

Mouse Atlas DiscoverySpace Tutorial



Example 1: Foregut vs. Hindgut

1. Find all the tags that are exclusive to the Foregut (SM107) and not in Hindgut (SM112) pg 2
2. Identify Tags that are up regulated in the Foregut when compared with the Hindgut pg 7
3. Map up regulated tags pg 9
4. Find GO Categories in the Hindgut library pg 13

Example 2: Gene of Interest

1. Retrieve tags for a gene of interest – FGF8 pg 16
2. Determine which libraries contain identified tags pg 18

Example 1: Foregut vs. Hindgut

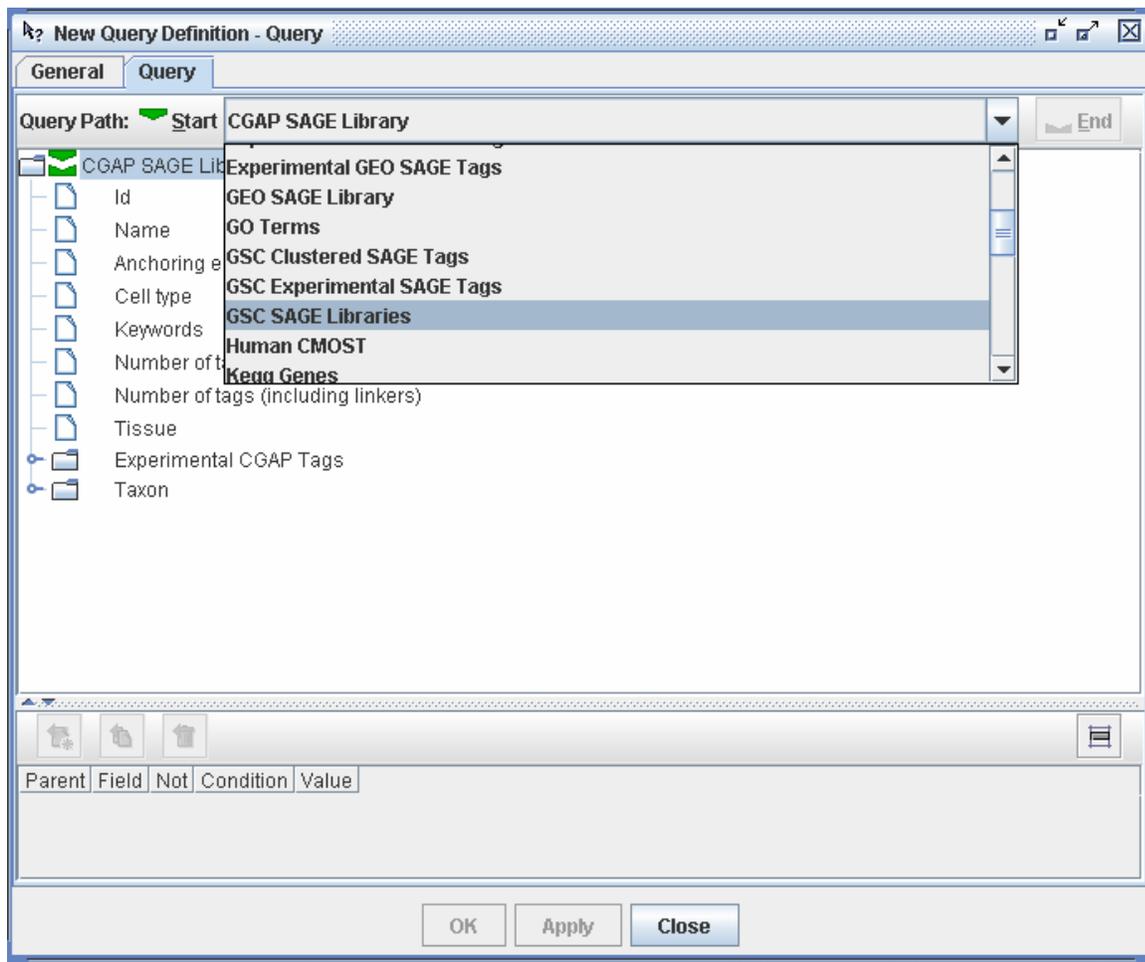
Task 1: find tags exclusively to hindgut and foregut libraries

1.1. Build query for Foregut (SM107)

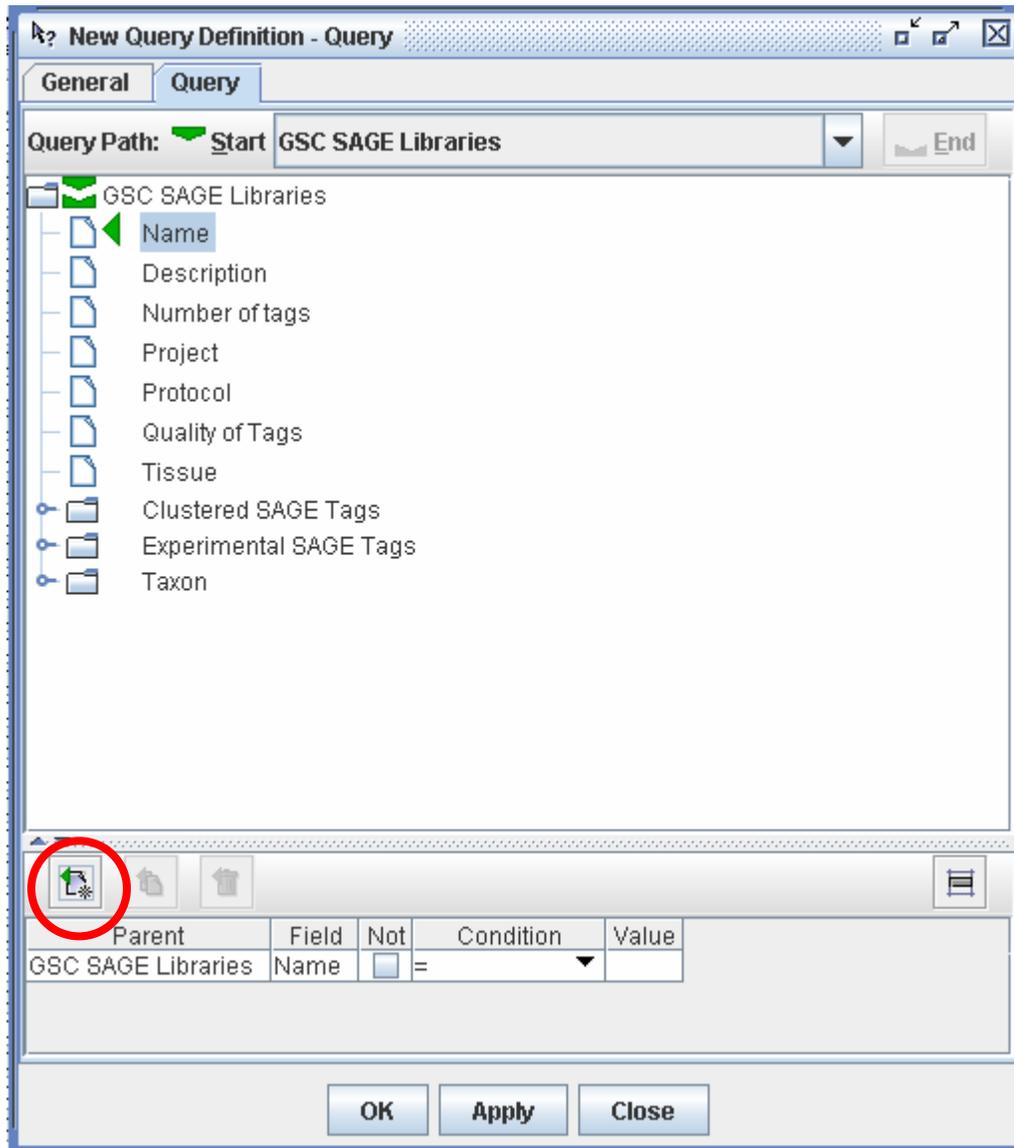
Create a new query by clicking the New Query button. This will open the Query Builder window.



Use the pull down menu to select GSC SAGE Libraries (Note: you must have set your user name and password to have access to these libraries. E-mail discoveryspace-support@bcgsc.ca if you do not have a password).



You can now begin to build your query. Understanding queries is crucial for successfully using DS4. It may feel overwhelming at first, but with a little practice, it will become clear.



