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-value = log2(/ (1 -)).

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)

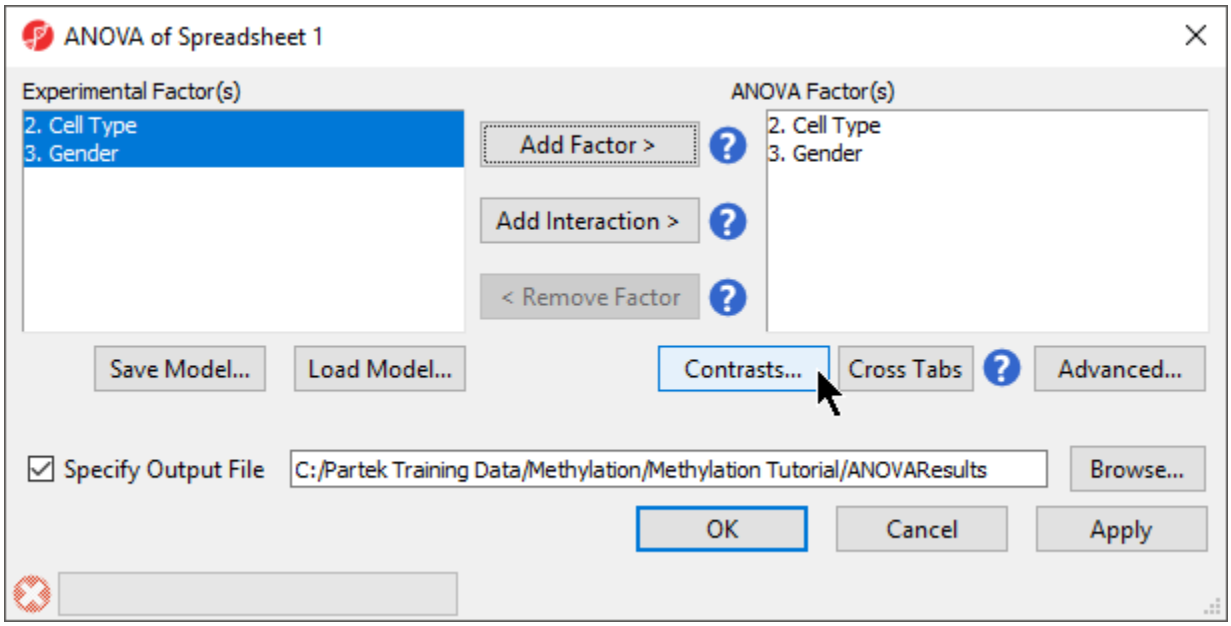

Figure 1. ANOVA setup dialog. Experimental factors listed on the left can be added to the ANOVA model.

- 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)

