

Identify putative gene signatures distinguishing mice exhibiting two different pathology results.

Objective: To select mice exposed to acute doses of hepatocarcinogens which are exhibiting pathology responses of interest. This search brings back three studies, and we will explore one performed in mice exposed to different hepatocarcinogens. Identify individual animals of interest based on their pathology diagnoses, navigate to microarray data for these animals and begin analysis. After visualizing the data, identify that fluor flips contribute more to the response than the signal in this particular study, and eliminate reverse hybridizations. Use CEBS to identify genes with altered expression between the two groups of animals with different pathologies.

Detailed work flow:

Go to the CEBS home page (www.cebs.niehs.nih.gov/prototype/)

Select the “Search by Subject Characteristics” link

Scroll down to find pathology, main diagnosis

Select: hypertrophy, infiltration, necrosis (hold down the Ctrl key for multi-select)

Submit the query (using the button at the bottom of page)

You should see the investigation “2003-responses to acute dosing with hepatocarcinogens in mice” among others. Click on the investigation title, and the associated study “Multiple Carcinogens” will appear. The case study focuses on this study.

Under Design Information (in the header above the list of investigations), click on “Timeline” next to the “Multiple Carcinogens” study to view the events on the study timeline. Details of protocols applied at different times during the study can be accessed from the timeline (click “protocol” found on the left of each line on the timeline). Click on “Design” to understand the major experimental factors identified by the depositor, and how these were used to define the group structure of the study. Additional Study details are found under “Details”.

Select the entire “Multiple Carcinogens” Study by ticking the check box to the left. Scroll down to the bottom of the page and select “Retrieve Pathology Results”. This will bring you to a listing of histopathology diagnoses for the different animals.

We would like to identify animals with either inflammation or necrosis.

Pathology results for female animals in the different Groups:

Group Name:	o-Nitrotoluene 5000ppm female				
Compound and dose	o-Nitrotoluene-HighDose				
Sex	Female				
Organ	Finding	Histological Site	Severity	Distribution	Infiltration Cell Type
Subject ID	o-Nitrotoluene mouse 10				
Liver	Infiltration		Minimal		Mixed population
Subject ID	o-Nitrotoluene mouse 11				
Liver	Infiltration		Minimal		Mixed population
Subject ID	o-Nitrotoluene mouse 7				
Liver	Infiltration		Minimal		Mixed population
Subject ID	o-Nitrotoluene mouse 8				
Liver	Infiltration		Minimal		Mixed population

Group Name:	o-Nitrotoluene 1250ppm female				
Compound and dose	o-Nitrotoluene-LowDose				
Sex	Female				
Organ	Finding	Histological Site	Severity	Distribution	Infiltration Cell Type
Subject ID	o-Nitrotoluene mouse 2				
Liver	Infiltration		Minimal		Mixed population
Subject ID	o-Nitrotoluene mouse 5				
Liver	Infiltration		Minimal		Mixed population
Subject ID	o-Nitrotoluene mouse 6				
Liver	Infiltration		Minimal		Mixed population

Group Name:	p-Nitrotoluene 5000ppm female				
Compound and dose	p-Nitrotoluene				
Sex	Female				
Organ	Finding	Histological Site	Severity	Distribution	Infiltration Cell Type
Subject ID	p-Nitrotoluene mouse 3				
Liver	Infiltration		Minimal		Mixed population
Subject ID	p-Nitrotoluene mouse 5				
Liver	Necrosis			Locally extensive	
Liver	Infiltration		Minimal		Mixed population
Liver	Congestion		Moderate		
Subject ID	p-Nitrotoluene mouse 6				
Liver	Necrosis			Focal	
Liver	Infiltration		Minimal		Mixed population

