## **Tutorial**

The GenePattern Tutorial introduces you to GenePattern by providing step-by-step instructions for analyzing gene expression. It takes approximately 40 minutes to complete.

All of the information you need to successfully complete this tutorial is contained in the tutorial. For users who like additional discussion along the way, the tutorial includes pointers to more information in other GenePattern guides. Feel free to follow these links or to ignore them, depending on your learning style.

#### Introduction

#### **Prerequisites**

To follow the hands-on instructions in this tutorial, you must have access to the following:

- A GenePattern server, such as the public GenePattern server available at http://genepattern.broadinstitute.org/gp/.
- The server must have the following GenePattern analysis modules installed: ComparativeMarkerSelection, ComparativeMarkerSelectionViewer, ExtractComparativeMarkerSelection, and HeatMapViewer.
- The following example data files: all\_aml\_train.res, all\_aml\_train.cls, all\_aml\_test.res, and all\_aml\_test.cls.

#### Scientific Scenario

The gene expression dataset used in the tutorial is from Golub and Slonim et al. (1999), which used clustering and prediction algorithms to find genes that distinguish between two subtypes of leukemia, ALL and AML. The dataset consists of 38 bone marrow samples (27 ALL, 11 AML) obtained from acute leukemia patients.

For the purposes of this tutorial, your goal is identify **marker genes** for the two subtypes of leukemia:

- 1. You use the ComparativeMarkerSelection (version 5) analysis to find the genes in the dataset file that are most closely correlated with the two subtypes of leukemia.
- 2. You use the ComparativeMarkerSelectionViewer (version 4) to examine the results of that analysis.
- 3. You use the ExtractComparativeResults (version 3) analysis to create a new dataset that contains the marker genes that you have identified.
- 4. You use the HeatMapViewer (version 8) to examine the expression levels of the marker genes and to confirm that the marker genes are clearly differentially expressed in the two subtypes of leukemia.

**Note**: If you are using a different version of any analysis, follow the instructions as closely as possible, but be aware that your results might not match those shown in the tutorial.

## **Starting GenePattern**

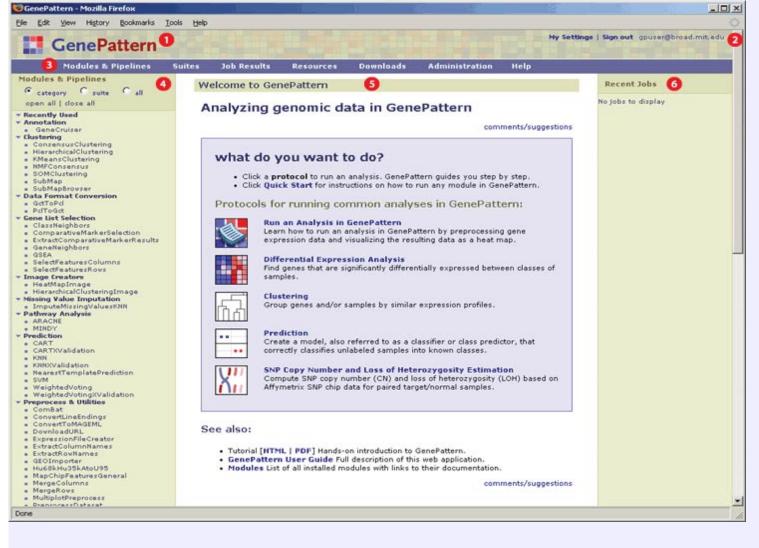
To start GenePattern:

- 1. Open a web browser, such a Mozilla Firefox, Internet Explorer, or Safari.
- Enter the URL of the GenePattern server that you want to use. For example, to use the public GenePattern server hosted by the Broad Institute, enter http://genepattern.broadinstitute.org/gp/.
  - The login page appears.
- 3. Enter your user name and password. If you do not have a GenePattern account, select *Click to register*.

  Whether a GenePattern server requires passwords depends on how it is configured. The public GenePattern server requires passwords. By default, a local GenePattern server does not.
- 4. Click Sign In. The GenePattern home page appears.

