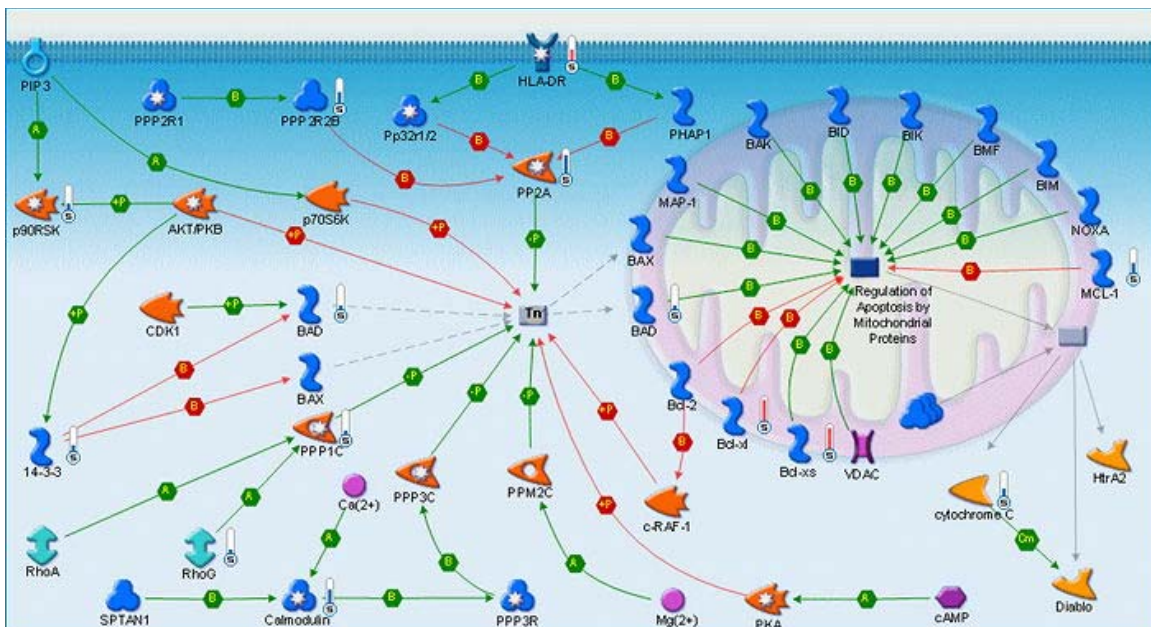




MetaCore Training Manual

Version 5.0



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Introduction

In this tutorial we will go through the step of analyzing a dataset using many of MetaCore's various tools; including maps, networks, pre-built networks, diseases, processes, etc. The dataset we will be using is from a psoriasis study, which is publicly available (1).

Background: Psoriasis is recognized as the most common T cell-mediated inflammatory disease in humans. Genetic linkage to as many as six distinct disease loci has been established but the molecular etiology and genetics remain unknown. To begin to identify psoriasis disease-related genes and construct in vivo pathways of the implicated processes, genome-wide expression screens of psoriasis patients need to be undertaken. Disease-related gene maps may provide new insights into the pathogenesis of psoriasis.

Methods: Four psoriasis patients' skin biopsies were sampled at two different time points, at the time of developed psoriatic lesion and at the time of recession. The samples were taken from the same exact skin spot from each patient, after which Affymetrix Human U95A microarray technology was utilized to evaluate the expression data. In this tutorial we will use this data represented as fold changes for each gene between psoriasis and normal-like skin for each patient, with corresponding p-values.

Objective: To apply whole-genome microarray data from Kulski et al (J Mol Med 2005) (1) to discover molecular pathways distinct for psoriasis, and to evaluate the molecular character of the disease using MetaCore™ functionality.

Getting started

This tutorial assumes that the data has already been uploaded to MetaCore. For more information about uploading data, please see the "How to use the Data Manager" tutorial available in the Help section of MetaCore. To begin analysis, first activate the data. When analyzing data make sure that the threshold is set appropriate to the data values in your file. The Threshold works as an absolute value, so for example, if the Threshold is set to 3, any genes with data values less than 3 and greater than -3 will be filtered out. The P Threshold filters out any genes with a p-value greater than the entered P threshold. After setting the desired thresholds, click OK. The effect of the thresholds can be seen in the Properties for the experiments and the experiments' statistics. To set the threshold select "Set threshold" from the Tools menu (**Figure 1**). For this data we will set the Threshold (corresponding to our uploaded fold change values) to 2 and the P-value Threshold to 0.05.

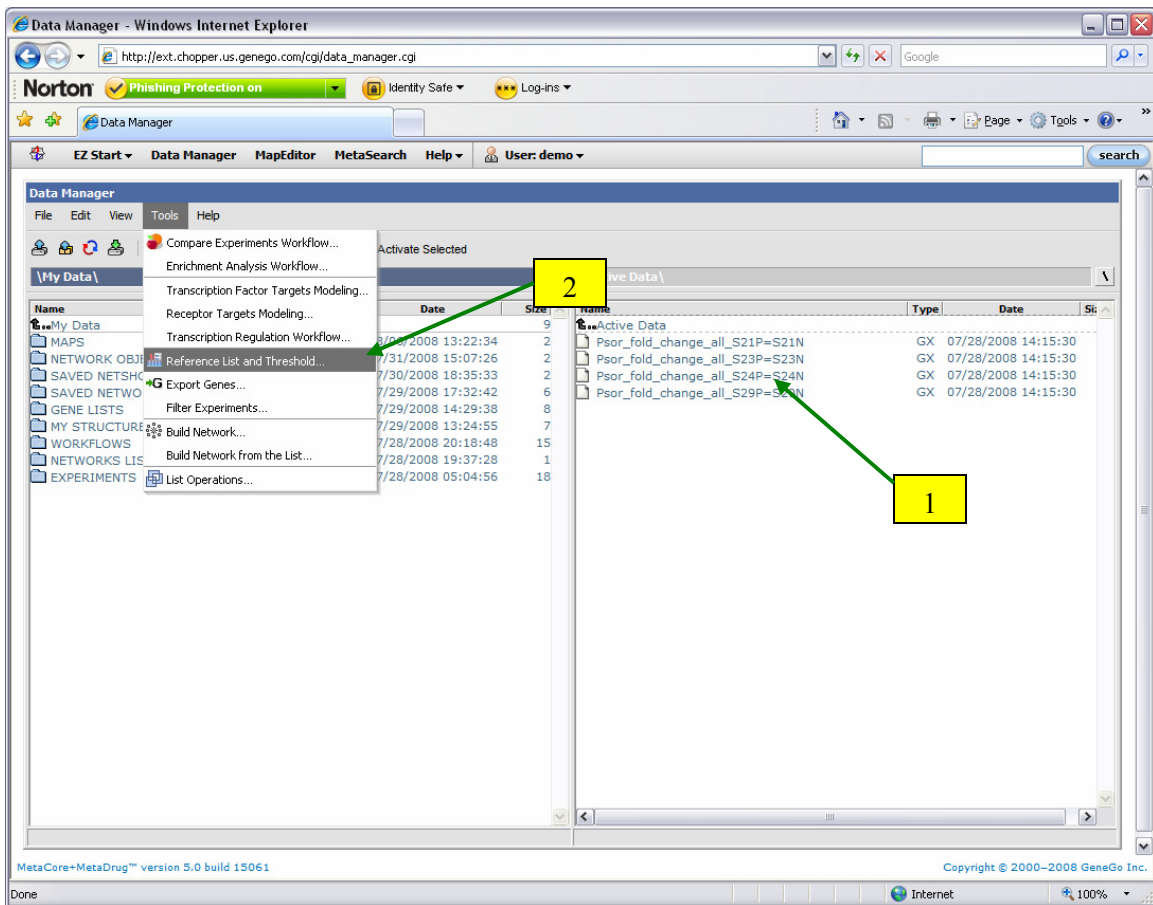


Figure 1: 1. Experiment activated. 2. Click here to set the thresholds.

Enrichment Analysis Workflow

The best place to start analyzing multiple data files is to use the Enrichment Analysis Workflow tool. In this case, we would like understand common functional themes in psoriasis pertaining to all samples. To compare the four samples and learn what is overrepresented among their common, similar, and unique genes, we select “Enrichment Analysis Workflow...” from the Tools menu. The “Enrichment Analysis Workflow” can also be accessed from the “Analyze Data” section of the EZ Start interface (Figure 2). On the page that appears, you can modify the threshold settings (or set them if you have not done so yet), and also choose what signals to use (up-regulated=“+”, down-regulated=“-“, or both). After choosing the settings, click the Apply button (Figure 3).

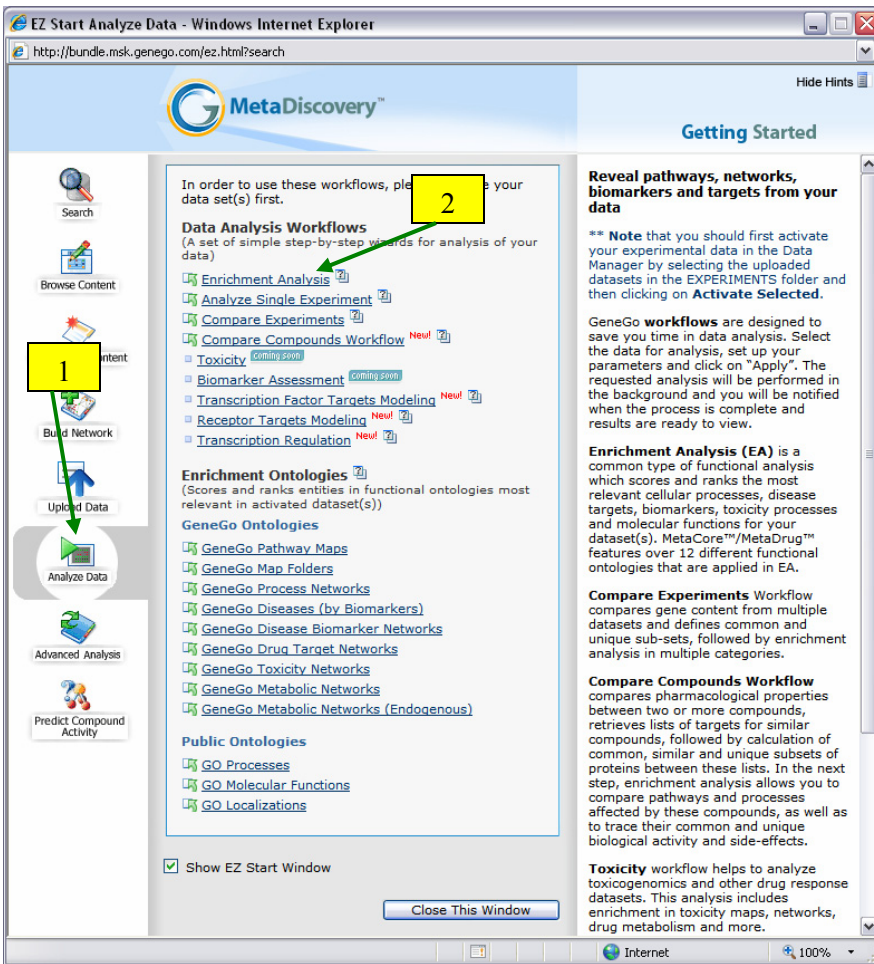


Figure 2: 1. Go to the Analyze Data section. 2. Click here to access the Enrichment Analysis Workflow.

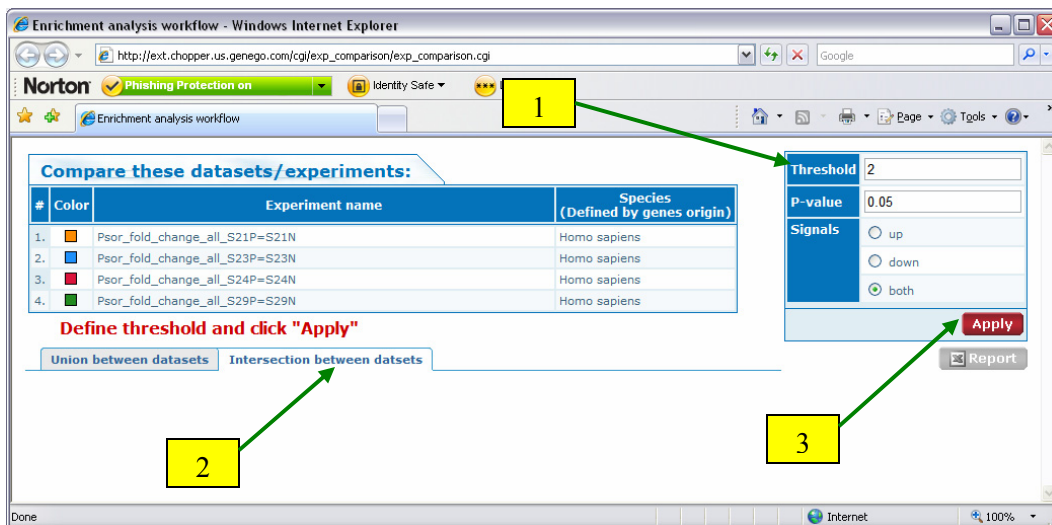


Figure 3: 1. Set thresholds here. 2. Select the “Intersection between datasets” tab. 3. Click Apply to start the comparison.

Here, we may want to find out what are the functional profiles of up-regulated genes in the psoriasis set, so we choose the “up” Signals to filter and sort by.

First, the intersections will be calculated. The intersections tell you how many of the network objects in your experiments are common to all experiments, how many are unique, and how many are similar (neither common to all experiments nor unique). One of the intersections calculated is the “best”, meaning that it contains the most common network objects. Next the distributions are calculated. The distributions are histograms showing the top 10 most significant results. A p-Value is calculated for the common and unique groups. The results are ranked by the $-\log(p\text{-Value})$. By default the most significant results for the common part is displayed. Mouse over a bar to see the $-\log(p\text{-Value})$ and the number of network objects in that group. Clicking the bar will allow you to build a network around the intersection objects, or view the map image in the case of the Distribution by canonical pathway maps. In this distribution we see which multistep pathways from literature consensus are most significantly enriched in our data set.

