Detect differentially methylated CpG islands

The approach described in previous sections relies on ANOVA to detect differentially methylated CpG sites and takes individual sites as a starting point for interpretation. Since ANOVA compares M values at each site independently, this strategy is robust to type I/type II probe bias.

An alternative could be to first summarize all the probes belonging to a CpG island region (i.e. island, N-shore, N-shelf, S-shore, S-shelf) and then use ANOVA to compare regions across the groups. Since the summarization will include both type I and type II probes, you may want to split the analysis in two branches and analyze type I and type II probes independently. To do this, we need to annotate each probe as type I or type II.

- Select the **mvalue** spreadsheet
- Select Transform from the main toolbar
- Select Create Transposed Spreadsheet... from the Transform drop-down menu (Figure 1)

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Figure 1. Creating a transposed spreadsheet

- Select Sample ID for Column: and numeric for Data Type:
- Select OK

A new temporary spreadsheet will be created with a row for each probe and columns for each sample.

- Right-click on column 1. ID to bring up the pop-up menu
- Select Insert Annotation
- Select Add as categorical
- Select Infinium_Design_Type and UCSC_CpG_Islands_Name from the Column Configuration options (Figure 2)

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Figure 2. Adding Infinium design type and CpG island annotations

• Select OK to add the Inifinium design type and UCSC CpG island name as categorical columns on the spreadsheet

Now, we can use the interactive filter to create separate spreadsheets for type I and type II probes.

- Select () to launch the interactive filter
 Select 2. Infinium_Design_Type from the drop-down menu if not selected by default
 Left-click the type I column to exclude it
 Right-click the temporary spreadsheet in the spreadsheet tree to bring up the pop-up dialog
 Color Cline (Cline O)
- Select Clone... (Figure 3)

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								-			

Figure 3. Creating a probe list with only Infinium type II probes

- Name the new spreadsheet female_only_typell_probes
- Select OK
- Save the created spreadsheet, we chose the file name *female_only_typell_probes* .
- Repeat process to create a spreadsheet for type I probes

The temporary spreadsheet is no longer needed so we can close it.

Close the temporary spreadsheet by selecting it in the file tree and selecting (
)

We can use these spreadsheets to generate lists of M values at CpG island regions

- Select spreadsheet female_only_typell_probesSelect Stat from the main toolbar
- Select Column Statistics... under Descriptive (Figure 4)

🤣 Partek Genomics Suite - mva	alue_typeii_j	probes (m	alue_ty	pell_pr	obes)								- 0	×
File Edit Transform View	Stat Filter	r Tools	Windo	w Cu	stom Help									
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gene-list (gene-	list.txt)	2.	cg23229	9610	п	chr 1:6844313-6	3.91045	3.86512	4.04818	3.83101	4.05214		Sample Box & Whiskers Chart	-
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muslus tuncii probes (mus	-Enrichr	4.	cg0545:	1842	п	chr 14:9358 1083	-4.28962	-4.12952	-4.13717	-4.26489	-4.11082		Anglusia	
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inteldes_type_only (inteld	co_cype.	6.	cg09838	8562	п	chr6:15244877-	-4.26049	-3.65329	-4.04391	-3.99152	-4.32209		Detect Differential Methylation	~
		7.	cg25458	8538	п		4.75172	4.83514	4.81975	4.92123	4.85547		View Sources of Variation	
		8.	cg0926	1072	п		1.1729	1.18209	0.958895	1.27574	1.2646		Create Marker List	~
		9.	cg02404	4579	п	chr 16:2521086-	2.64724	3.51785	3.38518	2.70609	3.00534		Classify Regions by Gene Section	
		10.	cg04118	8974	п		0.543575	1.21936	0.901688	0.582222	1.0349			
		11.	cg01236	5347	п		0.691399	1.28086	1.43729	0.927938	0.912946		Find Overlapping Genes	~
		12.	cg2258	5117	п		3.41818	3.28173	3.70497	3.58951	3.65894		Visualization	
		13.	cg25553	2317	п		4.27861	3.40747	4.18825	4.2146	3.69007		Cluster Based on Significant Genes	
		14.	cg2387	5663	п	chr 20: 17296125	1.15546	1.07433	1.59974	2.00537	1.14507		Chromosome View	
		15.	cg07659	9892	п		-3.11366	-1.84245	-2.0929	-2.78493	-2.5534		✓ Biological Interpretation	
		16.	cg1599	5909	п		4.19721	4.51626	4.05424	4.00055	4.35215		Gono Sat Analysis	
		17.	cg23728	8960	п		-0.533466	0.0497478	-0.285093	-1.21627	-0.397413			
		18.	cg11993	3619	п	chr 1: 11228 1052	0.285262	0.865634	0.354727	0.527648	0.530082		Pathway Analysis	~
		19.	cg0192	5883	п		1.62131	2.2407	2.51242	2.08669	1.86149			
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		1										· .		