Note: The pictures in the tutorial show GenePattern running in a Firefox web browser on a Windows PC. If you are using a different web browser or a

different operating system, your screen may look slightly different.



- Click the GenePattern icon to return to this home page at any time.
- The upper right corner shows your user name.
- The navigation bar provides access to other pages.
- The Modules & Pipelines panel lists the analyses that you can run. Click the all radio button to list them alphabetically.
- 👩 The center pane is the main display pane, which GenePattern uses to display information and to prompt you for input.
- The Recent Jobs panel lists the most recent analyses that you have run and their results files. When you start GenePattern for the first time, this panel is empty.

For more information: see Exploring the User Interface in the GenePattern User Guide.

## **Running Analyses**

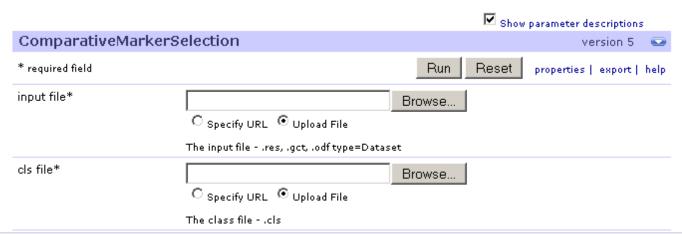
Now that you have started GenePattern, you are ready to analyze your data. In this section, you learn how to:

- Run the ComparativeMarkerSelection analysis to find marker genes -- the genes in the dataset that are most closely correlated with the two phenotypes (ALL
  and AML) in the dataset.
- Run the ComparativeMarkerSelectionViewer to examine the ComparativeMarkerSelection analysis results.
- Run the ExtractComparativeResults analysis to create a smaller dataset that contains only the marker genes.
- Run the HeatMapViewer to examine the expression levels of the marker genes.

### Running ComparativeMarkerSelection

Run the ComparativeMarkerSelection analysis to find the genes in the dataset that are most closely correlated with the two phenotypes (ALL and AML) in the dataset. To run the ComparativeMarkerSelection analysis:

1. In the Modules & Pipelines panel, under Gene List Selection, select the ComparativeMarkerSelection analysis. GenePattern displays the ComparativeMarkerSelection parameters.



- 2. For information about the module and its parameters, click help.
- 3. For the input file parameter, select all\_aml\_train.res (ftp://ftp.broadinstitute.org/pub/genepattern/datasets/all\_aml/all\_aml\_train.res).
  - To use a downloaded copy of the file, click the Browse button and select the local file. Leave the Upload File radio button selected indicate that you are selecting
    a file from the file system.
  - o To use the file from the FTP site, select the Specify URL radio button and enter the URL of the file.
- 4. For the cls filename parameter, select all\_aml\_train.cls (ftp://ftp.broadinstitute.org/pub/genepattern/datasets/all\_aml/all\_aml\_train.cls).
- 5. Click *Run* to start the analysis. GenePattern sends the analysis job to the server and displays the Job Status page. After a minute or two, the status icon



Analysis results are stored on the GenePattern server. The text shown in red tells you when the analysis results will be deleted. To save your analysis results, copy the files from the GenePattern server to your own directories, as described later in this tutorial.

6. Click Return to Modules & Pipelines Start to return to the home page.

## **Reviewing Result Files**

When you return to the GenePattern home page, the Recent Jobs pane shows the analysis job that you ran and the associated analysis results files:



- 1. Click the job number. GenePattern redisplays the status page. On the Job Status page, click Return to Modules & Pipelines Start to return to the home page.
- 2. Click the icon next to the analysis job to display commands that you use to work with the job:

Download Downloads a zip file containing all analysis results files for this job.

Reload Displays the analysis and its parameters, with the parameters set to the values used for this analysis job.

Delete Deletes the analysis job and its analysis results files from the GenePattern server.

Info Displays the parameter values and the analysis results files for this job.

