DNA Methylation Arrays
MSO Array
Illumina Methylation 450k Array

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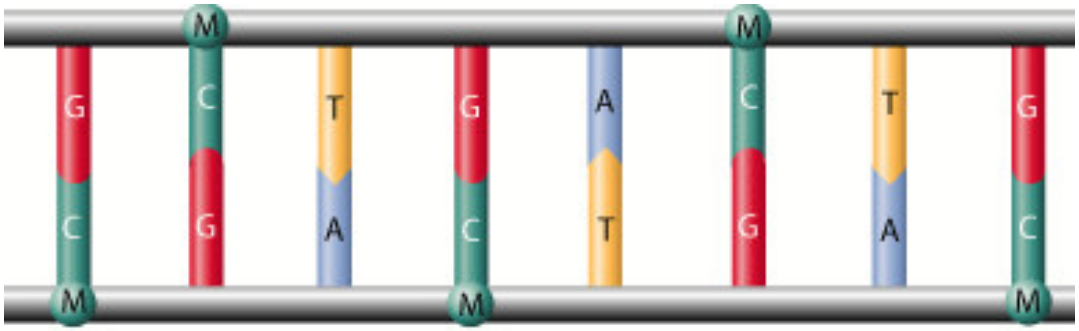
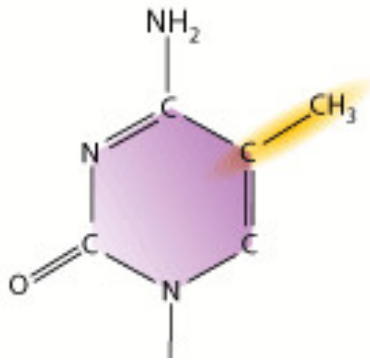
Overview

DNA Methylation

- **DNA Methylation:** Addition of methy group to cytosine, sometimes regulating the transcription
- **CpG island:** Part of genome where CG more frequently than average





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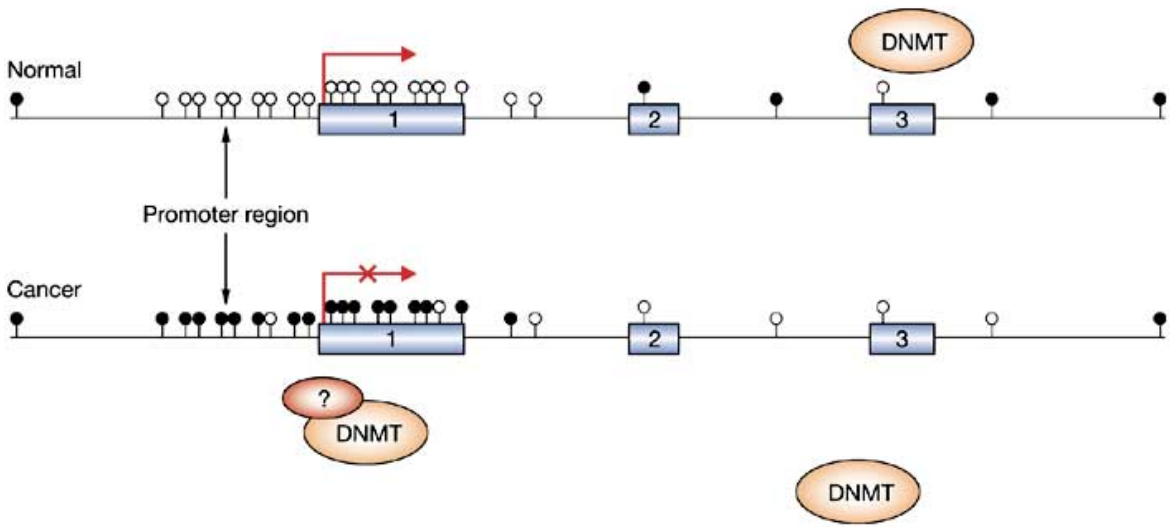
[1] http://extremelongevity.net/2012/06/12/dna-switches-discovered-to-decline-significantly-with-age/

