Outline

• Epigenetic phenomenon

• What is epigenetics?

• Epigenetic mechanisms

• Epigenome in health, in disease, and with environment

• Modern epigenomic technology and resources
The classics

Zinc, methionine, betaine, choline, folate, B₁₂

Morgan, Whitelaw, 1999
Waterland, Jirtle, 2004

Yellow Slightly
Mottled Heavily

Maternal genistein alters the fetal epigenome

Genistein supplementation and DNA methylation of the cytoband 10q23.3. The IAP retrotransposon (boxed with a gray outline) is represented upstream of the Agouti gene. Genistein supplementation increased DNA methylation of sites 4–9 within the transcription start site of the IAP murine retrotransposon upstream of the Agouti gene. A cryptic promoter (short arrow labeled A运势) drives ectopic agouti expression. CpG sites 1–9 are oriented in the 3´ to 5´ direction and are numbered and marked by gray boxes. The total number of offspring studied was from 15 unsupplemented litters (PS1A) of the murine agouti (AgoutiA运势) and genistein-supplemented (genistein-456789A运势=4运势4运势) littermates. Ends of the boxes indicate the interquartile range representing the 25th to 75th percentage distribution. Horizontal lines within each box indicate median; and dashed horizontal lines represent average percent methylation. The statistical significance of site 4 is an order of magnitude greater than that for sites 5–9. Moreover, the effect of IAP methylation on coat color was most pronounced at site 4 methylation principally mediates the offspring toward the pseudoagouti phenotype. Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C). Increased body weight is also positively correlated to ectopic agouti (methylated) (Morgan et al., 1999) (Figure 1B,C).
Conservation versus innovation – a complex model of evolution

Figure 5. The MT2B2-driven Cdk2ap1DN isoform is evolutionarily conserved in human (A and B) Preimplantation-specific Cdk2ap1DN isoforms are derived from species-specific promoters (A) but exhibit evolutionary conservation in protein sequences (B).

(A) Mouse Cdk2ap1DN originates from the MT2B2 promoter; human CDK2AP1DN originates from a promoter region containing an L2a and a Charlie4z hAT transposon element. Blue, canonical exons; red, alternative exons.

(B) Canonical Cdk2ap1 and Cdk2ap1DN isoforms are 97.4% and 98.8% identical, respectively, between mouse and human.

(C and D) Ectopic expression of CDK2AP1DN, but not CDK2AP1CAN, rescues defective cell proliferation in Cdk2ap1MT2B2/DMT2B2 morulae (C) and blastocysts (D), as demonstrated by BrdU incorporation and total cell number.

Figure 7. A model on the transposon-dependent gene regulation of Cdk2ap1 in mammalian preimplantation embryos
Conserved protein isoform, species-specific expression/phenotype

**Figure 7.** A model on the transposon-dependent gene regulation of Cdk2ap1 in mammalian preimplantation embryos

**QUANTIFICATION AND STATISTICAL ANALYSIS**

- Quantification and statistical analysis for experimental data
- Quantification of genes and retrotransposons
- Differential expression analysis on genes and retrotransposons
- Differential expression analysis on retrotransposon:gene junctions
- Differential expression of Cdk2ap1 isoforms

**ADDITIONAL RESOURCES**

Supplemental information can be found online at [https://doi.org/10.1016/j.cell.2021.09.021](https://doi.org/10.1016/j.cell.2021.09.021)

Modzelewski and Shao, Cell 2021
Epigenetic mutation upregulate oncogenes

Normal: 
- Epigenetic insult or misregulation
- No activity

Cancer: 
- Active cryptic promoter
- Epigenetic insult or misregulation

Oncogene or proto-oncogene

mRNA transcripts

Oncogene expression
50% tumors had at least one oncoexaptation

A total of 145 events involving 117 oncogenes across 4103 tumors (53%). On average, each event was in 49 samples with the highest occurring one in 901 samples.

