Medical Genomics Lecture 4: Epigenomics

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International Agency for Research on Cancer
World Health Organization

Outline

Part 1: Introduction to epigenomics

- **Concepts**: role of epigenetics, mechanisms, cancer epigenetics
- **Techniques**: detecting and measuring epigenetic modifications
- **Resources**: databases of regulatory annotations and epigenetic profiles

Part 2: DNA methylation arrays in cancer

- **Methodology**: processing DNA methylation arrays, normalisation methods
- **Research applications**: principal components analysis, differential methylation, deconvolution
- **Clinical application**: DNA methylation arrays for diagnosis

What is epigenetics?

If all the cells in a human body have essentially the same DNA, why do they divide, grow and function in an organ and tissue-specific way?

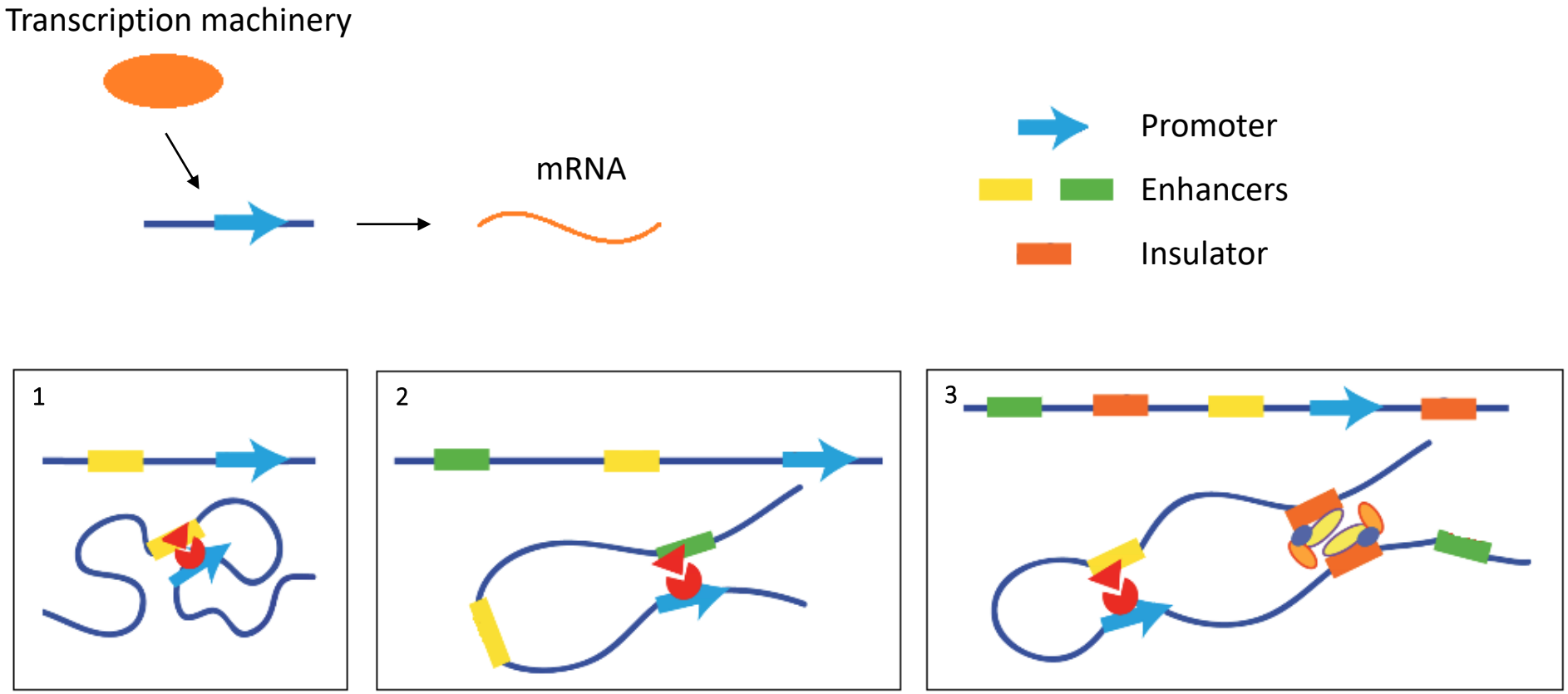
One of the mechanisms through which this occurs is **epigenetics**

Epigenetics is the study of modifications made to DNA and associated factors that:

- Do not change the DNA sequence itself
- Are maintained during cell division
- Cause stable changes in gene expression

What are these modifications?

Mechanisms of gene expression: regulatory elements



Promoters

Contain the TSS, RNA polymerase binding site and other transcription factor binding sites

Enhancers

Bind transcription factors to increase the likelihood of, or boost, transcription

Adapted from: Matharu N & Ahituv N. *PLoS Genetics* 2015; 11(12): e1005640

Mechanisms of gene expression: chromatin structure

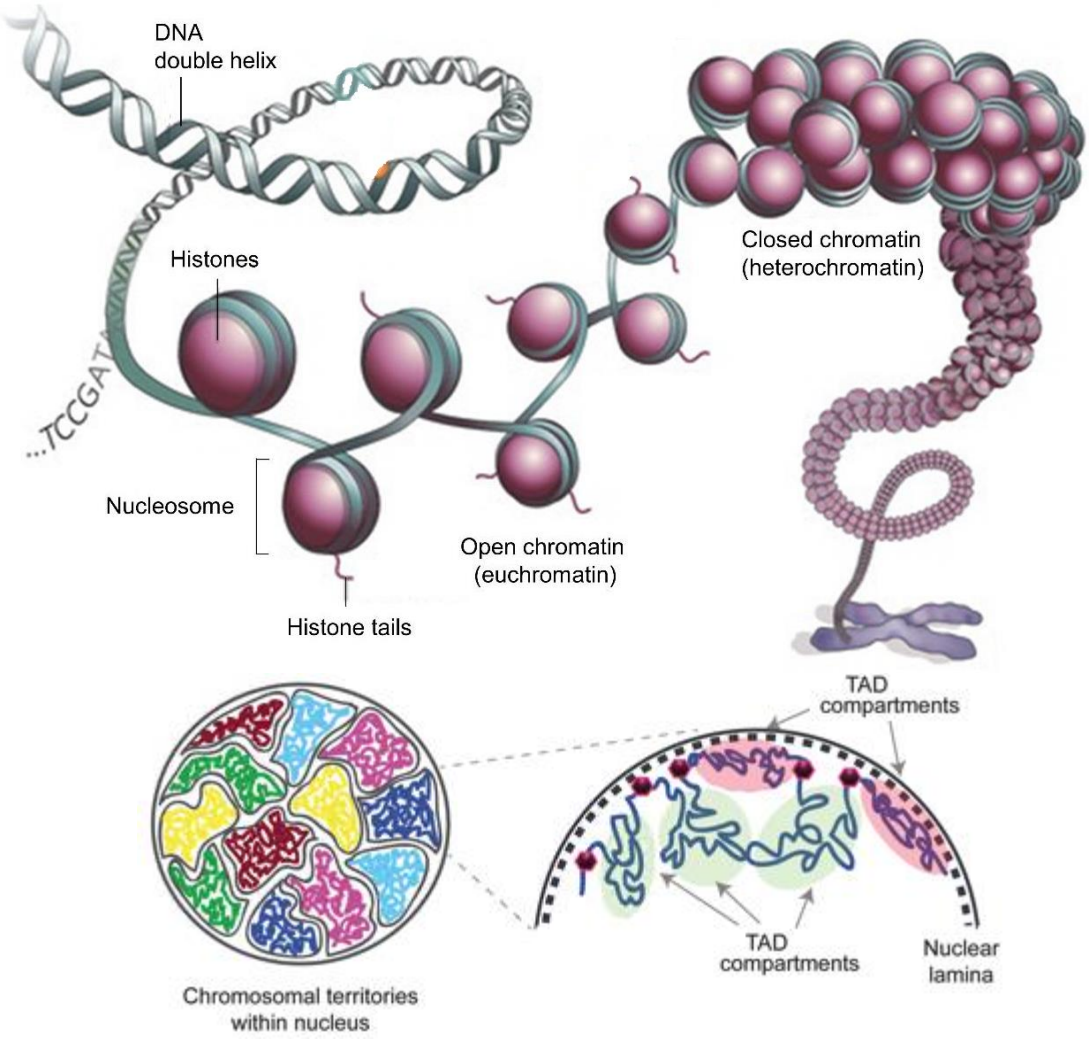
Chromatin folding is required to squeeze 2m of DNA into the cell nucleus

Primary structure

- Double helix winds around histone octamers to form the nucleosome
- A nucleosome contains 8 histone proteins (H2A, H2B, H3 and H4)
- Euchromatin contains space for transcription machinery

Higher-order structure

- The way in which chromatin loops and folds over itself into topological associated domains and chromosomal territories within the nucleus



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Adapted from: Marx V. *Nature* 2012; 491(17422): 143-147

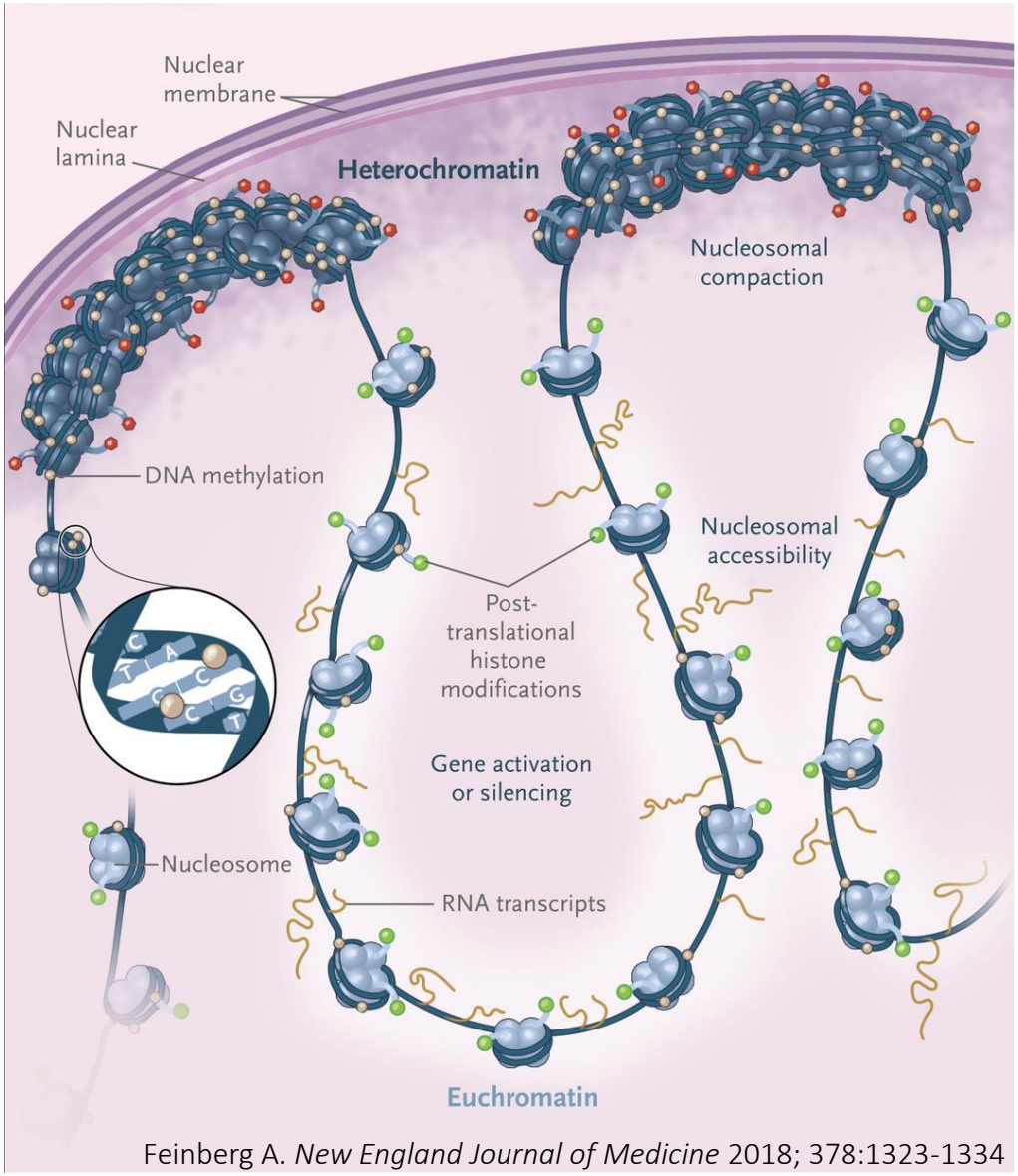
Adapted from: Matharu N & Ahituv N. *PLoS Genetics* 2015; 11(12):e1005640