Epigenetics

- **Epigenetics:** Heritable changes in gene action without changing DNA sequence
- Types:
  - DNA methylation
  - histone modification
  - ..
- *Why do we need this knowledge?*



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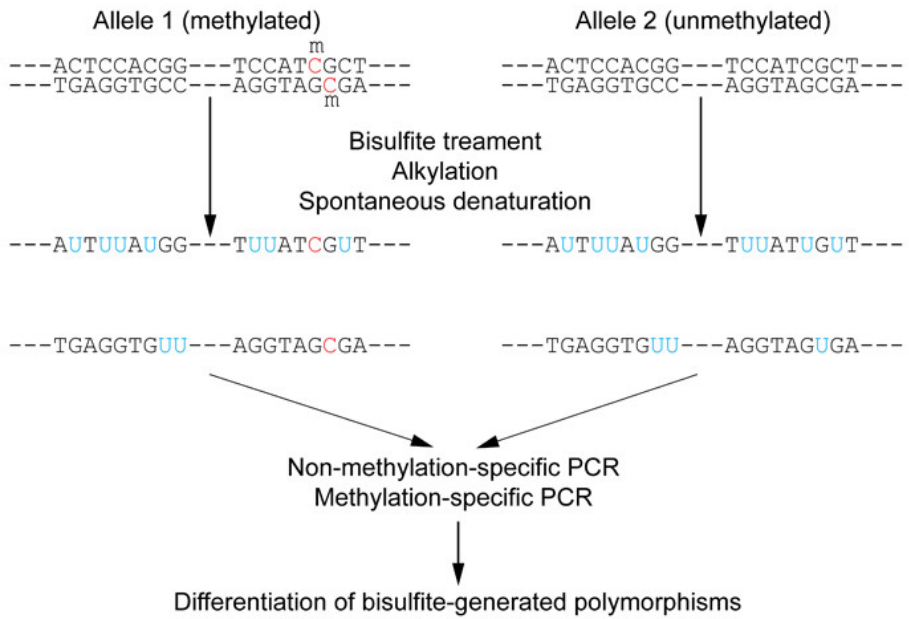
[2] http://www.nature.com/nrclinonc/journal/v2/n12s/fig_tab/ncponc0354_F2.html

DNA Bisulfite Treatment

Method

Chemical treatment of DNA to convert *unmethylated* cytosine to uracil and *methylated* cytosine to cytosine. So we can infer the methylation degree comparing thymine with cytosine



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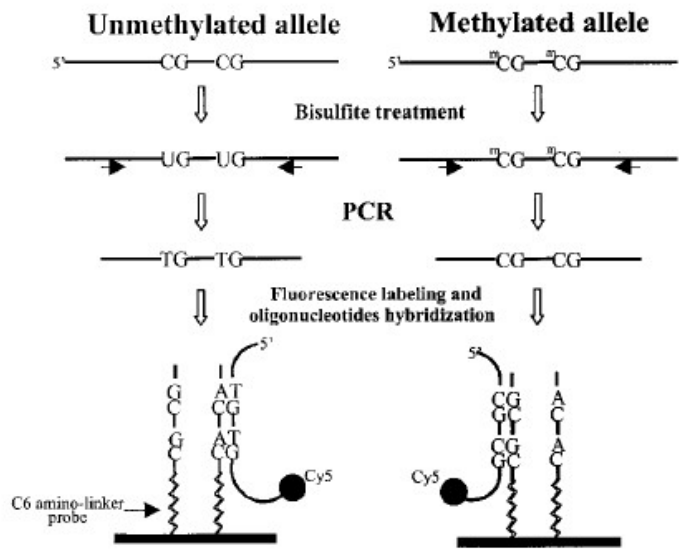
[3] http://en.wikipedia.org/wiki/File:Wiki_Bisulfite_sequencing_Figure_1_small.png

