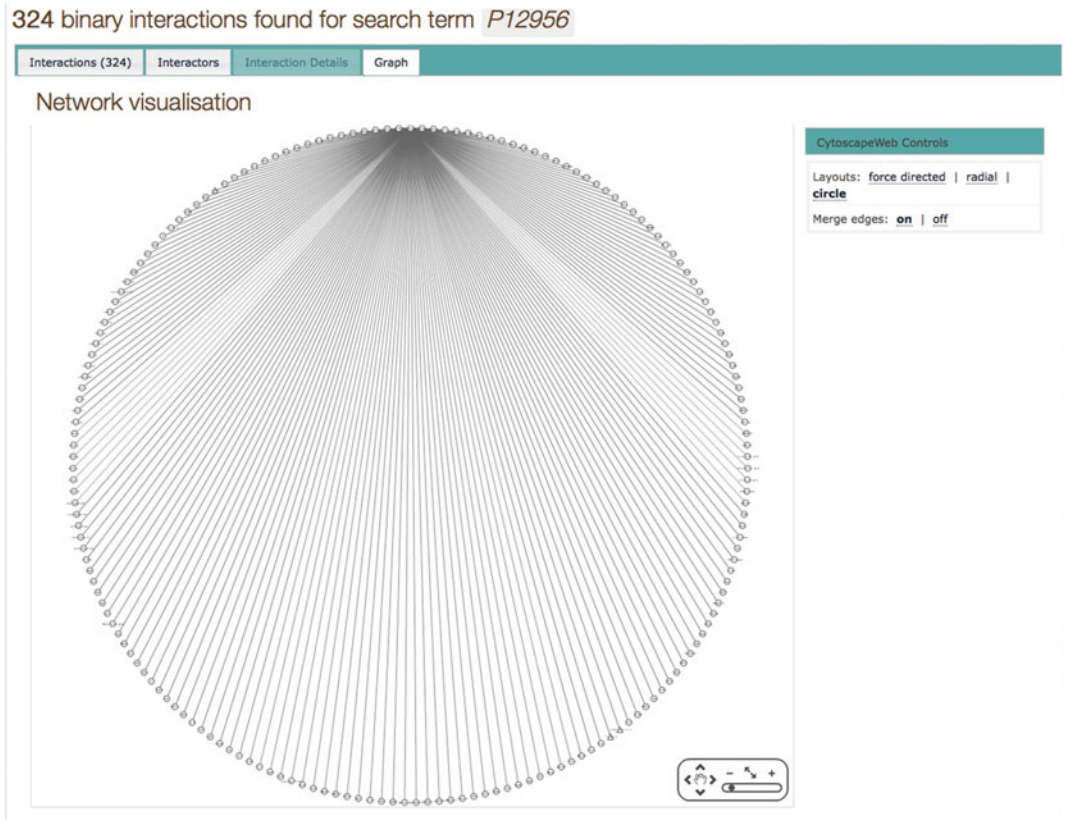


Fig. 16.35 Circle layout of the interaction map found for XRCC6 in IntAct database. XRCC6 protein query is located at the *top* of the map

corresponding HPRD ID 01236, Gene symbol NUMA1, Molecular Weight 238259 Da, Chromosome location 11q13, intracellular localization, domains and motifs, and sites of tissue gene expression (Fig. 16.41). The sequence of NUMA1 and its corresponding mRNA are obtained by clicking on Sequence tab (Fig. 16.42). A list of proteins that interact with NUMA1, and types of experiment and interactions (direct or in a complex) are shown in Fig. 16.43.

Alternatively, it is possible to search HPRD by browsing Molecule Class, Domains, Motifs, PTMs, and Localization by pushing the Browse key on the right of the main webpage (Fig. 16.39). Furthermore, access to Human Proteinpedia, Pathways, PhosphoMotif Finder, or downloading the complete HPRD are possible using the main menu.

16.8.6 BioGRID

The Biological General Repository for Interaction Datasets (BioGRID, http://thebiogrid.org), as many other protein-protein interactions databases, has as main goals to curate, organize and make it freely available. The funding partners of this important database are the National Institutes of Health (NIH), the

Fig. 16.36 Interaction map found for PSA3, SYWC, MCM4, SMAP, DDB1, EIF3, PYR1, MCM3, SSRP1 and METK2 proteins in IntAct database. Force directed layout of the network showing many more interactions that are contained in the IntAct database

Canadian Institutes of Health Research (CIHR), the Genome Canada, and GenomeQuébec. Many other institutions have joined efforts to BioGRID, including the Université de Montréal, Princeton University, Mount Sinai Hospital, University of Edinburgh, SGD, FlyBase, GeneDB, NCBI, WormBase, MaizeGDB, MINT, IntAct, String, MatrixDB, SIB, GO, UniProt, Reactome, Cytoscape, and many others that can be found in the BioGRID webpage. The current version of BioGRID database (3.4.131, December 2015) has information for several model organisms, including *A. thaliana, C. elegans, Candida albicans, Danio rerio, Dictyostellium discoideum, D. melanogaster, H. sapiens, Mus musculus, Neurospora crassa, Plasmodium falciparum, S. cerevisiae, Schizosaccharomyces pombe, Xenopus laevis*,

