Annotate samples

Each row of the spreadsheet (Figure 1) corresponds to a single sample. The first column is the names of the .idat files and the remaining columns are the array probes. The table values are -values, which correspond to the percentage methylation at each site. A -value is calculated as the ratio of methylated probe intensity over the overall intensity at each site (the overall intensity is the sum of methylated and unmethylated probe intensities).

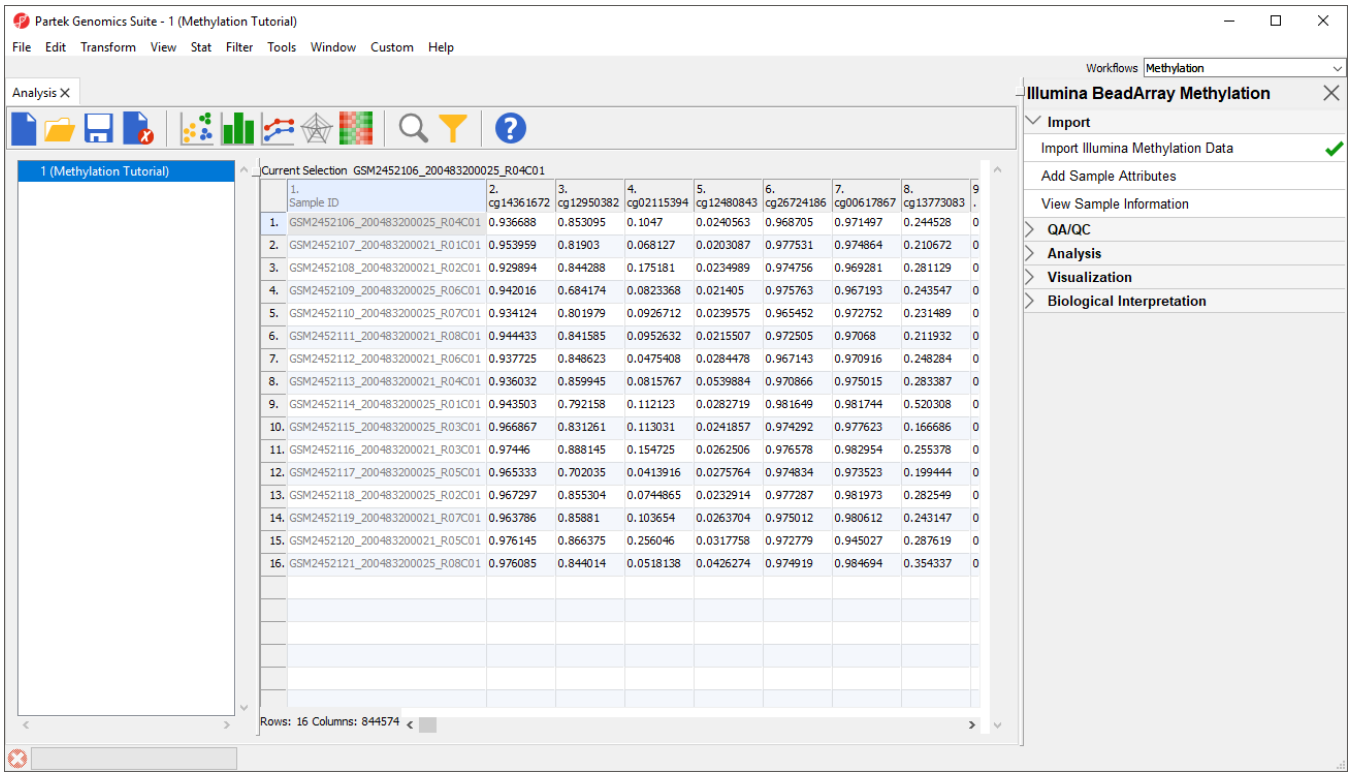

Figure 1. Spreadsheet after .idat file import: samples on rows (Sample IDs are based on file names), probes on columns, cell values are functionally normalized beta values (default settings)

Before we can perform any analysis, the study samples need to be organized into their experimental groups.

