CENTRE
NATIONAL DE
GENOTYPAGE

DNA methylation analysis as a tool to bridge the phenotype gap

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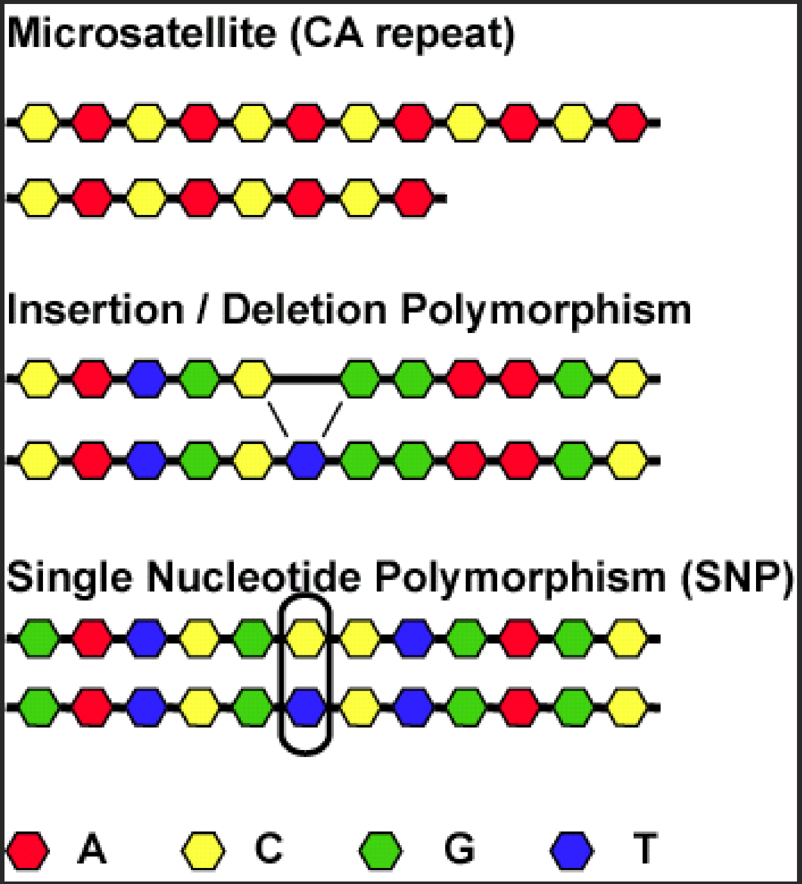
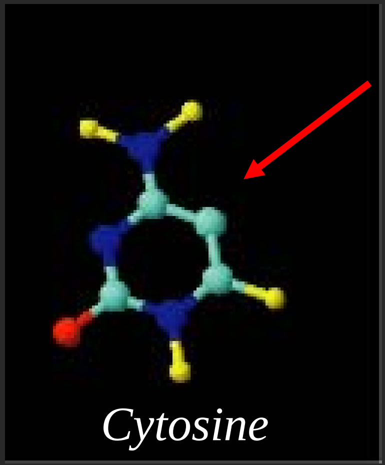
Evry, 12.5.2009

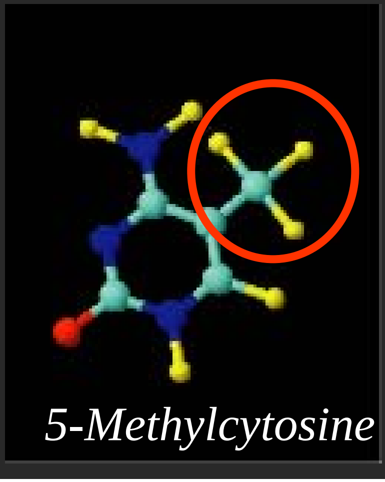
DNA based variations

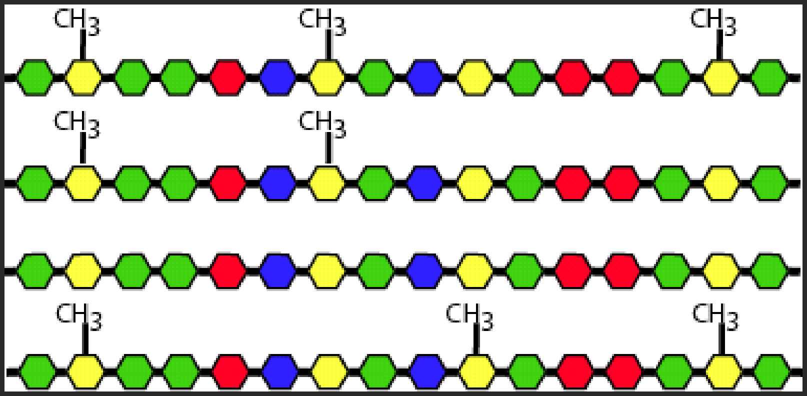
Genetic variations



Epigenetic variations


Cytosine


5-Methylcytosine



The case of the missing heritability

NATURE|Vol 456|6 November 2008

PERSONAL GENOMES **NEWS FEATURE**

Concordance rate of complex diseases in monozygotic twins

Breast cancer	15 %
Ulcerative colitis	20 %
Multiple sceloris	25-30 %
T2 diabetes	25-45 %
Schizophrenia	50 %
Alzheimer’s disease	40-70 %

Petronis TIGS 2001

Epigenetics

....is the study of a set of reversible heritable changes in gene function or other cell phenotypes that occur without a change in DNA sequence.

Epigenetics mediated by

- Polycomb / thritorax complexes
- snRNA (siRNA, miRNA and asRNA)
- Histone tail modifications
- Histone variants and nucleosomal occupancy
- DNA methylation

Environmental factors inducing DNA methylation changes

- Age
- Food (Agouti mouse model)
- Xenobiotic chemicals
- Endocrine disruptors (Vinclozin, Diethylstilberol)
- Maternal stress and nurturing
- Low dose radiation
- Smoking (lung cancer)

DNA methylation in disease

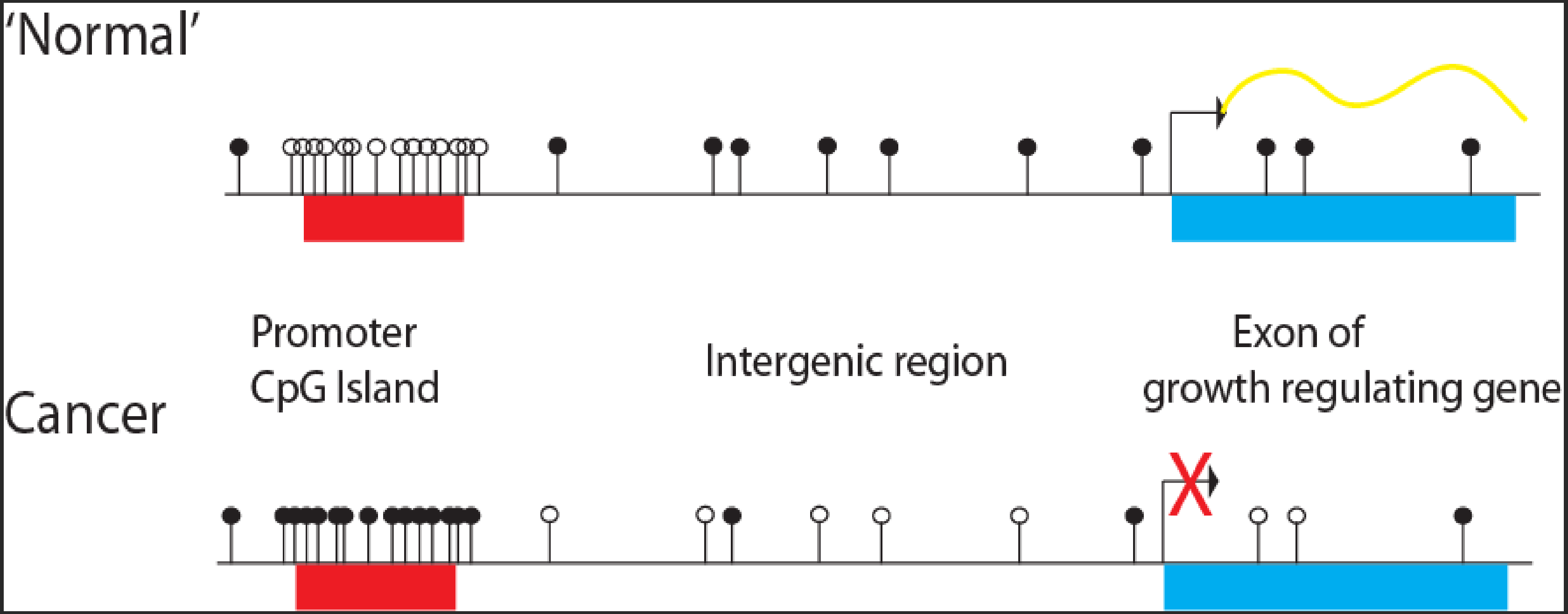
- Cancer

- Complex disorders
  - Type 2 Diabetes
  - Autoimmune diseases
  - Asthma ????