Group Name:	Oxazepam 2500ppm female				
Compound and dose	Oxazepam-HighDose				
Sex	Female				
Organ	Finding	Histological Site	Severity	Distribution	Infiltration Cell Type
Subject ID	Oxazepam mouse 13				
Liver	Necrosis		Minimal	Focal	
Liver	Infiltration		Minimal		Mixed population
Liver	Hypertrophy		Marked		
Subject ID	Oxazepam mouse 14				
Liver	Infiltration		Minimal		Mixed population
Liver	Hypertrophy		Marked		
Subject ID	Oxazepam mouse 15				
Liver	Infiltration		Minimal		Mixed population
Liver	Hypertrophy		Moderate		
Subject ID	Oxazepam mouse 17				
Liver	Hypertrophy		Marked		
Subject ID	Oxazepam mouse 18				
Liver	Infiltration		Minimal		Mixed population
Liver	Hypertrophy		Moderate-Marked		

Group Name:	Oxazepam 125ppm female
Compound and dose	Oxazepam-LowDose
Sex	Female

Organ	Finding	Histological Site	Severity	Distribution	Infiltration Cell Type
Subject ID	Oxazepam mouse 1				
Liver	Infiltration		Minimal		Mixed population
Subject ID	Oxazepam mouse 2				
Liver	Infiltration		Minimal		Mixed population
Subject ID	Oxazepam mouse 3				
Liver	Infiltration		Minimal		Mixed population
Subject ID	Oxazepam mouse 6				
Liver	Infiltration		Minimal		Mixed population

12 mice have diagnoses of infiltration only; 3 mice have necrosis and infiltration, 3 mice have only hypertrophy and infiltration. We will compare between these three groups.

Return to the Study page by using the back button. Click the study title to see the study groups. Expand the female groups (skipping the Acetaminophen since no microarray data were deposited for this group).

The individual mice can be selected by checking the tick box to the left of each group name. Uncheck Oxazepam mouse 17, the animal with only hypertrophy. (see screen at right)

At the bottom of the page select Individual Hybridizations to navigate to the microarray data for these animals.

While we selected 18 animals and retrieved 18 microarrays, only 9 animals are represented in the microarray data. Each hybridization was carried out using two technical replicates with the dyes swapped between samples in each replicate.

- Multiple_Carcinogens_B6C3F1_2003
 - + Acetaminophen 6000ppm female
 - o-Nitrotoluene 1250ppm female
 - o-Nitrotoluene mouse 2
 - o-Nitrotoluene mouse 5
 - o-Nitrotoluene mouse 6
 - o-Nitrotoluene 5000ppm female
 - o-Nitrotoluene mouse 10
 - o-Nitrotoluene mouse 11
 - o-Nitrotoluene mouse 7
 - o-Nitrotoluene mouse 8
 - + o-Nitrotoluene 5000ppm male
 - Oxazepam 125ppm female
 - Oxazepam mouse 1
 - Oxazepam mouse 2
 - Oxazepam mouse 3
 - Oxazepam mouse 6
 - + Oxazepam 125ppm male
 - Oxazepam 2500ppm female
 - Oxazepam mouse 13
 - Oxazepam mouse 14
 - Oxazepam mouse 15
 - Oxazepam mouse 17
 - Oxazepam mouse 18
 - + Oxazepam 2500ppm male
 - p-Nitrotoluene 5000ppm female
 - p-Nitrotoluene mouse 3
 - p-Nitrotoluene mouse 5
 - p-Nitrotoluene mouse 6

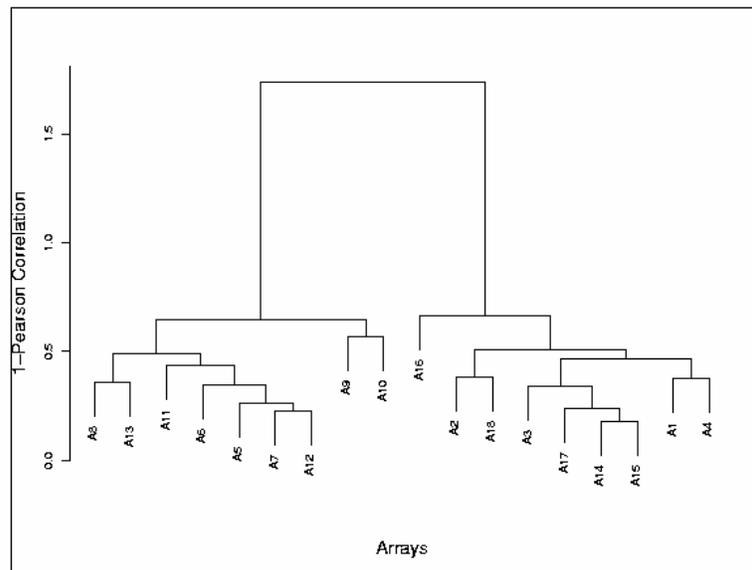
Microarray data analysis:

The experiment was performed with a two-color microarray platform and fluor-flip experimental design. Scroll down to the Retrieve Data button at the bottom of page and click on it. This takes you to a page where you can select the data preprocessing steps you wish to apply. Select Agilent preprocessing or chose your desired preprocessing method and then select Continue.

CEBS permits visualization of the data; to do this, select the “Visualize Preprocessed Data” button. This provides a series of global visualizations of the data. Scroll down to view the hierarchical clustering or multi-dimensional scaling plots.

Clustering of Arrays

This plot displays the relative similarities between arrays (based on Pearson correlation of global expression), and hierarchical clustering of arrays.



[See Legend for Array Information](#)

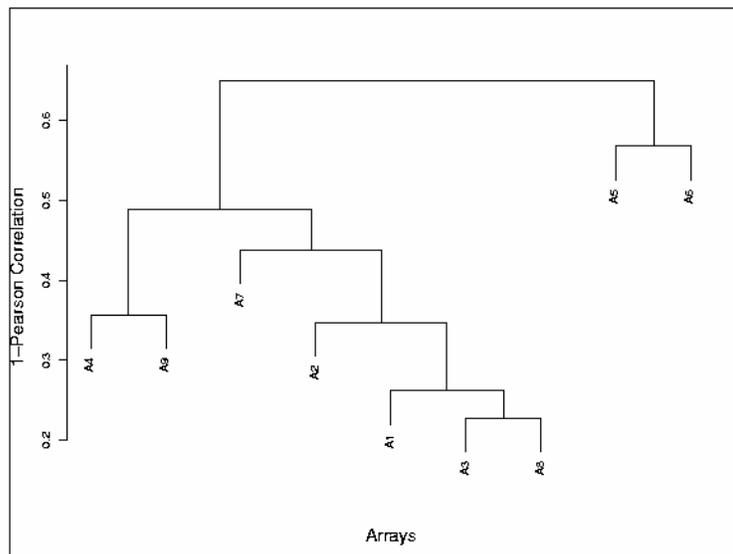
Note that the arrays fall into two main clusters in both the hierarchical clustering and multi-dimensional scaling. Using the legend at the bottom of the page, note that the two clusters are perfectly described by the forward and reverse fluor flips.

Return to the selection of microarray page (click the back button three times) and de-select the arrays with reverse fluor checked. (de-select the array using check box at far left, not the reverse label check box!).

Re-do the preprocessing step and continue on with array analysis (i.e. select “Analyze Preprocessed Data instead of visualization, or select “Continue Microarray Analysis” at the bottom of the visualization page).

Clustering of Arrays

This plot displays the relative similarities between arrays (based on Pearson correlation of global expression), and hierarchical clustering of arrays.



[See Legend for Array Information](#)

[Back to Preprocessing](#) | [Continue Microarray Analysis](#)