Top pathway maps suggest immune and inflammatory themes with possible involvement of IFNs-alpha/beta and STATs. This is an intuitively reasonable result for this disease, and it is most significant for common and similar genes (striped and clear bars). Map number 4 happens to be a custom map specific to this account, which was generated with use of an add-on module Map Editor.

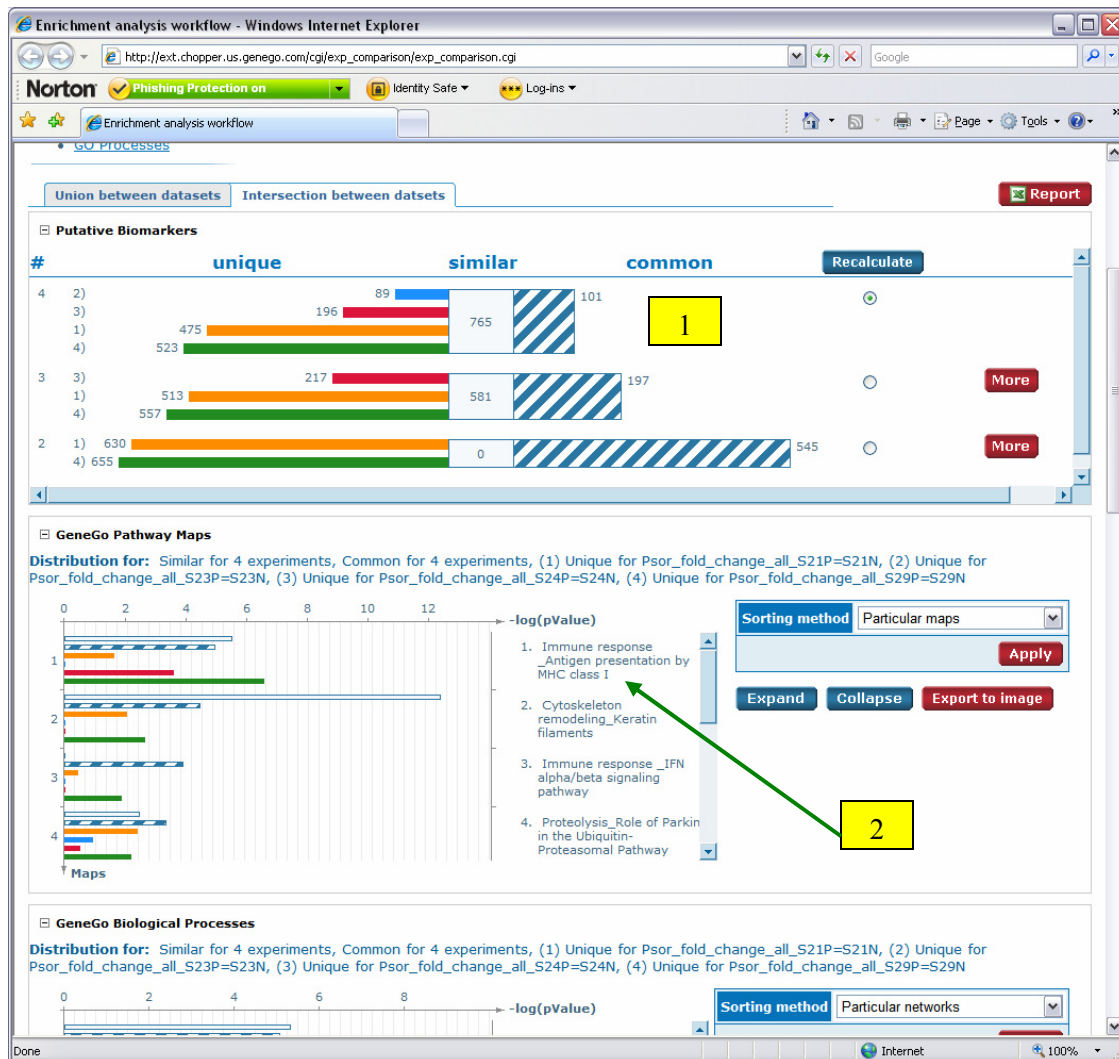


Figure 4: 1. Intersections displayed here. 2. Click on a name to open a map.

Now we will open the top significant map. Click on the map name to open it (**Figure 4**). The map name will open in a new window and the data values will be automatically mapped on it. Any data, which is placed into the Active Data window of the Data Manager will be automatically overlaid on any map or network that we open up. Data Values are represented by colored bars (thermometer-like icons) next to their associated genes.

There are many other visualization options available on maps. To access these, click the “Visualization Options” header. By selecting an option from one of the Marker categories, the objects associated with the selected category will be marked by a flashing icon. Here we will mark the objects related to the Psoriasis disease, the skin tissue, and all drug targets.

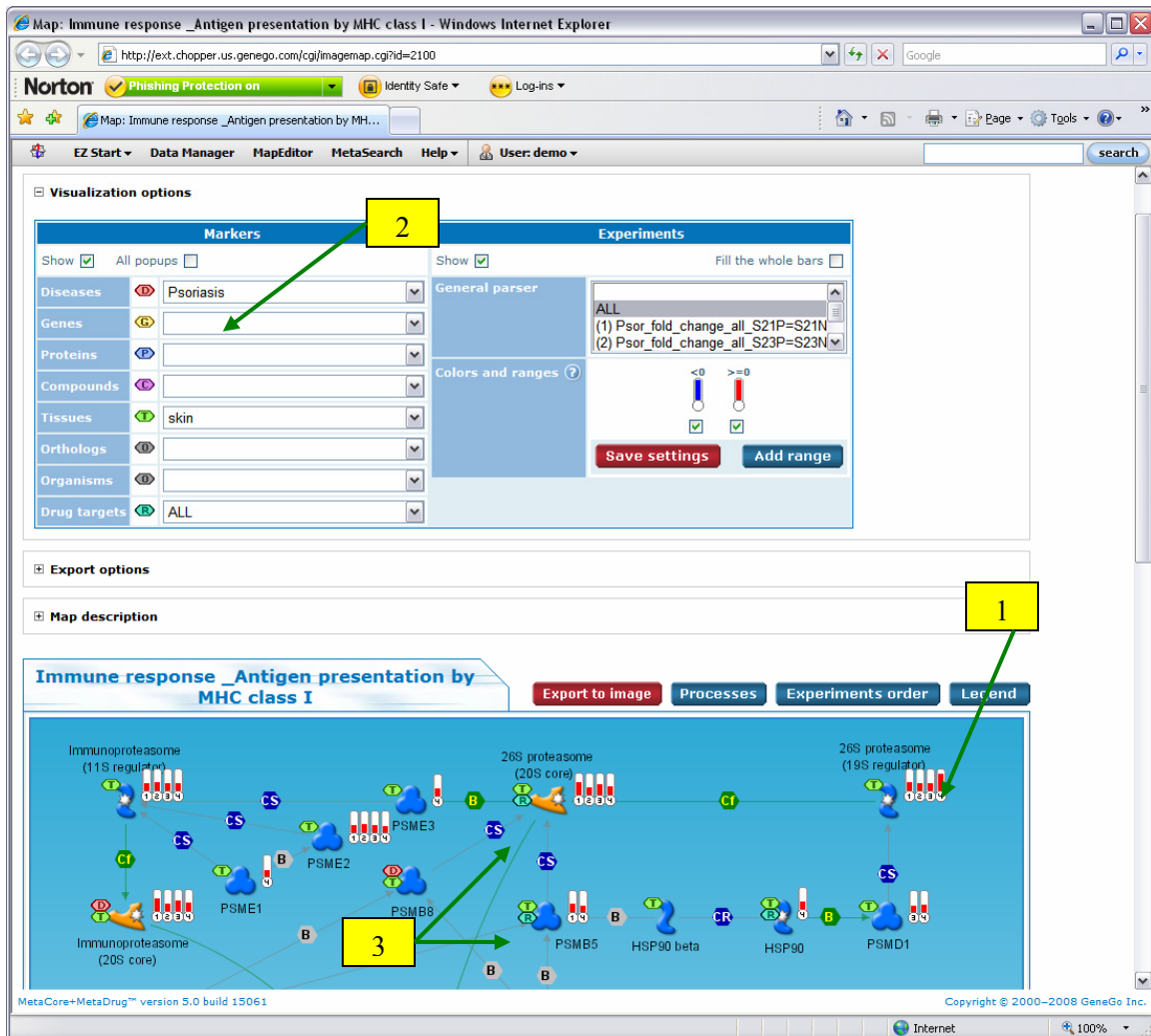


Figure 5: 1. Data visualized as thermometer icons. 2. Select visualization markers here. 3. Disease, tissue and drug target markers are next to their associated object.

The map image itself is interactive. You can click on the objects and interactions for more database information and literature references. After clicking on an interaction hexagon a window appears containing links to the PubMed articles where the interaction information was found. Additionally, from this window you can export selected references to EndNote, by clicking the red Export button.

Clicking on the objects will take you to the object information page. From here you can build a network around that object and you can go to the database page for the gene, protein, compound, and maps associated with the object. You can also see which GO processes and Diseases it is involved in, and see all other network objects that it interacts with, all summarized for up/downstream activation/inhibition, and mechanisms of interactions (including unspecified effects and mechanisms) with links to those objects and to the literature support behind each interaction info.

The screenshot displays a complex biological network visualization. The main window shows a 3D-like representation of an antigen-presenting cell with various proteins and their interactions. Key components include TAP2, Tapasin, SEC61 complex, MHC class I, and Calreticulin. A 'Links info' window is open, showing a table of PubMed references and experiment details. A 'Network objects. Proteins' window is also open, showing a list of Calreticulin protein entries for Human, Rat, and Mouse. Two yellow boxes with numbers 1 and 2 are overlaid on the network diagram, with green arrows pointing to the 'Links info' and 'Network objects' windows respectively.

From	To	Effect	Mechanism	Species
SEC61 complex	MHC class I	inhibition	transport	Homo sapiens, Rattus norvegicus, Mus musculus

References	Experiment details
1. Albring J, Koopmann JO, Hammerling GJ, Momburg F. Retrotranslocation of MHC class I heavy chain from the endoplasmic reticulum to the cytosol is dependent on ATP supply to the ER lumen. Molecular immunology 2004 Jan;40(10):733-41.	Organism: Homo sapiens
2. Wiertz EJ, Tortorella D, Bogoy M, Yu J, Mothes W, Jones TR, Rapoport TA, Ploegh HL. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature 1996 Dec 5;384(6608):432-8.	Organism: Homo sapiens
3. Schekman R. Cell biology: a channel for protein waste. Nature 2004 Jun 24;429(6994):817-8.	Organism: Homo sapiens

Name	Description
1 Calreticulin	

Name	Description
1 CALR_HUMAN	Calreticulin precursor
2 CALR_RAT	Calreticulin precursor
3 CALR_MOUSE	Calreticulin precursor

Figure 6: 1. Click on an interaction hexagon for PubMed links. 2. Click on an object for more object information.

Some of the flashing visualization markers are also clickable. Clicking on one of the drug target icons (flashing R) will take you to the compound details page. From here you can search the MDL DiscoveryGate software based on the compound. To do so, select a search method and click the “Search in DiscoveryGate” button. The DiscoveryGate software will then open and automatically perform the selected search. From the compound detail page you would also be able to build a network around it, or explore its other targets and learn how specific or broad the action of the compound may be.

The screenshot shows a web browser window titled "Drug details - Windows Internet Explorer". The address bar shows the URL: <http://ext.chopper.us.genego.com/cgi/chem.cgi?id=222769946>. The page content is organized into a table with the following sections:

- General Information**
 - Generic Name: Bortezomib
 - Drug Status: FDA approved, EU approved, Clinical trial drug
 - Synonyms/Brand Names: AIDS004352, Alanine alpha-aminoboronic acid, BO2, Boronic acid, [(1R)-3-methyl-1-[[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino]propyl]amino]butyl]-, Bortezomib, Bortezomib (JAN/USAN), Compound, DPBA, Dipeptidyl boronic acid derivative, LDP-341, LPD 341, LPD-341, MLN-341, N-[(1R)-1-(DIHYDROXYBORYL)-3-METHYLBUTYL]-N-(PYRAZIN-2-YLCARBONYL)-L-PHENYLALANINAMIDE, NCI60_029010, NSC-681239, NSC681239, PS-341, Peptidyl boronic acid derivative, Peptidyl boronic acid derivative (1), Ps 341, Pyz-Phe-boroLeu, Velcade, Velcade (TN), [(1R)-3-methyl-1-[[[(2S)-1-oxo-2-[[oxo-(2-pyrazinyl)methyl]amino]-3-phenyl]propyl]amino]butyl]boronic acid, [(1R)-3-methyl-1-[[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino]propyl]amino]butyl] boronic acid, [(1R)-3-methyl-1-[[[(2S)-3-phenyl-2-(pyrazin-2-ylcarbonylamino)propanoyl]amino]butyl]boronic acid, [(1R)-3-methyl-1-[[[(2S)-3-phenyl-2-(pyrazine-2-carbonylamino)propanoyl]amino]butyl]boronic acid, [3-methyl-1-(3-phenyl-2-pyrazin-2-ylcarbonylamino)propanoyl]amino]butyl]boronic acid, boronic acid, (1r)-3-methyl-1-[[[(2s)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino]propyl]amino]butyl]-, bortezomib [usan], component of Velcade, ldp 341, mg 341, mln-341, ps 341 (pharmaceutical), ps-341
 - Chemical IUPAC Name: [(1R)-3-methyl-1-[[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino]propyl]amino]butyl] boronic acid.
 - Compound Category: Xenobiotic
 - Chemical Formula: C19H25BN4O4
 - Chemical Structure:
- MOL File: [Download structure](#)
- Molecular Weight: 384.20
- State: Solid
- H2O Solubility: The solubility of bortezomib, as the monomeric boronic acid, is 3.3 to 3.8 mg/mL in a pH range of 2 to 6.5.
- MetaDrug: [Upload structure to MetaDrug](#)
- MDL databases: [Search in DiscoveryGate](#) Select Search Type: Automatic
- External Links
 - CAS Number: 179324-69-7
 - SMILES: [C@@H](CC(C)C)NC([C@H](C1CCCC1)NC(=O)C1CNCN1)=O)B(O)O
 - InChI: InChI=1/C19H25BN4O4/c1-13(2)10-17(20(27)28)14-11(18)16-4-6-4-3-5-7-14)23-19(26)16-12-21-8-9-22-16/h3-9,12-13,15,17,27-28H,10-11H2,1-2H3,(H,23,26)(H,24,25)/t15-17/m/0/s1

A green arrow points from the "Search in DiscoveryGate" button to a yellow box containing the number "1".