Types of epigenetic modifications

Chromatin remodelling

Histone modifications

DNA methylation



Feinberg A. *New England Journal of Medicine* 2018; 378:1323-1334

Chromatin remodelling

Changes to primary structure

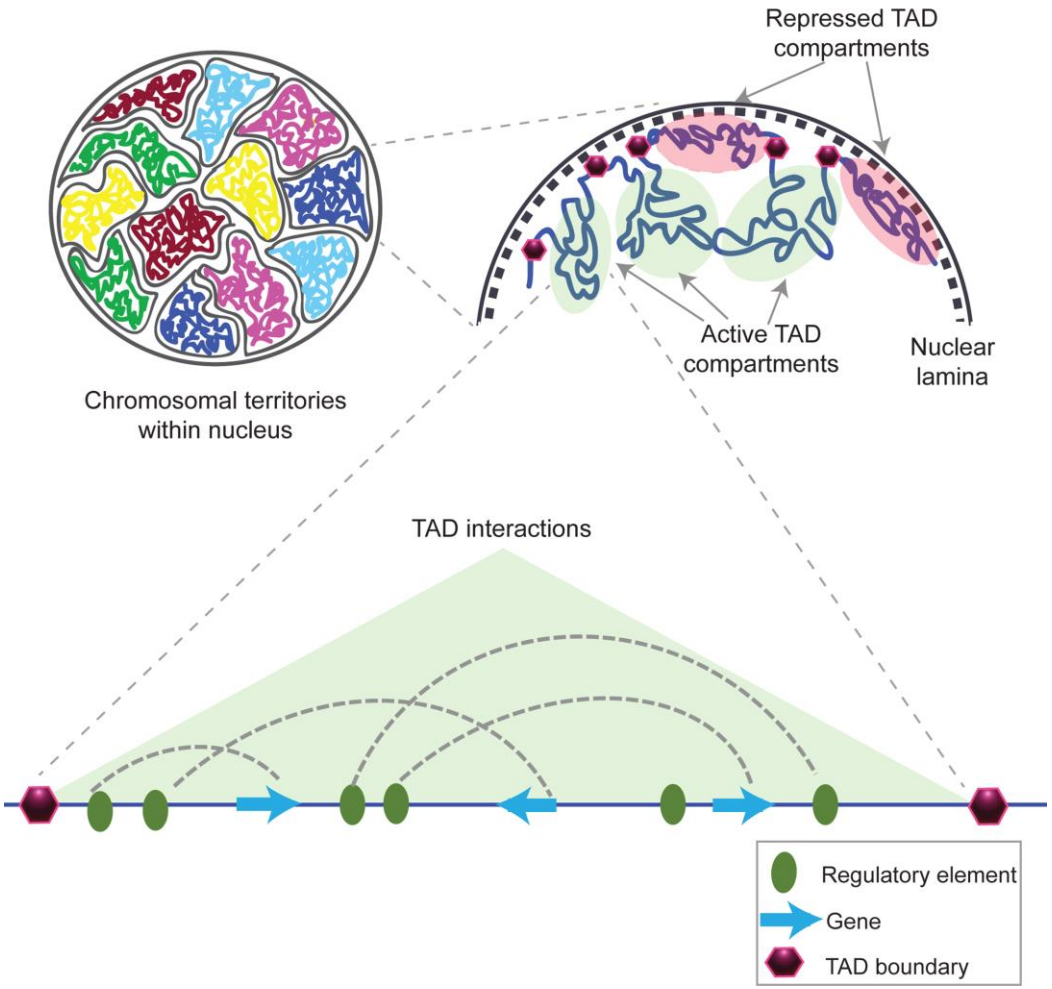
- heterochromatin ↔ euchromatin

Changes to higher-order structure

- Rearrangements of TADs and chromosomal territories
- Brings distant regions together which require the same active/repressed state

Controlled by:

- Histone modifications
- Chromatin remodelling complexes



Matharu N & Ahituv N. *PLoS Genetics* 2015; 11(12):e1005640

Histone modifications

Enzymatic addition and removal of molecules to amino acids on the tails of histones within the histone octamer (nucleosome)

P – phosphorylation

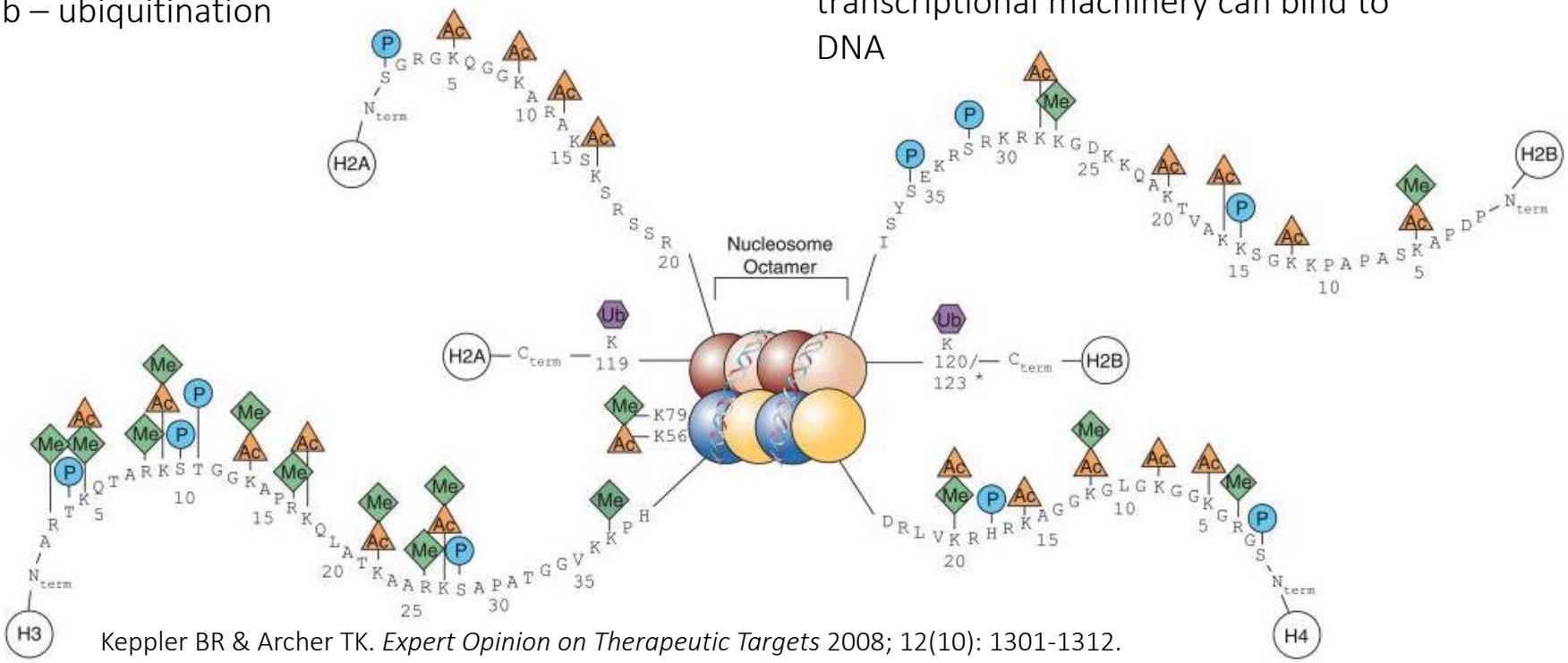
Ac – acetylation

Me – methylation

Ub – ubiquitination

Modifications can

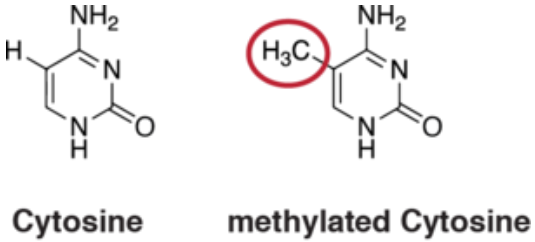
- Disrupt the contact between nucleosomes
- Disrupt the contact between the histones and DNA
- Recruit remodelling proteins
- Therefore change how tightly the chromatin is packed and how easily transcriptional machinery can bind to DNA



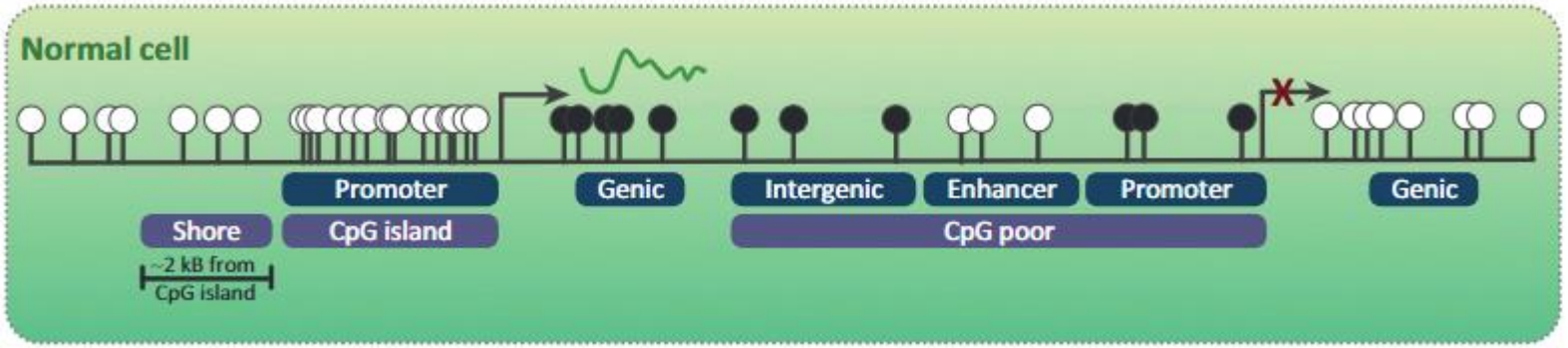
Keppler BR & Archer TK. *Expert Opinion on Therapeutic Targets* 2008; 12(10): 1301-1312.

DNA methylation

Methyl group added to the fifth carbon of a cytosine base, in the context of CG sequences



The effect of DNA methylation on gene expression depends on the location of the methylation



CpG sites in the promoter are typically unmethylated
- Suppression of gene expression is achieved instead through histone modifications
- Promoter hypermethylation does occur in imprinted genes and on the inactive X-chromosome

CpG sites outside the promoter are typically extensively methylated
- Maintains chromosomal stability

Gene bodies are also methylated - even in transcribed genes

DNA methylation maintained by DNTM1, DNMT3a and DNMT3b
Removed by TET family enzymes with the help of IDH

Stirzaker C *et al*. *Trends in Genetics* 2014; 30(2): 75-84

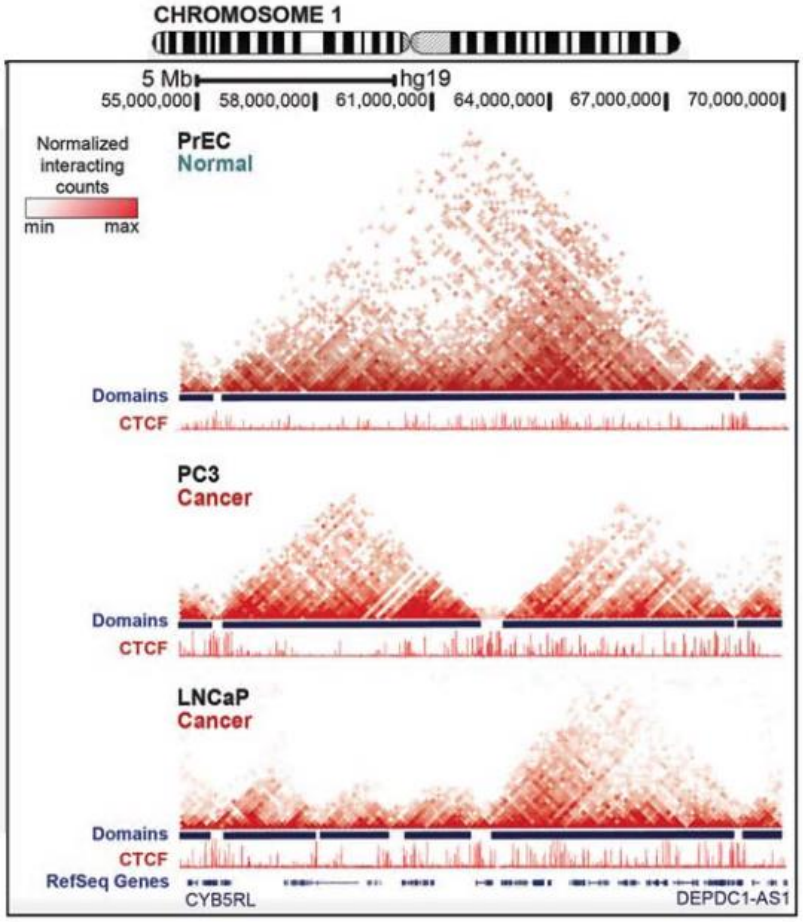
Epigenetics in cancer

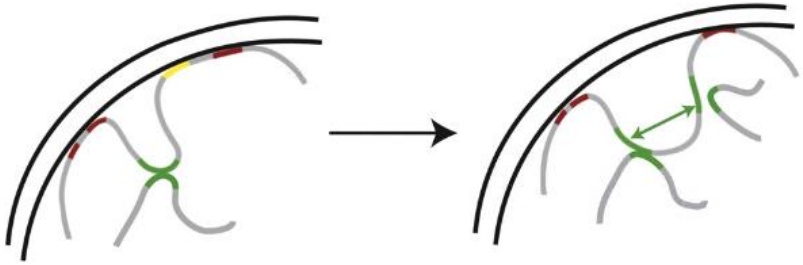
Classical model of cancer development:

- Cancer arises from single cells that accumulate genetic changes during subsequent divisions to form a sub-population of cells with selective advantage over non-tumourous cells
- Genetic changes allow for dysregulation of the cell's proliferative mechanisms, bypassing of checkpoints that regulate cell growth and division, and evasion of immune surveillance = tumour growth and malignancy

- But! Genetic changes aren't the only way a cell can acquire disrupted gene function – epigenetic mechanism also play a key role in the tumourigenic process

Chromatin structure in cancer





A notable feature of cancer cells is abnormal chromatin and nuclear architecture

Cancer cells retain the ability to segment their genomes into topological associated domains

- In cancer these TADs are more numerous and smaller
- Often associated with changes in copy number
- Produce novel cancer-specific chromatin interactions to alter gene expression
- Alterations to TAD structure increases genomic instability

- Mechanisms for change to TADs in cancer is unknown

Taberlay PC *et al*. *Genome Research* 2016; 26: 719-731.
Reddy KL & Feinberg AP. *Seminars in Cancer Biology* 2013; 23: 109-1135.

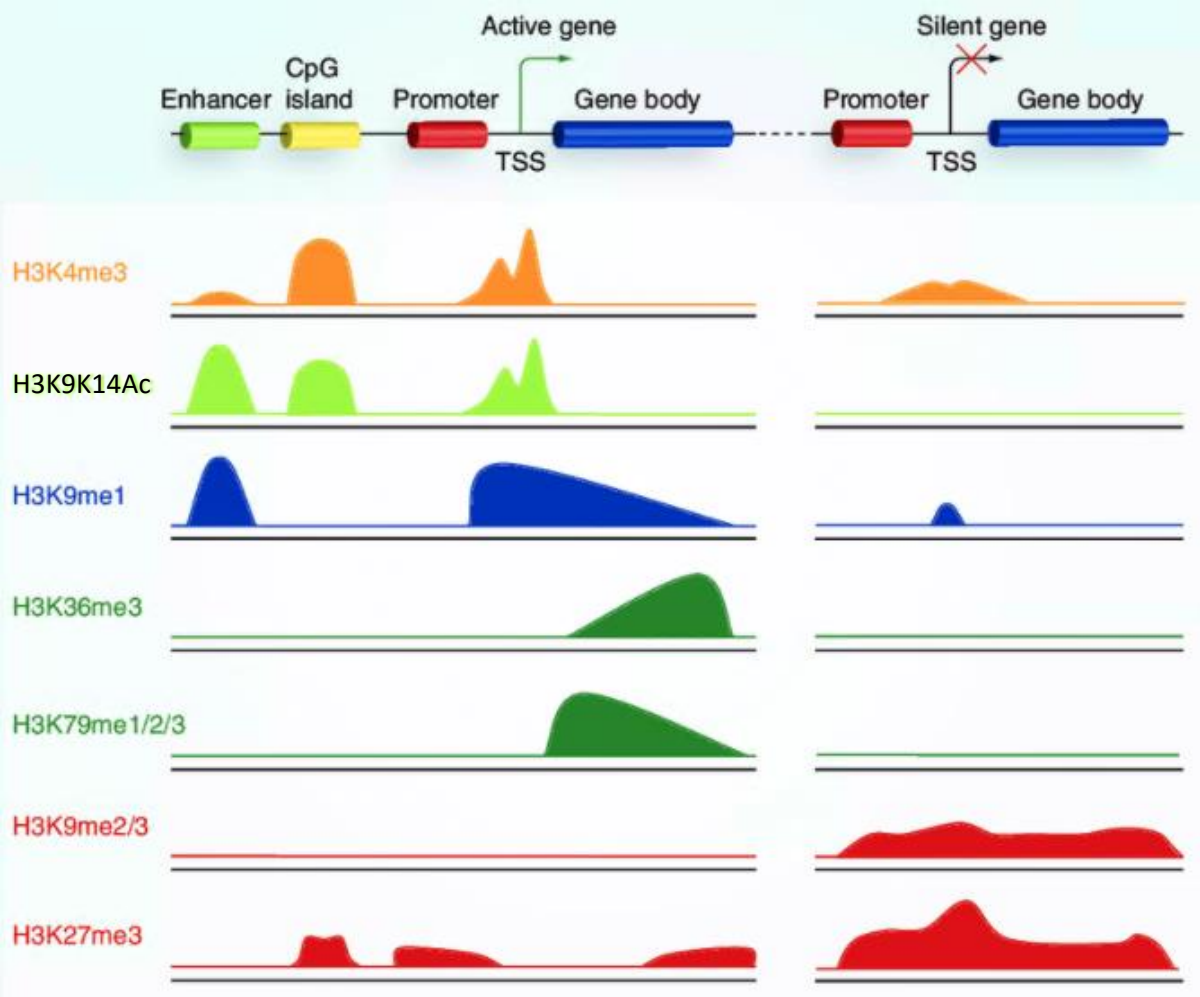
Histone modifications

Histone modifications facilitate or block transcription

Aberrant patterns of histone modifications in cancer result in activation and silencing of genes to promote tumourigenesis

Typically patterns are altered by acquisition of genetic changes to histone modifying enzymes

AML1-ETO fusion protein recruits HDAC1 → transcriptional repression

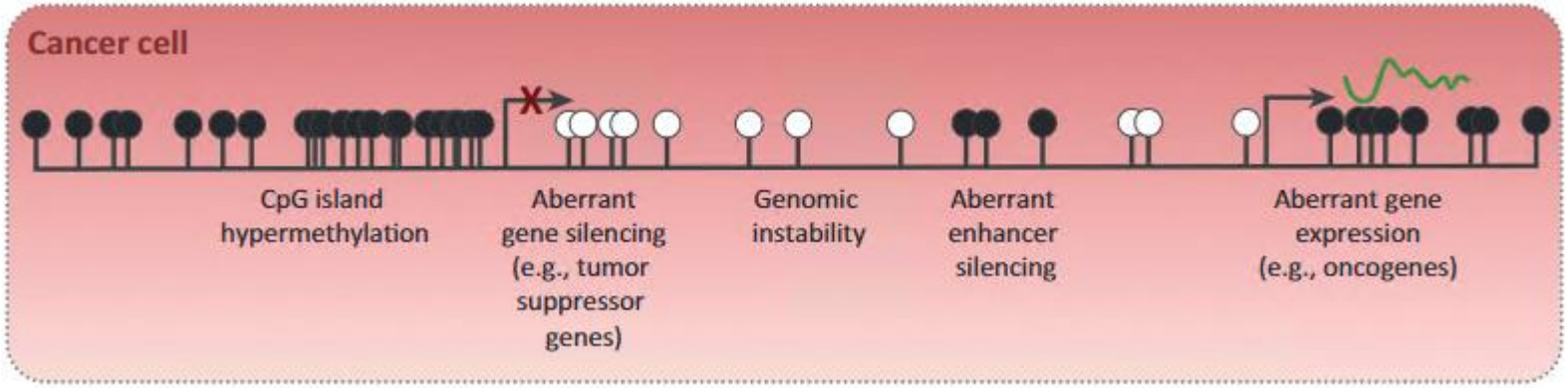


Siew Lim P, Shannon MF & Hardy K. *Epigenomics* 2010; 2(6): 775-795.

DNA methylation in cancer

Nearly all human neoplasms exhibit aberrant DNA methylation patterns

- Genome-wide hypomethylation
- Promoter-specific hypermethylation



Genome-wide hypomethylation

- Reactivation of transposable elements, chromosomal instability, aneuploidy, translocations, loss of imprinting

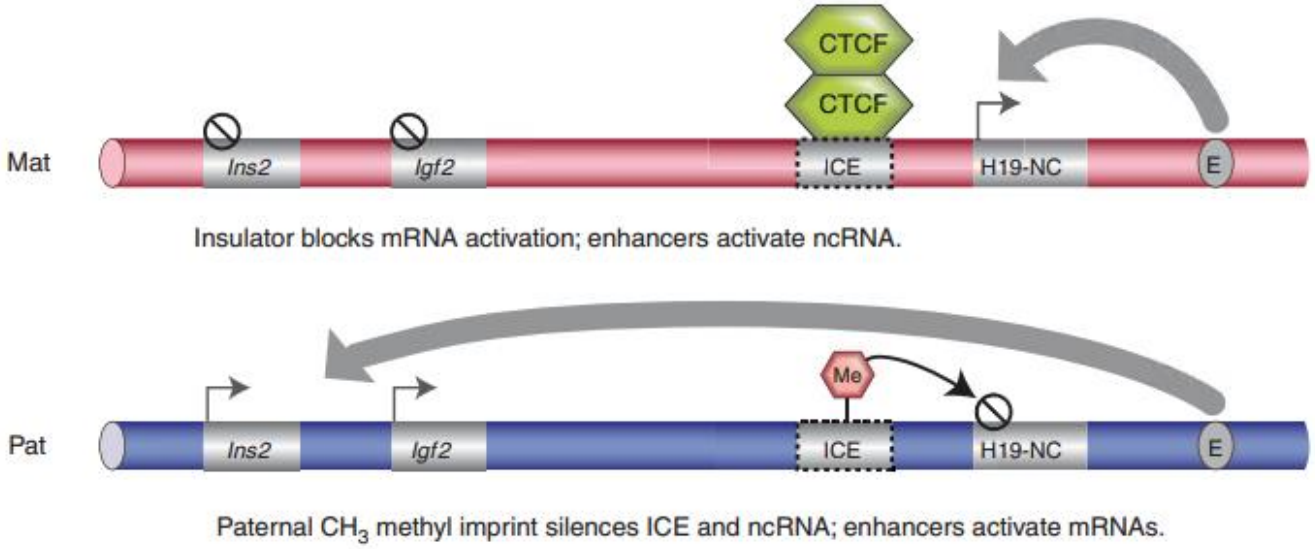
Promoer hypermethylation

- Silencing of tumour suppressor genes (2nd hit in tumourigenesis)

Stirzaker C *et al*. *Trends in Genetics* 2014; 30(2): 75-84

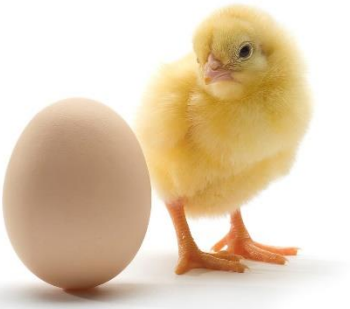
DNA methylation in cancer

Methylation of other regulatory regions such as insulators



Barlow DP & Bartolomei MS. *Cold Spring Harbor Perspectives in Biology* 2014; 6.

Cancer epigenetics



What comes first? Epigenetic or genetic changes…

- Benign tumours and pre-malignant lesions display genome-wide hypomethylation
  - May be triggered by chronic inflammation
- Methylation-induced silencing of TSGs can occur prior to mutations in known driver genes
  - May be triggered by environmental factors such as synthetic hormones, heavy metals and smoking

- However, genetic changes (mutations, CNVs etc) can alter expression of epigenetic modifiers leading to widespread epigenetic changes
  - IDH1/2 mutations in brain cancer → no α-ketoglutarate → no demethylation → hypermethylation of TSGs

How do we detect and measure epigenetic modifications?