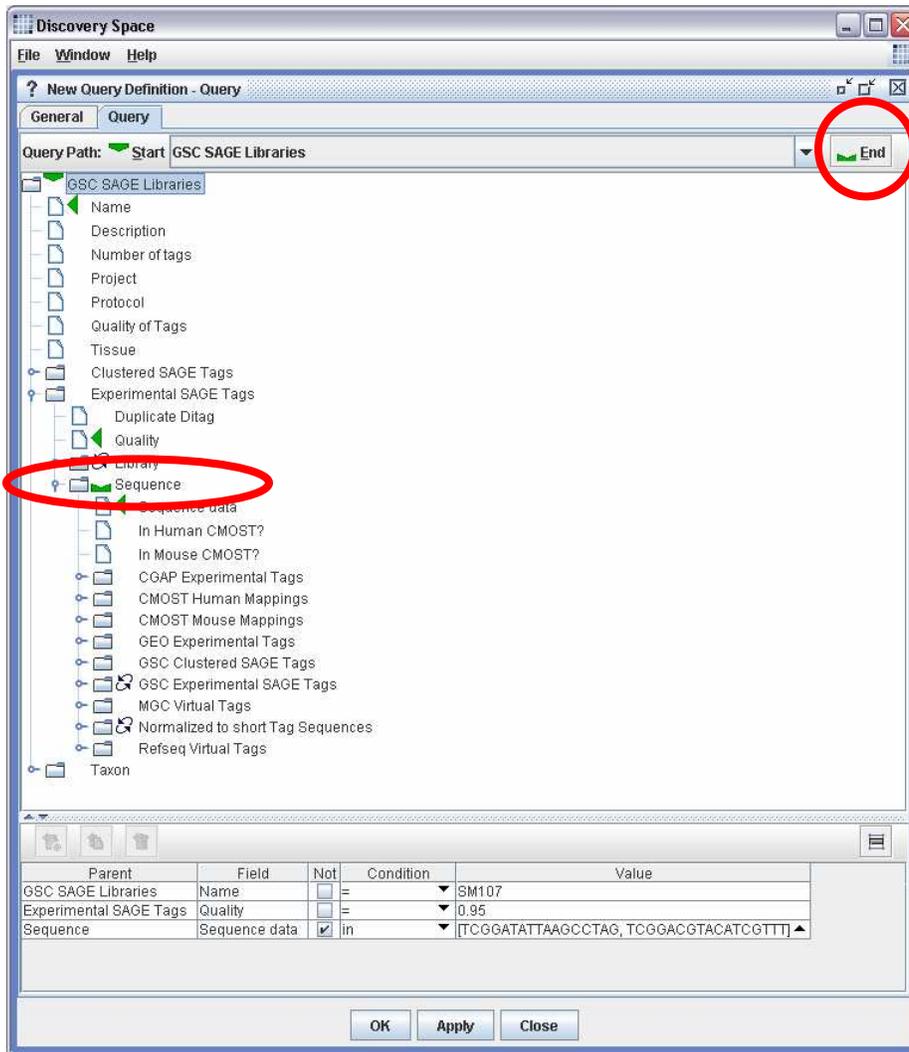
Click on each item you want to set a constraint for and click the “Set Parameter” button. In the bottom window you can set your constraint by selecting a condition and setting a value.

Parameters to set:

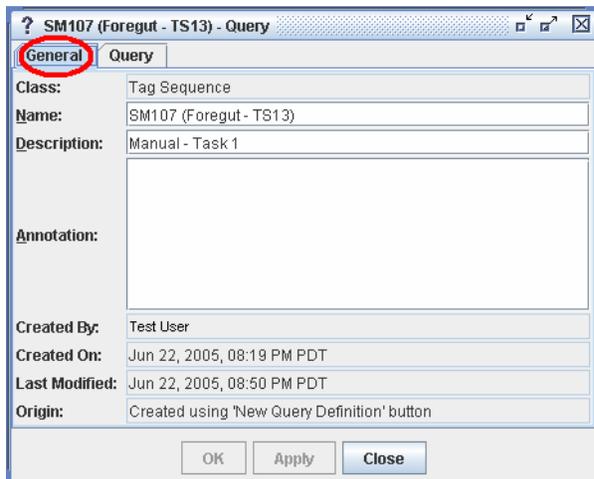
- GSC Libraries -> Name : set to library accession (SM107)
- Experimental SAGE Tags -> Quality : set to ≥ 0.95 (95%)
- Experimental SAGE Tags -> Sequence -> Sequence data: set to linkers using IN clause. Click NOT box to exclude linkers from your analysis.

The final and VERY IMPORTANT step is to use the END button (on upper right hand side of the Query builder) to set what kind of data the query is going to return. In this case we set it to Sequence to return tags for our library.

Click Sequence, then click on the End button.

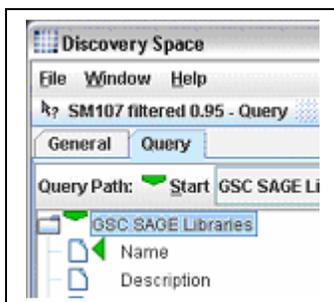
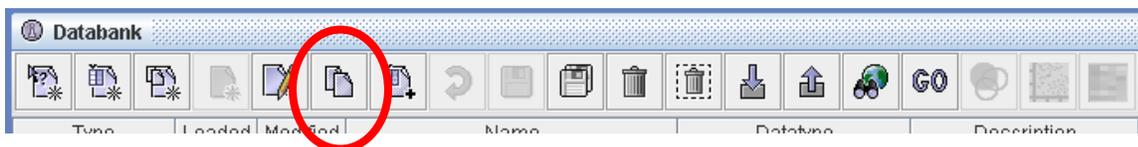


Our query is almost complete. Before clicking on “OK”, make sure that you will be able to identify this query for future reference. You can do this by going to the General tab at the top of the query generator window and adding as much information as you think would be useful.



The query is now complete. Click OK.

1.2. To create another query with the same parameters but for a different library, you can either repeat the previous step (1), or you can select your previous query in the databank and use the “Duplicate Selected Definitions” button on the databank to create an identical query for Hindgut (SM112).



All you have to do is change the Name parameter to SM112. The other constraints will remain unchanged.

Again, you should save this query using an appropriate name by using the General tab.

You now have two separate queries. One for Hindgut and one for Foregut.

Now go to the databank and look at the two queries. Notice they are both a query type. This indicates that if you work with this type of data you will be making a direct connection to the database. This is not necessarily a bad thing to do, but if many users are connecting in this fashion (using this data type) the queries will take a long time to return results.

Type	Loaded	Modified	Name	Datatype	Description	Origin	Last Modif
Query	<input type="checkbox"/>	<input checked="" type="checkbox"/>	SM107 (Foregut - TS13)	Tag Sequence	Manual - Tas...	Created using 'New Query Definition' butt...	Jun 22, 2005, 04
Query	<input type="checkbox"/>	<input checked="" type="checkbox"/>	SM112 (Hindgut - TS13)	Tag Sequence	Manual - Tas...	Duplicated from Definition 'SM107 (Foreg...	Jun 22, 2005, 04

IMPORTANT: To speed up further analysis, you should create data definitions from your queries. Highlight your queries and select the Create Data Definition button. These definitions should be renamed for future reference. Click the General tab and enter identifying information. The data definitions will be used in subsequent analyses.



1.3. In the databank, select these two **data** definitions and click the Venn Diagram button.



The Venn widget will appear. You can extract tags exclusive to each library.

Name	Count	Description
SM112 (Hindgut - TS13)	110552	Manual - Task 1
SM107 (Foregut - TS13)	102985	Manual - Task 1

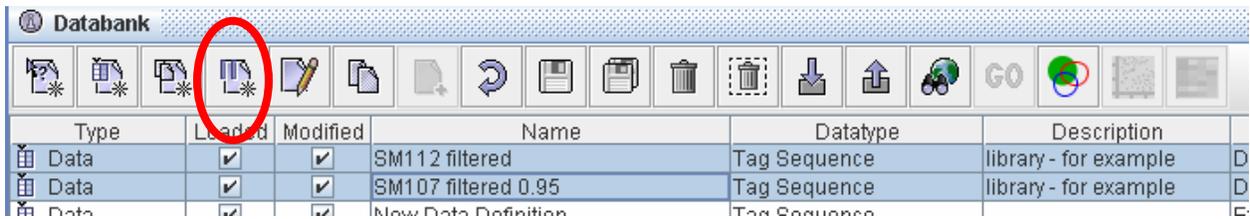
Item	Count	SM107 (Foregut - TS13)
TTTGGTTTAAGAAGAGG	21	21
GCCAACAGCATCGCCAG	11	11
GTTGTCCTTTTTCTCT	11	11
CACGCTCCCGGAAGGC	10	10
CGGTTCACCCGAAAGG	10	10
CTGTGCCCTCCAGGTA	10	10
CAAGGTGACAGGCCGGT	9	9
TTAAAGTAATGAAAAA	9	9
TTGGTAGCCGTAGCACA	9	9
ACCAACAGGTAGTTGGC	8	8
ACCATCCTCTGCCACC	8	8
ACTTCAGATCATCCAG	8	8
CGTTGATCTTGCACTT	8	8
CTATTAATTTTTGCCCT	8	8
GAAGGAGTCTTTGTATG	8	8
GCCGCTCTGGTTGCTCT	8	8
TAAGAAGGATTCACCG	8	8

You can drag your tags out anywhere on the desktop. This will create a data definition for those tags. Click the General tab to rename this data for future reference.