Figure 7: 1. Access MDL DiscoveryGate by using this button.

There are other export options available on the map page as well. It is possible to export a list of genes on the map. First click the “Export options” header. Then enter a name for the list and click the Export button. You can also export only those genes with experimental data by checking the box next to “With expression data only”. Genes from the map can be exported to Rosetta Resolver, GeneSpring GX and Spotfire DecisionSite by selecting the desired tool in the “Export to” row. Exporting the map image itself is also possible in 300 dpi publication-quality png format.

Figure 8: 1.Click here to access the export options. 2. Genes can be exported to third-party software here.

GeneGo Content Networks

Networks, such as those that depict proteins and interactions for different functional cellular processes, can also be accessed from the Enrichment Analysis Workflow results. MetaCore contains a number of pre-built networks. These are based on processes, disease biomarkers, and metabolic pathways. In the Distribution by GeneGo Processes, we see similar themes as were picked up by the canonical pathway maps ontology enrichment graph (except now among the top process we also see a proteolysis network). Click on a top process name to open the network (**Figure 9**).

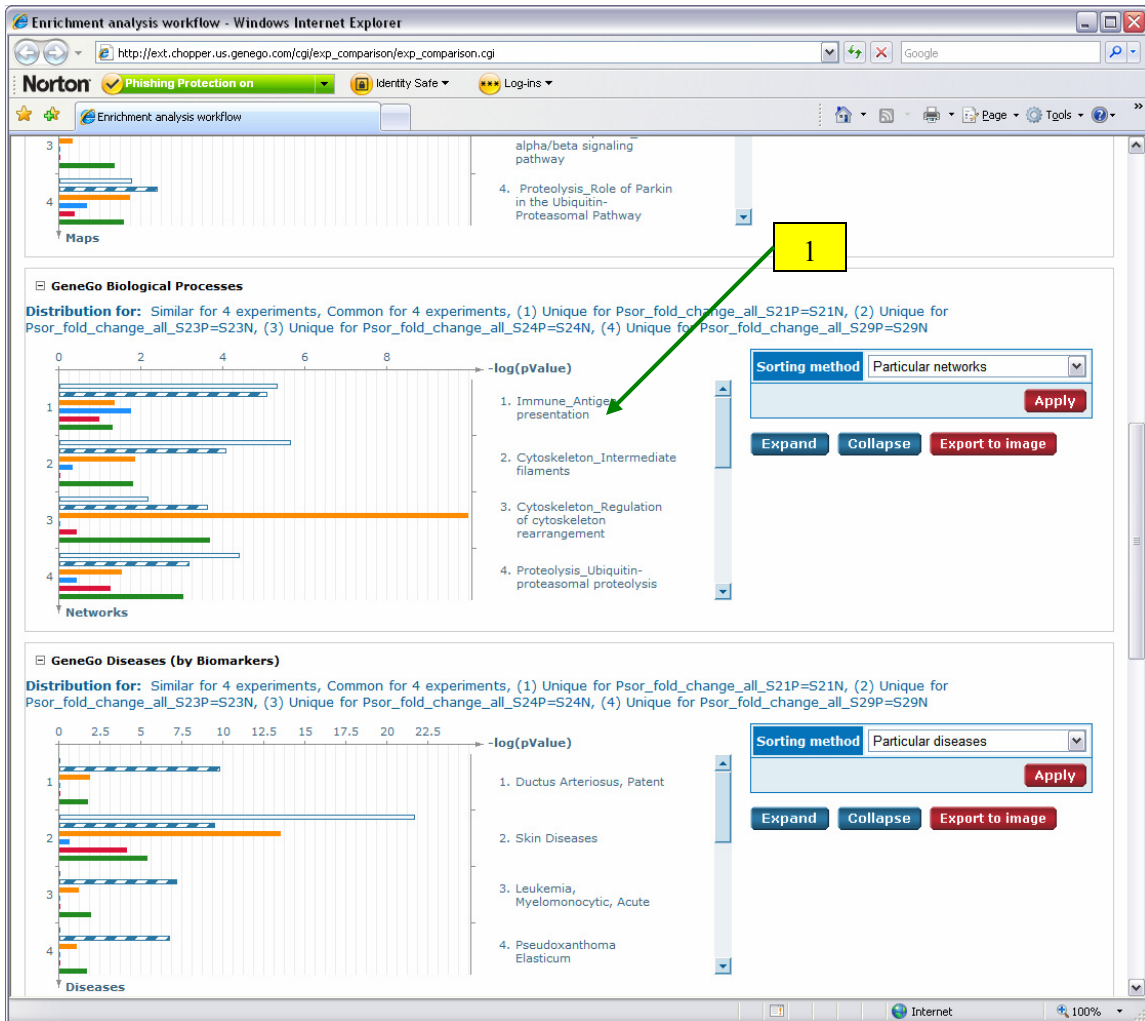


Figure 9: 1. Click here to open a GeneGo Process network.

Data values on networks are represented by red and blue circles. You can click on the circle to see the specific values (**Figure 10**). Networks are more interactive than maps; you can click and drag the objects to move them. You can also click the objects and interaction beads/hexagons for more information, just as with maps.

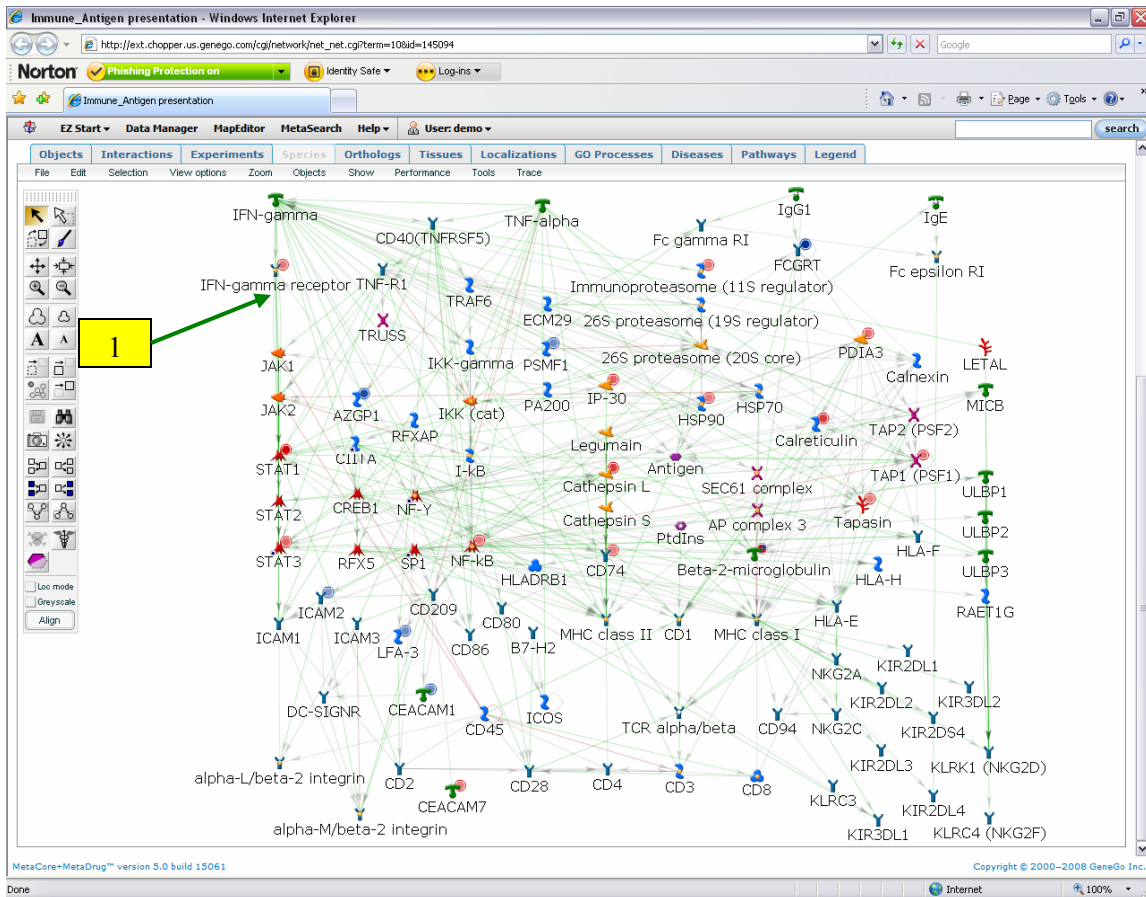


Figure 10: 1. Data represented by red and blue circles.

Sometimes networks can be more complex than maps. When this occurs, there are many features of the network interface that can help make networks less complex and easier to view and interpret. One such option is to hide the effect hexagons (that describe mechanisms of interactions). To do this, uncheck the 'Effects' option in the Show menu.

This will make edges appear as simple lines, but the overall effect information will be visible with green being activation, red – inhibition. Directionality of interactions is always available and will be easily traceable with arrows and with hover codes that enable you to mouse over any node and see all the up-stream interactions and nodes highlighted yellow, and downstream – light blue.

Another option to ease network view and manageability is to hide objects that do not have data values. To do this, open the Edit menu, then select the Selection sub-menu, and then click on the Hide non-expressed objects option. The objects that do not have expression values (or basically ANY values that came from the active data sets and passed the applied threshold) will be hidden. Hidden objects can be brought back by clicking the 'Show all hidden objects' button on the toolbar (**Figure 11**).

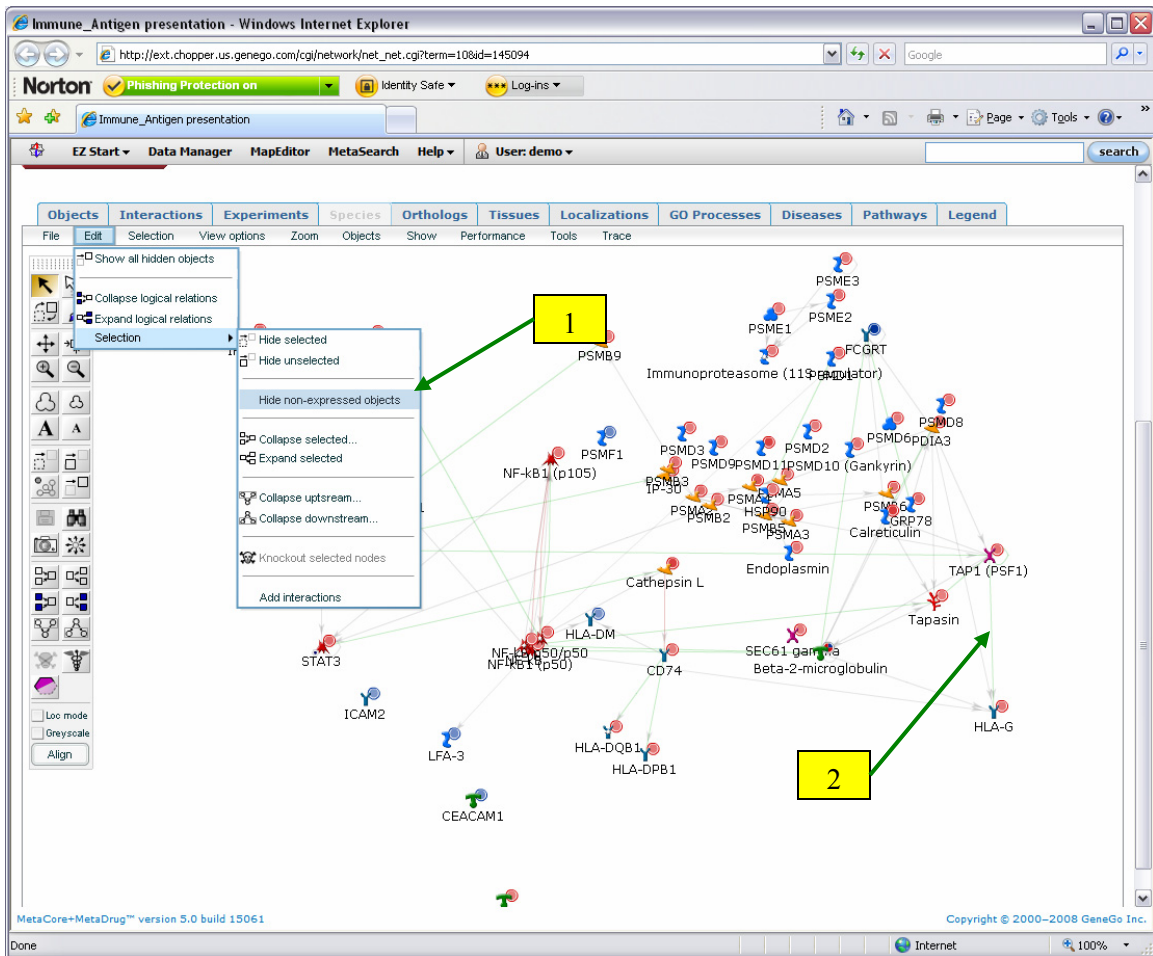


Figure 11: 1. Click here to hide the non-expressed objects. 2. Effect hexagon are not shown here.