Higher-order chromatin structure

- Chromosome conformation capture with 4C, 5C and Hi-C

Primary chromatin structure

- DNase-seq, ATAC-seq

Histone modifications

- ChIP-seq

DNA methylation

- Bisulphite sequencing, DNA methylation array

All of these techniques capture a profile for (mostly) one cell type at one time

- Implications for extrapolating results

Chromosome conformation capture

General principal

- Chromatin is cross-linked using formaldehyde so that DNA regions within spatial proximity are glued together with protein complexes
- The DNA is then fragmented and ends ligated

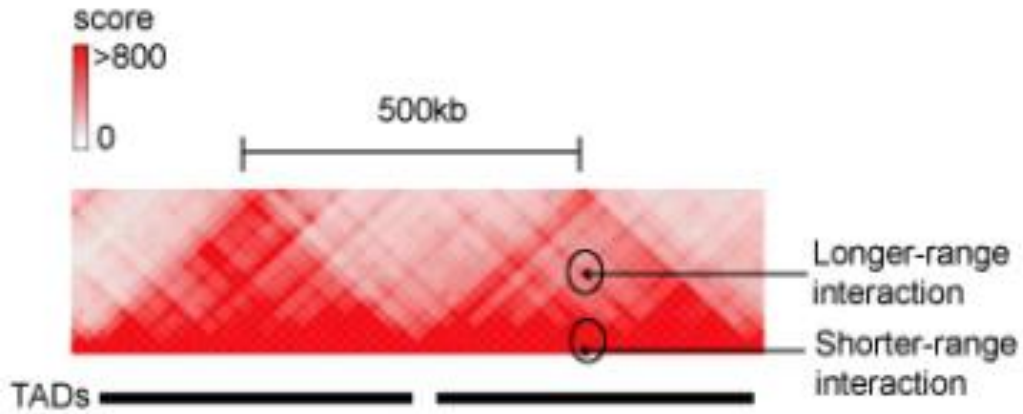
3C – locus-specific primers to amplify and sequence the region of interest

4C – one to all

5C – many to many

Hi-C – all to all

ChIA-PET: chromatin immunoprecipitation interaction assay with paired end tagging – enriching for particular types of interactions



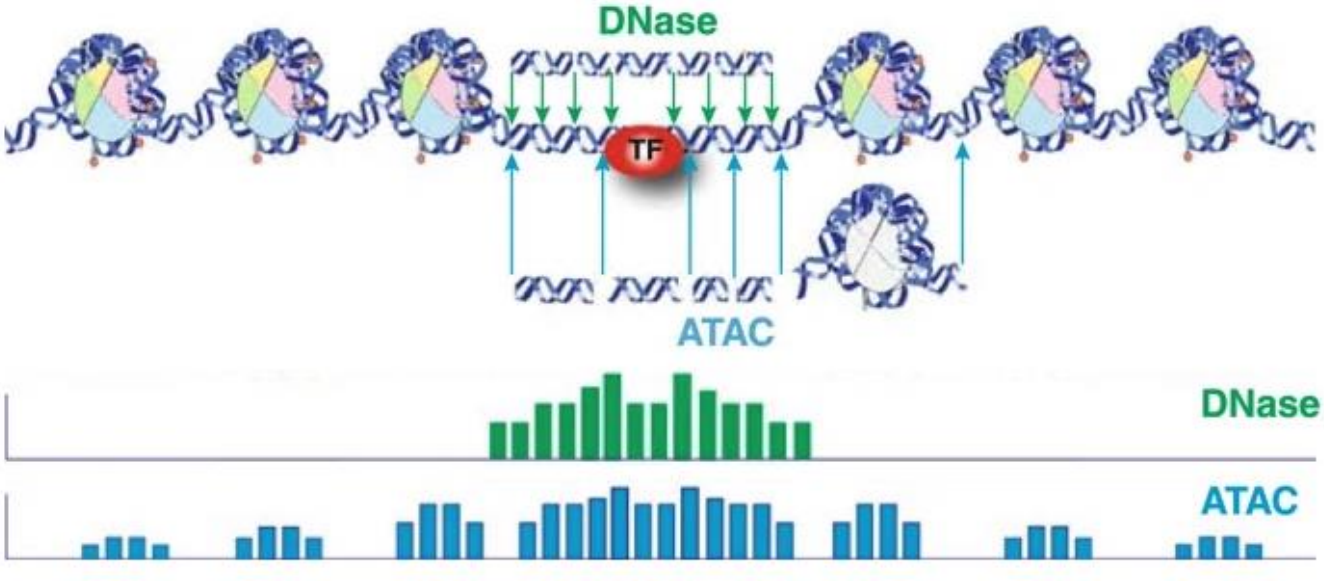
Primary chromatin structure

DNase-seq

- Identifies DNase I hypersensitivity sites
- DNase I hypersensitive sites appear as a result of transcription factor binding and the removal of nucleosomes – in active chromatin

Assay for Transposase Accessible Chromatin (ATAC-seq)

- "Tagmentation" fragmenting and tagging simultaneously with Tn5 transposase
- Identifies sites of nucleosome occupancy and nucleosome-free regulatory regions



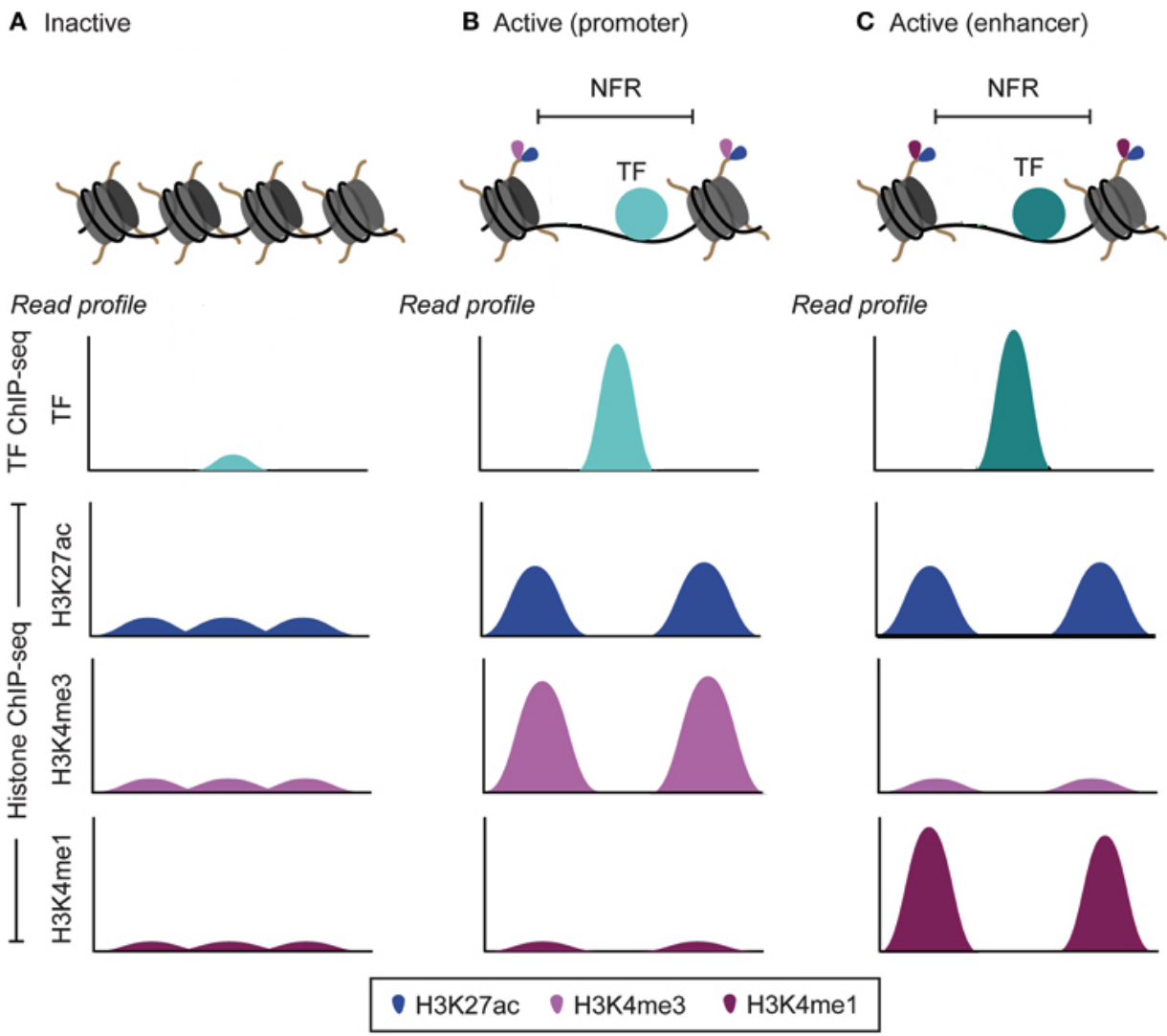
Tsompana M & Buck MJ. *Epigenetics & Chromatin* 2014; 7(33).

Histone modifications

Chromatin immunoprecipitation + sequencing (ChIP-seq)

Identifies all the binding sites for a particular protein (such as a TF), or histone modification

Some regions are promoter and enhancers in virtually all cell types, others can be cell-type specific

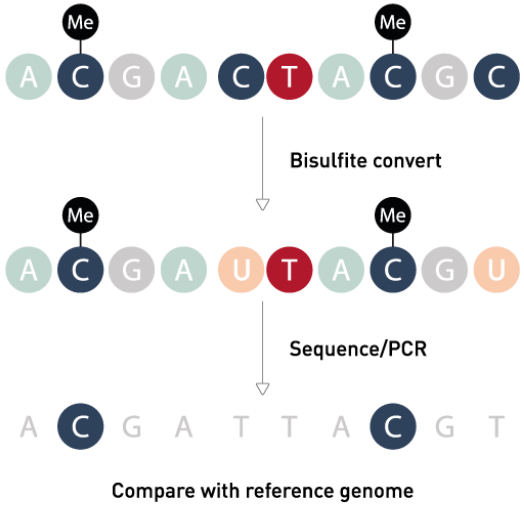


Pundhir S, Poirazi P & Gorodkin J. *Frontiers in Genetics* 2015; 6(188).

DNA methylation

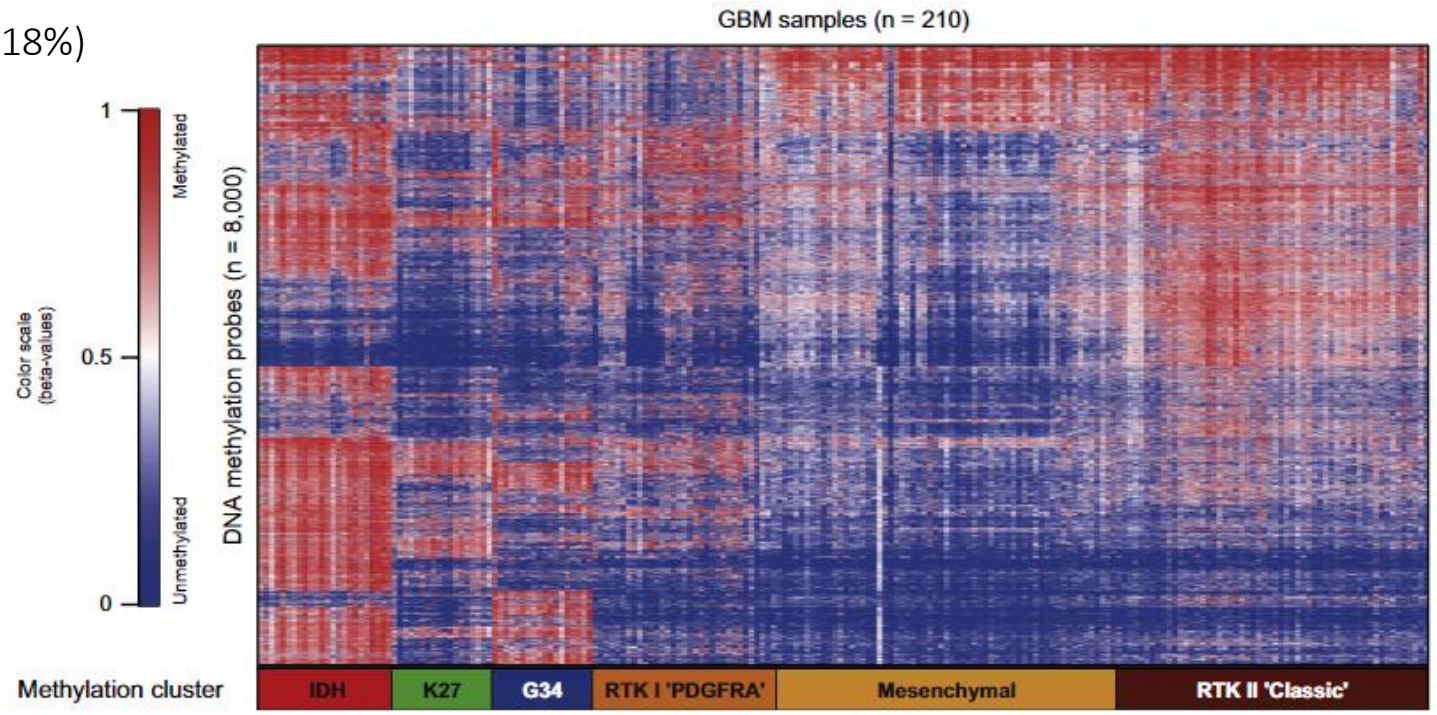
Bisulphite conversion techniques

- Whole genome bisulphite sequencing (~95%)
- Reduced representation bisulphite sequencing (~4%)
- Methylation microarrays (~2%)



