Perform gene set and pathway analysis

To perform gene set and pathway analysis, we need to create a list of genes that overlap with differentially methylated CpG loci.

- Select **LCLs_vs_B_cells_CpG_Islands** in the spreadsheet tree
- Select **Find Overlapping Genes** from the *Analysis* section of the workflow

The *Output Overlapping Features* dialog will open (Figure 1). This dialog allows you to choose the annotation database that will define where gene are located. By default the promoter region will be defined as 5000 base pairs upstream and 3000 base pairs downstream from the transcription start site.

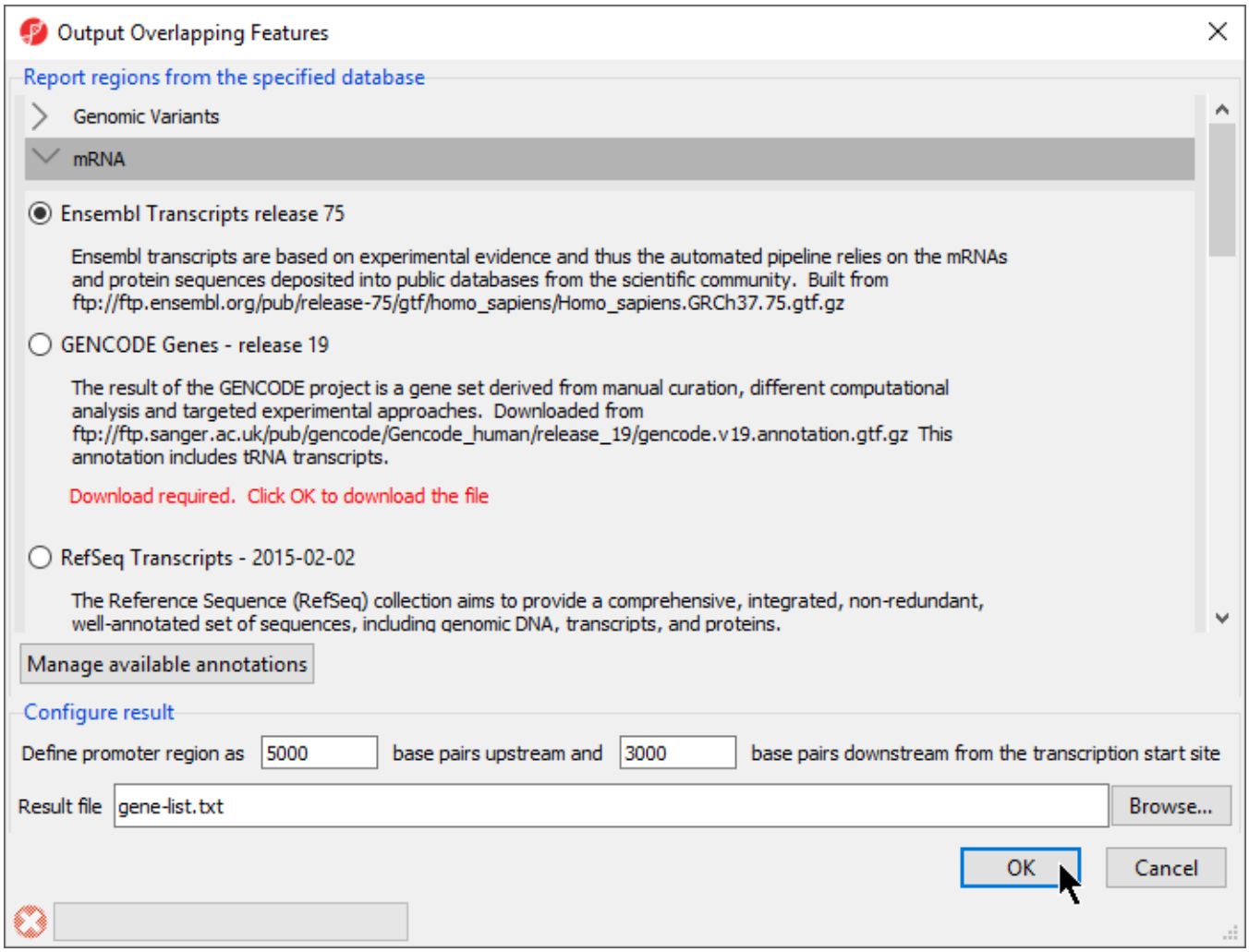
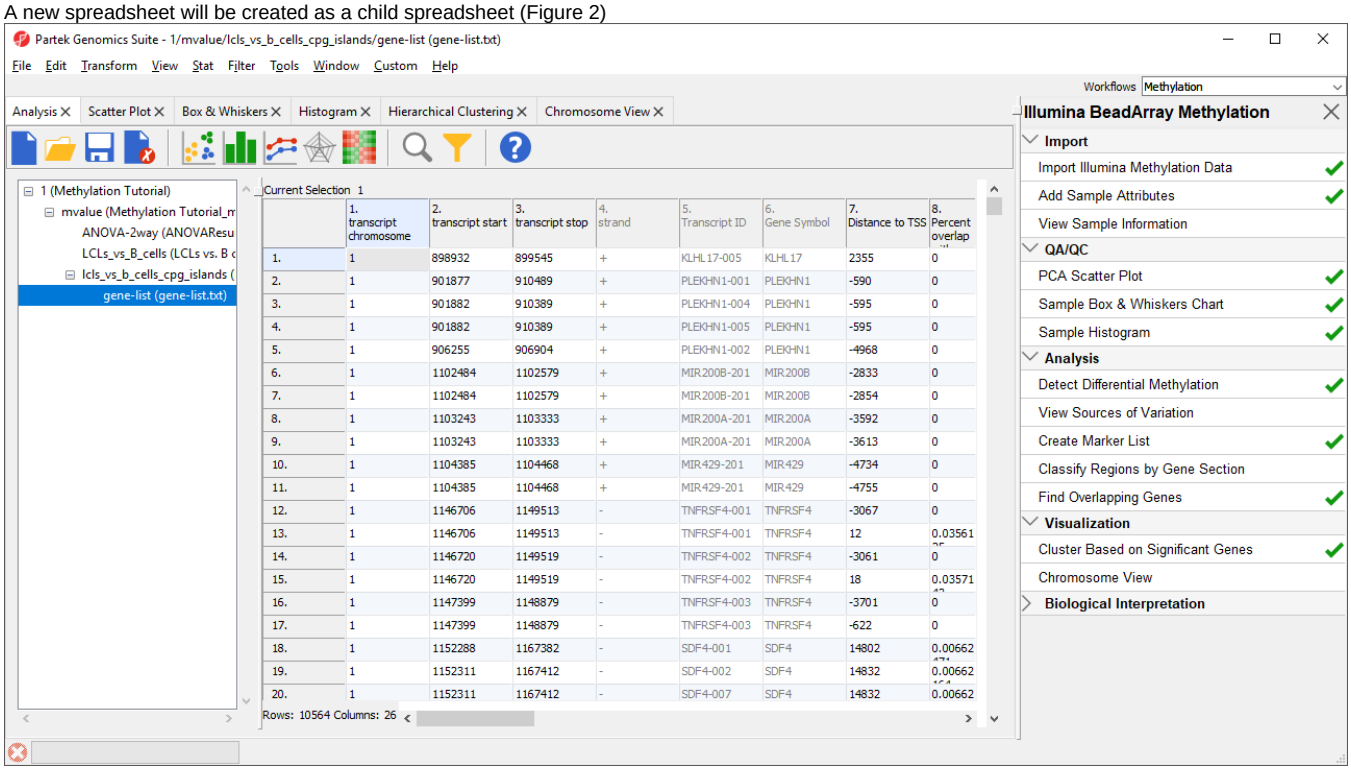


Figure 7. Selecting Finding Overlapping Genes form the main toolbar

- Select **Ensembl Transcripts release 75** from the *Report regions from the specified database* options
- You can select a name for the new list, we have named it *gene-list*
- Select **OK**