- Select **Add Sample Attributes** from the *Import* section of the *Illumina BeadArray Methylation* workflow
- Select **Add a Categorical Attribute** from the *Add Sample Attributes* dialog (Figure 2)

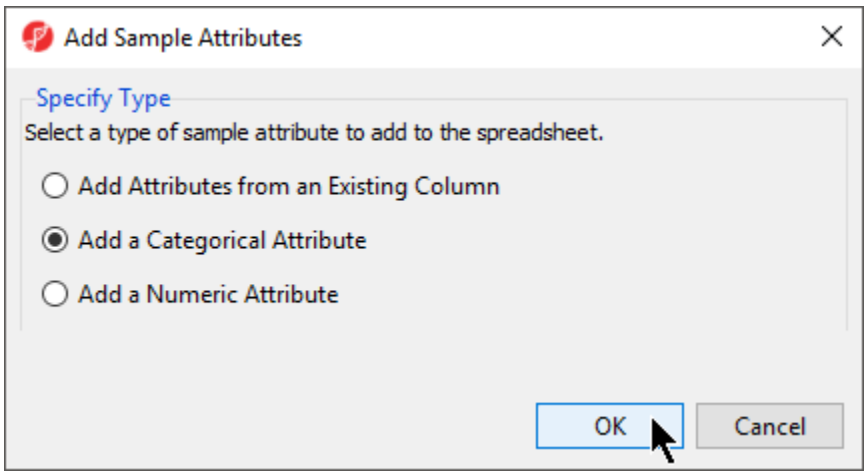

Figure 2. Adding sample attributes. Adding Attributes from an Existing Column can be used to split file names into sections, based on delimiters (e.g. _, -, space etc.). Adding a Numeric or Categorical Attribute enables the user to manually specify sample attributes

- Select **OK**

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The *Create categorical attribute* dialog allows us to create groups for a categorical attribute. By default, two groups are created, but additional groups can be added.

- Set *Attribute name:* to *Cell Type*
- Rename the groups *B cells* and *LCLs*
- Drag and drop the samples from the *Unassigned* list to their groups as listed in the table below

Sample ID	Cell Type
GSM2452106_200483200025_R04C01	B cells
GSM2452107_200483200021_R01C01	B cells
GSM2452108_200483200021_R02C01	B cells
GSM2452109_200483200025_R06C01	B cells
GSM2452110_200483200025_R07C01	B cells
GSM2452111_200483200021_R08C01	B cells
GSM2452112_200483200021_R06C01	B cells
GSM2452113_200483200021_R04C01	B cells
GSM2452114_200483200025_R01C01	LCLs
GSM2452115_200483200025_R03C01	LCLs
GSM2452116_200483200021_R03C01	LCLs
GSM2452117_200483200025_R05C01	LCLs
GSM2452118_200483200025_R02C01	LCLs
GSM2452119_200483200021_R07C01	LCLs
GSM2452120_200483200021_R05C01	LCLs
GSM2452121_200483200025_R08C01	LCLs

There should now be two groups with eight samples in each group (Figure 3).