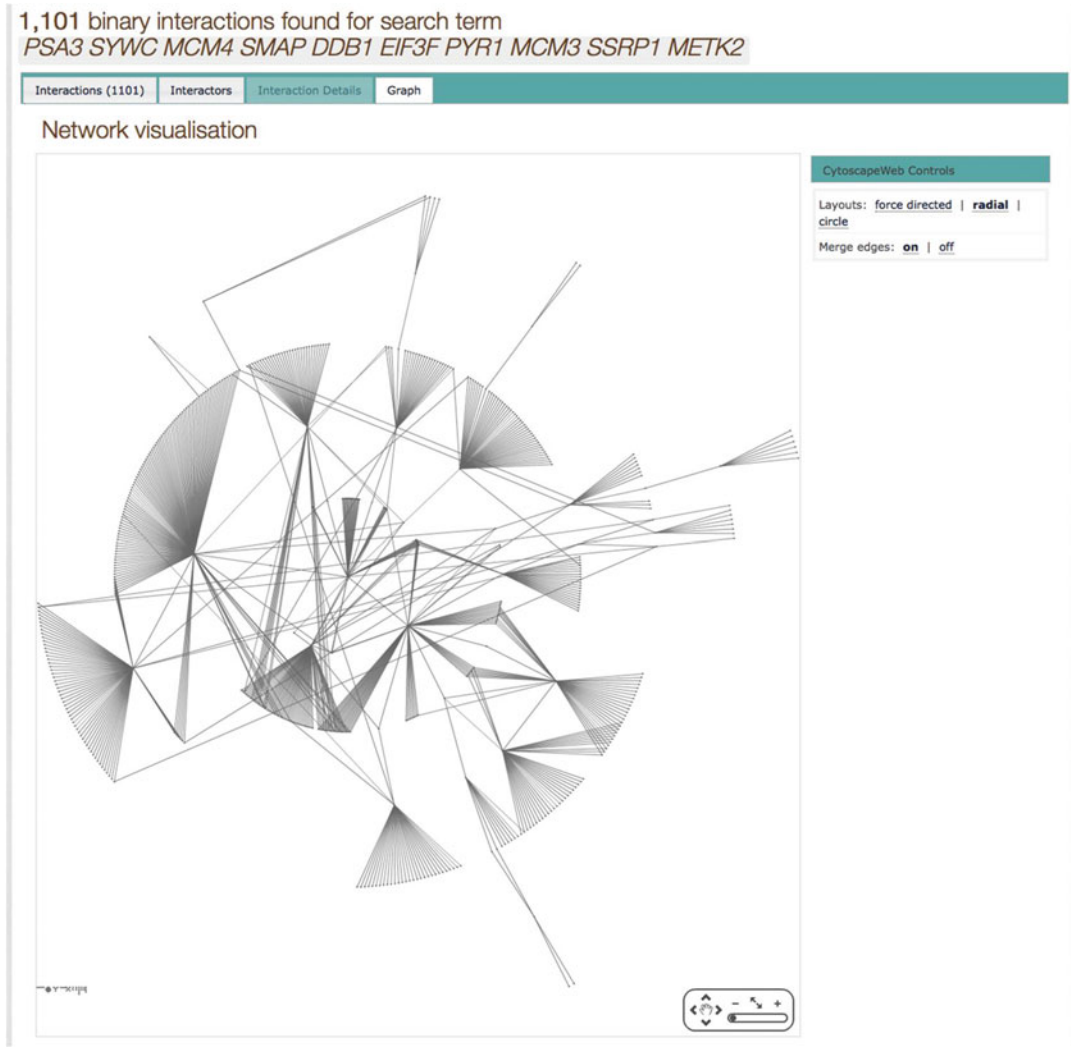


Fig. 16.37 Radial layout of the network found for PSA3, SYWC, MCM4, SMAP, DDB1, EIF3, PYR1, MCM3, SSRP1 and METK2 proteins in IntAct database

among other eukaryotic organisms. Furthermore, it has information of prokaryotic cells, such as *B. subtilis, E. coli, Mycobacterium tuberculosis, and Streptococcus pneumoniae*. Some viruses are included as well, e.g. Hepatitis C virus, Human Herpesvirus, Human Immunodeficiency virus, and Human Papillomavirus type 16 [44–46]. In its current version, the BioGRID database contains 749,213 non- redundant interactions, corresponding to 63,026 gene products and 45,623 unique publications. BioGRID database also includes 11,329 non-redundant interactions between 4851 unique chemical compounds and 2464 gene products accumulated from 8875 scientific publications. BioGRID also contains PTMs information. A total of 19,981 PTMs corresponding to 18,578 unassigned sites, 3165 unique proteins, 14,999 genes retrieved from 4317 publications are stored in this database.

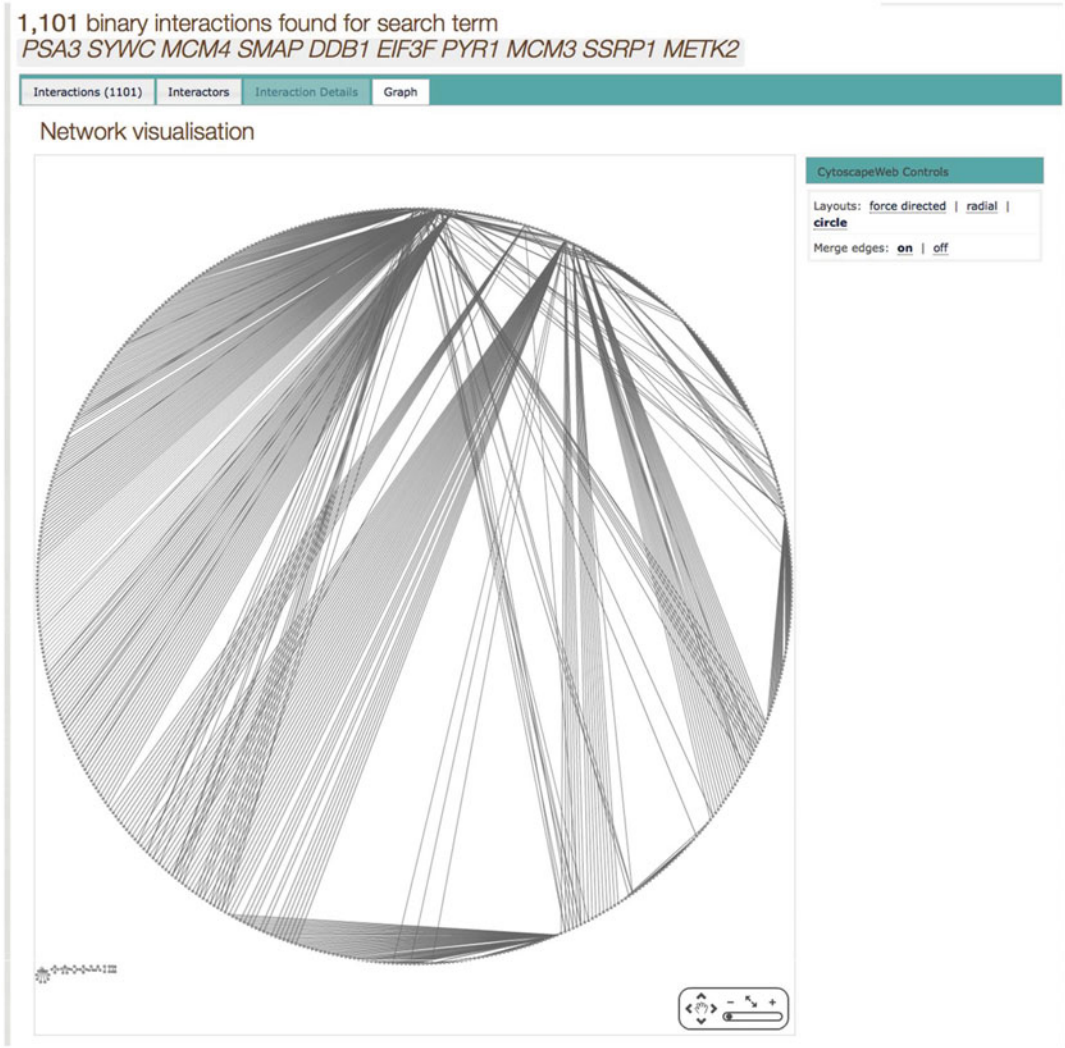


Fig. 16.38 Circle layout of the interaction map found for PSA3, SYWC, MCM4, SMAP, DDB1, EIF3, PYR1, MCM3, SSRP1 and METK2 proteins in IntAct database

To perform a search in BioGRID database, type your query (gene name, identifier or keywords) in the gene search window and select the species (Fig. 16.44). It is important to note that only one protein at a time can be searched. Alternatively, searches can be done by PubMed publication. However, searching of Multiple Genes or Publications will be available soon. As an example of a search, we selected the MCM6 protein, which was found overexpressed in both Luminal A and MDA-MB-231 breast cancer cell lines [18]. Results indicates that MCM6, the Minichromosome maintenance complex component 6, is involved in four GO Biological Processes:

1. DNA replication
2. DNA strand elongation involved in DNA replication
3. G1/S transition of mitotic cell cycle
4. Mitotic cell cycle