A new spreadsheet will be created as a child spreadsheet (Figure 2)



	1. transcript chromosome	2. transcript start	3. transcript stop	4. strand	5. Transcript ID	6. Gene Symbol	7. Distance to TSS	8. Percent overlap
1.	1	898932	899545	+	KLHL17-005	KLHL17	2355	0
2.	1	901877	910489	+	PLEKHN1-001	PLEKHN1	-590	0
3.	1	901882	910389	+	PLEKHN1-004	PLEKHN1	-595	0
4.	1	901882	910389	+	PLEKHN1-005	PLEKHN1	-595	0
5.	1	906255	906904	+	PLEKHN1-002	PLEKHN1	-4968	0
6.	1	1102484	1102579	+	MIR200B-201	MIR200B	-2833	0
7.	1	1102484	1102579	+	MIR200B-201	MIR200B	-2854	0
8.	1	1103243	1103333	+	MIR200A-201	MIR200A	-3592	0
9.	1	1103243	1103333	+	MIR200A-201	MIR200A	-3613	0
10.	1	1104385	1104468	+	MIR429-201	MIR429	-4734	0
11.	1	1104385	1104468	+	MIR429-201	MIR429	-4755	0
12.	1	1146706	1149513	-	TNFRSF4-001	TNFRSF4	-3067	0
13.	1	1146706	1149513	-	TNFRSF4-001	TNFRSF4	12	0.03561
14.	1	1146720	1149519	-	TNFRSF4-002	TNFRSF4	-3061	0
15.	1	1146720	1149519	-	TNFRSF4-002	TNFRSF4	18	0.03571
16.	1	1147399	1148879	-	TNFRSF4-003	TNFRSF4	-3701	0
17.	1	1147399	1148879	-	TNFRSF4-003	TNFRSF4	-622	0
18.	1	1152288	1167382	-	SDF4-001	SDF4	14802	0.00662
19.	1	1152311	1167412	-	SDF4-002	SDF4	14832	0.00662
20.	1	1152311	1167412	-	SDF4-007	SDF4	14832	0.00662

Figure 8. Annotating the differentially methylated CpG loci with genes

Partek Genomics Suite offers several tools to help interpret this list of genes. First, let's look at *Gene Set Analysis*.

- Select **Gene Set Analysis** from the *Biological Interpretation* section of the *Illumina BeadArray Methylation* workflow
- Select **GO Enrichment** for *Select the method of analysis*
- Select **Next >**
- Select **1/mvalue/lcls_vs_b_cells_cpg_islands/gene-list (gene-list.txt)** for the source spreadsheet
- Select **Next >**
- Select **Invoke gene ontology browser on the result** and leave the rest of the options set to defaults for *Configure the parameters of the test* (Figure 3)