Figure 4. Selecting column statistics

- Add Mean to the *Selected Measure(s)* panel
 Select Group By and set it to 3. UCSC_CpG_Islands_Name (Figure 5)

Column Statistics of Spreadsheet mvalue_typ	eii_probes X
Candidate Measure(s) Coefficient of Variation Max Median Min Obs Standard Deviation Sum	Selected Measure(s) -> <-
Column(s) All Response Variables ✓ ✓ Specify Output File C:/Partek Training Data/N	Trimmed Mean Range 10 % from Min 10 % from Max Methylation/Methylation Tutorial/descriptive_colum Browse OK Cancel
	-

Figure 5. Configuring column statistics

Select OK

The new temporary spreadsheet has one CpG island region per row (Figure 6), samples on columns, and the values in the cells represent the mean of M values of all the CpG probes in the region.

											Workflows Methylation	
Analysi: Scatter Plo Box & Whis	cer: His	togram Hiera	rchical Cluste	rin <u>c</u> Chromo	osome View Gene	Ontology Brow	/ser (1/mvalue/	lcls_vs_b_cells_	pg_islands/gene-	list/1	Illumina BeadArray Methylation	>
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LCLs_vs_B_cells (LCLs vs. B c		_			04C01	01C01	02C01	06C01	07C01		✓ QA/QC	
Icls_vs_b_cells_cpg_islands (1.	- Mean		Mean	2.05443	2.10913	2.27858	2.11875	2.13742		PCA Scatter Plot	•
gene-list (gene-list.bt)	2.	chr 10: 1000282	0 chr 10: 10002	B20 Mean	-0.452443	-0.305016	-0.0444587	-0.345506	-0.208762		Sample Box & Whiskers Chart	
11 (Bathway-Enrich	3.	chr 10: 1002274	chr 10: 10022	743 Mean	-1.68781	-1.56506	-1.60548	-1.56352	-1.65828		Sample Histogram	
2 (ptmp20) *	4.	chr 10: 1009921	5 chr 10: 10099	215 Mean	-3.03373	-2.67533	-2.80676	-2.88015	-2.87671		✓ Analysis	
mvalue_typeii_probes (mvalue_type	5.	Chr 10: 1009938	2 chr 10: 10099	382 Mean	-3.36568	-3.18153	-3.16685	-3.22943	-3.36071		Detect Differential Methylation	
mvalues_typei_only (mvalues_typel_	0.	Chr 10: 10 10890	1 chr 10: 10 108	901Mean	0.96552	1.09234	1.14689	1.01872	0.999664			
	/.	chr 10: 1011904	chr 10: 10119	Def Mean	-1.79927	-1.7/015	-1.7/242	-1.79002	-1.62516		View Sources of Variation	
	0.	- 1012000000	- chr 10: 10127	110 Marca	-4.29509	-2.30007	-3.09301	-3.44000	-3.2401		Create Marker List	•
	9.	chr10:1012011	chr 10:10120	272 Mean	2.57612	2.75555	2.73203	2.03303	2.76142		Classify Regions by Gene Section	
	10.	chr10:101282/	C 10120202	716Moon	0.131110	0.509313	0.223912	0.242412	0.636760		Find Overlapping Genes	
	12	chr10:1012871	chr10,10128	002Mean	E 52742	4 42912	E 21/77	E 4903E	4 56224		Visualization	
	12.	chr 10: 10 12900	c +01200220	160 Mean	-0.290255	-0.156008	-0.500575	-0.655652	-9.401646		Cluster Based on Significant Cones	
	14	chr 10: 1012924	chr 10: 10129	74° Mean	-4 74375	-4 47686	-4 44459	-4 88285	-4 65537		Cluster Dased on Significant Genes	
	15	chr 10: 1012930	1 cbr 10: 10129	301Mean	-3.50438	-3.14411	-2.99311	-3.56212	-3.53597		Chromosome View	
	16	chr 10: 1012944	4 chr 10: 10129	444 Mean	-4.989	-4.42077	-4.6901	-4.82142	-4.91756		Biological Interpretation	
	17.	chr 10: 1012974	chr 10: 10129	74° Mean	-1.558	-1.27888	-1.4096	-1.64813	-1.51386		Gene Set Analysis	•
	18	chr 10: 1012999	chr 10: 10129	999 Mean	-3.91073	-3.73482	-3.52735	-3.776	-3.57629		Pathway Analysis	•
	19.	chr 10: 1013797	chr 10: 10137	970 Mean	-2,17506	-2.04729	-1.95225	-1.97996	-2.05065			
~		0.101001445	0 10120144		2.2.000							

Figure 6. New spreadsheet with average M values for probes at each CpG island; probes not at CpG islands are collected into the first row "- Mean"

Note the first row, with label "- Mean". It corresponds to all the probes that map outside of UCSC CpG islands. As it is not needed for the downstream analysis, we will remove it.

- Right-click on the row header for Mean
- Select Delete to remove the row

The final step is to transpose the data back to its original orientation.