Fig. 16.39 Homepage of the Human Protein Reference Database HPRD

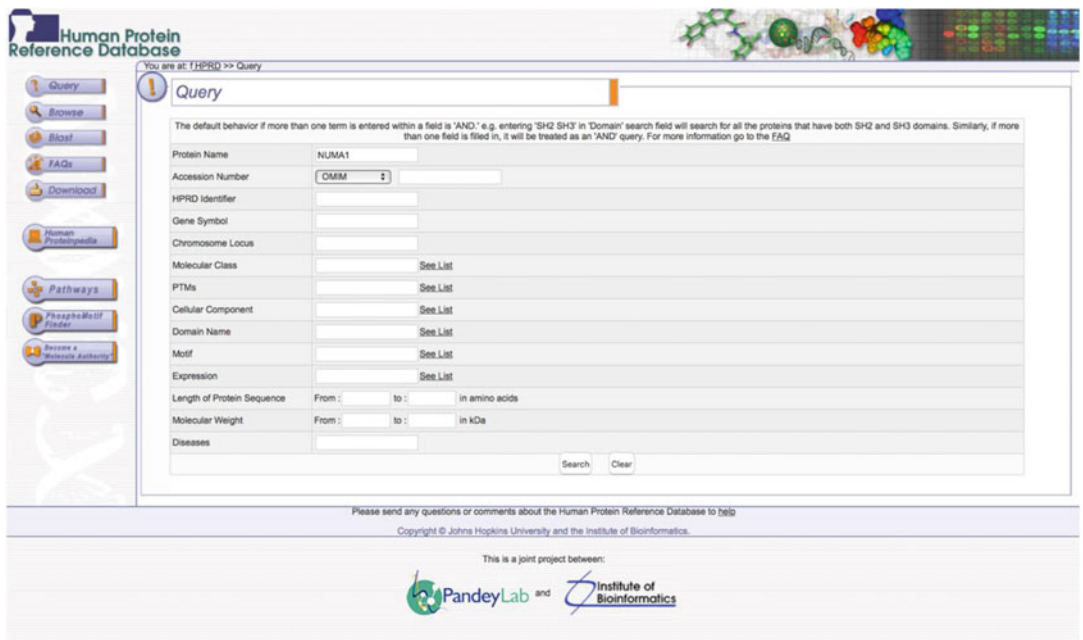


Fig. 16.40 Query webpage of the Human Protein Reference Database HPRD

Fig. 16.41 HPRD query result for the Nuclear Mitotic Apparatus Protein 1, NUMA1. This screenshot shows a putative PTM map as well as a summary for NUMA1 indicating the chromosome localization, subcellular localization, domains, and tissues where the protein is expressed

This protein is also involved in four GO Functions:

1. ATP binding
2. ATP-dependent DNA helicase activity
3. Identical protein binding
4. Protein binding

MCM6 is also part of three GO Components:

1. MCM complex
2. Nucleoplasm
3. Nucleus (Fig. 16.45, arrows 1–3)

In order of significance according to the number of physical interactions, MCM6 has 82 interactors which are MCM2, MCM4, MCM7, MCM10, MCMBP, MCM3, CDT1, TONSL, MCM5, HIST1H4A, SSRP1, ASF1B, CDKN2A, ASF1A, MMS22L, and ING5 (Fig. 16.45). When the interactions option is selected, a list of 142 interactions are displayed on screen, indicating the name of interactor, its role in the interaction, name of the species, code for the experimental evidence, source of the dataset, whether interaction is from high or low high throughput screening experiments, a

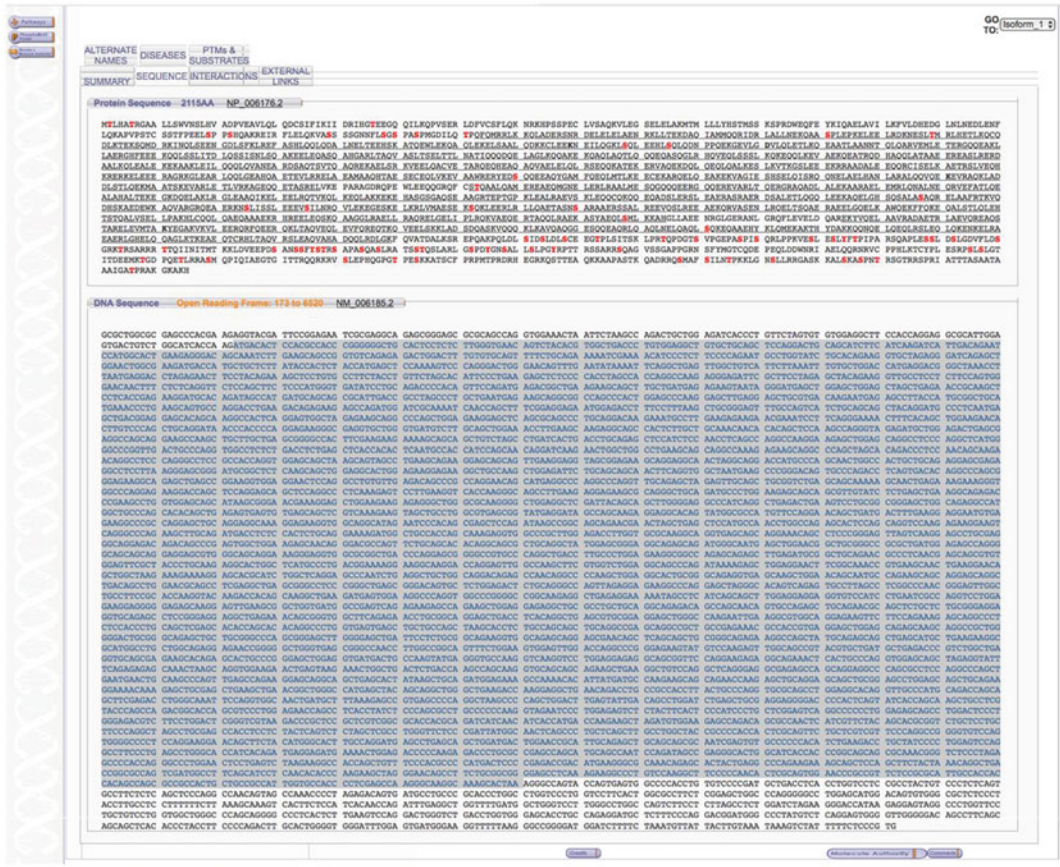


Fig. 16.42 Protein and DNA sequences for NUMA1 in HPRD

score for each interaction, the name of the person who curated the information, and additional notes (Fig. 16.46). When the Network tab is selected, three different layouts can be obtained: Concentric circles (Fig. 16.47), Single circle (Fig. 16.48), and Grid (Fig. 16.49). If the number of minimum evidence is changed to five for example, the number of interactions will drop (Fig. 16.50), thus reducing the complexity of the interaction map. When the PTM sites tab is selected, the amino acid sequence of the query is displayed and those residues with an identified PTM are highlighted in blue. Additional information such as the type of modification indicated as well as the source of information are also provided if PTM option is selected (Fig. 16.51). In the case of MCM6, there are 35 Lysine residues marked as ubiquitinated and two additional non-assigned PTMs (neddylation and sumoylation) (Fig. 16.52).

16.8.7 PIPs

The Human Protein-Protein Interaction Prediction (PIPs) is a specialized database containing a