You could also use advanced selection options (explained more on p22) to choose specific classes of proteins (like trans-factors, receptors, ligands, etc...) and hide all unselected objects that you do not choose to focus on.

Build Networks

Networks can also be built ‘on the fly’ by starting with a list of objects from your data that you are interested in and using one of our network building algorithms to construct a network. In some studies, such as comparisons between disease and normal, or between effects of one treatment agent vs. another, one may want to click on the thin unique bar(s) to build specific ‘signature networks’ for the unique genes for that set. Here, we want to focus on commonly up-regulated psoriasis genes, so we will click on the common group of objects from the Enrichment Analysis Workflow results. This will bring up a new window containing the network building algorithms.

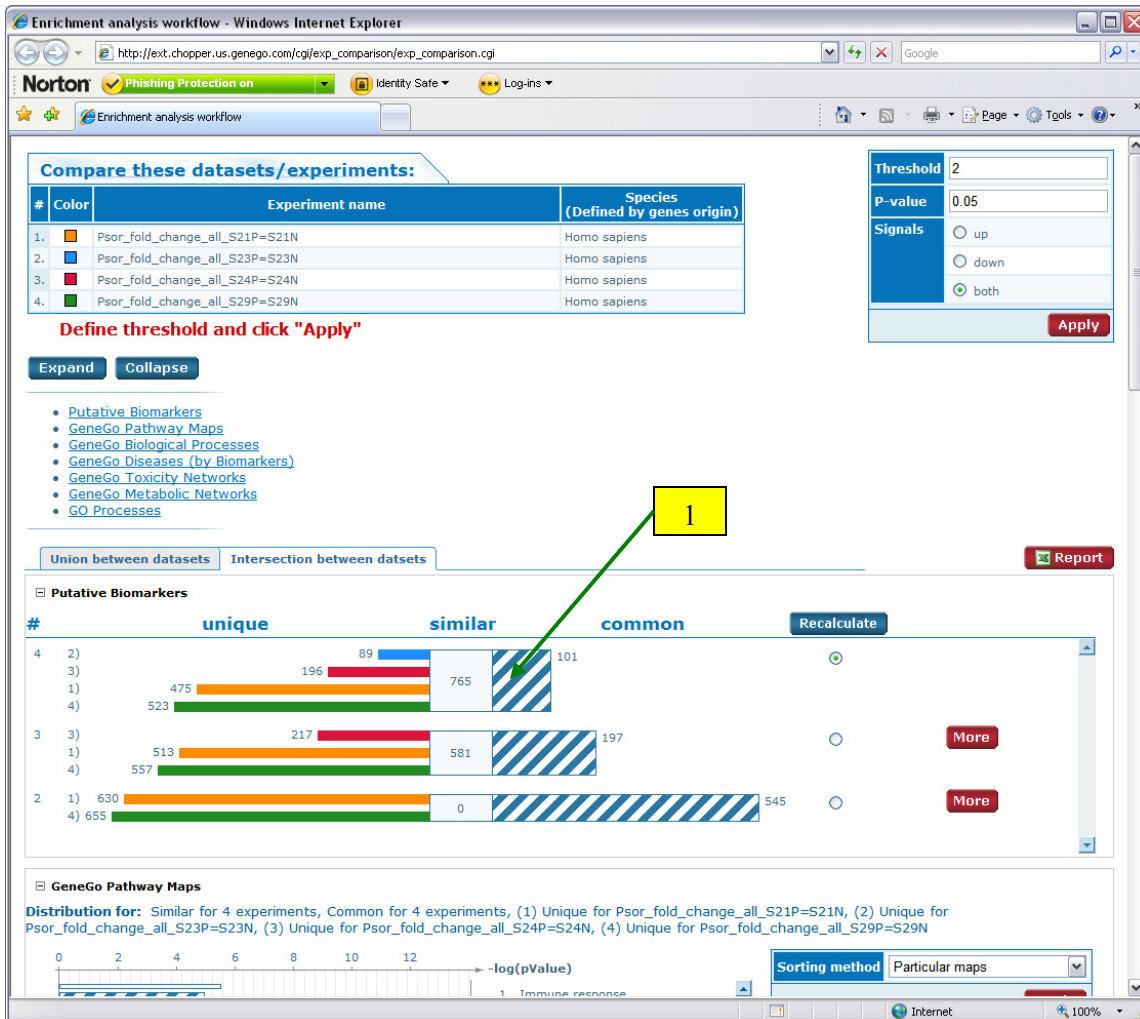


Figure 12: 1. Click here to build a network starting with the common objects.

Network Options

This page contains our network building algorithms. Analyze network creates a large network and breaks it up into smaller sub-networks which can be built separately. This algorithm is good for large sets of data. The Shortest paths algorithm tries to link the original genes with additional objects from the database. The Direct interactions algorithm creates a network only from the objects in the original list. Auto expand adds objects until it creates a fairly dense network, this is best used to see the interactions around a few nodes. Analyze transcription regulation works similar to analyze network, except the sub-networks created are centered on transcriptional factors. The Self regulations algorithm is like shortest paths except it tries to connect nodes with transcription factors. Finally, Expand by one interaction simply adds all the one-step interactions around each node from the list.

Two additional “Analyze network” algorithms (transcription factors and receptors) are also available. These options focus on the presence of either start-nodes or end-nodes of a certain pathway in the submitted list of genes.

The “transcription factors” mechanism favors network construction where the end-nodes / receptor targets of transcriptionally regulated pathways are present in the original gene list. It is irrelevant for this algorithm if the corresponding transcription factors are also present in the original gene list or not.

The “receptors” mechanism favors network construction where the end-point of a pathway leads to a receptor (through “receptor binding”) and the starting point of a pathway (a transcription factor, or ligands, etc…) is present in the original gene list, regardless of the presence of the end-point receptor in the list.

Another option available here is the ability to pre-filter a network based on tissue, sub-cellular localization, organism, object type, and/or interaction type. The resulting network then contains only those objects that are known to be expressed in the selected tissue, localization, and organism; or are of the selected object type(s) or connected by the selected interaction type(s). To use the pre-filters, uncheck the ‘Disabled’ box and click the Select button to select the pre-filter criteria. In this case, leave the pre-filters disabled.

On the Network Options page, you can also choose to use canonical pathway interactions. Checking the box to enable this feature will instruct the network building algorithms to try to use interactions that also appear on MetaCore’s canonical pathway maps. Interactions added in this manner will be highlighted on the resulting network.

Here we will use the Analyze network (transcription factors) algorithm (**Figure 13**).

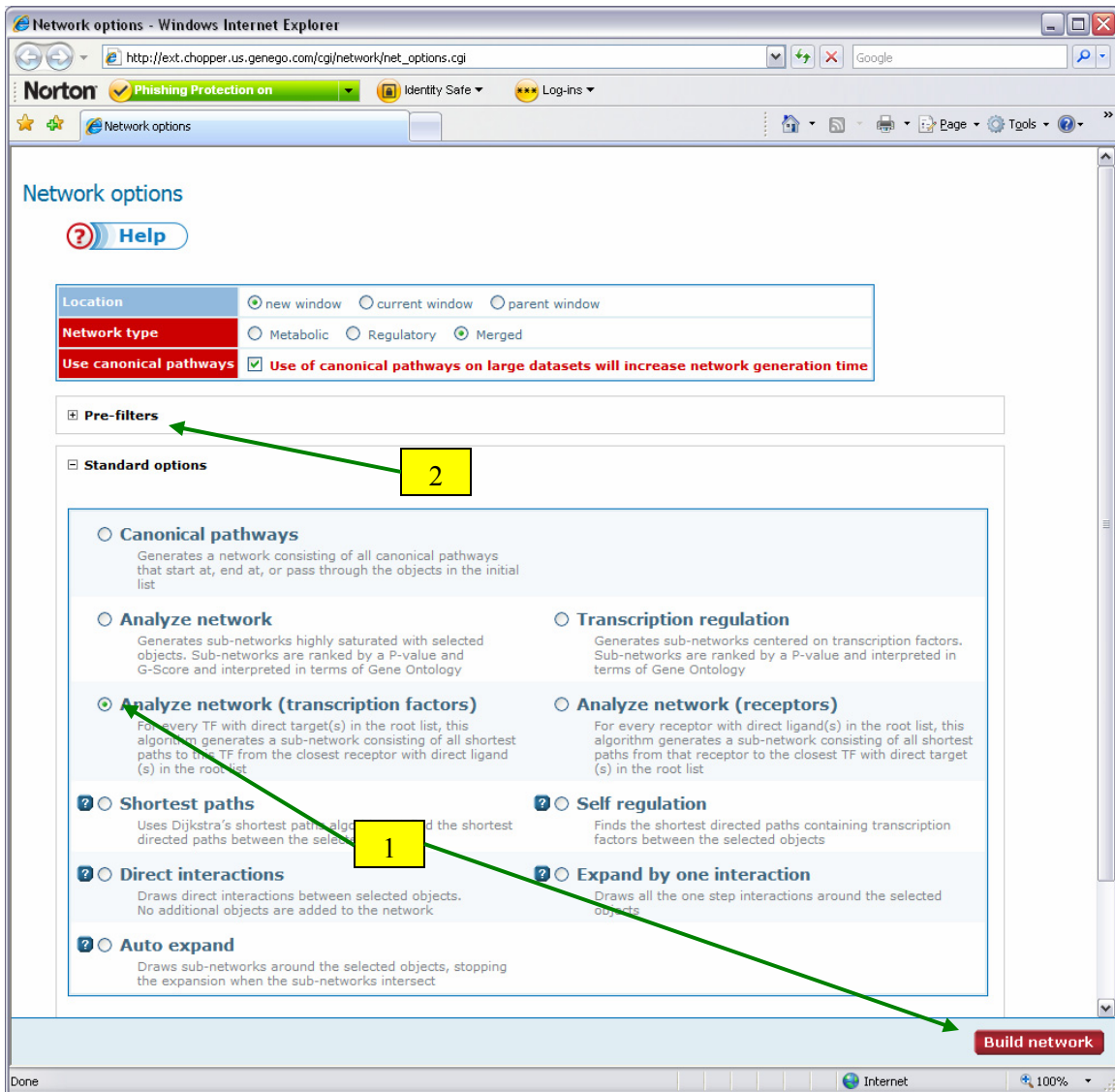


Figure 13: 1. Select Analyze network (transcription factors) and then click Build network. 2. Pre-filters are located here

Network List

All Analyze network algorithm sub-divide the resulting ‘master network’ with a large number of genes and return a list of generated sub-networks ranked by corresponding p-values. The table also lists the top five related GO processes for each network, the total number of objects in the network, the number of objects in the network from the original list (Target genes, also sometimes termed as “Root Nodes”), the number of canonical pathways implicated in each network, and a Z-Score. You can click the Scoring Procedures button and the “?” button next to Priority options for more information about the p-Value and Z-Score calculations. More information and details on algorithms and scoring procedures can be found by clicking the Questions and Answers link on top of the Data Manager page.

Networks are also named on the fly based on the main elements involved in each (hubs, nodes, etc...). By seeing the list of resulting networks, their main elements, and associated processes, one can get the idea of main resulting functional network themes before deciding which ones to open and explore.

By un-checking all the boxes and selecting a couple of networks of interest, these networks can be merged by clicking “Merge Selected.” The entire network list can also be saved (click Save button on top right) to be able go back and open /merge any of the networks at any time in the future from this list.

Click the link in the Network column to visualize networks one at a time (**Figure 14**).

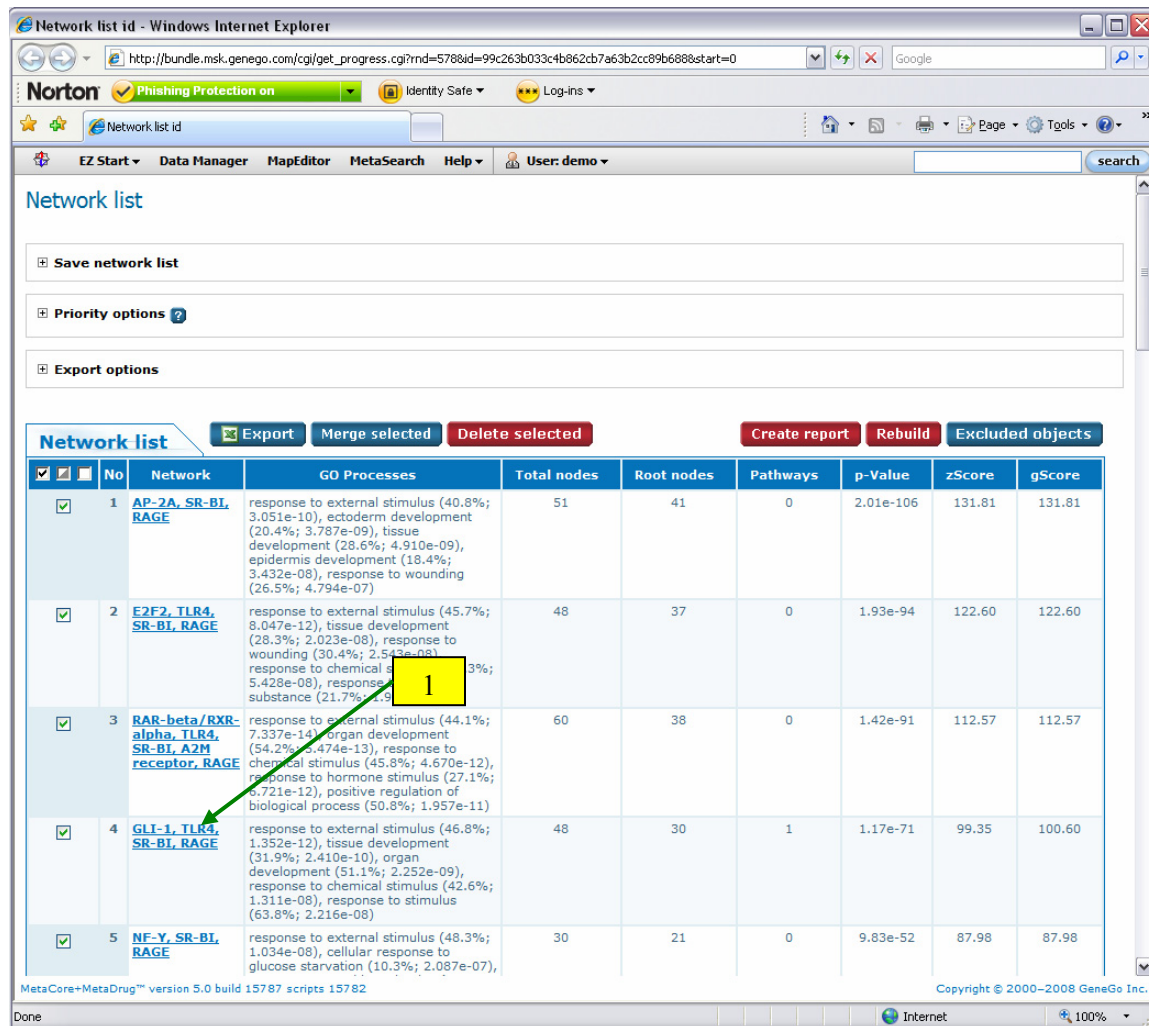


Figure 14: 1. Click here to view the network.