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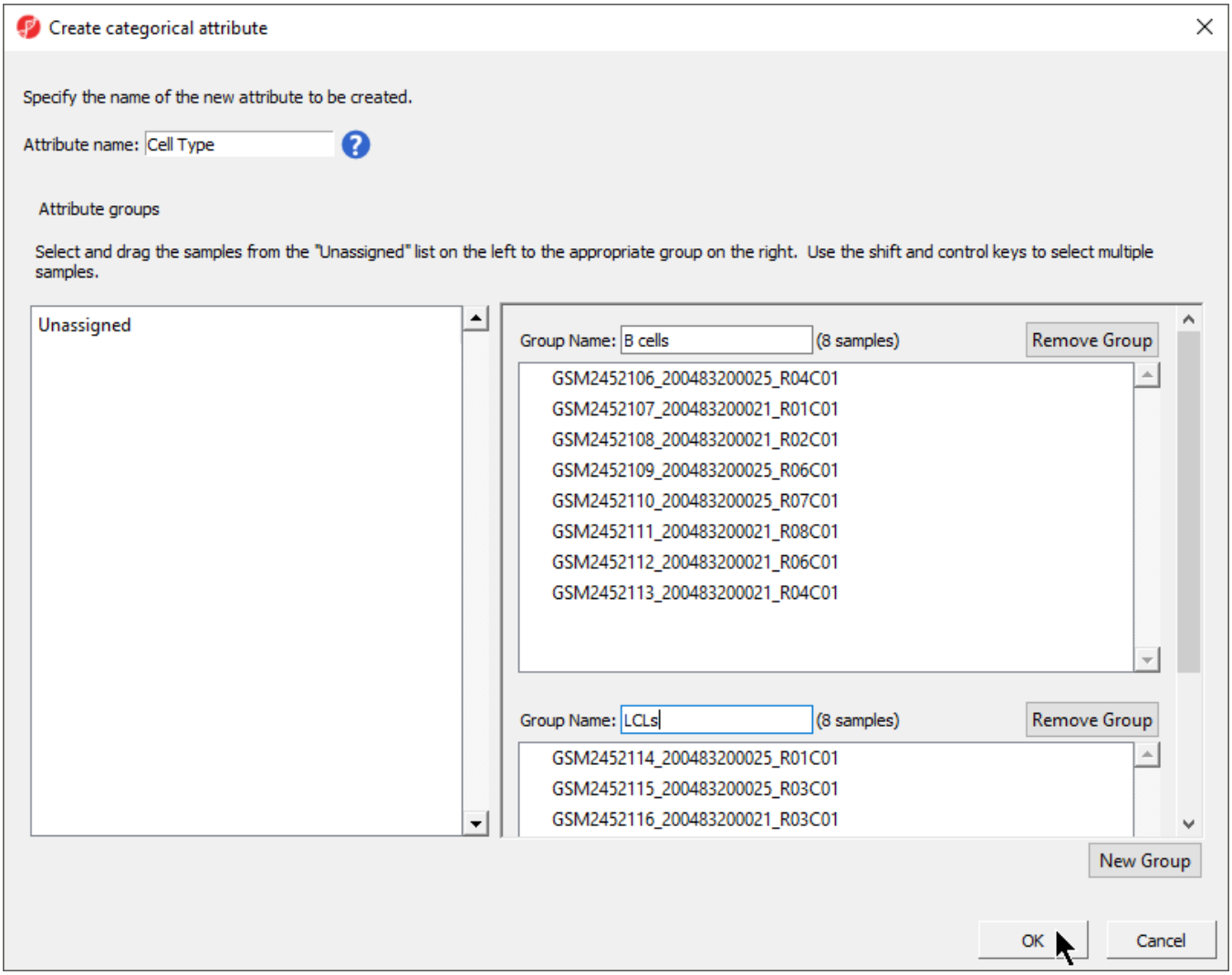

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Figure 3: Adding Cell Type attribute as a categorical group

- Select **OK**
- Select **Yes** from the *Add another categorical attribute* dialog
- Set *Attribute name:* to *Gender*
- Rename the groups *Male* and *Female*
- Drag and drop the samples from the *Unassigned* list to their groups as listed in the table below

Sample ID	Gender
GSM2452106_200483200025_R04C01	Female
GSM2452107_200483200021_R01C01	Female
GSM2452108_200483200021_R02C01	Male
GSM2452109_200483200025_R06C01	Female
GSM2452110_200483200025_R07C01	Female
GSM2452111_200483200021_R08C01	Female
GSM2452112_200483200021_R06C01	Female
GSM2452113_200483200021_R04C01	Male
GSM2452114_200483200025_R01C01	Female
GSM2452115_200483200025_R03C01	Female
GSM2452116_200483200021_R03C01	Male
GSM2452117_200483200025_R05C01	Female
GSM2452118_200483200025_R02C01	Female
GSM2452119_200483200021_R07C01	Female
GSM2452120_200483200021_R05C01	Female
GSM2452121_200483200025_R08C01	Male

There should now be two groups with four samples in *Male* and twelve samples in *Female* (Figure 4).