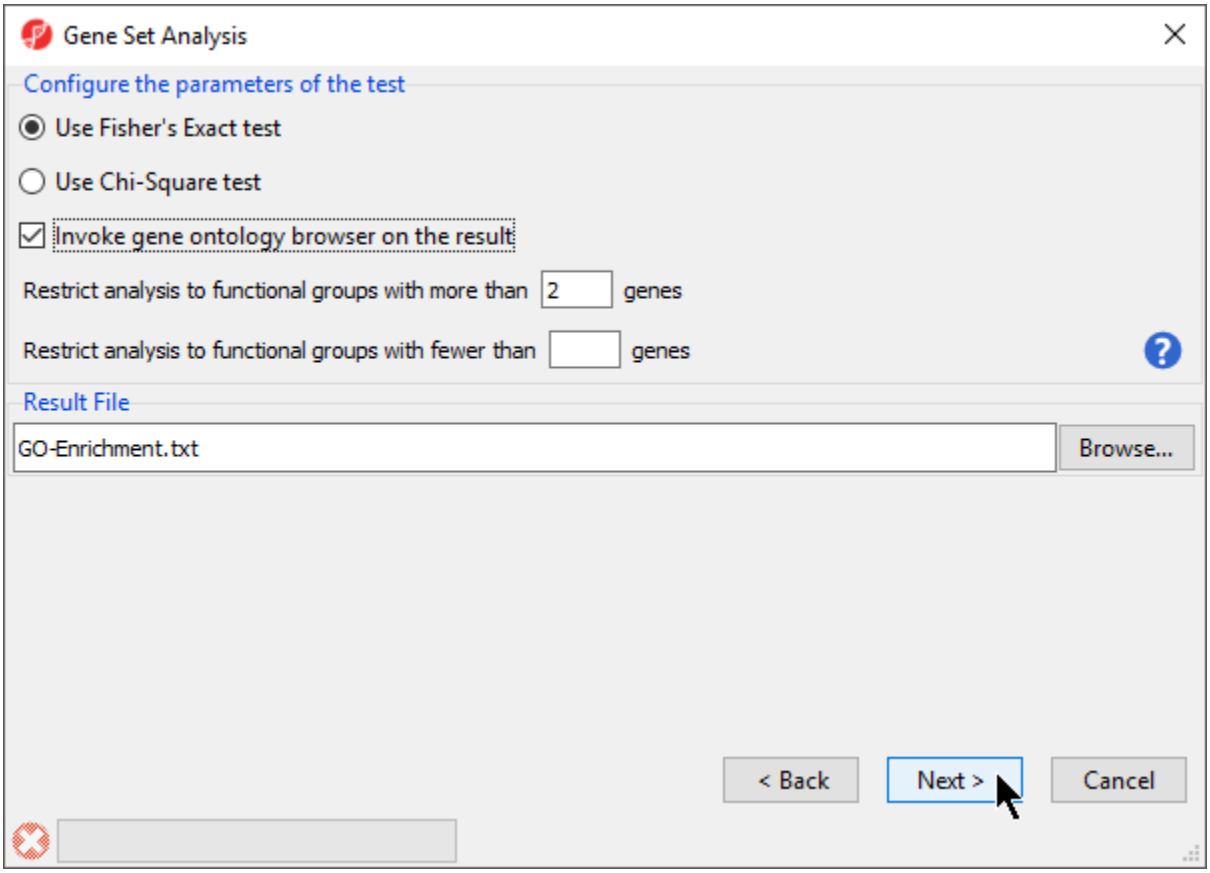


Figure 9. Configuring the parameters of the test

- Select **Next >**
- Select **Default Mapping File** for *Select the method of mapping genes to genes sets*
- Select **Next >**

A new spreadsheet will be created with categories ranked by enrichment score and the *Gene Ontology Browser* will launch to graphically display the results of the spreadsheet (Figure 4). The results show which gene sets are over represented in the list of genes overlapped by differentially regulated CpG loci between the experimental and control groups.