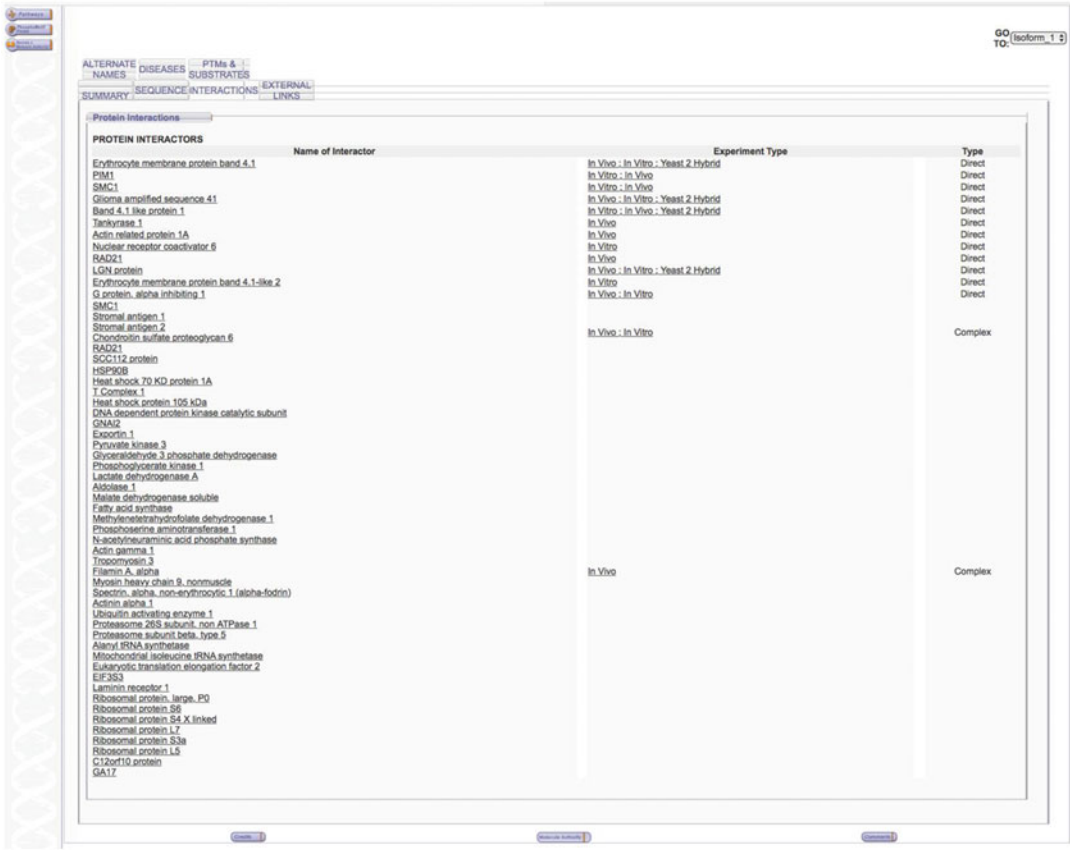


Fig. 16.43 List of protein interactors of NUMA1 queried in HPRD

catalogue of predicted human protein-protein interactions that have been probabilistically determined using a Bayesian model, which takes into account several modules: Expression, Orthology, Localization, Domain co-occurrence, PTMs co-occurrence, Disorder, and Transitive. Expression considers information from a number of gene expression profiles. Orthology uses the interactions that have been determined for orthologues from fly, human, worm and yeast. Localization is determined by using a human subcellular localization predictor (PSLT) in different subcellular compartments. Domain co-occurrence uses the information stored in InterPro (Protein sequence analysis and classification, http://www.ebi.ac.uk/interpro) and Pfam (Protein families, http://pfam.xfam.org) protein domain databases. PTM co-occurrence uses the information contained in HPRD and UniProtKB. Disorder refers to the prediction of intrinsic disorder of protein found in VLS2 prediction. Finally, Transitive is a module which involves the local topology of networks, considering all modules described above [47].

PIPs database is located at the University of Dundee and the current version (December 2015) contains 37,606 interactions with a score > 1.0, indicating a high probability of occurrence. To

Fig. 16.44 Homepage of the Biological General Repository for Interaction Databasets, BioGRID

perform a search, an ID in IPI, RefSeq or UniProtKB format must be entered in the search window. As an example, when TBP was used to initiate a query, results were displayed in several boxes each containing a number of interactions with a certain score. In this case, there are 65 interactions when a score value $\geq$ 1.0 was selected. For score values equal or larger than 2.5, 12.5, 25, 250, and 2500, there were 33, 15, 13, 7, and 3 interactions, respectively. When the number of interactions for a score $\geq$ 1.0 is selected, a list of interactors and the scores for each module used will be displayed on the screen.

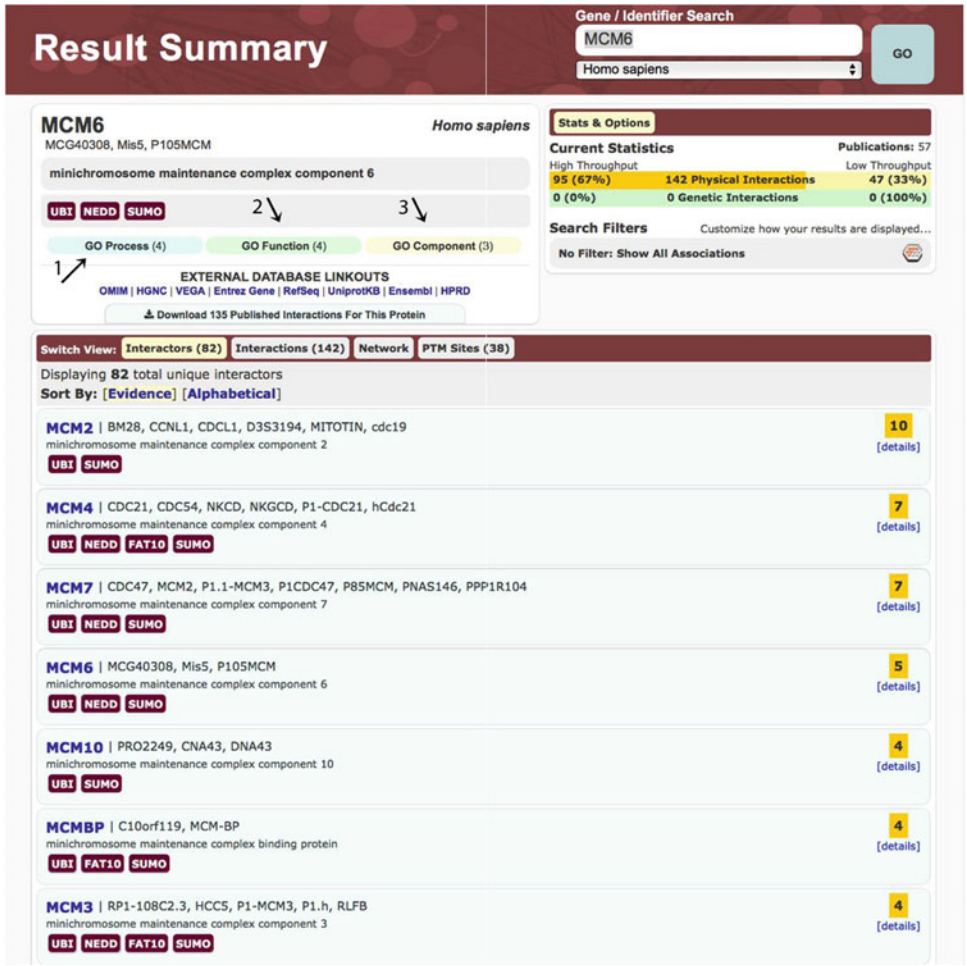
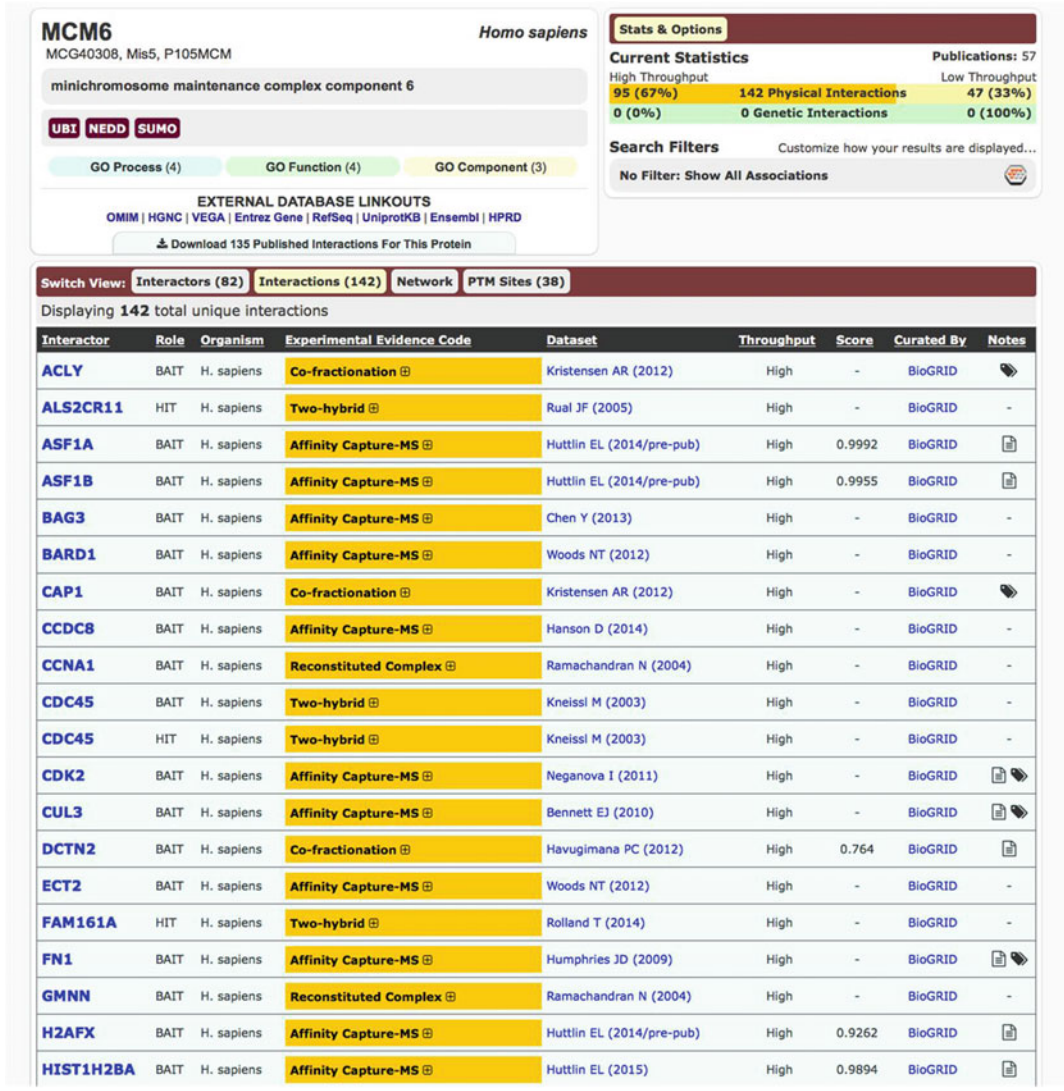


