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Instructions:

- For each step of the analytical pipeline provide the R code you used
- In some steps, student specific instructions are given; refer to the tables below for assignments

Student	**Surname**
1	Davide Abbondandolo
2	Tommaso Becchi
3	Federica Brando
4	Alessia Campo
5	Ludovica Cataneo
6	Alessandro Caula
7	Gaia Cervi
8	Simone Del Motto
9	Immanuela Antigone Engländer
10	Giorgia Gandolfi
11	Ana Cristina Gonzalez Sanchez
12	Fidan Gurbanova
13	Sahar Heidaribakavoli
14	Biagio Iacolare
15	Thomas James Isaac
16	Naiara Landeta González
17	Davide Lisi
18	Daniele Lucarelli
19	Gennaro Luciano
20	Vanessa Mucci
21	Matteo Orlandi
22	Fabiana Patalano
23	Lorenzo Pedroni
24	Saul Pierotti
25	Ilaria Pirona
26	Stefano Roncelli
27	Aigerim Rymbekova
28	Andrea Sambugaro
29	Óscar San José Rodríguez
30	Rosaria Tornisiello
31	

Step 3

Student	Address

1	18756452
2	71773431
3	42639338
4	46801437
5	13673406
6	61760464
7	18744490
8	64638362
9	10737353
10	39802405
11	18756452
12	29643447
13	31763489
14	44666390
15	45652402
16	45652402
17	10715421
18	25710468
19	21695377
20	64689504
21	20750401
22	41620492
23	13673406
24	59625465
25	72740437
26	10633381
27	52682510
28	32792369
29	59609499
30	59625465
31	

Step 5

Student	detPvalue threshold
1	0.05
2	0.05
3	0.05
4	0.05
5	0.05
6	0.05
7	0.05
8	0.05

9	0.05
10	0.05
11	0.05
12	0.05
13	0.05
14	0.05
15	0.05
16	0.01
17	0.01
18	0.01
19	0.01
20	0.01
21	0.01
22	0.01
23	0.01
24	0.01
25	0.01
26	0.01
27	0.01
28	0.01
29	0.01
30	0.01
31	0.01

Step 7

Student	Normalization
1	preprocessNoob
2	preprocessSWAN
3	preprocessQuantile
4	preprocessFunnorm
5	preprocessNoob
6	preprocessSWAN
7	preprocessQuantile
8	preprocessFunnorm
9	preprocessNoob
10	preprocessSWAN
11	preprocessQuantile
12	preprocessFunno

	rm
13	preprocessNoob
14	preprocessSWAN
15	preprocessQuantile
16	preprocessFunnorm
17	preprocessNoob
18	preprocessSWAN
19	preprocessQuantile
20	preprocessFunnorm
21	preprocessNoob
22	preprocessSWAN
23	preprocessQuantile
24	preprocessFunnorm
25	preprocessNoob
26	preprocessSWAN
27	preprocessQuantile
28	preprocessFunnorm
29	preprocessNoob
30	preprocessSWAN
31	preprocessQuantile

Step 9

Student	Normalization
1	t-test
2	Mann-Whitney test
3	t-test
4	Mann-Whitney test
5	t-test
6	Mann-Whitney test
7	t-test
8	Mann-Whitney test

9	t-test
10	Mann-Whitney test
11	t-test
12	Mann-Whitney test
13	t-test
14	Mann-Whitney test
15	t-test
16	Mann-Whitney test
17	t-test
18	Mann-Whitney test
19	t-test
20	Mann-Whitney test
21	t-test
22	Mann-Whitney test
23	t-test
24	Mann-Whitney test
25	t-test
26	Mann-Whitney test
27	t-test
28	Mann-Whitney test
29	t-test
30	Mann-Whitney test
31	t-test

Pipeline steps

1. Load raw data with minfi and create an object called RGset storing the RGChannelSet object

2. Create the dataframes Red and Green to store the red and green fluorescences respectively

3. Fill the following table: what are the Red and Green fluorescences for the address assigned to you? **Optional**: check in the manifest file if the address corresponds to a Type I or a Type II probe and, in case of Type I probe, report its color.

Sample	**Red fluor**	**Green fluor**	**Type**	**Color**

4. Create the object MSet.raw

5. Perform the following quality checks and provide a brief comment to each step:
   - QCplot
   - check the intensity of negative controls using minfi
   - calculate detection pValues; for each sample, how many probes have a detection p-value higher than the threshold assigned to each student?

Sample	**Failed positions**

6. Calculate raw beta and M values and plot the densities of mean methylation values, dividing the samples in DS and WT (*suggestion:*

subset the beta and M values matrixes in order to retain DS or WT subjects and apply the function mean to the 2 subsets).

7. Normalize the data using the function assigned to each student and compare raw data and normalized data. Produce a plot with 6 panels in which, for both raw and normalized data, you show the density plots of beta mean values according to the chemistry of the probes, the density plot of beta standard deviation values according to the chemistry of the probes and the boxplot of beta values. Provide a short comment regarding the changes you observe.

8. Perform a PCA on the beta matrix generated in step 7. Comment the plot.

9. Using the matrix of normalized beta values generated in step 7, identify differentially methylated probes between group DS and group WT using the functions assigned to each student. Note; it can take several minutes; if you encounter any problem you can run the differential methylated analysis only on a subset of probes (for example those on chromosome 1, 18 and 21)

10. Apply multiple test correction and set a significant threshold of 0.05. How many probes do you identify as differentially methylated considering nominal pValues? How many after Bonferroni correction? How many after BH correction?

11. Produce an heatmap of the top 100 differentially mehtylated probes

12. Produce a volcano plot and a Manhattan plot of the results of differential methylation analysis

Optional

As DS is caused by the trisomy of chromosome 21, try also to plot the density of the methylation values of the probes mapping on chromosome 21. Do you see a very clear difference between the samples? How many differentially methylated probes do you find on chromosome 21?