Legend for Array Information

Label	Experiment ID	Array Name	Sample Name
A1	839190001	CY5_P-nitrotoluene_5000ppm_Female_30_16011978011680	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_30_cubed_liver_RNA(CY5)
A2	839190001	CY5_P-nitrotoluene_5000ppm_Female_29_16011978011234	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_29_cubed_liver_RNA(CY5)
A3	839190001	CY5_Oxazepam_2500ppm_Female_18_16011978011466	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_18_cubed_liver_RNA(CY5)
A4	839190001	CY5_Oxazepam_2500ppm_Female_14_16011978011828	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_14_cubed_liver_RNA(CY5)
A5	839190001	CY5_Oxazepam_2500ppm_Female_13_16011978012445	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_13_cubed_liver_RNA(CY5)
A6	839190001	CY5_O-nitrotoluene_5000ppm_Female_41_16011978012231	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_41_cubed_liver_RNA(CY5)
A7	839190001	CY5_O-nitrotoluene_5000ppm_Female_38_16011978012057	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_38_cubed_liver_RNA(CY5)
A8	839190001	CY5_O-nitrotoluene_1250ppm_Female_35_16011978011472	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_35_cubed_liver_RNA(CY5)
A9	839190001	CY5_O-nitrotoluene_1250ppm_Female_32_16011978011640	CY3_Control_Female_Pool_RNA(CY3)
			CY5_TD_32_cubed_liver_RNA(CY5)

Note the numbering changes: P-nitrotoluene Females 29 and 30 are animals 5 and 6; O-nitrotoluene 5000 ppm Female 38 and 41 are animals 8 and 11; O-nitrotoluene 1250 ppm Females 32 and 35 are animals 2 and 5.

This information can be obtained from the first page listing the microarrays and the specimen from which each RNA were derived.

It is possible to carry out several interesting analyses at this point. Since the arrays are two-color, one can identify genes with altered expression compared to control. Additionally one can compare genes with different fold-change-relative-to-control between the two agents. To do the latter, select “Comparison of two groups of arrays”.

At the next screen toggle the animals experiencing infiltration (o-Nitrotoluene 32, 35, 38 and 41) to Group A and animals with necrosis (p-Nitrotoluene 29 and 30, and Oxazepam 13) to Group B.

Select “Continue” at the bottom of the page to retrieve the data for analysis. This leaves Oxazepam 14 and 18 out of the analysis.

Experiment	Array Name	Sample Name	Dye	Array Group A	Array Group B	Neither
839190001	CY5_P-nitrotoluene_5000ppm_Female_30_16011978011680	CY3_Control_Female_Pool_RNA	CY3	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
		CY5_TD_30_cubed_liver_RNA	CY5	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
839190001	CY5_P-nitrotoluene_5000ppm_Female_29_16011978011234	CY3_Control_Female_Pool_RNA	CY3	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
		CY5_TD_29_cubed_liver_RNA	CY5	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
839190001	CY5_Oxazepam_2500ppm_Female_18_16011978011466	CY3_Control_Female_Pool_RNA	CY3	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
		CY5_TD_18_cubed_liver_RNA	CY5	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
839190001	CY5_Oxazepam_2500ppm_Female_14_16011978011828	CY3_Control_Female_Pool_RNA	CY3	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
		CY5_TD_14_cubed_liver_RNA	CY5	<input type="radio"/>	<input type="radio"/>	<input checked="" type="radio"/>
839190001	CY5_Oxazepam_2500ppm_Female_13_16011978012445	CY3_Control_Female_Pool_RNA	CY3	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
		CY5_TD_13_cubed_liver_RNA	CY5	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>
839190001	CY5_O-nitrotoluene_5000ppm_Female_41_16011978012231	CY3_Control_Female_Pool_RNA	CY3	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
		CY5_TD_41_cubed_liver_RNA	CY5	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
839190001	CY5_O-nitrotoluene_5000ppm_Female_38_16011978012057	CY3_Control_Female_Pool_RNA	CY3	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
		CY5_TD_38_cubed_liver_RNA	CY5	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
839190001	CY5_O-nitrotoluene_1250ppm_Female_35_16011978011472	CY3_Control_Female_Pool_RNA	CY3	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
		CY5_TD_35_cubed_liver_RNA	CY5	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
839190001	CY5_O-nitrotoluene_1250ppm_Female_32_16011978011640	CY3_Control_Female_Pool_RNA	CY3	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
		CY5_TD_32_cubed_liver_RNA	CY5	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>