Fig. 16.45 Result summary for the Minichromosome Maintenance Complex Component 6, MCM6, queried in BioGRID. A total of 82 interactors were found in database

16.8.8 MPIDB

The Microbial Protein Interaction Database (MPIDB) at the Craig Venter Institute (http://jcvi.org/mpidb/about.php) is a database whose main goal is to gather information for all known protein interactions from microbial organisms [48]. The current version of MPIDB is 2009-11-18 and contains 24,295 interactions that have been experimentally determined for 250 species of bacteria. This number of interactions corresponds to 7810 proteins and 24,295 interactors. Like many other databases, MPIDB also imports information from other databases, including IntAct, Database of Interacting Proteins (DIP), The Biomolecular Interaction



Interactor	Role	Organism	Experimental Evidence Code	Dataset	Throughput	Score	Curated By	Notes
ACLY	BAIT	H. sapiens	Co-fractionation	Kristensen AR (2012)	High	-	BioGRID	
ALS2CR11	HIT	H. sapiens	Two-hybrid	Rual JF (2005)	High	-	BioGRID	-
ASF1A	BAIT	H. sapiens	Affinity Capture-MS	Huttlin EL (2014/pre-pub)	High	0.9992	BioGRID	
ASF1B	BAIT	H. sapiens	Affinity Capture-MS	Huttlin EL (2014/pre-pub)	High	0.9955	BioGRID	
BAG3	BAIT	H. sapiens	Affinity Capture-MS	Chen Y (2013)	High	-	BioGRID	-
BARD1	BAIT	H. sapiens	Affinity Capture-MS	Woods NT (2012)	High	-	BioGRID	-
CAP1	BAIT	H. sapiens	Co-fractionation	Kristensen AR (2012)	High	-	BioGRID	
CCDC8	BAIT	H. sapiens	Affinity Capture-MS	Hanson D (2014)	High	-	BioGRID	-
CCNA1	BAIT	H. sapiens	Reconstituted Complex	Ramachandran N (2004)	High	-	BioGRID	-
CDC45	BAIT	H. sapiens	Two-hybrid	Kneissl M (2003)	High	-	BioGRID	-
CDC45	HIT	H. sapiens	Two-hybrid	Kneissl M (2003)	High	-	BioGRID	-
CDK2	BAIT	H. sapiens	Affinity Capture-MS	Neganova I (2011)	High	-	BioGRID	
CUL3	BAIT	H. sapiens	Affinity Capture-MS	Bennett EJ (2010)	High	-	BioGRID	
DCTN2	BAIT	H. sapiens	Co-fractionation	Havugimana PC (2012)	High	0.764	BioGRID	
ECT2	BAIT	H. sapiens	Affinity Capture-MS	Woods NT (2012)	High	-	BioGRID	-
FAM161A	HIT	H. sapiens	Two-hybrid	Rolland T (2014)	High	-	BioGRID	-
FN1	BAIT	H. sapiens	Affinity Capture-MS	Humphries JD (2009)	High	-	BioGRID	
GMNN	BAIT	H. sapiens	Reconstituted Complex	Ramachandran N (2004)	High	-	BioGRID	-
H2AFX	BAIT	H. sapiens	Affinity Capture-MS	Huttlin EL (2014/pre-pub)	High	0.9262	BioGRID	
HIST1H2BA	BAIT	H. sapiens	Affinity Capture-MS	Huttlin EL (2015)	High	0.9894	BioGRID	

Fig. 16.46 List of interactions found for MCM6 in BioGRID

Network Database (BIND) and MINT. Search can be performed using the name of a protein (UniProtKB ID or locus name) or by selecting species name. Results will be displayed as a table containing the UniProtKB ID, name of protein, interactor, loci of query and interactor, species for query and interactor and the number of evidences for such interaction.

16.8.9 TAIR

The Arabidopsis Information Resource (TAIR) at Phoenix Bioinformatics (https://www.arabidopsis.org) is a database of information for plant research model *A. thaliana*.

This database contains the whole *A. thaliana* genome sequence, analysis, structure and