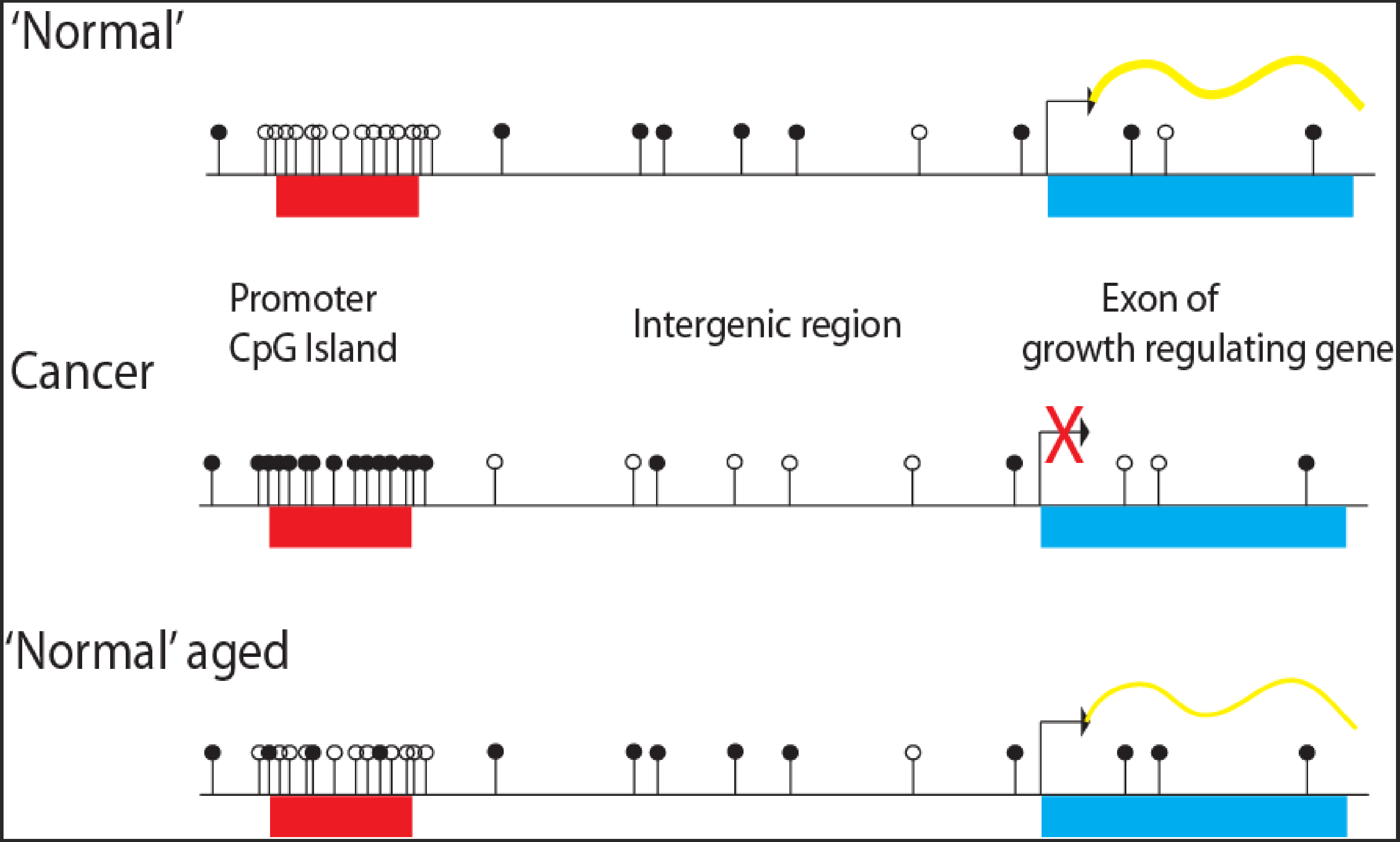
- Imprinting disorders / rare pediatric syndromes
  - Beckwith-Wiedemann syndrome
  - Silver Russel syndrome
  - Prader-Willi syndrome
  - Angelman syndrome

- Neurodevelopmental disorders
  - Fragile X
  - ICF
  - Rett syndrome

Aberrant methylation pattern



...but no black and white situation



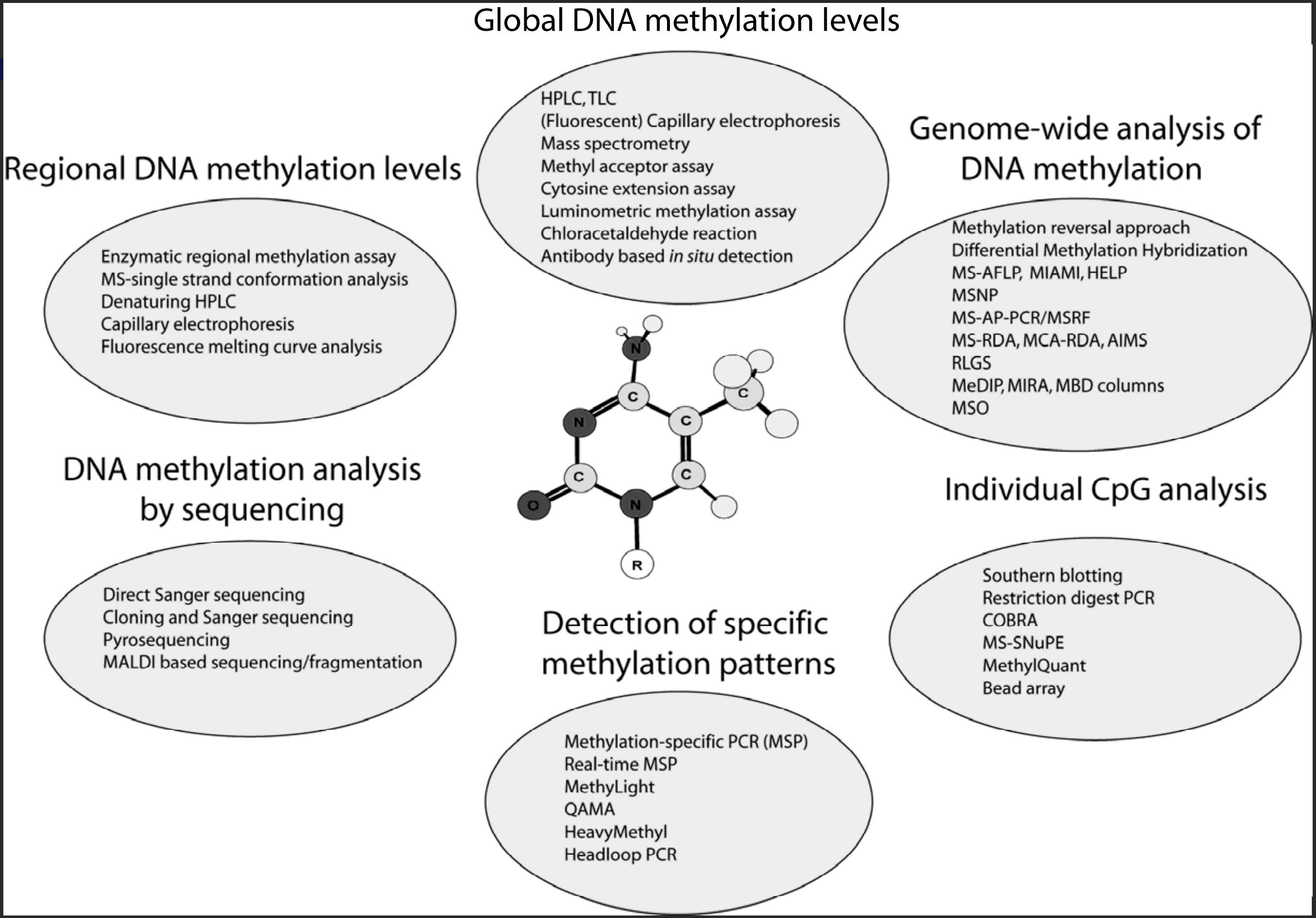
Why study DNA methylation

- Amplifiable
- Stable over time and over cell cycle
- Applicable to FFPE samples
- Positive signal
- Ratio C/T independent of starting material
- Confers an advantage to cells

