

Detect differentially methylated loci

To detect differential methylation between CpG loci in different experimental groups, we can perform an ANOVA test. For this tutorial, we will perform a simple two-way ANOVA to compare the methylation states of the two experimental groups.

- Select **Detect Differential Methylation** from the *Analysis* section of the *Illumina BeadArray Methylation* workflow

A new child spreadsheet, *mvalue*, is created when *Detect Differential Methylation* is selected. M-values are an alternative metric for measuring methylation. -values can be easily converted to M-values using the following equation: $M\text{-value} = \log_2 \left(\frac{I}{1 - I} \right)$.

An M-value close to 0 for a CpG site indicates a similar intensity between the methylated and unmethylated probes, which means the CpG site is about half-methylated. Positive M-values mean that more molecules are methylated than unmethylated, while negative M-values mean that more molecules are unmethylated than methylated. As discussed by [Du and colleagues](#), the -value has a more intuitive biological interpretation, but the M-value is more statistically valid for the differential analysis of methylation levels.

Because we are performing differential methylation analysis, Partek Genomics Suite automatically creates an M-values spreadsheet to use for statistical analysis.

- Select **2. Cell Type** and **3. Gender** from the *Experimental Factor(s)* panel
- Select **Add Factor >** to move *2. Cell Type* and *3. Gender* to the *ANOVA Factor(s)* panel (Figure 1)

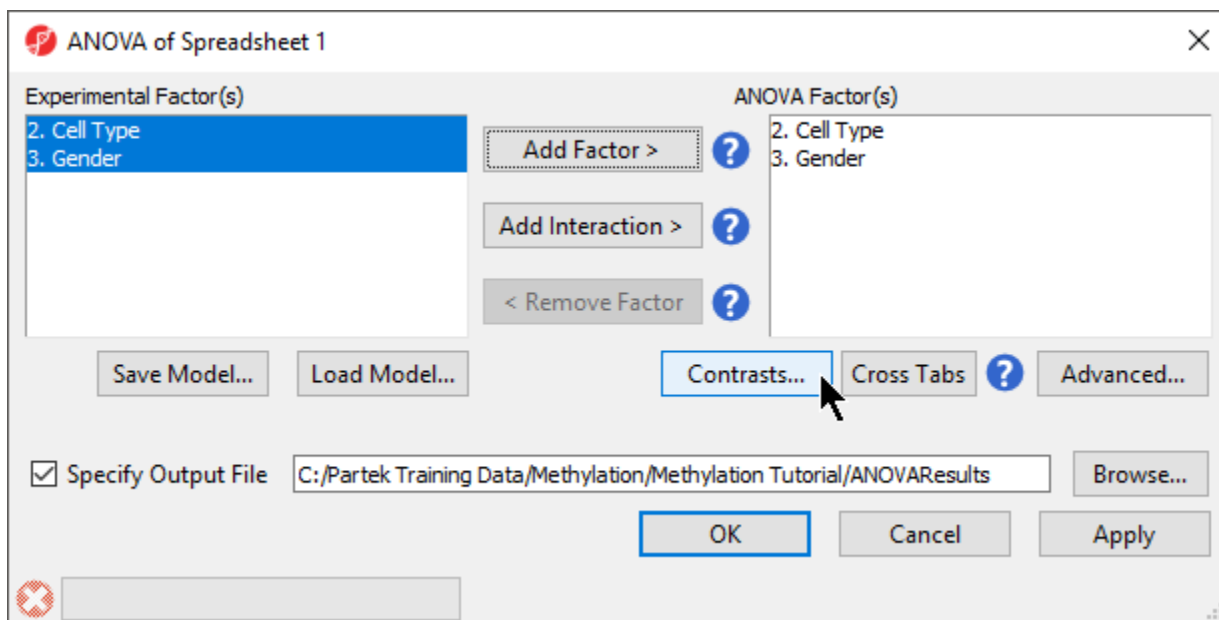


Figure 1. ANOVA setup dialog. Experimental factors listed on the left can be added to the ANOVA model.