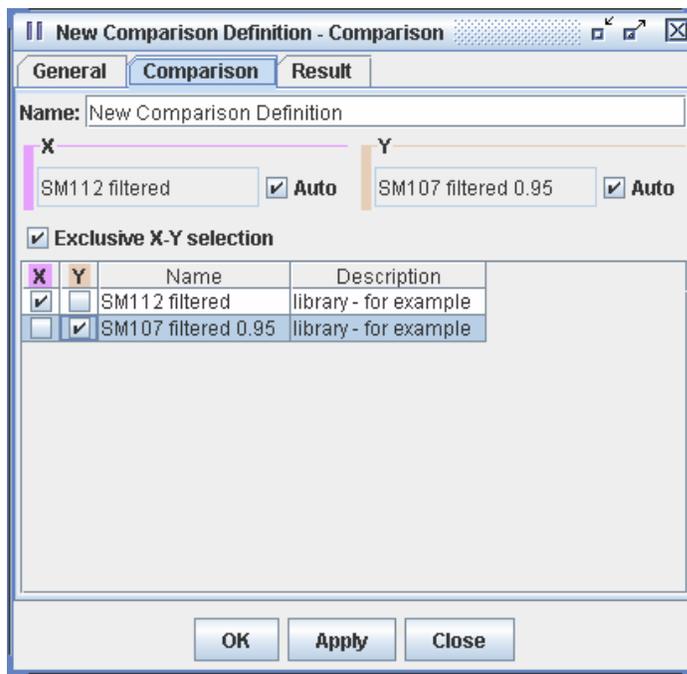
Class	Item	Quantity
Tag Sequence	TTTGGTTTAAGAAGAGG	21
Tag Sequence	GCCAACAGCATCGCCAG	11
Tag Sequence	GTTGTCCTTTTTCTCT	11
Tag Sequence	CACGCTCCCGGAAGGC	10
Tag Sequence	CGGTTCACCCGAAAGG	10
Tag Sequence	CTGTGCCCTCCAGGTA	10
Tag Sequence	CAAGGTGACAGGCCGGT	9
Tag Sequence	TTAAAGTAATGAAAAA	9
Tag Sequence	TTGGTAGCCGTAGCACA	9
Tag Sequence	ACCAACAGGTAGTTGGC	8

Task 2: find tags up regulated in Foregut vs. Hindgut

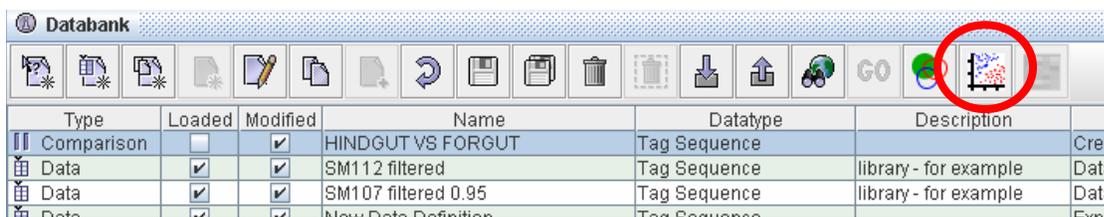
Highlight your two library tag Data Definitions and select the Comparison button.



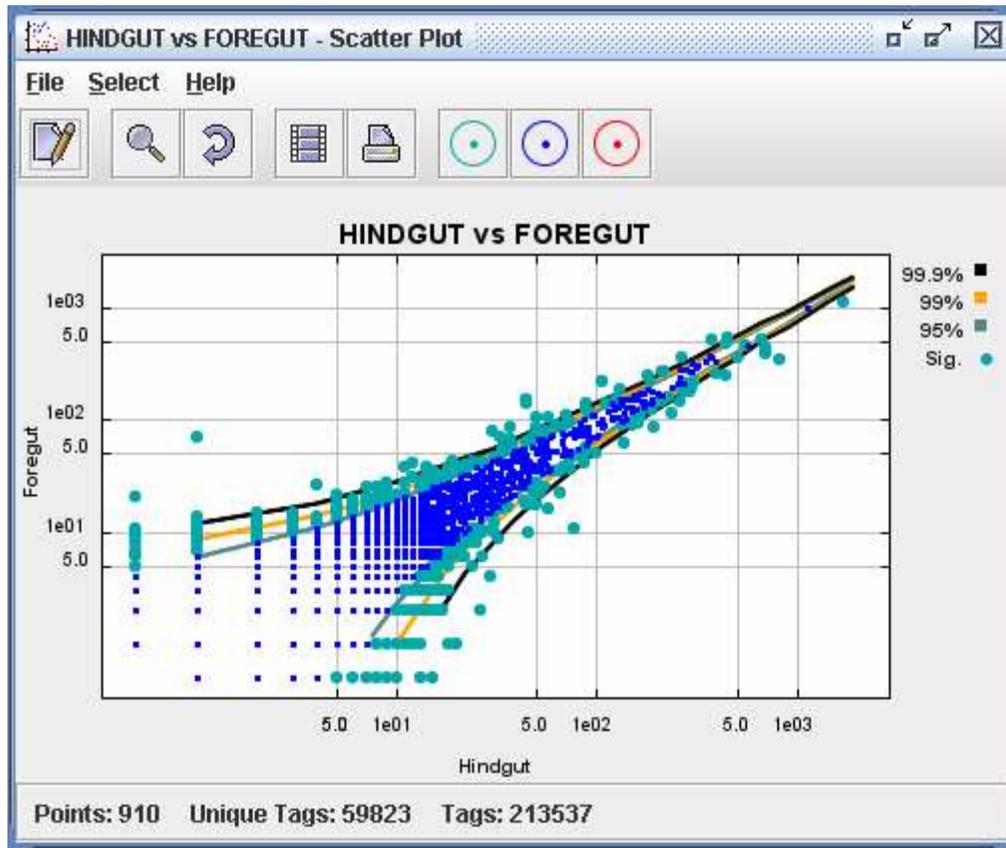
Select which library you want for each axis and rename the comparison. Close the widget.



Now highlight the comparison in the databank and select the Scatterplot button.



This will open the scatterplot diagram. Selected tags of interest can be dragged anywhere on the desktop to create an additional data definition. Select the up regulated tags and save them as 'UPREG'

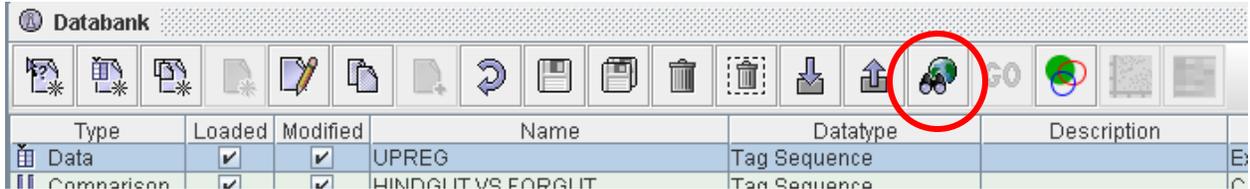


Good job! By completing this example you have learned how to use the comparison widget and scatterplot.