MSO Array (Method)

In this approach [Raad S. Gitan et al.], for first time they combine:

- Bisulfite modification
- PCR amplification
- Microarray technology

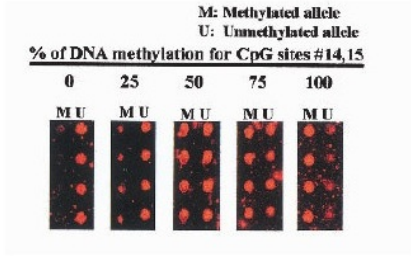
so they can infer methylation degree comparing thymine to cytosine in highthroughput(!)



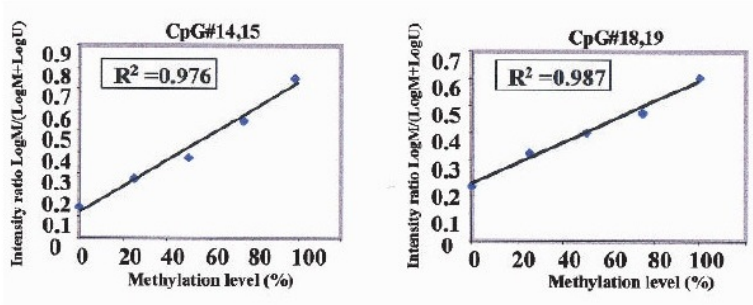
MSO Array (Test and control experiment)

Test

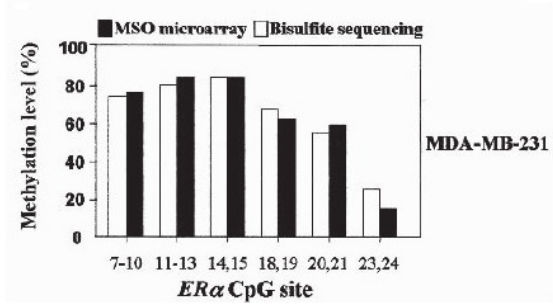
- 15 CpG sites in first exon of ERa gene
- For each site one methylated and one unmethylated probe
- Measure the intensity ratio $\frac{\log(M)}{\log(M)+\log(U)}$
- They show a linear relationship between the DNA methylation percentage and the intensity ratio



Test results



Control results



Control

Compare results with bisulfite sequencing

Methylation 450k Array (Introduction)

Microarray features:

- Investigation of $>$ 485,000 methylation sites for human genome
- Covering 99% of RefSeq genes with average of 17 CpG sites
- Capture of 96% of CpG islands