Working with the Network

There are many other tools that can be used on the network interface. These include selecting specific types object for filtering/hiding, filtering experiments, viewing pathways, drug targets, processes, and diseases. Just as with maps, you can export lists of

genes to MetaCore and to other third-party software. You can also obtain statistics about the network in a tabular format and save the network to MetaCore or as an image file.

Advanced Select Tool

With this tool you can select specific object types. After selecting them you can export the genes from the selected objects, or use the selection for filtering. For example, to see what happens to the network when the transcription factors are removed, select 'Transcription factor' from the list and click the Select button. Then click the 'Hide selected objects' button on the toolbar. All the transcription factors on the network will then be hidden.

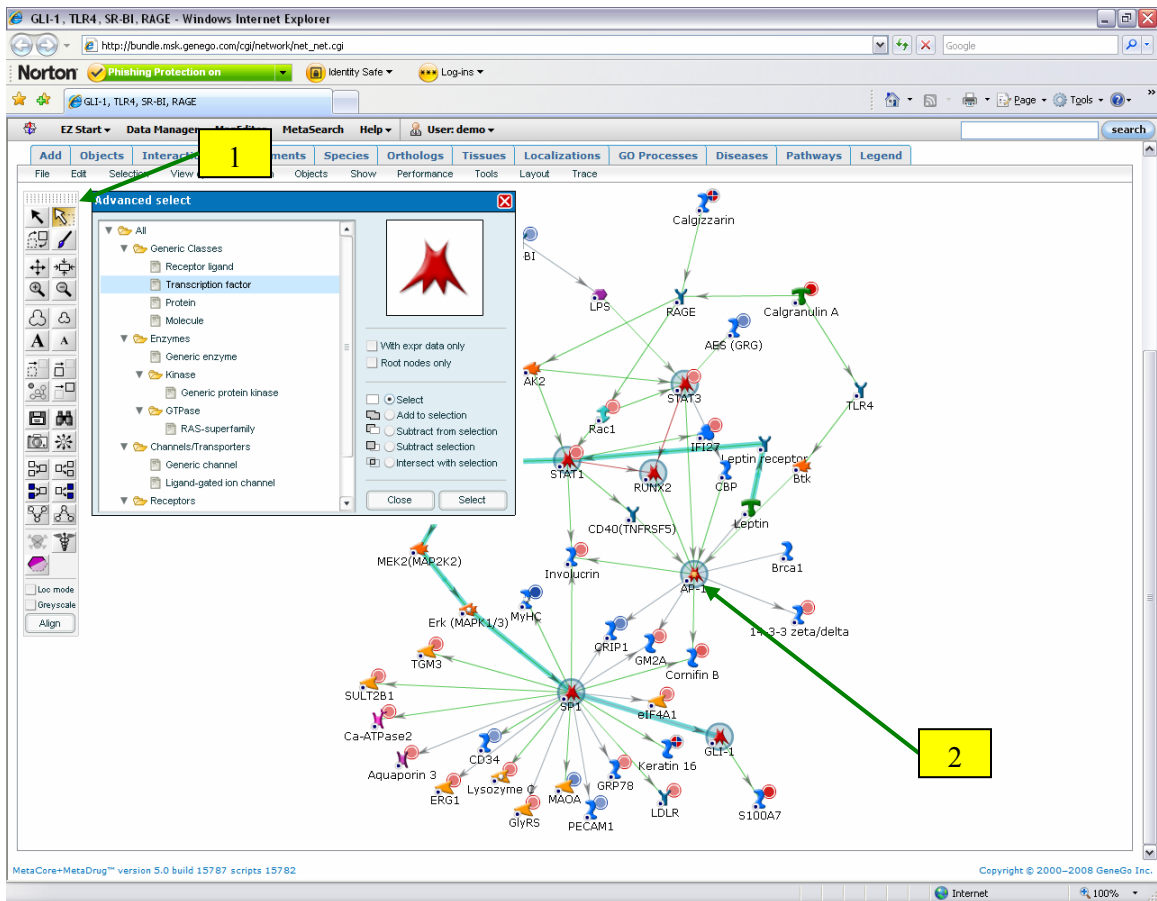


Figure 15: 1. Click here to open the Advanced Select tool. 2. Transcription factors selected.

Filter Experimental Data

It is also possible to filter experimental data and visually compare similarly or differentially regulated genes. Click the Experiments tab; select the experiments to view; and click Apply. Objects with experimental data only from the checked experiment(s) will display the blue and red circles next to them. If you un-check all experiments, the network will only contain objects themselves, but no data (Figure 16). The Experiments filter also has the option to animate the data. This will display the data circles for each

selected experiment individually and toggle through them. This provides a useful visual illustration of the differences and similarities in gene regulation on this network between different samples. If at this point we wanted to also overlay samples from another data set or simply change threshold settings to see which additional genes light up, we could activate or set thresholds for the data in Data Manager, and then get back to this network window and click the red button “Apply current Experiment Data” to visualize current sample or newly updated filtering conditions on the network.

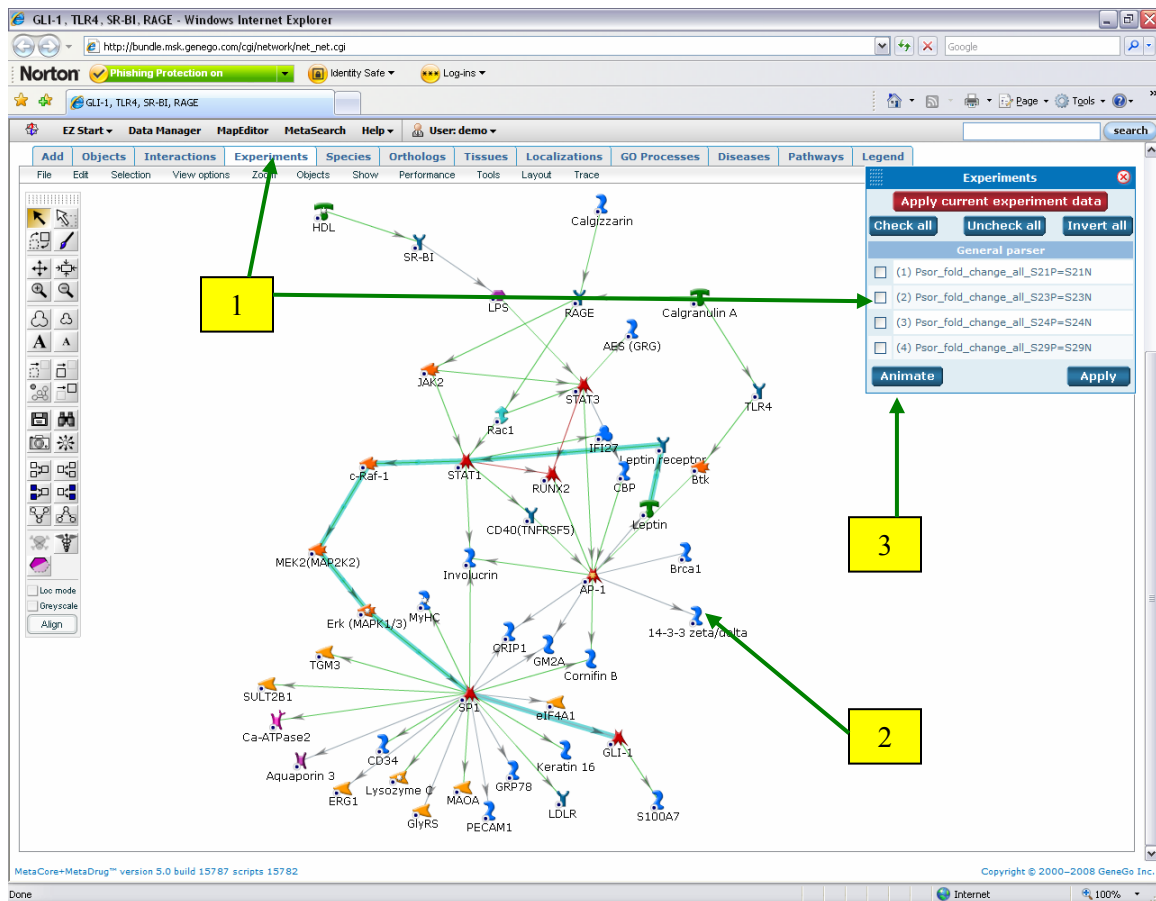


Figure 16: 1. Use the experiments tab to filter out experimental data. 2. Blue and red circles no longer appear. 3. Click here to cycle through the selected experiments.

Trace Pathways on a Network

If the “Use canonical pathways” option was checked on the Network Options page and canonical pathways are present on the network (turquoise colored interaction arrows), you can use the Pathways tab to automatically trace the individual canonical pathways present on the network. The Pathways tab contains the list of all the canonical pathways on the network. Click on a pathway to highlight its interactions in green. Clicking the “Animate” button will cycle through all the pathways on the network. You can also click the “View source” button to see the map or pre-built network that the selected pathway is from (**Figure 17**).

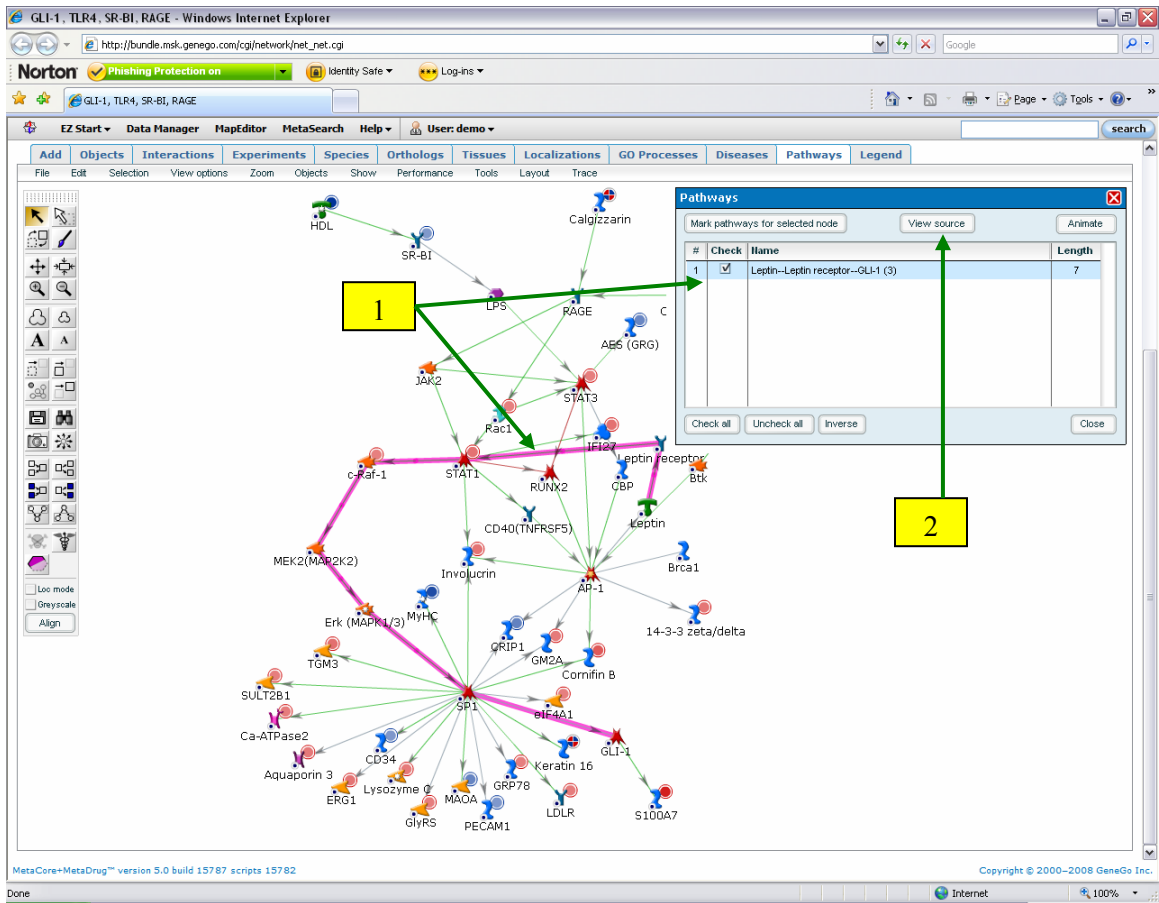


Figure 17: 1. Selected pathway is highlighted in green. 2. Click here to see the source map or pre-built network.

Marking Drug Targets on a Network

Press the Drug Targets button to mark the objects on the network that have drug targets. Objects with drug targets will be marked with a grey circle (**Figure 18**).