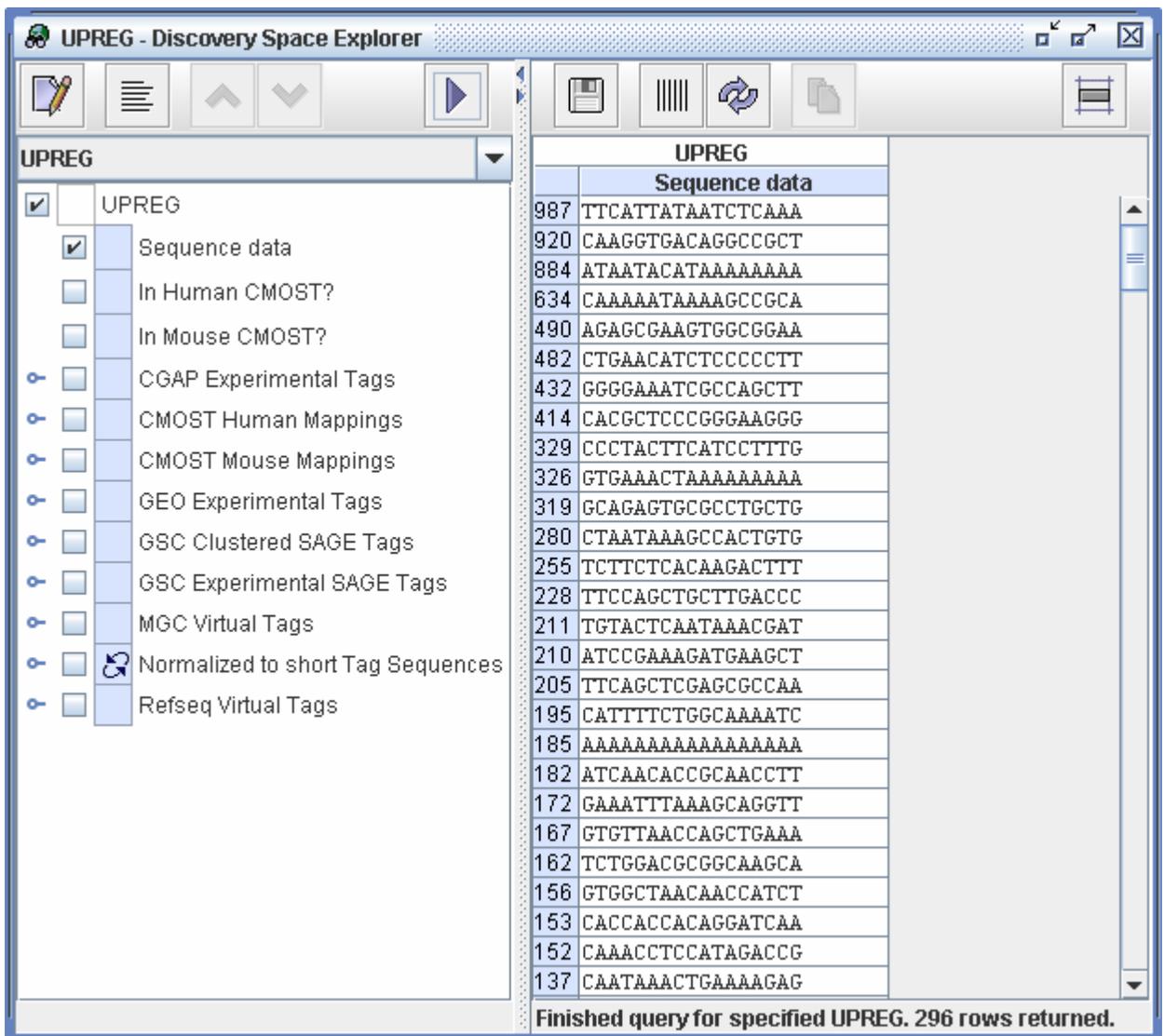
Task 3: Map up regulated tags

Ensure you have dragged tags out from the scatterplot into a data definition before you start this example.

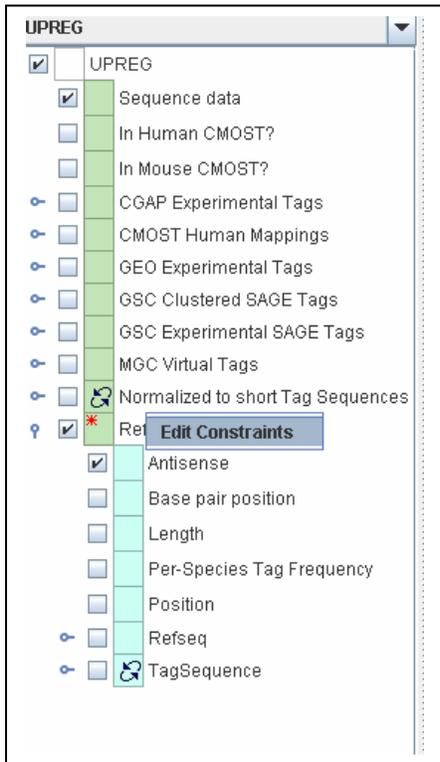
Highlight your data definition and click the explorer button.



This will open the Explorer widget which will allow you to map your tags. This widget is central to DS4.

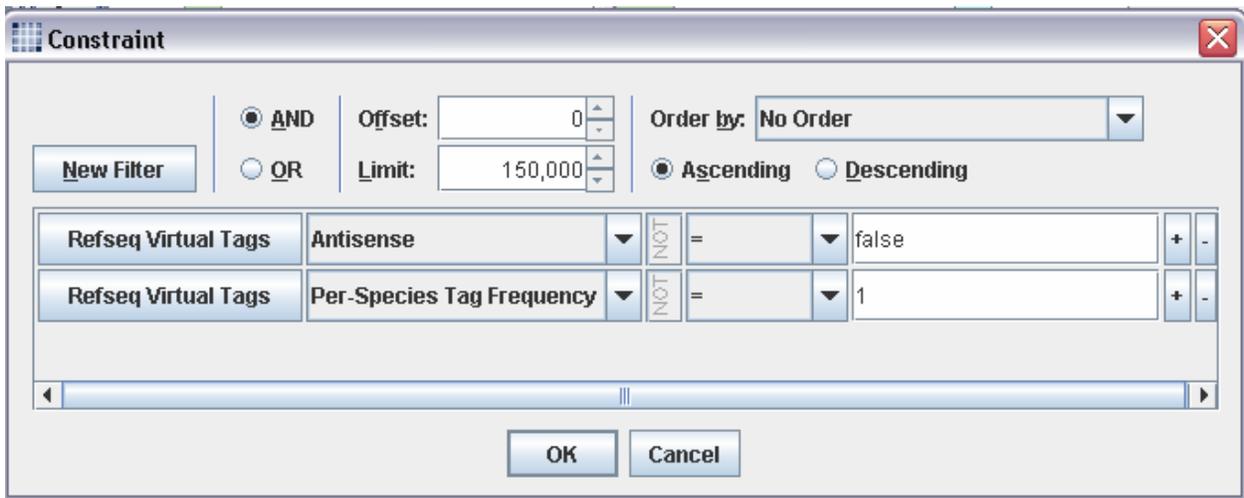


In order to map our tags, we have to set some constraints similar to those set with the Query Builder.



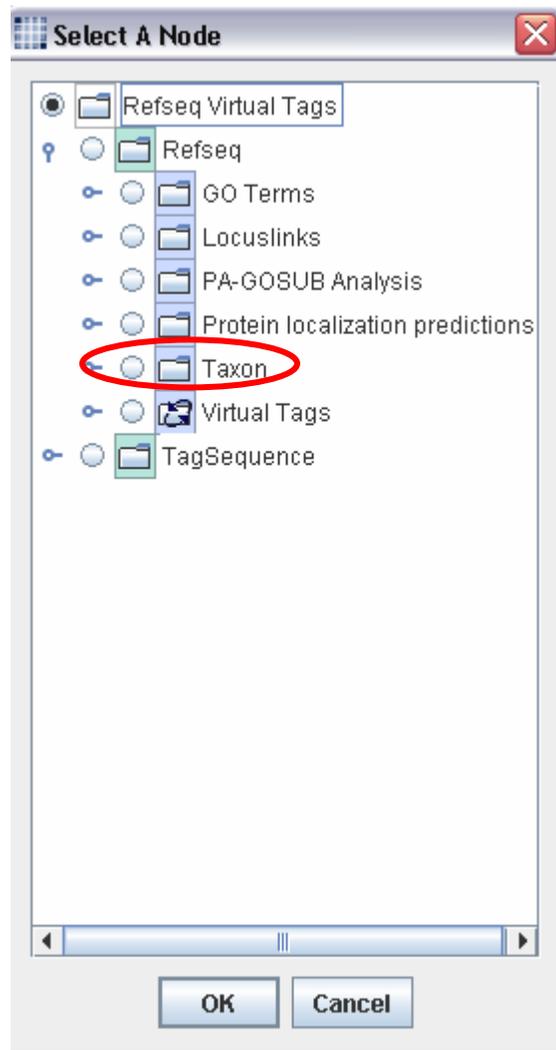
Right click on RefSeq. This will allow you to open the constraints window.

You might want to exclude antisense mappings and only look at unambiguous matches.