DNA methylation and cancer

- Early diagnosis
- Cancer subtype classifcation
- Response to treatment
- Prognostic biomarker
- Monitoring of treatment

DNA methylation techniques



Global DNA methylation levels

- HPLC, TLC
- (Fluorescent) Capillary electrophoresis
- Mass spectrometry
- Methyl acceptor assay
- Cytosine extension assay
- Luminometric methylation assay
- Chloracetaldehyde reaction
- Antibody based *in situ* detection

Regional DNA methylation levels

- Enzymatic regional methylation assay
- MS-single strand conformation analysis
- Denaturing HPLC
- Capillary electrophoresis
- Fluorescence melting curve analysis

Genome-wide analysis of DNA methylation

- Methylation reversal approach
- Differential Methylation Hybridization
- MS-AFLP, MIAMI, HELP
- MSNP
- MS-AP-PCR/MSRF
- MS-RDA, MCA-RDA, AIMS
- RLGS
- MeDIP, MIRA, MBD columns
- MSO

DNA methylation analysis by sequencing

- Direct Sanger sequencing
- Cloning and Sanger sequencing
- Pyrosequencing
- MALDI based sequencing/fragmentation

Individual CpG analysis

- Southern blotting
- Restriction digest PCR
- COBRA
- MS-SNuPE
- MethylQuant
- Bead array

Detection of specific methylation patterns

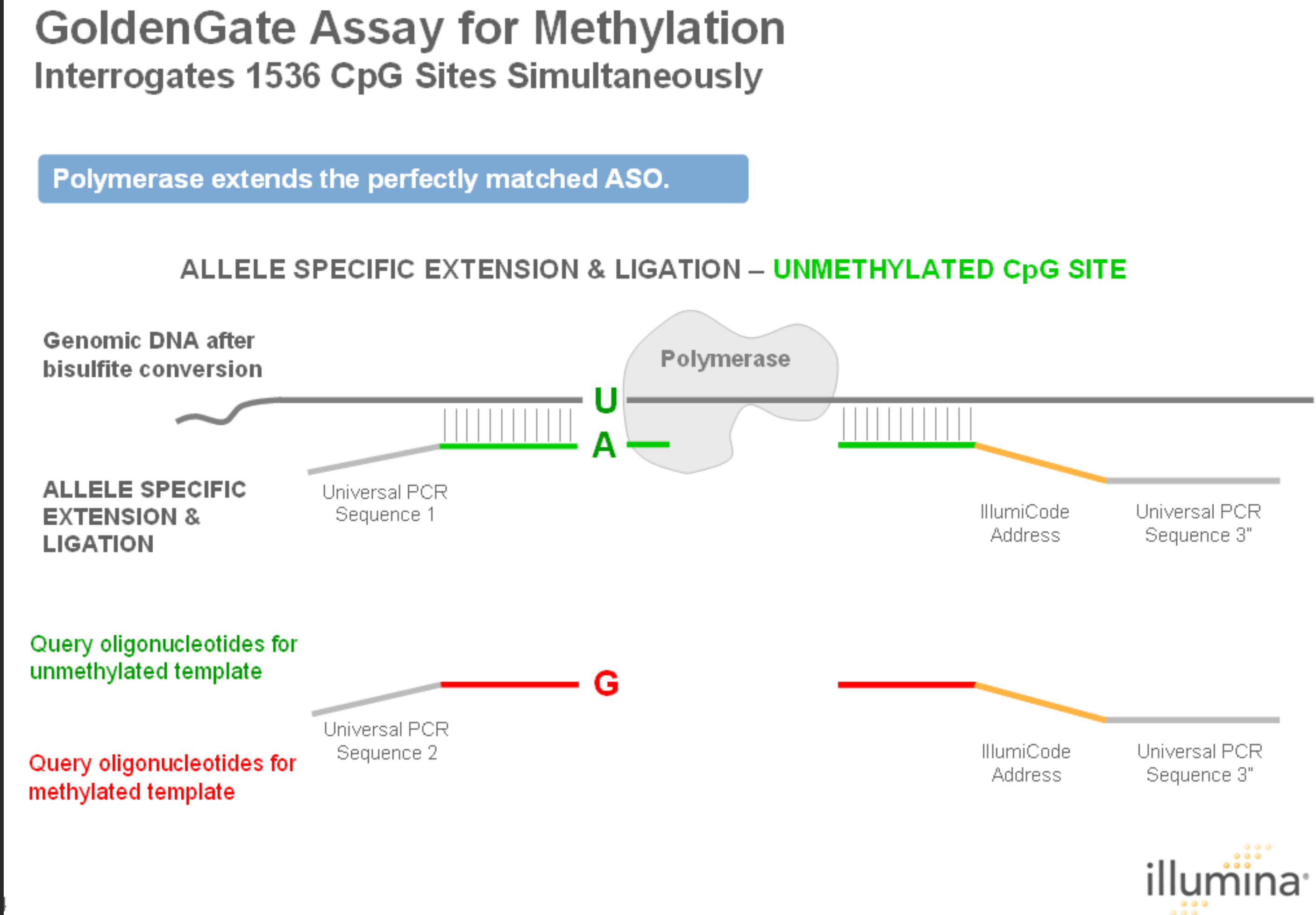
- Methylation-specific PCR (MSP)
- Real-time MSP
- MethyLight
- QAMA
- HeavyMethyl
- Headloop PCR

Tost, Epigenetics, 2008, Horizon Scientific Press

Platforms at CNG

- Analysis of gene-specific DNA methylation patterns:
  - Pyrosequencing
  - Real-Time PCR
  - High-resolution melting analysis
  - Mass spectrometry
- Genome-wide analysis of DNA methylation patterns:
  - NimbleGen
  - Illumina (Bead Array/Infinium)
  - Illumina/Solexa sequencing

Illumina Bead Array Methylation analysis



GoldenGate Assay for Methylation

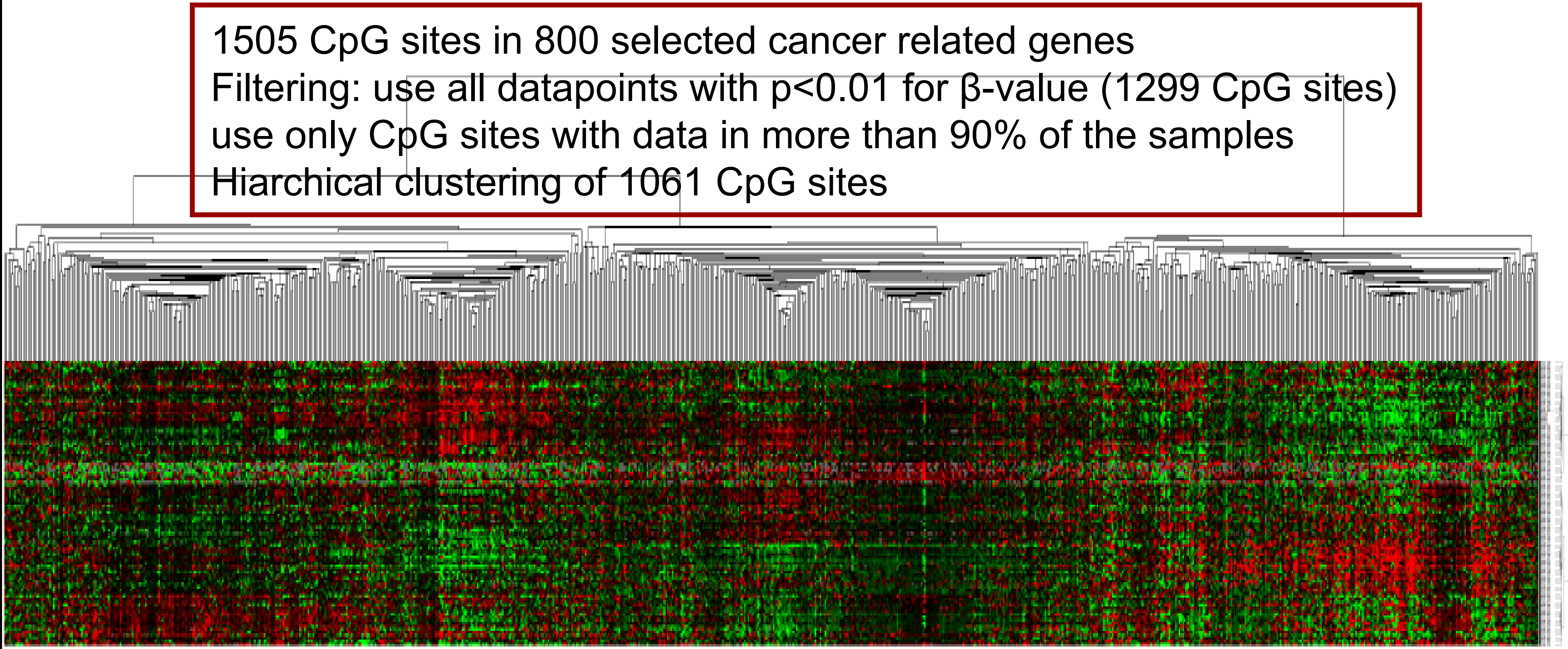
Interrogates 1536 CpG Sites Simultaneously