View Java Code Displays the command line that you would use to run this job in the Java, MATLAB, or R programming environments.

View MATLAB Code These commands are useful for programmers who want to access GenePattern from one of these programming

View R Code environments or from their own applications.

3. Click the icon next to an analysis results file to display commands that you use to work with the file:

Delete Deletes the file from the GenePattern server.

Save Downloads the file from the GenePattern server.

Create Pipeline Creates a GenePattern pipeline that reproduces this analysis results file. Pipelines are discussed later in this tutorial.

Lists analyses that commonly use this type of file as an input parameter.

Select an analysis to display its parameters with this results file specified as the first input parameter.

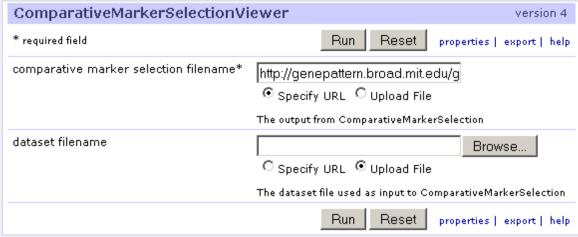
4. Click the analysis results file, al l\_aml\_train. comp. marker. odf, to display it in a text viewer. The amount of information it contains makes the file difficult to understand. This file, like most analysis results file, is not intended to be viewed as a text file, but rather intended to be used as input to subsequent analyses.

5. Click the *Back* button in your web browser to return to the home page.

#### Running the ComparativeMarkerSelectionViewer

After running the ComparativeMarkerSelection analysis, run the ComparativeMarkerSelectionViewer to examine the analysis results. To run the ComparativeMarkerSelectionViewer:

- 1. In the Recent Jobs pane, click the icon next to your all l\_aml\_train.comp. marker. odf results file.
- 2. Select *ComparativeMarkerSelectionViewer*. GenePattern displays the ComparativeMarkerSelectionViewer parameters. The *comparative marker selection filename* parameter is automatically set to the all\_aml\_train.comp. marker.odf results file.



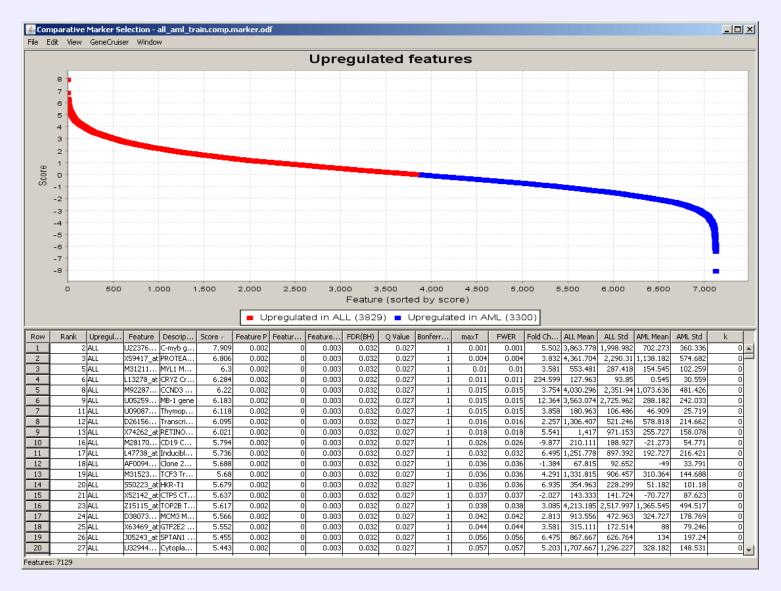
- 3. For the *dataset filename* parameter, click *Browse* and select the all\_aml\_train.res file.
- 4. Click Run. GenePattern displays the Job Status page.

Unlike most analyses, viewers run on your desktop PC rather than on the GenePattern server. The first time you run a viewer on your desktop, the following security message may appear.