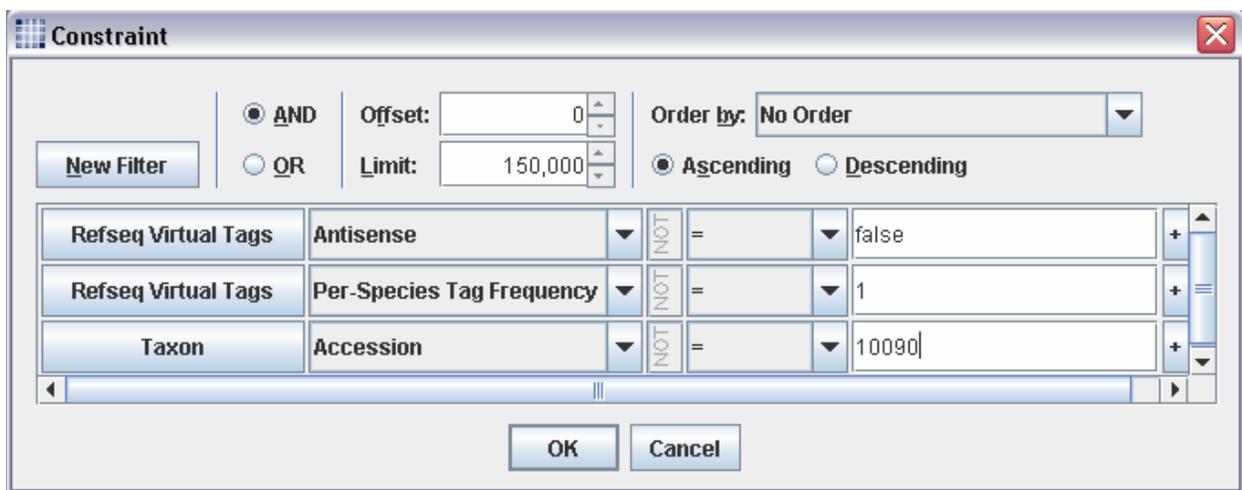
You also might want to only look at mouse matches. Click New Filter. Click the RefSeq Virtual Tags button.

This will open the Select a Node window.



By selecting the Taxon item you can narrow your mappings down by species.

In our case, we set the Taxon accession to 10090 for mouse.



Sequence data	Antisense	Per-Species...	Refseq Virtual Tags			
			Accession	Definition	Accession	Scientific name
987 TTCATTATAATCTCAAA	false	1	NM_008972	Mus musc...	10090	Mus musculus
920 CAAGGTGACAGGCGCT						
884 ATAATACATAAAAAAAA						
634 CAAAAATAAAACCGCA						
490 AGAGCGAAGTGGCGGAA	false	1	NM_018860	Mus musc...	10090	Mus musculus
482 CTCACATCTCCCCCTT						
432 GGGGAAATCGCCAGCTT						
414 CACGCTCCGGGAAGGG						
329 CCCTACTTCATCCTTTG	false	1	NR_001592	Mus musc...	10090	Mus musculus
326 CTCAAACTAAAAAAA						
319 GCAGAGTGGCCCTGCTG						
280 CTAATAAAGCCACTGTG	false	1	NM_007990	Mus musc...	10090	Mus musculus
255 TCTTCTCACAAAGACTTT	false	1	NM_026055	Mus musc...	10090	Mus musculus
228 TTCCAGCTGCTTGACCC	false	1	NM_023418	Mus musc...	10090	Mus musculus
211 TGTACTCAATAAACGAT						
210 ATCCGAAAGATGAAGCT						
205 TTCAGCTCGAGCGCCAA	false	1	NM_011300	Mus musc...	10090	Mus musculus
195 CATTITCTGGCAAAATC	false	1	NM_010514	Mus musc...	10090	Mus musculus
185 AAAAAAAAAAAAAAAAAA						
182 ATCAACACCGCAACCTT						
172 GAAATTTAAAGCAGGTT						
167 GTGTTAACCGCTGAAA						

Hit the run button and you will map your tags!!! As you can see, the tag counts are in the same table as the mappings.

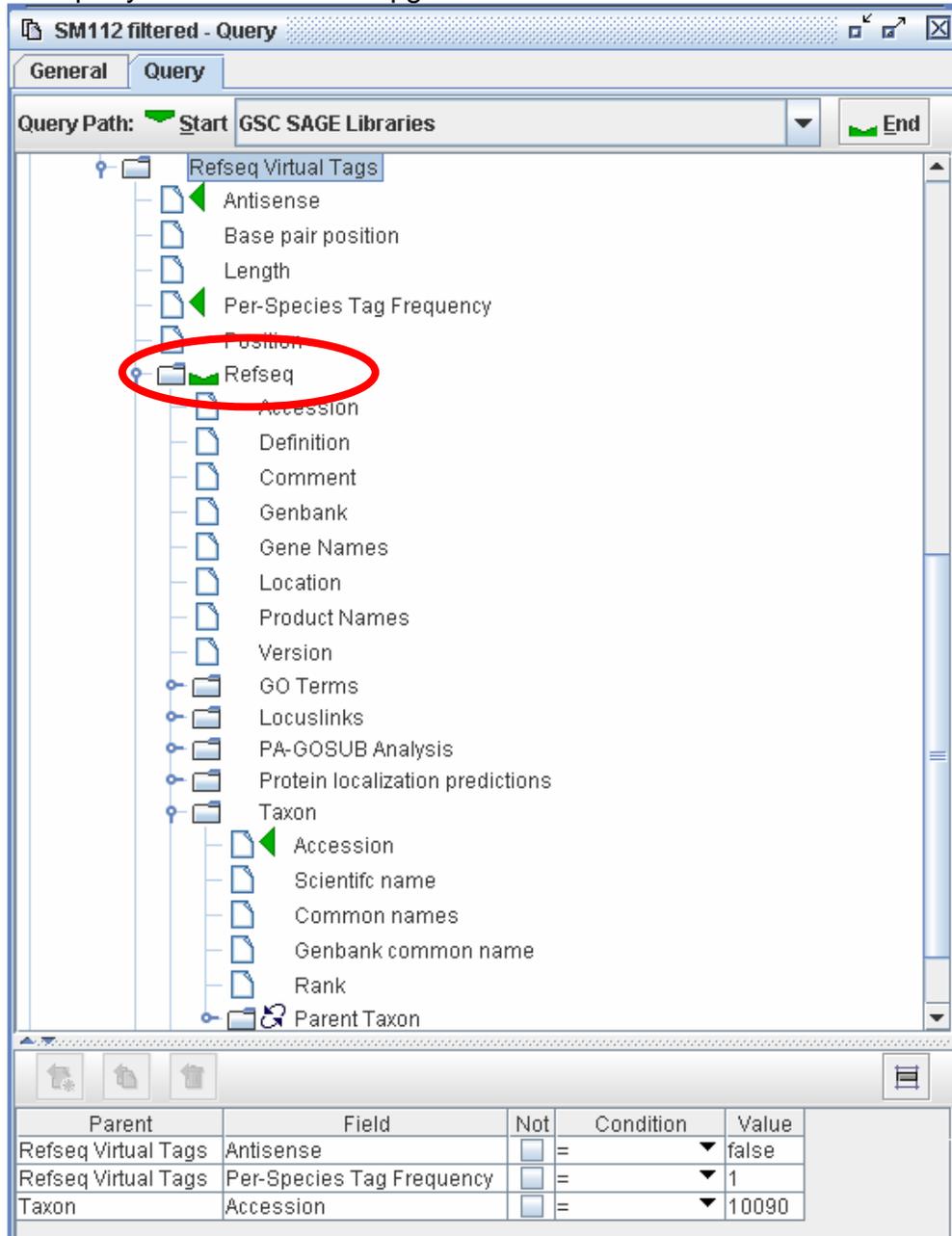
Task 4: Find GO Categories in Hindgut Library

In order to use the GO Browser, we must retrieve data that can be inputted into GO. As you may know, GO accepts RefSeq, MGC, and Ensembl accession numbers. Therefore, we do not want tag data, we want gene data.

Since we want to retrieve gene data for the Hindgut library, we can duplicate our query that retrieved the tag data for Hindgut and modify it to retrieve gene data.

4.1. Duplicate Hindgut query as in task 1. You must use your query definition NOT your data definition. The data definition only contains your tag data, you need the actual query so you can modify it.