Polymerase extends the perfectly matched ASO.

ALLELE SPECIFIC EXTENSION & LIGATION – UNMETHYLATED CpG SITE

Illumina Golden Gate Methylation Cancer Panel

91 MicMa tumors
4 Normal breast tissue

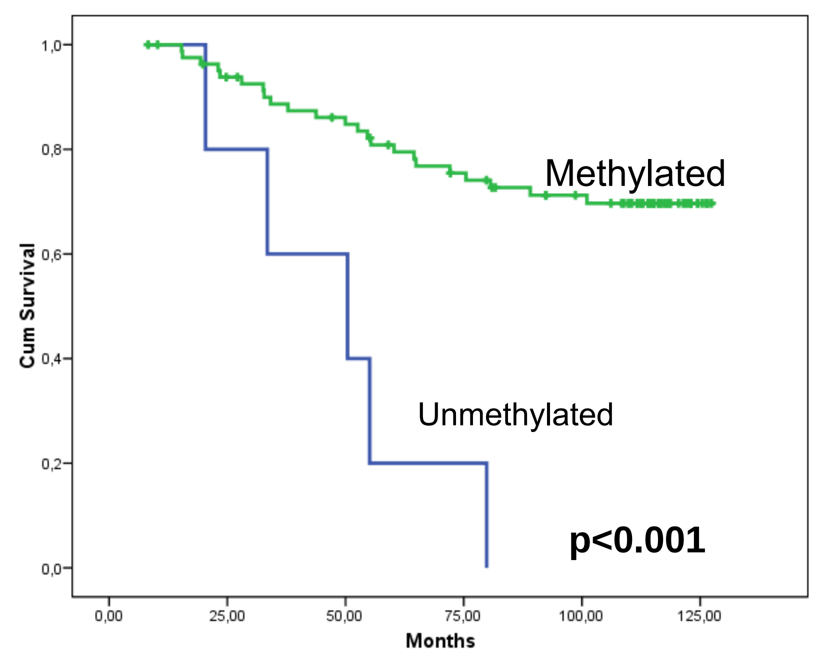


1505 CpG sites in 800 selected cancer related genes
Filtering: use all datapoints with $p<0.01$ for β-value (1299 CpG sites)
use only CpG sites with data in more than 90% of the samples
Hiarchical clustering of 1061 CpG sites

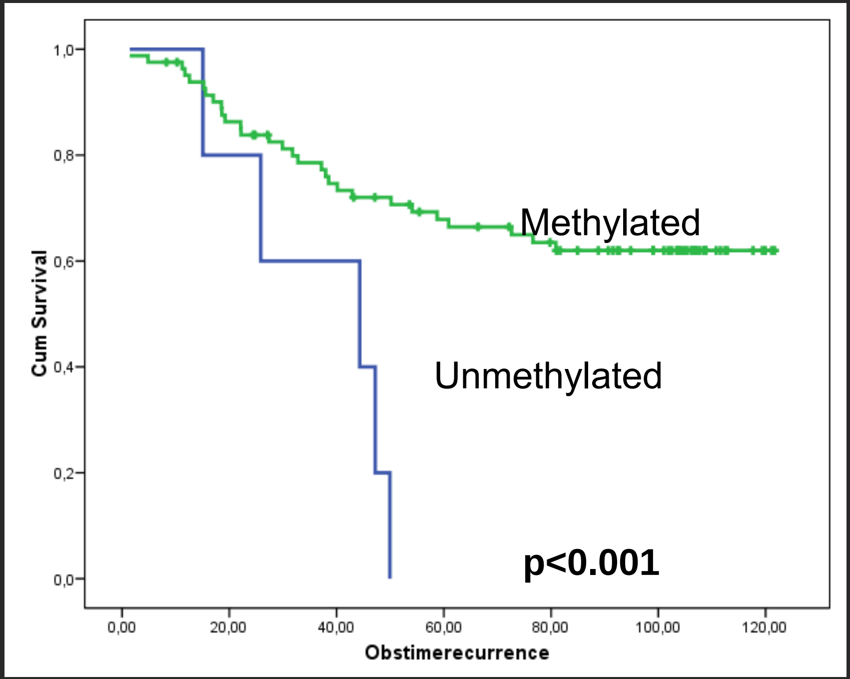
69 genes significantly associated with overall breast cancer survival using SAM

10 genes with p<0.001

Example 1, Overall survival



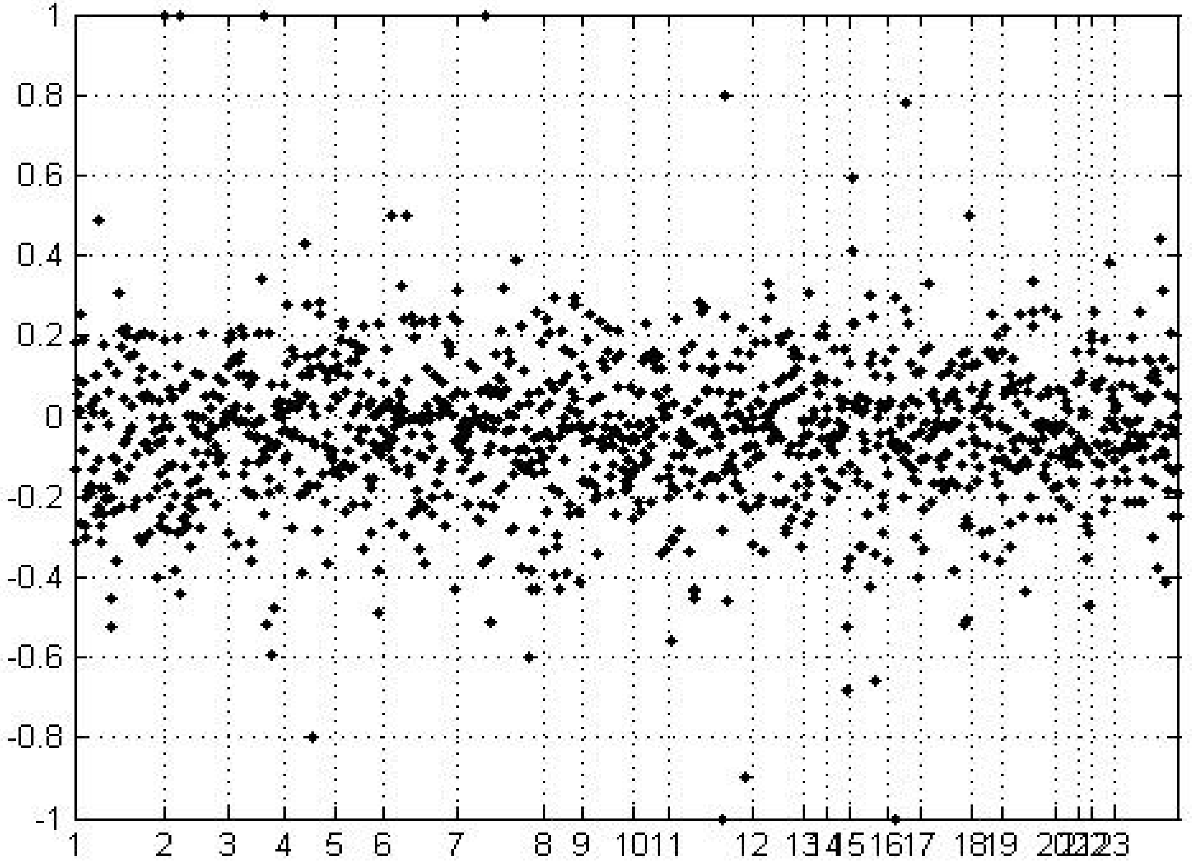
Example 2, Overall survival



Integrate methylation and mRNA expression data

Significant corrrelations between methylation and expression (*in cis*)