5. If the security message appears, click *Run* to continue.

The ComparativeMarkerSelectionViewer appears:



**Tip**: To change the width of a column, click and drag the edge of the column heading.

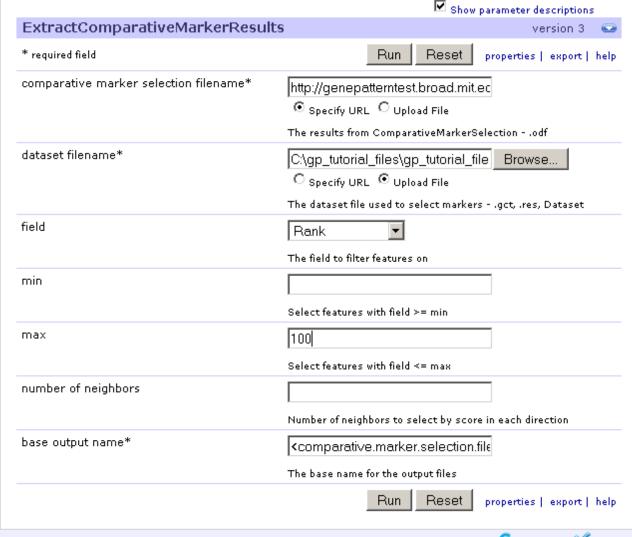
- 6. In the ComparativeMarkerSelectionViewer:
  - The Score column shows the value of the metric used to correlate gene expression and phenotype. A high score indicates correlation with the first phenotype (upregulated in ALL) and a low score indicates correlation with the second phenotype (upregulated in AML).

- The middle columns, FDR through FWER, provide different ways to measure the significance of the score. The lower the value the more significant the result.
   For example, you might choose to measure significance using the false discovery rate (FDR) and set a significance cutoff of FDR < .05. Using this measure, you would focus on genes with the lowest and highest scores, where the measure of significance for the score was an FDR < .05.
  </p>
- 7. To close the ComparativeMarkerSelectionViewer, select *File>Exit*.
- 8. In GenePattern, click *Return to Modules & Pipelines Start* to return to the home page.

#### Running ExtractComparativeResults

Now that you have examined the ComparativeMarkerSelection analysis results, you want to create a new dataset that contains only the most promising marker genes from the results file for further analysis. To run the ExtractComparativeResults analysis:

- 1. In the Recent Jobs pane, click the icon next to the ComparativeMarkerSelection results file, all\_aml\_train.comp.marker.odf.
- 2. Select *ExtractComparativeMarkerResults*. GenePattern displays the ExtractComparativeMarkerResults parameters. The *comparative marker* selection filename parameter is automatically set to the all\_aml\_train.comp.marker.odf results file.
- 3. For the *dataset filename* parameter, click *Browse* and select the all\_aml\_train.res file.
- 4. Enter the following parameter values to extract the top 100 features (genes) in the analysis results file:
  - o For the field parameter, select Rank.
  - For the max field, enter 100.



5. Click *Run*. GenePattern displays the Job Status page. After a few seconds, the status icon changes from running to complete and GenePattern displays the analysis results files.



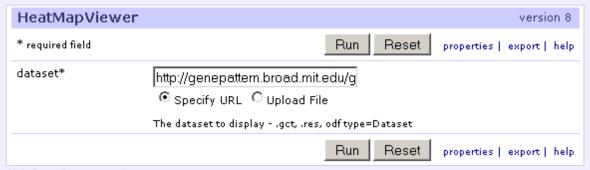
6. Leave the Job Status page displayed.