Jang, Shah, et al, Nat Genetics, 2019
Oncoexaptation: from prognostic to cancer evolution

Jang, Shah, et al, Nat Genetics, 2019
Where is the Queen?
Workers are more methylated
Inhibition of Dnmt3 phenocopy Royal Jelly

A  Effect of Dnmt3 RNAi on larval development

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RNAi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workers</td>
<td>238</td>
<td>73</td>
</tr>
<tr>
<td>Queen-likes</td>
<td>74</td>
<td>188</td>
</tr>
</tbody>
</table>

In hive reared larvae, (whole body)

Worker larva: 58%
Queen larva: 48%
The King of Butterflies
The Monarch
Same Genome

Different Epigenome

Different Phenotype
What is Epigenetics/Epigenomics?

- A mitotically or meiotically heritable state of different gene activity and expression (phenotype) that is independent of differences in DNA sequence (genotype) – based on Conrad Waddington, 1942

- The sum of the alterations to the chromatin template that collectively establish and propagate different patterns of gene expression (transcription) and silencing from the same genome.

- Epigenetic changes influence the phenotype without altering the genotype.

- While epigenetics often refers to the study of single genes or sets of genes, epigenomics refers to more global analyses of epigenetic changes across the entire genome.
The Epigenome

The complete number, location, and types of epigenetic modifications that occur in a given cell.

The epigenome

<table>
<thead>
<tr>
<th>DNA methylation</th>
<th>DNA accessibility</th>
<th>Histone modifications</th>
<th>Polycomb complex</th>
</tr>
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<tbody>
<tr>
<td>DNA</td>
<td>DNA</td>
<td>Histone</td>
<td>DNA binding proteins RNA</td>
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<tr>
<td>methylation</td>
<td>accessibility</td>
<td>modifications</td>
<td>proteins</td>
</tr>
</tbody>
</table>
Epigenetic Landscape

Conrad Hal Waddington (1905–1975)
Developmental biologist
Paleontologist
Geneticist
Embryologist
Philosopher
Founder for systems biology
Inheritance, broad definition of epigenetics

A

Trans epigenetic signal

B

Cis epigenetic signal
Epigenetics Mechanisms

RNA Interference

Gene Expression

Histone Modifications
Nucleosome Positioning

DNA Methylation
Epigenetic mechanisms

• DNA methylation
  • Normal cells: role in gene expression and chromosome stability
  • Cancer cells: consequences of aberrant hypo- and hyper-methylation

• Histone modification
  • Normal cells – the histone code
  • Cancer cells – consequences of altered histone modifying enzymes

• Interaction between DNA methylation, histone modifications and small RNAs

• Cell/tissue type specificity

• Gene/Environment interaction, disease susceptibility
What is DNA Methylation?
The 5\textsuperscript{th} base

\[ \text{Cytosine} \leftrightarrow 5\text{-Methyl cytosine} \]

\[ \text{DNMT + SAM} \rightarrow \text{Methylation} \rightleftharpoons \text{Demethylation} \]

\[ \text{DNA demethylase (?)} \]
# History of DNA 5-mC

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
<th>Scientists</th>
</tr>
</thead>
<tbody>
<tr>
<td>1951</td>
<td>DNA 5-mC first reported</td>
<td>Wyatt</td>
</tr>
<tr>
<td>1968</td>
<td>Activity of a DNA 5-mC writer detected</td>
<td>Kalousek &amp; Morris</td>
</tr>
<tr>
<td>1975</td>
<td>Model for maintaining 5-mC across cell divisions proposed by 2 independent groups</td>
<td>Riggs Holliday &amp; Pugh</td>
</tr>
<tr>
<td>1980</td>
<td>DNA 5-mC is associated with gene repression using 5-azacytidine</td>
<td>Jones &amp; Taylor</td>
</tr>
<tr>
<td>1982</td>
<td><em>De novo</em> DNA methylation detected</td>
<td>Jahner et al.</td>
</tr>
<tr>
<td>1983</td>
<td>1st DNA 5-mC writer, Dnmt1, purified</td>
<td>Bestor &amp; Ingram</td>
</tr>
<tr>
<td>1987</td>
<td>DNA methylation of promoters associated with gene repression</td>
<td>Kovesdi et al.</td>
</tr>
<tr>
<td>1989</td>
<td>1st DNA 5-mC reader, MeCP1, discovered</td>
<td>Meehan et al.</td>
</tr>
<tr>
<td>1993</td>
<td>DNA 5-mC is associated with gene repression using dnmt1 knockout mice</td>
<td>Li et al.</td>
</tr>
<tr>
<td>1998</td>
<td>Function of Dnmt3a and Dnmt3b (<em>de novo</em> methylation of proviral DNA and repetitive sequences) determined</td>
<td>Okano et al.</td>
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<tr>
<td>1998</td>
<td>Additional DNA 5-mC readers, MeCP2, MBD1, MBD2 &amp; MBD4, discovered</td>
<td>Hendrich &amp; Bird</td>
</tr>
<tr>
<td>2002</td>
<td>Function of Dnmt3L (<em>de novo</em> methylation of maternal imprinted genes) determined</td>
<td>Hata et al.</td>
</tr>
<tr>
<td>2007</td>
<td>DNA methylation of gene bodies associated with gene expression</td>
<td>Hellman &amp; Chess</td>
</tr>
<tr>
<td>2009</td>
<td>DNA 5-mC erasers, TET1-3, discovered</td>
<td>Tahiliani et al.</td>
</tr>
</tbody>
</table>