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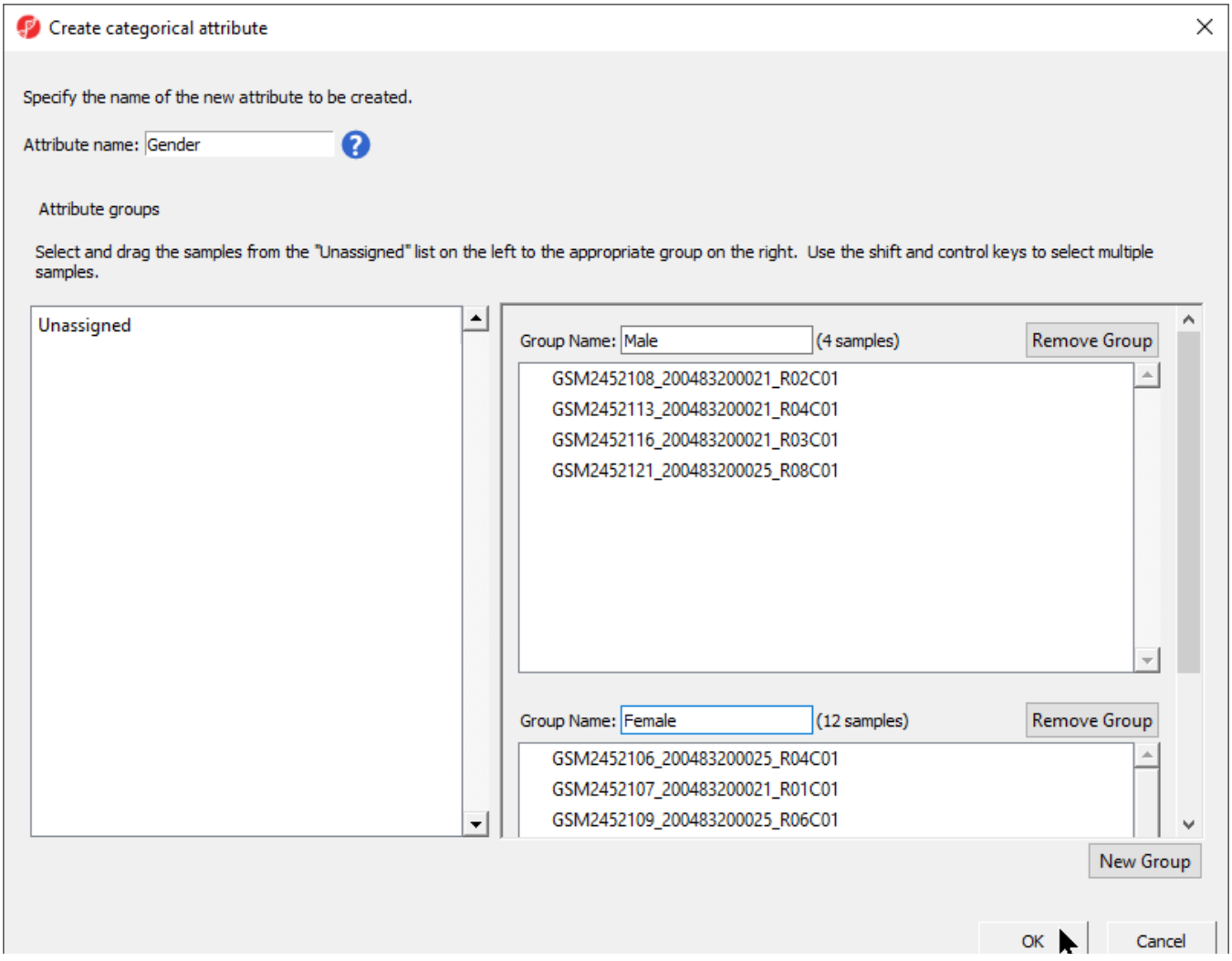