Non BC techniques

- MBDCap-seq (~18%)



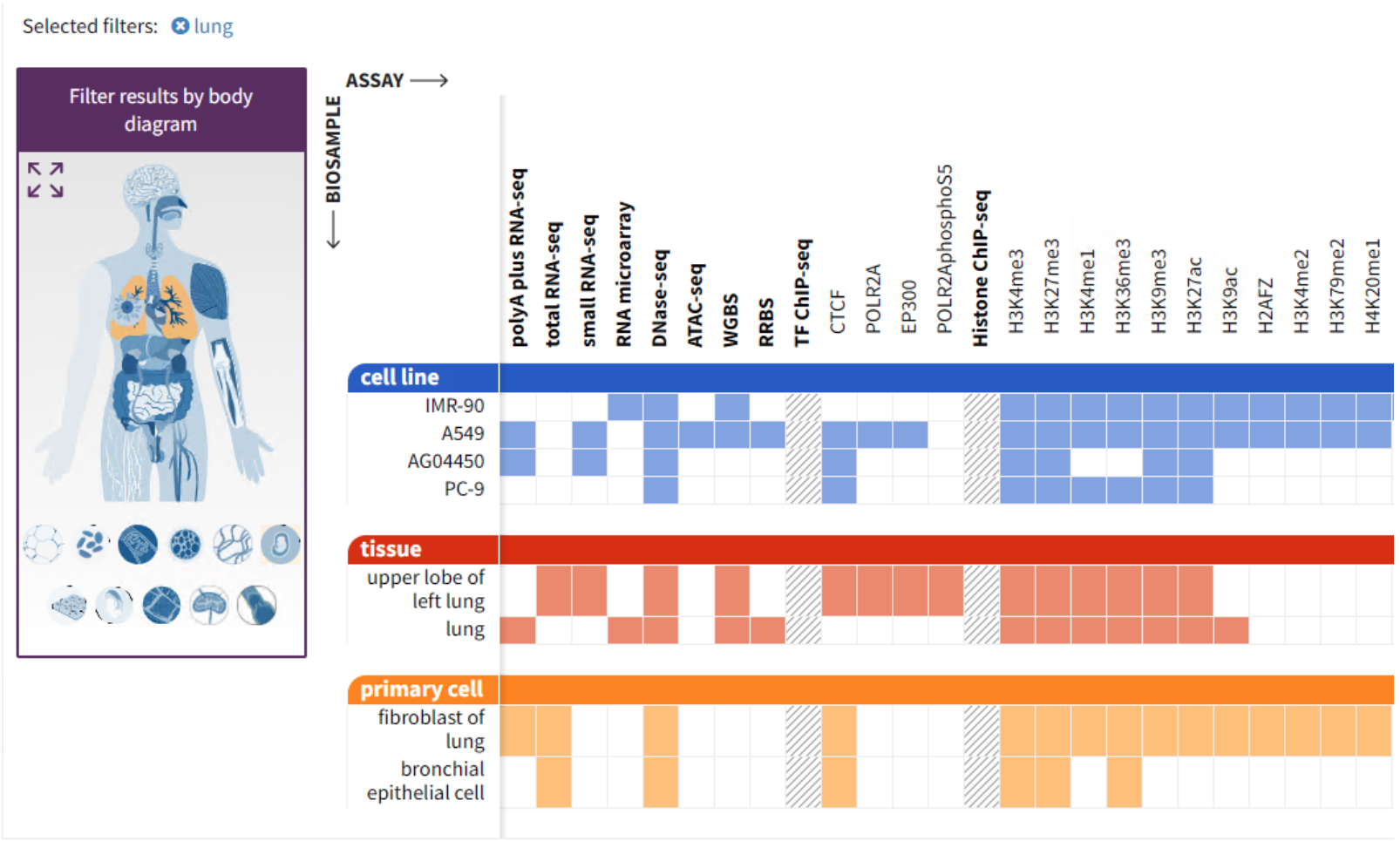
Sturm D *et al. Cancer Cell* 2012; 22(4): 417-418.

Epigenetic profile resources

International Human Epigenome Consortium

- Blueprint, ENCODE, Roadmap

TCGA, GEO



Summary

- Epigenetic mechanisms alter gene expression without changing the underlying DNA sequence
- They are dynamic, responding to the environment, and ensure that a cell behaves the way it's supposed to for its particular tissue type

- Epigenetic mechanisms are disrupted in cancer, they both lead to, and are a product of, cancer development

Outline

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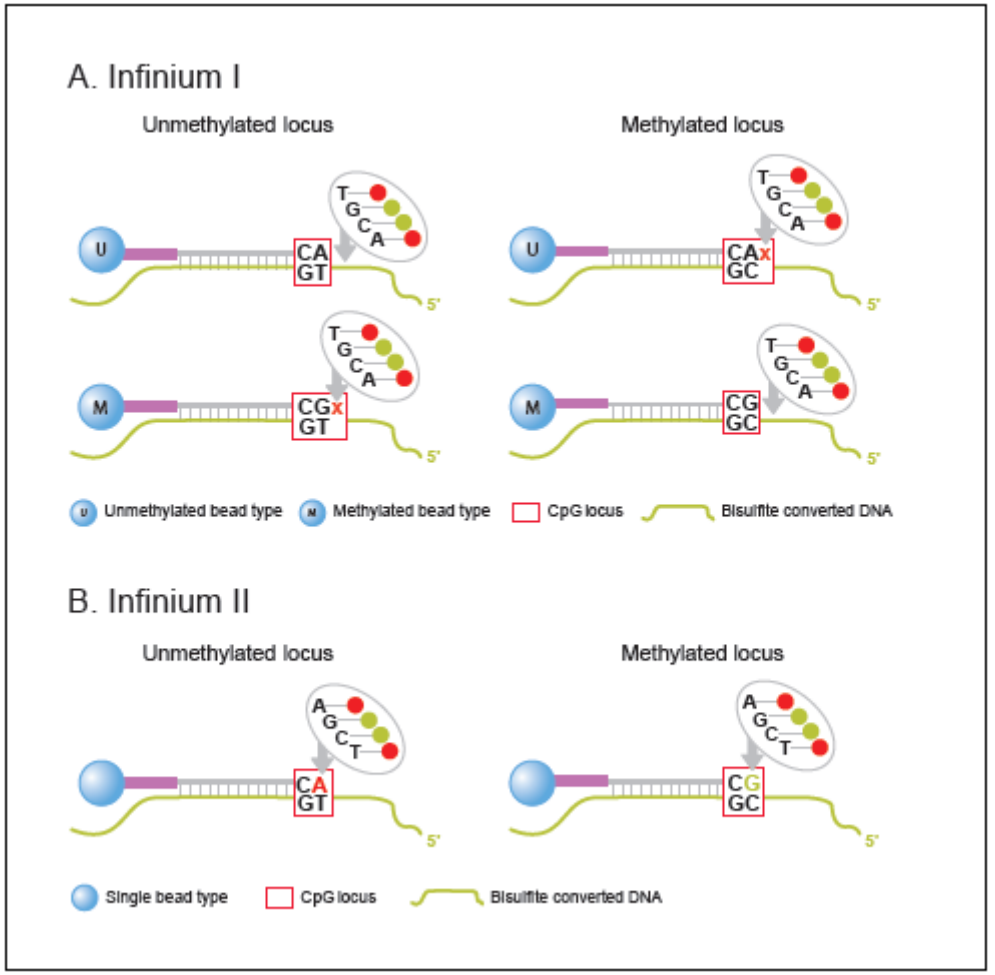
Illumina Infinium HumanMethylationEPIC Array

1. Bisulphite converted DNA is hybridised to oligonucleotide coated beads

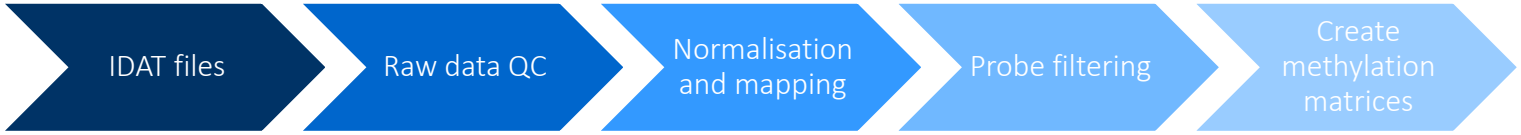
   Two types of beads exist: Infinium I and Infinium II

2. Array is fluorescently stained

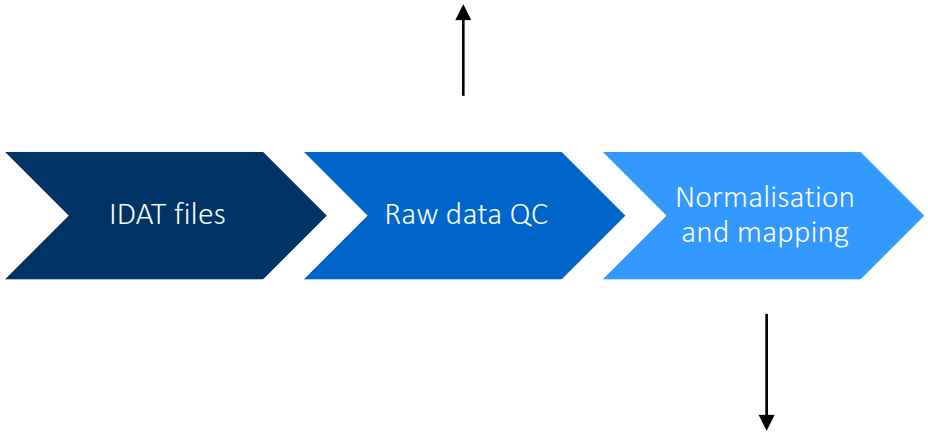
3. Intensity of methylated and unmethylated probe signals captured into IDAT files



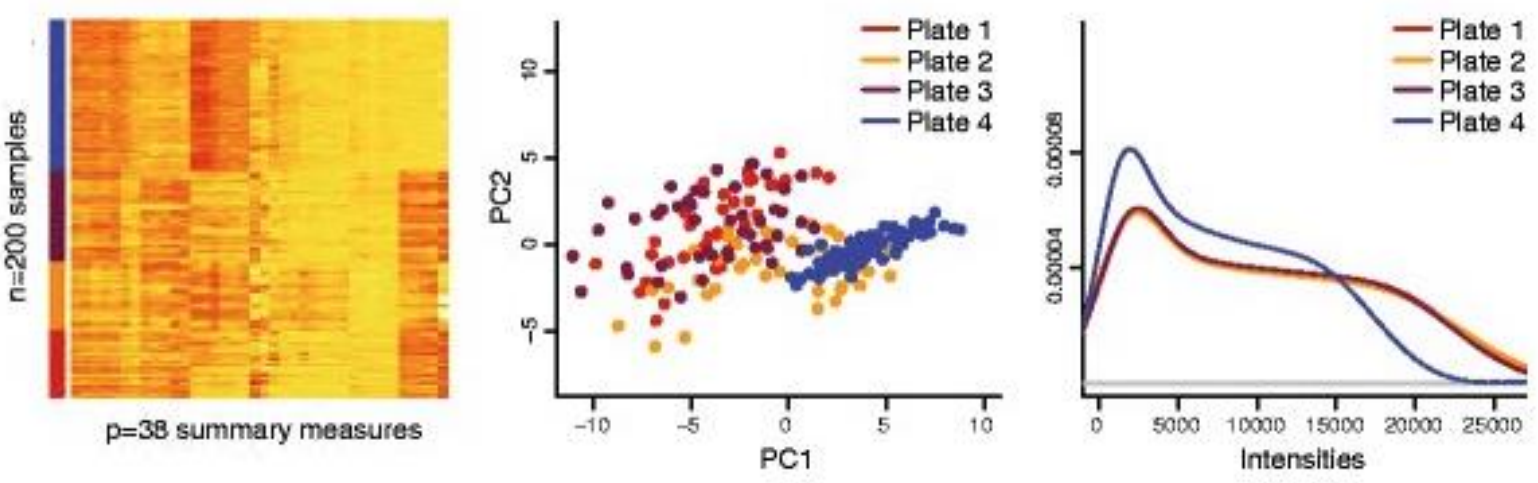
Processing IDAT files to methylation matrices



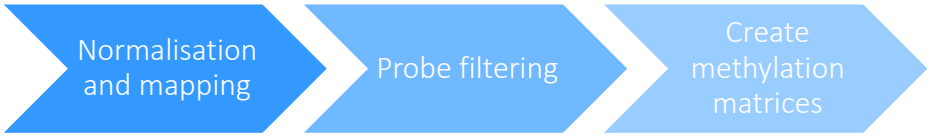
`plotCtrl`: examine efficiency of technical steps
`getQC`: examine signal intensity
`detectionP`: examine probe detection level



Reduce within and between array technical effects



Fortin J-P *et al*. *Genome Biology* 2014; 15(11): 503-520.