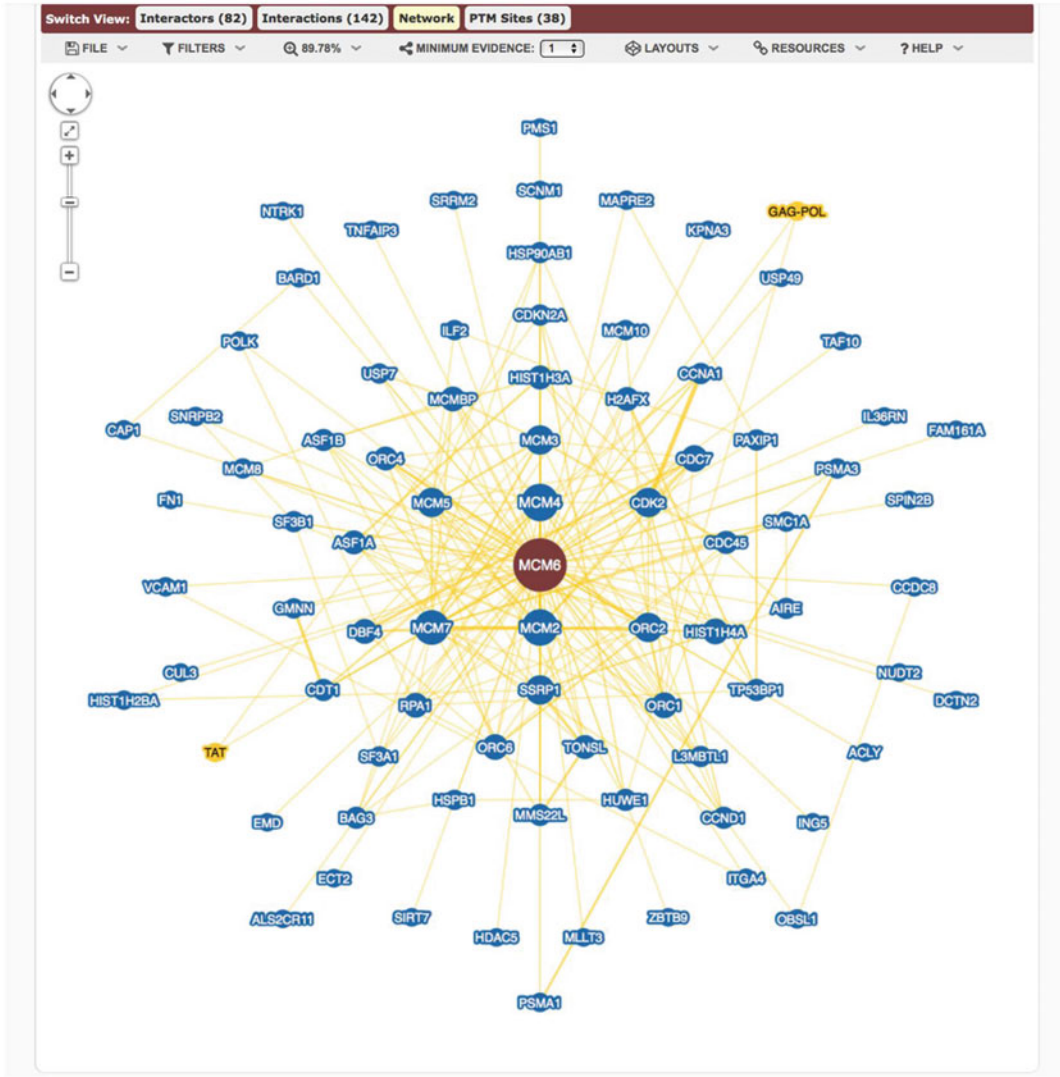


Fig. 16.47 Map of interactions for MCM6 in BioGRID database. Layout of interaction map is shown in concentric circles, where query protein is at the center

annotation of genes, information for all proteins encoded in its genome, data from gene expression experiments, genome maps, pathways, and other information useful to the scientific community [49]. Like other databases, experts from TAIR curate information using published experiments before entering them in this database. Search in TAIR can be performed in several ways: DNA/Clones, Ecotypes, Genes, Gene Ontology, Plant Ontology, Keywords, Locus, Markers, Microarray element, Microarray expression, People/Labs, Polymorphism/Alleles, Protein, Protocols, PMIDS, Seed/Germplasm, and Text. TAIR webpage also contains tools for

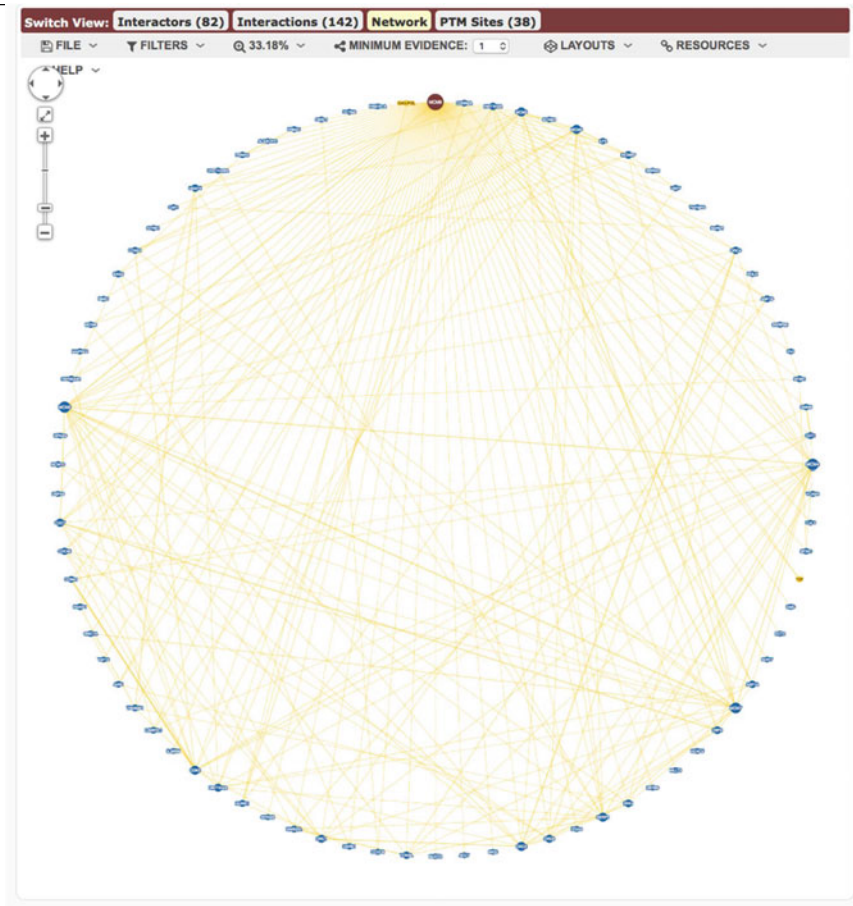


Fig. 16.48 Map of interactions for MCM6 in BioGRID database. Layout of interaction map is shown as a *single circle*, where MCM6 query protein is located at the *top* of the map

analysis of sequences, as well as viewers for maps and sequences. It is recommended to register in TAIR to download the whole genome sequence.

16.8.10 GeneCards

The Human Gene Database (GeneCards, http://www.genecards.org) is another useful database covering the human genome [50–53]. This database was created by scientists at the Weizmann Institute of Science and LifeMap Sciences. Search can be done using keywords, symbols, aliases, or identifiers. Information that can be retrieved from this database include:

1. Aliases for query
2. Links to HGNC (HUGO Gene Nomenclature Committee, http://www.genenames.org), Entrez Gene at NCBI, Ensembl (genome databases for vertebrates and other