### Running HeatMapViewer

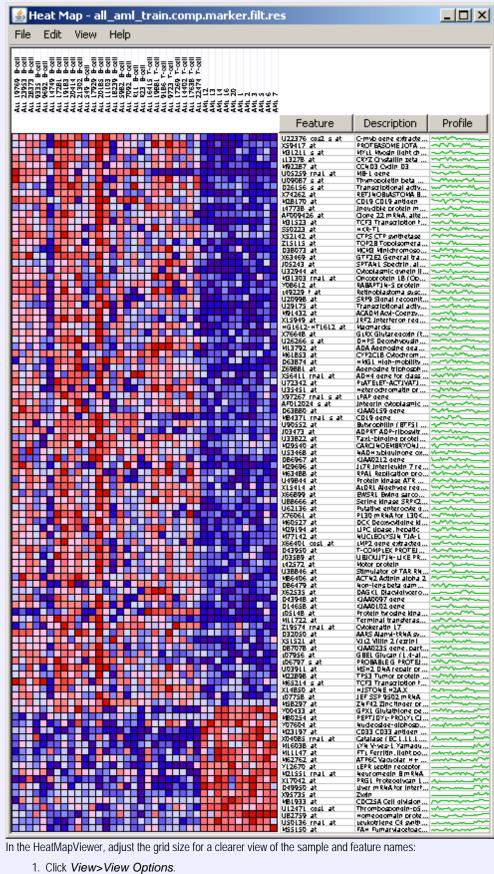
The HeatMapViewer displays expression values in a color-coded heat map. The largest expression values are displayed in red (hot) and the smallest values are displayed in blue (cool). Intermediate values are displayed in different shades of red and blue. The color-coding provides a quick coherent view of gene expression levels.

To display your new dataset in the HeatMapViewer:

- 1. On the Job Status page, click the icon next to the results file from the ExtractComparativeMarkerResults analysis, all\_aml\_train.comp. marker. filt.res.
- 2. Select H*eatMapViewer*. GenePattern dislays the HeatMapViewer parameters. The *dataset filename* parameter is automatically set to the all\_aml\_train.comp. marker. filt.res results file.



3. Click *Run*. The HeatMapViewer appears:



- 4. In the HeatMapViewer, adjust the grid size for a clearer view of the sample and feature names:
  - 1. Click View>View Options.
  - 2. Click-and-drag the *Row Size* or *Column Size* slider to modify the grid size.
  - 3. Close the View Options window.
- 5. To close the HeatMapViewer, select File>Exit.
- 6. In GenePattern, click Return to Modules & Pipelines Start to return to the home page.

## **Using Pipelines**

run multiple analyses. You can define a pipeline to run multiple analyses against a single dataset or to run a sequence of analyses, where the output from one analysis becomes the input for a subsequent analysis. Modules run from a pipeline work exactly the same as those run directly from GenePattern.

In this tutorial, you have run two analyses: Comparative Marker Selection and Extract Comparative Marker Results. The analysis results file from the first analysis became the input file for the second analysis. Running these two analyses produced a new dataset that contains the 100 genes in your dataset (all\_aml\_train.res) that are most closely correlated with phenotypes in your class file (all\_aml\_train.cls).

In this section, you will

- Create a pipeline that duplicates your analysis results.
- Edit the pipeline so that it operates on any set of data (res and cls) files.

For more information: see Working with Pipelines in the GenePattern User Guide.

#### **Creating a Pipeline Based on Previous Results**

You can create a pipeline in one of two ways:

- · You can create an empty pipeline and then add to it the analysis modules that you want the pipeline to run.
- You can create a pipeline based on an analysis results file. In this case, GenePattern adds to the pipeline the analysis modules used to create that results file:
  - 1. It adds the module that created the results file.
  - 2. It checks the module's input file parameter.
  - 3. If the input file for the module was the output file of a previous module, the GenePattern adds the previous module and returns to step 2; otherwise, there are no more modules to be added to the pipeline.

To create a pipeline based on the ExtractComparativeMarkerResults results file:

- 1. In the Recent Jobs pane, click the icon next to the results file from the ExtractComparativeMarkerResults analysis, all\_aml\_train.comp.marker.filt.res.
- 2. Select *Create Pipeline*. GenePattern displays the new pipeline.

The new pipeline includes the module that created the results file, ExtractComparativeMarkerResults. It also includes the ComparativeMarkerSelection analysis because the input file for ExtractComparativeMarkerResults was the output file from the ComparativeMarkerSelection analysis. The parameters for both analyses are set to the values used to generate the results file. (You will need to scroll to see the entire pipeline.)