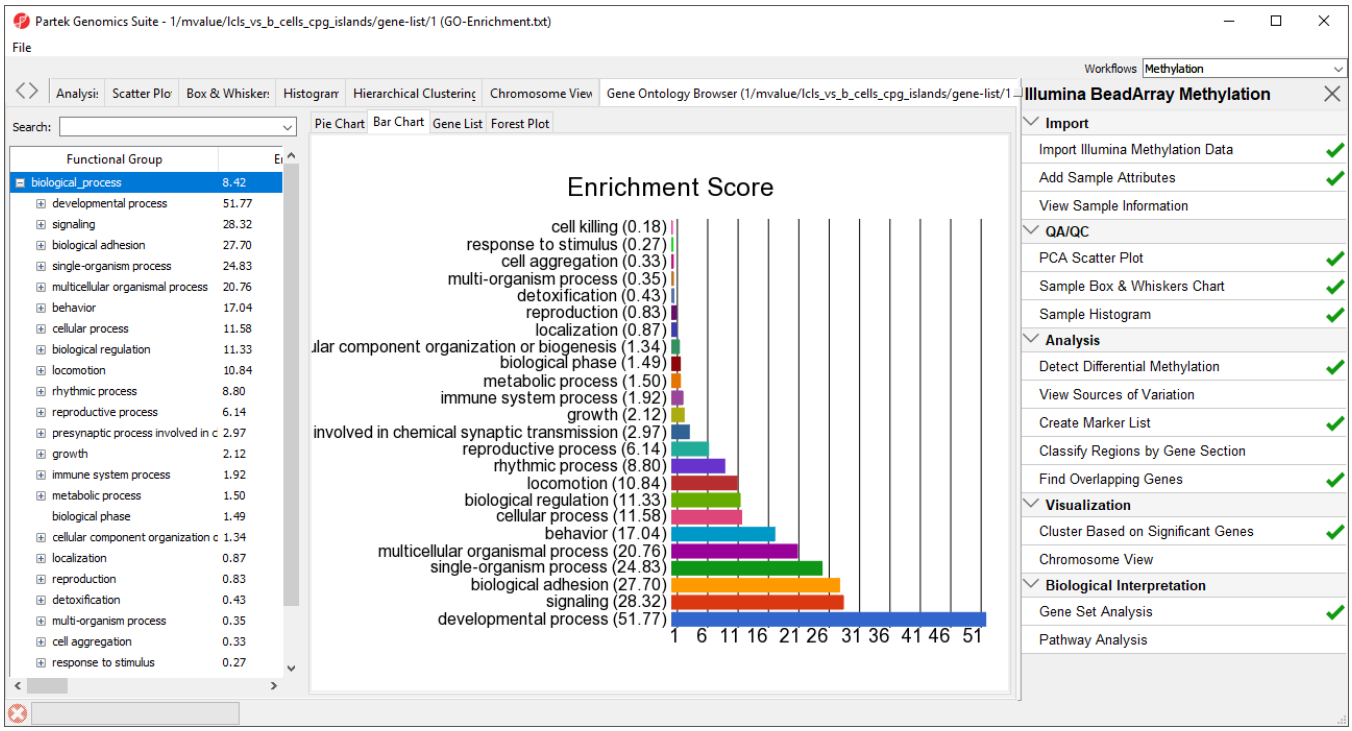


Figure 10. GO enrichment browser showing gene groups overrepresented in the list of genes which overlap with differentially methylated CpG loci

To get a better idea whether genes associated with these GO terms have increated or decreased methylation, we can view the Forest Plot.

- Select the **Forest Plot** tab

Go terms are listed by the number of significantly up-regulated genes, with the percent up-regulated and down-regulated shown in red to green bars. Here, we see that most GO terms show increased methylation in their associated genes (Figure 4).