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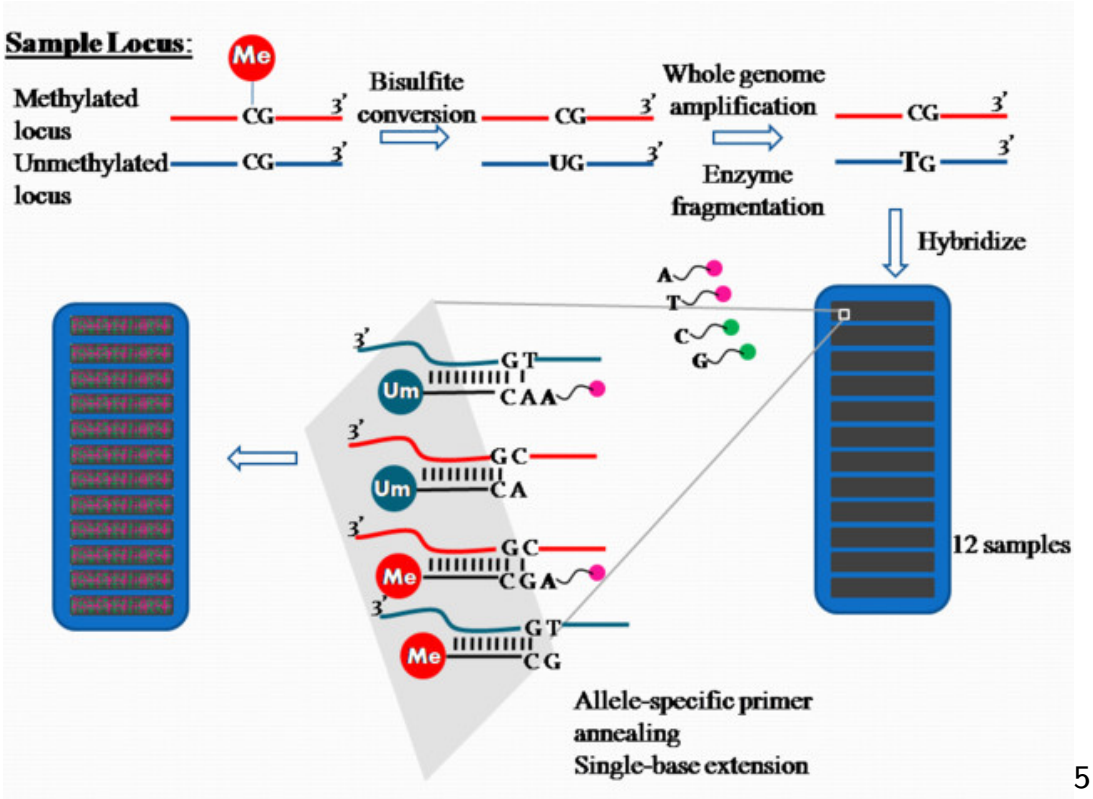
[4] http://www.illumina.com/products/methylation_450_beadchip_kits.ilmn

Methylation 450k Array (Method)

Goal:

Evaluate cytosine methylation by quantitative genotyping of single polymorphism C/T



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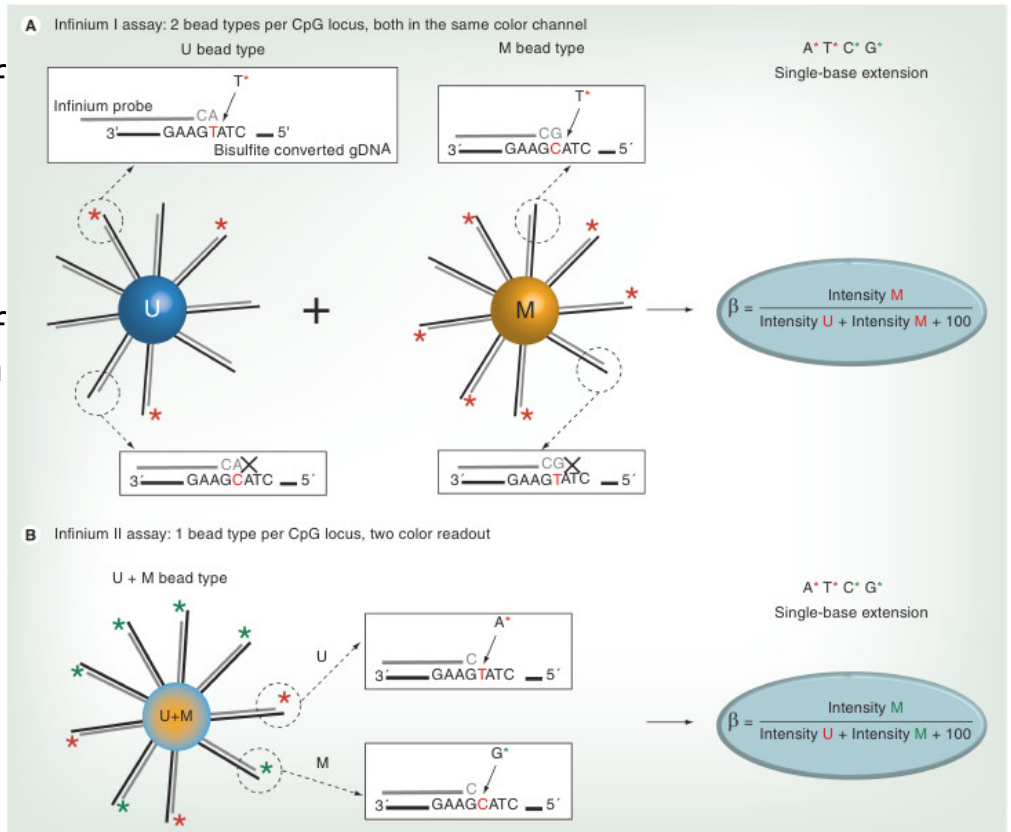
Quite similar with first approach..