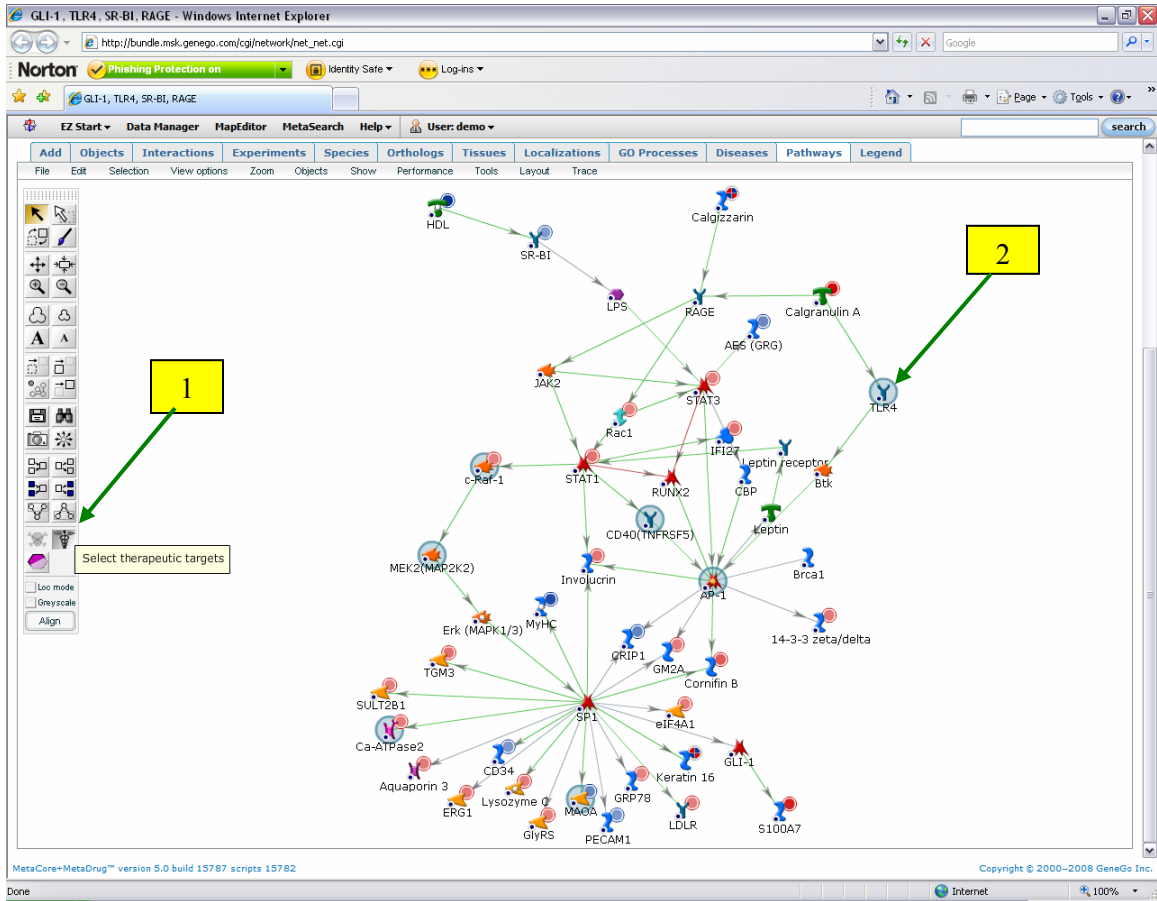


Figure 18: 1. Click the Drug Targets button. 2. Objects with drug targets will be marked in grey.

The GO Processes Tab

Click the GO Processes tab to see the top 12 functional processes associated with the network. After clicking the GO Processes tab, a dialog box will appear asking if you wish to continue, click OK. The coverage percentage and p-Value for each process are also listed. You can select genes involved in (a) particular process(es) by clicking “mark selected”. Any time we make a selection we can choose to hide unselected objects and thereby focus on the portion of network dealing particularly with selected processes or diseases or drug targets or tissue, or orthologs. We could choose keratinocyte differentiation from the list here, because it is relevant to psoriasis and is also one of the top scoring processes, and then select all the corresponding genes, and hide unselected ones to filter out less relevant processes.

We can also trace a process by selecting it and clicking the Trace Selected button. The process will be traced automatically using the trace objects feature (**Figure 19**).

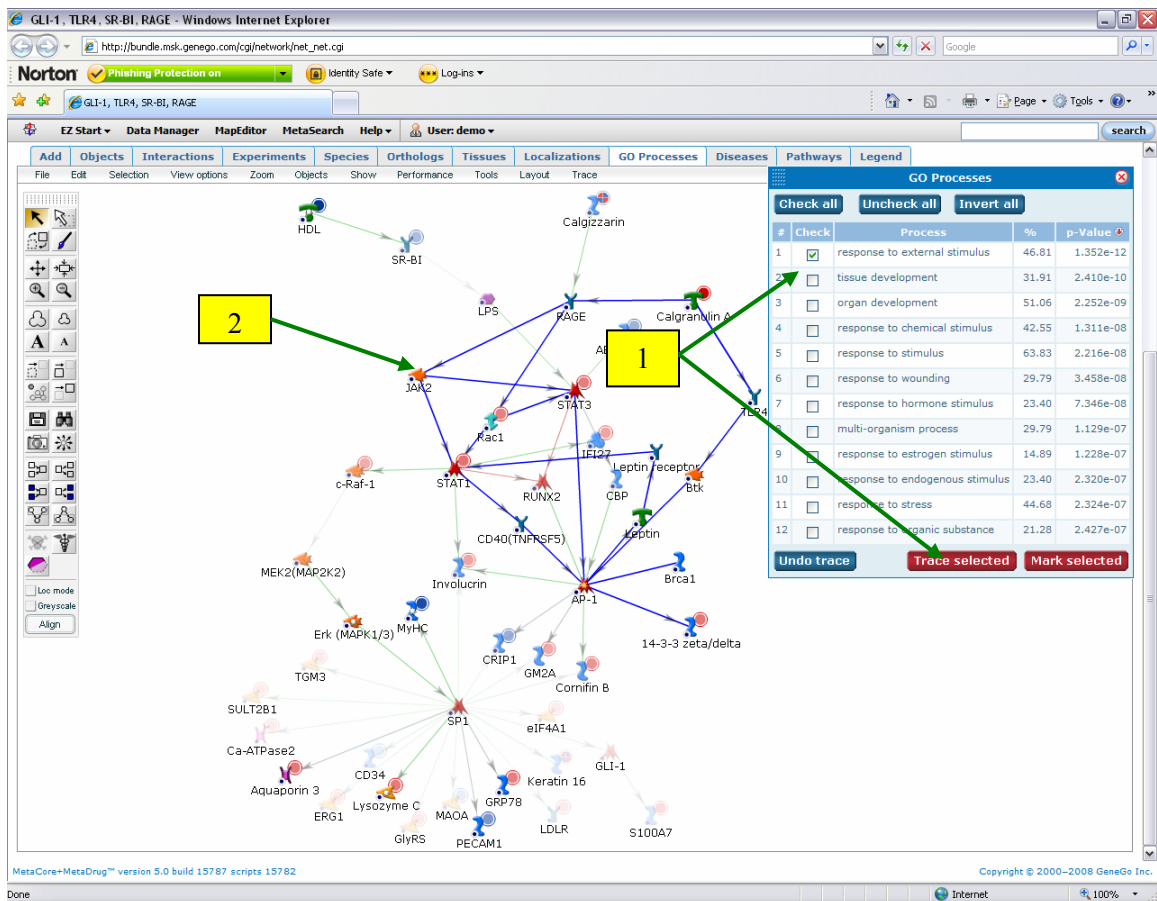


Figure 19: 1. Check a process and click Trace Selected. 2. The process will be traced automatically.

The Diseases Tab

Click the Diseases tab to see the top 12 diseases associated with the network. This tool works similar to the Processes tab. After clicking the Diseases tab, a dialog box will appear asking if you wish to continue, click OK. The coverage percentage and p-Value for each disease are also listed. You can trace a disease by selecting it and clicking the Trace Selected button. The disease will be traced automatically using the trace objects feature (**Figure 20**). If we wanted to narrow down our network to specified disease genes, we would use the Mark Selected option and then hide all unselected nodes. Here, for instance, we could concentrate only on skin diseases by selecting the genes in the top significant disease category and hiding all un-selected nodes.

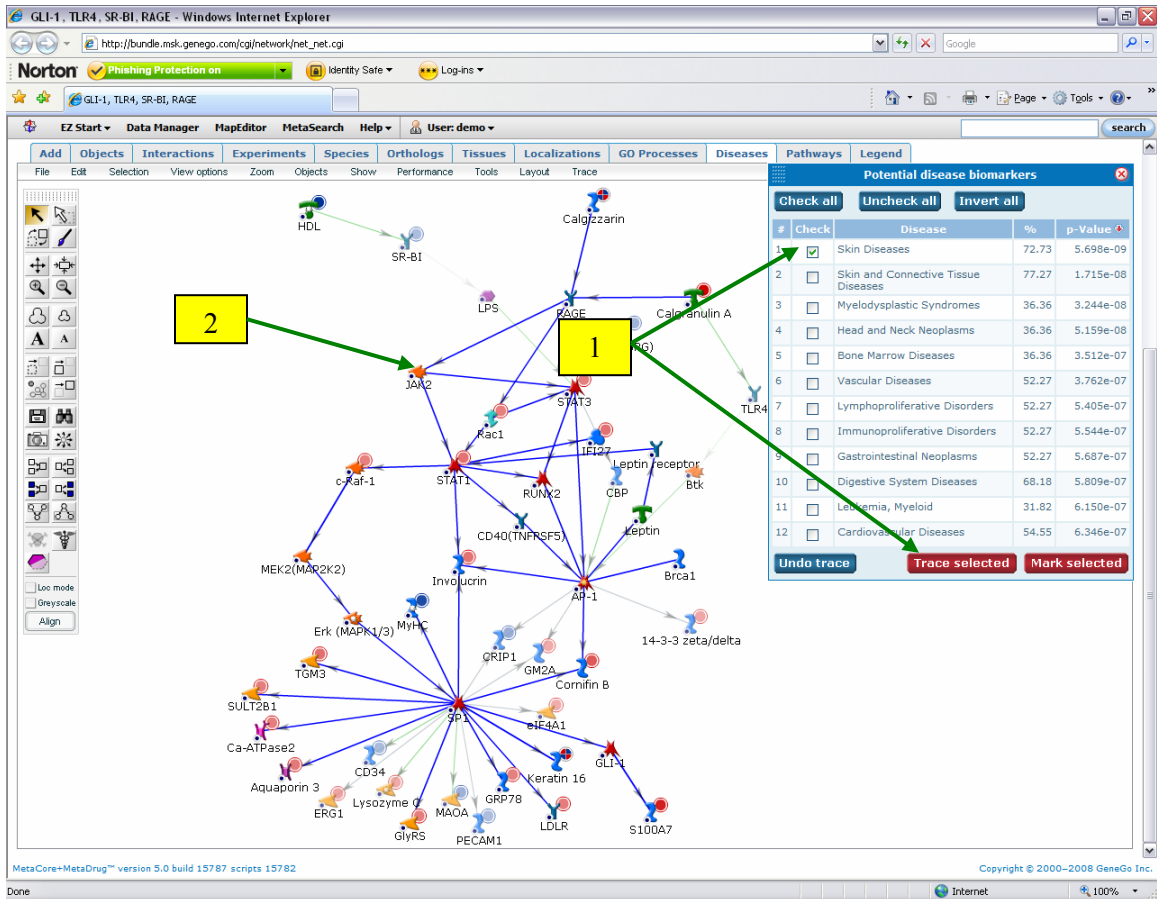


Figure 20: 1. Check a disease and click Trace Selected. 2. The disease will be traced automatically.

Exporting a List of Genes from a Network

As with maps, it is possible to export a list of genes from a network. Here, we will export a list of genes from a selected disease. First, open the diseases tab and select a disease. Then, click Mark Selected. In the export section (above the network), choose Genes in the 'Export as' row, check the 'Advanced mode' box and choose Selected nodes next to 'Which nodes'. Finally, give the list a name and click the Export button (**Figure 21**). In addition to exporting to MetaCore, you can export genes to Rosetta Resolver, GeneSpring

GX, Spotfire DecisionSite, and to Excel. Excel table output with default options will include all network genes. Those network genes that have data values mapped onto them will have those sample ID's and values exported in the table and marked with different shades of red and blue, according to the sign and magnitude of values in those cells.