Pipeline name*:	: job11957	job11957		
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Privacy: Varaion commo		private •		
Version comme	····  0	0		
Daarum antation:				
Documentation:		Browse		
LS	ID: urn:lsid:7070.genepatt.node25	broad.mit.edu:genepatternmodules:205:1		
▼ 1. Comparat	tiveMarkerSelection:		version 5 (latest)	
	Feature-Specific P-Value, and Bonfer			
	documentation:	ComparativeMarkerSelection.pdf		
prompt when run	parameter name	value descrip		
	input file:		ut fileres, .gct, .odf type=Dataset	
	cls file:	<genepatternurl>getFile.jsp?task=<lsid>&amp;file=all_aml_train.res The class</lsid></genepatternurl>	ss filecls	
	cis ille.	Browse Browse The class  GenePatternURL> getFile jsp?task= <lsid> Mile=all_amt_train.cls</lsid>	ss lifecis	
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	complete:	no 🔽 Whethe	r to perform all possible permutations	
	balanced:	no 🔽 Whethe	r to perform balanced permutations	
	random seed:	773948241 permuta		
	smooth p values:		r to smooth p-values	
	phenotype test:	one versus all (Note: n	perform when cls file has more than two classes ot fully corrected for multiple hypothesis testing)	
	output filename:	<input.file_basename>.c The nar</input.file_basename>	ne of the output file	
		Add Another Module Delete ComparativeMarkerSelection		
		Add Antonic Module		
▼ 2. ExtractCo	mparativeMarkerResults:		version 3 (latest)	
	Creates a derived dataset and featur	list file from the results of ComparativeMarkerSelection		
	documentation:	ExtractComparativeMarkerResults.pdf		
rompt when run	parameter name	value	description	
	comparative marker selectio	filename: Browse	The results from ComparativeMarkerSelection -	
		or use output from 1. ComparativeMarkerSelection 🔻 1st output	.odf	
	dataset filename:	Browse	The dataset file used to select	
		⟨GenePatternURL⟩ getFile isp?task=⟨LSID⟩ &file=all_aml_train.res  or use output from Choose module  ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼ ▼	markersgct, .res, Dataset	
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	base output name:	<comparative.marker.sel< td=""><td>The base name for the output files</td></comparative.marker.sel<>	The base name for the output files	

3. By default, the pipeline name is the job number. Change the pipeline name to MyComparativeMarkerSelection by editing the *Pipeline name* field.

### **Adding Modules to a Pipeline**

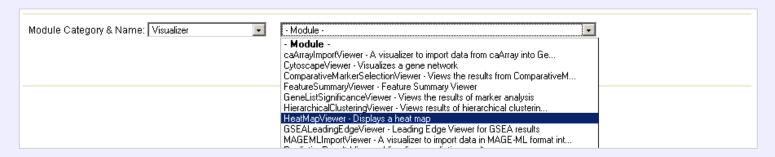
The pipeline contains the two analysis modules used to create the analysis results file: Comparative Marker Selection and Extract Comparative Marker Results. In your original analysis, after creating the analysis results file, you used the Heat Map Viewer to review the results.

To add the Heat Map Viewer module to your pipeline:

- 1. Scroll to the end of the pipeline definition.
- 2. Click Add Another Module to add a module after the ExtractComparativeMarkerResults analysis. GenePattern prompts you for the module to add:



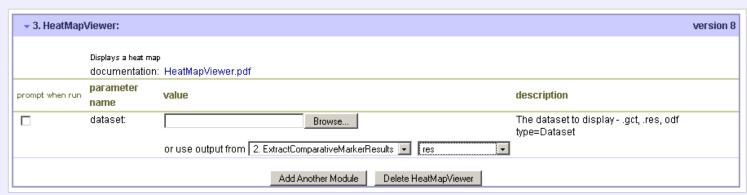
3. Select Visualizer as the category and HeatMapViewer as the module:



GenePattern adds the HeatMapViewer module to the pipeline:



4. For the *dataset filename* parameter, select the analysis results file from the ExtractComparativeMarkerResults module. To do so: next to *use output from*, choose **ExtractComparativeMarkerResults** as the module and **res** as the output file:



- 5. Click **Save** to save the pipeline. GenePattern displays a status page.
- 6. Click Continue to Modules & Pipelines Start to return to the home page. GenePattern displays the pipeline and its parameters (if any).