[5] http://en.wikipedia.org/wiki/File:Illuminamethylationworkflow.png

Methylation 450k Array (Technology)

Infinum Assays:

- Infinium 1: assuming methylations of CpG depends on the methylation of each neighbors (50 bp)
- Infinium 1: 2 probes per CpG site + same color channel
- Infinium 2: assuming methylations of CpG independent on the methylation of each neighbors
- Infinium 2: 1 probe per CpG site + red and green channel



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[a] Figure 2 of [Sarah Dedeurwaerder et al.]

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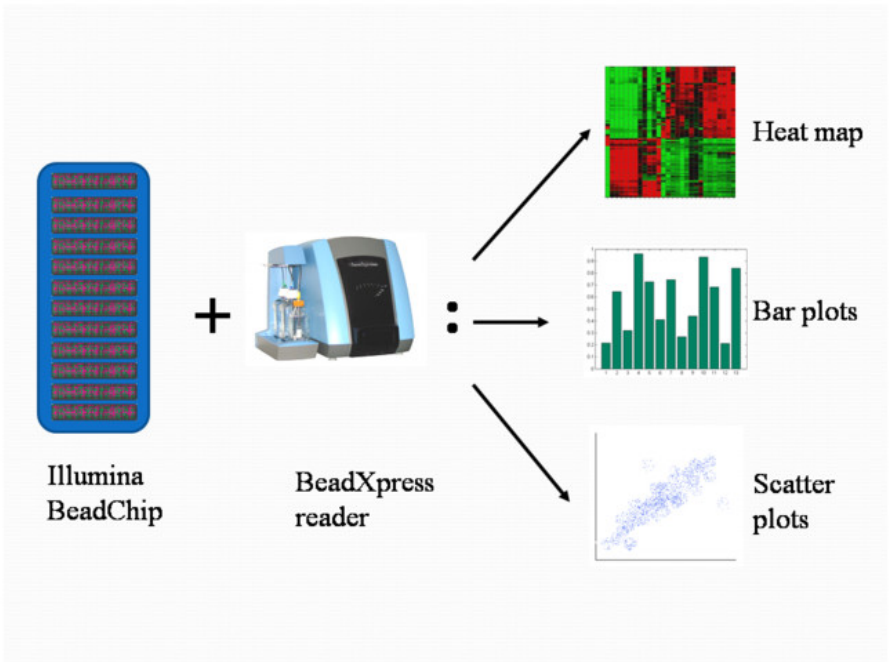
[6] http://res.illumina.com/documents/products/technotes/technote_hm450_data_analysis_optimization.pdf

Methylation 450k Array (Data Analysis)

After normalization of scanned images, visualization and analysis like:

- Correlation of methylation
- Visualization of each site relative methylation level
- Clustering of data for profiling