Chen et al., *Cell Chemical Biology* (2016)
Two classes of DNA methyltransferases (DNMTs)

The diagram illustrates two classes of DNA methyltransferases:

**De novo**
- $\text{DNMT3A}$ and $\text{DNMT3B}$

**Replication**

**Maintenance**
- $\text{DNMT1}$

*Jones and Liang, 2009*
*Nature Review Genetics*
DNA Methylation is Heritable

DNA methyltransferases

5'-CpG-3' 3'-GpC-5'

DNMT: DNA methyltransferase
SAM: S-adenosyl-methionine
SAH: S-adenosyl-L-homocysteine

One-carbon donors

SAM
SAH
DNA demethylation pathways

**Passive**

![Diagram of passive DNA demethylation pathway](image)

**Active**

![Diagram of active DNA demethylation pathway](image)

BER = base excision repair pathway

DNA methylation is not distributed evenly in the mammalian genome

- In human somatic cells, 60%-80% of all CpGs (~1% of total DNA bases) are methylated

  - Most methylation is found in “repetitive” elements

- “CpG islands”, GC-rich regions that possess a high density of CpGs, remain methylation-free

  - The promoter regions of ~70% of genes are embedded in CpG islands
Function of DNA Methylation in Mammalian System

- Host defense – endogenous parasitic sequence (repeats, etc.)
- Imprinting
- X chromosome inactivation
- Heterochromatin maintenance, chromosome stability, telomere length
- Gene expression controls
Normal pattern and function of DNA methylation
DNA methylation changes during developmental epigenetic reprogramming
Normal Patterns of DNA Methylation

CpG islands and gene expression

Pericentromeric regions - stability

- Methylation
- Unmethylation
Mechanisms of gene silencing by methylation

**Direct mechanism:**

Inhibition of transcription factor binding (e.g. CTCF, UBF)

Not a universal mechanism since not all transcription factor binding sites contain CG dinucleotides

**Indirect mechanism:**

Inhibition mediated by methyl-CpG binding proteins MeCP1/ MeCP2

Recruitment of corepressor complexes including histone deacetylases (HDAC)

Change in chromatin conformation
DNA methylation in cancer
Point mutations
Deletions
CpG island hypermethylation
Chromosomal instability
Gene amplification
Genomic hypomethylation
Translocations
Copy number changes
Deletions
Gene amplification
Epigenetic
Tumorigenesis
Genetic
Tumorigenesis
Tumor Suppressor Gene Inactivation in Human Cancer

Knudsen's 2-hit hypothesis, 1971
Cancer is a genetic and epigenetic disease

Aberrant DNA Methylation in Cancer

CpG islands hypermethylation

Pericentromeric hypomethylation

Methylated CpG

Unmethylated CpG
Different regions of the genome are hypermethylated or hypomethylated in cancer.
"Epigenetic cancer therapy"

**normal**
- Tumor suppressor: ON

**tumor**
- Tumor suppressor: OFF

**Diagram:**
- Normal state: Tumor suppressor is ON.
- Tumor state: Tumor suppressor is turned OFF.
- Treatment steps:
  - Normal state: 5-azacytidine and Dnmt inhibitors turn ON tumor suppressor.
  - Tumor state: SAHA and HDAC inhibitors turn ON tumor suppressor.
DNA methylation and other diseases
Imprinting Diseases: 
Angelman and Prader-Willi Syndromes