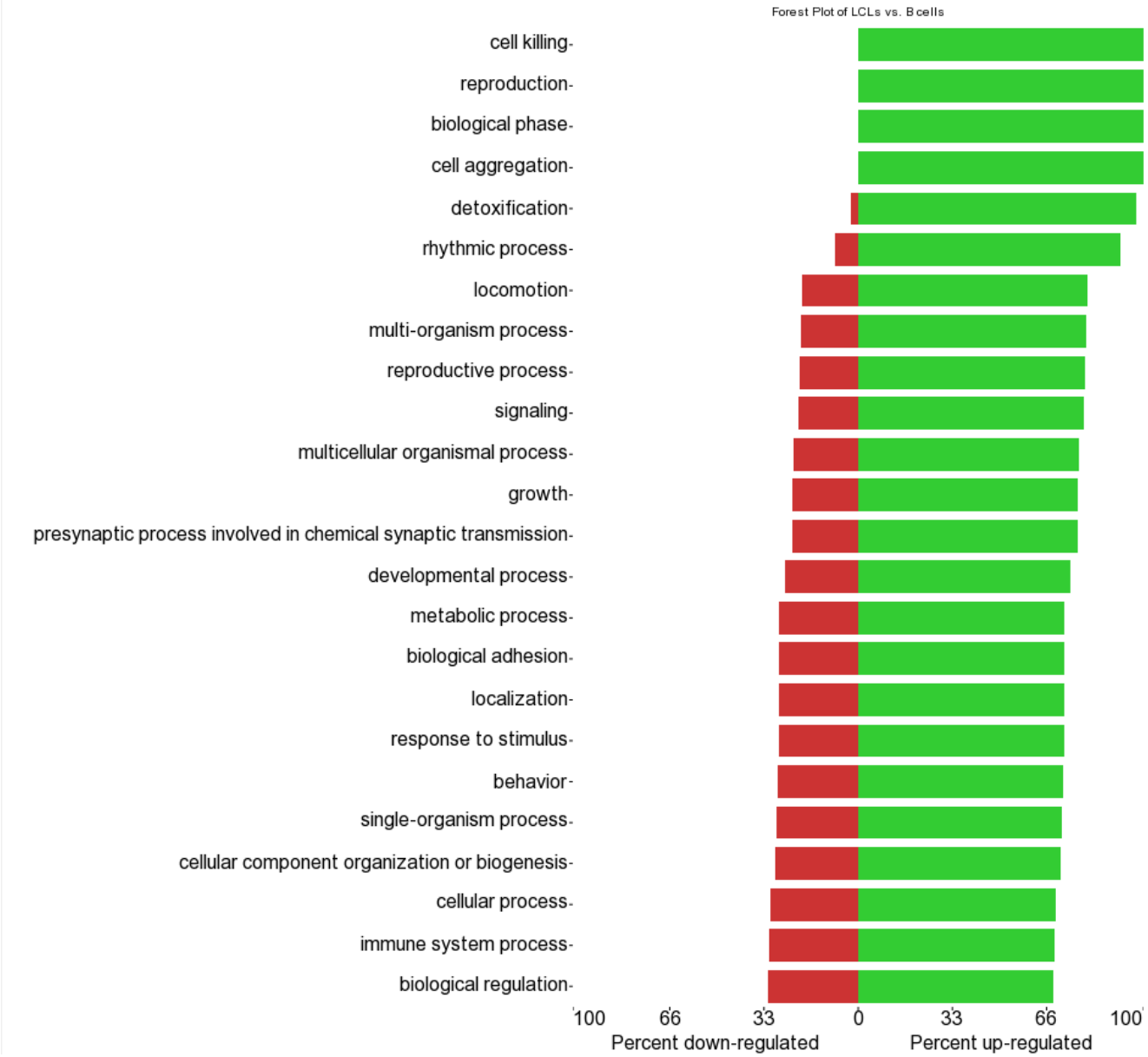


Figure 11. Gene Ontology Forest Plot

Next, we can perform *Pathway Analysis* to see which pathways are over represented in the gene overlapped by differentially regulated CpG loci.

- Select **gene-list** from the spreadsheet tree
- Select **Pathway Analysis** from the *Biological Interpretation* section of the *Illumina BeadArray Methylation* workflow
- Select **Pathway Enrichment** for *Select the method of analysis*
- Select **Next >**
- Select **1/mvalue/lcls_vs_b_cells_cpg_islands/gene-list (gene-list.txt)** for the source spreadsheet
- Select **Next >**
- Leave the default selections for the *Configure parameters of the test* panel
- Select **Next >**
- Leave the default selections for the *Result File* and *Select the parameters* panels
- Select **Next >** to run the analysis

The *Pathway-Enrichment* spreadsheet will be added to the spreadsheet tree in Partek Genomics Suite and the Partek® Pathway™ software will open to provide visualization of the most significantly enriched pathway as a pathway diagram (Figure 5). The color of the gene boxes reflects p-values of the associated differentially methylated CpG loci (bright orange is insignificant, blue is highly significant). The *Color by* option can be changed another column from the *gene-list.txt* spreadsheet, such as Difference.