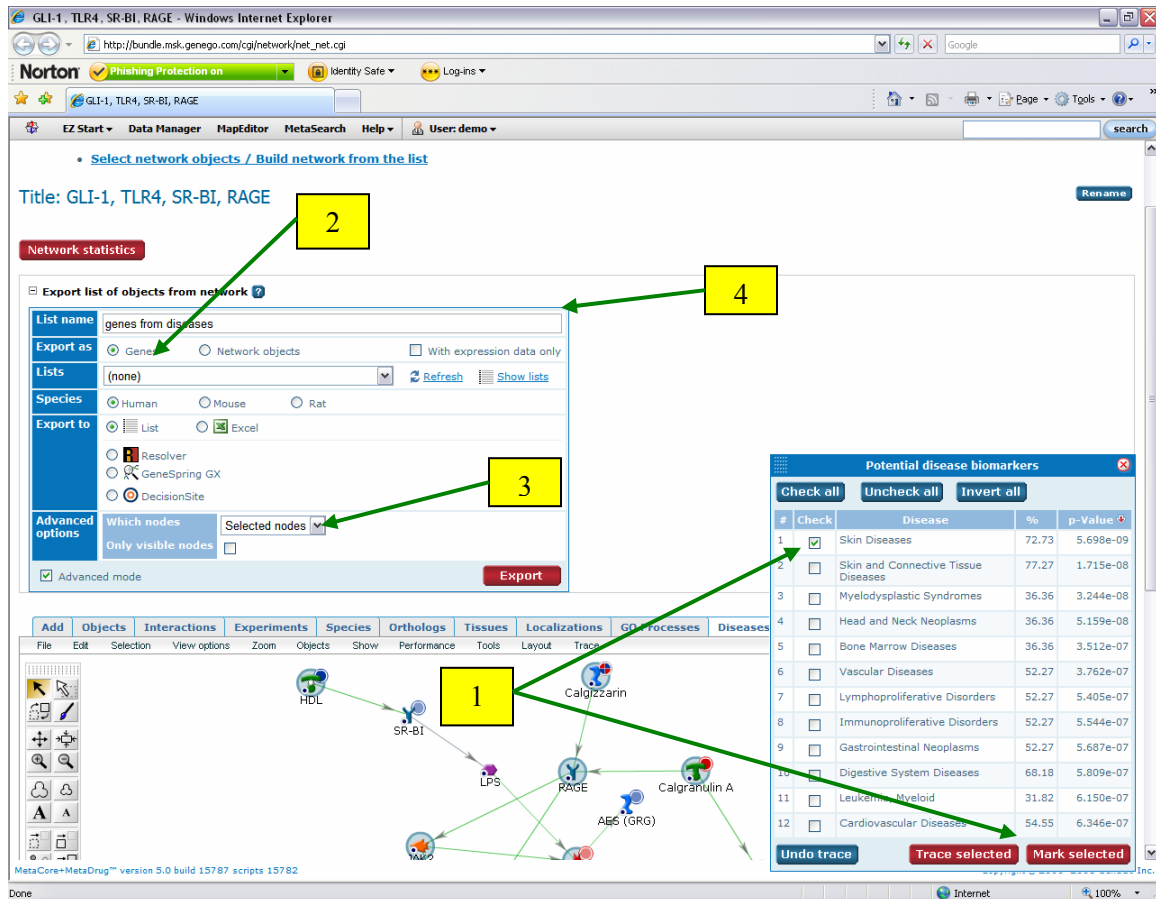


Figure 21: 1. Choose a disease and click Mark Selected. 2. Choose Genes. 3. Export selected nodes. 4. Enter a name and click Export.

Network Statistics

Various statistics about a network can be seen by clicking the Network Statistics button or by choosing Network Statistics from the File menu. This allows you to see a tabular overview of the network including information about how the network was built; any experimental data on the network; how active different objects are in the network; and the GO processes, diseases and tissues related to the network (**Figure 22**).

The top section of the statistics page displays basic information about the network including its name and description (a description can be added by clicking the "Rename" button on the network page). Click on a section hyperlink to go directly to that section or click a section header to open the information panel. The first section shows what options were used when building the network including the algorithm used, any chosen pre-filters and the advanced options used. All the nodes with experimental data are listed as well.

The table includes the network object, its related genes, the gene description, and the data value (**Figure 23**). All of the objects on the network are listed in the Hubs and Nodes sections. A Hub is a node that has five or more edges on the network. Two numbers are listed in the edges column. The first number is the total number of edges for that node and the second number is the number of hidden edges for that node on the network. An edge is hidden if one or more of the nodes connected to it is hidden or if the nodes connected by the edge are in a group.

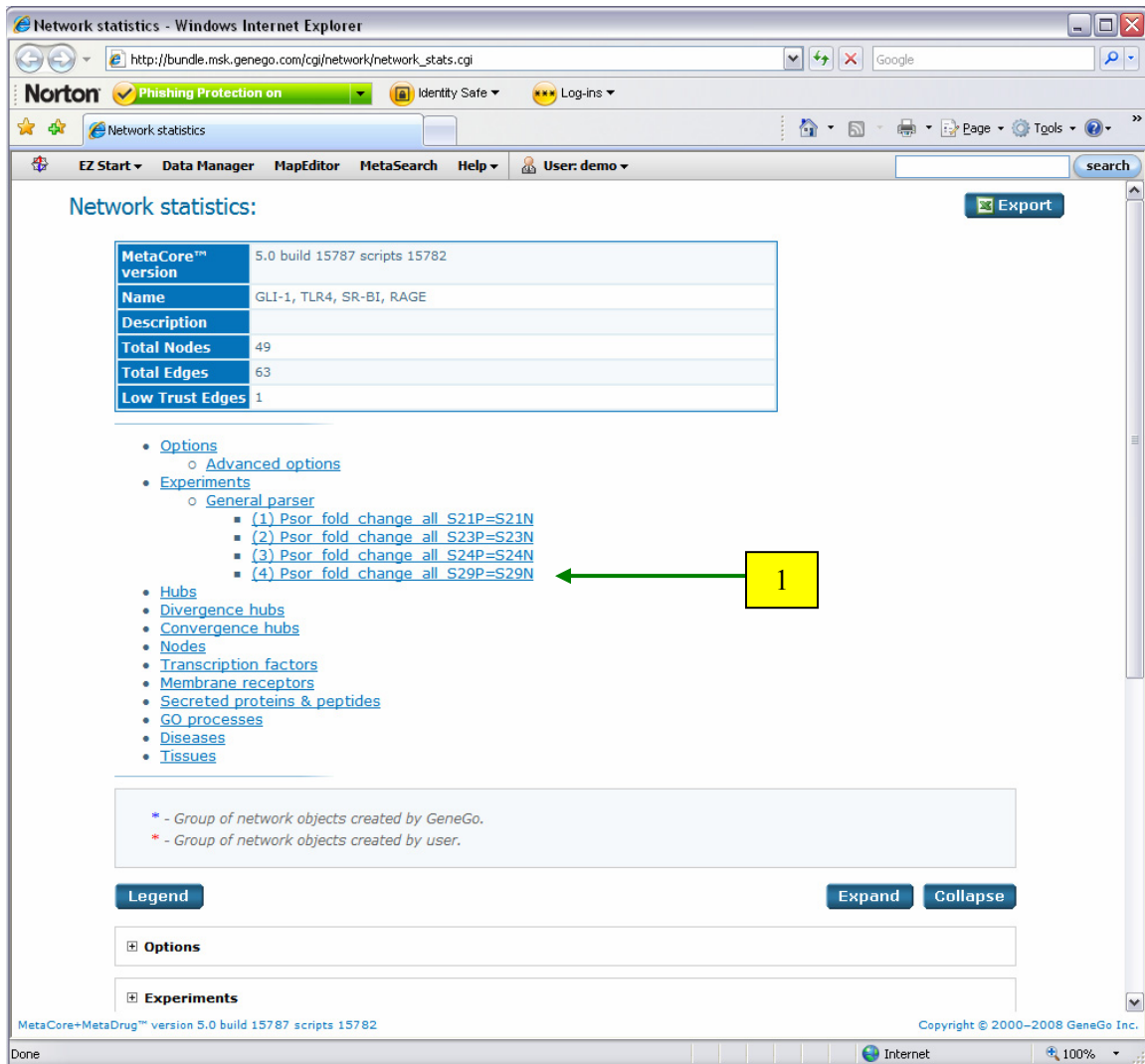


Figure 22: 1. Click a link to jump to that section.

The screenshot shows a web browser window with the URL `http://bundle.msk.genego.com/cgi/network/network_stats.cgi`. The browser's address bar and search bar are visible. The main content area displays a navigation menu with items like 'EZ Start', 'Data Manager', 'MapEditor', 'MetaSearch', and 'Help'. Below this, there are expandable sections: 'Options', 'Experiments', and 'General parser'. Under 'General parser', there is a sub-section '(1) Psor_fold_change_all_S21P=S21N' which contains a table of network objects.

#	Visible	Network object	Name	Description	Signal
1	<input checked="" type="checkbox"/>	14-3-3 zeta/delta	1433Z_HUMAN	Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathway. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.	7.963
2	<input checked="" type="checkbox"/>	AES (GRG)	AES_HUMAN	Acts as dominant repressor towards other family members. Inhibits NF-kappa-B-regulated gene expression. May be required for the initiation and maintenance of the differentiated state.	-3.805
3	<input checked="" type="checkbox"/>	Aquaporin 3	AQP3_HUMAN	Forms a water-specific channel that provide kidney medullary collecting duct with high permeability to water, thereby permitting water to move in the direction of an osmotic gradient. May function as a water and urea exit mechanism in antidiuresis in collecting duct cells. Also slightly permeable to urea and glycerol. May play an important role in gastrointestinal tract water transport and in glycerol metabolism (By similarity).	5.993
4	<input checked="" type="checkbox"/>	Ca-ATPase2	AT2A2_HUMAN	This magnesium-dependent enzyme catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum lumen. Isoform SERCA2A is involved in the regulation of the contraction/relaxation cycle.	4.499
5	<input checked="" type="checkbox"/>	Calqizzarin	S10A8_HUMAN		3.599
6	<input checked="" type="checkbox"/>	Calgranulin A	S10A8_HUMAN	Expressed by macrophages in chronic inflammations. Also expressed in epithelial cells constitutively or induced during dermatoses. May interact with components of the intermediate filaments in monocytes and epithelial cells.	17.07

At the bottom of the page, there is a footer with the text: 'MetaCore+MetaDrug™ version 5.0 build 15787 scripts 15782 Copyright © 2000–2008 GeneGo Inc.'

Figure 23: 1. Click the headers to open each section. 2. The Experiments section lists the objects with data. A description of the object and the data value are listed as well.

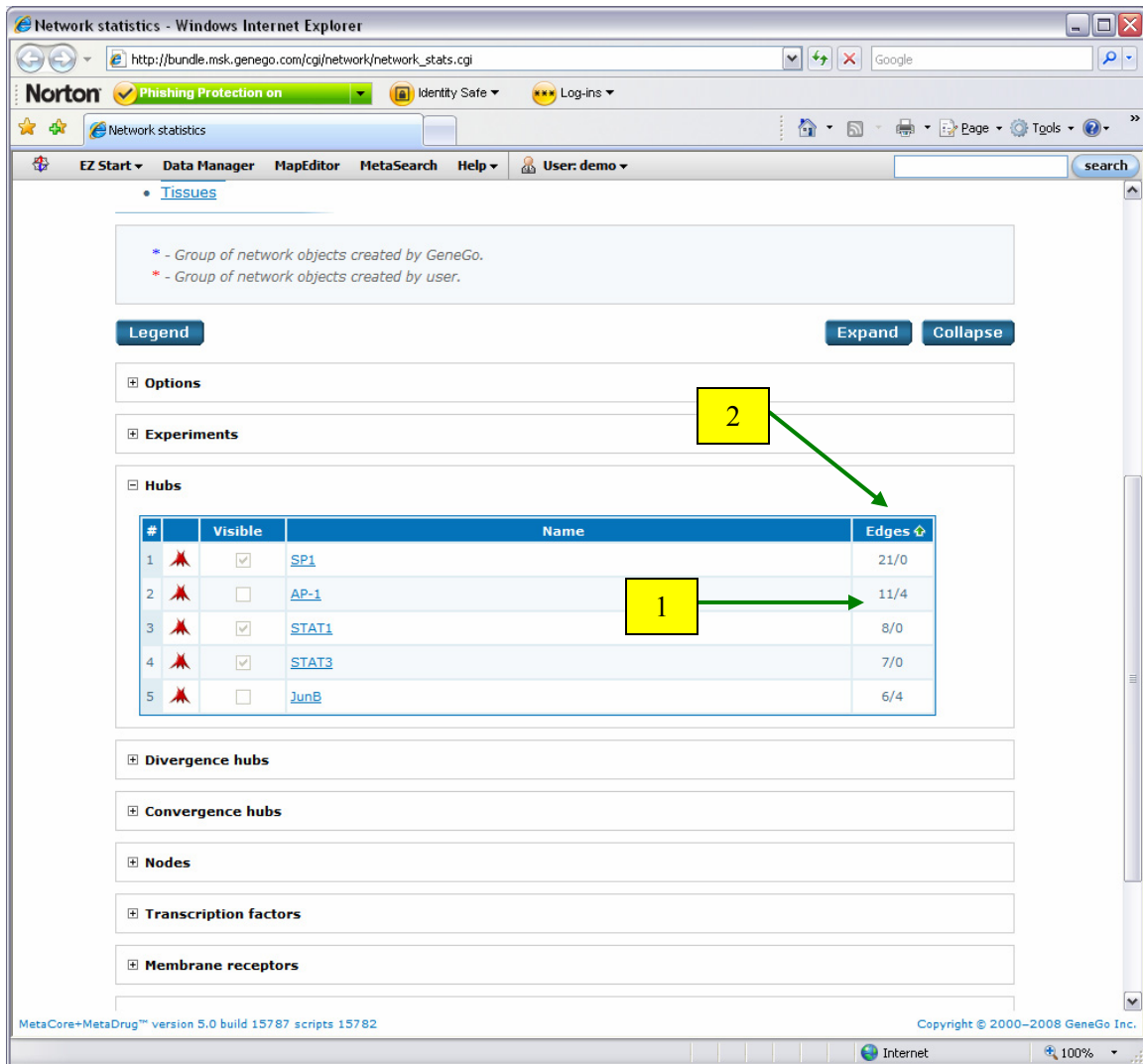


Figure 24: 1. Visible edges / Hidden edges are listed for each Node and Hub. 2. Click the “Edges” header to sort the table.

Saving Networks and Images

Networks can be saved in two formats, Networks and Netshots. Netshots retain the network exactly as it is with any expression data mapped. Saved Networks, however, do not retain the expression data and will reflect any subsequent changes to the objects or interactions on the network as the MetaCore database is updated. So, if you save a network as both a Network and a Netshot you can see exactly how it looked when it was created (with the Netshot) and how it may have changed with subsequent MetaCore updates (with the Network). To save a network, choose “Save” or “Save as” from the File menu. Then, in the window that appears, enter a name and optional description; choose whether to save it as a Network or a Netshot; and then click “Save”. From this window you can also choose to retain or discard any hidden objects by checking or unchecking the “Save hidden nodes and edges” box (Figure 25).