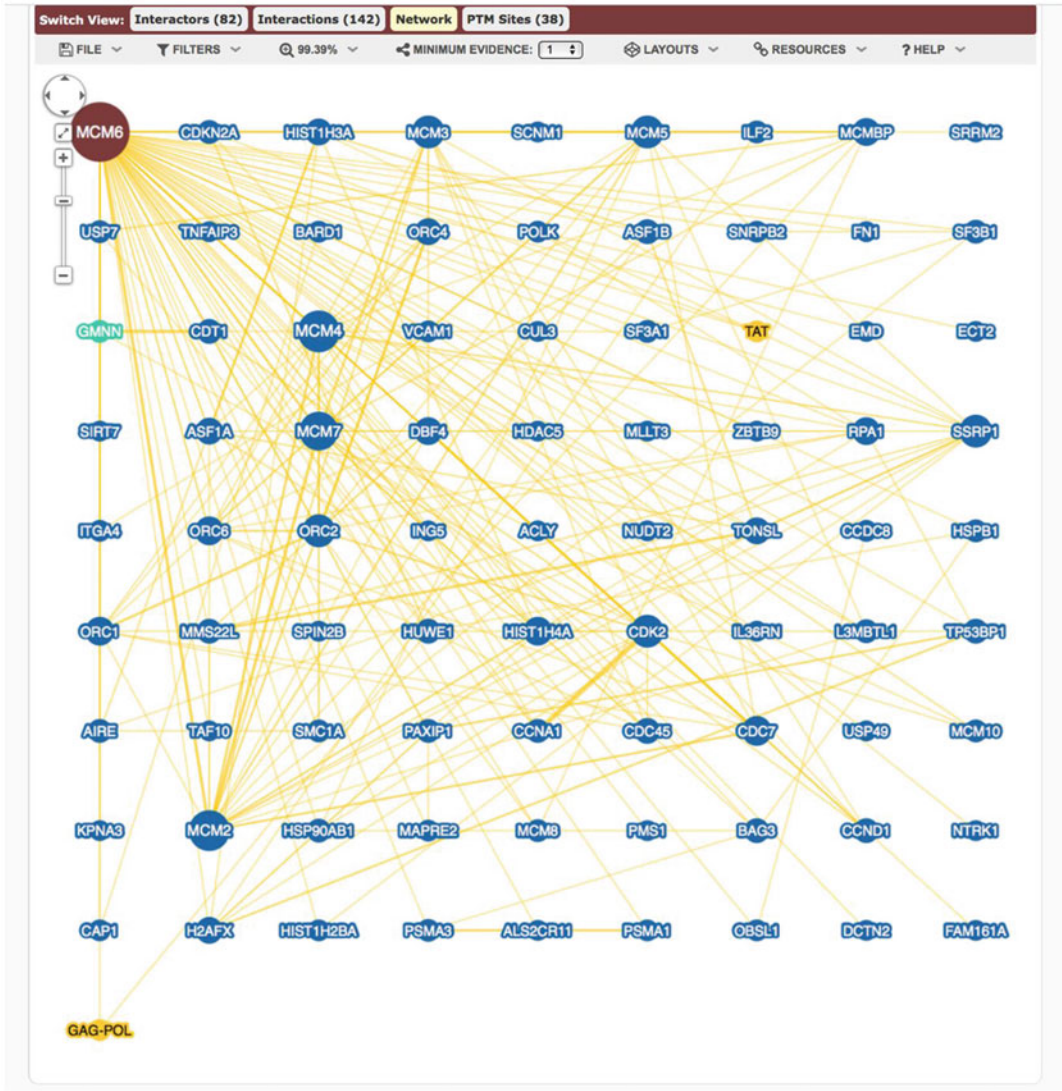


Fig. 16.49 Grid layout of the map of interactions for MCM6 in BioGRID database. MCM6 query protein is located at the *top left corner* of the map

eukaryotic species, http://www.ensembl.org/index.html), OMIM http://www.omim.org), and UniProtKB

3. Summaries of queries retrieved from different sources
4. Genomics data for query, including Regulatory Elements, Genomic location, Genomic region view, and RefSeq DNA sequence
5. Protein information such as Protein ID, Length in amino acids, Molecular Mass, Quaternary structure, Three dimensional structure from OCA (Brower-database for protein structure/function, http://oca.weizmann.ac.il/oca-docs/oca-home.html), Proteopedia (The free, collaborative D-encyclopedia of proteins & other

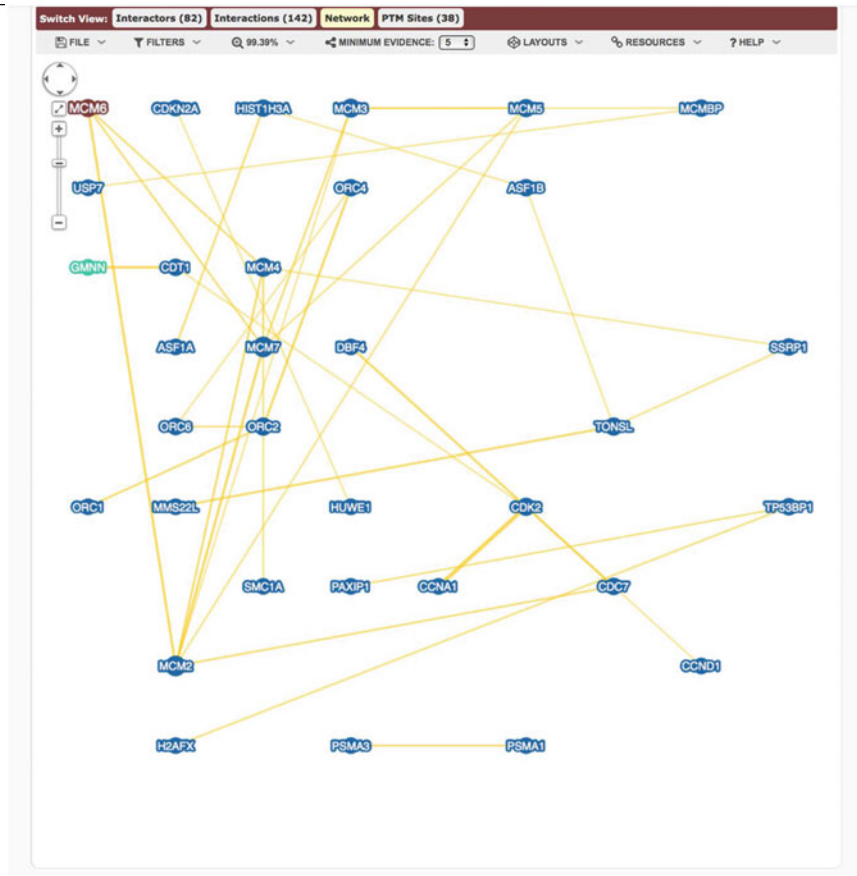


Fig. 16.50 Grid layout of the map of interactions for MCM6 in BioGRID database using a minimum value of 5 as evidence

molecules, http://proteopedia.org/wiki/index.php/Main_Page), Alternative splice forms, Data of protein expression in Proteomics DB (https://www.proteomicsdb.org/proteomicsdb/#overview), PaxDB (Protein Abundance Across Organisms, http://pax-db.org/#!home), MOPED (Multi-Omics Profiling Expression Database, https://www.proteinspire.org/MOPED/mopedviews/proteinExpressionDatabase.jsf), MaxQB (The MaxQuant DataBase, http://maxqb.biochem.mpg.de/mxdb/), and PTMs, (6) Domains in InterPro (Protein sequence, analysis and classification, http://www.ebi.ac.uk/interpro), ProtoNet (Automatic Hierarchical Classification of Proteins, http://www.protonet.cs.huji.ac.il/requested/cluster_card.php?global=protonet|no|6|61|lifetime|1|2|2&cluster=4023630&releaseid=6&firstEnterTimeClient=&blast=11053692|274977&clusteringNum=61)

6. Functions retrieved from UniProtKB, Enzyme Number; Gene Ontology; Phenotypes; Animal models for query; links to CRISPR products, miRNAs, siRNAs, shRNAs, clone products, etc.