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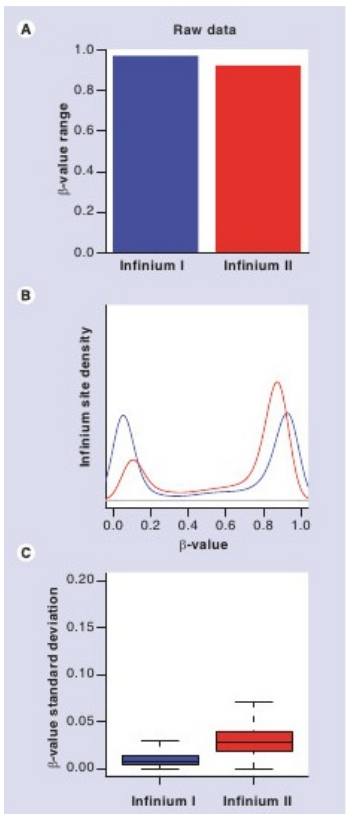
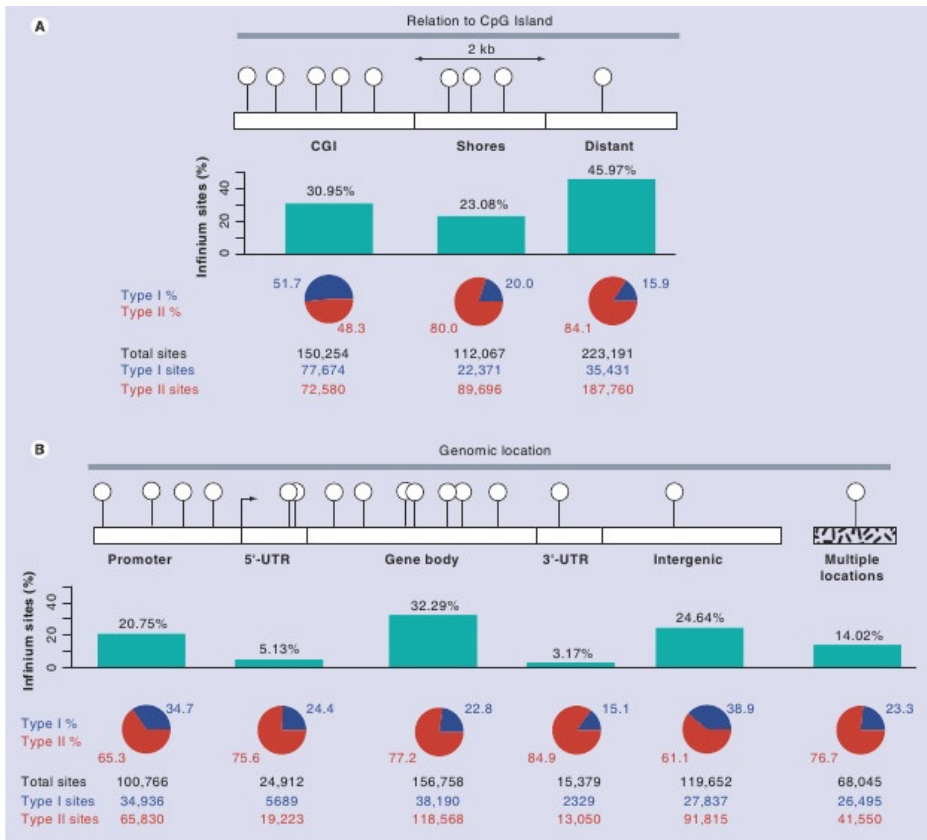
[7] http://en.wikipedia.org/wiki/File:Dataoutput.png

Assessment of Infinium 1 & 2 Assays

Using HCT116 WT and double-knockout and 16 breast tissue samples [Sarah Dedeurwaerder et al.]