After correction for multiple testing (FDR <0.01)

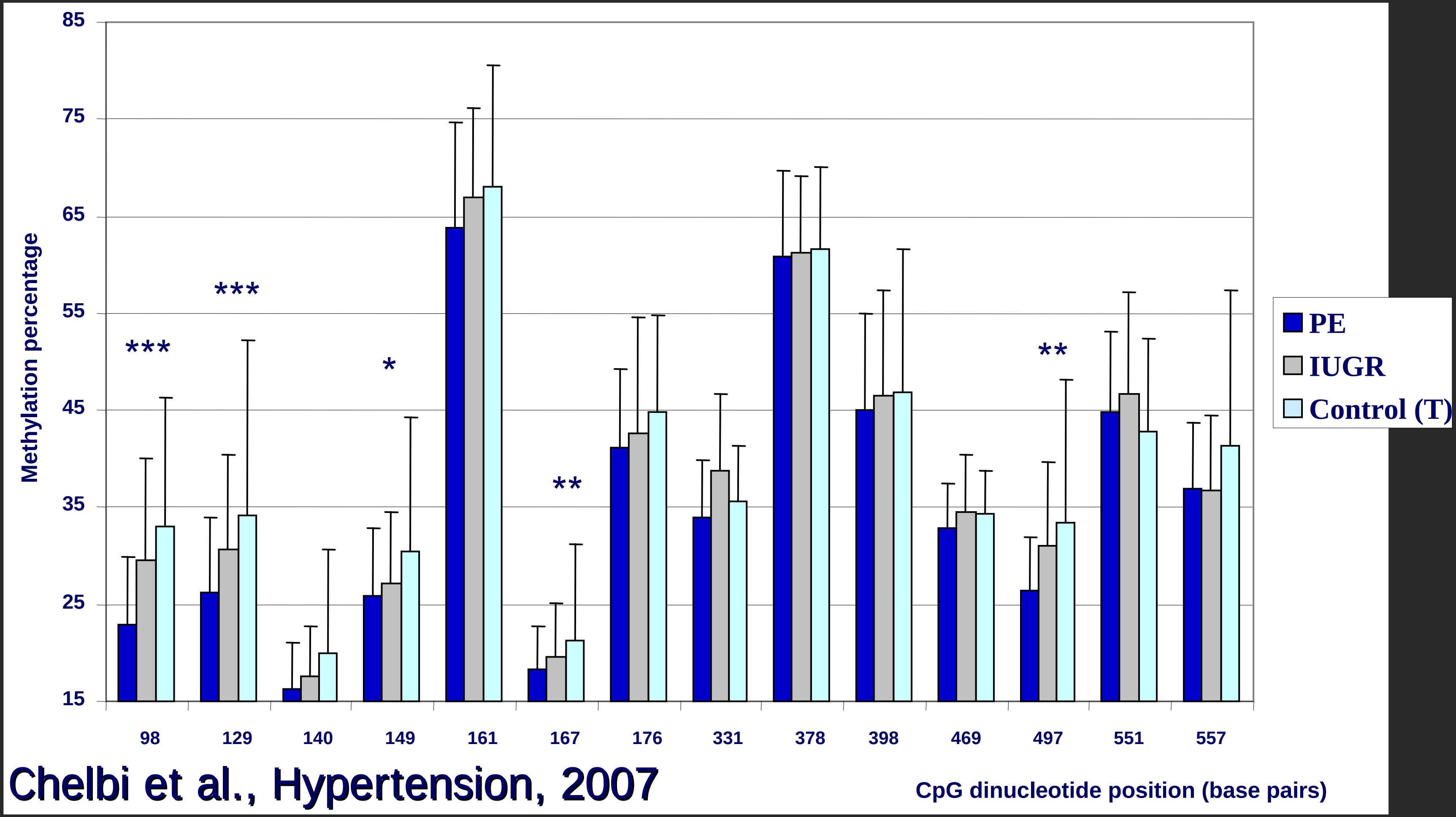


Epigenetics of complex disease

The beginning of an era....

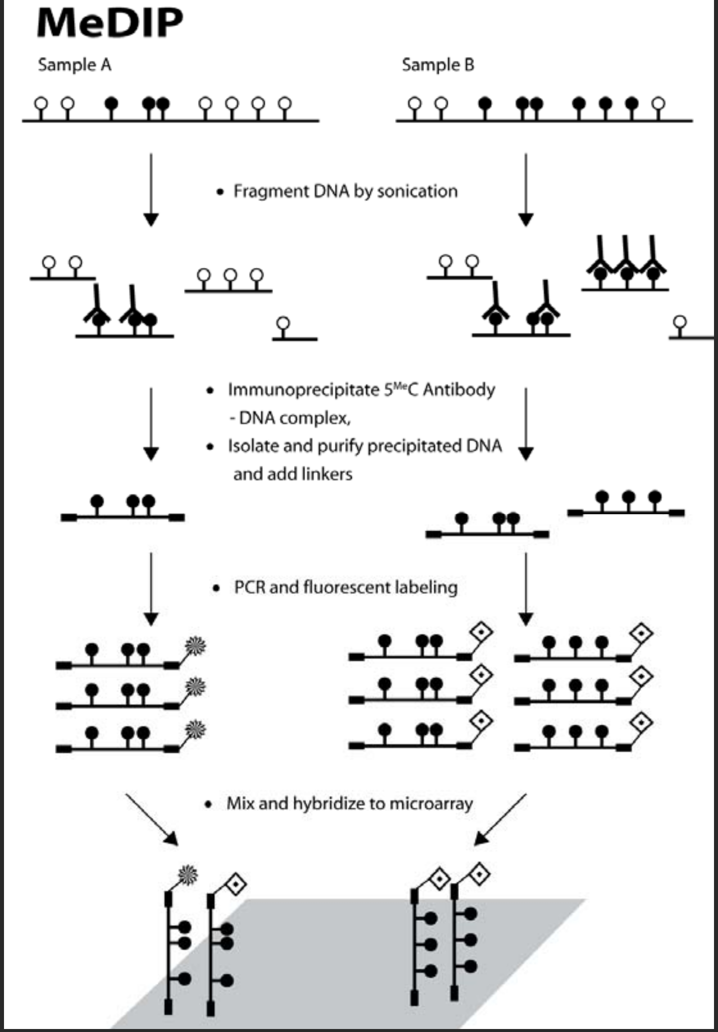
Epigenetics constitutes a new framework for the integration of previously unrelated molecular, epidemiological and clinical data