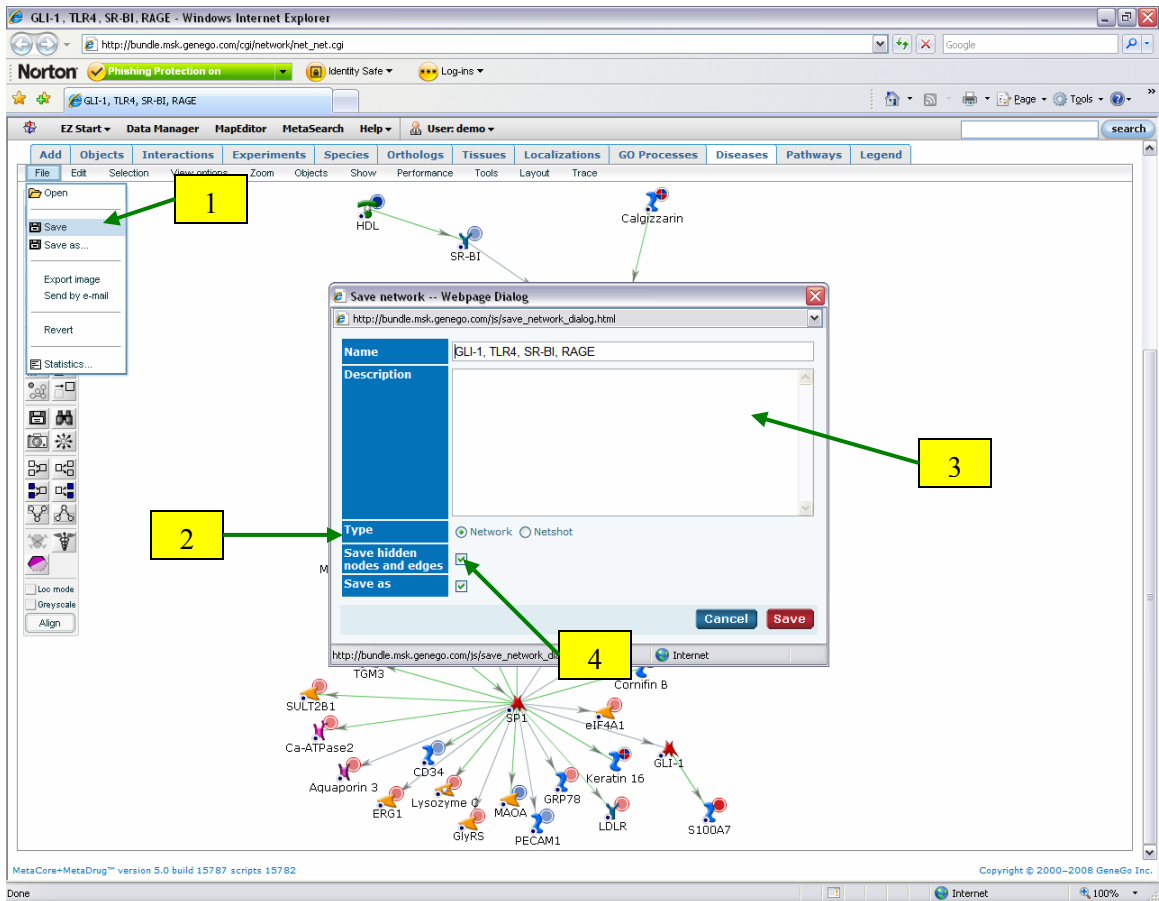


Figure 25: 1. Click here to save the network. 2. Choose Network or Netshot here. 3. Enter a name and optional description. 4. Choose to save or discard hidden objects here.

High quality images (300dpi) of networks can be saved as well. To save an image of a network choose the “Export image” option from the file menu. In the box that appears, choose a resolution and click OK (**Figure 26**). Then browse for a location on your computer to save the file. The image file type is PNG.

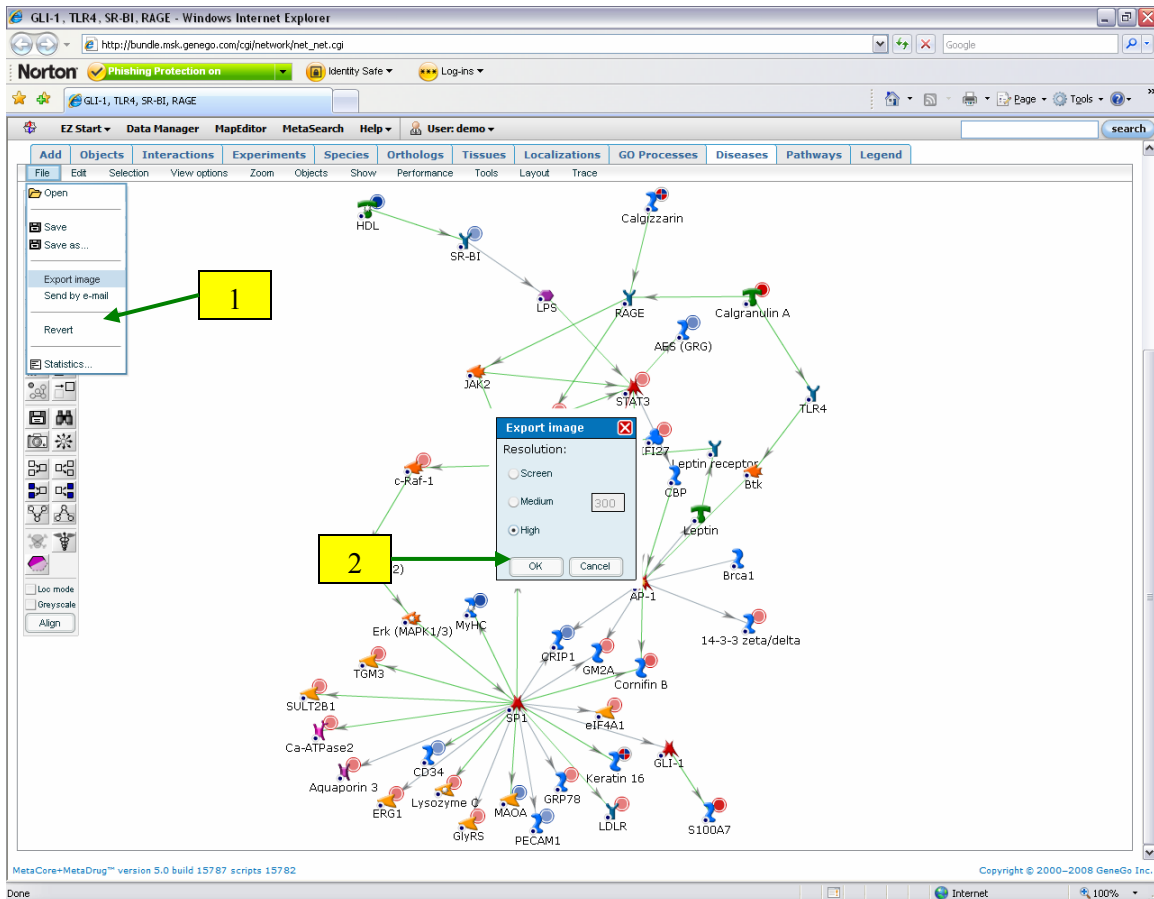


Figure 26: 1. Click here to export an image file. 2. Choose a resolution and click OK.

Data Sharing

With the Data Manager, you can share experiments, gene lists, and saved networks with other users or groups. There are two ways to access the share tool. You can either click Share from the File menu after selecting an experiment by Left-Clicking on it, or you can Right-Click on an experiment and select Share in the context menu. The window that comes up will display some information about the experiment and a field to search for users to share it with. Enter a username in the search field and click Search. Select a user in the results field and click “Add selected to list”. The user will appear in the “Shared with” section (**Figure 27**).

There are six share levels available. The most permissive level is on the left and the least permissive level is on the right. The different levels are:



Allow

- OW - Owner Rights. This level will allow the user it is shared with to share it to others and to delete it.
- WA - Write Access. This level will allow the user it is shared with to rename it and to overwrite it.
- RA - Read Access. This level will only allow the user it is shared with to view the experiment(s) and related maps, networks, etc.

Deny

- OW - Owner Rights. Choose this level if you have, for example, allowed ownership rights to an entire folder, but would like to deny ownership right to some individual experiments within the shared folder.
- WA - Write Access. Choose this level to deny write access to individual experiments with a shared folder.
- RA - Read Access. Choose this level to totally restrict all access to a file within a shared folder.

Additionally, if you wish to completely give up your ownership of a file to another user, first select to Allow Owner Rights (OW) of the file to the other user and then check the box next to "Refuse from ownership" and click OK. You will then no longer have ownership rights to the file.

Experiments you own that are shared with other users will be marked with a blue hand icon in the Data Manager (). Experiments owned by another user that are shared with you, can be found in the Shared Data folder and they will be marked with a green hand icon (.

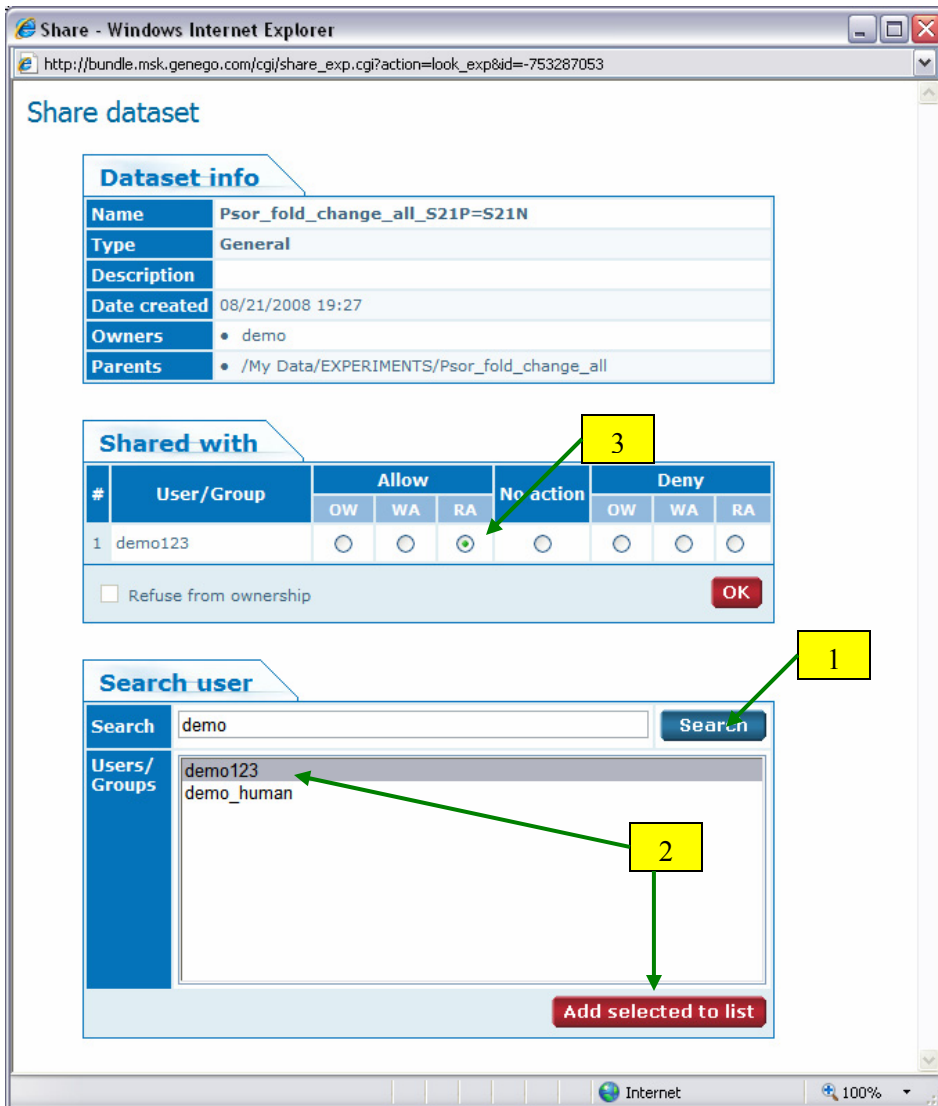


Figure 27: 1. Search for the user to share with here. 2. Select a user and click “Add selected to list”. 3. Set the sharing level and click OK.

Results

This network alone shows the activation and interplay of two key processes in psoriasis. Key regulators that are overexpressed have major implications in immune-system-regulated inflammation /response to stimuli, and keratinocyte differentiation /proliferative processes, as can be also inferred from the canonical pathway edges associated with them (thick turquoise lines). A few significantly overexpressed genes, such as S100A7 and Calgranulin-B (S100A9) have known documented associations with psoriatic processes (2). Some other genes are also documented psoriasis markers (STAT1, SP1, AP1 proteins, and RAGE, which is a receptor that binds S100A9)(3-6), and many genes on this network serve as targets for known compounds (gray circles). The fact that these markers are up-regulated and all present on the same sub-network, which also happens to be one of the most significant here, corroborates the importance of these processes in psoriasis pathogenesis. This is a likely scenario in auto-immune

inflammatory disease that leads to proliferation of inflamed keratinocytes to the skin surface in a chaotic uncontrolled fashion, causing acute epidermal hyperplasia with the 'raw' wound-like appearance, which eventually develops into scales, flaking off which in turn exposes yet another level on inflamed injured skin.

Conclusions

Two quick analysis steps in MetaCore™ (Enrichment Analysis Workflow and analyze network) led us to identification of key regulators of psoriasis-related processes. Enrichment analysis and network construction allowed us to evaluate statistically significant genes, which distinguish psoriatic samples from normal skin. In addition, examining the information extracted from the highest-ranked common processes in MetaCore™ has helped validate the implicated disease pathways, which recapitulate the inflammatory and proliferation/differentiation processes in psoriasis. Upon further analysis, MetaCore may enable future identification of novel candidate gene leads that may serve as novel therapeutic intervention points or possible predictive markers of disease pathogenesis.

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