4.2. Edit the query to retrieve RefSeq genes.



The screenshot shows a software interface for managing queries. The window title is "SM112 filtered - Query". It has two tabs: "General" and "Query". The "Query" tab is active, showing a tree view of query fields. The "Query Path" is set to "Start GSC SAGE Libraries" and "End". The tree view includes a folder "Refseq Virtual Tags" with sub-items: Antisense, Base pair position, Length, Per-Species Tag Frequency, Position, and Refseq. The "Refseq" item is circled in red. Below the tree view is a table with the following data:

Parent	Field	Not	Condition	Value
Refseq Virtual Tags	Antisense	<input type="checkbox"/>	=	▼ false
Refseq Virtual Tags	Per-Species Tag Frequency	<input type="checkbox"/>	=	▼ 1
Taxon	Accession	<input type="checkbox"/>	=	▼ 10090

Since we are doing GO Analysis, we need to retrieve gene data. Therefore, set the END button to RefSeq.

We need to narrow down the genes we return just as in Task 3. Therefore, we set the following parameters:

RefSeq Virtual Tags -> Antisense : set to “false”

RefSeq Virtual Tags -> Per-Species Tag Frequency : set to “1”

RefSeq Virtual Tags -> RefSeq -> Taxon -> Accession : set to 10090

This will return mouse genes that unambiguously map our tags. Make sure you rename this new query.

We are now ready to use the GO Browser!

4.3. Highlight your new gene query and click the GO button.



This will open the GO Browser. Please refer to the GO Browser primer for detailed instructions of its use.

You will notice two things different in DS4.

- 1) Tag counts are imported into the GO Browser (left-most column)
- 2) There is an option to “Score using Counts”. This will calculate percentages based on tag counts. If you deselect this box, the percentages will be calculated based on number of genes.

The image shows the 'GO SM112 filtered - genes - GO Analysis' window. At the top, there is a 'Select function..' dropdown menu and a checked checkbox for 'Score using Counts'. Below this is a table titled 'Set of imported records' with columns for 'Count', 'Type', 'Identifier', and 'Description'. The table lists 20 records of RefSeq genes from Mus musculus. At the bottom of the window, a status bar indicates '6470 records imported. 6470 records now in set'.

Count	Type	Identifier	Description
475	Refseq Gene ...	NM_018853	Mus musculus ribosomal protein, large, P1 (Rplp1), mRNA.
385	Refseq Gene ...	NM_024277	Mus musculus ribosomal protein S27a (Rps27a), mRNA.
381	Refseq Gene ...	NM_008972	Mus musculus prothymosin alpha (Ptma), mRNA.
323	Refseq Gene ...	NM_009098	Mus musculus ribosomal protein S8 (Rps8), mRNA.
264	Refseq Gene ...	NM_026020	Mus musculus ribosomal protein, large P2 (Rplp2), mRNA.
261	Refseq Gene ...	NM_007393	Mus musculus actin, beta, cytoplasmic (Actb), mRNA.
252	Refseq Gene ...	NM_010699	Mus musculus lactate dehydrogenase 1, A chain (Ldh1), mRNA.
244	Refseq Gene ...	NM_052835	Mus musculus ribosomal protein 10 (Rpl10), mRNA.
238	Refseq Gene ...	NM_008143	Mus musculus guanine nucleotide binding protein, beta 2, related sequence 1 (Gnb2-rs1), mRNA.
221	Refseq Gene ...	NM_013765	Mus musculus ribosomal protein S26 (Rps26), mRNA.
218	Refseq Gene ...	NM_011292	Mus musculus ribosomal protein L9 (Rpl9), mRNA.
196	Refseq Gene ...	NM_009084	Mus musculus ribosomal protein L37a (Rpl37a), mRNA.
196	Refseq Gene ...	NM_010330	Mus musculus embigin (Emb), mRNA.
194	Refseq Gene ...	NM_018860	Mus musculus ribosomal protein L41 (Rpl41), mRNA.
186	Refseq Gene ...	NM_007687	Mus musculus cofilin 1, non-muscle (Cf1), mRNA.
175	Refseq Gene ...	NM_025592	Mus musculus ribosomal protein L35 (Rpl35), mRNA.
174	Refseq Gene ...	NM_027015	Mus musculus ribosomal protein S27 (Rps27), mRNA.
171	Refseq Gene ...	NM_009093	Mus musculus ribosomal protein S29 (Rps29), mRNA.
163	Refseq Gene ...	NM_008302	Mus musculus heat shock protein 1, beta (Hspcb), mRNA.
155	Refseq Gene ...	NM_022891	Mus musculus ribosomal protein L23 (Rpl23), mRNA.
152	Refseq Gene ...	NM_016774	Mus musculus ATP synthase, H+ transporting mitochondrial F1 complex, beta subunit (Atp5b), nuclear gene enc...
149	Refseq Gene ...	NM_172086	Mus musculus ribosomal protein L32 (Rpl32), mRNA.

4.4. Select “Show associated terms” from the drop down menu.

The screenshot shows the 'GO SM112 filtered - genes - GO Analysis' window. On the left, a table titled 'Set of imported records' lists genes with their counts and types. On the right, a dropdown menu is open, showing options like 'Show associated terms', 'Show only directly associated terms', etc. The 'Score using Counts' checkbox is checked.

Count	Type
475	Refseq Gene ...
385	Refseq Gene ...
381	Refseq Gene ...
323	Refseq Gene ...
264	Refseq Gene ...
261	Refseq Gene ...
252	Refseq Gene ...

This will display the GO categories and the percentage of transcripts in each category.

IMPORTANT:

Direct Association: the lowest branch a gene is categorized in

Ancestral Association: gene is in this category, but has also been put into lower branches

For example: a transcription factor (Direct Association) is also in DNA binding (Ancestral Association).

The screenshot shows the 'Terms associated with the selected records' table. It includes columns for Accession, Name, Term Type, Depth, and two association metrics: Direct Association and Ancestral Association. Red bars represent the percentage of transcripts for each term.

Accession	Name	Term Type	Depth	Direct Associa...	Ancestral Ass...
all	all	universal	0	0.000	85.870
GO:0003674	molecular_function	function	1	0.000	76.069
GO:0008150	biological_process	process	1	0.000	69.912
GO:0005575	cellular_component	component	1	0.000	72.279
GO:0000004	biological_process unknown	process	1	3.209	3.209
GO:0007610	behavior	process	2	0.024	0.259
GO:0005554	molecular_function unknown	function	2	2.759	2.759
GO:0030528	transcription regulator activity	function	2	0.186	3.466
GO:0007275	development	process	2	0.820	5.503
GO:0031012	extracellular matrix	component	2	0.000	0.739
GO:0045182	translation regulator activity	function	2	0.002	2.453
GO:0003774	motor activity	function	2	1.016	1.035
GO:0003824	catalytic activity	function	2	1.569	25.053
GO:0016032	viral life cycle	process	2	0.091	0.140
GO:0005198	structural molecule activity	function	2	1.834	15.657
GO:0050789	regulation of biological process	process	2	0.000	10.935
GO:0043234	protein complex	component	2	0.000	25.694
GO:0009987	cellular process	process	2	0.000	32.188
GO:0005488	binding	function	2	0.862	44.766
GO:0004871	signal transducer activity	function	2	0.458	5.094
GO:0003767	co-chaperone activity	function	2	0.022	0.022
GO:0005576	extracellular	component	2	0.512	8.286
GO:0016209	antioxidant activity	function	2	0.756	0.935
GO:0030188	chaperone regulator activity	function	2	0.015	0.015
GO:0007582	physiological process	process	2	0.120	64.382
GO:0002224	enzyme regulator activity	function	2	0.022	2.444