Placental pathologies: *SERPINA3* as a marker for pre-eclampsia

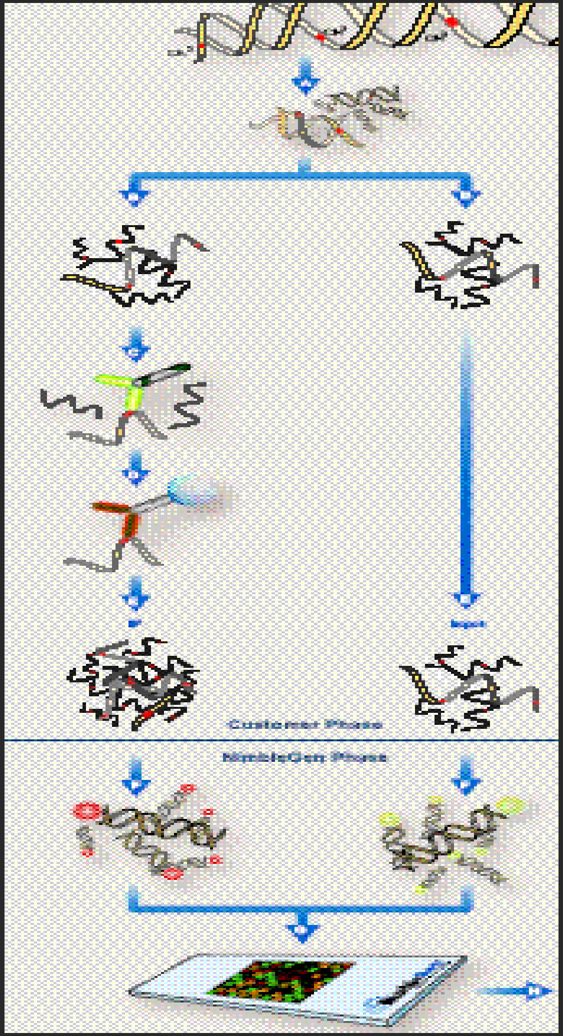


Chelbi et al., Hypertension, 2007

Methylated DNA immunoprecipitation (MeDIP)

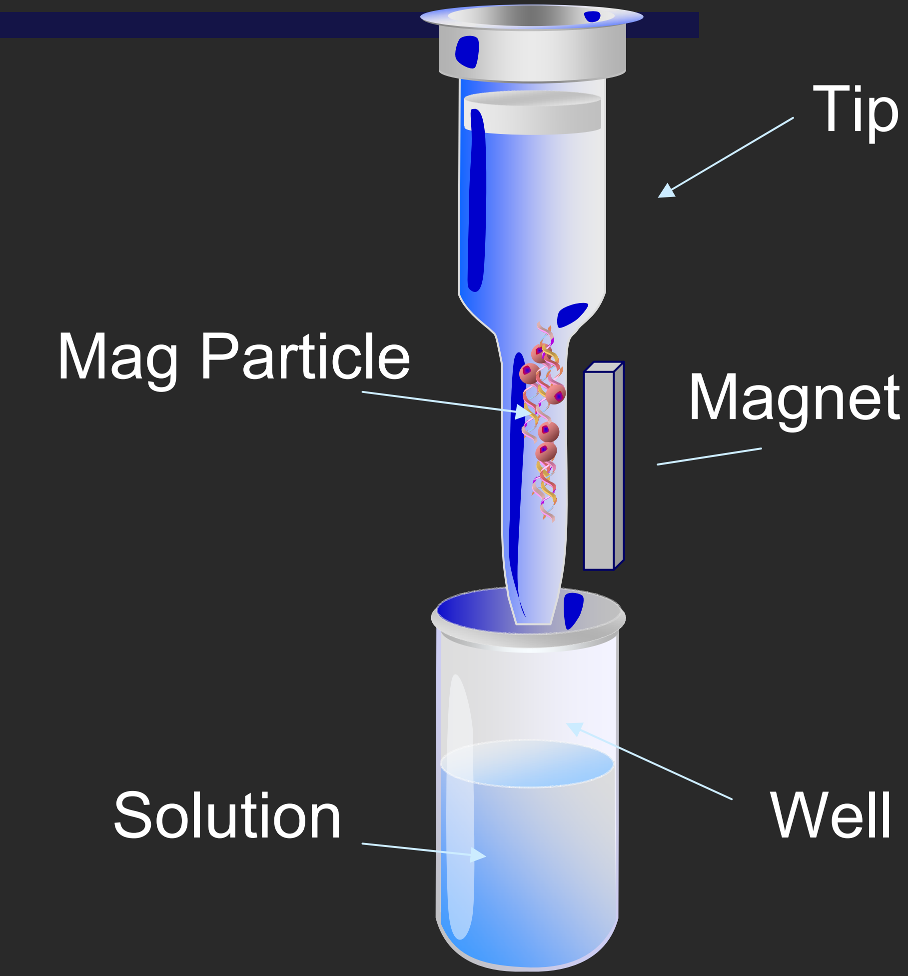


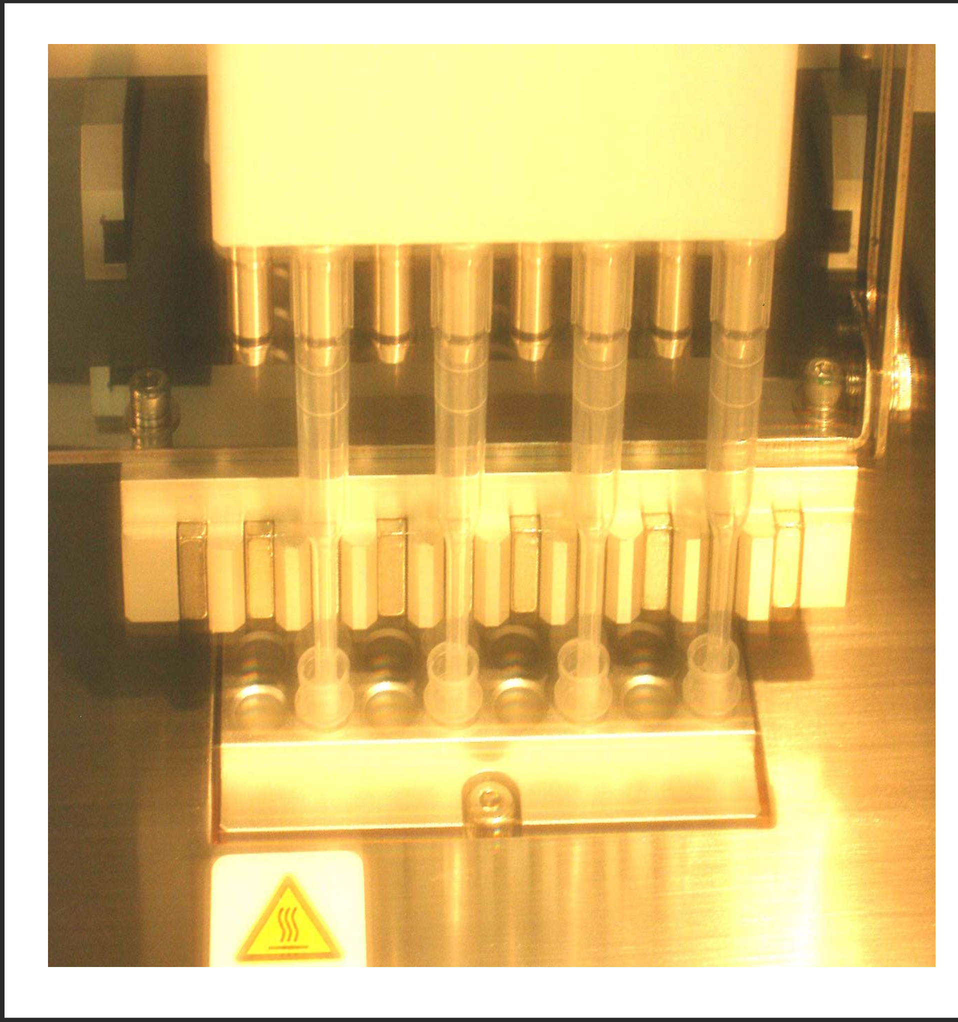
- Fragment DNA by sonication
- Immunoprecipitate $5^{Me}C$ Antibody - DNA complex,
- Isolate and purify precipitated DNA and add linkers
- PCR and fluorescent labeling
- Mix and hybridize to microarray