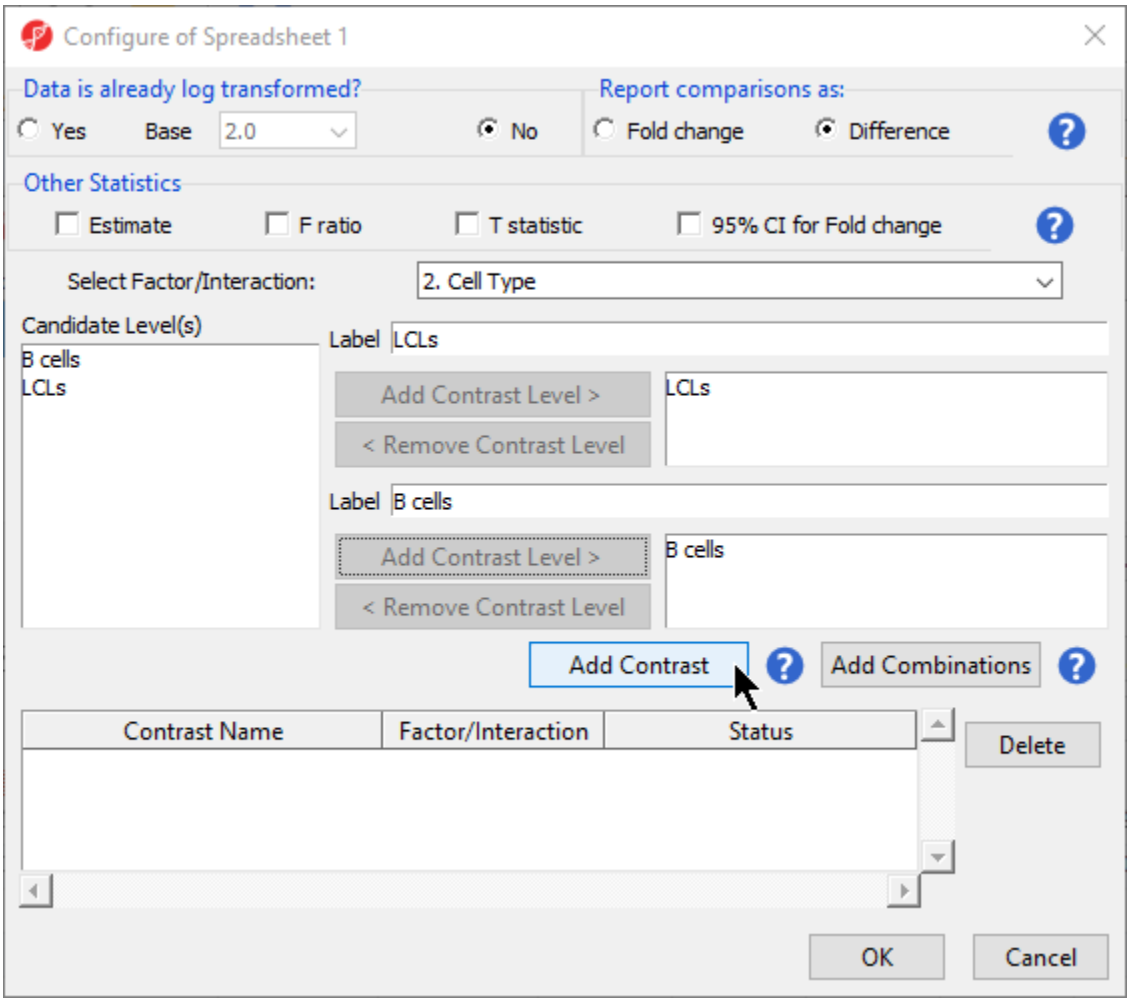

Figure 2. Configuring ANOVA contrasts

- 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* i
- Select **MAPINFO** for *Physical Position*
- Select **Close**