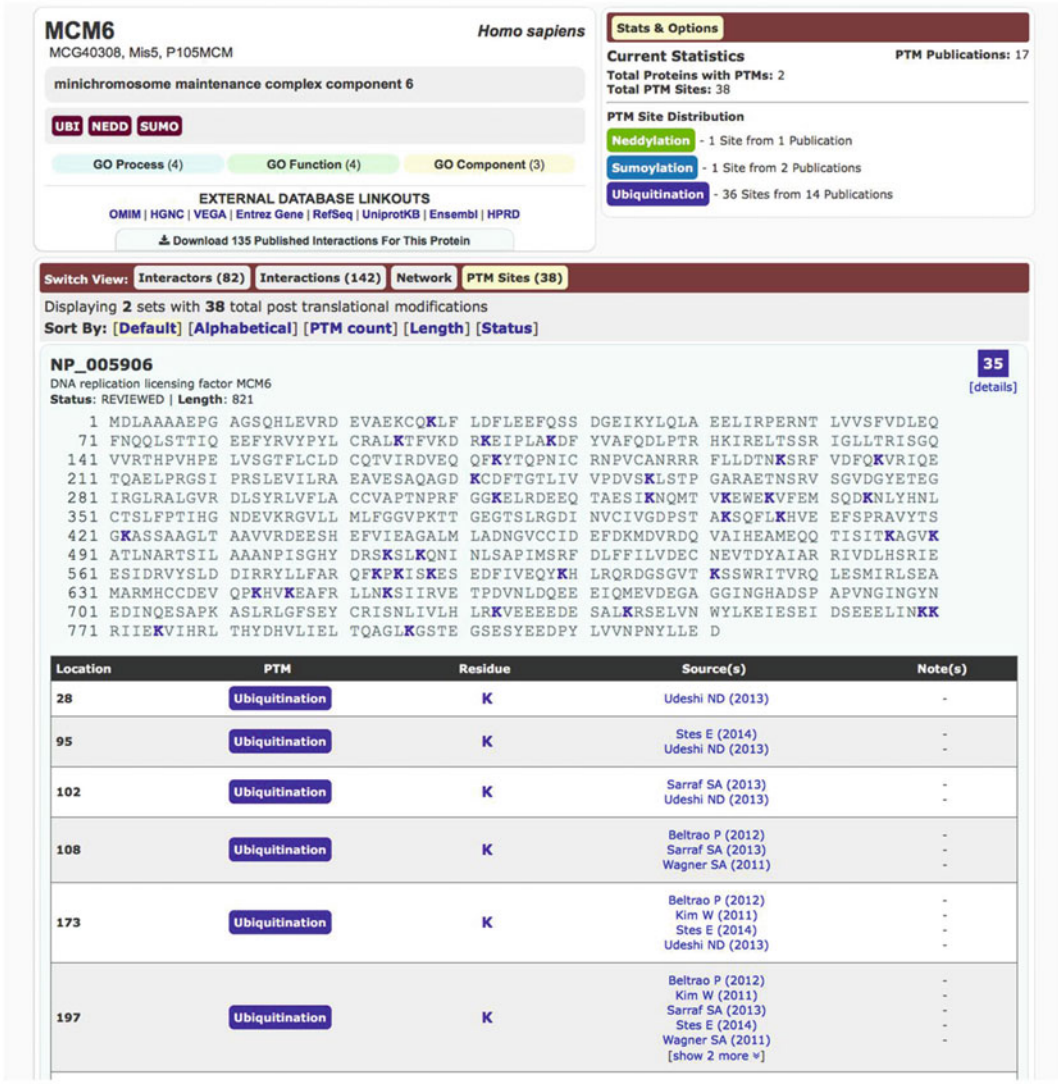


Fig. 16.51 PTMs reported for MCM6 in BioGRID database. There are a few sites shown to carry ubiquitination for MCM6. Reference is also provided

7. Localization of genes in chromosomes and subcellular location of proteins
8. Pathways
9. Drugs for query
10. Transcripts: Reference sequence (RefSeq), Enseml, Unigene Clusters
11. Expression in tissues: GeneAnalytics (http://geneanalytics.genecards.org/?utm_source=genecards&utm_medium=banner&utm_campaign=genecards&utm_content=banner_expression)
12. Orthologs
13. Paralogs
14. Variants
15. Disorders in MalaCards (The Humans Disease Database, http://www.malacards.org)
16. Publications

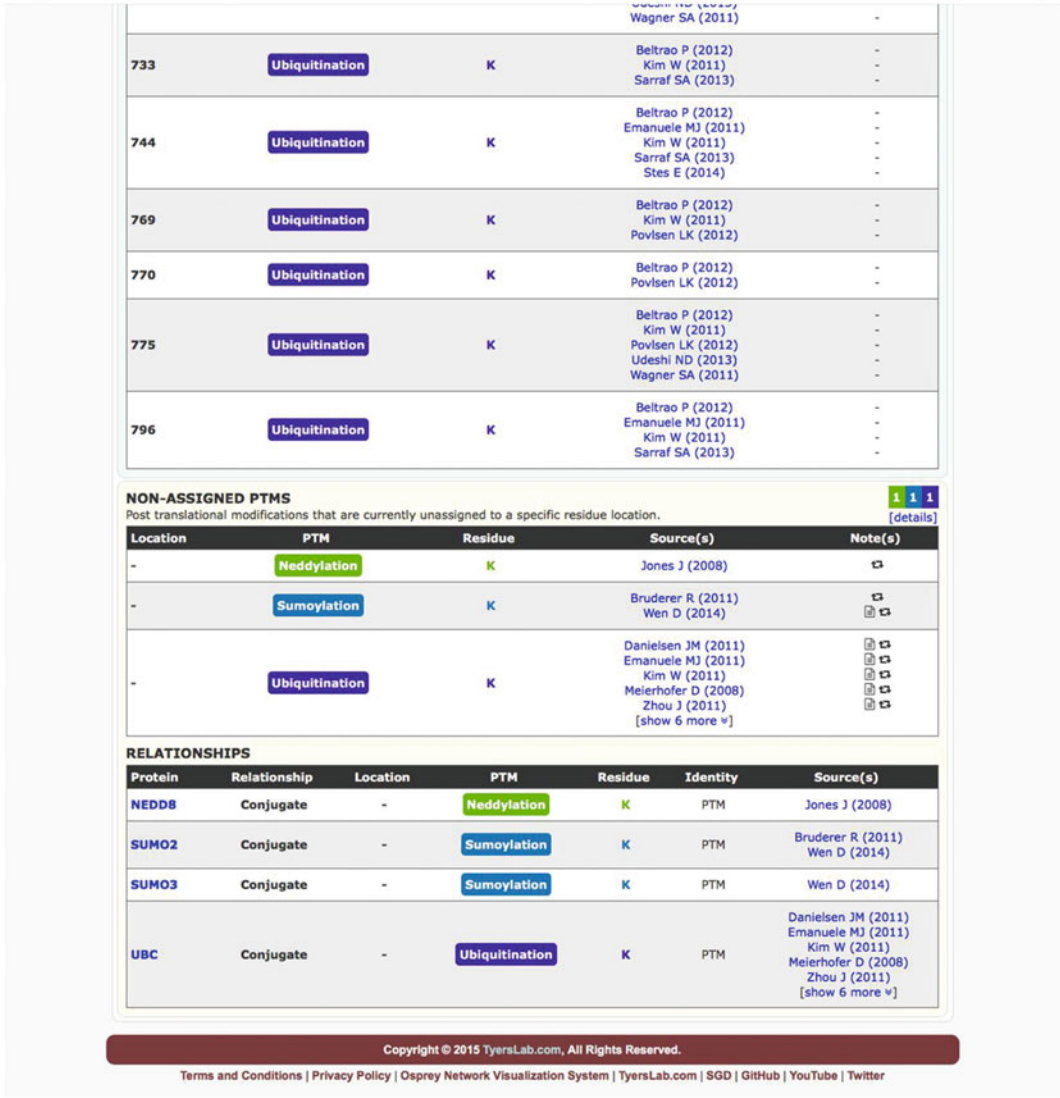


Fig. 16.52 PTMs reported for MCM6 in BioGRID database. Other PTMs are also shown in this figure for MCM6, including neddylation, sumoylation, as well as other ubiquitination sites

In addition, there are a lot of links to companies that might have products for the protein of interests, such as antibodies, immunofluorescence, animal models, silencing, etc.

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