- Select Transform from the main toolbar
- Select Create Transposed Spreadsheet... from the Transform drop-down menu
- Select 2. Level for Column: and numeric for Data Type:
- Select OK

The layout of the new transposed spreadsheet is as follows: one sample per row with CpG island regions on columns; cell entries correspond to mean methylation status of the region (Figure 7). The column with a blank value for the column header is the average of all probes not associated with CpG island regions. You can delete this column if you like.

										Workflows Methylation	
Analysi: Scatter Plo Box & Whisk	er: Histogram	Hierarchical C	lusterin <u>c</u>	Chromosome View	Gene Ontolo	ogy Browser (1/	mvalue/lcls_vs_l	b_cells_cpg_isla	nds/gene-list/1	Illumina BeadArray Methylation	\rightarrow
) 🧀 🔲 💽 🗍 🛃			-	2						∽ Import	
			•							Import Illumina Methylation Data	•
1 (Methylation Tutorial)	Current Selectio	n GSM2452106_	200483200	025_R04C01					<u> </u>	Add Sample Attributes	
 mvalue (Methylation Tutorial_rr ANOVA-2way (ANOVAResu 		1. ID	2.	3. chr 10: 10002820 4-100028508	4. chr 10: 1002274 8-100227832	5. 43 chr 10: 1009921 6-100992687	6. 5 chr 10: 1009938 0-100994188	7. 2 chr 10: 10 10890 0-10 1090655	8. 1chr 10: 10 1190451	View Sample Information	
LCLs_vs_B_cells (LCLs vs. B c	1.	00483200025_F	2.05443	-0.452443	-1.68781	-3.03373	-3.36568	0.96552	-1.79927	✓ QA/QC	
 lcls_vs_b_cells_cpg_islands (2.	GSM2452107_2	2.10913	-0.305016	-1.56506	-2.67533	-3.18153	1.09234	-1.77613	PCA Scatter Plot	•
gene-list (gene-list.txt)	3.	GSM2452108_2	2.27858	-0.0444587	-1.60548	-2.80676	-3.16685	1.14689	-1.77242	Sample Box & Whiskers Chart	•
1 (GO-Enrichment.tx	4.	GSM2452109_2	2.11875	-0.345506	-1.56352	-2.88015	-3.22943	1.01872	-1.79662	Sample Histogram	
2 (ntmp20) *	5.	GSM2452110_2	2.13742	-0.208762	-1.65828	-2.87671	-3.36071	0.999664	-1.62516		
3 (2 transpose)	6.	GSM2452111_2	2.04699	-0.266601	-1.60396	-2.94236	-3.45777	0.963269	-1.80612	Detect Differential Mathedation	
mvalue_typeii_probes (mvalue_type	7.	GSM2452112_2	2.07176	-0.469106	-1.77053	-2.87873	-3.23787	1.2805	-1.71871	Detect Differential Methylation	`
mvalues_typei_only (mvalues_typel	8.	GSM2452113_2	1.95091	-0.302441	-1.86453	-3.19654	-3.45512	0.997016	-1.88915	View Sources of Variation	
	9.	GSM2452114_2	1.62144	-0.258133	-0.637555	-0.300348	-1.7773	1.44752	-1.34595	Create Marker List	•
	10.	GSM2452115_2	1.35925	-0.887169	-0.668727	-2.57721	-2.48109	0.893183	-1.55009	Classify Regions by Gene Section	
	11.	GSM2452116_2	2.01658	0.457735	-1.05521	-1.93489	-1.51255	1.6345	-1.33885	Find Overlapping Genes	
	12.	GSM2452117_2	1.85826	0.81323	-1.07283	-1.82702	-1.88291	1.17994	-1.3158	Viewelization	
	13.	GSM2452118_2	2.07284	0.330575	-1.13665	-2.31668	-2.39114	1.42563	-1.48644		
	14.	GSM2452119_2	1.84127	0.294067	-0.938683	-2.19766	-2.29883	1.46088	-1.33229	Cluster Based on Significant Genes	
	15.	GSM2452120_2	1.77765	-0.43748	-1.15973	-2.79582	-2.86336	1.40771	-1.3692	Chromosome View	
	16.	GSM2452121_2	2.12012	0.661712	-1.17047	-2.57019	-2.9632	1.25039	-1.45982	$^{\checkmark}$ Biological Interpretation	
										Gene Set Analysis	•
										Pathway Analysis	

Figure 7. Spreadsheet with average M values of probes in each CpG island for each sample

- Right-click the transposed spreadsheet, 2_transpose
- Select Save as... from the pop-up menu
- Name it mvalues_typell_probes_CpG_islands
- Close the source temporary spreadsheet by selecting it in the spreadsheet tree and selecting (
)

The *mvalues_typell_probes_CpG_islands* spreadsheet can be used as a starting point for ANOVA and other analyses. You can also repeat the steps above to create an equivalent spreadsheet for type I probes.

« Perform gene set and pathway analysis Optional: Add UCSC CpG island annotations »

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: ★★★★ 34 rates