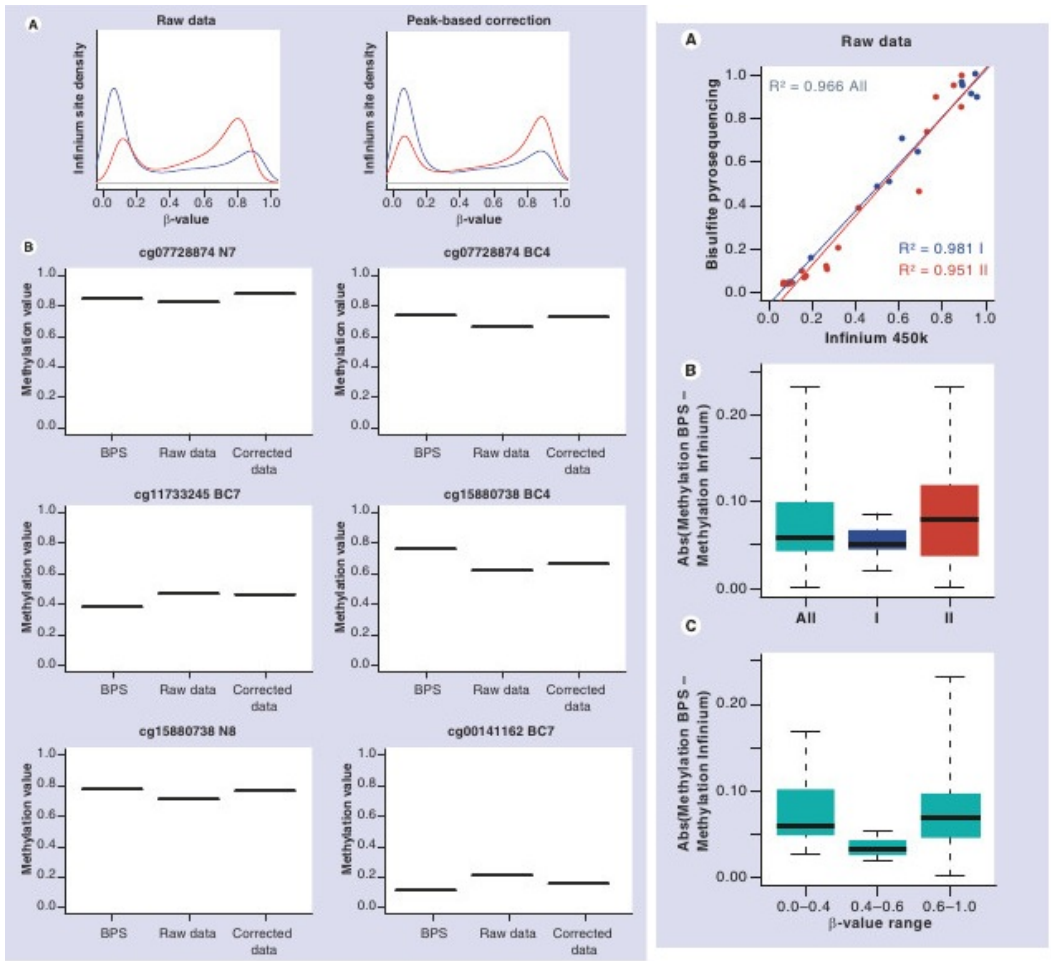
- Investigate the distribution and its variance for 2 assays
- Compare the ability of recovering methylation from different genomic areas





Test and Control of Infinium 1 & 2 Assays

- Test the assays on breast samples
- Control 450k results with bisulfite sequencing



Summary

Advantages:

- High throughput
- Covering most of RefSeq genes and CpG broad region
- High accurate and reproducible (after correction)

Disadvantages:

- Raw data **need** correction before downstream analysis

Leading to:

GWAS $\rightarrow$ EWAS!

References

Raad S. Gitan et al. (2002)

Methylation-Specific Oligonucleotide Microarray: A New Potential for High-Throughout Methylation Analysis.

Genome Research 12, 158 - 164.

Sarah Dedeurwaerder et al. (2011)

Evaluation of Infinum Methylation 450K technology

Epigenomics 3(6), 771-784.