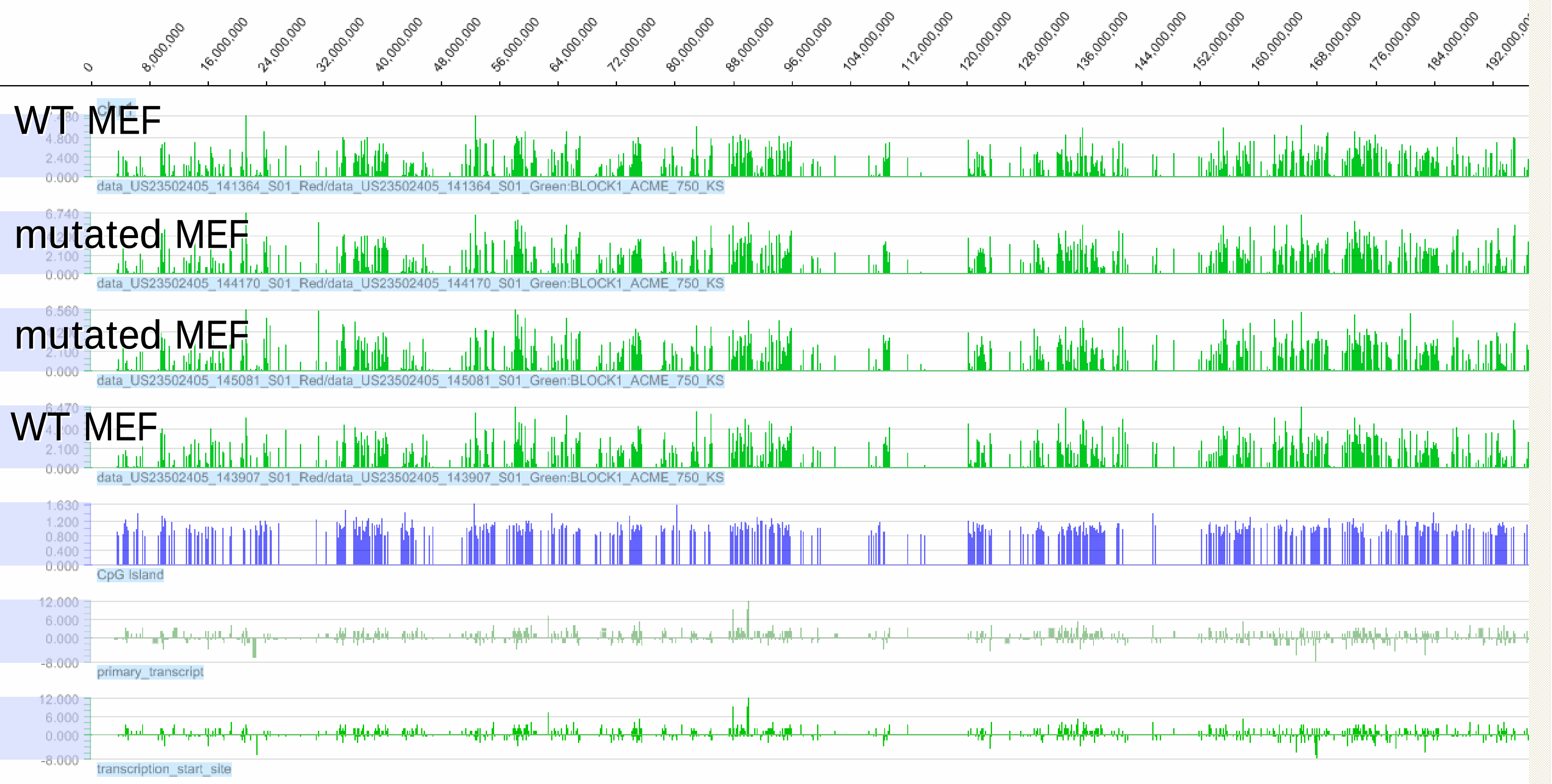
Automation of Immunoprecipitation

Magnet of the robot

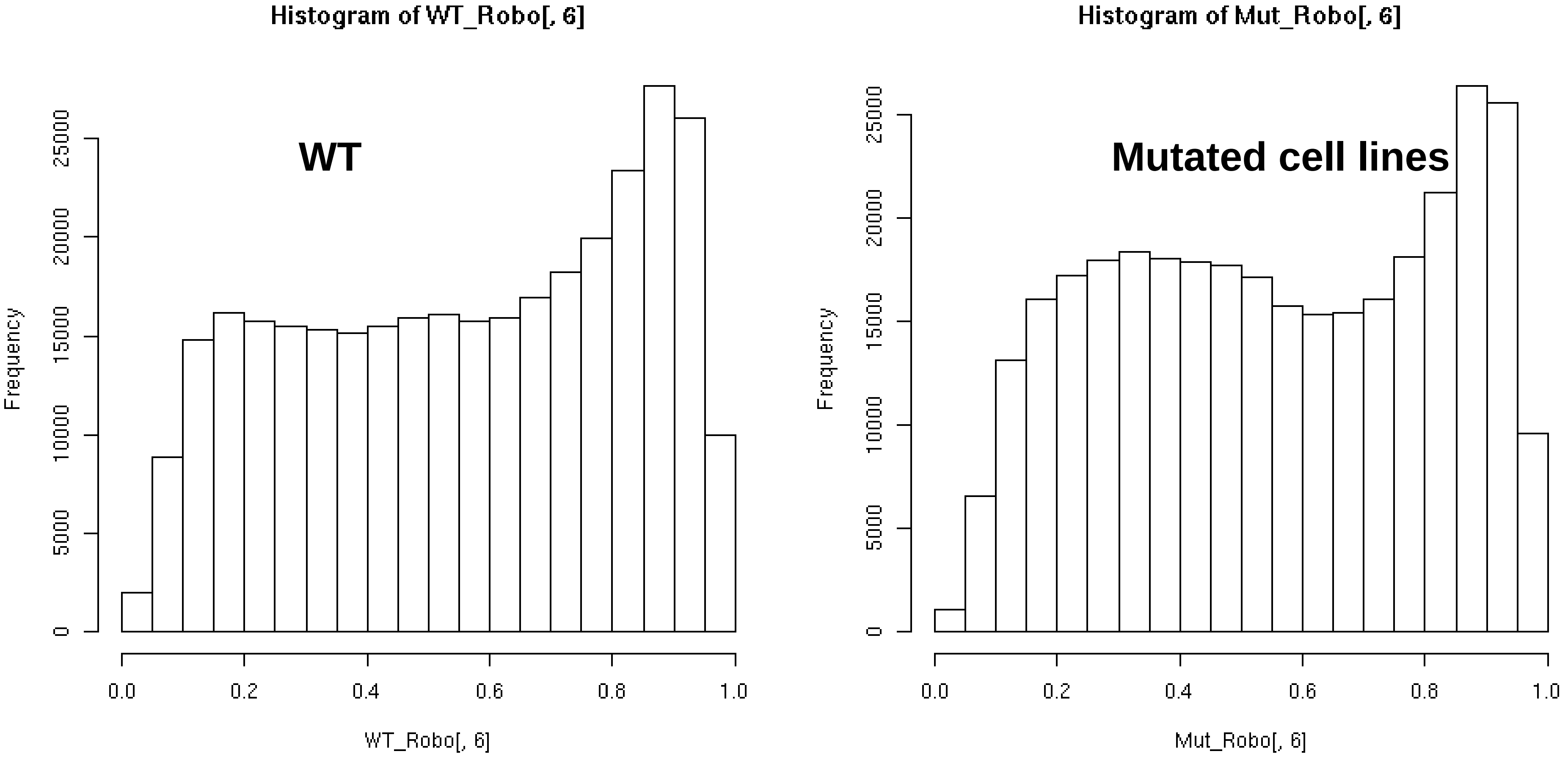




MeDIP – MEFs NimbleGen Promoter-CpG island tiling array (385k)