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

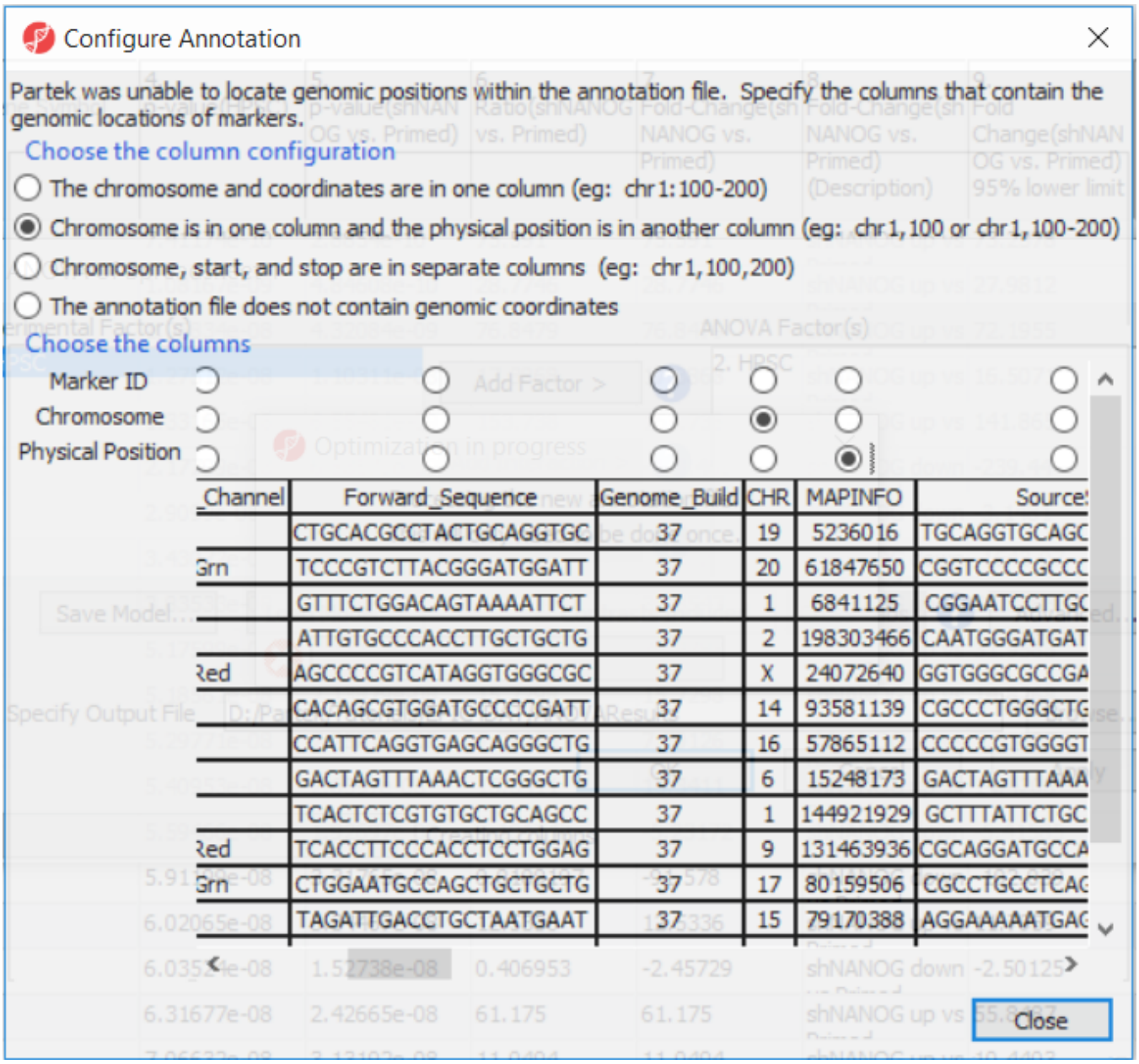

Figure 3. Processing the annotation file. User needs to point to the columns of the annotation file that contain the probe identifier as well as the chromosome and coordinates of the probe.

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*).

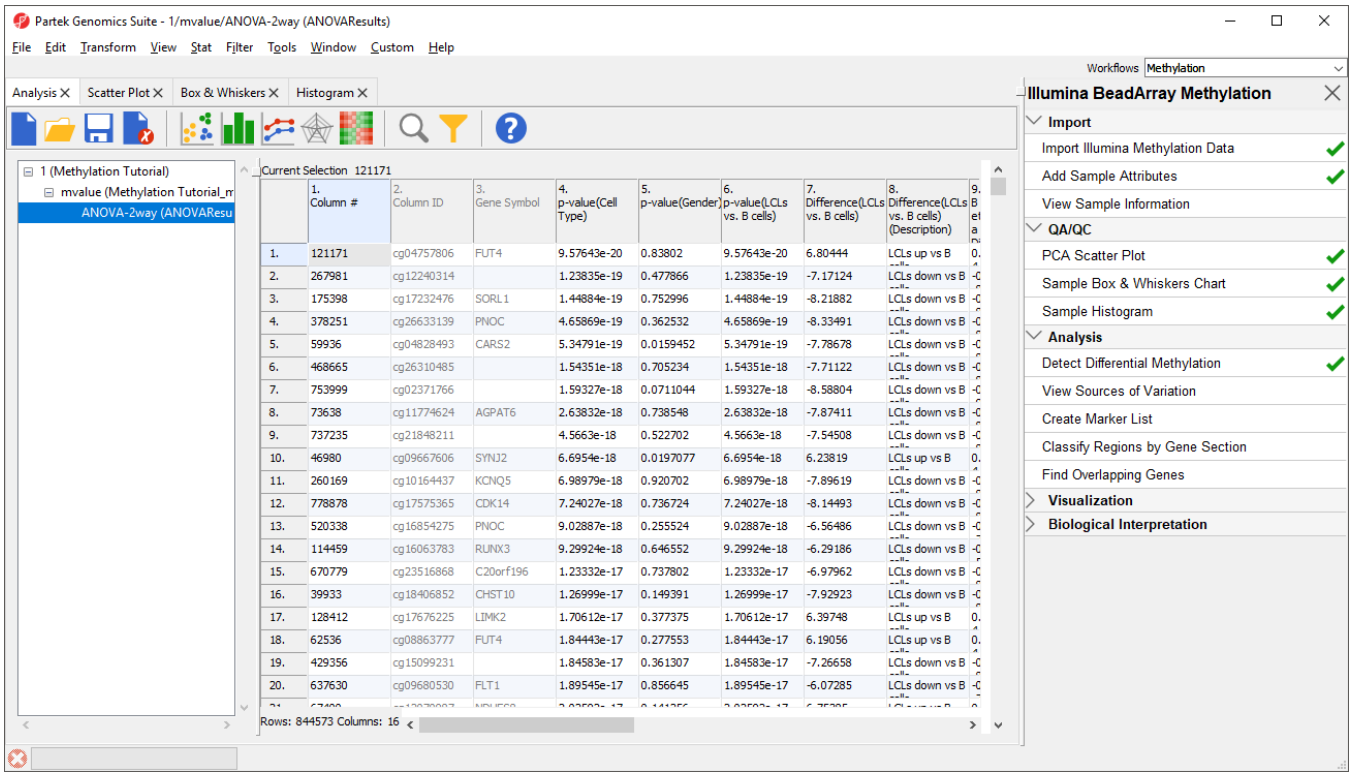

Figure 4. ANOVA spreadsheet. Each row is a result of an ANOVA at a given CpG locus (identified by the Column ID column). The remaining columns contain annotation and statistical output

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