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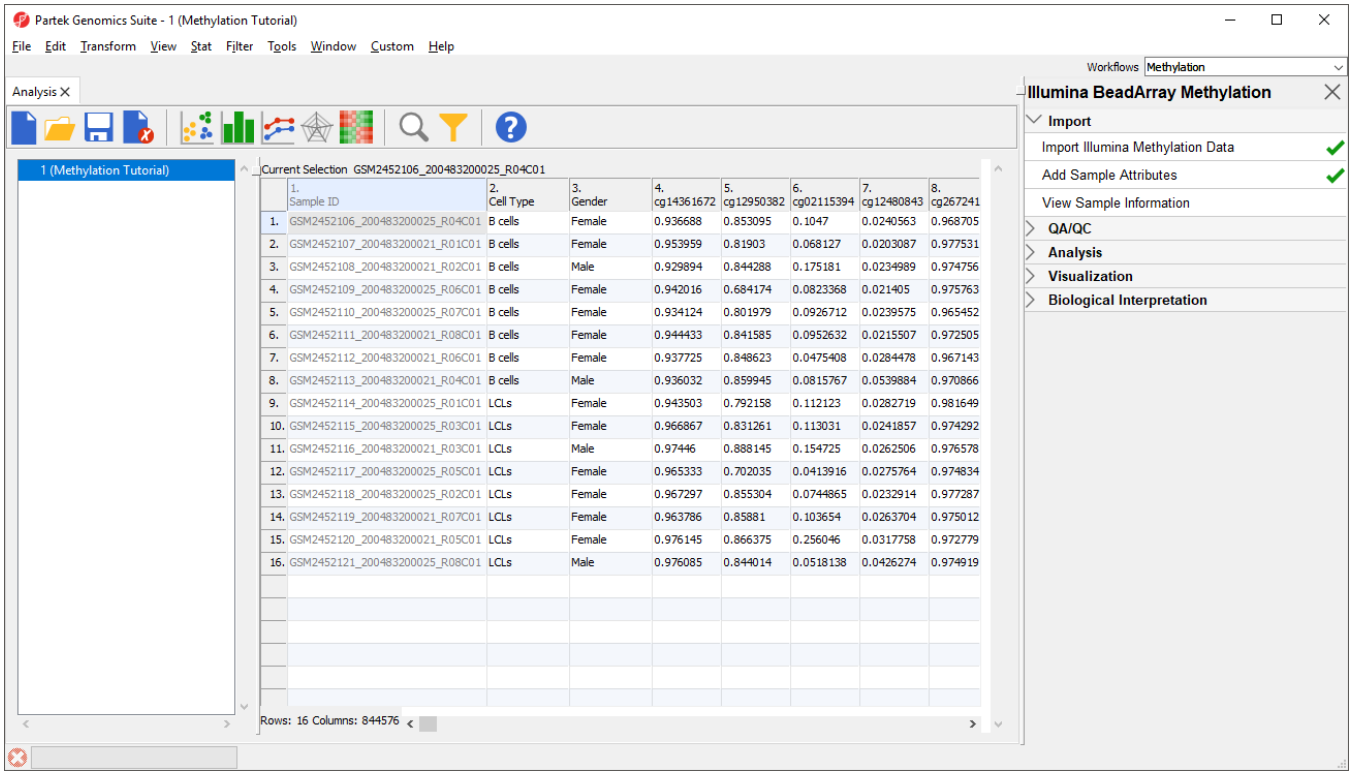
Figure 4. Adding Gender attribute as a categorical group

- Select **OK**
- Select **No** from the *Add another categorical attribute* dialog
- Select **Yes** to save the spreadsheet

Two new columns have been added to spreadsheet *1 (Methylation)* with the cell type and gender of each sample (Figure 5).



	1. Sample ID	2. Cell Type	3. Gender	4. cg14361672	5. cg12950382	6. cg02115394	7. cg12480843	8. cg267241
1.	GSM2452106_200483200025_R04C01	B cells	Female	0.936688	0.853095	0.1047	0.0240563	0.968705
2.	GSM2452107_200483200021_R01C01	B cells	Female	0.953959	0.81903	0.068127	0.0203087	0.977531
3.	GSM2452108_200483200021_R02C01	B cells	Male	0.929894	0.844288	0.175181	0.0234989	0.974756
4.	GSM2452109_200483200025_R06C01	B cells	Female	0.942016	0.684174	0.0823368	0.021405	0.975763
5.	GSM2452110_200483200025_R07C01	B cells	Female	0.934124	0.801979	0.0926712	0.0239575	0.965452
6.	GSM2452111_200483200021_R08C01	B cells	Female	0.944433	0.841585	0.0952632	0.0215507	0.972505
7.	GSM2452112_200483200021_R06C01	B cells	Female	0.937725	0.848623	0.0475408	0.0284478	0.967143
8.	GSM2452113_200483200021_R04C01	B cells	Male	0.936032	0.859945	0.0815767	0.0539884	0.970866
9.	GSM2452114_200483200025_R01C01	LCLs	Female	0.943503	0.792158	0.112123	0.0282719	0.981649
10.	GSM2452115_200483200025_R03C01	LCLs	Female	0.966867	0.831261	0.113031	0.0241857	0.974292
11.	GSM2452116_200483200021_R03C01	LCLs	Male	0.97446	0.888145	0.154725	0.0262506	0.976578
12.	GSM2452117_200483200025_R05C01	LCLs	Female	0.965333	0.702035	0.0413916	0.0275764	0.974834
13.	GSM2452118_200483200025_R02C01	LCLs	Female	0.967297	0.855304	0.0744865	0.0232914	0.977287
14.	GSM2452119_200483200021_R07C01	LCLs	Female	0.963786	0.85881	0.103654	0.0263704	0.975012
15.	GSM2452120_200483200021_R05C01	LCLs	Female	0.976145	0.866375	0.256046	0.0317758	0.972779
16.	GSM2452121_200483200025_R08C01	LCLs	Male	0.976085	0.844014	0.0518138	0.0426274	0.974919

Figure 5. Annotated beta values spreadsheet

« Import and normalize methylation data Perform data quality analysis and quality control »

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