- Select **Contrasts...**
- Leave *Data is already log transformed?* set to **No**
- Leave *Report comparisons as* set to **Difference**

For methylation data, fold-change comparisons are not appropriate. Instead, comparisons should be reported as the difference between groups.

- Select **2. Cell Type** from the *Select Factor/Interaction* drop-down menu
- Select **LCLs**
- Select **Add Contrast Level >** for the upper group
- Select **B cells**
- Select **Add Contrast Level >** for the lower group
- Select **Add Contrast** (Figure 2)

Figure 2. Configuring ANOVA contrasts

- Select **OK** to close the *Configuration* dialog


The *Contrasts...* button of the *ANOVA* dialog now reads *Contrasts Included*

- Select **OK** to close the *ANOVA* dialog and run the ANOVA

If this is the first time you have analyzed a MethylationEPIC array using the Partek Genomics Suite software, the manifest file may need to be configured. If it needs configuration, the *Configure Annotation* dialog will appear (Figure 3).

- Select **Chromosome is in one column and the physical location is in another column** for *Choose the column configuration*
- Select **Ilmn ID** for *Marker ID*
- Select **CHR** for *Chromosome*
- Select **MAPINFO** for *Physical Position*
- Select **Close**

This enables Partek Genomics Suite to parse out probe annotations from the manifest file.


Configure Annotation
×

Partek was unable to locate genomic positions within the annotation file. Specify the columns that contain the genomic locations of markers.

Choose the column configuration

- ☐ The chromosome and coordinates are in one column (eg: chr1:100-200)
- ☒ Chromosome is in one column and the physical position is in another column (eg: chr1,100 or chr1,100-200)
- ☐ Chromosome, start, and stop are in separate columns (eg: chr1,100,200)
- ☐ The annotation file does not contain genomic coordinates

Choose the columns

☐ Marker ID
 ☐ Chromosome
 ☐ Physical Position

☐ Add Factor >

☐ 2. HMSC

Channel	Forward_Sequence	Genome_Build	CHR	MAPINFO	Source
	CTGCACGCCTACTGCAGGTGC	37	19	5236016	TGCAGGTGCAGC
Grn	TCCCGTCTTACGGGATGGATT	37	20	61847650	CGGTCCCCGCC
	GTTTCTGGACAGTAAATTCT	37	1	6841125	CGGAATCCTTGC
	ATTGTGCCACCTTGCTGCTG	37	2	198303466	CAATGGGATGAT
Red	AGCCCCGTCATAGGTGGGCGC	37	X	24072640	GGTGGGCGCCGA
	CACAGCGTGGATGCCCCGATT	37	14	93581139	CGCCCTGGGCTG
	CCATTCAGGTGAGCAGGGCTG	37	16	57865112	CCCCCGTGGGGT
	GACTAGTTTAAACTCGGGCTG	37	6	15248173	GACTAGTTTAAA
	TCACTCTCGTGTGCTGCAGCC	37	1	144921929	GCTTTATTCTGC
Red	TCACCTCCCACCTCCTGGAG	37	9	131463936	CGCAGGATGCCA
Grn	CTGGAATGCCAGCTGCTGCTG	37	17	80159506	CGCCTGCCTCAG
	TAGATTGACCTGCTAATGAAT	137336	15	79170388	AGGAAAAATGAC

Save Model...
Specify Output File
Close

Figure 3. Processing the annotation file. User needs to point to the columns of the annotation file that contain the probe identifier as well as the chromosome and coordinates of the probe.

The results will appear as *ANOVA-2way (ANOVAResults)*, a child spreadsheet of *mvalue*. Each row of the spreadsheet represents a single CpG locus (identified by *Column ID*).