#### **Running the Pipeline**

On the home page in the Modules & Pipelines pane, notice that the pipeline names are color-coded; the pipelines you create are displayed in red.

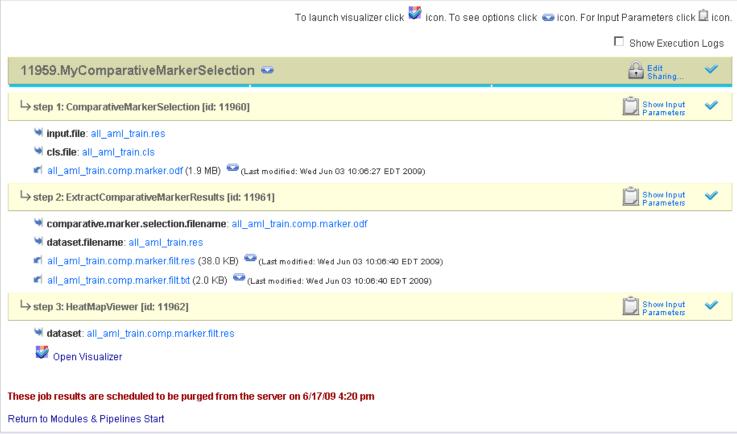
To run the pipeline:

- 1. Click Run. GenePattern displays the Job Status, runs each module, displays the analysis results, and opens the HeatMapViewer.
- 2. Close the HeatMapViewer.
- 3. In GenePattern, click *Return to Modules & Pipelines Start* to return to the home page.

The Recent Jobs pane shows the pipeline job, which lists each analysis run and its analysis result files.

To review the pipeline results in the HeatMapViewer:

1. Click the job number to redisplay the Job Status page.



- 2. Click the *Open Visualizer* icon. GenePattern opens the HeatMapViewer.
- 3. Close the HeatMapViewer.
- 4. In GenePattern, click *Return to Modules & Pipelines Start* to return to the home page.

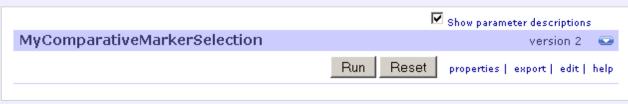
### Adding Parameters to a Pipeline

You have created a pipeline that duplicates your original analysis: it runs the Comparative Marker Selection analysis on the all\_aml\_train data (res and cls) files, uses the analysis results as input to the Extract Comparative Marker Results analysis, and then displays the analysis results using the Heat Map Viewer.

You can make the pipeline more generally useful by having it prompt you for the data (res and cls) files to be analyzed, rather than simply analyzing the all\_aml\_train data files.

To edit the pipeline:

1. In the Modules & Pipelines pane, select your pipeline. GenePattern displays the pipeline:

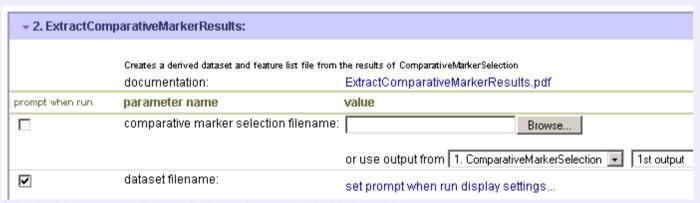


2. Click *Edit*. GenePattern displays the pipeline definition for editing.

- 3. The data (res and cls) files to be analyzed are named in the *input file* and *cls file* parameters of the Comparative Marker Selection analysis. Modify the pipeline to have it prompt you for these parameter values:
  - 1. Click on the *Prompt when run* check box next to the *input file* parameter of the Comparative Marker Selection analysis. GenePattern removes the parameter value from the pipeline definition.
  - 2. Click on the *Prompt when run* check box next to the *cls file* parameter of the Comparative Marker Selection analysis. GenePattern removes the parameter value from the pipeline definition.