Removal of:

- Poor performing probes
- Cross-reactive probes
- SNP-associated probes

- Sex chromosome probes

Beta values

$$\beta = \frac{M}{M+U+100}$$

M values

$$\mathrm{M} = \log_2\left(\frac{M}{U}\right)$$

Replacing –Inf/+Inf values

Applications of DNA methylation array data for research

What can we learn from running tumour samples on DNA methylation arrays for research?

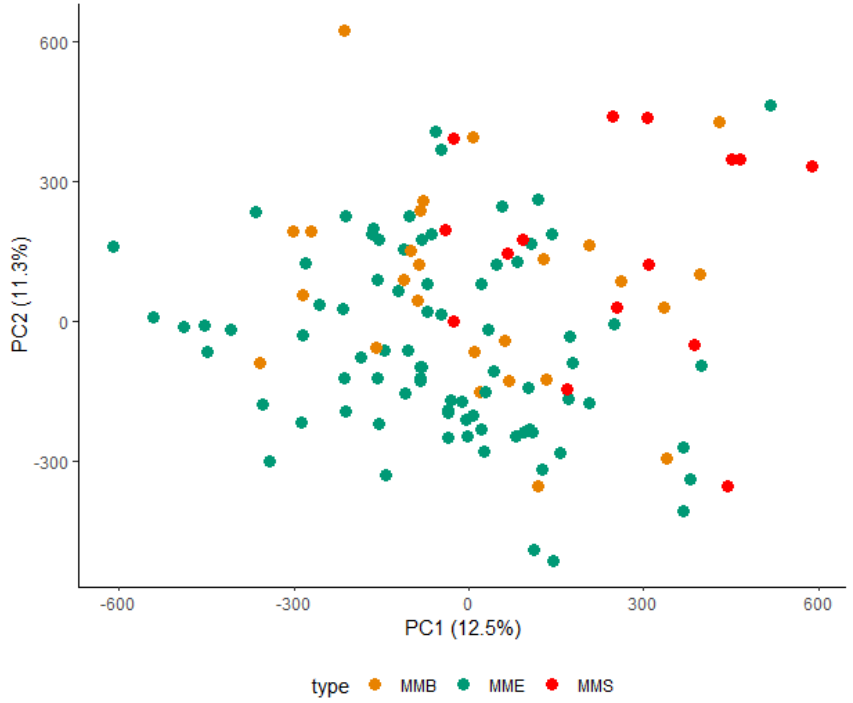
Methods

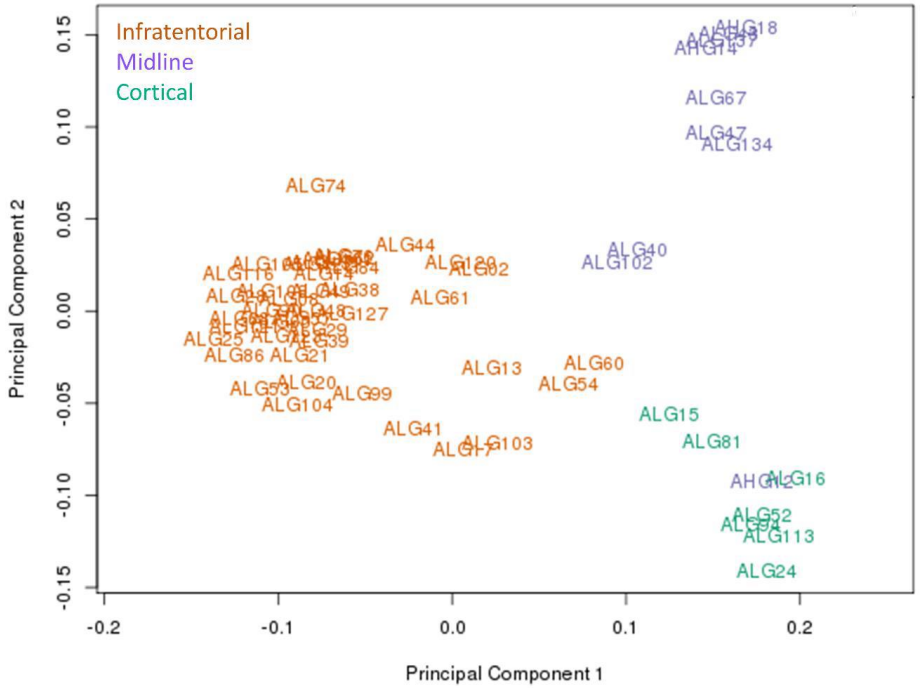
- Principal components analysis and beta density plots
- Identify differentially methylated sites and regions
- Deconvolution of blood and neural cell types
- Estimating tumour purity
- Predicting smoking status

Principal components analysis

PCA allows us to:

- Examine clinical and technical sources of variation within the data
- Identify interesting drivers of DNA methylation profile

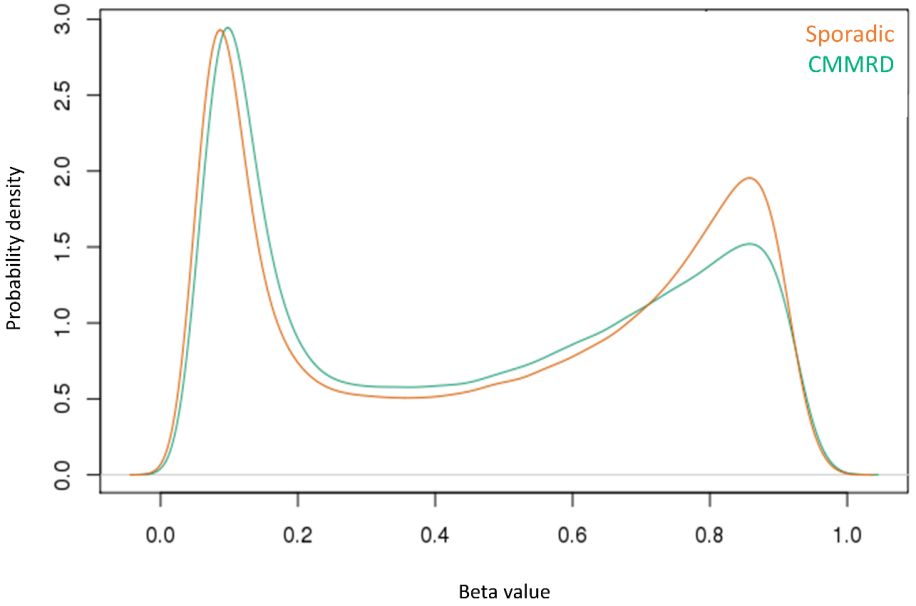


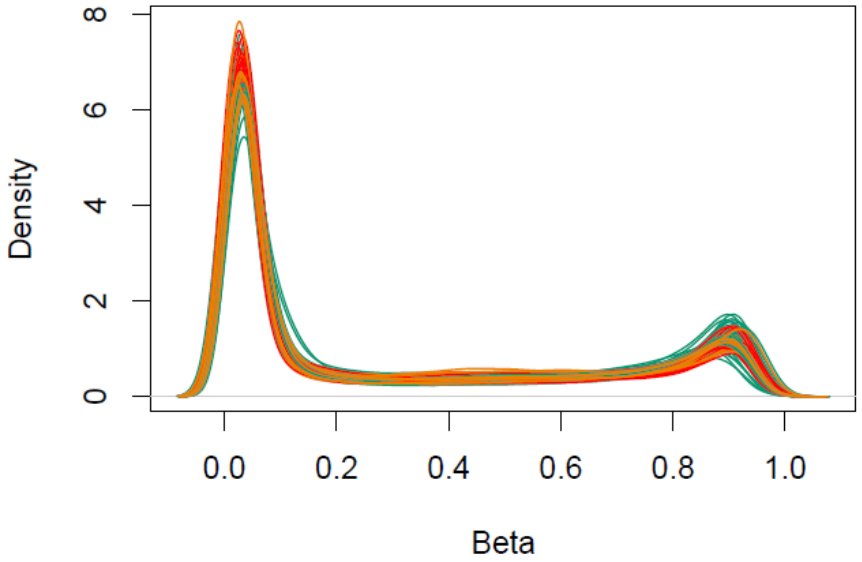


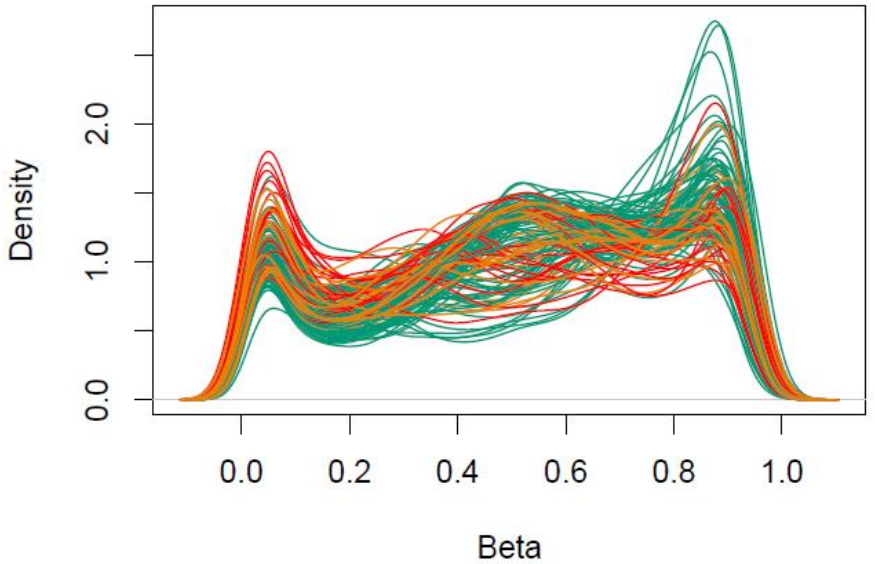
Exploring beta density

Another method to examine global profile is beta density plotting (minfi package)