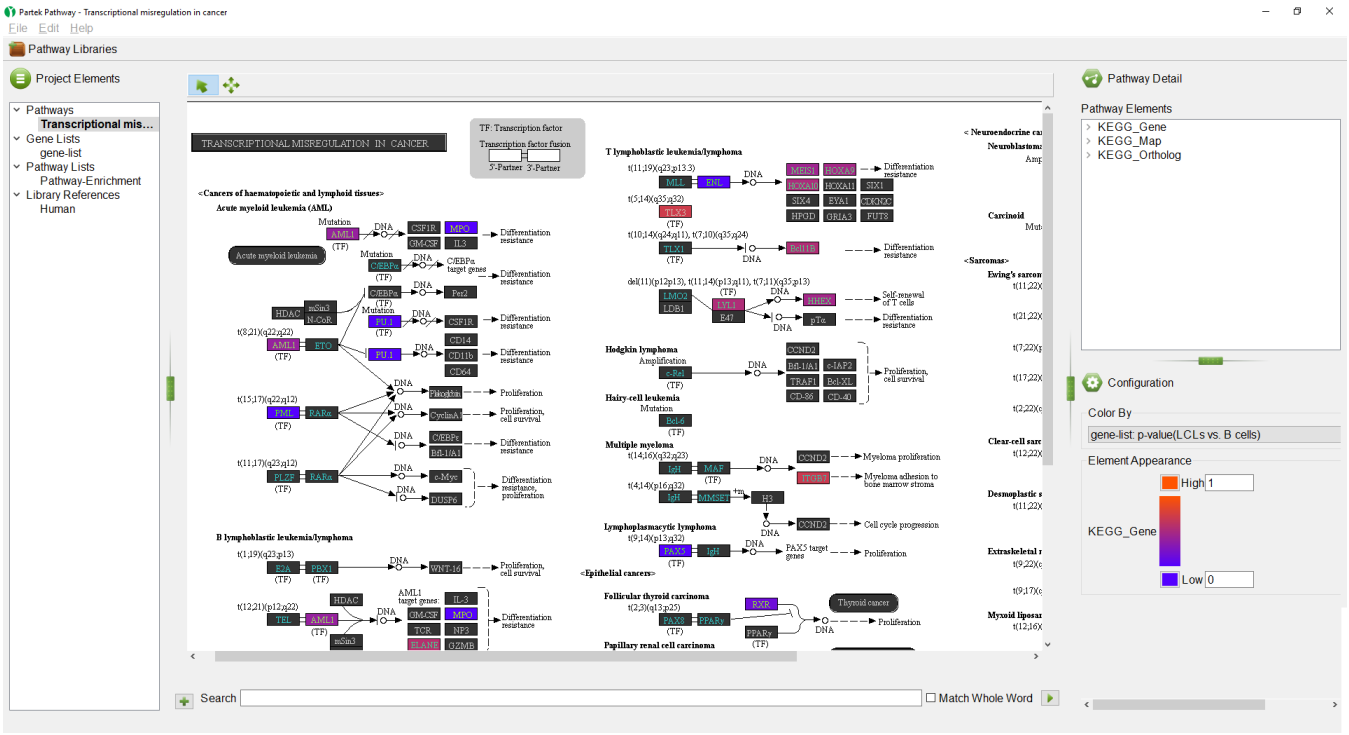


Figure 12. : Partek Pathway illustrating one of the pathways overrepresented in the list of genes overlapping the differentially methylated CpG sites.

The *Pathway-Enrichment* spreadsheet can also be viewed in Partek Pathway by switching to the *Pathway-Enrichment* section of the menu tree on the left-hand side of the window. From the spreadsheet view, you can select a pathway name to visualize that pathway. Alternatively, you can open a pathway visualization in *Partek Pathway* from the *Pathway-Enrichment* spreadsheet in Partek Genomics Suite by right-clicking on a row and selecting **Show pathway...** from the pop-up menu. Please note that if you have closed Partek Pathway and have reopened it, you will need to import a gene list if you want to color the visualization by attributes form the gene list. For more information about using *Partek Pathway*, checkout our Partek Pathway Tutorial.

« Visualize methylation at each locus Detect differentially methylated CpG islands »

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