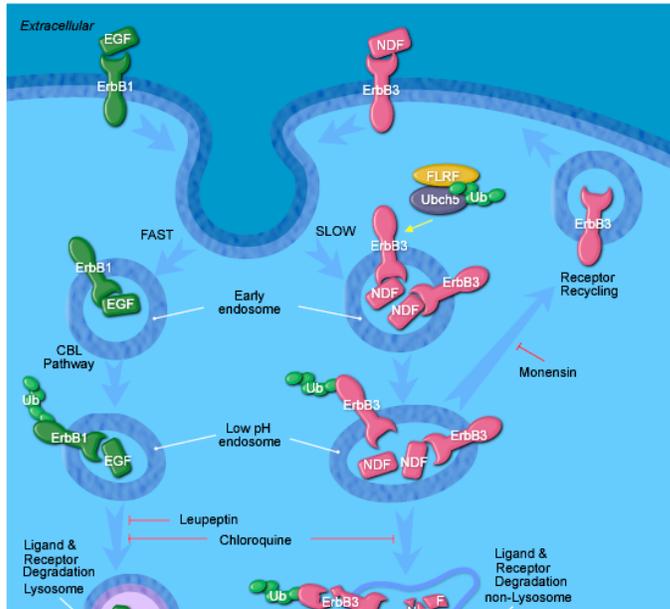
< Back R Reset > Continue

The next step is to define the statistical criteria that will be used to identify “significant change”. Select 1.4 as the minimal fold change and default p-values (or threshold / method of your choice). The values used return 70 genes. To see the gene list select “View Expression Report”. This list can be sorted by clicking on the header of any column. Additionally clicking on the link for each gene name brings up the gene - information page for each gene, with links to other external annotation sources, homology predictions and GO categories.

Return to the selection page using the back button and then select “Perform Analysis Using BioCarta”. This page shows all currently-linked BioCarta pathways. Click on the “Enrichment” header to sort by the degree of enrichment.

Gene category name	Total	Up	Down	Change	Enrichment	Fisher exact test p-value	View detailed expression reports
Neuroregulin receptor degradation protein-1 Controls ErbB3 receptor recycling	7	1	0	1	43.50612	0.02276	Genes Diagram
Lissencephaly gene (LIS1) in neuronal migration and development	13	0	1	1	23.42637	0.04187	Genes Diagram
Role of ERBB2 in Signal Transduction and Oncology	21	1	0	1	14.50204	0.06677	Genes Diagram

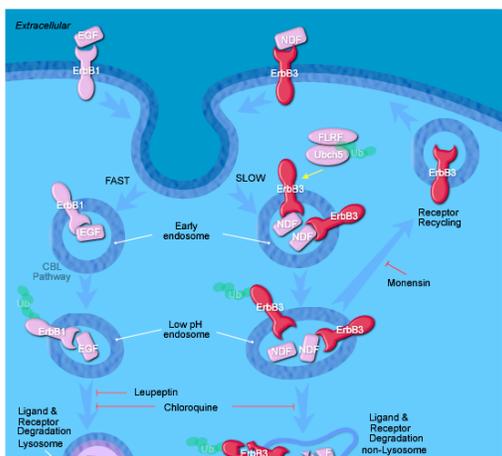
Clicking on the “Diagram” link at the far right brings up a BioCarta diagram of the pathway. The diagram for neuroregulin receptor degradation is given below:



You may need to download the SVG viewer to see the pathway diagram. Click on the link above the spot where the diagram is trying to appear, and then select “Viewer Download” under “SVG Viewer” at the left. Scrolling down the page brings you to a list of systems and viewers, select the one you want and download and install it.

Once you see the image, CEBS can overlay it with a legend that identifies genes whose products are sampled on the array, and those with altered expression in the comparison you are making. This is done by clicking on the +/- Expression link next to the diagram.

- stands for gene(s) without detected change.
- stands for up-regulated gene(s) (1.4 fold).
- stands for down-regulated gene(s) (1.4 fold).
- stands for gene(s) represented by multiple features, but they demonstrate different trends (increased, decreased, or unchanged) of changes.



[Genes On Chip](#)
[Expression +/-](#)
[Reset](#)

[Pathway Summary Report](#)

This analytical work flow can be applied to any study in CEBS that contains both histopathology and microarray data.