Partek Genomics Suite - 1/mvalue/ANOVA-2way (ANOVAResults)

File Edit Transform View Stat Filter Tools Window Custom Help

Analysis X Scatter Plot X Box & Whiskers X Histogram X

1 (Methylation Tutorial)
mvalue (Methylation Tutorial_m)
ANOVA-2way (ANOVAResults)

Current Selection 121171

	1. Column #	2. Column ID	3. Gene Symbol	4. p-value(Cell Type)	5. p-value(Gender)	6. p-value(LCLs vs. B cells)	7. Difference(LCLs vs. B cells)	8. Difference(LCLs vs. B cells) (Description)	9. Beta Difference
1.	121171	cg04757806	FUT4	9.57643e-20	0.83802	9.57643e-20	6.80444	LCLs up vs B	0.00000
2.	267981	cg12240314		1.23835e-19	0.477866	1.23835e-19	-7.17124	LCLs down vs B	-0.00000
3.	175398	cg17232476	SORL1	1.44884e-19	0.752996	1.44884e-19	-8.21882	LCLs down vs B	-0.00000
4.	378251	cg26633139	PNOC	4.65869e-19	0.362532	4.65869e-19	-8.33491	LCLs down vs B	-0.00000
5.	59936	cg04828493	CARS2	5.34791e-19	0.0159452	5.34791e-19	-7.78678	LCLs down vs B	-0.00000
6.	468665	cg26310485		1.54351e-18	0.705234	1.54351e-18	-7.71122	LCLs down vs B	-0.00000
7.	753999	cg02371766		1.59327e-18	0.0711044	1.59327e-18	-8.58804	LCLs down vs B	-0.00000
8.	73638	cg11774624	AGPAT6	2.63832e-18	0.738548	2.63832e-18	-7.87411	LCLs down vs B	-0.00000
9.	737235	cg21848211		4.5663e-18	0.522702	4.5663e-18	-7.54508	LCLs down vs B	-0.00000
10.	46980	cg09667606	SYNJ2	6.6954e-18	0.0197077	6.6954e-18	6.23819	LCLs up vs B	0.00000
11.	260169	cg10164437	KCNQ5	6.98979e-18	0.920702	6.98979e-18	-7.89619	LCLs down vs B	-0.00000
12.	778878	cg17575365	CDK14	7.24027e-18	0.736724	7.24027e-18	-8.14493	LCLs down vs B	-0.00000
13.	520338	cg16854275	PNOC	9.02887e-18	0.255524	9.02887e-18	-6.56486	LCLs down vs B	-0.00000
14.	114459	cg16063783	RUNX3	9.29924e-18	0.646552	9.29924e-18	-6.29186	LCLs down vs B	-0.00000
15.	670779	cg23516868	C20orf196	1.23332e-17	0.737802	1.23332e-17	-6.97962	LCLs down vs B	-0.00000
16.	39933	cg18406852	CHST10	1.26999e-17	0.149391	1.26999e-17	-7.92923	LCLs down vs B	-0.00000
17.	128412	cg17676225	LIMK2	1.70612e-17	0.377375	1.70612e-17	6.39748	LCLs up vs B	0.00000
18.	62536	cg08863777	FUT4	1.84443e-17	0.277553	1.84443e-17	6.19056	LCLs up vs B	0.00000
19.	429356	cg15099231		1.84583e-17	0.361307	1.84583e-17	-7.26658	LCLs down vs B	-0.00000
20.	637630	cg09680530	FLT1	1.89545e-17	0.856645	1.89545e-17	-6.07285	LCLs down vs B	-0.00000

Rows: 844573 Columns: 16

Workflows Methylation

Illumina BeadArray Methylation

Import

- Import Illumina Methylation Data ✓
- Add Sample Attributes ✓
- View Sample Information

QA/QC

- PCA Scatter Plot ✓
- Sample Box & Whiskers Chart ✓
- Sample Histogram ✓

Analysis

- Detect Differential Methylation ✓
- View Sources of Variation
- Create Marker List
- Classify Regions by Gene Section
- Find Overlapping Genes

Visualization

Biological Interpretation

Figure 4. ANOVA spreadsheet. Each row is a result of an ANOVA at a given CpG locus (identified by the Column ID column). The remaining columns contain annotation and statistical output

For each contrast, a p-value, Difference, Difference (Description), Beta Difference, and Beta Difference (Description) are generated. The Difference column reports the difference in M-values between the two groups while the Beta Difference column reports the difference in beta values between the two groups.

« Perform data quality analysis and quality control Create a marker list »

Additional Assistance

If you need additional assistance, please visit [our support page](#) to submit a help ticket or find phone numbers for regional support.



Your Rating:  Results:  33 rates