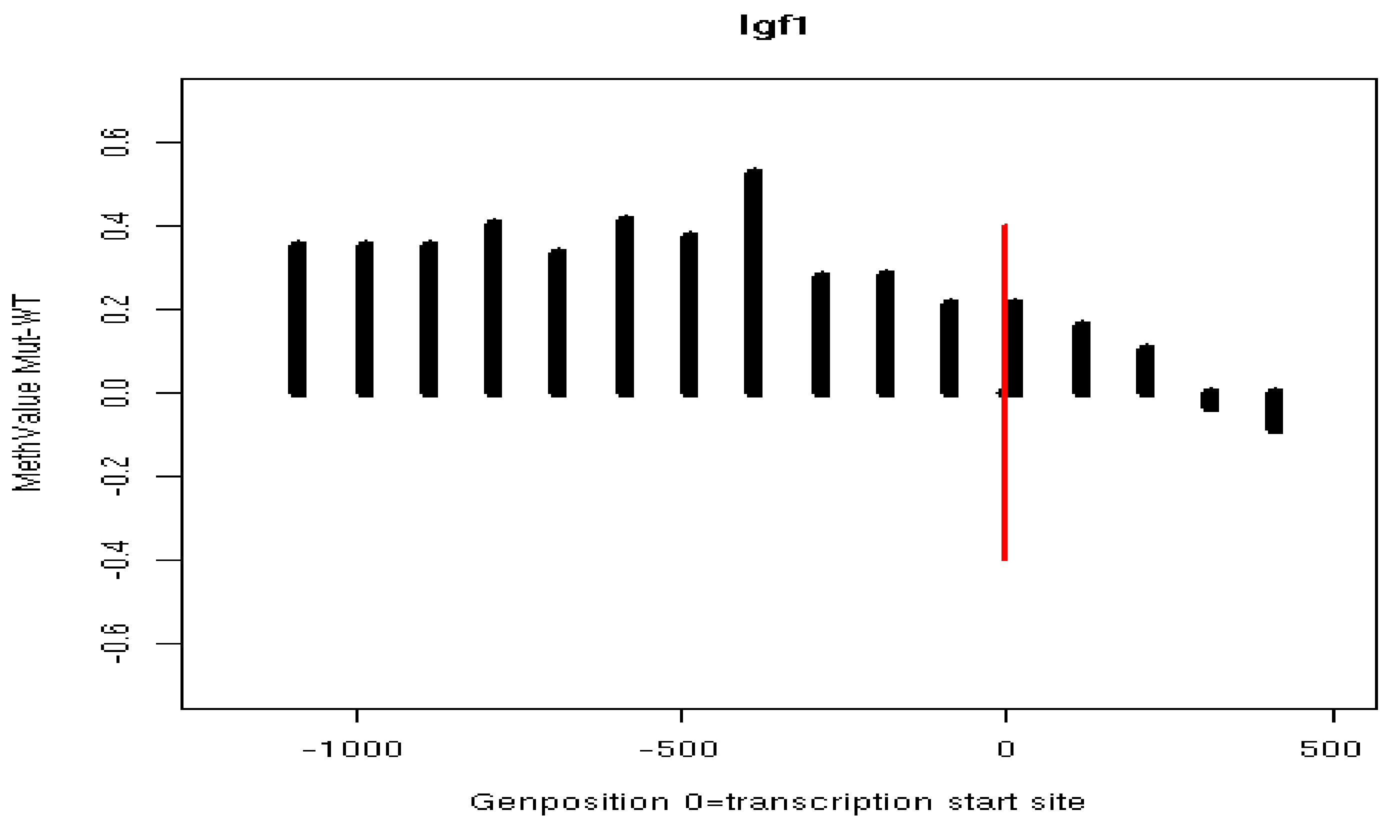


Batman-calibrated data using five experiments per phenotype



Down, TA *et al.* Nat. Biotech, 2008

Igf1



Functional importance validated by expression analysis (8.5 fold)

Acknowledgments

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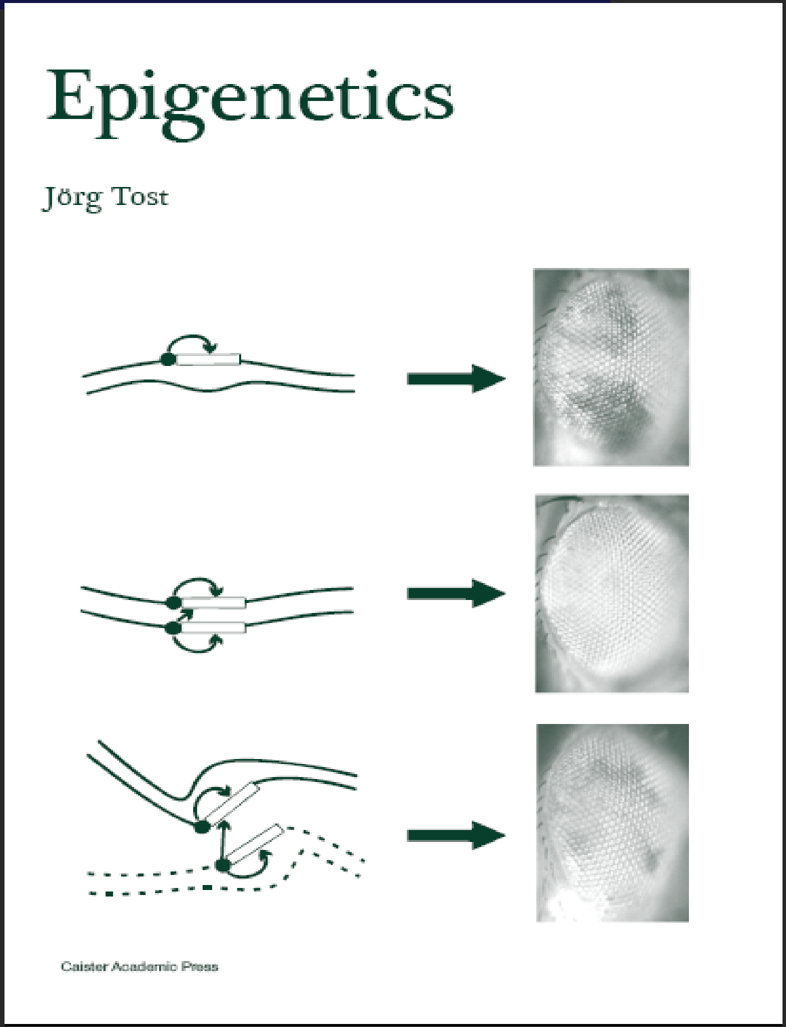
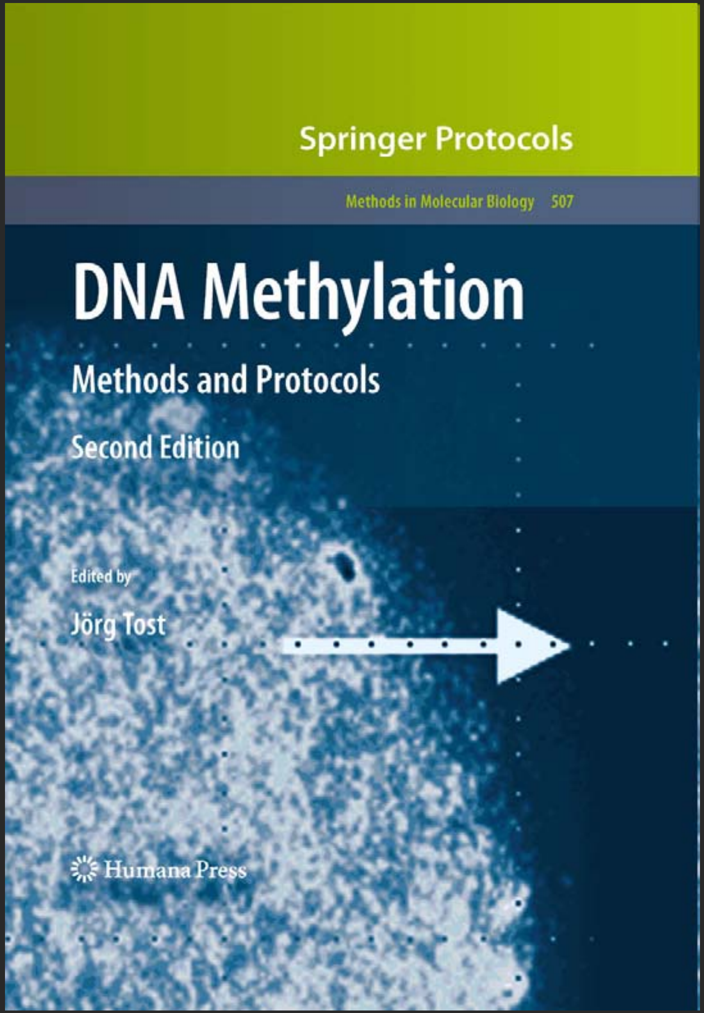
Further reading:



Epigenetics

Jörg Tost

Caister Academic Press



Springer Protocols

Methods in Molecular Biology 507

DNA Methylation

Methods and Protocols

Second Edition

Edited by

Jörg Tost

Humana Press