▼ 1. ComparativeMarkerSelection:				
	Computes significance values for features using several metrics, including FDR(BH), Q-Value, maxT, FWER, Feature-Speci P-Value, and Bonferroni.  documentation: ComparativeMarkerSelection.pdf			
prompt when run	parameter name	value		
V	input file:	set prompt when run display settings		
V	cls file:	set prompt when run display settings		
	confounding variable cls file:	Browse		

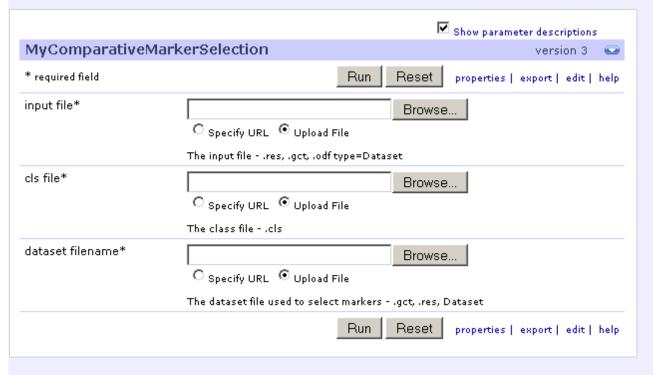
- 3. Optionally, click set prompt when run display settings to modify the default text of the prompt.
- 4. The data (res) files to be analyzed is also named in the *dataset filename* parameter of the Extract Comparative Marker Results analysis. Modify the pipeline to have it prompt you for this parameter value also:
  - 1. Click on the *Prompt when run* check box next to the *dataset filename* parameter of the Extract Comparative Marker Results analysis:



- 2. Optionally, click set prompt when run display settings to modify the default text of the prompt.
- 5. Scroll to the end of the pipeline definition.
- 6. Click Save. GenePattern displays a message confirming that the pipeline has been saved.
- 7. Click Continue to Modules & Pipelines Start to return to the home page.

### Running the Edited Pipeline

When you edit a pipeline and return to the home page, GenePattern displays the pipeline and its parameters:



To run the edited pipeline:

- 1. For the *input file* parameter, select all\_aml\_test.res.
- 2. For the *cls file* parameter, select all\_aml\_test.cls.
- 3. For the *dataset filename* parameter, select all\_aml\_test.res.
- 4. Click Run. The Status page appears and GenePattern runs each module. After a few moments, GenePattern displays the HeatMapViewer.
- 5. Close the HeatMapViewer.
- 6. In GenePattern, click *Return to Modules & Pipelines Start* to return to the home page.

## Saving and Deleting Result Files

As described earlier in the tutorial, analyses are run on the GenePattern server and analysis results files are stored on the server. Server storage is temporary and analysis results files are deleted after they have been on the server for a certain length of time (by default, one week).

To save your analysis results files, you must copy each file from the server to a more permanent location. If you do not need your analysis results, you can delete them at any time.

To save an analysis results file:

- 1. In the Recent Jobs pane, click the icon next to a results file.
- 2. Click Save.

To delete an analysis results file:

- 1. In the Recent Jobs pane, click the icon next to a results file.
- 2. Click Delete.

To save or delete a job and all of its analysis results files, click the icon next to the job and click *Download* or *Delete*.

## **Exiting from GenePattern**

To exit from GenePattern, click the Sign out link in the top right corner of the title bar and then close the web browser window.

# **Learning More About GenePattern**

Thank you for taking this time to learn about GenePattern!

As you continue to work with GenePattern, please visit http://genepattern.org, which provides an overview of the GenePattern analysis modules, as well as links to the GenePattern software and documentation.

We welcome your feedback. If you have suggestions, comments, or questions that are not answered by the documentation, contact the GenePattern help desk (gp-help (at) broadinstitute.org).