Angelman Syndrome
• “Happy puppet”
• Severe mental retardation
• Absence of speech
• Happy disposition
• Excessive laughing
• Hyperactive, with jerky repetitive motions
• Red cheeks, large jaw and mouth

Prader Willi Syndrome
• Small hands and feet
• Underactive gonads, tiny external genitals
• Short stature
• Mentally retarded
• Slow-moving
• Compulsive overaters
• Obese
Imprinting Diseases:  
Angelman and Prader-Willi Syndromes

Chr15 deletion, ~4Mb

Loss of maternal contribution
UBE3A
Angelman Syndrome

Loss of paternal contribution
SNRPN
Prader-Willi Syndrome
Genomic imprinting

• Imprinting is unique to mammals and flowering plants. In mammals, about 1% of genes are imprinted.
• For imprinted genes, one allele is expressed and the other is silent.
• This is typically controlled epigenetically. The expressed alleles are unmethylated and associated with loosely packed chromatin.
• Imprinted genes bypass epigenetic reprogramming.
• Imprinting is required for normal development.
Why imprinting?

• The Genetic Conflict Hypothesis
  • Many imprinted genes are involved in growth and metabolism.
  • Paternal imprinting favors the production of larger offspring, and maternal imprinting favors smaller offspring

• Imprinted genes are under greater selective pressure.
  • No back up!
  • Any variation in single gene is expressed
  • Closely related species have different imprinting patterns
    • Liger and Tigon

• Imprinted genes are sensitive to environmental signals.
What we don’t know about imprinting

• What targets a gene for imprinting?
  • Why are some genes expressed from both alleles and other expressed from only one allele?

• How are the imprints imposed?
  • Do males and females have different mechanisms for imprinting genes?
Rett Syndrome

- X-linked trait
- Mainly girls affected
- Normal at birth
- At 6–18 months, begin losing purposeful movement
- Persistent wringing of hands
- Loss of speech, gait
- Mental retardation ensues

Rett’s is due to defect in MeCP2

- Methyl-cytosine binding protein 2 (MeCP2) binds methylated DNA and recruits binding of a histone deacetylase

- Normal role is tightening chromatin packing, leading to gene silencing
Mouse model for Rett’s

• Mice have a gene that is homologous to MeCP2

• Knocking out the gene in mouse gives a phenotype similar to human Rett’s

• This model offers good experimental system for studying the human disease

Male mice with MeCP2 knockout develop normally for a while (middle), but at 6 weeks of age, they begin to develop neurological symptoms, such as hindlimb clasping (right).
Why is the phenotype neurological?

• The phenotype suggests that the targets are genes in the brain

• Normal neurological differentiation requires silencing of MeCP2 gene target(s)

• The target(s) of MeCP2 are not known

LETTER

L1 retrotransposition in neurons is modulated by MeCP2

Alysson R. Muotri¹*, Maria C. N. Marchetto²*, Nicole G. Coufal², Ruth Oefner², Gene Yeo³, Kinichi Nakashima⁴ & Fred H. Gage²
DNA Methylation with age, environment
Differences in DNA methylation patterns between identical twins increase with aging.

Fraga et al, PNAS 102:10604, 2005
Maternal Bisphenol A (BPA) Exposure

- Monomer that makes up polycarbonate plastic
- Endocrine active compound
- Found in commonly used products
- Present in 95% of humans tested
- Some animal studies reveal negative health outcomes
Maternal BPA Exposure

![Graph showing percent of offspring with different coat patterns under control and BPA diets.](image)

- Control Diet (n=60)
- BPA Diet (n=73)

p-value = 0.0074
Maternal Genistein Supplementation

- Plant phytoestrogen
- Found in soy and soy products
- Selective estrogen receptor modulator
- Worldwide exposure varies by diet
- Chemoprevention and decreased adipocyte deposition
Maternal Genistein Supplementation

Maternal Nutritional Supplementation

- Control Diet (n=60)
- BPA Diet (n=73)
- Genistein Supplemented BPA Diet (n=39)

Percent of Offspring

- Yellow
- Slightly Mottled
- Mottled
- Heavily Mottled
- Pseudo-agouti

p-value=0.96 (control versus genistein supplementation)
Oxidative derivatives of DNA 5-mC

5-hydroxymethylcytosine (5-hmC)  
5-formylcytosine (5-fC)  
5-carboxylcytosine (5-fC)
Many unanswered questions remain

• Who are the 5-hmC readers?