3553 associated Terms found

Example 2: Gene of Interest

Task 1: Retrieve tags for a gene of interest – FGF8

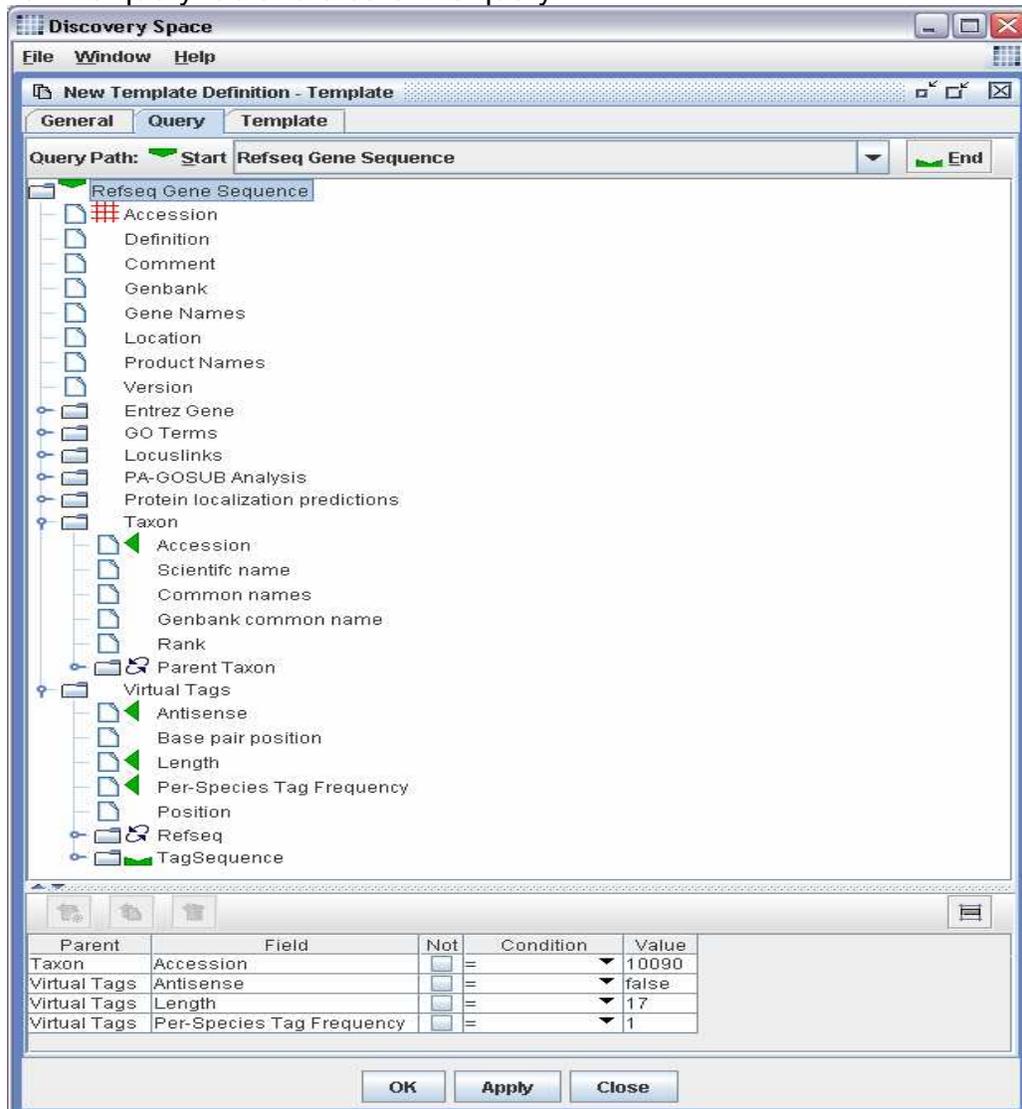
1.1 Build a query to return tags for a given gene. This time we will use the Template builder.

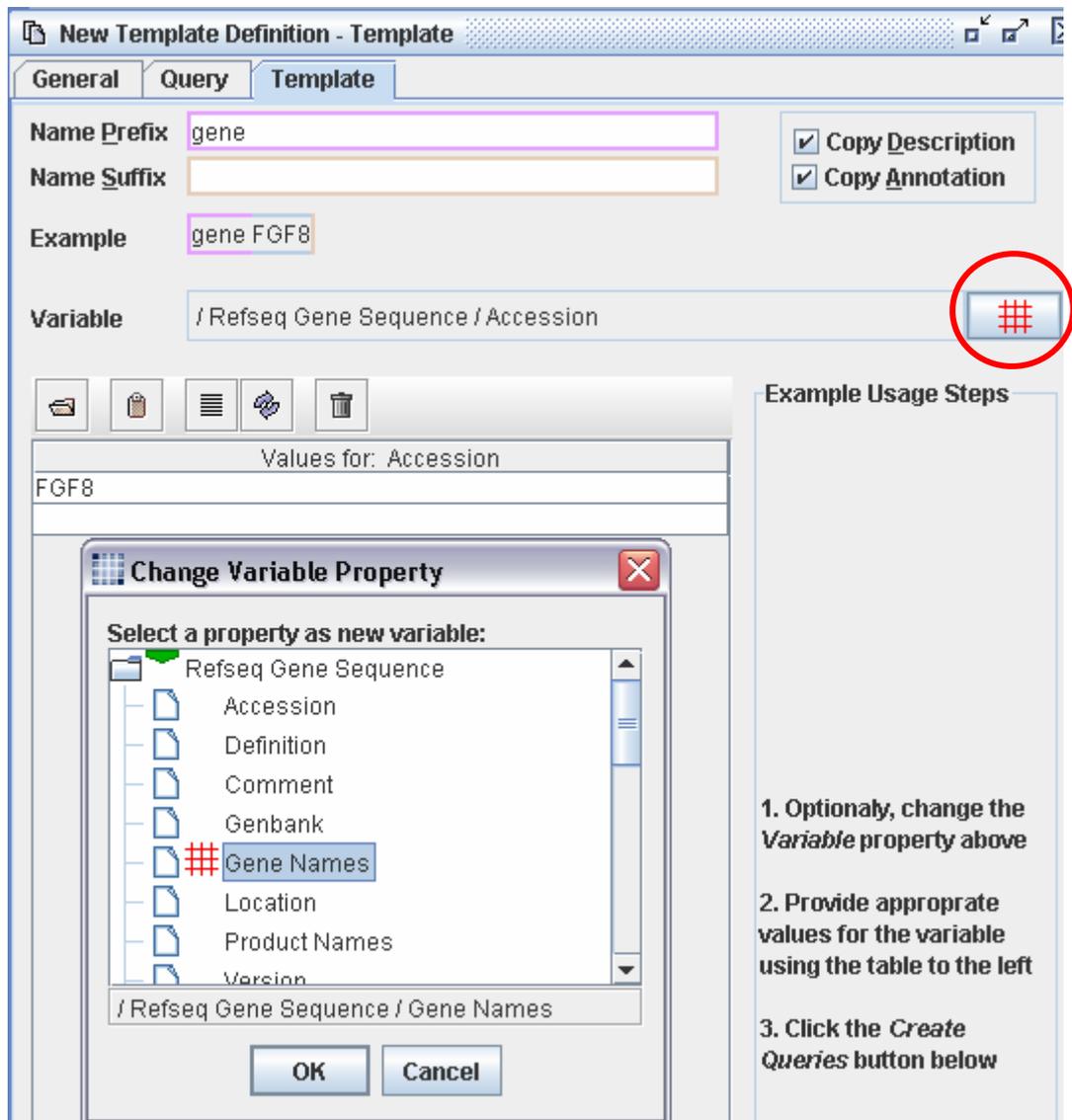
Click the Template button to create a Template.



You should be comfortable with queries after completing Example 1. In this example we want to return tags from a given gene. We will use RefSeq Gene Sequence to retrieve all longSAGE mouse unambiguous tags. Notice the END function is on the TagSequence item.

Click the query tab and create this query:





We have to set the variable. This allows us to run this query as many times as we like to create an identical query for different gene names. You can then input FGF8 into Values for: Gene Names column.

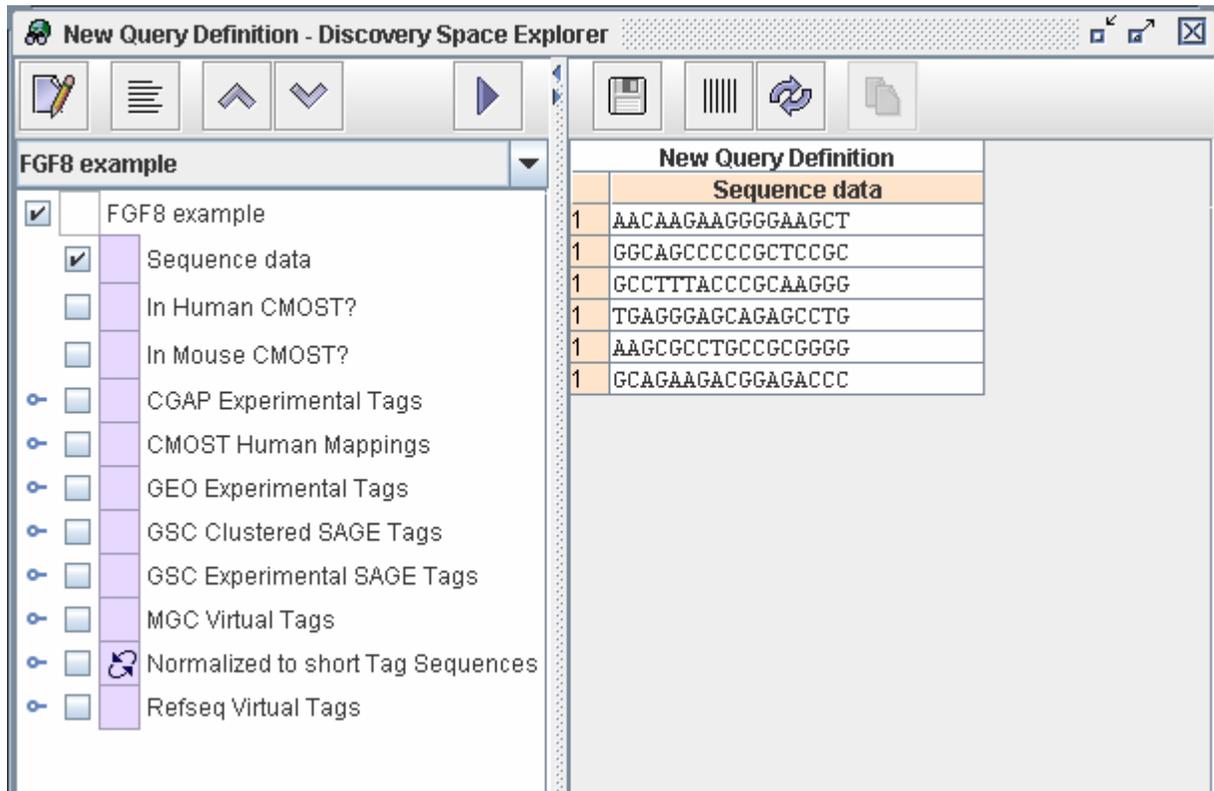
You can set Name Prefix and Name Suffix to help label your queries. In this example, the query will be named 'gene FGF8'.