- Compare between groups
- Compare between regions



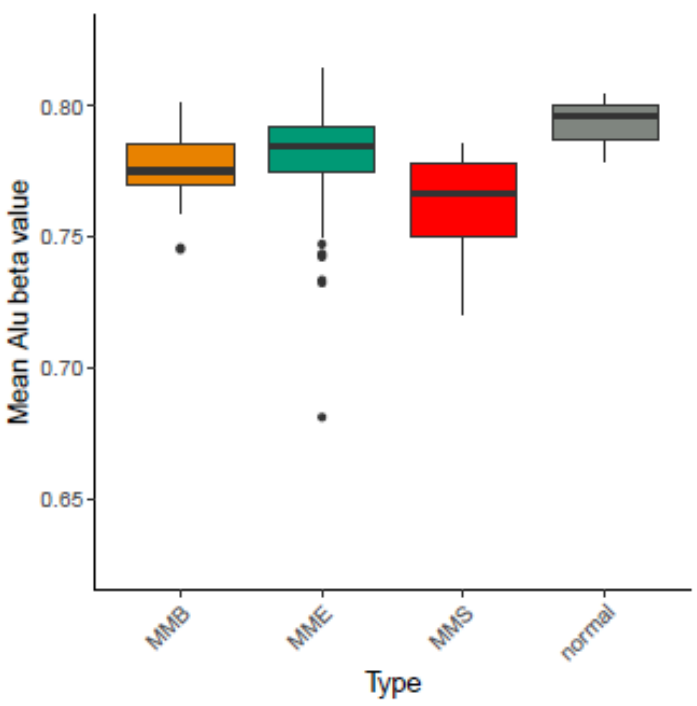


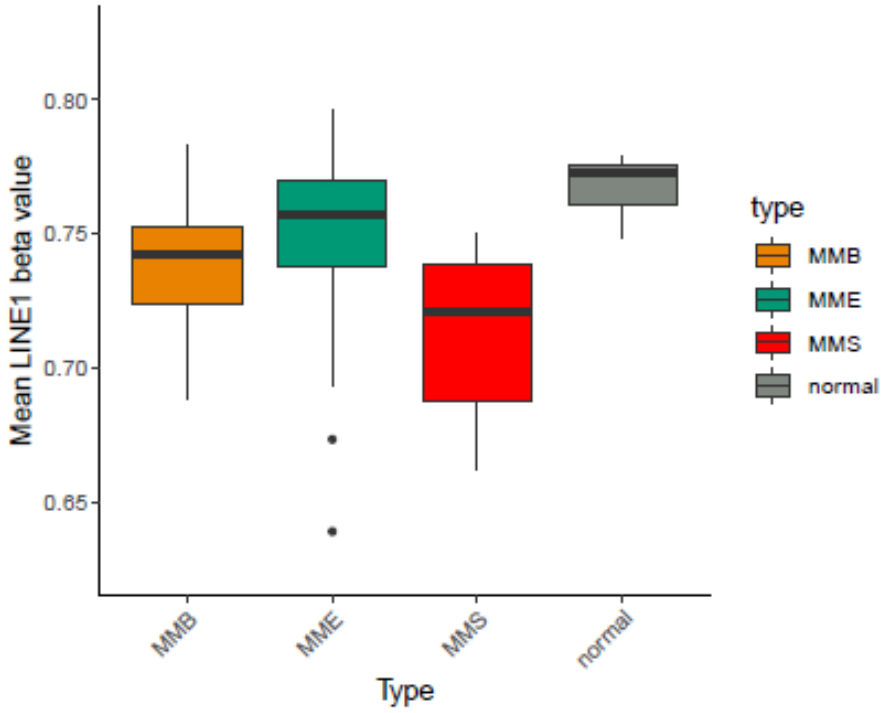


Estimating global methylation

Using REMP (R package) to identify CpG sites within Alu and LINE1 elements

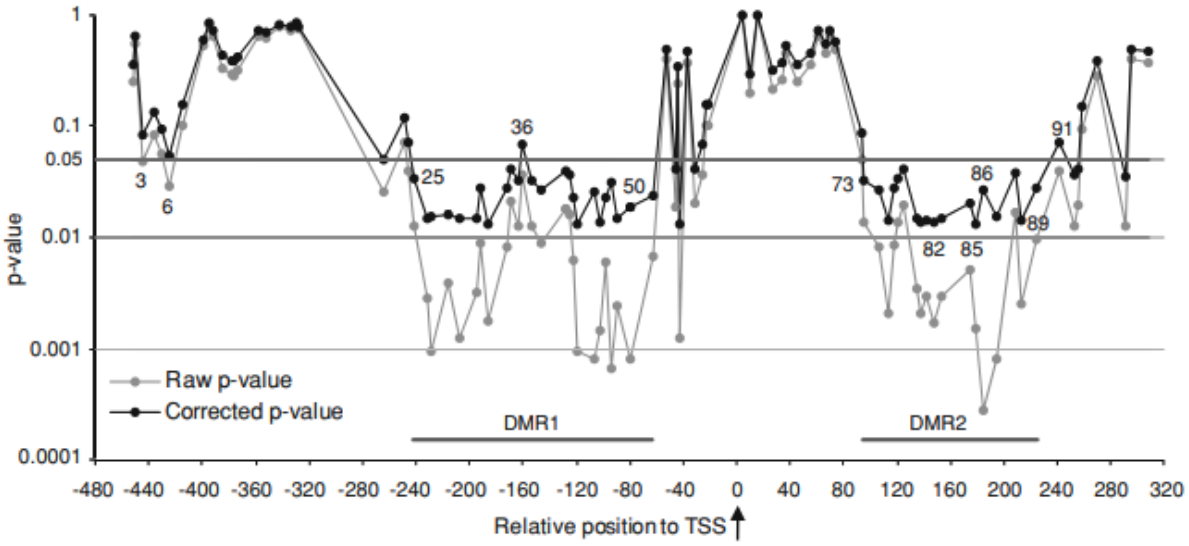
Average methylation at LINE1 or Alu can be used as a proxy for global methylation

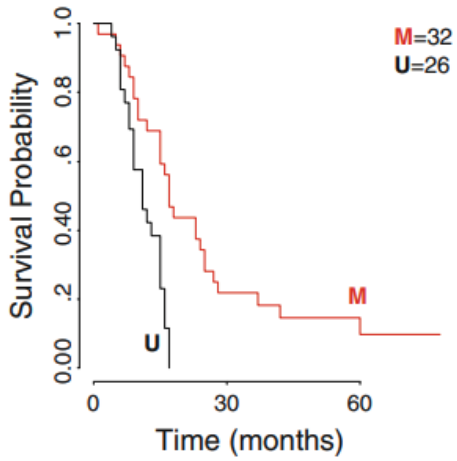


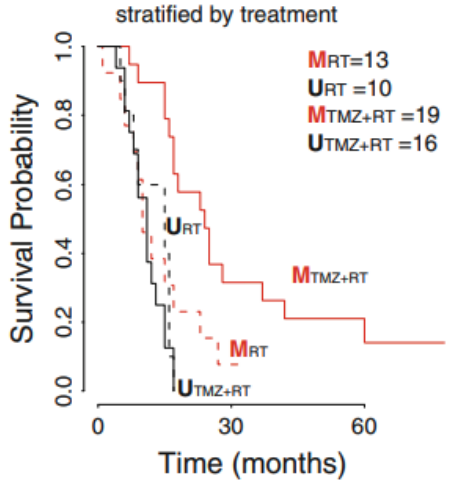


Differentially methylated sites and regions

Use linear regression analysis (limma package) to identify DMPs and DMRs







↑ DMR methylation

↓

↓ MGMT expression

Temozolomide induces DNA alkylation

↓

No repair of DNA damage

↓

Tumour cell death

Malley DS *et al*. *Acta Neuropathologica* 2011. 121: 651-661.

Bady P *et al. Acta Neuropathologica* 2012. 124: 547-560.

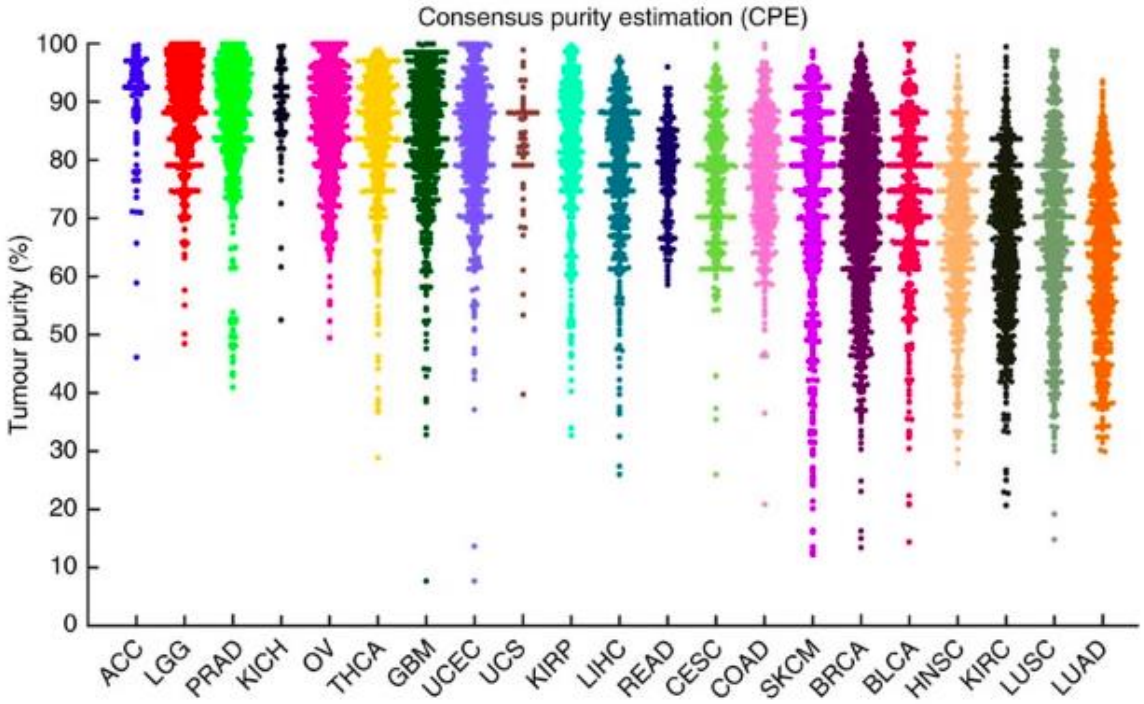
Estimating tumour purity

Tumour purity varies for intrinsic and extrinsic factors, for example:

- Immune and stromal infiltration within a tumour
- Amount of normal tissue surrounding tumour biopsy

LUMP – leukocyte unmethylation to infer tumour purity

- 44 CpG sites as the junction between 30,106 sites consistently unmethylated in leukocytes (< 5%) and 174,696 consistently methylated sites (>30%) in 21 cancer types



- Purity estimate = mean(LUMP 44 sites)/0.85

International Agency for Research on Cancer

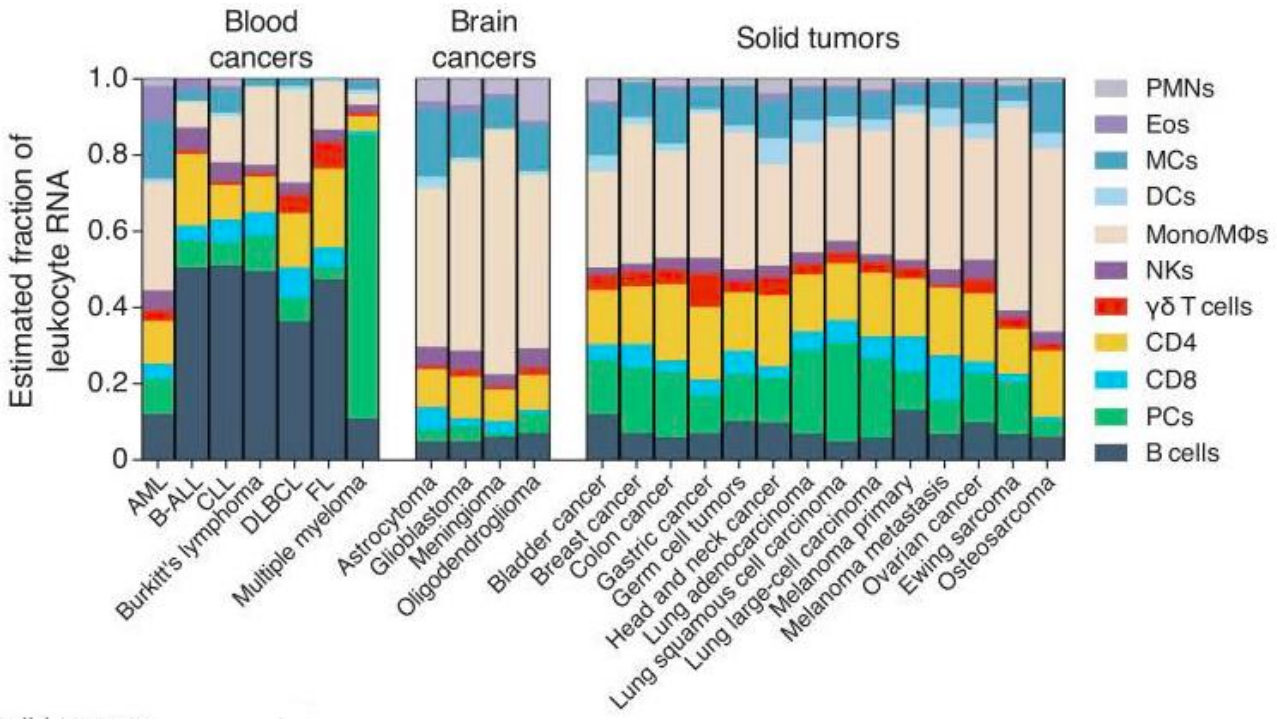
World Health Organization

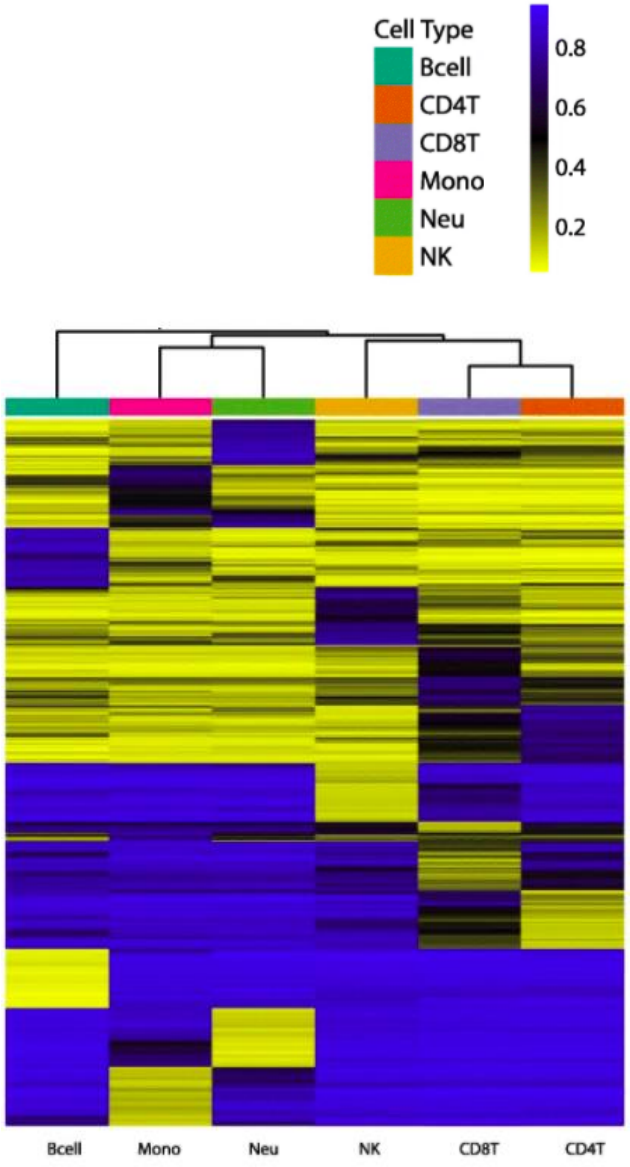
Aran D, Sirota M & Butte A. *Nature Communications* 2015. 6(8971).