• How is 5-hmC maintained during DNA replication?

• What are the physiological context and relative importance of the various pathways involving 5hmC?

• How does 5-hmC affect transcriptional regulation?

• What are the dynamics of 5-hmC during development?

• What role does 5-hmC play in cancer?
DNA methylation + Histone modification → Chromatin
Chromatin: DNA plus protein in cells with nuclei

- 2 each of histones: H2A, H2B, H3 and H4

Nucleosome

146 bp of DNA
The Nucleosome core particle

A
Post-translational Histone Modifications

H3 tail Modifications:

- Acetylation
- Methylation

Active

HDACs
KMTases
HATs

Repressive

histone H3
Epigenome provides an annotation of the genome
Chromatin modifications demarcate functional elements in the genome

Zhou, Goren and Bernstein, Nature Rev Genetics, 2011
Chromatin modification patterns reveal various genomic features.

Rivera & Ren, Cell 2013

**Table 3. Distinctive Chromatin Features of Genomic Elements**

<table>
<thead>
<tr>
<th>Functional Annotation</th>
<th>Histone Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoters</td>
<td>H3K4me3</td>
</tr>
<tr>
<td>Bivalent/Poised Promoter</td>
<td>H3K4me3/H3K27me3</td>
</tr>
<tr>
<td>Transcribed Gene Body</td>
<td>H3K36me3</td>
</tr>
<tr>
<td>Enhancer (both active and poised)</td>
<td>H3K4me1</td>
</tr>
<tr>
<td>Poised Developmental Enhancer</td>
<td>H3K4me1/H3K27me3</td>
</tr>
<tr>
<td>Active Enhancer</td>
<td>H3K4me1/H3K27ac</td>
</tr>
<tr>
<td>Polycomb Repressed Regions</td>
<td>H3K27me3</td>
</tr>
<tr>
<td>Heterochromatin</td>
<td>H3K9me3</td>
</tr>
</tbody>
</table>
Histone Modifications in Relation to Gene Transcription

- Bisulfite-Seq
- H3K27ac
- H3K4me1
- H3K4me3
- H3K36me3
- H3K27me3
- H3K9me3

RefSeq genes: SRPK1, SLC26A8, MAPK14
Predicting non-coding RNA?

• From sequence?
  • Not clear which properties can be exploited
  • Sequence features such as promoters are too weak

• Histone modifications + conservation worked
Figure 1 | Intergenic K4–K36 domains produce multi-exonic RNAs.
How to detect epigenetic marks?
Restriction Landmark Genome Scanning (RLGS)

Unmethylated

- NotI digest

Partial Methylation

- NotI digest

Homozygous Methylation

- NotI digest

EcoRV, 1-D, HinfI, 2-D
Scaling to high coverage, high resolution

- Enrichment based methods
  - MeDIP-seq
  - MBD-seq/MethylMiner

- Restriction enzyme based methods
  - MRE-seq
  - HELP, Methyl-MAPS, Methyl-seq

- Bisulfite based methods
  - MethylC-seq
  - RRBS, bisulfite padlock

- Direct reading of modified nucleotides
  - SMRT
  - Nanopore sequencing
Enriching for methylated DNA targets
MeDIP-seq and MBD-seq

Me
CCGG
GGCC

Sonicate gDNA
Size selection, end repair
Adapter ligation
Denaturation

Me
Me
Me

IP

Size selection

Me
Me
Me
Typical MeDIP data on a genome browser

5' CpG islands are unmethylated

3' CpG island is partially methylated
Taking advantage of methylation dependent restriction enzymes
MRE-seq

Digest gDNA (here with Hpa II)

Combine parallel digest
Size selection, end repair
Adapter ligation
Size selection
Typical MeDIP/MRE data on a genome browser

- **Methylated**
- **Unmethylated**

5’ CpG islands are unmethylated

3’ CpG island is partially methylated
The image depicts a graphical representation of the MLH1 promoter region on chromosome 3. The diagram shows CpG islands and CpG sites within a 5 kb interval, marked by positions ranging from 37033000 to 37041000. The text highlights different types of MLH1 promoter modifications:

- **Normal**
- **Type II**
- **Type I**

The diagram includes various lines representing different densities and modifications, such as Endo Normal Medip Density, Endo Normal MRE CpG, Endo 1099 Medip Density, Endo 1099 MRE CpG, and so on, each differentiated by type and modification level.
Allele-specific methylation at imprinted genes

Chr 7:
SNURF/SNURP
MeDIP-seq
MRE-seq
imprinted

Chr 7:
MEST
MeDIP-seq
MRE-seq
imprinted

Chr 18:
SMAD4
MeDIP-seq
MRE-seq
not imprinted

DMR
Gold standard: bisulfite sequencing
Bisulfite sequencing

![Chemical structures of cytosine, cytosinesulfonate, uracilsulfonate, and uracil.](image)

**Bisulfite Treatment**

- ATTACGATCGATAT-3′
  - ATTA<sup>m</sup>GCTAGCTATA-5′

**PCR**

- ATTACGATGATAT-3′
  - Original top (OT)
  - TAATGCTAATTATA-5′
  - Complementary to original top (CTOT)

- ATTACGATCAATAT-3′
  - Complementary to original bottom (CTOB)
  - TAATGCTAGTTATA-5′
  - Original bottom (OB)

_Grehl et al., Epigenomes, 2018_
Sequencing Quality Check & remove adapters → Align to the genome → Remove PCR bias: Deduplication (WGBS)

Methylation Calling: uses coverage and quality scores

Remove PCR bias: Coverage Filtering (RRBS)

Check conversion rates

Filtering for known C/T SNPs

Results as Tables, BED files, summary statistics

Differential Methylation

Methylation segmentation

Visualization & Clustering/PCA

Wreczycka et al., Journal of Biotechnology, 2017
RRBS: Reduced Representations Allow Enrichment of CpG Dinucleotides

98% of the genome
1 CpG/100bp
majority methylated

<2% of the genome
1 CpG/10bp short stretches (~1000bp)
majority unmethylated
5-mC detection with the Infinium BeadChip

**Pros**
- Relatively inexpensive
- Single-base resolution
- Internal quality controls
- Highly reproducible ($r > 0.98$)
- PCR-free protocol
- Works with FFPE samples

**Cons**
- Only covers a subset of the methylome
- Bisulfite treatment can damage DNA
- Dependent on bisulfite conversion
### 5-mC detection with the Infinium BeadChip

<table>
<thead>
<tr>
<th>Array</th>
<th>Year released</th>
<th># of Sites</th>
<th>Targeted sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>HumanMethylation2 7k</td>
<td>2008</td>
<td>&gt; 27k</td>
<td>• &gt;14K RefSeq genes</td>
</tr>
<tr>
<td>HumanMethylation4 50k</td>
<td>2011</td>
<td>&gt; 450k</td>
<td>• 99% of RefSeq genes</td>
</tr>
<tr>
<td>Infinium MethylationEPIC</td>
<td>2015</td>
<td>&gt; 850k</td>
<td>• &gt; 90% of sites in the 450k</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• FANTOM5 and ENCODE enhancers (350k sites)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• CpG sites outside of CpG islands</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• ENCODE open chromatin</td>
</tr>
</tbody>
</table>

**Context of the new EPIC probes**

![Image of Infinium BeadChip](image.png)
Direct detection of modified nucleotides
Single-molecule real-time (SMRT) sequencing

SMRT sequencing discriminates between different bases by analyzing variations in polymerase kinetics

**Pros**
- Single-base resolution
- Measures absolute levels of many modified nucleotides
- "Raw" DNA is used
- Long reads

**Cons**
- Suboptimal accuracy
- Low throughput

Genome-wide detection of cytosine methylation by single molecule real-time sequencing

O. Y. Olivia Tse\(^{a,b,1}\), Peiyong Jiang\(^{a,b,1}\), Suk Hang Cheng\(^{a,b,1}\), Wenlei Peng\(^b\), Huimin Shang\(^b\), John Wong\(^a\), Stephen L. Chan\(^a\), Liona C. Y. Poon\(^1\), Tak Y. Leung\(^1\), K. C. Allen Chan\(^{a,b,1}\), Rossa W. K. Chiu\(^{a,b}\), and Y. M. Dennis Lo\(^{a,b,2}\)

Figure adapted from Clark et al., *Nucleic Acids Research* (2011) | Kinney et al., *Epigenetic Alterations in Oncogenesis* (2013)
Nanopore sequencing

Nanopore amperometry methods can discriminate between C, 5-mC, and 5-hmC due to differences in current profiles.