NOTE: We can now reuse this query over and over to retrieve tags for different genes!

Click Apply. Click Create queries.

This creates your specified queries in the Databank. Highlight your new query and select the Explorer button.

When the query is run, it will return tags.



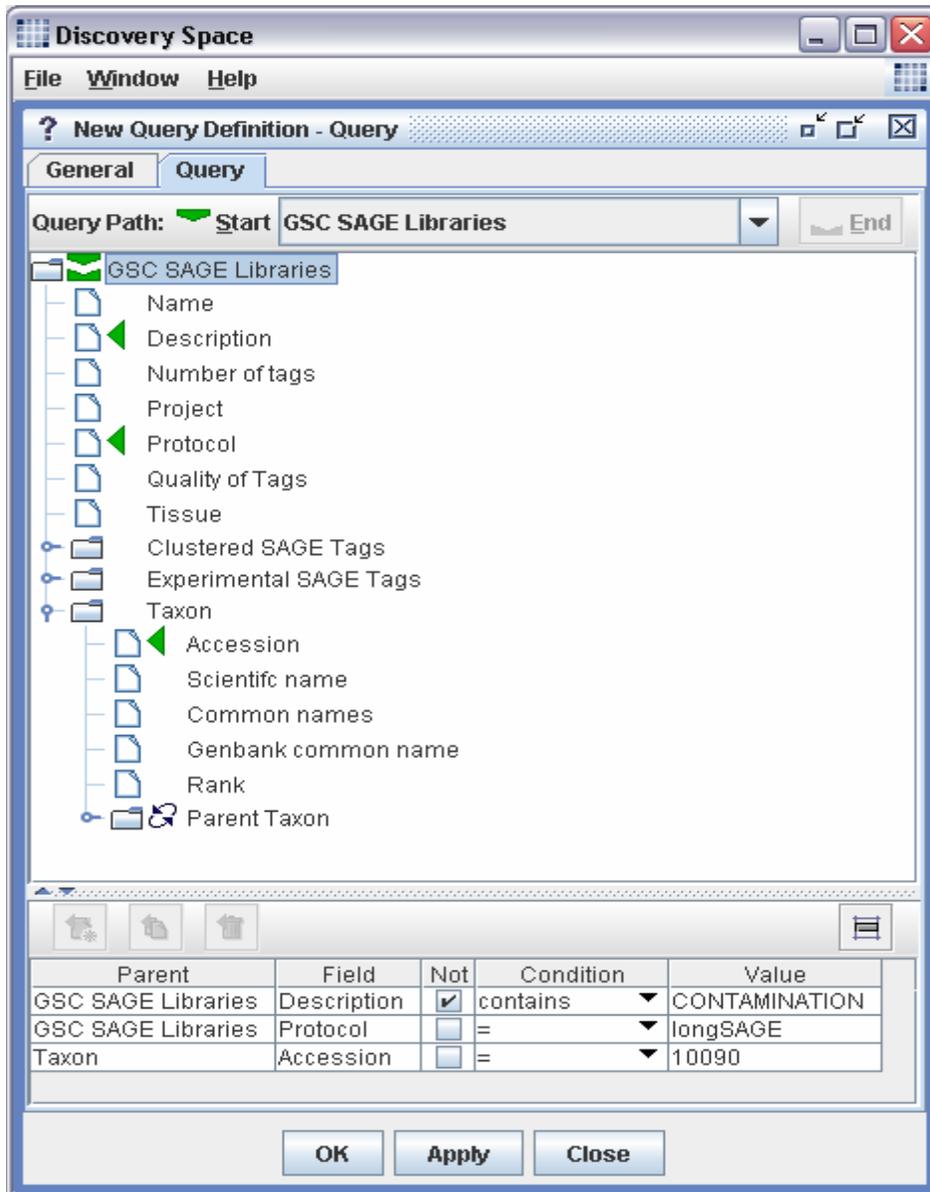
Task 2: Determine which libraries contain these tags

2.1. Create query of all available libraries

We need an additional query to return available libraries. This query will be used to open the Search For Tags in Libraries widget.

For our example, let's look at all longSAGE mouse libraries in the GSC Libraries.

Let's also remove the contaminated libraries.



2.2. Highlight the GSC SAGE Libraries query and click the Search For Tags in Libraries button.



Now highlight the tags from your Tag Sequence query and drag them into the Search for Tags widget.

Click the libraries on the left column.

Tag Search

Available

Name	Description	Protocol
<input type="checkbox"/> SM090	Whole Intest...	LongSAGE
<input type="checkbox"/> SM096	Large Intesti...	LongSAGE
<input type="checkbox"/> SM095	xxxxx	LongSAGE
<input type="checkbox"/> SM099	xxxxx	LongSAGE
<input type="checkbox"/> SM102	xxxxx	LongSAGE
<input type="checkbox"/> SM091	xxxxx	LongSAGE
<input type="checkbox"/> SM103	xxxxx	LongSAGE
<input type="checkbox"/> SM104	xxxxx	LongSAGE
<input type="checkbox"/> SM100	xxxxx	LongSAGE
<input type="checkbox"/> SM106	xxxxx	LongSAGE
<input checked="" type="checkbox"/> SM107	xxxxx	LongSAGE
<input type="checkbox"/> SM108	xxxxx	LongSAGE
<input type="checkbox"/> SM109	xxxxx	LongSAGE
<input type="checkbox"/> SM105	xxxxx	LongSAGE
<input type="checkbox"/> SM114	xxxxx	LongSAGE
<input checked="" type="checkbox"/> SM112	xxxxx	LongSAGE
<input type="checkbox"/> SM106a	xxxxx	LongSAGE
<input type="checkbox"/> SM117	xxxxx	LongSAGE
<input type="checkbox"/> SM116	xxxxx	LongSAGE
<input type="checkbox"/> SM113	xxxxx	LongSAGE
<input type="checkbox"/> SM118	xxxxx	LongSAGE
<input type="checkbox"/> SM122	xxxxx	LongSAGE
<input type="checkbox"/> SM124	xxxxx	LongSAGE
<input type="checkbox"/> SM116a	xxxxx	LongSAGE
<input type="checkbox"/> SM104_f30	xxxxx	LongSAGE
<input type="checkbox"/> SM104_l22	xxxxx	LongSAGE
<input type="checkbox"/> SM119	xxxxx	LongSAGE
<input type="checkbox"/> SM120	xxxxx	LongSAGE
<input type="checkbox"/> SM133	xxxxx	LongSAGE
<input type="checkbox"/> SM139	xxxxx	LongSAGE
<input type="checkbox"/> SM140	xxxxx	LongSAGE
<input type="checkbox"/> SM137	xxxxx	LongSAGE
<input type="checkbox"/> SM134	xxxxx	LongSAGE
<input type="checkbox"/> SM138	xxxxx	LongSAGE
<input type="checkbox"/> SM142	xxxxx	LongSAGE

Results

Absolute Quality Cutoff: 0.99

		Name	Description	Total Tags
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			
		SM112	xxxxx	110586
		SM107	xxxxx	103008

You might want to search public libraries for your tags. You can create a query for GEO libraries and repeat these steps.