Deconvolution of different cell types

Using deconvolution methods (minfi package) we can identify proportions of:

- Whole blood cell types
- Umbilical cord blood cell types
- Frontal cortex cell types





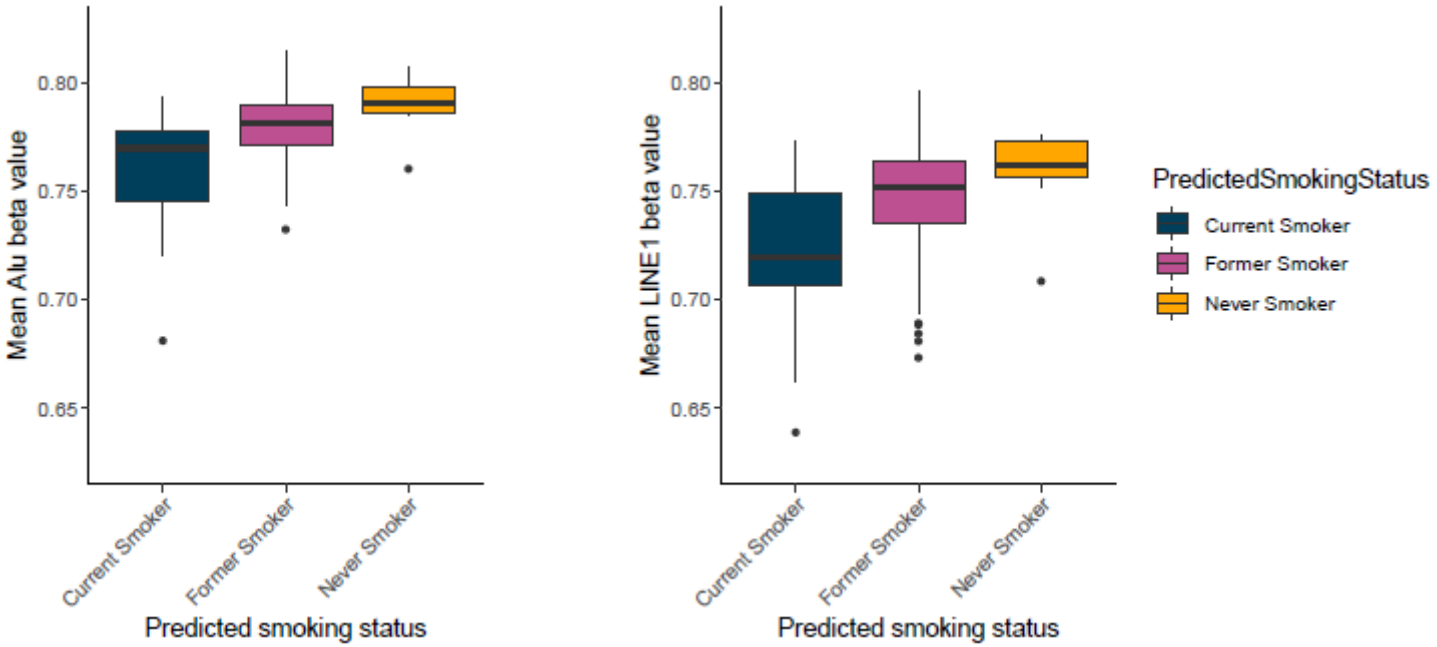
Gentles AJ *et al*. *Nature Medicine* 2015; 21(8): 938-945.

Salas LA *et al. Genome Biology* 2018. 19(64).

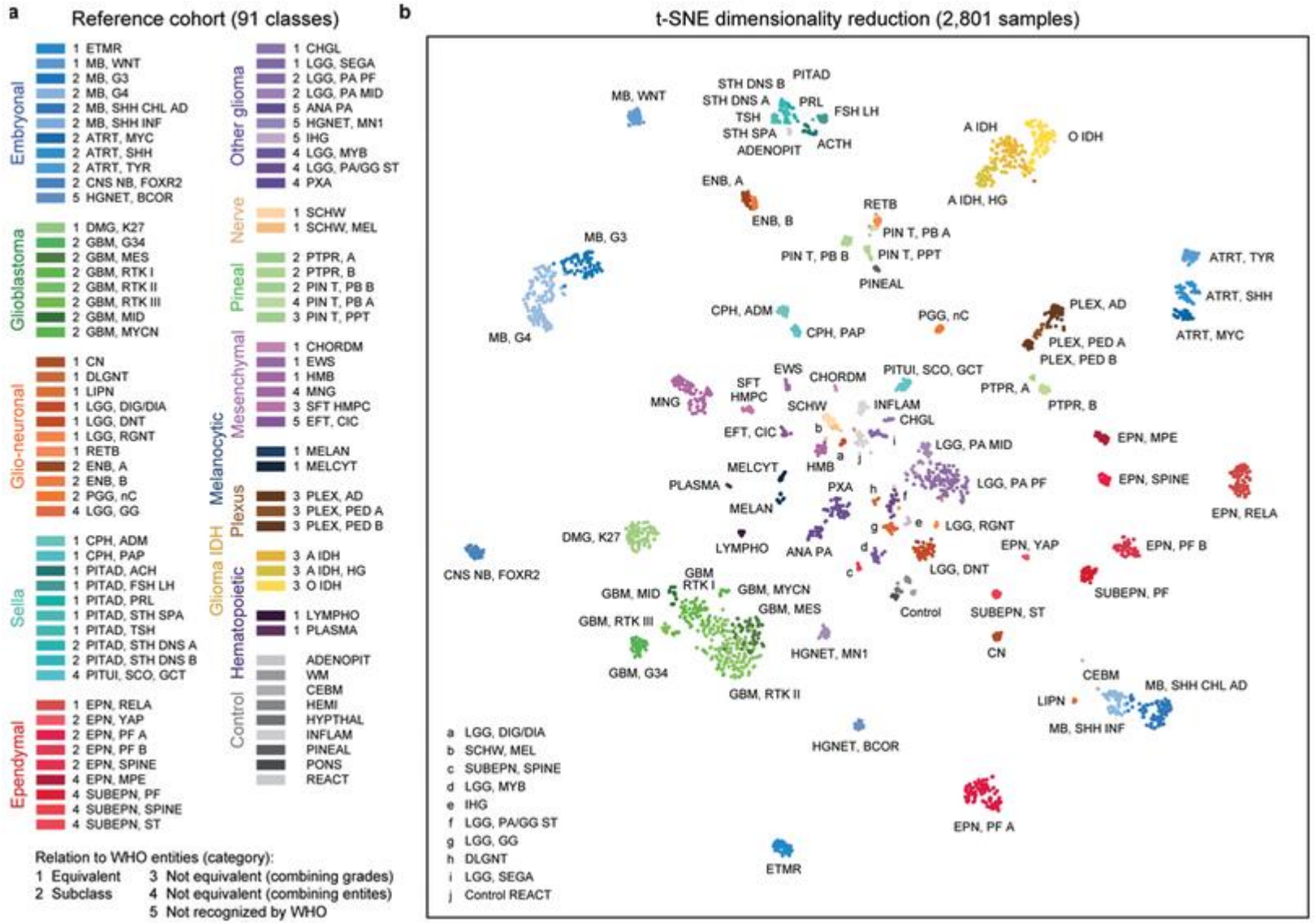
Predicting smoking status

Smoking has a large impact on DNA methylation level in blood, and lung tissue

EpiSmokeR (R package) can be used to predict current, former and never smoking status

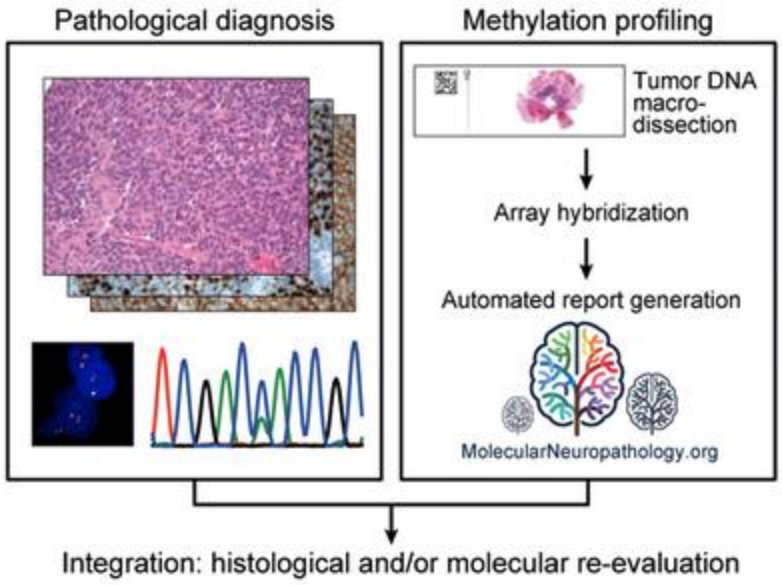


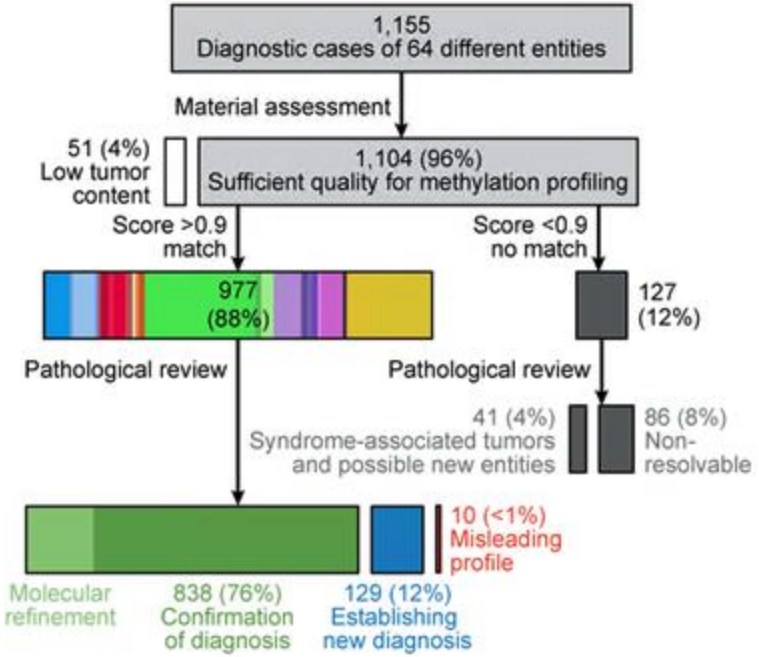
Applications of DNA methylation array data for clinical oncology



Capper D *et al. Nature* 2018. 55(7679): 469-474.

Applications of DNA methylation array data for clinical oncology





Capper D *et al. Nature* 2018. 55(7679): 469-474.

Summary

- DNA methylation arrays are a useful tool in cancer research and clinical oncology