- **Pros**
  - Single-base resolution
  - Measures absolute levels of many modified nucleotides
  - “Raw” DNA is used
  - Long reads

- **Cons**
  - Suboptimal accuracy
  - Low throughput
Modern DNA Methylationomics

Methylation-sensitive Restriction Digest

5-meC Antibody Immunoprecipitation

Methylated DNA Affinity Column

Bisulfite (C to T; mC to C)

5-meC Antibody Immunoprecipitation

Methylated DNA Affinity Column

Bisulfite (C to T; mC to C)

MRE-seq

MeDIP-seq

MBD-seq (MethylMiner)

MethylC-seq

RRBS

Direct sequencing (Pol. kinetics)

SMRT Nanopore

Sequencing Platform

Detection Reagent
Technologies for Interrogating Chromatin States

ChIP-seq

Chromatin IP

input

crosslink
sonicate

Zhang et al. Genome Biol 2008

σ = 120 bp

Location with respect to the center of Watson and Crick peaks (bp)

Tag percentage (%)
Chromatin-IP Sequencing

- K4me1
- K4me2
- K4me3
- K27me3

A. Align reads

B. Infer positions of ChIP fragments

C. Count fragments at each genomic position

Alignable

ES (V6.5)

H3K4me3

Oct4/ Tcf19/Cchcr1

H3K27me3

Psors1a

“active”

“repressive”
Histone methylation and transcriptional state

**Transcribed gene**
- H3K4^me3
- H3K36^me3

**Silent developmental gene**
- K4me3
- K27me3

**Constitutive heterochromatin**
- K9me3
- K20me3

**'Poised' developmental gene**
- K4me3
- K27me3

FoxP1

Olig1

Histone methylation and transcriptional state
Mapping nucleosome positions

http://www.plosgenetics.org/article/info:doi/10.1371/journal.pgen.1003036
Genomic distribution of nucleosomes

• The presence of NFRs demonstrated that open promoter states are stable and common, even at genes that are transcribed so infrequently

Cizhong Jiang, B Franklin Pugh, Nature Review Genetics, 2009 vol. 10 (3) pp. 161-72
Genes, regulatory DNA, and epigenetic features - promoters - enhancers - silencers - insulators - etc.
Digital DNaseI profiling

Precise delineation of the accessible regulatory DNA compartment
Digital DNaseI profiling: direct access to regulatory sequences
ATAC-seq
(assay for transposase-accessible chromatin)
Other interesting topics

• RNA component
  • RNAi, miRNA, X inactivation, HOTAIR, PiwiRNA
  • RNA methylation

• Reprogramming

• Cloning

• Population epigenetics

• Evolution of DNA methylation

• Evolution of epigenome
THE EPIGENETICS ALIGNMENT CHART

**MECHANISM PURIST**
(Epigeneitics must be chemical modifications on top of DNA)

**MECHANISM NEUTRAL**
(Epigeneitics must be functional changes to the genome)

**MECHANISM REBEL**
(Epigeneitics are any change that doesn’t affect nucleotide sequence)

**TIMESCALE PURIST**
(Epigeneitics must persist across generations of organisms)

**TIMESCALE NEUTRAL**
(Epigeneitics must persist across cell division)

**TIMESCALE REBEL**
(Epigeneitics can persist for any amount of time)

Imprinting is epigenetics

X inactivation is epigenetics

Histone modifications are epigenetics

Transposable element silencing is epigenetics

Chromosome positioning is epigenetics

Chromatin accessibility is epigenetics

RNAi is epigenetics

Mitotic bookmarking by TFs is epigenetics

RNAs and proteins are epigenetics

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Engaging today’s epigenomic technologies and resources
Accessing the community resource

http://epigenomemegateway.wustl.edu
The Reference Human Epigenome